Print: ISSN -2424-6921
Online: ISSN - 2424-693X

International Journal of Minor Fruits, Medicinal and Aromatic Plants (IJMFM&AP)

Publisher
Dr. S. N. Ghosh, India

Place of publication
Green Tower, 2nd Floor, Flat No C/6,
3 No. Priyanath Chatterjee Street, Belghoria,
Kolkata-700 056, West Bengal, India.

Volume 10 Number 2 December,2024

International Journal of Minor Fruits, Medicinal and Aromatic Plants

Print ISSN: 2424-6921 and On line ISSN: 2424-693X

Journal CODEN Code: IJMFCQ Website: https//www.ijmfmap.in/

Registration Number of Journal (Received from RNI, Government of India): WBENG/2017/76033

Received from IJIFACTOR indexing (2023): International Journal Impact Factor: 4.9 and Journal Ranking: A+++

Received from Index Copernicus International (ICI): Index Copernicus Value (ICV) for 2022 is 100.

Journal received NAAS (National Academy of Agricultural Sciences) rating 4.77 (validity from 01.01.2024 to 31.12.2026)

Journal is indexed in SCOPUS

Journal is abstracted by CABI

Journal is indexed in Indian Citation Index (ICI), J-Gate, IJIFACTOR, EBSCO,

Index Copernicus International (ICI), CAS (Chemical Abstracts Service, a not-for-profit division of The American Chemical Society) and Scope Data Base

Journal is the Part of ICI World of Journals (Index Copernicus International (ICI)

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(Registration number: No. S0032741 of 2022-2023)

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International Journal of Minor Fruits, Medicinal and Aromatic Plants
Print ISSN: 2424-6921 and On line ISSN: 2424-693X
Website: https://www.ijmfmap.in/

Plagiarism of all articles, published in December, 2024 issue, have been checked by a special Software provided by iThenticate

Volume 10 **Number 2** **December 2024**

Review article

Technological interventions in exploration of underutilized berries for multilevel values

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Receipt: 10.10.2024 *Revised: 25.11.24* *Acceptance: 27.11.24*
DOI: 10.53552/ijmfmap.10.2.2024.1-9

ABSTRACT

Berries are associated with numerous health benefits, potentially addressing a variety of diseases. Polyphenols, particularly anthocyanins, have garnered significant attention in the literature for their health-promoting properties.In vitro studies have shown that anthocyanins and other polyphenols present in berries may have a number of potential anti-cancer and heart disease advantages, including antioxidant, anti-inflammatory, and cell regulatory actions. Berry phenolic compounds can be used as natural antibacterial agents in a variety of applications, including food and medicine. Ozone therapy stimulated a defence mechanism against oxidative damage in blueberry fruit stored at 4°C. Extracts from blueberries significantly decrease skin inflammation and too represent a promising new defence against harm from cutaneous pollution. Because of their advantages in avoiding urinary tract infections, among the berries, cranberries have long attracted attention (UTIs). The ability of those cranberry products tends to prevent UTI and act as a non-antibiotic substitute could significantly impact public health by lowering the overall amount of antibiotics administered for UTI.Such health benefits emphasize the significant potential of berries in functional food and therapeutic applications.

Keywords:Anthocyanin, berries, health benefits,ozone treatment, phenolic compounds.

INTRODUCTION

The production of berries for food processing has been steadily rising, indicating a strong demand for products with added value, especially from consumers who remain health-conscious. Blueberries, raspberries, blackberries, cranberries, and blackcurrants were chosen at a stage that been almost fully ripe in order to maintain their sweetness and appropriate flavour. After all, those berries were processed to create a range of finished or semi-finished products, including puree and juice. These fruits remain as an excellent source for blending because to their intense flavour and colour including their structure makes them simple to juice using either cold or hot pressing.

Like other fruits, berries tend to have a number of vitamins that remain essential for overall health. Ascorbic acid, vitamin C, remain abundant in many berry species, although only a small number of the fruits may meet the recommended daily allowance (RDA) of human. Berries perform good antioxidant activity, making them a clear dietary source of vitamin C which operate as a cofactor in hydroxylation processes that necessary for the production of collagen that play a part in the production of hormones, strengthening immune system, platelet aggregation, thrombus formation, iron absorption and too protect against

osteoporosis, heart disease and several categoriesof cancer. Berries are rich in folic acid, a water-soluble vitamin B, which helps reduce the risk of heart disease and cancer through mechanisms such as reducing homocysteine levels, catalyzing the synthesis of nitric oxide, and preserving DNA integrity. Additionally, folic acid been necessary to prevent malformed neural tubes in newborns (Beattie *et al.*, 2005; Golovinskaia and Wang, 2021).

Underutilized berries

Underutilized berries were those which remain neither grown commercially on large scale nor traded widely. Berries are distinguished by their tiny (5 to 25 mm) pieces with relatively soft flesh, irregular shape and absence of an inner core or peel. Seeds remain small and simple to separate while juicing. These fruits were however readily polluted when grown on or near the ground. The fragile fleshes are also more vulnerable to physical harm and insect attack. The tiny unit size also makes it challenging to identify and remove blemished or defective pieces. Underutilized berries included to bayberries, blueberries, raspberries, cranberries, blackberries, currants, wineberries, barberries, bilberries, gooseberries, cloudberries, huckleberries, and nannyberries. The best natural antioxidants were found in blueberries, which also a great source of fiber, manganese, vitamin C, vitamin K, and vitamin A. These berries can be purchased fresh or processed into dried or infused berries, puree, juice, individually fast frozen (IQF) fruit, or dried fruit. Following that, they may be added to a range of consumer items including jellies, jams, pies, muffins, snack foods, pancakes and morning cereals. Berries also tend to be observed as a potential source of phytochemicals oftenincludes antioxidants, anti-cancer agents, anti-neuro degenerative agents, anti-inflammatory agents. In chronic smokers, blueberry has decreased the oxidative stress biomarkers. For three weeks, consuming one cup of blueberries daily will lower the chance of heart disease, as well as diabetes, eye problems, Alzheimer's disease and cancers of the mouth, breast, colon and prostate as reported by Golovinskaia and Wang (2021); Paredes-López*et al.* (2010).

Reason for being underutilized berries in India

Berries were underutilized in India because they fail to thrive in our climatic conditions, and they require specific chilling hours to flower or fruit. The disease management of these berries have been also difficult since these crops were closely grown to ground which leads to many contaminations. The post-harvest management remain not that easy because they were very much delicate skinned and face lot of bruising damage during transport thatresults several post-harvest loss which ultimately leads to decline in productivity.

Nutrient Composition of underutilized berries

Berries observed to be fortified with numerous sources of essential vitamins, minerals, and dietary fibers. They are high in natural sugars but low in calories and fat. Raspberries, blackberries and blackcurrants provide vitamin C, dietary fiber, potassium and folates. Blueberries have the lowest amount of vitamin C among these berries, while strawberries have the highest ranging from 9.7 to 60 mg/100g. Strawberries, blackberries, and raspberries were theprodigious providers of potassium (Vitamin B9) and folate. Cranberries remain high in vitamin E, while blueberries and blackberries high in vitamin K. Blackberries are rich in antioxidants such as lutein, zeaxanthin, and beta-carotene. Blackcurrants contain the highest levels of calcium, iron, phosphorus, and potassium among those berries (Golovinskaia and Wang, 2021). Bayberry and yellow Himalayan raspberry-based health beverage possess high content of various antioxidants(Hare Krishna and Attri, 2016).

Phytochemicals in Berries

Conjugated anthocyanidinswere the anthocyanins responsible for distinctive and vivid colour palette of dark berries. There are six major anthocyanidins in the plant kingdom: pelargonidin, peonidin, petunidin, delphinidin, cyanidin, and malvidin. Glucose, sophorose, rutinose, rhamnose, galactose, arabinose and xylose were among the sugars with which they form conjugates. Anthocyanin profiles of tissues that recognisable were used in identification of adulteration with juices and wines. The presence of delphinidin, cyanidinrutinosides and glucosidesexpressthe way that distinguishes blackcurrants from other fruits.

The bog whortleberry of *Vacciniumuliginosum*, bilberry of*Vacciniummyrtillus* and elderberries were found to have most quercetin (15.8 mg/100 g) respectively. Myricetin (8.9-20.3 mg/100 g) remain as the most prevalent flavanol in blackcurrant cultivars, followed by quercetin and kaempferol. Different berry species, colour, flavour and potential health benefits can all be influenced by the polyphenol content of the berries. A large family of substances known as polyphenolsranges in structure and potential bioactivity. Berries have a range of colours from red to purple to blue because anthocyanins, a kind of polyphenol pigment been present as reported byKristo and Sikalidis (2022).

Anthocyanins

Natural pigments called anthocyaninsrender many fruits and vegetables their distinctive colours and have several positive health effects on humans and other living things. In addition to other disorders, these anthocyaninswere crucial for the treatment of diabetes, cancer, neurological and cardiovascular diseases. They also have antioxidant, anti-inflammatory and antibacterial characteristics. Blueberries, blackberries and black raspberries contain far greater anthocyanin concentrations than red raspberries, cranberries and blackcurrants which observed to have tremendous therapeutic value (Kristo and Sikalidis, 2022).

The most common berries that contain these compounds includescyanidin, pelargonidin, delphinidin, malvidin, peonidin, and petunidin. When sugars and anthocyanidinsremain mixed, anthocyaninswere created. Several anthocyaninsobserved to be found in urine after berries or berry extract consumption, however at extremely low levels - typically 0.1% or lessof the eaten dosage. Anthocyanin levels in human plasma peak 0.5–1 hour after ingestion and return to baseline within 6–8 hours. Thus, unlike flavanol glycosides, glycosylated anthocyanins can enter the circulation. This may be the case because human small intestine b-glucosidases do not break down quercetinglucosides or anthocyanin glucosides. Improved analytical methods now starting to show the presence of reduced amounts of methylation and sulphated metabolites in urine after consumption, even though historically only unmodified anthocyanins have been detected in urine following supplementation (Kristo and Sikalidis, 2022; Kalt, 2014).

Proanthocyanidins

The finest sources of proanthocyanidins (condensed tannins) remain with those berries such as cranberries, blackcurrants, and blueberries. As the fruit turns from red to black as it ripens, proanthocyanidin concentration fluctuates, peaking but then drastically declining in the last stage. Studies on blackberries revealed that proanthocyanidin and anthocyanin levels vary with development stage. Astringency, sourness, bitterness, sweetness, saliva viscosity, aroma, and colour composition attributed to proanthocyanidins. The PAC level of cranberries and blueberries be extremely high. Cranberries have more PACs than other berries. These remainless blueberries in PAC. PACs have anti-inflammatory,

antibacterial, antiviral, anti-inflammatory, vasodilator, antioxidant and anti-carcinogenic effects (Golovinskaia and Wang, 2021).

Flavanols

Berries include phenolic compounds called flavanols, which include kaempferol, quercetin and myricetin. These compounds have antibacterial, anticancer and antioxidant qualities and help prevent cardiovascular diseases. Quercetin and myricetin remain abundant in blueberries. The primary flavanol in cranberries and black raspberries bequercetin. These berries also contain myricetin glycosides, but in less amounts. Nine quercetin compounds and three kaempferol derivatives included in blackberries. Red raspberries have the anti-oxidants quercetin and kaempferol. Bilberries and bog whortleberries were found to have the highest levels of quercetin (Golovinskaia and Wang, 2021).

Phenolic acid

Antioxidative and anticancer characteristics possessed by the hydroxycinnamic and hydroxybenzoic acids p-coumaric, caffeic, ferulic, and p-hydroxybenzoic, gallic and ellagic. In addition to various phenolic acids, the cranberry peel includes significant amounts of ursolic acid in the aglycone form (of which phydroxycinnamic acid is the most important). Ferulic, caffeic and gallic acids have been found in blueberries. The potent antioxidant chlorogenicacid remains one of the important phenolics found in blueberries. Red raspberries include both hydroxybenzoic acids and hydroxycinnamic acids. Blackberries have a total ellagic acid concentration of 319.7 mg/100 g, according to acid hydrolysis. Ellagic acid contains a variety of biological characteristics, including the ability to neutralise free radicals, stop the development of cancer cells and have anti-inflammatory and antibacterial actions (Golovinskaia and Wang, 2021; Dou *et al*., 2022).

Ellagitannins

A growing body of studies suggests that eating foods high in ellagic acid and ellagitannins may be beneficial to human health. Ellagitanninswere the complex derivatives of ellagic acid as a member of the hydrolyzable tannin family. They have antibacterial, anti-inflammatory, anti-carcinogenic and anti-Helicobacter pylori (*H. pylori*) qualities. Ellagatannins ripe in raspberries and blackberries.Elagitannins are the main hydrolyzable tannins present in blackberries, along with sanguiin H6 and lambertianin. The two main ellagitannins identified in raspberries were C lambertiantrimer and the sanguiin H6 dimer (Golovinskaia and Wang, 2021; Lee, 2017).

Stilbenes

Berries include a particular type of phenolic, non-flavonoid chemicals called stilbenes. Resveratrol been the component in berries were most well-known. The biological and pharmacological characteristics of stilbenesremain diverse and too have positive impacts over human health. These characteristics include antioxidant, anticancer and neuroprotective effects. Berries like blueberries and cranberries contain stilbenes (Golovinskaia and Wang, 2021).

Oxidative stress suppression

High levels of free radical formation tend to oxidative stress, which has been connected to many degenerative diseases and the speeding up of ageing. Berry antioxidants interact with substances that actively scavenge oxygen radicals, including carotenoids, phenolic compounds, and vitamin C. Berries have four times the antioxidant content of other fruits and 10 times the antioxidant content of vegetables. According to studies, blueberries have powerful phenolic components and vitamin C levels were strongly associated with their antioxidant potential. Raspberries have a high level of radical scavenger activity and contain a lot of antioxidants. The primary

antioxidant in raspberries, p-coumaric acid, is present in the freeze-dried aqueous extracts of many raspberry cultivars. Additionally, polyphenols, ellagitannins and anthocyanins shown antioxidant and anti-tumour growth properties. According to studies, anthocyanins and ellagitannins account for 75% of raspberries antioxidant potential. Along with other bioactive components, the two main antioxidants in black raspberries, cyanidin 3-rutinoside and cyanidin 3-xylosylrutinoside have been connected to the prevention and treatment of numerous malignancies which shown positivity in human research (Golovinskaia and Wang, 2021; Kalt, 2014).

Neutraceutical rich fruits	**Nutraceutical factors**
Blue berries, black berries, cranberry, raspberry, black currant	*Flavonoids* (Anthocyanidins): Cyanidin 3-glycosides, Malvidin, Delphinidin, Pelargonidin
Berries and cherries	*Flavanols*: Morin, ProcyanidinsProdelphinidins, Catechin, Epicatechin and their gallates
Berries, currants, cherries	*Anthoxanthins*(Flavonols): Myricetin, Fisetin, Quercetin, Kaempferol, Isorhamnetin
Raspberry, blueberry, cranberry	*Phenolic acids*: Chlorogenic acid, Ferulic acid, p-coumaric acid Ellagic acid, Gallic acid
Raspberries, blackberry	*Tannins*: Catechin, Epicatechin polymers, Ellagitannins, Proanthocyanidins, Tannic acids

Verma*et al*.(2024)

Berries as cancer preventer

Scavenging reactive oxygen species (ROS), which cause oxidative damage to cellular macromolecules like DNA and RNA, be one of the most crucial components of antioxidant activities. Oxidative stress remains one of the primary causes of increased carcinogenicity because it promotes the accumulation of oxidative DNA damage, which aids in the development of tumors. The formation of cancer is typically seen as a microevolutionary process requiring the combined effects of several events, *viz.,* (1) creation of a somatic cell's DNA mutation; (2) encouragement of tumorigenic cell clone multiplication and (3) advancement of malignant transformation of the tumour into cancer.

Several distinct human cancer cell lines show antiproliferative activity in response to anthocyanins and ellagic acid. Ellagitannins and anthocyanins, which have chemo-preventive potential, remain abundant in black raspberries. Freeze-dried black raspberries have been demonstrated in studies to lessen the development of colon and oesophageal cancer due to toxins. The development of several tumour cell lines, including those from the colon, prostate, breast, mouth, cervix, ovary and skin that entirely inhibited by bilberry extract when tested *in vitro*. A diet rich in blueberries may help prevent breast cancer caused by oestrogen (Golovinskaia and Wang, 2021).

According to *in vitro* research, certain berry extracts limit the development and multiplication of cancer cells, cause cell death and have an impact on angiogenesis by preventing the production of the vascular endothelial growth factor (VEGF). It has been proposed that it could control these processes. Results from animal models, however, were not definitive, for instance, rats fed tumour initiator-containing diets with freeze-dried black raspberries saw a decrease in the development, occurrence and variety of oesophageal tumours. Blueberries,

however, did not have the same effects(Golovinskaia and Wang, 2021; Kostecka-Gugala*et al*., 2015).

Diabetes

By preventing the rise in blood glucose levels, the physiologically active phytochemicals anthocyanins and proanthocyanidins, which were abundant in berries tends to cure diabetes and other metabolic illnesses. When compared to other berry extracts, red raspberry extracts have the strongest amylase inhibition properties. According to fractionation experiments, the raspberry extract's active component against -glucosidasebe the unbound anthocyanin-enriched fraction. The bound fraction, however, was beginning to accumulate amylase inhibitors. This demonstrates that proanthocyanidins significantly reduces amylase activity. A slower rate of glucose absorption in the stomach has been associated with the soluble fibres polydextrose and glucan present in cranberries. Cranberry flavonoids increase glycaemic response while reducing intestinal glucose absorption. Amylase and glucoamylase activity can be inhibited by proanthocyanidins and ellagitannins found in cranberry extract. By removing reactive carbonyl radicals, cranberry procyanidins can help prevent the glycation of human hemoglobin and serum albumin (McDougall and Stewart, 2012).

Benefits in immune system

It has been demonstrated that consuming 330ml of berry juice daily for two weeks improves natural killer cell lytic activity, increases lymphocyte responsiveness to mitogen activation and causes human volunteers to release cytokines unique to T lymphocytes. It was not known if these conceivably advantageous benefits were exclusive to berries or a more widespread reaction to increasing fruit consumption. According to Beattie *et al*. (2005) and Bader *et al*. (2022), an obtrusive and persistent *Escherichia coli* was suppressed by cranberry concentrate because it reduced the bacteria's adherence and invasion. This was in accordance with the findings of Khoo*et al*. (2022).

Prevention of urinary tract infection

Numerous studies have been conducted on the impact of cranberries on the condition of the urinary system since the 1920s. It was long thought that cranberries tendency to make urine more acidic because of intake explained their capacity to cure urinary tract infections (UTI). Cranberry proanthocyanidins, on the other hand, seem to stop p-fimbriated*E. coli* from adhering to the uroepithelial cells. Similar traits may be seen in other fruit kinds, such as the blueberry, a species of *Vaccinium*. The effects of cranberry juice and tablets have been the subject of numerous published clinical investigations. Numerous studies have demonstrated that cranberry juice or pills do have some protective effects, even if there were insufficient evidence to recommend their usage in the treatment or prevention of UTIs (Liska*et al*., 2016; Raz*et al*., 2004).

The rich nutritional content of dietary fiber, vitamin C, vitamin K and the necessary mineral manganese makes blackberries unique. Fruit acid, flavonoids and tannins were found in the leaf, whereas saponins and tannins been found in the root. The U.S. Department of Agricultural Research Service observed that young blackberry leaves had a high concentration of antioxidants, or the capacity to absorb oxygen radicals, in a study published in the "Journal of Agricultural Food Chemistry" in February 2000 (Ayoub, Maha 2015). In Europe, *R. fruticosus* has been used to treat diabetes (Verma*et al*., 2014). Consuming meals high in phytonutrients on a regular basis can help prevent cardiovascular diseases. Additionally, anthocyaninsbeen used to create packaging films that serve as freshness indicators for food (Feng*et al*., 2022).

Response of antioxidative defence System to ozone treatment in stored blueberry

Ozone remains as a material with strong oxidative properties. Numerous studies suggest that ozone can be utilized to prolong the commercial life of berry fruits while ensuring their high nutritional and processing value, as well as their microbiological safety. The study examined the amounts of antioxidant enzyme activity, reactive oxygen species (ROS) and protein oxidative damage in blueberry fruit that was ozonated and that was not during storage. Over the first 21 days of storage, ozonated fruit displayed increased antioxidant enzyme activity than non-ozonated fruit, including glutathione peroxidase, superoxide dismutase, and phenylalanine ammonia-lyase. The amount of hydrogen peroxide and superoxide anion radicals in ozonated fruit was significantly lower than in untreated material because of the improved ROS detoxifying system activity. However, after 21 days of storage, ozone treatment caused proteins to undergo oxidative changes, which may be the reason why the enzymes required for cellular defence against oxidative stress remain less active. This illustrates the need for more food science research on the use of ozonation technologies in stimulating the postharvest shelf life of soft fruits (Piechowiak, *et al*., 2020).

Prevention of ozone induced cutaneous inflamassome actions with blueberry extracts

O_3 has the ability to damage tissue, but primarily because of its interaction with the stratum corneum's (SC) cutaneous lipids, which produces molecules such hydrogen peroxide (H_2O_2) and lipid peroxidation products (4-Hydroxynonenal), which tends to trigger an inflammatory reaction. Although different blueberries have been used medicinally and topically to lessen wrinkles, telangiectasias, and skin aging, the potential protective mechanisms of topical application against pollution-induced damage have never been assessed, especially for vaccinium species, according to the Traditional Ecological Knowledge of Native American pharmacopeia. Since ozone exposure has already been connected to the development and exacerbation of inflammatory skin illnesses, it was thought that using blueberry extracts would be able to avoid redox imbalance and, hence, block the ozone-induced activation of the cutaneous inflammasome. Without the aid of blueberry extract, ozone was unable to enhance the oligomerization of the components of the inflammasome, as well as the expression of genes and proteins linked to the inflammasome. Additionally, blueberry extract pre-treatment was successful in decreasing oxidative markers associated with ozone exposure and speeding up the healing of epithelial wounds (Pambianchi*et al*., 2020).

Osmotic dehydration of berries

The degree of osmotic dehydration of berries been significantly influenced by the concentration of the hypertonic solution. A drop in mass indicates that the 70°Brix solution achieved the most severe osmotic dehydration and the best dehydration time. The blackberries lost 16% and 21% of their moisture after 6 hours, 4% and 7% of their moisture in 1.5 hours, 3% and 6% of their moisture after 6 hours, and 4% and 8% of their moisture after 7 hours. As a result, the berries lose less moisture when a hypertonic sucrose solution with the target concentration of 60°Brix had beenused. Additionally, it was discovered that lengthening the time of osmotic dehydration that not recommended since it causes the berries to become mushy and distorted, which remain unacceptable. A longer period of dehydration also causes the sensory indications to change negatively (Gribova *et al*., 2021).

Conclusion

Folic acid and vitamin C, two essential vitamins were abundant in berries. They include many phytochemicals as well. These exhibit a range of *in vitro* characteristics that point to possible health

advantages. Before further information regarding berry anthocyanins and flavanols*in vivo* absorption and metabolic destiny be available, it would be prudent to refrain from attributing to berries any extra health benefits beyond those that presently recognized to exist in all fruits and vegetables. However, locally grown fresh or frozen berries were an underutilized and possibly healthy dietary option in areas where consumption of plant-based meals remains frequently low. In recent years, there has been significant research progress in understanding the bioactive compounds in berries and their practical applications. The antioxidant chemicals bioavailability in berries and similar components from other fruits in general, as well as stronger proof of their benefits on consumer health, appear to be the main areas of research which triggers the necessity of nutritional security in futuristic generations of human.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review article

Content analysis, development and standardisation of choleretic agents based on medicinal herbal raw materials of *Tanacetum* and *Achillea*

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Received : 12.11.24 Revised :05.12.24 Accepted : 07.12.24
DOI: 10.53552/ijmfmap.10.2.2024.10-23

ABSTRACT

The purpose of the study was to collect data on Achillea and Tanacetum species common in Kyrgyzstan, their active components, and therapeutic characteristics. A review of scientific papers and other sources of information on the Internet, including databases and national resources, on the subject of pyzhma and yarrow, with an emphasis on their chemical composition, prevalence, biological activity, and traditional use, was conducted. A search was performed for keywords and plant species with data analysis in Microsoft Excel for systematisation and validation. The study covered the distribution, chemical composition and therapeutic properties of pyzhma and yarrow in Kyrgyzstan. The chemical composition of plants contains essential oils, flavonoids, and sesquiterpenes, causing their choleretic, antispasmodic and hepatoprotective properties. The content of active ingredients in wild forms of pyzhma is higher, making them more valuable for medical use. The study shows that the Asteraceae family is widespread in Kyrgyzstan and thrives in diverse conditions, with different chemical components having hepatoprotective, choleretic, and anti-inflammatory effects, useful in medical practice.

Keywords: *Achillea*, biological activity, chemical composition, choleretic property, plant extracts, *Tanacetum*,

INTRODUCTION

More than half of modern medicinal products are synthesised from herbal remedies (Khatib *et al.*, 2023). Their wide range of biological activity and a variety of phytochemical compounds make them suitable for the creation of new drugs. Various species of pyzhma (*Tanacetum*) and yarrow (*Achillea*) grow in Kyrgyzstan, which are traditionally used in folk medicine. The chemical

composition and biological activity may vary depending on the species, plant organ, place of growth, and environmental conditions (Lechkova *et al.*, 2023). One of the main problems is the lack of information about the chemical composition and active ingredients of plants growing in Kyrgyzstan. Current scientific evidence supporting the efficacy and safety of these plants is also limited. The examination of the chemical composition and pharmacological properties of pyzhma and yarrow will expand the understanding of the mechanisms of their action and the potential of their choleretic properties for therapeutic use. In addition, the exploration and use of local plant resources can be of substantial economic importance. This will not only expand scientific knowledge but may also lead to the creation of new medicines.

At the moment, studies on the variation of the chemical composition of pyzhma and yarrow, the mechanisms of their action on the biliary system, methods of standardisation of extracts, and their safety, toxicity, pharmacokinetics, and clinical efficacy remain relevant. The purpose of the study was to systematise and analyse existing data on pyzhma and yarrow species in Kyrgyzstan, their chemical composition and biological properties, and to evaluate their use in medical practice.

PROCEDURE ADAPTED FOR COLLECTION OF INFORMATION

A comprehensive review of information resources on the Internet was performed to conduct this study, including papers, databases such as PubMed, Scopus, Semantic Scholar, Google Scholar, and the sites of the National Academy of Science of Kyrgyz Republic, I.K. Akhunbaev Kyrgyz State Medical Academy, Kyrgyz-Turkish Manas University, Kyrgyz National University named after Jusup Balasagyn. National botanical resources, reports, and other publications related to the species, chemical composition, and biological properties of pyzhma and yarrow were also used.

After determining the plant species growing on the territory of the republic, the search additionally included such species as *Achillea millefolium, Achillea Setacea, Achillea asiatica, Achillea filipendulina*, *Achillea collina*, *Tanacetum vulgare, Tanacetum parthenium*. The selection of relevant publications was conducted according to the following inclusion criteria: works published in peer-reviewed journals, books, monographs, reports, conference materials, and clinical trials describing studies on the use of samples of various types of medicinal plants *Tanacetum* and *Achillea* collected from different territories of Kyrgyzstan. Specific gathering sites included regions with diverse ecosystems such as pastures, steppes, mountain slopes, and river valleys. Studies on the growth of pyzhma and yarrow in Central Asia and Kyrgyzstan, as well as data on the active components of these plants, their therapeutic properties, and active substances, were also included. Special attention was paid to studies of choleretic activity, namely experiments on laboratory animals using liver perfusion techniques to measure bile flow and a comparative analysis of the choleretic effect of extracts with known hepatoprotectors. The content analysis of the collected information included the systematisation of data on the species, chemical composition, and biological properties of pyzhma and yarrow.

Special attention was paid to papers describing research in Kyrgyzstan over the past 5 years, however, due to insufficient data, the search was expanded

to publications issued more than 5 years ago. The object of the study included various types of yarrow and pyzhma common in Kyrgyzstan, regions and places of their growth, active components of plants, therapeutic properties, extraction methods, and features of plant raw materials.

The biological activity of plants was analysed in the context of their therapeutic effects, with an emphasis on choleretic function and general effects on the hepatobiliary system. The information obtained was entered into Microsoft Excel spreadsheets to facilitate the structuring of the data. The tables were systematised by plant species and included information on the chemical composition and distribution, features of the growing landscape, and research materials.

The data were analysed by two researchers to increase the reliability of the results. The units of analysis included a plant species with choleretic properties that grows on the territory of the Kyrgyz Republic, the region of growth, chemical composition (content of active substances), collection and storage of plant raw materials, methods of synthesis of nanoparticles of plant raw materials, author and date of publication, forms of medicines, safety issues of medicines, experimental data (research results on animals or people).

FINDINGS

The prevalence of pyzhma and yarrow in Kyrgyzstan

One of the leading families in the Kyrgyz Republic is *Asteraceae Dumortier*. According to Zhailybayeva *et al.* (2023), the *Asteraceae Dumortier* family, which includes plants such as pyzhma and yarrow, accounts for 16.9% of the total number of species in the country and includes 141 species. It is shown that each *Achillea* species has its own preferences in terms of landscape types and growth conditions, from meadows and steppes to mountain slopes and shores. Most *Achillea* species prefer open and semi-mountainous landscapes such as pastures, steppes, grasslands, and slopes, which indicates their ability to adapt to diverse ecosystems.

Pyzhma grows in various conditions, from reclaimed lands to ruderal sites, which can affect the chemical composition and quality of plant materials. Nurzyńska-Wierdak *et al.* (2022) established that different harvesting sites can affect the morphological parameters of pyzhma, such as plant height and inflorescence mass. The highest content of flavonoids and phenolic acids was identified in raw materials from ruderal and reclaimed sites.

The examination of *Achillea filipendulina*, conducted in the northern foothills of the Alai range in the Kara-Suu and Nookat regions, focused on the vegetation cover and its floral composition. It was determined that the association of gundelia milkweed yarrow is located in the Kichik tract at an altitude of 1800-1900 m. The projective vegetation was 55-60%, while the yarrow was 15-20%. The study showed that the natural reserves of yarrow and other medicinal plants vary depending on the height and composition of plant communities. Information about the condition of these plants is important for their rational use and protection (Mero *et al*., 2023). It was established that *Tanacetum vulgare* is widespread in Central Asia and Kyrgyzstan, inhabiting various landscapes.

The chemical composition of pyzhma and yarrow, which are found on the territory of Kyrgyzstan

The active components of *Achillea* species are presented in detail in Table 1.

Table 1: Active substances of *Achillea* (yarrow) species

Achillea species	**Chemical composition**	**Source**
Achillea millefolium/ Yarrow ordinary/ kadimki kaz tandai	Dicaffeoylkine acids, luteolin-7-O-β-D-glucuronide, sesquiterpenes (hamazulene), monoterpenes (camphor, thujol), flavone glycosides (apigenin, luteolin), alkaloids (achillein, betaine, choline, trigonellin), sesquiterpenoids (acetoxyartabsin, acetylbalkanolide, achillicin, achillin, austricin, balkhanid, dihydroacetoxitamacin, hydroxyachylin, leucodine, millefin, millefolid), vitamin K, flavonoids (artemethin, vitexin, vicenin, isovitoxin, isoramnetin, isoerentin, casticin, cosmosin, quercetin, orientin, certisin), essential oils (furfural, isobutyl acetate, kadinen, humulene, copaene, caryophyllene, isoartemisiacetone, limonene, alpha-thujone, eucalyptol, pinene, camphene, farnesene, borneol, myrcene, sabinene, terpineol, cumin aldehyde, bornyl acetate, terpinol-4, cymol, γ-terpinene, azulene, menthol, terpinolene, aljoyen, α-terpinene), carbohydrates (inositol, glucose, arabinose, galactose), organic acids (aconite, amber), phenolic carboxylic acids (salicylic, caffeic).	Liu *et al.* (2020), Berdigulova *et al.* (2022)
Achillea Setacea/ Yarrow bristly/ Katuu tuktuu kaz tandai	Alkaloids (betaine, choline, trigonellin, achillein), glycosides, sesquiterpenes, sesquiterpenoids (acetoxyartabsin, acetylbalkanolide, achillicin, achillin, austricin, balhanide, dihydroacetoxitamacin, hydroxyachilin, leucodine, millefin, millefolide), flavonoids (artemethin, vitexin, vicenin, isovitoxin, isoramnetin, isoerentin, casticin, cosmosin, quercetin, luteolin, orientin, certisin, apigenin), essential oils (furfural, isobutyl acetate, kadinen, humulene, copaene, caryophyllene, isoartemisiacetone, limonene, alpha thujone, eucalyptol, pinene, camphene, farnesene, borneol, myrcene, sabinene, terpineol, cumin aldehyde, bornyl acetate, terpinol-4, cymol, γ-terpinene, azulene, menthol, terpinolene, aljoyen, α-terpinene), resins, tannins, vitamins C and K, organic acids (aconite, amber), phenolic carboxylic acids (salicylic, caffeic), 1,8-cineol, carbohydrates (inositol, glucose, arabinose, galactose).	Eisenman *et al.* (2013), Liu *et al.* (2020)
Achillea asiatica/ Yarrow Asian/	Essential oils (furfural, isobutyl acetate, kadinen, humulene, copaene, caryophyllene, isoartemisiacetone, limonene, alpha thujone, eucalyptol, pinene, camphene, farnesene, borneol, myrcene, sabinene, terpineol, cumin aldehyde,	Eisenman *et al.* (2013), Liu *et al.*

Chinese/ Golian/ Asia kaz tandayy	bornyl acetate, terpinol-4, cymol, γ-terpinene, azulene, menthol, terpinolene, aljoyen, α-terpinene, limonene), sesquiterpene lactones, sesquiterpenoids (acetoxyartabsin, acetylbalkanolide, achillicin, achillin, austricin, balhanide, dihydroacetoxitamacin, hydroxyachylin, leucodine, millefin, millefolide), alkaloids (betaine, choline, trigonellin, achillein), flavonoids (artemetin, vitexin, vicenin, isovitoxin, isoramnetin, isoerentin, casticin, cosmosin, quercetin, luteolin, orientin, certisin, apigenin), phytoncides, bitter and astringent substances, resins, vitamins C and K, carotene, carbohydrates (inositol, glucose, arabinose, galactose), organic acids (aconite, amber), phenolcarboxylic acids (salicylic, caffeic).	(2020)
Achillea filipendulina / Yarrow meadowsweet/ fern-like/ Tabylgylbyr actu kaz tandai	Contains 0.07-0.26% oil (furfural, isobutyl acetate, kadinen, humulene, copaene, caryophyllene, isoartemisiacetone, limonene, alpha thujone, eucalyptol, pinene, camphene, farnesene, borneol, myrcene, sabinene, terpineol, cumin aldehyde, bornyl acetate, terpinol-4, cymol, y-terpinene, azulene, menthol, terpinolene, aljoyen, a-terpinene), sesquiterpenoids (acetoxyartabsin, acetylbalkanolide, achillicin, achillin, austricin, balhanide, dihydroacetoxitamacin, hydroxyachylin, leucodine, millefin, millefolide), flavonoids (quercetagetine, kentaureidine, artemethin, vitexin, vicenin, isovitoxin, isoramnetin, isoerentin, casticin, cosmosin, quercetin, luteolin, orientin, certisin, apigenin), traces of alkaloids, asparagine, amino acids and nitrogen-containing substances, carbohydrates (inositol, glucose, arabinose, galactose), organic acids (aconite, amber), phenolic carboxylic acids (salicylic, caffeic).	Sharopov (2015), Liu *et al.* (2020)
Achillea collina/ Yarrow of the hill.	No data available	Botanical Garden named after E.Z. Gareev of the National Academy of Sciences of the Kyrgyz Republic (2022)

The identified active components of *Tanacetum* (pyzhma) species are presented in Table 2.

Table 2: Active substances of *Tanacetum* (pyzhma)

Species	Chemical composition	Study
Tanacetum vulgare/ Chrysanthemum vulgare/ Common pyzhma	Alkaloids, glycosides, essential oils (including α-thujone, β-thujone, L-camphor, thujol, borneol, pinene, γ-terpinene, artemisium ketone, chrysanthenyl acetate, chrysanthenol, chrysanthenone, umbellone, sabinene and 1,8-cineol), terpenoids (a-amyrin), flavonoids (such as luteolin quercetin, apigenin, diosmetin), tannins, phenolic acids, sesquiterpene lactones (arbusculin-A, thanacetin, germacrene D, crispolide), flavonoid derivatives, caffeic acid, sterols (j3-sitosterol cholesterol, stigmasterin, taraxasterin and campesterin), and triterpenes.	Bussmann *et al.* (2020), Sharopov (2015), Medical Economics Company (2000)
Tanacetum partheniu m/ Feverfew	Parthenolide, thanetine, camphor, chrysanthenyl acetate, chrysanthenol, chrysanthenone, artemisia ketone, artemisia alcohol and 1,8-cineol.	Sharopov (2015)

The main part of the active components of *Tanacetum* and *Achillea* species are essential oils, flavonoids, and glycosides, which have choleretic, hepatoprotective, and antispasmodic effects. In addition, the hepatobiliary system is also influenced by phytosterols, tannins, resins, and other components present in pyzhma and yarrow (Raudone *et al.*, 2024; Eisenman *et al.*, 2013; Makhovska *et al.*, 2020). The inclusion of local plant species in pharmacopoeias and the development of standards will enhance their use in medical practice.

Therapeutic properties of yarrow *(Achillea)*

It was established that all *Achillea* species growing on the territory of the Kyrgyz Republic have a similar chemical composition and traditional use. *Achillea millefolium*, one of the most famous and widely used plants of the genus *Achillea*, is distinguished by its numerous medicinal properties. In particular, mono- and dicofeoylquinic acids contained in this plant play a key role in the manifestation of its hepatoprotective and choleretic effects. These components also substantially increase the overall antioxidant activity of *Achillea millefolium* extracts (Raudone *et al.*, 2024). In the study by Benedek *et al.* (2006), an analysis of *Achillea* extract by HPLC (high performance liquid chromatography) showed that it contains 48.8% dicofeylquinic acids (DCCA) with certain percentages for 3.4-DCCA, 3.5-DCCA, and 4.5-DCCA, 3.4% luteolin-7-bDO-glucuronide. The extract showed a dose-dependent increase in bile flow in liver perfusion experiments, with the *Achillea* fraction showing a two to three-times higher increase compared to cinarin alone. The DCCA in the *Achillea* fraction is likely responsible for this choleretic effect. The study suggested that the traditional use of yarrow in the treatment of hepatobiliary disorders is justified by the content of DCCA in it and their substantial effect on the flow of bile. In addition, yarrow tea containing DCCA can have a substantial choleretic effect. *Achillea millefolium* extracts reduce contractions of various smooth muscles, including the ileum, gallbladder ducts, pulmonary artery, trachea, uterus, and vas deferens. The aqueous alcohol extract

reduces contractions caused by acetylcholine and potassium chloride (Zubtsova *et al.*, 2019).

The plant exhibits pronounced hemostatic, analgesic, diuretic, antipyretic, and anti-inflammatory properties, which makes it a valuable tool in the treatment of various diseases. In medical practice, inflorescences and shoots of yarrow are used to obtain a therapeutic effect (Berdigulova *et al.*, 2022; Barda *et al.*, 2021). A decoction of inflorescences is used for hepatitis, angiocholitis, gallbladder diseases, acute disorders of the gastrointestinal tract, to regulate the menstrual cycle, and gynaecological pathologies (Eisenman *et al.*, 2013). Infusions of herbs without flowers are used in the treatment of rheumatism (Zubtsova and Skliar, 2023).

Achillea filipendulina decoctions have traditionally been used to treat a wide range of diseases, including gastrointestinal disorders, gout, sciatica, nasal congestion, cardiovascular diseases, abdominal pain, cough, arthritis, and malaria. In addition, this plant has been used as a laxative, diuretic, antipyretic, expectorant, antitussive, and anthelmintic agent. Externally, *Achillea filipendulina* was used to treat wounds and scabies.

Examining essential oils and lipids of two *Achillea* species grown in Uzbekistan, Asilbekova *et al.* (2019) identified that the essential oil from *Achillea filipendulina* flowers had a yield of 1.2% by dry weight and included 84 components, among which santolin alcohol (50.1%) and (Z)-chrysanthenyl acetate (13.8%) prevailed. The total lipid yield from this plant was 4.4% of the dry weight. The essential oil from the aboveground part of *Achillea millefolium* had a yield of 1.0% and included 82 components, the most substantial of which were 1,8-cineol (14.3%) and bornyl acetate (4.4%). The total lipid yield was 3.6% of the dry weight. The study showed that environmental conditions substantially affect the composition of essential oils.

The antibacterial properties of the plant manifest against both gram-positive and gram-negative bacteria. The flavonoid quercetagetin demonstrates anti-HIV activity by acting as an inhibitor of HIV reverse transcriptase and integrase. Therewith, kentaureidine exhibits cytotoxicity by inhibiting tubulin polymerisation (Sharopov, 2015).

Achillea Setacea has a wide range of medicinal properties, including antimicrobial, anti-inflammatory, and hemostatic effects. Studies have shown that essential oil is released from the aboveground parts of this plant, which is a carrier of many bioactive components. In particular, 51 active components were found in the composition of this oil, among which sesquiterpenes are especially prominent. These compounds demonstrate substantial anti-inflammatory activity, which makes *Achillea Setacea a* promising agent for use in traditional and modern medicine (Eisenman *et al.*, 2013).

A similar study was conducted by Barda *et al.* (2021), reviewing the current state of knowledge about the phytochemistry of the genus *Achillea* and comparing traditional applications with modern pharmacological data. Over the past decade, 31 species of *Achillea* have been examined, 141 chemical compounds have been identified, including flavonoids, phenolic and quinic acids, sesquiterpenoid lactones, etc. The traditional uses of 24 types are discussed, including the treatment of spasms, gastrointestinal and hepatobiliary disorders, haemorrhages, pneumonia, rheumatism pain, wound healing, and diuretic, anti-inflammatory, antipyretic, and the treatment of menstrual and gynaecological disorders.

Therapeutic properties of pyzhma *(Tanacetum)*

Tanacetum vulgare (common name pyzhma) has many useful properties due to the content of phenolic acids, flavonoids, terpenoids, and fatty acids, which make its extracts especially valuable in medical and pharmacological practice. These extracts exhibit strong antioxidant activity, effectively inhibit enzymes such as tyrosinase and amylase, and have neuromodulatory effects by stimulating the dopamine transporter and the release of norepinephrine. However, at high concentrations (50-100 micrograms/ml), the extracts exhibit cytotoxicity, which requires caution when using them (Ak *et al.*, 2021; Berganayeva *et al.*, 2023). The main components of pyzhma essential oil include camphor and trans-chrysanthenyl acetate, which contribute to its biological activity (Ivanović *et al.*, 2022). *Tanacetum vulgare* is known for its antioxidant, antimicrobial, attractant, and insecticidal properties, which makes it useful in various fields of medicine (Sharopov, 2015). Herbs and inflorescences of wild yarrow have a substantially higher concentration of essential oil compared to cultivated varieties. According to various sources, the essential oil content in a wild plant ranges from 0.07% to 0.5%, whereas in cultivated species, this indicator is about 0.3% (Sodombekov *et al.*, 2023; Sytnik *et al.*, 2023). Thus, wild yarrow is more valuable for medical and pharmacological applications due to its higher content of essential oils.

Dried pyzhma flowers (30 g-50 g) are taken as an infusion or liquid extract, as an anthelmintic, carminative, and choleretic agent (Toktonaliev, 2019). In a study conducted by Abdurakhmanova *et al.* (2019), clinical trials of the anthelmintic drug Gelrem, developed on the basis of medicinal plants of Uzbekistan, were reviewed. This preparation included extracts of pyzhma pseudocyst, wormwood, and flower buds of cloves. The tests were conducted at the Tashkent Pediatric Medical Institute on 20 patients suffering from enterobiosis, ascariasis, and giardiasis. The results showed that Gelrem has high therapeutic efficacy and good tolerability. Clinical improvement was observed in all patients: appetite improved in 100% of patients, abdominal pain decreased in 79%, and itching in the anus disappeared in 93%. Laboratory tests confirmed the complete disappearance of intestinal parasites: pinworm, ascaris, and dwarf tapeworm eggs were not found in any patient after the course of treatment. Giardia disappeared in 9 out of 11 patients.

In vitro and animal studies have shown that pyzhma extracts have antispasmodic effects on rabbit intestines and choleretic activity in dogs. It is assumed that these effects may be related to the presence of caffeic acid, which is known for its ability to stimulate bile production (Medical Economics Company, 2000; Far *et al.*, 2023).

The choleretic and cholekinetic activity of new pharmaceutical compositions containing a thick extract of pyzhma flowers (GECPO) and essential oils of lavender, mint, and cloves was thoroughly examined in 2022 under the guidance of Yurchenko and Mishchenko (2022). During experiments on healthy animals, it was established that all tested compositions substantially increased bile secretion, and their effectiveness was substantially higher compared to the drug “Holelesan”. The greatest choleretic effect was demonstrated by the composition of GECPO in combination with lavender essential oil, which indicates its particularly high effectiveness.

A Decoction from the leaves of *Tanacetum parthenium* is used in gynaecology for dysmenorrhea, infections, abdominal pain, as an

abortifacient. The litholytic properties of pyrethrum have also been observed (Khatib *et al.*, 2023). *Tanacetum parthenium*, which is part of the drug "Feverfew", contains sesquiterpene lactones, among which the main active component is parthenolide. Flavonoids and volatile oils are also present in the composition of the plant. Parthenolide is found in the surface leaf glands in concentrations from 0.2% to 0.5%. This component has a multifaceted effect, including anti-inflammatory, analgesic, antioxidant, cytotoxic, and antispasmodic. Such properties make it effective in the treatment of fever, migraines, toothache, stomach aches, infertility, and rheumatoid arthritis. In the experiments conducted by Lechkova *et al.* (2023) on rats, it was determined that *Tanacetum parthenium* essential oil is safe at doses up to 1 g/kg of body weight for no more than one month. The antispasmodic effect of parthenolide helps to relax the smooth muscles of the biliary tract and improve the outflow of bile. In addition to parthenolide, flavonoids such as luteolin, quercetin, apigenin, and diosmetin and terpenoids, including L-camphor and thujol, have a substantial effect on the walls of the bile ducts. These substances exhibit pronounced antispasmodic properties, which help to relax and improve the patency of the biliary tract.

Drug safety and plant extraction

Although herbal medicines are usually made from plant materials, this does not make them absolutely safe. The development and standardisation of such medicines require careful and accurate analysis of the content of active substances and their standard concentrations. This is critically important to ensure both the effectiveness and safety of these drugs. For example, the *Asteraceae* family is characterised by a high concentration of thujones in essential oils. This concentration can vary substantially depending on the organ of the plant and the stage of its development. Studies show that thujones have dose-dependent genotoxic and carcinogenic properties (Corvino *et al.*, 2023).

In addition, an important aspect is the quality control of raw materials, including analysis of the content of harmful elements. Thus, in the study by Chekirov *et al.* (2018), soil and *Achillea millefolium* samples collected every 3 km at seven stations along the Alamedin River were analysed. The use of inductively coupled plasma mass spectrometry (ICP-MS) allowed determining the content of heavy metals and mineral elements. The results of the study showed differences in the concentrations of calcium (Ca), copper (Cu), potassium (K), magnesium (Mg), and nickel (Ni) in different plant organs and soil. High levels of heavy metals have been recorded in urbanised areas, indicating a substantial anthropogenic impact.

Both dry and fresh parts of the plant can be used for the synthesis of active components. The leaves, flowers, and stems of pyzhma and yarrow are suitable for extracting active substances (Zaychenko *et al.*, 2019). The dry vegetable raw materials are pre-ground to the desired size, which increases its surface area and contributes to more efficient extraction of active substances. After that, sieving and cleaning are conducted to remove unnecessary impurities (Maharramova, 2021). Extraction occurs when the solvent interacts with plant tissue, resulting in the dissolution of phytochemicals having a similar polarity to the solvent. In the plant extract, phytochemicals can also act as biocatalysts.

The active components of plants can be extracted by various methods, including maceration, reflux extraction,

ultrasonic, and microwave treatment, percolation, hydrodistillation, Soxlet extraction, and boiling. Distilled water, ethanol, and methanol are most often used in the extraction of *Tanacetum* and *Achillea* (Nizhenkovska *et al.*, 2018). For the analysis of essential oils and lipids of two types of *Achillea*, Asilbekova *et al.* (2019) applied the method of hydrodistillation using the Clevenger apparatus. Neutral lipids were extracted using hexane, whereas polar lipids – a mixture of chloroform and methanol. The obtained extracts were analysed by thin-layer chromatography (TLC), column chromatography (CC), and gas chromatography with a mass spectrum detector (GC-FID).

Farajpour *et al.* (2024) used the method of hydrodistillation using the Clevenger apparatus (by vapour condensation) to obtain essential oil from the aboveground parts of thirty-five samples of three *Achillea* species. Ground to a fine powder and dried above ground parts of plants (100 grammes each) were subjected to hydrodistillation for three hours.

The study conducted by Al-Rimawi *et al.* (2024) consisted of obtaining an extract from the dried powder of *A. fragrantissima* by multistage extraction. In this case, 2 grammes of powder prepared from the leaves, stems, and flowers of *A. fragrantissima* were soaked in 150 ml of distilled water, ethanol, and 1% sodium bicarbonate solution. The extraction process was conducted for 20 minutes. The mixture was left at room temperature for 12 hours to improve the extraction of active substances from plant material. The extracts were then filtered to remove plant particles and concentrated using an IKA WEREKRV06-ML rotary evaporator. The concentrated extracts were freeze-dried in a Labconco dryer until a constant weight was achieved. The finished dried extracts were stored in the refrigerator until use.

The highest concentration of tannins in *Achillea millefolium* was recorded during budding, amounting to 7.1-2.6%. The peak content of flavonoids was noted during the flowering period (0.186-0.028%), which makes this period optimal for the collection of the *Herba Millefolii* drug. In *Tanacetum vulgare*, the highest content of tannins (8.75-4.3%) and flavonoids (0.160-0.063%) was also identified during budding. The content of tannins of at least 4% and flavonoids of at least 0.02% is recommended for Herba Tanaceti (Ivancheva *et al.*, 2000).

The stages of collecting flowers of feverfew substantially influenced the content and composition of the essential oil. Omidbaigi *et al.* (2007) established that the highest average essential oil content (0.55%) was obtained from plants harvested at the stage of fruit formation, while the lowest average content (0.35%) was extracted from flowers harvested at the budding stage.

CONCLUSIONS

The results of the study showed that for the Kyrgyz Republic, the characteristic species of the *Asteraceae* family are *Achillea millefolium, Achillea setacea, Achillea asiatica, Achillea filipendulina, Achillea collina, Tanacetum vulgare, and Tanacetum parthenium*. Species of the genus *Achillea* are found in Turkestan-Alai province, Kyrgyz Ala-Too, Tien Shan, Pamir-Alai, Alai ranges, and other regions. The characteristic landscapes were steppe, meadow, shrubs, adyr zones, river valleys, and roadsides.

Essential oils such as thujol, thujone, camphor, borneol, humulene, furfural, azulene, limonene, chrysanthemol, 1,8-cineol, etc., were responsible for the choleretic properties of plants. In large quantities, pyzhma and yarrow contain flavonoids quercetin,

casticin, certisin, apigenin, diosmetin, cosmosin, etc., which are also characterised by choleretic, hepatoprotective, and antispasmodic properties. Sesquierpenes (achillin, austricin, balhanide, dihydroacetoxitamacin, hydroxyachilin, leucodine) with a powerful anti-inflammatory effect also have a huge therapeutic potential. *Achillea* has hemostatic, antipyretic, diuretic, antibacterial, anthelmintic, and laxative properties. The *Tanacetum* species is characterised by antioxidant, antimicrobial, anthelmintic, choleretic, litholytic, anti-inflammatory, and analgesic properties. The extraction of pyzhma and yarrow can be done with fresh or dried plant organs. Flowers, leaves, and stems of pyzhma and yarrow are used. After grinding and filtration, the process of synthesis of nanoparticles from plants occurs by maceration, boiling, percolation, and other methods.

In the future, it is necessary to continue research aimed at systematising this knowledge and confirming it through clinical trials. It is advisable to collect and analyse plant samples from different regions and environmental conditions to identify possible differences in chemical composition and biological activity.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Response of PGRs and chemical substance in seeds dormancy breaking and seedling growth of custard apple (*Annona squamosa* L.) cv. Local cultivar

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Receipt: 27.10.2024 *Revised: 18.11.24* *Acceptance: 20.11.24*
DOI: 10.53552/ijmfmap.10.2.2024.24-31

ABSTRACT

Custard apple seeds take a long time to germinate due to their hard and thick seed coat which requires breaking. There are many plant growth regulators (PGRs) available for the purpose. An experiment was carried out to know the most suitable PGR for breaking seed dormancy and faster growth of seedlings during February to May 2023 at Krishi Vigyan Kendra, Anjora, Durg, Chhattisgarh, India. There were seven treatments having two PGRs and one chemical (GA_3, NAA, KNO_3,) and each PGR had two levels (500 and 1000 ppm) of seed soaking treatment for assessing seed germination and seedling growth related attributes. The Treatment GA_3 @1000 ppm was found the best among all treatments with respect to minimum number of days required for seed germination (35.02 DAS), 50% seed germination (49.44 %), seeds germination percentage (85.69%), survival percentage (91.43 %) and seedling height (15.29, 20.71 and 24.74 cm at 60, 75 and 90 DAS, respectively). Furthermore, seedling growth parameters such as number of leaves plant^{-1} (7.95, 9.31 and 10.38), collar diameter (0.29 & 0.39 cm) and root length (21.91 and 29.76 cm) at 60 and 90 DAS stages were also noted best in treatment GA_3 @1000 ppm. Additionally, the best performance of GA_3 @1000 ppm was also observed at 60 and 90 DAS in terms of fresh shoot weight (0.97 and 2.40 g), fresh root weight (0.15 and 0.44 g) and shoot and root ratio (0.60 and 1.17). Therefore, it was inferred from present experiment that GA_3 @1000 ppm should be used for breaking seed dormancy, optimum seed germination and better seedling growth related parameters in custard apple.

Keywords: Custard apple, dormancy, germination, PGRs, plant survival, seedling growth, shoot & root weight,

INTRODUCTION

Custard apple is a woody and semi-deciduous shrub and tree belonging to the family Annonaceae. It is propagated by different methods like seeds, grafting, budding, cutting etc. In India, farmers are propagating custard apple using many methods in which good and healthy planting material is very important. Custard apple seeds take a long time to germinate due to their hard and thick seed coat, which requires breaking, for which various methods were used by several researchers. Since the seed coat is hard and thick, there are many plant growth regulators (PGRs) available for breaking the seed dormancy (Sunder *et al.,* 2024, Pavithra *et al.,* 2018, Pandey and Bahadur, 2024). Seed germination is affected by many factors, including the type of substrate used, environmental factors such as oxygen, water,

temperature, and light. Some researchers observed that PGRs was effective in encouraging custard apple seed germination and growth (Rawat and Pandey 2019; Pravin *et al.*, 2021 and Rana *et al.*, 2020). Without the use of growth regulators, seeds showed poor response to germination and growth (Deeksha *et al.*, 2020). Seeds extracted from ripe fruits are used for sowing as the viability may be as short as few days, months, or at most a year and the seed viability depends upon storage environment. Recommended conditions for storing custard apple seeds are: temperatures between 15 and 20° C, low oxygen and ethylene tensions coupled with 10% carbon dioxide and a relative humidity of 85%–90% in the storage atmosphere. Pre-sowing seed treatment is a very useful method to improve seed germination and subsequent seedling growth in custard apple fruit species. Seed treatment is important for getting uniform and quick germination and to avoid the problem of uneven and irregular germination for obtaining plants for planting or for use as rootstock.

MATERIALS AND METHODS

The experiment of dormancy breaking of custard apple was carried out at Krishi Vigyan Kendra, Anjora, Durg, Chhattisgarh, India, under net house during February to May months, 2023. The experimental site is located in plains zone of Chhattisgarh at 20°54' and 21°32' north latitude & 81°10' and 81°36' east longitude. The district is 317 meters above mean sea level.

Seed of custard apple (*Annona squamosa* L.) were collected from local cultivars in Kanker District area of KVK, Kanker, Chhattisgarh. Seeds were extracted from freshly harvested fruits and stored for experiment. Rooting media were prepared which comprised of garden soil, sand and rotted FYM @ 2:2:1 ratio and were filled in poly bags (12cm x 10cm) of 50 microns thickness and seeds were soaked in PGRs and chemicals along with control (Table 1) for about 24 hours and then sown in pre-filled growing medium one seed per poly bags with 2-2.5 cm depth). All the poly bags were then kept under net house. The experiment was set up with randomized block design having three replications and 30 seeds were sown in each replicate, the total number of seeds in the experiment was 540.

Table 1: Plant growth regulators (PGRs) and chemical supplements

Symbol	Treatment details	Solution
T_0	Control	Distilled water
T_1	KNO_3 (0.5 %)	5g-Liter water^{-1}
T_2	KNO_3 (1 %)	10 g-Liter water^{-1}
T_3	GA_3 (500 ppm)	500 mg-Liter water^{-1}
T_4	GA_3 (1000 ppm)	1000 mg-Liter water^{-1}
T_5	NAA (250 ppm)	200 mg-Liter water^{-1}
T_6	NAA (500 ppm)	500 mg-Liter water^{-1}

After seed sowing evenly light watering was given to poly bags with rose can regularly and fungicide was sprayed with SAAF powder (Carbendazim 12% and Mancozeb 63% WP) 2g-L^{-1} during fungal infection.

Observations were recorded at 60, 75 and 90 DAS (days after sowing) on the parameters *viz.*, days taken to sprouting of seed germination, days taken to 50% seed germination, germination percentage (%),

survival percentage (%), seedling height (cm), number of leaves plant^{-1}, collar diameter (cm), root length (cm), fresh shoot weight (g), fresh root weight (g) and shoot and root ratio. Statistical analysis of data was carried out using MS-Excel, OPSTAT (Online statistical analysis software) for each observed character under study. Data investigation was analysed using randomised block design (RBD) (Gomez and Gomez,1985).

RESULT AND DISCUSSION

Seed germination and days taken to sprouting of seed germination (DAS): The statistical analysis of the observations on seed germination parameters has been presented in Table 2. A different pre-sowing seed treatment of custard apple seeds was found to have significant effect on seed germination. GA_3 at 1000 ppm took the fewest days (35.02) to start seed germination, followed by GA_3 (500 ppm) (35.36) and NAA (500 ppm) (37.44) in that order. While maximum time was taken under control treatment. Days needed for 50% of the seeds to germinate were significantly impacted by the various treatments. The least number of days (49.44) were needed for GA_3 (1000 ppm). But GA_3 (500 ppm) came next (52.20), and the control needed the most days (66.38).

The germination of custard apple seeds was significantly impacted by several treatments (Table 2). The GA_3 (1000 ppm) recorded the highest germination percentage (85.69%) when compared to other treatments, while the control group was lowest percentage (60.59%). Similar results were also reported by different researchers in custard apple Patel *et al.* (2017); Singh and Maheshwari, (2017); Lawhale *et al.* (2020); Pravin *et al.* (2021) and Rawat and Pandey (2019).

Survival percentage (%)

Significant variation in survival percentage was observed in the present study (Table 2) with the highest survival percentage (91.43%) recorded with soaking in GA_3 (1000 ppm) followed by GA_3 (500 ppm) (91.31%) and NAA (500 ppm) (90.04%) treatments. The lowest survival percentage (77.97%) was observed with control treatment. Rana *et al.* (2020), Jain *et al.* (2017), Yadav *et al.* (2018), and Rajput and Sharma (2020) all corroborated the current custard apple and Suja *et al.* (2016) in walnut seed germination and seedling growth under Kashmir valley conditions.

No. of leaves plant^{-1}

The number of leaves in plant^{-1} affected by various pre-sowing seed treatments is given in (Table 3). After 60, 75, and 90 days of sowing, it was found that the plants in the GA_3 (1000 ppm) treatment had more leaves (7.95, 9.31, and 10.38) than those in the GA_3 (500 ppm) (7.65, 8.80, and 10.01). The control was less leaves plant^{-1} (4.72, 6.31 and 7.08 respectively). Kumawat *et al.* (2014) in papaya seeds and Halder *et al.* (2023) in Indian olive seeds also noted that treating seeds with various chemicals resulted in the largest number of leaves and leaf size.

Collar diameter (cm)

Data regarding collar diameter at 60 and 90 days after sowing are presented in (Table 3). The maximum collar diameter at 60 and 90 days after sowing was recorded in GA_3 (1000 ppm) with the value of 0.29 and 0.39 cm, respectively, whereas it was followed by (0.28 and 0.37 cm,) in GA_3 (500 ppm). While, the minimum collar diameter as 0.26 and 0.33 cm under control. Similar results have been reported by Palepad *et al.* (2017), Rawat and Pandey (2019), Rajput and Sharma (2020) in custard apple and Muralidhara *et al.* (2023) in sapota.

Root length (cm)

Data on how various seed treatments effect root length are shown in (Table 3). At 60 and 90 DAS, the GA_3 (1000 ppm) treatment produced the longest root length of seedlings (21.91 & 29.76 cm), followed by NAA (500 ppm) with values (21.55 & 25.97 cm). The shortest root length (16.51 and 20.07 cm) was measured in control. Palepad *et al.* (2016); Singh and Maheswari (2017); Mane *et al.* (2019); Ara *et al.* (2022) and Dadhaniya *et al.* (2020) all noted that the elongation of the cells in the sub-apical area of roots was caused by the increase in osmotic uptake of nutrients brought on by the administration of various hormones.

Seedling height (cm)

Seedling height (cm) for treatments at 60, 75, and 90 days after sowing were recorded (Table 4). The highest seedling height was recorded in GA_3 (1000 ppm) (15.29, 20.71, and 24.74 cm, respectively), followed by GA_3 (500 ppm) (15.06, 19.79 & 23.44 cm, respectively). The lowest seedling heights were observed under control (10.10, 15.19, and 17.86 cm, respectively). Similar findings in custard apple have been reported by Patel *et al.* (2016); Bhowmick and Santhoshkumar (2023); Dey *et al.* (2022); Rawat and Pandey (2019); Lawhale *et al.* (2020) and Pravin *et al.* (2021).

Seedling fresh shoot and root weight (g)

The various pre-sowing treatments had a substantial impact on the fresh weight of the shoots and root of custard apple seeds, as shown in (Table 4). Following 60 and 90 days of seeding, the GA_3 (1000 ppm) showed the highest fresh weight of shoots (0.97 & 2.40 g) respectively. The treatment (control) resulted in a minimum fresh weight of shoots (0.62 & 1.37 g).

GA_3 (1000 ppm) produced the highest fresh weight of root (0.15 & 0.44 g) 60 and 90 days after sowing, followed by GA_3 (500 ppm) (0.14 & 0.43 g) respectively. In the control, the lowest fresh weight of root (0.07 & 0.21 g) was noted. Possible reason of increased fresh shoot & root weight (g) could be the overall growth of shoots and increased rate of photosynthesis, which result in higher fresh weight. The outcomes closely match the custard apple research conducted by Rajput and Sharma 2020; Rawat and Pandey 2019; Yadav *et al.*, 2018 and Patel *et al.*, 2016.

Shoot and root ratio

Analysis of the data revealed that the shoot to root ratio of seedlings (Table 4) was significantly impacted by the various seed treatments applied 60 and 90 days after sowing. Maximum in the GA_3 (1000 ppm) (0.60 & 1.17) and lowest shoot and root ratio of seedlings (0.42 & 0.75) was observed in control. Similar results are in agreement with the findings of Kumawat *et al.* (2014) in papaya, Rai *et al.* (2018) in khirni, Dev *et al.* (2020) in saucer-berry, Boricha *et al.* (2020) in guava and Lalitha *et al.* (2020) in aonla.

CONCLUSION

It is concluded that among the different pre-sowing treatments, GA_3 at 1000 ppm had the highest performance in terms of germination, seedling growth, and survival. For farmers/nurserymen point of view, a concentration of 1000 ppm of GA_3 is recommended to obtain beneficial results.

ACKNOWLEDGEMENTS

Author (s) express heartfelt gratitude to the staff members and workers of Krishi Vigyan Kendra, Anjora, Durg, who provided proper guidance and advice at every stage of experimentation.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2: Effect of PGRs in seed germination parameters of custard apple cv. Local cultivar

Treatments	Days taken to sprouting of seed germination (DAS)	Days taken to 50% seed germination (DAS)	Germination percentage (%)	Survival percentage (%)
Control	41.94	66.38	60.59	77.97
KNO_3 (0.5 %)	38.14	58.77	68.33	81.97
KNO_3 (1 %)	37.78	57.04	69.42	82.89
GA_3 (500 ppm)	35.36	52.20	83.98	91.31
GA_3 (1000 ppm)	35.02	49.44	85.69	91.43
NAA (250 ppm)	37.46	56.70	75.41	89.11
NAA (500 ppm)	37.44	56.35	77.13	90.04
SE(m)±	**0.958**	**1.522**	**1.615**	**1.965**
C.D.at 5%	**2.933**	**4.662**	**4.945**	**6.019**

Table 3: Effect of PGRs in No. of leaves plant^{-1}, collar diameter (cm) and root length (cm) of custard apple cv. Local cultivar

Treatments	No. of leaves plant^{-1}			Collar diameter (cm)		Root length (cm)	
	60	75	90	60	90	60	90
Control	4.72	6.31	7.08	0.26	0.33	16.51	20.07
KNO_3 (0.5 %)	6.42	7.57	8.92	0.27	0.34	17.86	23.63
KNO_3 (1 %)	6.55	7.57	9.19	0.27	0.35	18.07	24.34
GA_3 (500 ppm)	7.65	8.80	10.01	0.28	0.37	21.55	25.97
GA_3 (1000 ppm)	7.95	9.31	10.38	0.29	0.39	21.91	29.76
NAA (250 ppm)	7.14	8.59	9.88	0.27	0.35	20.71	25.85
NAA (500 ppm)	7.22	8.66	9.95	0.28	0.36	21.61	26.42
SE(m)±	**0.276**	**0.241**	**0.301**	**0.010**	**0.014**	**0.659**	**0.458**
C.D.at 5%	**0.844**	**0.737**	**0.920**	**N/A**	**N/A**	**2.017**	**1.404**

Table 4: Effect of PGRs in seedling growth parameters of custard apple cv. Local cultivar

Treatments	Seedling height (cm)			Fresh shoot weight (g)		Fresh root weight (g)		Shoot and root ratio	
	60	75	90	60	90	60	90	60	90
Control	10.10	15.19	17.86	0.62	1.37	0.07	0.21	0.42	0.75
KNO_3 (0.5 %)	13.77	17.88	20.88	0.78	1.60	0.12	0.25	0.50	0.82
KNO_3 (1%)	13.84	18.36	21.70	0.81	1.62	0.12	0.26	0.52	0.83
GA_3 (500 ppm)	15.06	19.79	23.44	0.94	2.29	0.14	0.43	0.58	1.15
GA_3 (1000 ppm)	15.29	20.71	24.74	0.97	2.40	0.15	0.44	0.60	1.17
NAA (250 ppm)	14.63	18.84	22.32	0.84	1.94	0.13	0.32	0.54	0.97
NAA (500 ppm)	14.89	19.35	22.87	0.89	2.14	0.14	0.33	0.56	1.05
SE(m)±	**0.427**	**0.542**	**0.774**	**0.026**	**0.065**	**0.004**	**0.012**	**0.047**	**0.202**
C.D.at 5%	**1.306**	**1.660**	**2.372**	**0.079**	**0.198**	**0.013**	**0.037**	**N/A**	**N/A**

Influence of *jeevamrit* and *kunapajala* on growth and herbage yield of sweet basil (*Ocimum basilicum* L.) under Mollisol region of Uttarakhand

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Receipt: 21.09.2024 *Revised: 10.11.24* *Acceptance: 13.11.24*
DOI: 10.53552/ijmfmap.10.2.2024.32-41

ABSTRACT

A field experiment was carried out during the Kharif season of 2019 at the Medicinal Plants Research and Development Centre (MRDC), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, to investigate the impact of jeevamrit and kunapajala on the herbage yield and quality of sweet basil (Ocimum basilicum L.). The experiment was laid out in Randomized Block Design with eight treatments replicated thrice. Treatments i.e. T_1: Recommended dose of fertilizer (RDF) (120:60:40) kg/ha, T_2: 15 t/ha farmyard manure (FYM), T_3: 500 l/ha kunapajala, T_4: 1000 l/ha kunapajala, T_5: 500 l/ha kunapajala + 7.5 t/ha FYM, T_6: 500 l/ha jeevamrit, T_7: 1000 l/ha jeevamrit, T_8: 500 l/ha jeevamrit+ 7.5 t/ha FYM. The results revealed that the treatment T_1 obtained highest plant height (109.67 cm), number of branches (20.50), leaf: stem ratio (0.85), fresh weight (615.74 g/plant), dry matter accumulation (116.71 g/plant), crop growth rate (10.29 g/m^2/day) as well as herbage yield (271.86 q/ha) but was statistically at par with treatment T_8. Keeping in view the harmful effects of chemical fertilizers, the use of these eco-friendly fermented organic liquid manures provides alternate production technologies.

Keywords: Dry matter, *jeevamrit*, k*unapajala, Ocimum basilicum*, organic, yield

INTRODUCTION

Sweet Basil (*Ocimum basilicum* L.) of Lamiaceae family is one of the most important medicinal and aromatic plants grown in India. It is a warm, tropical industrial crop with a short growing period of 75-90 days. The leaves and seeds of sweet basil are economically important parts of the whole plant. The different plant parts of the sweet basil contain heterogeneous group of aromatic compounds of immense value of flavour and fragrance (Corrado *et al.,* 2020). The essential oil of basil contains mono-terpenes, phenol, sesqui-terpenes, eugenol, methyl-eugenol, thymol, methyl-cinnamate, linalool, methyl chavicol, Citral 'A' and 'B', alcohol, camphor, etc., which are liable for the unique pleasant odour and flavors (Hallmann *et al*., 2024). The oil of sweet basil finds different uses in the cosmetic and perfumery industries and also in indigenous system of medicine. Its oil is utilized for flavouring food stuff in confectionary, thermogenic, cardiotonic, condiments, depurative, dental cream and mouth freshener and other countless indigenous and ayurvedic health care system (El-Mahrouk *et al*., 2024). Its extract can be utilized as bio-insecticide, fungicide, antifeedants and preparation of food products. A little work has been accomplished so far on mineral nutrition of different medicinal and aromatic crops including sweet basil (*Ocimum basilicum* L.). The organic fertilization is not just a cost effective and ecofriendly, but improves soil environment, yield and oil

quality of medicinal and aromatic plants. Not all nutrients in the soil are immediately available to plants. They must first be converted into an accessible form through the action of microorganisms naturally present in the soil. However immoderate use of chemical products has disturbed the flora as well as fauna along with the population of micro-organisms and population of earthworms is almost negligible. Therefore, it is essential to activate and sustain populations of various soil microorganisms and enhance earthworm biomass through innovative natural or traditional methods, such as the use of desi cow dung, cow urine, and organic matter. Ancient Indian texts describe more scientifically and clinically formulated liquid biofertilizers under the general name "*kunapajala*," as noted by Surapala (1996) in the *Vrikshayurveda*. These biofertilizers stimulate biological activity in the soil and make nutrients available to crops (Kanali, 2016). Organic liquid manure, such as *jeevamrit*, is a rich bio-formulation containing beneficial microbial consortia (Pathak and Ram, 2013). *Jeevamrit* also enhances soil nitrogen content by promoting non-symbiotic nitrogen fixation. In light of these findings, the present study, titled "Effect of *jeevamrit* and *kunapajala* on growth and herbage yield of sweet basil under the Foothills of Shivalik Himalayan, India," was conducted.

MATERIAL AND METHODS

Experimental Site: The field experiment on Sweet basil variety CIM-Saumya was carried out at the Medicinal Plants Research and Development Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar (Uttarakhand) during the Kharif season of 2019. Pantnagar is situated in the Tarai belt, approximately 30 km south of the foothills of the Shivalik range of the Himalayas, at a longitude of 79° 29′ E and an altitude of 243.83 meters above mean sea level, within a subtropical humid climate. The soils of the Tarai region belong to the Mollisol order and have a sandy clay loam texture. The soil used in the experiment was sandy clay loam, neutral in pH, with medium organic carbon content (0.68%), low available nitrogen (186.60 kg/ha), and medium levels of phosphorus (18.90 kg/ha) and potassium (201.23 kg/ha).

Experimental details: The experiment was designed in Randomized Block Design with 8 treatments replicated thrice. A total of 24 plots each with gross plot size of 5.0 x 3.2 m and a net plot size of 3.5 x 2.4 m were made.

Treatment details: T_1: RDF ((N_{120}:P_{60}:K_{40}) kg/ha); T_2: FYM @ 15 t/ha, T_3: *Kunapajala* @ 500 l/ha, T_4: *Kunapajala* @ 1000 l/ha, T_5: *Kunapajala* @ 500 l/ha + FYM @ 7.5 t/ha, T_6: *Jeevamrit* @ 500 l/ha, T_7: *Jeevamrit* @ 1000 l/ha, T_8: *Jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha

Cultural operations: The experimental field was ploughed by disc plough followed by two harrowing and leveled by using wooden plank. Plots were made, each separated by bunds of 60 cm width and 20 cm height. Well decomposed FYM was incorporated in the field 10 days before the planting was done. The recommended dose of fertilizer (120:60:40) kg/ha in the form of urea, Diammonium phosphate (DAP) and Muriate of potash (MOP) were applied in the field. Uniform amount of *jeevamrit* (200 l) and *kunapajala* (200 l) were applied in the plots before planting of the seedlings. After that, both the formulations were applied at regular intervals of 15, 30, 45, and 60 days after transplanting up to the final harvest of the crop. A total of 2 irrigations were given during the entire growth period. Manual weeding and mulching were done to check the weed flora. The crop was harvested at 80 days after planting manually with the help of sickle by cutting plant at ground level in the plots leaving a border of 50 cm.

Preparation and application of organic sources of nutrients

Jeevamrit was prepared using the method developed by Padmashri Shri Subhash Palekar (Palekar, 2006), while *kunapajala* was prepared following the method outlined by Nene (2012).

Jeevamrit

Jeevamrit is a rich microbial formulation prepared by providing the un-restricted growth of microbes through the fermentation process. The components used were cow dung, pulse flour, cow urine, jaggery and virgin forest soil (for inoculation). It can be used at a regular interval of 15-30 days with irrigation water. This bio- formulation can be used within 6-7 days of preparation. A quantity of 200 litres is sufficient for an acre area. 3-4 sprays are sufficient for one crop cycle. *Jeevamrit* can be used for drenching the mulch material before its application. It is also effective in quickly decomposition of residues in the field if given with irrigation water applied for field preparation. 3-4 times more area can be covered, if micro irrigation is adopted with the same amount of *jeevamrit*.

Materials required for preparation of 200 litres *jeevamrit* (for an acre of land)

S. No.	Ingredients	Quantity	S. No.	Ingredients	Quantity
1.	*Desi* cow dung	10 kg	4.	Virgin forest soil	100 g
2.	Pulse flour	2 kg	5.	Jaggery	2 kg
3.	*Desi* cow urine	5 litres	6.	Water	200 litres

Procedure

All the ingredients *i.e.*, *desi* cow dung, pulse flour, cow urine, virgin forest soil and jiggery were mixed in the required amounts as mentioned above in 200 litres of water and rotated clockwise twice a day and stored for 6-7 days for fermentation, than *jeevamrit* was applied with irrigation water.

Kunapajala

According to Y.L. Nene, the organic source '*kunapajala*' has the potentiality to play the role of providing immunity and promoting growth in the plant system. The *kunapajala* bioformulation proposed by Y. L. Nene and S. L. Choudhary consists of nine products: *Desi* cow dung, *desi* cow urine, cane jaggery, germinated blackgram pulse, mustard oil cake, finely chopped local weeds (broadleafy only), Virgin forest soil and plain water. It can be used at an interval of 15-30 days with irrigation water. Soil drenching and foliar application of *kunapajala* from the beginning up to 40 days of its preparation will be helpful for plant and soil so that we can utilize its total potential.

Materials required for preparation of 200 litres *kunapajala* (for an acre of land)

S. No.	Ingredients	Quantity	S. No.	Ingredients	Quantity
1.	*Desi* cow dung	20 kg	5.	Mustard oil cake	2 kg
2.	*Desi* cow urine	10 litres	6.	Finely chopped local weeds (broad leafy only)	20 kg
3.	Jaggery	2 kg	7.	Water	100 litres
4.	Germinated pulse (urd bean)	2 kg	8.	Virgin forest soil	100 gm

A quantity of 200 litres is sufficient for an acre area. 2-3 sprays are sufficient for one crop cycle. *Kunapajala* can be used for drenching the mulch before its application. It

is effective in quickly decomposition of residues in the field if drenched with irrigation water given for field preparation. If micro irrigation is adopted, 3-4 times more field area can be covered with the same amount of *kunapajala*.

Procedure

All the ingredients *i.e.*, *desi* cow dung, cane jaggery, *desi* cow urine, germinated blackgram pulse, mustard oil cake, finely chopped local weeds (broad leafy only), and Virgin forest soil were mixed in the required amounts in 100 litres of water and rotated clockwise twice a day and stored for 6-7 days for fermentation, than *kunapajala* was applied with irrigation water but recommendation for application is from 20 to 40 days after preparation. As much quantity as require, it can be made in advance and can be used for a long time. The older it is the batter it is for use.

Observations

Plant height per metre square per plant (cm) with wooden scale, number of branches per metre square per plant, Leaf-stem ratio (at harvest) of three plants, fresh weight in grams per metre square (gm) and dry weight after oven drying at 65 ± 5°C for 48-72 hours in grams per metre square (gm) of three plants were recorded manually at 30, 60 days after planting and at harvest and averaged.

Total fresh herbage yield

$$\text{Total herbage yield (q ha}^{-1}) = \frac{\text{Weight of plants harvested from net plot area (kg)}}{\text{net plot area} \times 100} \times 10000$$

Growth analysis

Mean crop growth rate

Crop growth rate was calculated by given formula:

$$\text{CGR} = \frac{W_2 - W_1}{t_2 - t_1} (\text{g m}^{-1}\text{day}^{-1})$$

Where, W_1 and W_2 are dry matter productions at times t_1 and t_2 respectively. It is expressed as gram of dry matter produced per square meter per day (g m^{-1} day^{-1}) (Redford, 1967).

Mean relative growth rate

Relative growth rate (RGR) was calculated by using the given formula:

$$\text{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} (\text{mg g}^{-1}\text{day}^{-1})$$

Where, W_1 and W_2 are the dry weights of plant at time t_1 and t_2 respectively. It is expressed as mg of dry matter which is produced by gram of existing dry matter in a day (mg/g/day) (Redford, 1967).

Statistical analysis

The experimental data were analyzed using the standardized procedure for a randomized block design (RBD) with the assistance of a computer program for R.B.D (STPR-3), developed by the Department of Mathematics and Statistics, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

RESULT AND DISCUSSION

Plant height

The data (Table 1) found that all the treatments had a direct and significant influence on the plant height. At 30 DAP it was observed that treatment T_1 recorded the highest plant height (41.87 cm) which was significantly highest among all the treatments. A similar trend was observed at 60 DAP, treatment T_8 in which the plant height was lower than T_1 at 30 DAP it was *at par* with T_1 at 60 DAP, The lowest plant height during both at 30 and 60 days (32.67 cm and 67.16 cm respectively) was observed in treatment T_2. At harvest, a significant difference was observed in the plant height as affected by different treatments among which T_1 remained significantly highest (109.67 cm) and was *at par* with T_8 (104.56 cm) and also found *at par* with T_5 (99.53

cm) in which the plant height was lower than T_1 at 30 and 60 DAP while all the other treatments were lower. Among the different treatments of fermented liquid manure, the better performance was observed in treatment T_8 and T_5. Solid application of *jeevamrit* or *kunapajala* on soil showed fair results but non availability of organic matter (Farmyard manure) for microbes restricted the continuous availability of essential nutrients to plant resulted lower plant height in treatments T_3, T_4, T_6 and T_7. Supportive evidence was given by research conducted by Al-mansour *et al.* (2018) in sweet basil and Dey *et al.* (2019). Also, similar results were reported by Chauhan (2019) in bramhi where application of *jeevamrit* @5000 l/ha combined with FYM @2.5 ton/ha reported highest shoot length, no. of shoots and no. of leaf/m^2.

Number of branches/plant

Like plant height, numbers of branches were also considerably affected by different treatments and with the advancement of crop age (Table 1). The number of branches was significantly higher in treatment T_1 (17.53 branches/plant) over the rest of the treatments at 30 DAP and it was found statistically *at par* with T_8 treatment (16 branches/plant) followed by T_5 treatment (15.85 branches/plant) while all the other treatments (T_2, T_3, T_4, T_6 and T_7) showed lower no. of branches. Similar results were observed at 60 DAP. At harvesting stage the no. of branches were significantly higher in treatment T_1 (21.81 branches/plant) which is *at par* with treatment T_8 (20.50 branches/plant) and the rest of the results were similar to as on 30 and 60 DAP. Combined application of liquid manures with FYM resulted in more number of branches. It can be related to the fact that liquid manures (product of microbial consortia) when applied in the soil helps to enhance the microbial population that in turn helps in quick decomposition of FYM and helps to mineralize the organic stock of nitrogen in the soil. Similar results were reported by Chauhan (2019) in bramhi where application of *jeevamrit* @5000 l/ha combined with FYM @2.5 ton/ha reported highest number of shoots.

Leaf-stem ratio at harvest (on fresh weight basis)

The factor that decides the harvesting stage of basil is leaf-stem ratio (Table 1). At harvest, the maximum leaf-stem ratio was recorded in treatment T_1 (0.85) significantly higher than the other treatments which was *at par* with the treatments T_8 (0.80) and T_5 (0.76). The lowest leaf-stem ratio was recorded in treatment T_2 (0.62). In all the growth attributes discussed above, it is evident that treatment T_1 showed the highest results at all the growth stages of crop followed by T_8. It was also observed that T_8 and T_5 were *at par* during most of the crop stages for different growth attributes. The solid application of farmyard manure or single application of fermented liquid manures (*jeevamrit* and *kunapajala*) showed less crop growth in all the growth stages. The similar result has been corroborated by Patel *et al*. (2018). Ankad *et al.* (2018) also found similar results in *Withania somnifera* where *kunapajala* treatments were higher in leaf area index and total leaf area.

Fresh weight (g/plant)

There was an increasing trend of dry matter with increased dose of liquid manures but combined application of liquid formulations with FYM showing its significant effect on the same as compare to solid application (Table 2). Fresh weights taken at 30 DAP showed that T_1 treatment showed the highest fresh weight (114.72 g/ plant) which was significantly higher than rest of the treatments. At 60 DAP, the trend in fresh weight as affected by different treatments was the same like 30 DAP. The observations recorded at harvest showed significant yield advantage of treatment T_1 (615.7 g/plant) over various treatments. The lowest fresh biomass was observed in treatment T_2 (449.6 g/plant). Use of liquid organic products like *jeevamrit*, *beejamrit* and *kunapajala* results in higher growth, yield, and higher quality of

crop and improve the soil biological as well as physico-chemical properties (Devakumar *et al*., 2008 and Tharmaraj *et al*., 2011).

Dry matter accumulation

Dry matter has a direct correlation with the fresh weight of the crop and is directly influenced by the treatment combinations at all the growth stages of crop (Table 2). At 30 DAP, the treatment T_1 (16.2 g/plant) was found statistically *at par* treatment T_8 (14.8 g/plant) and treatment T_5 (14.2 g/plant). The lowest dry matter accumulation was recorded in T_2 (12.07 g/plant). At 60 DAP, T_1 (65.3 g/plant) had a significant effect on the dry matter accumulation by plant and was *at par* with T_8 (61.4 g/plant) and T_5 (60.2 g/plant) Solely applied liquid manures had less dry matter accumulation as compared to integrated application of FYM+ *jeevamrit* or *kunapajala*. The lowest accumulation of dry matter was recorded in treatment T_2 (48.5 g/plant). At harvest, the treatments differed significantly from each other and it was recorded that treatment T_1 (116.7 g/plant) was also *at par* with T_8 (110.7 g/plant), T_5 (108.9 g/plant) and T_7 (105.6 g/plant). Treatment T_2 (92.2 g/plant) gave the lowest values of dry matter accumulation at harvest. Since the dry weight is directly related to the fresh herbage yield of the crop is also affected by the same parameters as the former. In an experiment conducted by Sharma *et al.* (2010) in carnation Siddappa (2015) in fieldbean and Kaur (2019) in wheat by applying bioenhancer also showed the same results.

Mean crop growth rate (CGR)

The CGR was significantly affected by the treatments (Table 3) and when recorded at the initial stage (30-60 DAP), the highest value was recorded in T_1 (8.19 g/m^2/day) which was significantly *at par* with T_8 (7.78 g/m^2/day), T_5 (7.68 g/m^2/day) and T_7 (7.46 g/m^2/day). It was observed that treatment T_3 (6.14 g/m^2/day) T_4 (6.76 g/m^2/day) and T_6 (6.31 g/m^2/day), showed the lower crop growth rate. A rapid increase in crop growth rate was observed at 60-85 days interval. Treatment T_1 (10.29 g/m^2/day) showed highest crop growth rate which was significantly higher than all the others treatment and it was found statistically *at par* with treatment T_8 (9.84 g/m^2/day) and T_5 (9.74 g/m^2/day). The solely applied *jeevamrit* and *kunapajala* treatments showed lower crop growth rate as compare to combined application with FYM. The lowest crop growth rate was observed in T_2 (8.73 g/m^2/day). Natural solutions (*jeevamrit* and *kunapajala*), which may not provide enough nutrients in applied area, but they help in the quick soil fertility build-up through increased activity of soil microflora and fauna (Yadav and Mowade, 2004).

Mean relative growth rate (RGR)

All the treatments positively affected the relative growth rate of the crop at all the growth stages (Table 3). At 30-60 days, it was observed that the relative crop growth rate was highest in treatment T_7 (48.31 mg/g/day) which was significantly higher than most of treatments. Treatment T_7 was *at par* with the all the remaining treatments *viz*., T_5 (48.26 mg/g/day), T_8 (47.57 mg/g/day), T_1 (46.78 mg/g/day) and T_2 (46.67 mg/g/day). At 60-85 days period, it was found that the growth rate was maximum in treatment T_3 (26.30 mg/g/day) which was significantly superior than other treatments. The lowest relative crop growth rate was observed in T_1 (23.25 mg/g/day). This can be attributed to the deficiency of nutrients in absence of organic manures, as *jeevamrit* may not directly provide enough nutrients, required by crop but it supports the crop growth initially. It needs organic source for microflora build up, in former and continual supply of microbial inoculums (*jeevamrit* and kuanapajala) to soil in later stages, which might have brought down the population of soil microbial flora and fauna, hence low rate of mineralization of native nutrients. Similar resuls were also obtained by Yadav and Vijayakumari (2004).

Fresh biomass yield

All the treatments had a positive and significant influence on the crop fresh biomass yield (Fig. 1). The application of NPK (120:60:40 kg/ha) *i.e.*, T_1 recorded significantly the highest fresh herbage yield (271.86 q/ha). It was closely followed by *jeevamrit* @ 500 L + FYM @ 7.5 t/ha (256.07 q/ha) *i.e.* T_8 followed by *kunapajala* @ 500 L + FYM @ 7.5 t/ha (244.03 q/ha) *i.e.* T_5 and these were found statistically *at par* to T_1 (271.86 q/ha). The lowest fresh herbage yield was recorded in T_2 applied with FYM (15 t/ha) alone (199.57 q/ha) and application of different doses of *jeevamrit* and *kunapajala* alone or their combination with FYM *i.e.* T_3, T_4, T_5, T_6, T_7, T_8 showed significantly higher fresh herbage yield as compare to FYM alone. The results obtained during the course of experiment indicated that there was a significant increase in fresh herbage yield which is due to more contribution of yield attributing parameters. Many successful attempts to show the beneficial effects of fermented liquid formulation on crop growth were done by Balakumbahan *et al.* (2010) in *Acorus calamus*. Similarly, Chauhan (2019) recorded higher fresh biomass yield in bramhi at different doses of *jeevamrit* combined with FYM.

CONCLUSION

Based on these findings and to ensure a balance between crop production and soil health, it can be concluded that applying *jeevamrit* at 500 l/ha combined with half of the recommended dose of farmyard manure (7.5 t/ha) is effective for providing nutrients to crops, enhancing fresh herbage yield, and maintaining soil health over the long term. This approach could serve as an alternative production technique for organic farmers and offer a new perspective for the scientific community to further refine and validate traditional farming practices in today's context, aiming to produce safe food, sustain soil health, and protect the environment.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Plant height (cm), Number of branches (per plant) and leaf: stem ratio as influenced by different treatments

Treatments details	Plant height (cm)			Number of branches (per plant)			Leaf: stem
	30 DAP	60 DAP	At harvest	30 DAP	60 DAP	At harvest	
T_1: RDF (N_{120}:P_{60}:K_{40}) kg/ha	41.87	77.17	109.67	17.53	20.50	21.81	0.85
T_2: FYM @ 15 t/ha	32.67	67.16	84.67	13.00	14.83	16.00	0.62
T_3: *Kunapajala* @ 500 l/ha	33.33	68.07	91.56	14.88	17.42	17.98	0.66
T_4: *Kunapajala* @ 1000 l/ha	34.20	68.99	93.06	15.50	17.73	18.67	0.71
T_5: *Kunapajala* @ 500 l/ha + FYM @ 7.5 t/ha	35.80	71.77	99.53	15.85	18.37	19.37	0.76
T_6: *Jeevamrit* @ 500 l/ha	33.73	70.05	92.22	15.30	17.55	18.08	0.68
T_7: *Jeevamrit* @ 1000 l/ha	35.07	71.13	98.10	15.75	18.23	18.83	0.74
T_8: *Jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha	37.07	74.89	104.56	16.00	18.57	20.50	0.80
S.Em±	1.70	1.76	3.95	0.66	0.76	0.84	0.04
CD at 5%	4.97	5.16	11.55	1.92	2.21	2.46	0.11

Table 2: Fresh weight of basil (g/plant) and dry matter accumulation of basil (g/plant) as influenced by different treatments

Treatments details	Fresh weight of basil (g/plant)			Dry matter accumulation (g/plant)		
	30 DAP	60 DAP	At harvest	30 DAP	60 DAP	At harvest
T_1: RDF (N_{120}:P_{60}:K_{40}) kg/ha	114.72	534.48	615.74	16.21	65.27	116.71
T_2: FYM @ 15 t/ha	84.88	385.61	449.66	12.07	48.50	92.17
T_3: *Kunapajala* @ 500 l/ha	90.42	412.59	476.57	12.83	49.67	95.87
T_4: *Kunapajala* @ 1000 l/ha	94.95	448.43	517.98	13.43	53.97	100.57
T_5: *Kunapajala* @ 500 l/ha + FYM @ 7.5 t/ha	100.85	477.18	551.18	14.17	60.23	108.9
T_6: *Jeevamrit* @ 500 l/ha	93.74	427.79	494.14	12.87	50.73	96.76
T_7: *Jeevamrit* @ 1000 l/ha	100.30	461.86	533.49	13.74	58.52	105.63
T_8: *Jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha	104.11	500.71	578.36	14.77	61.44	110.67
S.Em±	5.49	21.14	24.01	0.78	2.42	2.75
CD at 5%	16.08	61.85	70.24	2.28	7.08	8.04

Table 3: Crop growth rate (g/m^2/day) and relative growth rate (mg/g/day) as affected by different treatments

Treatments details	Crop growth rate (g/m^2/day)		Relative growth rate (mg/g/day)	
	30-60 DAP	60-85 DAP	30-60 DAP	60-85 DAP
T_1: RDF (N_{120}:P_{60}:K_{40}) kg/ha	8.18	10.29	46.78	23.34
T_2: FYM @ 15 t/ha	6.07	8.73	46.67	25.72
T_3: *Kunapajala* @ 500 l/ha	6.14	9.24	45.10	26.30
T_4: *Kunapajala* @ 1000 l/ha	6.76	9.32	46.38	24.90
T_5: *Kunapajala* @ 500 l/ha + FYM @ 7.5 t/ha	7.68	9.74	48.26	23.75
T_6: *Jeevamrit* @ 500 l/ha	6.31	9.20	45.71	25.84
T_7: *Jeevamrit* @ 1000 l/ha	7.46	9.42	48.31	23.63
T_8: *Jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha	7.78	9.84	47.57	23.72
S.Em±	0.33	0.23	0.57	0.61
CD at 5%	0.96	0.68	1.67	1.78

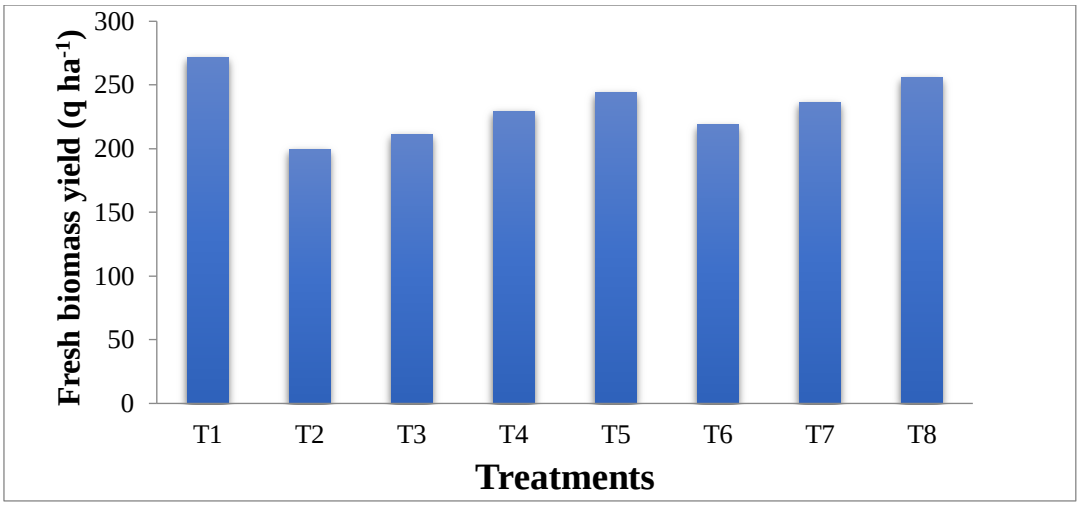

Fig. 1. Fresh biomass yield (q/ha) as affected by different treatment

Enhancing sweet basil (*Ocimum basilicum* L.) yield, soil health and economic returns using *Jeevamrit* and *Kunapajala* in the Shivalik Himalayan Region of India

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Receipt: 26.09.2024 *Revised: 15.11.24* *Acceptance:17.11.24*
DOI: 10.53552/ijmfmap.10.2.2024.42-51

ABSTRACT

A field experiment was conducted during the Kharif season of 2019 at Medicinal Plants Research and Development Centre (MRDC) of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand to study the effect of jeevamrit and kunapajala on herbage yield and quality of sweet basil (Ocimum basilicum L.). The experiment was laid out in Randomized Block Design with eight treatments replicated thrice. Treatments i.e. T_1: recommended dose of fertilizer (RDF) (120-60-40) kg/ha, T_2: 15 t/ha farmyard manure (FYM), T_3: 500 l/ha kunapajala, T_4: 1000 l/ha kunapajala, T_5: 500 l/ha kunapajala + 7.5 t/ha FYM, T_6: 500 l/ha jeevamrit, T_7: 1000 l/ha jeevamrit, T_8: 500 l/ha jeevamrit+ 7.5 t/ha FYM. Results revealed that application of 15 t/ha FYM showed lowest bulk density (1.552 g cc^{-1}) and highest organic carbon content (0.860%). The maximum available N (212.75 kg/ha), P (24.31 kg/ha) and K (203.53 kg/ha) was recorded under treatment T_1. Significantly highest population of bacteria (20.01×10^4 CFU/g soil), fungi (5.00×10^4 CFU/g soil) and actinomycetes (9.00×10^4 CFU/g soil) recorded in T_8. Treatment T_1 recorded highest net return (₹ 2,54,810/ha) as well as herbage yield (271.86 q/ha).

Keywords: *Jeevamrit, Kunapajala*, net return, *Ocimum basilicum*, organic, yield

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) of the Lamiaceae family is one of the most significant medicinal and aromatic plants cultivated in India. It comprises nine subspecies, either annual or perennial, primarily from the basilicum and sanctum species, known for their use in essential oils and fragrances (Chandel *et al.*, 2024; Corrado *et al.*, 2020). This crop thrives in warm tropical climates and has a short growing period of 75-90 days. India accounts for approximately 60% (3000 ha) of the global cultivation area (5000 ha) and produces around 70% (350 tons) of the world's annual basil oil output (500 tons) (Sanganeria, 2010). The leaves and seeds are the most economically valuable parts of the plant. Various parts of sweet basil contain a diverse group of aromatic compounds, highly valued for their flavor and fragrance (Corrado *et al*., 2020). Its essential oil is rich in compounds such as monoterpenes, phenols, sesquiterpenes, eugenol, methyl eugenol, thymol, methyl cinnamate, linalool, methyl chavicol, Citral 'A' and 'B', alcohol, and camphor, which contribute to its distinctive aroma and flavor profile (Hallmann *et al*., 2024). The oil of sweet basil finds different uses in the cosmetic and perfumery industries and also in indigenous system of medicine. Its oil is utilized for flavouring food stuff in

confectionary, thermogenic, cardiotonic, condiments, depurative, dental cream and mouth freshener and other countless indigenous and ayurvedic health care system (El-Mahrouk *et al*., 2024). Its extract can be utilized as bio-insecticide, fungicide, antifeedants and preparation of food products. Basil's oil has stimulant, stomachic, demulcent and expectorant action. The leaves are acrid, aromatic, bitter, appetizing, carminative, digestive, anthelmintic and cardiotonic (Spence, 2024). A little work has been accomplished so far on mineral nutrition of different medicinal and aromatic crops including sweet basil (*Ocimum basilicum* L.). The organic fertilization is not just a cost effective and ecofriendly, but improves soil environment, yield and oil quality of medicinal and aromatic plants. The nutrients present in the soil don't remain in available form for the plants, they first need to be converted into the available form. However immoderate use of chemical products has disturbed the flora as well as fauna along with the population of micro-organisms and population of earthworms is almost negligible. For the liquid biofertilizers, a better option is described in ancient Indian literature with more scientific and clinical formulation under the generic name "*kunapajala*" given by Surapala (Surapala, 1996) in 'Vrikshayurveda' literature. These promote biological activities in the soil as well as make the nutrients available to crops. Organic liquid manure *i.e. jeevamrit* is a rich bio-formulation which contains consortia of beneficial microbes (Pathak and Ram, 2013). *Jeevamrit* can also add nitrogen to the soil by increasing non-symbiotic nitrogen fixation. Different levels of nitrogen-fixing rhizobia have been observed to increase during preparation of *jeevamrit* to 4,400% of the starting mixture (Smith *et al*., 2020). Therefore, keeping these facts in view, the present study entitled "*Jeevamrit* and *Kunapajala* role in enhancing yield, soil health, and economics of Sweet Basil (*Ocimum basilicum* L.) in the foothills of Shivalik Himalayan, India" was carried out.

MATERIALS AND METHODS

Experimental site: The field experimentation on Sweet basil variety CIM-Saumya was conducted at Medicinal Plants Research and Development Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand during the *Kharif* season of 2019. Pantnagar lies in *tarai* belt, 30 kms Southward to the foothills of Shivalik range of Himalayas at 79°, 29′ E longitude and at an altitude of 243.83 meter above mean sea level under subtropical humid climate. The soils of *tarai* region are of order Mollisol, sandy clay loam in texture. The experimental soil was sandy clay loam in texture, neutral in reaction, medium in organic carbon (0.68%), low in available nitrogen (186.60 kg/ha) and medium in phosphorus (18.90 kg/ha) and potassium (201.23 kg/ha).

Experimental details: The experiment was designed in Randomized Block Design with 8 treatments replicated thrice. A total of 24 plots each with gross plot size of 5.0 x 3.2 m and a net plot size of 3.5 x 2.4 m were made.

Treatment details: FYM @ 15 tonnes/ha as a control and different doses of *jeevamrit* and *kunapajala* along with a basal application of FYM @ 7.5 t/ha were applied with total eight treatments. T_1: RDF (N_{120}-P_{60}-K_{40}) kg/ha); T_2: FYM @ 15 t/ha, T_3: *Kunapajala* @ 500 l ha^{-1}, T4: *Kunapajala* @ 1000 l ha^{-1}, T_5: *Kunapajala* @ 500 l ha^{-1} + FYM @ 7.5 t/ha, T_6: *Jeevamrit* @ 500 l ha^{-1}, T_7: *Jeevamrit* @ 1000 l ha^{-1}, T_8: *Jeevamrit* @ 500 l ha^{-1} + FYM @ 7.5 t/ha

Cultural operations: The experimental field was ploughed by disc plough followed by two harrowing and leveled by using wooden plank. Well decomposed FYM was incorporated in the field 10 days before the planting. The recommended dose of fertilizer (120:60:40) kg/ha in the form of urea, diammonium phosphate (DAP) and muriate of potash (MOP) were applied in the field. Uniform amount of *jeevamrit* (200 l) and *kunapajala* (200 l) were applied in the plots before planting of the seedlings. After that, both the formulations were applied at regular intervals of 15, 30, 45, and 60 days after transplanting

up to the final harvest of the crop. A total of 2 irrigations were given during the entire growth period. Manual weeding and mulching were done to check the weed flora. The crop was harvested at 80 DAP manually with the help of sickle by cutting plant at ground level in the plots leaving a border of 50 cm.

Preparation of organic sources of nutrients

Beejamrit and *jeevamrit* were prepared by the method developed by Padmashri Shri Subhash Palekar (Palekar, 2006) who is a strong promoter of natural farming and *kunapajala* was prepared by the method developed by Nene (2012)

Observations

Fresh herbage yield

$$\text{Total herbage yield (q ha}^{-1}) = \frac{\text{Weight of plants harvested from net plot area (kg)}}{\text{net plot area} \times 100} \times 10000$$

Bulk density (g/cc)

Bulk density (g/cc) was measured from the pre-noted weight of the dried sample and the total soil volume (Black, 1971).

$$\text{Bulk density} = \frac{\text{Dry weight of soil}}{\text{Total volume of soil}}$$

Soil Analysis

Organic carbon: Organic carbon content in soil was measured by using by using Modified Walkley and Black method (Jackson, 1973)

Available nitrogen: Subbiah and Asija (1956) method was followed for the estimation of available nitrogen.

Available phosphorus: Available phosphorus estimation in soil was done by Olsen's method (Olsen *et al*., 1954). The intensity of blue color was recorded at 660 nm on Spectrophotometer.

Available potassium: Available potassium determination in soil was done by using Flame photometer (Perur *et al*., 1973).

Microbial analysis

Present study is based on to find out the changes in population dynamics of microbes before and after the soil drenching of biozyme (*jeevamrit* and *kunapajala*) and other organic sources as given by Wollum (1982).

Statistical analysis

Experimental data was analyzed by adopting the standardized procedure for randomized block design (RBD) with the help of computer having analysis for R.B.D (STPR-3), programmed by the Department of Mathematics and Statistics, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar.

RESULT AND DISCUSSION

Yield

All the treatments had a positive and significant influence on the crop fresh biomass yield (Fig. 1). The application of NPK (120:60:40 kg/ha) *i.e.*, T_1 recorded significantly the highest fresh herbage yield (271.86 q/ha). It was closely followed by *jeevamrit* @ 500 L + FYM @ 7.5 t/ha (256.07 q/ha) *i.e.* T_8 followed by *kunapajala* @ 500 L + FYM @ 7.5 t/ha (244.03 q/ha) *i.e.* T_5 and these were found statistically *at par* to T_1 (271.86 q/ha). The lowest fresh herbage yield was recorded in T_2 applied with FYM (15 t/ha) alone (199.57 q/ha) and application of different doses of *jeevamrit* and *kunapajala* alone or their combination with FYM *i.e.* T_3, T_4, T_5, T_6, T_7, T_8 showed significantly higher fresh herbage yield as compare to FYM alone. The results obtained during the course of experiment indicated that there was a significant increase in fresh herbage yield which is due to more contribution of yield attributing parameters. Many successful attempts to show the beneficial effects of fermented liquid formulation on crop growth were done by Balakumbahan *et al.* (2010) in *Acorus calamus*. Similarly, Chauhan (2019) recorded higher fresh biomass yield in bramhi at different doses of *jeevamrit* combined with FYM.

Soil fertility

Soil bulk density

The soil bulk density was significantly influenced by different treatments (Table 1). The lowest bulk density was observed in T_2 (1.552 g/cc) followed by T_8 (1.602 g/cc) followed by T_5 (1.612 g/cc) as T_2, T_8 and T_5 treatments comprised of organic manures. The highest bulk density was recorded in T_1 (1.643 g/cc) which was significantly higher than solely applied FYM T_2 and organic formulations combined with FYM treatments T_8 and T_5. Among the different liquid manures treatments, the lowest bulk density was recorded in treatment T_8 (1.602 g/cc). The bulk density of soil in case of organic manure is lowest as compared to the other treatments because the microbial population is higher in the former and microbes feed on the carbon, evolved CO_2 which creates air spaces that makes the soil more porous and absorptive which in turn decreases the bulk density of the soil. Similar findings were also reported by Weber *et al.* (2007).

Organic carbon

Organic carbon was considerably affected by all the treatments (Table 1). The treatments comprising of organic source *i.e.*, FYM in different concentrations had higher values of organic carbon in comparison with the inorganic treatment and liquid manure formulation. Treatment T_2 had the highest organic carbon content (0.860%) which was significantly higher over all the other treatments except T_8 (0.783%) and T_5 (0.780%) to which it was *at par* with. The other treatments comprising of liquid manures had organic carbon content in the medium range. The highest organic carbon content among the liquid manures treatments was obtained in the soil drenched *jeevamrit* and FYM mixture *i.e.*, T_8 (0.783%) which showed a decreasing trend with the solid application of *jeevamrit i.e.*, T_7 (0.75%), T_6 (0.73%) similar trend was recorded in case of *kunapajala*. The lowest organic carbon content was observed in RDF treatment T_1 (0.69%) which was 19.7% lower than treatment T_2. Similar finding was reported by (Chandel *et al.*, 2024).

Available NPK

The significant influence of different treatments was observed on the available nitrogen, phosphorus and potassium in the soil (Table 1). The maximum available nitrogen was recorded in treatment T_1 (212.75 kg/ha) which was significantly superior to all the treatments except T_8 (198.63 kg/ha) to which it was *at par*. The lowest available nitrogen was found in treatment T_2 (180.80 kg/ha) which was 15.05% lower than treatment T_1. The higher amount of available nitrogen was recorded when *jeevamrit* or *kunapajala* combined with FYM as compare to solely applied *jeevamrit* or *kunapajala* or FYM because of FYM acts as organic food for microbial consortia present in liquid manures that help in mineralization and increased nitrogen availability as well as other nutrients. The high amount of available nitrogen was reported in RDF because of the split application of nitrogen that increased the soil fertility and its availability to the plant system **(**Chandel *et al.*, 2024). The treatment T_1 had the highest available phosphorus (24.31 kg/ha) which was significantly superior to all the other treatments. The lowest content of available phosphorus was observed in treatment T_3 (15.57 kg/ha) which was 35.90 % lower than treatment T_1. The T_1 treatment had the highest available potassium content (203.53 kg/ha) which was significantly greater than most of the treatments except T_8 (197.84 kg/ha). The lowest available potassium content was recorded in treatment T_3 (189.39 kg/ha) which was 7.11% lower than treatment T_1.

Bacterial population

Different treatments had a remarkable effect on the soil bacterial population after crop harvest (Table 1). Significantly highest population of bacteria was recorded in T_8 with application of *jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha (20.01×10^4 CFU/g soil) was *at par* with T_2 (17.70×10^4 CFU/g soil), T_5 (16.83×10^4 CFU/g soil) and T_7 (14.93×10^4 CFU/g soil). These treatments were

significantly superior to the T_1 (8.04×10^4 CFU/g soil). Significantly lower microbial activity with application of *jeevamrit* (T_6) and *kunapajala* alone (T_3) might be attributed to the absence of source of organic carbon for further multiplication of bacteria, fungi and actinomycetes and a higher microbial activity with application of FYM (T_2) might be due to the presence of microbial inoculums (*jeevamrit*). Similar results were also obtained by Ravusaheb (2008) in sesame, Shwetha (2008) in soybean. The results are also in accordance with the findings of Siddappa (2015) in filed bean, where higher population of bacteria, fungi and actinomycetes was recorded with *jeevamrit* @ 1500 L ha^{-1} followed by *jeevamrit* @ 1000 L ha^{-1}.

Fungi population

Fungi population in the soil after harvest of basil was recorded significantly highest (Table 1) in T_8 with application of *jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha (5.00×10^4 CFU/g soil). It was statistically equal to FYM 15 t/ha (4.67×10^4 CFU/g soil). Significantly lower fungi population was recorded in T_1 with application of recommended dose of NPK (1.47×10^4 CFU/g soil). A similar observation as that of bacterial population was recorded here wherein the fungal population decreased with the decreased dose of liquid manures from treatment T_7 to T_6 in *jeevamrit* and from treatment T_7 to T_6 in *kunapajala*. The treatment T_3 (1.93×10^4 CFU/g soil) and T_6 (2.00×10^4 CFU/g soil) which is a sole organic liquid manure application without the FYM had lower fungal population as compared to T_5 (3.90×10^4 CFU/g soil), T_8 (5.00×10^4 CFU/g soil). Kumbar *et al*. (2016) found that the population of soil microbes increased with the application of biozyme *viz*., bacteria (47.44×10^6 CFU/g soil), fungi (31.22×10^4 CFU/g soil) and actinomycetes (31.44×10^4 CFU/g soil). Similar results were also reported by Chauhan (2019) in bramhi, where she reported 64.42% less microbial count in RDF as compared to *jeevamrit* @ 5000 l/ha + FYM @ 2.5 t/ha.

Actinomycetes population

Significantly higher population was noted with T_8 *i.e*., application of *jeevamrit* @ 500 l/ha + FYM @ 7.5 t/ha (9.00×10^4 CFU/g soil) which was significantly superior to the rest of the treatments (Table 1). The actinomycetes population in liquid manures treatments was found to decrease as the dose of formulation decreased *i.e.*, it was highest in treatment T_7 (4.00×10^4 CFU/g soil) and lowest in treatment T_6 (2.10×10^4 CFU/g soil) similar trend was recorded in *kunapajala* treatments *i.e*. highest in T_4 (2.33×10^4 CFU/g soil) and lowest in T_3 (2.10×10^4 CFU/g soil). Significantly lower actinomycetes population was recorded in T_1 with application of suggested dose of NPK (1.70×10^4 CFU/g soil). The results are also matched with the findings of Kaur (2019), where he reported significantly higher population of bacteria (32.69×10^6 CFU/g soil), fungi (24.86×10^3 CFU/g soil) and actinomycetes (6.02×10^2 CFU/g soil) in plot treated with *jeevamrit* @ 20 per cent at two weeks interval among all treatments.

Total microbial population

Total microbial population in soil after harvest of basil recorded significantly superior in T_8 (34.01×10^4 CFU/g soil) which was highest than all other treatments (Table 1). It was statistically *at par* with T_2 (30.07×10^4 CFU/g soil) followed by T_5 (25.83×10^4 CFU/g soil). Among the liquid manures treatments, the highest total microbial population was recorded in treatment T_7 (18.4×10^4 CFU/g soil) followed by T_6 (13.00×10^4 CFU/g soil). The treatment T_1 comprising of RDF had the lowest population of microbes (11.20×10^4 CFU/g soil) which was 67.03% less than treatment T_8 which has the microbial formulation combined with FYM. As *jeevamrit* contains immense amount of microbial load which multiplies in the soil and functions as a tonic to improve the microbial activity in the soil (Palekar, 2006) and FYM has favourable effects on the soil properties which might have lowered the bulk density and improved soil aeration and also provided carbon as energy source to the microbes present in liquid manures for their rapid multiplication and survival. Devakumar *et al*.

(2014) revealed that use of handful of virgin soil for *jeevamrit* preparation performed as a source of initial inoculums of fungi, bacteria and actinomycetes, P- solubilizers and N-fixers. Consequently, more no. of beneficial microorganisms was observed in liquid manure formulation. These findings are also resembled with the study of Papen *et al.* (2002).

Economics

Cost of cultivation

Cost of cultivation (Table 2) varied with different treatments wherein, the highest cost of Cultivation was recorded in FYM @ 15 t/ha (₹ 81,520/ha) followed by *Kunapajala* @ 500 t/ha + FYM @ 7.5 t/ha (₹ 69,440/ha) and *jeevamrit* @ 500 t/ha + FYM @ 7.5 t/ha (₹ 69423/ha). The lower doses of *jeevamrit* or *kunapajala* had low cultivation cost which increases with increasing dose of formulation (T_4, T_7) but the amount of applied FYM increased the cost of cultivation in treatments T_5 (₹ 69,440/ha) and T_8 (₹ 69,423/ha). The minimum cultivation cost among treatments was found in treatment T_6 consisting of *jeevamrit* @ 500 t/ha (₹ 55,673/ha).

Gross returns

The gross returns are directly related to the total oil yield of each treatment and so vary significantly with each other (Table 2). The highest gross returns were obtained from T_1 *i.e.*, recommended dose of fertilizers (₹ 3,15,333/ha). Followed closely by the *jeevamrit* combined with FYM (₹ 3,04,788/ha) and followed by *kunapajala* combined with FYM (₹ 2,84,845/ha). The lowest gross return was obtained in treatment T_2 *i.e.*, FYM @ 15 t/ha (₹ 2,21,614/ha).

Net return

The maximum net return (Table 2) was obtained in treatment T_1 *i.e.*, Recommended dose of fertilizers (₹ 2,54,810/ha) which was followed by treatment *jeevamrit* @ 500 t/ha + FYM @ 7.5 t/ha (₹ 2,35,365/ha). Among different treatments of solely applied liquid manures, higher dose of *jeevamrit* + FYM *i.e.*, T_7 had the higher net returns (₹2,72,556/ha). The lowest net returns were obtained in treatment T_2 (₹1,40,094/ha) which is the FYM @ 15 t/ha treatment.

Benefit- Cost ratio (B: C ratio)

The maximum B: C ratio (Table 2) was obtained in treatment T_1 (4.21) which was highest among all while minimum B: C ratio was obtained in treatment T_2 (1.72). Highest benefit-cost ratio was seen in RDF as compared to other treatments because the gross returns (directly related to the total herbage yield) may be higher but the cost associated with liquid manure and FYM is also higher than RDF. A low B: C ratio is obtained in FYM combination because the yield is less and so are the gross returns in this treatment. Similar findings were also obtained by Amareshwari and Sujathamma (2015) and Manjunatha *et al.* (2009). It was noted that application of *jeevamrit* is one of the cheapest and efficient natural substitutes along with other organic manures like FYM, *ghanjeevamrit* in integrated approach for high crop yield and profitability, besides improving the nutrient status of the soil. Kasbe *et al.* (2015) also found application of liquid manure to be cost effective treatment. Similar results were obtained by Chauhan (2019) in bramhi where, she reported that application of biozyme integrated with FYM recorded higher benefit-cost ratio.

CONCLUSION

Based on the above findings, it can be concluded that *jeevamrit* @ 500 l/ha combined with half of the recommended dose of farmyard manure (7.5 t/ha) is sufficient to supply nutrients to crops, increases fresh herbage yield, maintain soil health and enhance profitability. It can be an alternate production technology to organic farmers and new vision to scientific community for further refinement and validation of age-old farming practices in present scenario to produce safe food, sustain soil health and to save the environment.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Bulk density, bacteria, fungi, actinomycetes and total microbial population in soil (0-15 cm), organic carbon content and available NPK after harvest of sweet basil as influenced by the treatments

Treatments details	B.D. (g/cc)	Organic carbon(%)	Available NPK (kg/ha)			Bacteria $\times 10^4$ CFU/g	Fungi $\times 10^4$ CFU/g	Actinomycetes $\times 10^4$ CFU/g	Total Count $\times 10^4$ CFU/g
			N	P	K				
T_0: Initial microbial population	1.570	0.68	186.60	18.90	201.23	5.16	0.83	0.72	6.71
T_1:RDF (N_{120}:P_{60}:K_{40}) kg/ha	1.643	0.69	212.75	24.31	203.53	8.04	1.47	1.70	11.21
T_2:FYM @ 15 t/ha	1.552	0.86	180.73	18.36	193.21	17.70	4.67	7.70	30.07
T_3:*Kunapajala* @ 500 t/ha	1.641	0.71	183.39	15.57	189.39	8.93	1.93	2.10	12.97
T_4:*Kunapajala* @ 1000 t/ha	1.632	0.72	187.25	16.17	190.92	14.30	2.07	2.33	18.70
T_5:*Kunapajala* @ 500 t/ha + FYM @ 7.5 t/ha	1.612	0.78	193.80	18.66	194.21	16.83	3.90	5.10	25.83
T_6:*Jeevamrit* @ 500 t/ha	1.640	0.73	185.27	15.87	190.12	10.87	2.00	2.10	14.97
T_7:*Jeevamrit* @ 1000 t/ha	1.632	0.75	188.17	16.77	191.02	14.93	2.57	3.47	20.97
T_8:*Jeevamrit* @ 500 t/ha + FYM @ 7.5 t/ha	1.602	0.78	198.63	19.06	197.84	20.01	5.00	9.00	34.01
S.Em±	0.002	0.03	5.86	1.57	2.39	1.76	0.24	0.38	1.87
CD at 5%	0.007	0.08	17.13	4.59	6.99	5.16	0.70	1.11	5.46

Table 2. Economics of the crop as influenced by different treatments

Treatments details	Total cost (₹/ha)	Gross returns (₹/ha)	Net returns (₹/ha)	B: C ratios
T_1:RDF (N_{120}-P_{60}-K_{40}) kg/ha	60524	315333	254810	4.21
T_2:FYM @ 15 t/ha	81520	221614	140094	1.72
T_3:*Kunapajala* @ 500 t/ha	55690	227401	171711	3.08
T_4:*Kunapajala* @ 1000 t/ha	56695	255791	199096	3.51
T_5:*Kunapajala* @ 500 t/ha + FYM @ 7.5 t/ha	69440	284845	215405	3.10
T_6:*Jeevamrit* @ 500 t/ha	55673	241966	186293	3.35
T_7:*Jeevamrit* @ 1000 t/ha	56660	272556	215896	3.81
T_8:*Jeevamrit* @ 500 t/ha + FYM @ 7.5 t/ha	69423	304788	235365	3.39

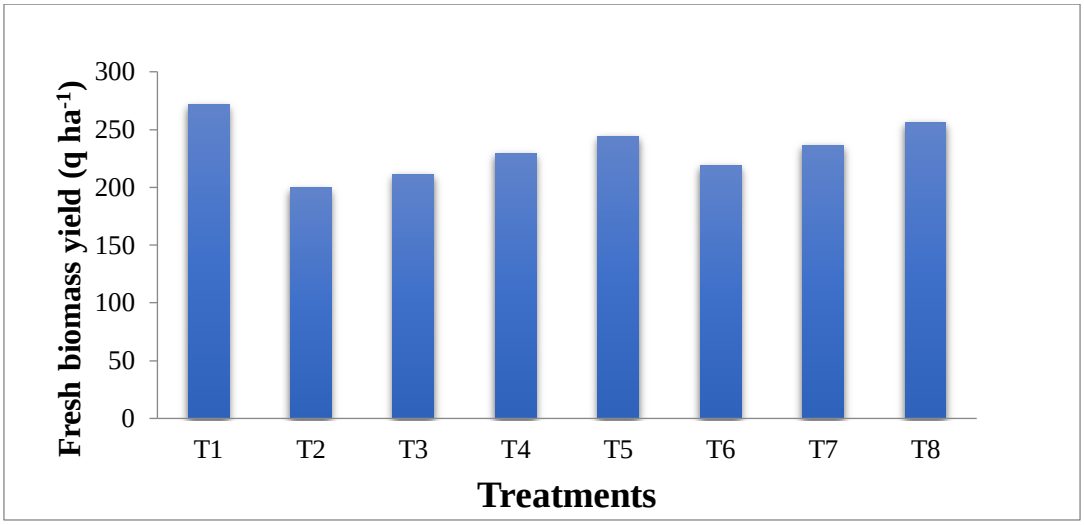

Figure 1. Fresh biomass yield (q/ha) as affected by different treatment

Seed propagation of nutritionally rich selected underutilized tropical fruit species

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Receipt: 26.06.2024 *Revised:25.08.2024* *Acceptance:27.08.2024*

DOI: 10.53552/ijmfmap.10.2.2024.52-59

ABSTRACT

Tropical countries harbor numerous fruit species with unexplored commercial potential. To reintroduce these species into cultivation, it is crucial to establish effective propagation systems to ensure continuous supply. This research aims to determine the suitability of seeds for the mass propagation of 10 underutilized yet nutritionally rich fruit species in Sri Lanka. We investigated key seed characteristics of the 10 fruit species, including viability, water imbibition, germination, and the necessity of dormancy-breaking methods, to develop standard seed propagation protocols. Seed viability was assessed using three different concentrations of triphenyl tetrazolium chloride (TTC) solution, and viability percentages were calculated. Water imbibition, moisture content, and in vitro and in vivo germination percentages were measured for all species. Except for Antidesma ghaesembilla, all seeds were viable. The highest water absorption was recorded for Syzygium caryophyllatum over 48 hours. Microcos paniculata exhibited the highest seed moisture percentage (75.1±0.66 %), while Ziziphus oenoplia had the lowest (6.8±0.03 %). Under in vitro conditions, seeds of S. caryophyllatum and Cynometra cauliflora showed 100 % germination, followed by Antidesma alexiteria with 13.3 %. In vivo, S. caryophyllatum, A. alexiteria, Baccauria motleyana, C. cauliflora, and Phoenix pusilla exhibited more than 50 % germination. However, dormancy-breaking methods were unsuccessful for ungerminated seeds both in vivo and in vitro conditions. The propagation of S. caryophyllatum, A. alexiteria, B. motleyana, C. cauliflora, and P. pusilla through seeds can be recommended as a suitable method for large scale propagation and commercialization.

Keywords: Seed germination, seed propagation, seed viability, underutilized

INTRODUCTION

South, Southeast, and East Asia are rich in diverse tropical fruits and their wild relatives (Sebastian and Prasad, 2014). Many of these fruits are considered "underutilized" because their full potential has not been realized, despite their commercial development prospects (Tontisirin, 2014). These fruit crops can be described as having value but are not widely grown, rarely found in the market, and not cultivated commercially (Hare Krishna *et al*., 2019).

Sri Lanka boasts a rich diversity of around 230 fruit species spanning 57 plant families, many of which are classified as underutilized (Pushpakumara and

Heenkenda, 2007). Over 60 varieties of such fruits have been identified, which are predominantly cultivated in forests, marginal lands, and home gardens (Dahanayake, 2015; Malkanthi, 2017). Recognizing indigenous tropical fruits with commercial potential is crucial for researchers, farmers, and industries seeking opportunities to promote and commercialize these fruits (Dahanayake, 2015; Ratnayake *et al*., 2019). However, the unchecked harvesting of these fruits from the wild poses a threat to their survival in their natural habitats. Additionally, most underutilized fruits lack standardized vegetative propagation methods tailored to various agro-climatic zones, hindering their mass-scale commercialization.

Seed propagation is known to enhance genetic diversity within a species, which is crucial for both conservation and crop improvement efforts (Keerthika *et al.,* 2020; Bohra *et al.,* 2021). Despite this, the sexual propagation of underutilized fruit plants by seeds remains largely unexplored globally (Waman and Bohra, 2019). Moreover, there is a lack of extensive research on the quality, germination, viability and dormancy of seeds from these underutilized fruits (Maldonado-Peralta *et al.,* 2016). Ten underutilized fruit plants which have received little scientific attention in recent years, were studied under the research project "Smart Village as a Strategy for Socio-economic Development of Rural Communities in Sri Lanka" funded by Accelerating Higher Education Expansion and Development)AHEAD(Development Oriented Research)DOR(grants. To address these issues, in this research, seed characteristics such as viability, water imbibition, germination, and the need for dormancy-breaking methods were studied for 10 selected underutilized fruits plants to develop standard seed propagation systems.

MATERIALS AND METHODS

Selection of underutilized fruit plants and collection of seeds

The fruit plants for the research were selected following a reconnaissance survey of Sri Lanka. The fruit species examined in this research included *Syzyzgium caryopyllatum*, *Microcos paniculata*, *Antidesma ghaesembilla*, *Antidesma alexiteria*, *Baccaurea motleyana*, *Cynometra cauliflora*, *Phoenix pussilla*, *Psidium guineense*, *Ziziphus oenoplia* and *Elaeocarpus angustifolius.* Seeds were collected from mature, ripe fruits of a healthy, fully grown single mother plant. The extraction process involved manually chopping the flesh around the seeds. The seeds were then screened using the floating method. The floating seeds were discarded. The remaining seeds were chosen for further experiments and were stored at room temperature (25±2 ^{o}C) until needed.

Viability of seeds

The seeds were soaked in 50 ml of water for 16 hours at room temperature (25±2 ^{o}C). After soaking, they were sectioned longitudinally with a sharp blade and stained with 0.01 %, 0.05 %, and 1 % 2,3,5-triphenyl tetrazolium chloride (TTC) solution for 5 hours at 38 ^{o}C. Three seeds from each species were stained at each TTC concentration, with three replicates for each treatment. Following staining, the TTC solution was discarded, and the seeds were rinsed with tap water. Seeds that turned red were considered viable, and the viability percentage was calculated following Sourabh, (2020).

$$\text{Viability percentage} = \frac{\text{Number of viable seeds}}{\text{Total number of seeds}} X\ 100$$

Seed water imbibition

Ten seeds of each species were placed in a petri dish lined with filter paper. A volume of 10 ml of distilled water was added onto the filter paper, and the setup was maintained at room temperature. Each species had three replicate petri dishes. At intervals of 0, 3, 6, 9, 12, 24, 36, and 48 hours each replicate seed was gently blotted to dry, weighed, and returned to its respective petri dish. The water uptake by each seed after each soaking period was then determined using the formula outlined by Shalimu *et al*. (2012).

$$\text{Imbibition percentage} = \frac{\text{Mass of the imbibed seeds}}{\text{Mass of the nonimbibed seeds}} X\ 100$$

Moisture content of the seeds

Ten seeds from each species were weighed and then subjected to drying in an oven at 80 °C for 48 hours. The percentage of seed moisture was determined using the equation provided by Shalimu *et al*. (2012).

$$\text{Seed moisture percentage} = \frac{\text{Fresh mass of the seeds}}{\text{Dry mass of the seeds}} X\ 100$$

Germination percentage of seeds

Germination tests were conducted both *in vitro* and *in vivo*. A hundred seeds of each species were soaked in water, and any floating seeds were discarded. The remaining seeds were then utilized to assess germination percentages both *in vivo* and *in vitro*.

***In vivo* seed germination and breaking seed dormancy**

For the experiment, three sets of eight seeds from each plant species were employed. These seeds were sown in seed trays filled with topsoil and watered three times weekly. Germination was determined by observing radicle protrusion from the seeds (Dinesh and Sushma, 2012). The count of germinated seeds was recorded over a period of 12 weeks. The *in vivo* seed germination percentage was calculated using the equation provided by Dinesh and Sushma (2012).

$$\text{Seed germinaton percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} X\ 100$$

Seed dormancy breaking techniques were applied to the non-germinating seeds of *M. paniculata*, *A. ghaesembilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius*. For these experiments, three sets of eight seeds from each of the mentioned plants were utilized. The seeds were first soaked in distilled water for 48 hours and then planted in trays filled with topsoil (Hossain *et al*., 2011). Mechanical scarification of the seeds was performed by rubbing them with sandpaper before planting in similar trays (Dinesh *et al*., 2019). Additionally, the seeds were treated with freshly squeezed lime (*Citrus aurantifolia*) juice for 15 hours to break dormancy, followed by planting in topsoil-containing trays (Eşen *et al*., 2009). The trays were watered three times weekly, and seed germination was monitored for a period of 12 weeks.

***In vitro* seed germination and breaking seed dormancy**

Fifteen seeds from each species underwent surface sterilization using ethanol (70 %) for 1 minute, followed by soaking in chlorox (5 %) for 10 minutes. Subsequently, the seeds were rinsed with sterilized distilled water. Five seeds were then placed in each Petri dish containing a moistened filter paper, with three replicate petri dishes utilized for each treatment. Germination was determined by observing radicle protrusion from the seeds, and the count of germinated seeds was recorded over an 8-week period (Elhindi *et al*., 2016). The *in vitro* seed germination

percentage was calculated using the equation provided by Elhindi *et al*. (2016).

$$\text{Seed germinaton percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} X\ 100$$

Economical and readily available techniques for seed dormancy breaking were applied to the surface-sterilized seeds that failed to germinate from *M. paniculata*, *A. ghaesembilla*, *P. pussilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius*. Following the method outlined by Elhindi *et al*. (2016), the seeds underwent surface sterilization. Subsequently, the seed dormancy breaking methods; soaking in water for 48 hours, mechanical scarification, and soaking in lime juice were applied as detailed earlier. Five seeds from each species were placed in petri dishes containing moistened filter paper, with three replicates used for each treatment. After 8 weeks, the seed germination percentage was calculated.

Analysis of data

One-way ANOVA and Tukey's pairwise comparison tests were conducted to assess significant differences among the means of seed viability percentages, seed imbibition percentages, seed moisture contents (Shalimu *et al*., 2012), as well as *in vivo* and *in vitro* seed germination percentages.

RESULTS AND DISCUSSION

Viability of seeds

Seeds of all fruit plants except *A. ghaesembilla* were viable. The seeds of the other nine fruit species showed viability in at least one TTC treatment. The viability of the seeds of *A. alexiteria*, *B. motleyana*, *C. cauliflora*, *P. pussilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius* ranged from 83.33% to 100%. The viability of the seeds of *S. caryophyllatum* was 66.8%. The lowest seed viability was observed in *M. paniculata* at 16.67%. There were significant differences in seed viabilities among TTC concentrations for *S. caryophyllatum*, *A. alexiteria*, *C. cauliflora*, *P. pussilla*, *P. guineense*, and *Z. oenoplia* (Table 1).

Table 1. Viability percentages of seeds for each fruit plant species.

Fruit species	0.01% TTC*	0.05 % TTC*	1 % TTC*
S. caryophyllatum (A)	66.8 ^{a} ± 0	50.0 ^{b} ± 0	5.56 ^{c} ± 4.54
M. paniculata (B)	16.67 ± 0	16.67 ± 0	16.67 ± 0
A. ghaesembilla (C)	0	0	0
A. alexiteria (D)	22.22 ^{c} ± 4.54	72.22 ^{b} ± 4.54	100.00 ^{a} ± 0
B. motleyana (E)	94.44 ± 4.54	100.00 ± 0	100.00 ± 0
C. cauliflora (F)	0 ^{b}	0 ^{b}	100.00 ^{a} ± 0
P. pussilla (G)	100.00 ^{a} ± 0	77.78 ^{b} ± 4.54	0.00 ^{c} ± 0
P. guineense (H)	0.00 ^{c} ± 0	61.11 ^{b} ± 4.54	94.44 ^{a} ± 4.54
Z. oenoplia (I)	16.67 ^{b} ± 9.86	83.33 ^{a} ± 9.86	0.00 ^{b} ± 0
E. angustifolius (J)	72.22 ± 9.07	88.89 ± 9.07	83.33 ± 0

*Means sharing the same letters are not significantly different at $P < 0.05$ (n=3).

In this study, three concentrations of TTC (0.01 %, 0.05 %, and 1 %) were used to enhance the accuracy in determining the germination potential of seeds. Interestingly, different TTC concentrations resulted in varying seed viability percentages within the same species indicating that the concentration of TTC can significantly influence the results of viability tests. Notably, seeds of *A.*

ghaesembilla exhibited 0% viability across all three TTC concentrations tested. This lack of viability could suggest either the inherent non-viability of the seeds or potential inadequacies in the testing conditions, TTC concentration, staining period and temperature that might affect the effective staining of the embryo (Victoria, 2006). These findings emphasize the importance of optimizing TTC test conditions for each species to obtain accurate assessments of seed viability. Further investigation is needed to determine whether the lack of viability in *A. ghaesembilla* seeds is due to true non-viability or suboptimal test conditions.

Seed water imbibition

Water absorption by seeds of *P. guineense* (H) and *M. paniculata* (B) ceased after 6 and 12 hours, respectively (Figure 1). Seeds of *A. alexiteria* (D) and *E. angustifolius* (J) stopped absorbing water after 24 hours. Seeds of *A. ghaesembilla* (C), *B. motleyana* (E), *C. cauliflora* (F), *Z. oenoplia* (I), and *P. pussilla* (G) ceased water absorption after 36 hours. Seeds of *S. caryophyllatum* (A) ended absorbing water after 48 hours.

Moisture contents of seeds

There was a significant difference in seed moisture percentages among the 10 fruit plant species (Table 2). The highest seed moisture percentage was recorded in *M. paniculata* (B) at 75.1±0.66 %, followed by *P. guineense* (H) at 73.8±2.40 %. The lowest values were observed in *B. motleyana* (E) at 18.1±1.90 % and *Z. oenoplia* (I) at 6.8±0.03 %. The seed moisture contents of the remaining fruit species ranged between 29.2±0.02 % and 50.6±0.42 %.

Germination of seeds *in vivo* and breaking seed dormancy

Under *in vivo* conditions, only seeds of *S. caryophyllatum*, *A. alexiteria*, *B. motleyana*, *C. cauliflora*, and *P. pussilla* exhibited germination rates of more than 50 % (Table 2). The highest seed germination percentage was observed in *P. pussilla* (79.2 ±4.2 %), while the lowest was recorded in *B. motleyana* (50.0 ±0.0 %). Seeds of *M. paniculata*, *A. ghaesembilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius* did not germinate successfully under *in vivo* conditions. Furthermore, the applied seed dormancy breaking methods were not effective for any species under *in vivo* conditions.

Germination of seeds *in vitro* and breaking seed dormancy

Only seeds of *S. caryophyllatum*, *A. alexiteria*, and *C. cauliflora* germinated under *in vitro* conditions (Table 2). The germination percentage for *S. caryophyllatum* and *C. cauliflora* seeds was 100 %, while for *A. alexiteria* seeds, it was 13.3 %. Seeds of *M. paniculata*, *A. ghaesembilla*, *B. motleyana*, *P. pussilla*, *P. guineense*, *Z. oenoplia*, and *E. angustifolius* did not successfully germinate under *in vitro* conditions. Furthermore, the applied seed dormancy breaking methods were ineffective for any species under *in vitro* conditions.

Based on the TTC test conducted in this study, the seeds of all fruit plant species were viable except for *A. ghaesembilla*. Consequently, seed propagation of *A. ghaesembilla* is not recommended at this time; however, further study is needed. Seeds from five species: *S. caryophyllatum*, *A. alexiteria*, *C. cauliflora*, *B. motleyana*, and *P. pussilla*, successfully germinated *in vivo*, suggesting seed propagation as a viable method for large-scale cultivation of these species. Among these, only three species (*S. caryophyllatum*, *A. alexiteria*, and *C. cauliflora*) also showed germination *in vitro*. Additionally, our previous research demonstrated successful propagation of *A. alexiteria* and *S. caryophyllatum* though stem cuttings using media commonly

employed by farmers indicating the suitability of both seeds and stem cuttings for large-scale propagation of these two species (Somasiri *et al.,* 2023). None of the seed dormancy breaking methods applied was successful for any species under both *in vivo* and *in vitro* conditions. Seed germination is influenced by seed genetics, hormones, and environmental factors during seed maturation (Abubakar and Attanda, 2022). Seed dormancy can be viewed as a barrier preventing germination of intact, viable seeds under favorable conditions. In this study, all seeds except those of *A. ghaesembilla* were viable despite not germinating. This highlights the need for further research into methods for breaking seed dormancy, as well as exploring various potting media and environmental conditions such as light intensity, temperature, and soil moisture, to enhance the germination potential of these species.

CONCLUSIONS

The fruit plant species studied exhibited different levels of seed viability. Propagation of *S. caryopyllatum*, *A. alexiteria*, *B. motleyana*, *C. cauliflora* and *P. pusilla* can be promoted by employing seed propagation for large scale production. However, the selected seed dormancy breaking methods were not successful in promoting germination of the seeds of the other tested fruit plant species indicating the need of further research to identify effective dormancy breaking techniques to enhance their germination and propagation potential.

ACKNOWLEDGEMENTS

This work was supported by the Accelerating Higher Education Expansion and Development - Development Oriented Research grant (AHEAD DOR 12).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2. Moisture and germination percentages of seeds for each fruit plant species.

Fruit species	Seed moisture percentage*	Seed germination *in vivo* (%)*	Seed germination *in vitro* (%)*
S. caryophyllatum (A)	28.3 ^{c} ± 0.69	58.3 ^{ab} ± 11.0	100.0 ^{a} ± 0.0
M. paniculata (B)	75.1 ^{a} ± 0.66	0.0 ^{c} ± 0.0	0 ^{c} ± 0.0
A. ghaesembilla (C)	29.2 ^{c} ± 0.02	0.0 ^{c} ± 0.0	0 ^{c} ± 0.0
A. alexiteria (D)	48.8 ^{b} ± 0.49	54.2 ^{b} ± 4.2	13.3 ^{b} ± 0.0
B. motleyana (E)	18.1 ^{d} ± 1.90	50.0 ^{b} ± 0.0	0 ^{c} ± 0.0
C. cauliflora (F)	50.6 ^{b} ± 0.42	66.7 ^{ab} ± 4.2	100 ^{a} ± 0.0
P. pussilla (G)	30.4 ^{c} ± 0.22	79.2 ^{a} ± 4.2	0 ^{c} ± 0.0
P. guineense (H)	73.8 ^{a} ± 2.40	0.0 ^{c} ± 0.0	0 ^{c} ± 0.0
Z. oenoplia (I)	6.8 ^{e} ± 0.03	0.0 ^{c} ± 0.0	0 ^{c} ± 0.0
E. angustifolius (J)	34.3 ^{c} ± 4.70	0.0 ^{c} ± 0.0	0 ^{c}± 0.0

* Means sharing the same letters are not significantly different.

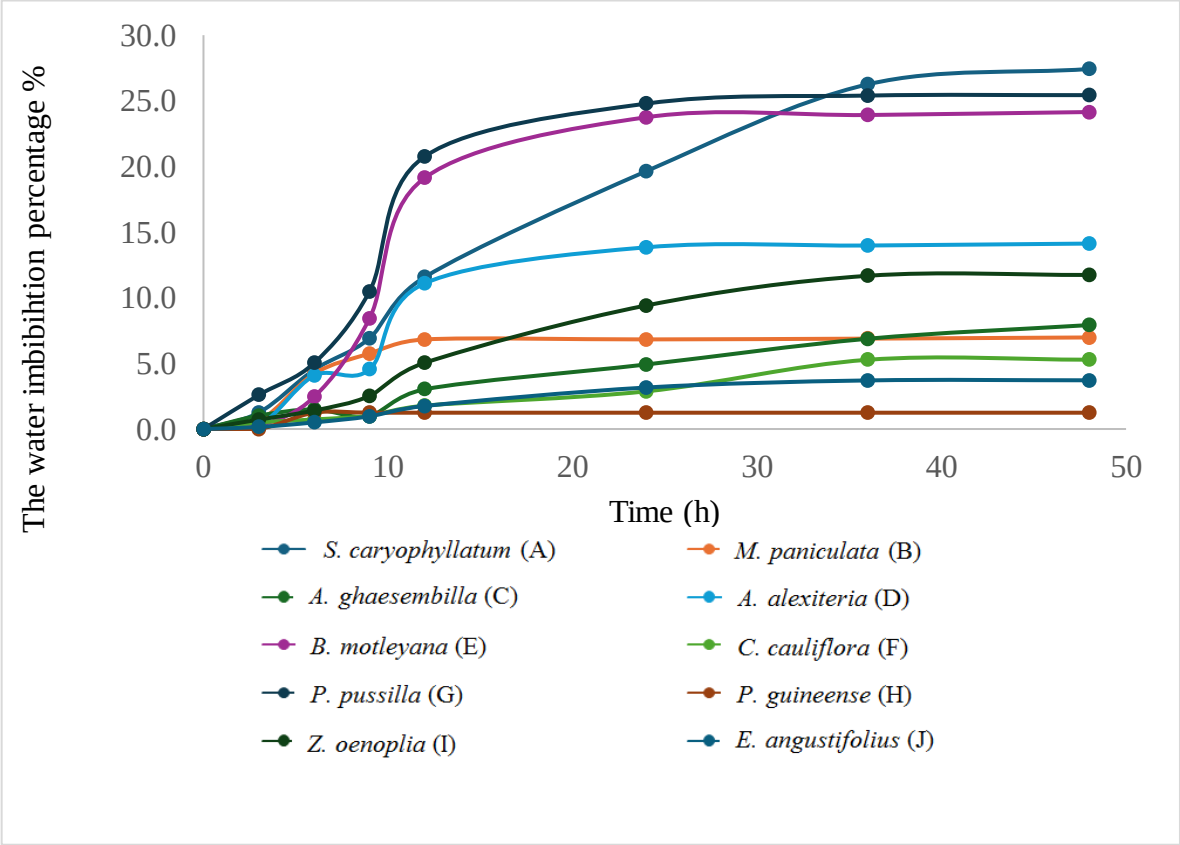

Figure 1: **The 48-hour water imbibition percentages of the 10 seed species.**

Results of the study of aboriginal varieties of pear in the Guba region of Azerbaijan

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Receipt: 19.08.2024 Revised: 25.09.2024 Acceptance:27.09.2024
DOI: 10.53552/ijmfmap.10.2.2024.60-66

ABSTRACT

Research was conducted to study local pear varieties of folk selection in the mountain and foothill villages of the Guba region of the Republic of Azerbaijan. The biodiversity, adaptation to local environmental conditions and potential of local varieties and forms of pear in the Guba region were studied. The native pear varieties discovered during our research have been described, propagated, and will soon be included in the genetic collection of our institute. About thirty varieties and forms of pears of folk selection were discovered. Most of these varieties are known to very few people due to their rarity. The majority of them have not yet had their biomorphological and economic characteristics described. Taking into account their uniqueness, rarity and economic contribution to the lives of local farmers and rural communities, we carried out a primary pomological description of 11 varieties, some of which were described for the first time. Most of these varieties are resistant to biotic and abiotic stress factors of the environment, very productive, the fruits have excellent taste qualities. They store well, are transportable, and are used fresh as well as for making dried fruits, jams and compotes. In the future, they can be used in the selection of new varieties as donors.

Keywords: Breading, Guba region, landraces, pear, productivity

INTRODUCTION

The Guba region is one of the main horticultural regions of the Republic of Azerbaijan. Here, various fruit plants, including numerous varieties, forms, and their wild ancestors, are grown by farmers on garden plots and are naturally widespread. These varieties and forms, which constitute the living history of our nation's agriculture, are distinguished by high productivity, quality, disease and pest resistance, drought and soil adaptability, and the ability for long-term fruit storage. They are used by the local population not only fresh but also for making jams, fruit syrups and dried fruits, which provide additional income (Hasanov and Aliev 2011). However, the number of indigenous varieties and forms, which represent a rich genetic resource of the local population, has sharply declined in recent years. Therefore, the collection, preservation, study, passportization, expansion of collections, and utilization of cultural and natural genetic diversity of fruit plants in the Guba region, alongside varieties of other fruit crops, is one of the most important and urgent tasks of today(Musayev and Akparov 2014).

Studying the genetic diversity of pear plants in the Guba region allows for the identification of

positive forms that can serve as donors in breeding new, high-yielding, and resilient cultivated varieties. Therefore, the collection and evaluation of existing genetic resources of fruit plants in this region, as well as enhancing the number of valuable agronomic traits applicable in agriculture, are key issues of contemporary importance. Musayev and Hajiyev **(**2023) reported that among 27 Caucasian pears, 19 pear species are growing in Azerbaijan flora and they have a number of natural hybrids in nature and courtyards.

Economic and biological indicators of efficiency, such as high yield, large fruits with superior taste quality, ability to ripen at different times, relative resistance to various biotic and abiotic stress factors, and suitability for various uses, have been evaluated for different pear varieties using modern methods to describe their pomological characteristics. Researching the genetic diversity of all fruit plants in the Guba region will not only help answer some phylogenetic questions but also enable the use of discovered forms with positive biological and agronomic characteristics, as well as resistance to various biotic and abiotic factors, as parental and donor forms for breeding new, productive, and resilient varieties. The abundance of pear species and locally selected varieties in Azerbaijan confirms that this region is the primary center of origin and domestication of this crop (Maghradze *et al*., 2012).

MATERIALS AND METHODS

The materials for the study were aboriginal varieties and forms of pears of folk selection in the orchards of local farmers in the Guba region. Phenological phases, growth, biomorphological description and productivity, fruit quality traits, resistance to disease and pests were studied by using the common description methods of fruit plants as described by (Lobanov, 1980; Sedova and Ogoltsova 1999). Farmers cultivating pears were invited to participate in the study. Samples of fruits and leaves were collected from various pear varieties and forms. Samples were collected at different times during the ripening season to encompass a wide range of phenotypic characteristics. Analysis of biomorphological features such as fruit shape and size, leaf shape and size, above-ground structure, etc., was conducted. Data on morphological differences between different pear varieties and forms were recorded. Assessment of the resistance of collected samples to major pear diseases and pests. Fruit quality assessment was conducted based on external characteristics (color, shape), organoleptic properties (taste, aroma). Interviews and surveys with farmers were conducted to gather additional information on the cultivated pear varieties and forms, their adaptation to local conditions, and the challenges faced by farmers during cultivation. These methods provided a comprehensive understanding of the biodiversity, adaptation, and potential of local pear varieties and forms in the Guba area.

RESULTS AND DISCUSSION

The geographic coordinates of the cultivated areas, biological and economic indicators, pomological features of local varieties of folk selection of pear plants, discovered in the territory of the Guba region of the Republic of Azerbaijan are presented below.

Garpyz armud (Synonyms: Unknown):
An ancient Azerbaijani variety of folk selection, widespread in the foothill villages of the Guba and Gusar regions of the Republic of Azerbaijan. Geographical coordinates of cultivation - N 41°22.5290', E 48°21.7570', at an altitude of 1002.1 meters above sea level. The trees are tall with a drooping crown shape. Fruits of this variety are small-sized, weighing 50-55 grams, and are yellow or yellow-pink in color, elongated-oval in shape. The flesh is white and juicy. The fruits have a pleasant watermelon-like flavor, which is the origin of the variety's name. It is a highly productive variety, with an average yield of 300-400 kg per tree. The fruit ripening period begins from

the second decade of September depending on the climatic conditions of the growing season. 'Garpyz armud' fruits are consumed fresh. **Advantages of the variety**: disease resistance, high fruit quality and good productivity. **Disadvantage of the variety**: small fruit size.

Dagur armud (Synonyms: Unknown):
An ancient Azerbaijani variety of folk selection, found in the foothill villages of the Guba and Gusar regions of the Republic of Azerbaijan. Geographical coordinates of cultivation - N 41°22.5310', E 48°21.7630', at an altitude of 985.1 meters above sea level. The trees are tall with a drooping crown shape. The fruit is small, round in shape, weighing 130-150 grams. The average yield per tree is 300-400 kg. The fruit ripens from the second decade of August depending on the climatic conditions of the growing season. Besides being consumed fresh, 'Dagur armud' fruits have been traditionally used by the local population to make dried fruits, jams, various compotes, and "Doshab" (a type of local fruit preserve). **Advantages of the variety**: disease resistance, high fruit quality and good productivity. **Disadvantage of the variety**: small fruit size.

Gefeyi armud (Synonyms: Unknown):
It is an ancient Azerbaijani variety of folk selection. Common in the foothill villages of the Guba and Gusar regions of the Republic of Azerbaijan. Geographical coordinates of cultivation - N 41°22.5280', E 48°21.7670', at an altitude of 992.1 meters above sea level. The trees are tall with a drooping crown shape. The fruit is very large, oval in shape, weighing 350-400 grams. The trees are highly productive, with an average yield of 300-400 kg per tree. The fruit ripens from September 10-30 depending on the climatic conditions of the growing season. In addition to being consumed fresh, 'Gefeyi armud' fruits have been traditionally used by the local population to make jams, various compotes, and "Doshab". **Advantages of the variety**: disease resistance, high fruit quality and good productivity.

Abbasbegi armud (Synonyms: Agagermaz', 'Bal armud):
An ancient Azerbaijani variety of folk selection, widely grown in the lowland and foothill regions of the Republic of Azerbaijan. Geographical coordinates of cultivation - N 41°22.5240', E 48°21.7630', at an altitude of 999.1 meters above sea level. The trees are tall, long-lasting, and have a spherical crown shape. The fruits are elongated-pear-shaped. The skin is thin, smooth, light green, turning yellowish, and lemon-colored when fully ripe. The flesh is white, very juicy, melting, sweet, refreshing, and very tasty. The fruits are medium-sized, with a height of 105 mm, width of 57 mm, fruit stalk length of 55 mm, and weight of 90-110 grams. The fruits do not drop. The fruit ripening period starts from the second decade of August depending on the climatic conditions of the growing season. The flesh does not darken when stored. It is a highly productive variety, with an average yield of 250-350 kg per tree. In addition to being consumed fresh, 'Abbasbegi' fruits have been traditionally used by the local population to make dried fruits, jams, various compotes, and "Doshab". **Advantages of the variety**: excellent fruit quality, abundant and annual fruiting. **Disadvantage of the variety:** susceptible to powdery mildew.

Jirnadiri (Synonyms: Unknown):
An ancient Azerbaijani variety of local selection. Developed in the Guba-Khachmaz zone of Azerbaijan. Widely spread in the lowland and foothill villages of the Azerbaijan Republic, especially in the foothills of the Guba and Kusar regions. Geographical coordinates of the cultivation site - N 41022.5390 E 48021.7550 at an altitude of 1000.6 meters above sea level. Trees of this variety are tall, long-lived, with an oval crown, densely leafy and densely branched. The fruits are small, relatively sweet in taste, with crisp flesh, elongated pear-shaped, 42 mm in length, 40 mm in width, 28 mm fruit stalk length, fruit weight 55-60 g on average. The fruits ripen in the second to third decade of August

depending on the climatic conditions of the growing season. The skin of the fruits is greenish-yellow. When fully ripe, they turn yellow with a weak red blush on the sun-exposed side. Very productive, with an average yield of 200-300 kg per tree. Highly resistant to diseases. The fruits do not drop prematurely. Besides fresh consumption, 'Jirnadiri' fruits have been traditionally used by the local population for making dried fruits, jams, etc. **Advantages of the variety**: disease resistance, longevity of the tree, high productivity, good quality of dried fruits. **Disadvantage of the variety:** small fruit size.

Ahmadgazi armud (Synonyms: Unknown):
An ancient Azerbaijani variety of folk selection. Developed in the Guba-Khachmaz zone of Azerbaijan. It is widespread in the lowland and foothill villages of the Azerbaijan Republic, particularly in the foothills of the Guba and Kusar regions. Trees of this variety are medium-sized, with a wide pyramidal crown. The fruits are of medium size, 90 mm in length, 55 mm in width, with a fruit stalk length of 40 mm, and weigh between 120-170 g. The fruits are elongated pear-shaped, with smooth, yellowish skin and a pale pink blush on the sunny side. The flesh is white with a creamy tint, sweet-tart in taste, juicy, aromatic, crisp, and free of stone cells. 'Ahmadgazi armud' is a fast-growing variety of pear that ripens in summer, but later than 'Abbasbayi' and 'Jirnadiri'. It is very productive, with an average yield of 150-200 kg per tree. The fruit ripens after the third decade of August depending on the climatic conditions of the growing season. The fruits do not drop prematurely. 'Ahmadgazi armud' fruits are primarily used fresh.
Advantages of the variety: resistance to pear scab and leaf spot, high fruit quality, and good productivity.

Dash armud (Synonyms: 'Gish armudu'):
An ancient Azerbaijani variety of folk selection. Developed in the Guba-Khachmaz zone of Azerbaijan. It is widespread in the lowland and foothill villages of the Azerbaijan Republic, particularly in the foothills of the Guba and Kusar regions districts. Geographical coordinates of the cultivation site - N 41022.5340 E 48021.7430 at an altitude of 994.3 meters above sea level. Trees of this variety are tall, with a weeping crown shape. The fruit is very large, 95 mm in height, 80 mm in width, with a fruit stalk length of 15 mm, and an average fruit weight of 280-320 g. It is a highly productive variety, with an average yield of 150-300 kg per tree. The fruits ripen after the third decade of November depending on the climatic conditions of the growing season. 'Dash armud' fruits are consumed fresh. The fruits do not drop prematurely. They are highly suitable for long-term storage and can be stored until the following spring.
Advantages of the variety: disease resistance, high fruit quality, good storability, and productivity.

Sapyburma armud (Synonyms: 'Uzunsaplaqli armud'):
An ancient Azerbaijani variety of folk selection. Developed in the Guba-Khachmaz zone of Azerbaijan. It is widespread in the mountainous villages of the Guba region of the Azerbaijan Republic. Geographical coordinates of the cultivation site - N 41022.5220 E 48021.7540 at an altitude of 998.6 meters above sea level. Trees of this variety are tall, with a weeping crown shape. The fruit is small, with a long fruit stalk, 40 mm in height, 40 mm in width, with a fruit stalk length of 50 mm, and weighs between 80-120 g. The fruit is round in shape, initially greenish-yellow, turning yellow, pale white, and darkening to brown as it ripens. It is a productive variety, with an average yield of 100-200 kg per tree. The fruits ripen from September to October, depending on the climatic conditions of the growing year. The fruits do not drop prematurely. Besides fresh consumption, 'Sapyburma armud' fruits have been traditionally used by the local population for making dried fruits, jams, etc.
Advantages of the variety: disease resistance, high fruit quality and good productivity.

Alpangaly armud (Synonyms: ‘Alpan armudu’)
An ancient Azerbaijani variety of folk selection. Developed in the Guba-Khachmaz zone of Azerbaijan. It is widespread in the mountainous villages of the Guba region of the Azerbaijan Republic. Trees of this variety are tall, with a weeping crown shape. The fruits are small, egg-shaped, 40 mm in height, 50 mm in width, with a fruit stalk length of 20 mm, and weigh between 40-50 g. The color of the fruits ranges from green to greenish-yellow, with a white pulp. It is a productive variety, with an average yield of 100-200 kg per tree. The fruits ripen from August to September, depending on the climatic conditions of the growing year. Besides fresh consumption, ‘Alpangaly armud’ fruits have been traditionally used by the local population for making dried fruits, jams, various compotes, and "Doshab".

Advantages of the variety: disease resistance, high fruit quality and good productivity.

Zargava armud (Synonyms: Unknown):
An ancient Azerbaijani variety of folk selection. Developed in the Guba-Khachmaz zone of Azerbaijan. It is widespread in the mountainous villages of the Guba region of the Azerbaijan Republic. Geographical coordinates of the cultivation site - N 41013.0810 E 48037.3410 at an altitude of 619.7 meters above sea level. Trees of this variety are tall, with a weeping crown shape. The fruit is large, pear-shaped, weighing between 220-300 g, with a fruit height of 100 mm, width of 80 mm, and fruit stalk length of 30 mm. The color of the fruits is green with green-pink spots, and the flesh is white. The fruits are very juicy. It is a productive variety, with an average yield of 200-300 kg per tree. The fruits ripen in November, depending on the climatic conditions of the growing season. The fruits do not drop prematurely. ‘Zargava armud’ fruits are consumed fresh.
Advantages of the variety: disease resistance, high fruit quality and good productivity.

Iri mayveli gish armudu (Synonyms: Iri Armud)
An ancient Azerbaijani variety of folk selection. Developed in the Guba-Khachmaz zone of Azerbaijan. Common in the foothill villages of the Guba region of the Azerbaijani Republic. Geographical coordinates of cultivation - N 41°13.0970', E 48°37.3830', at an altitude of 634.7 meters above sea level. Trees of this variety are tall with a drooping crown shape. The fruits are very large, pear-shaped, weighing 700-900 grams. It is a high-yielding variety, with an average yield of 200-300 kg per tree. Fruits ripen in January-February of the following year depending on the climatic conditions of the growing season. The fruits do not drop. Due to their late ripening, these fruits are suitable for long-term storage. **Advantages of the variety**: high taste quality of the fruits, good storage ability and high yield.

During the research, we described the sequence of ripening periods for local pear varieties in mountain villages, which was of great significance to the economic life of the local farmers (Table-1). Most of this varieties are currently under threat of extinction, protection of which has not only agricultural value, but also they have the historical and cultural importance.

CONCLUSIONS

Research on local varieties and forms of pear in the gardens of local farmers in the Guba area has revealed significant diversity and adaptation to local conditions. The following conclusions were drawn:
1. Biomorphological diversity: A wide range of biomorphological characteristics was observed among local varieties and forms of pear, indicating their genetic richness and adaptation to various climatic and soil conditions.
2. Resistance and fruit quality: Local pear varieties and forms demonstrated high resistance to diseases and pests, confirming their potential for sustainable and environmentally friendly fruit production.

3. Breeding of promising forms: Breeding identified promising pear forms based on high productivity, fruit quality (color, taste, aroma), as well as their commercial attractiveness and durability.

4. Significance for agriculture: Local pear varieties and forms have high potential for the development of local agriculture, providing environmentally friendly products to local residents and generating economic benefits through sales in markets.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Ripening period of local pear varieties

Name of the varieties	Ripening period	Name of the varieties	Ripening period
Bildirchinbudu	1-5 July	Axuni	1-15 September
Gorkhmazi	1-5 July	Davudi	1-15 September
Galyani	10-15 July	Giradim	1-15 September
Idrisi	20-30 July	Shikhahmedi	1-15 September
Shakarpara	1-5 August,	Shaftali	1-15 September
Chichi	5-15 August	Halvayi	1-15 September
Cirnadiri	5-20 August	Cirhunduru	5-15 September
Tursh	20-30 August	Zahra	5-20 September
Peykali	20-30 August	Nurunburun	5-20 September
Nargila	20-30 August	Kurdaki	20-30 September
Tumsuz Nargila	20-30 August	Goy Armud	20-30 September
Abasbayi	20-30 August	Sini Armud	1-10 October
Khamzeyi	20-30 August	Gara Armud	1-15 October
Nararmud	1-15 October	Shamakhizari	1-15 October
Chaggalboghan	20-30 October	Gomgomi	1-10 November (fruits stay on the tree till December)

Rare and endangered woody plants of the Sary-Chelek State Biosphere Reserve

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Receipt: 07.10.2024 *Revised: 22.10.2024* *Acceptance:27.10.24*
DOI: 10.53552/ijmfmap.10.2.2024.67-73

ABSTRACT

The research conducted in Sary-Chelek State Biosphere Reserve focuses on assessing the status of rare and endangered woody plants. Through field studies and literature analysis, the species composition, population size, and spatial distribution of these species were investigated. This article presents the results of an in-depth study of rare and endangered woody plants such as Semenov Fir (Abies semenovii B. Fedtsch.), Usunakhmat grape (Vitis usunachmatica Vass.), Crabapple (Malus sieversii (Ledeb.) M.Roem.), Nedzvetsky's Apple (Malus niedzwetzkyana Dieck.), and Persian Rowan (Sorbus persica Hedl.), found within the Sary-Chelek State Reserve. Extensive long-term field studies conducted by the authors have yielded substantial data on the status of rare species populations. The analysis of the obtained data, combined with a review of the literature, allowed for a detailed characterization of the ecological features of these rare woody plants. Based on the results, recommendations were developed for biodiversity conservation and effective strategies for protecting these species. Proposed measures include the establishment of special protected zones, monitoring population status restoring degraded ecosystems, and conducting educational outreach among the local community. These findings are significant for developing long-term management plans for the reserve and can be utilized in creating regional biodiversity conservation programs.

Keywords: Biodiversity, endangered tree species, environment protection, rare woody plants, Sary-Chelek State Reserve

INTRODUCTION

The Kyrgyz Republic is a mountainous, sparsely forested country located in the eastern part of the Central Asian region, with approximately 90% of its territory situated at an elevation of more than 1500 meters above sea level (Anon., 2023). According to data from the Global Forest Resources Assessment (FAO, 2020), 92% of the country's forests serve soil protection and water conservation functions. All forests in the Kyrgyz Republic are under state ownership and are considered a national asset. The total area of the state forest fund is 2619.7 thousand hectares. The forests of Kyrgyzstan represent a vast genetic reservoir of biodiversity and are one of the primary centers of origin for many modern cultivated plants. This is evidences by the fact that nearly all species of valuable fruit-bearing plants native to Central Asia are found here in their wild state. These forests serve as a gene pool for numerous species, not only of woody and shrub plants but also of herbaceous flora. Additionally, they provide a source of income for local communities and play an essential role in the national economy.

Given the vast biological diversity of vegetation, which holds significant scientific and practical value, the Sary-Chelek Nut-Fruit Reserve was established. This was done by decree №118 of the Council of Ministers of the Kyrgyz SSR on May 5, 1959, with the aim of preserving and promoting the further development of this unique ecosystem. In 1979, by the decision of the Presidium of the International Coordinating Council of the "Man and the Biosphere" Program of the UNESCO Council for Reserves and Protected Areas, the Sary-Chelek Reserve was included in the list of biosphere reserves worldwide (Arkit 2019; Sputnik, 2016). This network of protected areas, representing the world's major ecosystem types, is designed for the conservation of nature and for conducting scientific research in the interest of humanity. Additionally, this network will serve as a model for assessing human impact on the surrounding environment. In June 2016, by the decision of the UNESCO Commission, the Sary-Chelek Reserve was inscribed on the UNESCO World Heritage List (Bishkek, 2020). According to botanical-geographical zoning, Sary-Chelek Reserve is part of the Chatkal Floristic Region. The flora of the Reserve was first studied by Borlakov (1966) and the initial list of reserve's plant species was compiled. The flora of the reserve includes 62 families, 376 genera, and 969 plant species (Borlakov, 1966; Borlakov *et al*., 1971). According to data from the "Kyrgyz Forest and Hunting Management" State Institution (Bishkek, 2020) 677 species of higher plants grow within the reserve. Of these, 72 species are trees and shrubs belonging to 20 families. The reserve is home to 11 vulnerable plant species: 5 woody and shrub species and 6 herbaceous species, all of which are included in the Red Book of Kyrgyzstan. Rare and endangered plant species not only require an effective biodiversity conservation strategy but also demand thorough study of their bio-ecological characteristics. Research and the development of propagation methods for these plants contribute not only to the preservation of unique natural heritage but also lead to a shift in the principles of biological resource utilization, making them more sustainable and less depleting. The objects of this study are vulnerable woody and shrub species growing within the territory of the Sary-Chelek State Biosphere Reserve.

METHODS

The research was conducted through surveys of local residents, reserve staff, and on-foot expeditions. During the studies, the habitat conditions of vulnerable species were identified. General information on the distribution of the studied species was collected, and a standard geobotanical description was carried out. The description involved the individual examination of each identified specimen, with coordinates recorded using a Garmin eTrex 10 GPS navigator. To assess species vulnerability, the IUCN categories and criteria were used (Anon., 2024). In accordance with the methodology of the International Union for Conservation of Nature, the geographic distribution, population size, and habitats of the species were specified. Within the territory of the Sary-Chelek Reserve, five species of rare and endangered woody plants *viz*., Semenov Fir (*Abies semenovii* B. Fedtsch.), Usunakhmat Grape (*Vitis usunachmatica* Vass.), Nedzvetsky's Apple (*Malus niedzwetzkyana* Dieck.), Crabapple (*Malus sieversii)* and Persian Rowan (*Sorbus persica* Hedl.) were taken for the study.

RESULTS AND DISCUSSION

Semenov Fir (*Abies semenovii* B. Fedtsch.)
It is listed in the IUCN Red List as *Abies sibirica subsp.semenovii*. According to IUCN classification and criteria, it was originally categorized as Critically Endangered (CR), but in 2010 it was reclassified as Least Concern (LC) (IUCN Red List of Threatened Species,a). In the Red Book of the Kyrgyz Republic, it is listed as a vulnerable species (VU) (Anon. 2016), while in the Red Book of Woody Plants of Central Asian it holds a critically endangered

status (CR B1ab(v)) (Anon., 2009). The results of molecular-genetic studies (nuclear markers: allozymes and AFLP, chloroplast markers: SSR) clearly indicate that Semenov Fir is a distinct species with a limited distribution range (Semerikova and Semerikov, 2011; Semerikova *et al*.,2012; Semerikova (2016); Orlova *et al*., 2016) which necessitates more active conservation measures. Despite this, in the IUCN Red List, Semenov Fir (*Abies semenovii* B. Fedtsch.) is still listed as a subspecies of Siberian Fir (*Abies sibirica subsp. semenovii*). The factors contributing to the vulnerability of *A. Semenovii* include not only increased anthropogenic pressure but also competition from other coniferous and deciduous species.

Semenov Fir. is a narrow endemic of the western Tien Shan, found only in Kyrgyzstan, primarily in the Aksy and Toktogul districts of the Jalal-Abad region. Small populations of this species also occur on the slopes of the Talas range (Besh-Tash Gorge). The total area of its range is 4326.7 hectares, of which 279.1 hectares are located within the Sary-Cheleek State Biosphere Reserve. In the Sary-Chelek State Biosphere Reserve, Semenov Fir grows at various altitudes, with the lower limit of its range at 1,250 meters above sea level (Bak-Chop area) and upper limit at 2,600 meters above sea level (Makmal area). Under natural conditions, Semenov Fir regenerates by seeds, although occasional rooting of lower branches, forming new shoots upon contact with the soil, has also been observed. In the Sary-Chelek Reserve, optimal conditions for seed regeneration of the fir occur in stands located at altitudes between 1,600 and 2,000 meters above sea level, particularly on north-facing slopes.

Usunakhmat Grape (*Vitis usunachmatica* Vass.)

It is listed in the Red Book of Kyrgyzstan with the status of vulnerable (VU). It grows in the lowest part of the reserve on a southern slope. The fruits, ranging from 5 to 10mm in diameter, vary in color from black-violet and pink to greenish-pink and green, with juicy flesh. The taste ranges from sweet to sweet-and-sour, sometimes with a slight astringency. The seeds are pear-shaped in reverse. In the conditions of the Sary-Chelek State Biosphere Reserve, the plant flowers in mid-May, and the fruits ripen in the third decade of August. Seed-based regeneration has been observed.

Crabapple *Malus sieversii* (Ledeb.)

It is listed in the Red Book of Kyrgyzstan with the status of Least Concern (LC), indicating a lower level of vulnerability (Anon.2016). It is included in the IUCN Red List and the Red Book of Woody Plants of Central Asia, classified as a vulnerable (VU) under criterion (IUCN Red List of Threatened Species (b); Anon.2009). This polymorphic fruit species is one of the secondary forest-forming species in the nut-fruit forests of Kyrgyzstan. According to DNA studies, it is the progenitor of many modern apple varieties (Velasco *et al.*, 2010). The tree reaches a height of 3 to 5 meters and has a compact crown. It grows throughout the reserve at altitudes ranging from 1,200 to 2,000 meters above sea level. In the reserves, depending on the growing conditions, it blooms from the third decade of April to the first half of May. The fruits ripen from the second half of August to mid-September. It reproduces by seeds and root suckers. In the Sary-Chelek State Biosphere Reserve, the population of Crabapple (*Malus sieversii* (Ledeb.) M.Roem.) remains stable. Observations indicated that degradation of this species is not occurring, which signifies favorable ecological conditions within the reserve. Effective conservation measures and the absence of significant anthropogenic impacts contribute to maintaining a healthy population of Crabapple.

Nedzvetsky's Apple (*Malus niedzwetzkyana* Dieck.)

It is a very rare, endemic, and endangered species. It is listed in the IUCN Red List with the status of Endangered (EN) due to critically low population numbers (IUCN

Red List of Threatened Species (c). It is also included in the Red Book of Woody Plants of Central Asia with the Status of Endangered (EN B2ab (iii,v)), indicating a high degree of extinction threat for this species (Annon., 2009). In the Red Book of Kyrgyzstan, it is assessed as Vulnerable (VU), highlighting the need for conservation measures (Anon., 2016). The bark of perennial branches has a reddish-brown hue, while in one-year-old branches it appears dark purple. The branches are thornless. The leaves are dense, oblong or obovate in shape, dark green with a reddish tint, and range from 7 to 10 cm in length. The flowers are light pink or purple. The fruits are small, spherical, slightly elongated, and either red or violet-red, with a waxy coating and pinkish-red flesh. In the Sary-Chelek State Reserve, *Malus niedzwetzkyana* Dieck. is found at altitudes ranging from 1,255 meters (Dendrosad) to 2,000 meters (Kyla-Kol) above sea level. It occurs as individual trees or in small clusters consisting of three to sixteen trees. At lower altitudes (1,200-1,300 meters above sea level) *Malus niedzwetzkyana* individuals are rare and appear as isolated trees. The majority of Nedzvetsky's apple trees are found at elevations between 1,400 and 1,700 meters above sea level, within the belt of walnut-fruit forests

During field studies in the reserve, 52 specimens of *Malus niedzwetzkyana* were identified across various slope exposures. According to the data presented in Figure 1, the vast majority of individuals (60%) are concentrated on southeast-facing slopes, while only 2% are found on south-facing slopes. This uneven distribution is likely due to the more favorable microclimatic conditions on southeast exposures, which promote optimal photosynthesis and reduce the risk of plant overheating.

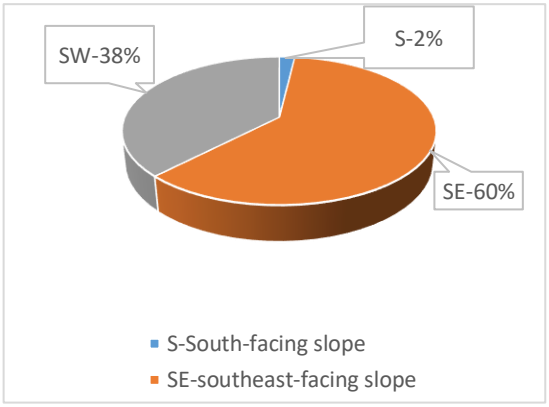

Fig.1: Distribution of *Malus niedzwetzkyana* Dieck. Individuals by slope exposure

Nedzvetsky's apple (*Malus niedzwetzkyana* Dieck.) is recognized for its diverse flesh colors, including light pink, pinkish-red, and deep red hues (Fig. 2).

Fig. 2: Variation in flesh color of *Malus niedzwetzkyana* fruit

Some fruits exhibit a uniform deep red flesh, while others display a gradient from lighter pink to more intense red. This characteristic not only enhances the aesthetic

appeal of Nedzvetsky's apple but also provides invaluable material for fruit breeders in developing cultivated varieties with red fruits and red flesh. Fruit ripening in *Malus niedzwetzkyana* Dieck occurs at different times depending on the altitude. At lower elevations (Dendrosad, 1,255 meters above sea level), fruit ripening takes place in September, while at an altitude of 2,000 meters above sea level (Kyla-Kol), ripening is observed by the end of September.

The number of viable seeds in the fruits of *Malus niedzwetzkyana Dieck* varies from 4 to 15. According to Firsov *et al.*, (2019), this variability is attributed to the unevenness of fertilization processes and the subsequent development of ovules. Some ovules are eliminated at early stages, while the development of other seeds may cease after fertilization. Additionally, the morphological formation of seeds within a single fruit is often heterogeneous. Through morphometric analysis of the seeds *Malus niedzwetzkyana Dieck*, it was determined that the average weight of one thousand seeds is 23.3g. The viability of the seeds, assessed using the indigo carmine staining methods, revealed that the percentage of viable seeds is 82%.

The Persian Rowan *(Sorbus persica* Hedl)

One of the rare and endangered species occurring within the territory of the Sary-Chelek State Biosphere Reserve is the Persian Rowan. It is an endemic species that is infrequently encountered and is listed in the Red Book of Kyrgyzstan with a status of (VU) as a vulnerable species (Anon. 2016). In the Red Book of Woody plants of Central Asia, it is classified as a species of least concern (Anon. 2009). In the Sary-Chelek State Biosphere Reserve, Persian rowan (*Sorbus persica* Hedl.) is found at altitudes ranging from 1,876 to 1,962 meters, both in groups and as solitary specimens. It grows on various slopes with varying degrees of steepness. The primary concentration of Persian rowan is observed on the northeastern and northwestern slopes, predominantly along the southern shores of the Sary-Chelek and Tuyuk-Kol lakes (Fig. 3). Most of the trees are of seed origin and fall into the mature or old-growth categories. Typically, the rowan forms small clusters consisting of 10-20 trees in limited areas.

Fig. 3: P1~P14 – Habitat locations of the identified Persian rowan in Sary-Chelek State Reserve

Persian Rowan (*Sorbus persica* Hedl.) is characterized by simple leaves measuring 5–8.5 cm in length and 3.5–5 cm in width. The leaf margin exhibits 4–6 lobes with either blunt or pointed tips, and the base tapers in a wedge shape. The leaf blade is elliptical or

elongated-elliptical, appearing almost glabrous on the upper surface, while the underside is covered with a white, woolly pubescence. The leaf margins feature oblique triangular teeth, and the petioles are hairy, measuring 1–2 cm in length. The inflorescence of the Persian Rowan is characterized by a complex umbel bearing numerous small white flowers. The diameter of the inflorescence varies from 6 to 12 cm. The flowers emit a characteristic scent that attracts pollinating insects. The fruit is a berry, either spherical or ellipsoid in shape, with a smooth, orange-red skin. The morphological and flavor characteristics of the fruits can very significantly among populations. The plant exhibits polymorphic life forms, ranging from tree to shrub. The heigh of the trees can reach between 5 and 12 meters. In the conditions of the Sary-Chelek State Biosphere Reserve, the fruits of the Persian rowan mature at the end of October. Notably, the seed productivity is low, with a clean seed yield of only 3.4% of the total fruit mass. The fruits often contain underdeveloped seeds or empty seed cavities. The average weight of 1,000 seed is 27.7±4.8 g.

CONCLUSION

The study of rare and endangered tree species in the Sary-Chelek State Biosphere Reserve has revealed several important characteristics. The findings highlight the necessity of developing and implementing comprehensive conservation measures to preserve the biodiversity of the reserve. Such measures include the establishment of special protected zones, monitoring the status of rare species populations, restoring disturbed ecosystems, and conducting educational outreach among the local population. The conservation of rare and endangered tree species is not only of scientific importance but also holds practical value, as these species represent vital genetic resources and play a key role in maintaining ecological balance.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Studies on combination of different forms of potassium and micronutrients on fruit yield and post-harvest quality of guava (*Psidium guajava* L.)

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Receipt: 25.08.2024 *Revised: 25.10. 2024* *Acceptance: 27.10.2024*
DOI: 10.53552/ijmfmap.10.2.2024.74-82

ABSTRACT

An investigation was carried out to find out combination of different forms of potassium and micronutrients on fruit yield and post-harvest quality of guava (Psidium guajava L.) was carried out at the Department of Horticulture, College of Agriculture Parbhani during the year 2019-20. The field experiment was laid out in Randomized Block Design with thirteen treatments and three replications. The treatments were: T_1- KH_2PO_4 at 1% + $FeSO_4$ at 0.5%, T_2- KH_2PO_4 at 1.5% + $FeSO_4$ at 0.5%, T_3 - KH_2PO_4 at 1% + $ZnSO_4$ at 0.5%, T_4 - KH_2PO_4 at 1.5% + $ZnSO_4$ at 0.5%, T_5 - K_2SO_4 at 1% + $FeSO_4$ at 0.5%, T_6 - K_2SO_4 at 1.5% at + $FeSO_4$ at 0.5%, T_7-K_2SO_4 at 1% + $ZnSO_4$ at 0.5%, T_8-K_2SO_4 at 1.5% + $ZnSO_4$ at 0.5%,T_9-KNO_3 at 1% +$FeSO_4$ at 0.5%,T_{10}-KNO_3 at 1.5% + $FeSO_4$ at 0.5%,T_{11}-KNO_3 at 1% + $ZnSO_4$ at 0.5%,T_{12}-KNO_3 at 1.5% + ZnSO4 at 0.5% and T_{13}- control through foliar application which was sprayed two times after fruit set at 15 days interval. Results of the study indicated that maximum number of fruits per tree (160.33), fruit retention (80.60 %), yield per tree (39.4 kg), yield per hectare (10.95 Mt per ha) and minimum fruit drop (19.84 %), maximum fruit weight (246.3 g), fruit volume (220.6 ml), fruit length (7.86 cm) and fruit diameter (8.06 cm) were more in treatment T_{12} i.e., KNO3 at 1.5% + ZnSO4 at 0.5%. Better fruit quality and more shelf life (8.4 days) and minimum physiological loss in weight (11.77 %), fruit decay (24.7 %) during at ambient storage was also recorded under above treatment.

Keywords: Forms of potassium, guava**,** micronutrients, quality, yield,

INTRODUCTION

Guava (*Psidium guajava* L.) is considered to be one of the exquisite, nutritionally valuable and remunerative fruit. Guava has gained considerable eminence on an account of its high nutritive and medicinal values and also for its aroma and flavour. Since it is a rich source of vitamin C (260-300 mg/100 g) which is three to five times more than oranges and ten times more than tomatoes, it is an ideal fruit crop for nutritional security. High concentrations of pectin in guava fruits play a significant role in the reduction of cholesterol and thereby decrease the risk of cardiovascular disease. Micronutrients are essentially as important as macronutrients to have better growth, quality and yield in plants. Their requirement by plants is in trace amounts. Foliar application of micronutrients and growth regulators play a vital role in improving the quality of the produce and increased the growth, yield and quality parameters in guava (Balakrishnan, 2000; Yadav *et al*., 2011). Today, due to increased demand for quality produce the interest of growers in production of high quality fruits is increasing. There is also need to improve post-harvest quality of guava fruits. Hence, considering the need, the present investigation "Studies on combination of different forms of potassium and

micronutrient on fruit yield and post-harvest quality of guava (*Psidium guajava* L.)" was taken.

MATERIAL AND METHODS

The present study was carried out on rainy season guava crops at the experimental orchard at Khanapur Tal. Khanapur Dist. Parbhani, and Post-harvest qualities was carried out at Post Graduation Laboratory of Department of Horticulture, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani during the year 2019-20. The age of the guava plants, cv. Sardar was six years; planted at 6m x 6m spacing.The experiment was laid out in Random Block Design with thirteen treatments and replicated thrice. The treatments were: T_1- KH_2PO_4 at 1% + $FeSO_4$ at 0.5%, T_2- KH_2PO_4 at 1.5% + $FeSO_4$ at 0.5%, T_3 - KH_2PO_4 at 1% + $ZnSO_4$ at 0.5%, T_4 - KH_2PO_4 at 1.5% + $ZnSO_4$ at 0.5%, T_5 - K_2SO_4 at 1% + $FeSO_4$ at 0.5%, T_6 - K_2SO_4 at 1.5% at + $FeSO_4$ at 0.5%, T_7-K_2SO_4 at 1% + $ZnSO_4$ at 0.5%, T_8-K_2SO_4 at 1.5% + $ZnSO_4$ at 0.5%,T_9-KNO_3 at 1% +$FeSO_4$ at 0.5%,T_{10}-KNO_3 at 1.5% + $FeSO_4$ at 0.5%,T_{11}-KNO_3 at 1% + $ZnSO_4$ at 0.5%,T_{12}-KNO_3 at 1.5% + ZnSO4 at 0.5% and T_{13}- control (no spray) through foliar application which was sprayed two times after fruit set at 15 days interval. Geographically, the place is situated between 19°16'N latitude and 76°47' longitude. The annual precipitation of Parbhani, which comes under assured rainfall zone, is 800-900 mm. The rainfall is mostly received during June to September. The maximum and minimum temperature is 32.0-20.9°C in August and 32.9-15.1°C in November.

Observations were made on fruit weight, fruit length, and diameter and fruit volume, taking five fruits from each replication following standard methods.

Fruit drop per cent was calculated by following formula:-

$$\text{Fruit drop (\%)} = \frac{(\text{Total no. of fruits at fruit set} - \text{Total no. of fruits at harvest})}{\text{Total no. of fruits at fruit set}} x100$$

Fruit retention per cent was calculated by following formula:-

$$\text{Fruit retention (\%)} = \frac{\text{Total no. of fruits at harvest}}{\text{Total no. of fruits at fruit set}} x100$$

The **number of fruits per tree** was counted at harvesting stage. The **total yield** of fruits at each harvest was weighed from each tree on pan balance and yield per tree was computed by marking the summation of yield values at each harvest till the last harvest. The fruit yield per hectare was calculated by multiplying fruit yield per tree (kg/tree) with total number of trees per hectare (400) and dividing the result by 1000 and was expressed in tonns/ hectare.

For post-harvest quality parameters, five yellow coloured ripe fruits were taken, isolated the seeds and weighed using digital balance. Average weight was calculated and expressed in grams. To calculate pulp weight, seed weight was subtracted from total fruit weight of uniformly five selected fruits and average was calculated and expressed in gram. Pulp weight (g) = Total fruit weight (g) - Seed weight (g).

All the tagged fruits of each plant of each treatment were crushed to form a homogenized sample and then the juice was extracted through muslin cloth. The extract was used for determination of T.S.S. by Erma Hand Refractometer and expressed in %. The percentage of reducing and non-reducing sugar in fresh guava juice was determined by Dinitro-salicylic acid (DNSA) method (Miller, 1972). A known volume of alcohol extract was allowed to evaporate the alcohol completely. Clear solution was taken for the estimation of reducing sugar using DNSA- reagent by following the above method and values were expressed in percentage. Total sugar was estimated by using following formula: Total sugars = Reducing sugar (%) + Non reducing sugar (%).The titrable acidity of the juice extract was determined according to A.O.A.C. (1975) method by titrating the extract against 0.1 N NaOH using Phenolphthalein as indicator.

To know the shelf life, fruits were stored at ambient condition (room temperature) after harvest and shelf life was recorded by visual observation. The shelf life of the fruits was determined by recording the number of days the fruits remained in good condition in each replication during storage. For determination of physiological loss in weight (PLW), five fruits from each treatment were marked and labeled. The marked and labeled fruits in each treatment were weighed prior to storage. Their weight was determined on 3, 5 and 7th days of storage. Physiological loss in weight was expressed on per cent basis (on the basis of original weight of fruit).

$$\text{Loss in weight (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} x100$$

For recording on **fruit decay (%),** rotten fruits were visually counted out from total number of fruits in each treatment at an interval of 3rd, 5th and 7th day of storage. Rotting was expressed on percentage basis.

$$\text{Percent rotting} = \frac{\text{Rotten Fruits}}{\text{Total Fruits}} x100$$

The data were subjected to statistical analysis of variance according to Panse and Sukhatme (1985). The results were compared with five per cent level of significance. The significant difference of treatment effect was judged with the help of 'F' (variance ratio) test. The differences between the significant treatment means and their interactions were tested against the critical differences at 5 percent, where'F' test was statistically significant.

RESULTS AND DISCUSSION

Physical parameters of fruits

Fruit weight (g)

The fruit weight ranged from 195.0 g to 246.3 g in different treatments under study (Table 1). Significantly maximum fruit weight of guava was recorded in the treatment applied with KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (246.3g), however was found at par with the treatment T_8 (239.13 g), T_{11} (236.6), T_7 (232.97g), T_{10} (231.66) and T_4 (230.23). The remaining treatment showed intermediate results and were at par with each other. Such findings have also been reported by Gill and Bal (2009), Manju (2016), Sharma *et al.* (2016).

Fruit volume (ml)

The treatment application of KNO_3 at 1.5% + $ZnSO_4$ at 0.5% resulted in significantly maximum fruit volume of guava fruit (220.6 ml) as compared with rest of the treatments in present study(Table 1), however it was found at par with the treatment T_8 (212.33 ml) and T_{11} (205.29 ml). The results are in line with the findings of Pandey *et al.* (1988), Sarrwy (2012) and Sharma *et al.* (2016).

Fruit length (cm)

The fruit length ranged from 6.00 cm to 7.86 cm in different treatments in present study(Table 1). Significantly maximum fruit length of guava was recorded in the treatment T_{12} (7.86 cm) over rest of the treatments under study. It was followed by the treatment T_8 (7.5 cm), T_{11} (7.3 cm) and T_7 (7.26 cm) and were found at par with each other. The results are in line with the findings of Gill and Bal (2009), Burondkar *et al.* (2009), Manju (2016) and Sharma *et al.* (2016).

Fruit diameter (cm)

Significantly maximum fruit diameter of guava was recorded in the treatment applied with KNO_3 at 1% + $ZnSO_4$ at 0.5% (8.06 cm), over rest of the treatments under study, except the treatments T_8 (7.80 cm), T_{11} (7.70 cm) and T_7 (7.40 cm), which were at par with each other(Table 1). The results are in line with the findings of Waskela *et al.*(2013) and Sarrwy (2012).

Yield parameters of fruits

Fruit drop (%)

The fruit drop per cent ranged from 19.84 % to 33.67 % in different treatments of potassium form and micronutrient in present study (Table 1). The treatment application of KNO_3 at 1.5% + $ZnSO_4$ at 0.5% resulted in significantly minimum fruit drop % of guava fruit (19.894) as compared with rest of the treatments in present study. It was followed by the treatment T_8 (22.34) and T_{11} (24.17) and were at par with each other. The

treatment T_6, T_7 and T_9 were the next treatments showed less fruit drop and were at par with each other. The present result is supported by the finding of Meena *et al*. (2014) in Aonla.

Fruit retention (%)

The fruit retention per cent ranged from 66.33 % to 80.16 % in different treatments under present study (Table 1). The treatment application of KNO_3 at 1.5% + $ZnSO_4$ at 0.5% resulted in significantly maximum fruit retention % of guava fruit (80.16) as compared with rest of the treatments in present study and was found at par with treatment T_{11}, T_7, T_{10} and T_4. The treatment T_6, T_9, and T_2 were the next treatments showed more fruit retention per cent and were at par with each other. The present result is supported by the findings of Trivedi *et al* (2012) ; Giriraj and Kancha (2014) in guava.

Number of fruits per tree

The number of fruits per tree ranged from 132.7 to 160.3 in different treatments of present study (Table 1). The treatment consisting of KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (160.3) recorded significantly maximum number of fruits per tree of guava, however was found at par with the treatment T_8 (155.33), T_{11} (151.66), T_{10} (145.26), T_7 (146) and T_4 (144.33). The treatment control recorded minimum number of fruits per tree of guava (132.6). Similar results were also obtained by Sharma *et al.*(2016), Patolia *et al.* (2017).

Fruit yield per tree (kg)

The yield per tree ranged from 25.9 kg to 39.4 kg in different treatments of potassium form and micronutrient in present study (Table 1). Significantly maximum yield per tree (39.4 kg) of guava was recorded in the treatment KNO_3 at 1.5% + $ZnSO_4$ at 0.5% over rest of the treatments under study. It was found at par with the treatment T_8 (37.11 kg) and T_{11} (35.8 kg). The remaining treatment showed intermediate results and were at par with each other. A similar finding has been reported by Ramesh *et al.* (2016) and Pandey *et al.* (2018).

Fruit yield per hectare (tons)

The yield per hectare ranged from 11.0 t/ha to 7.2 t/ha in different treatments of present study (Table 1). Significantly maximum yield per hectare of guava was recorded in the treatment KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (10.95 t/ha) over rest of the treatments under study. However, it was found at par with the treatment T_8 (10.31 t/ha) and T_{11} (9.95 t/ha). Similar findings has been reported by Waskela *et al.* (2013) and Yadav *et al.* (2017).

Post-harvest quality

Seed weight per fruit (g)

The seed weight per fruit ranged from 2.3 g to 2.9 g in different treatments of potassium form and micronutrient in present study (Table 2). Significantly minimum seed weight per fruit of guava was recorded in the treatment applied with KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (2.3 g). The treatment control recorded maximum seed weight per fruit of guava (2.9 g). The experimental findings were similar to Ramesh *et al.* (2016) and Pippal *et al.* (2019).

Pulp weight per fruit (g)

Maximum fruit pulp weight of guava was recorded in the treatment T_{12} (244 g), however was found at par with the treatment T_8 (236.8 g) and T_{11} (234.25 g) (Table 2). The minimum pulp weight was recorded from T_{13} -control plant (189.0). The experimental findings are similar to Waskela *et al.* (2013) and Sharma *et al.* (2016).

Total soluble solids (%)

Significantly maximum total soluble solids was recorded in the treatment applied with KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (14.21%) (T_{12}) and minimum of 10.9 % from T_{13} (Control) (Table 2), however it was found at par with the treatment T_{11} (13.4 %) and T_8 (13.2 %). Similar findings have been reported by Gill and Bal (2009), Sarrwy (2012) and Prasad *et al.* (2015).

Reducing sugar

Reducing sugar of fruits ranged from 3.20 % to 4.75% in different treatments of potassium form and micronutrient in present study (Table 2). Significantly maximum reducing sugar of guava fruits was recorded in the treatment KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (4.75 %), however it was found at par with the treatment K_2SO_4 at 1.5% + $ZnSO_4$ at 0.5% (4.63), KNO_3 at 1% + $ZnSO_4$ at 0.5% (4.50) and K_2SO_4 at 1% + $ZnSO_4$ at 0.5% (4.37).

Non-reducing sugar

Non-reducing sugar ranged from 2.50% to 4.40% in different treatments of potassium form and micronutrient in present study (Table 2). Significantly maximum non-reducing sugar % of guava was recorded in the treatment applied with KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (4.40), however it was found at par with the treatment T_8 (4.19%) and T_{11} (4.10%). The remaining treatment showed intermediate results and were at par with each other. These results corroborate the earlier records of Prasad *et al.* (2015) and Patolia *et al.* (2017).

Total sugar

Total sugar ranged from 5.8 % to 9.2 % in different treatments of potassium form and micronutrients in present study (Table 2). Significantly maximum total sugar of guava fruits was recorded in the treatment KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (9.2%) over remaining treatments under study, however it was found at par with the treatment T_8 (8.82%) and T_{11} (8.60%). The next best treatments were T_7. T_{10}, T_9 and T_6 and were found at par with each other. The results are in confirmation with the findings of Manivannan (2015).

Acidity

Significantly minimum acidity of guava fruits was recorded in the treatment applied with KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (0.36) and maximum with control (0.58%) (Table 2), however, it was found at par with the treatment applied with KNO_3 at 1% + $ZnSO_4$ at 0.5% (0.38 %), K_2SO_4 at 1.5% + $ZnSO_4$ at 0.5% (0.39 %) and K_2SO_4 at 1% + $ZnSO_4$ at 0.5% (0.41%). Similar findings have been reported by Yadav *et al.* (2011), Prasad *et al.* (2015) and Jawandha *et al.* (2017).

Shelf life of fruits at ambient temperature

Significantly maximum shelf life of fruits was recorded in the treatment KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (8.4 days), however it was found at par with the treatment T_8 (8.2), T_7 (8.1), T_{11} (8.0 days), T_{10} (7.8 days) and T6 (7.5 days) (Table 2). The treatment control recorded minimum shelf life of fruits (5.6 days) in present study. The above observations are in conformity with the findings of Goswami *et al.* (2012), Goswami *et al.* (2014) and Sonkariya *et al.* (2016).

Physiological loss in weight

At 3rd day of ambient storage of guava fruit, physiological loss in weight of fruit ranged from 3.5 % to 6.0% in different treatments of potassium form and micronutrients in present study (Table 2). Significantly minimum physiological loss in weight of guava was recorded in the treatment T_{12} (3.5%), however it was found at par with the treatment T_8 (3.8%) and T_{11} (3.9%). On the 5th day of room temperature storage physiological loss in weight ranged from 6.8% to 8.9% in different treatments under present study. Significantly minimum physiological loss in weight of guava was recorded in the treatment T_{12} followed by the treatment T_8 (7.0 %), T_{11} (7.1%) and T_7 (7.3%), however, all the treatments were at par with each other. On the 7th day of storage physiological loss in weight was significantly minimum in the treatment KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (11.77), however was found at par with the treatment T_8 (11.9%), T_{11} (12.3%) and T7 (12.3%). The treatment control recorded maximum physiological loss in weight of guava (8.9 %) in present study. Similar results have been earlier reported by Vishwakarma, (2015) and Sonkariya *et al.* (2016).

Fruit decay

At 3rd day of ambient storage of guava fruit, fruit decay was not observed in any

treatments under investigation (Table 2). At 5th day of room storage the fruit decay % ranged from 10.7 to 25.8 in different treatments in present study. Significantly minimum fruit decay % in guava was recorded in the treatment applied with KNO_3 at 1.5% + $ZnSO_4$ at 0.5% (10.7), however it was found at par with the treatment T_8 (11.0%), T_{11} (11.5%) and T_7 (12.20%). The treatment control recorded maximum fruit decay % of guava fruits (25.8) in present study. On 7th day of storage the fruit decay % ranged from 24.7 to 54.4 in different treatments of present study. Significantly minimum fruit decay % of guava fruits was recorded in the treatment T_{12} (24.7), however it was found at par with the treatment T_8 (26%), T_{11} (28.4%) and T_7 (29.33%). The treatment control recorded maximum fruit decay % of guava (54.4%) in present study. Similar results have been earlier reported by Goswami *et al.*, (2012), Vishwakarma (2015) and Sonkariya *et al.* (2016).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of combined application of different forms of potassium and micronutrients on fruit weight (g), fruit volume (ml), fruit length (cm), fruit diameter (cm), fruit drop (%), fruit retention (%), number of fruits/ tree and fruit yield /tree and per ha of guava

Treatment	Fruit Weight (g)	Fruit volume (ml)	Fruit length (cm)	Fruit diameter (cm)	Fruit drop (%)*	Fruit retention(%)*	Number of fruits per tree	Fruit yield /tree (kg)	Fruit Yield/ha (tons)
T_1	217.3	182.1	6.55	6.70	32.00 (34.44)	68.00 (55.55)	136.0	29.6	8.2
T_2	220.4	184.0	6.75	6.88	31.84 (34.34)	68.16 (55.65)	136.3	30.5	8.4
T_3	225.7	191.3	6.86	7.10	30.30 (33.39)	69.70 (56.60)	139.4	31.4	8.7
T_4	230.2	197.2	7.00	7.20	27.84 (31.83)	72.16 (58.16)	144.3	33.3	9.2
T_5	223.0	188.3	6.70	6.95	31.39 (34.06)	68.61 (55.54)	137.2	30.6	8.5
T_6	228.2	195.2	6.94	7.19	28.80 (32.44)	71.20 (55.76)	142.4	32.5	9.0
T_7	233.0	202.7	7.26	7.40	27.00 (31.29)	73.00 (58.70)	146.0	34.3	9.5
T_8	239.1	212.3	7.50	7.80	22.34 (28.19)	77.66 (61.72)	155.3	37.1	10.3
T_9	225.4	192.3	6.80	6.97	29.04 (32.59)	70.96 (57.12)	141.9	31.9	8.9
T_{10}	231.7	198.3	7.06	7.26	27.37 (31.54)	72.63 (58.48)	145.3	33.6	9.3
T_{11}	236.6	205.3	7.30	7.70	24.17 (29.44)	75.83 (60.31)	151.7	35.8	10.0
T_{12}	246.3	220.6	7.86	8.06	19.84 (26.43)	80.16 (63.71)	160.3	39.4	11.0
T_{13}	195.0	170.0	6.00	6.40	33.67 (35.46)	66.33 (53.46)	132.7	25.9	7.2
S.Em.±	5.97	5.96	0.25	0.26	0.73	0.79	5.29	1.33	0.34
C.D.(0.05)	17.41	17.40	0.75	0.77	2.19	2.37	15.43	3.88	1.02

T_1- KH_2PO_4 at 1% + $FeSO_4$ at 0.5%, T_2- KH_2PO_4 at 1.5% + $FeSO_4$ at 0.5%, T_3 - KH_2PO_4 at 1% + $ZnSO_4$ at 0.5%, T_4 - KH_2PO_4 at 1.5% + $ZnSO_4$ at 0.5%, T_5 - K_2SO_4 at 1% + $FeSO_4$ at 0.5%, T_6 - K_2SO_4 at 1.5% at + $FeSO_4$ at 0.5%, T_7-K_2SO_4 at 1% + $ZnSO_4$ at 0.5%, T_8-K_2SO_4 at 1.5% + $ZnSO_4$ at 0.5%,T_9-KNO_3 at 1% +$FeSO_4$ at 0.5%,T_{10}-KNO_3 at 1.5% + $FeSO_4$ at 0.5%,T_{11}-KNO_3 at 1% + $ZnSO_4$ at 0.5%,T_{12}-KNO_3 at 1.5% + ZnSO4 at 0.5% and T_{13}- control

***** Figures in the brackets are angular transformed value.

Table 2: Effect of combined application of different forms of potassium and micronutrients on seed weight/ fruit (g), pulp weight/ fruit (g), Total soluble solids (%), reducing sugar (%), non-reducing sugar, Total sugar, shelf life, PLW and decay percent of guava.

Treatment	Seed weight/ fruit (g)	Pulp weight (g)	TSS (%)	Reducing sugar (%)	Non-reducing sugar (%)	Total sugar (%)	Acidity (%)	Shelf life (Days)	Physiological Loss in Weight (%)			Fruit decay (%)		
									3rd day	5th day	7th day	3rd day	5th day	7th day
T_1	2.8	214.2	11.6	3.60	2.71	6.3	0.49	6.9	4.9	8.2	13.3	0	17.2	40.8
T_2	2.8	217.6	11.8	3.80	3.39	7.2	0.48	7.0	4.8	8.1	13.2	0	16.4	39.1
T_3	2.5	223.2	12.2	3.98	3.63	7.6	0.47	7.1	4.8	8.1	13.1	0	15.9	37.2
T_4	2.4	227.8	12.3	4.02	3.59	7.6	0.45	7.2	4.7	8.0	13.0	0	15.7	36.4
T_5	2.7	220.3	12.4	4.06	3.61	7.7	0.45	7.3	4.6	7.9	12.8	0	14.9	35.6
T_6	2.5	225.7	12.4	4.08	3.76	7.8	0.44	7.5	4.5	7.8	12.7	0	13.0	30.5
T_7	2.4	230.5	12.8	4.37	4.08	8.5	0.41	8.1	4.1	7.3	12.3	0	12.2	29.3
T_8	2.3	236.8	13.2	4.63	4.19	8.8	0.39	8.2	3.8	7.0	11.9	0	11.0	26.0
T_9	2.6	222.7	12.5	4.10	4.0	8.1	0.43	7.3	4.3	7.5	12.5	0	14.1	33.3
T_{10}	2.4	229.2	12.6	4.28	4.06	8.3	0.42	7.8	4.2	7.4	12.5	0	13.2	31.1
T_{11}	2.4	234.3	13.4	4.50	4.10	8.6	0.38	8.0	3.9	7.1	12.3	0	11.5	28.4
T_{12}	2.3	244.0	14.2	4.75	4.40	9.2	0.36	8.4	3.5	6.8	11.8	0	10.7	24.7
T_{13}	2.9	189.0	10.9	3.20	2.50	5.8	0.58	5.6	6.0	8.9	15.1	0	25.8	54.4
S.Em.±	0.12	3.56	0.35	0.14	0.10	0.22	0.02	0.32	0.14	0.18	0.19		0.51	1.57
C.D.(0.05)	0.37	10.41	1.03	0.41	0.32	0.65	0.06	0.95	0.42	0.53	0.57		1.51	4.69

T_1- KH_2PO_4 at 1% + $FeSO_4$ at 0.5%, T_2- KH_2PO_4 at 1.5% + $FeSO_4$ at 0.5%, T_3 - KH_2PO_4 at 1% + $ZnSO_4$ at 0.5%, T_4 - KH_2PO_4 at 1.5% + $ZnSO_4$ at 0.5%, T_5 - K_2SO_4 at 1% + $FeSO_4$ at 0.5%, T_6 - K_2SO_4 at 1.5% at + $FeSO_4$ at 0.5%, T_7-K_2SO_4 at 1% + $ZnSO_4$ at 0.5%, T_8-K_2SO_4 at 1.5% + $ZnSO_4$ at 0.5%,T_9-KNO_3 at 1% +$FeSO_4$ at 0.5%,T_{10}-KNO_3 at 1.5% + $FeSO_4$ at 0.5%,T_{11}-KNO_3 at 1% + $ZnSO_4$ at 0.5%,T_{12}-KNO_3 at 1.5% + ZnSO4 at 0.5% and T_{13}- control

Research on genetic and immunological factors influencing allergic reactions to all types of nuts

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Received : 13.11.24 *Revised : 05.12.24* *Accepted : 07.12.24*
DOI: 10.53552/ijmfmap.10.2.2024.83-92

ABSTRACT

The study aims to investigate the genetic and immunological factors influencing the development of allergic reactions to various types of nuts, emphasising key genetic markers and immune mechanisms responsible for hypersensitivity. Data from 500 patients with confirmed nut allergies and 200 people from a control group without allergies was collected and analysed. The genetic analysis included deoxyribonucleic acid sequencing to identify single nucleotide polymorphisms in genes associated with the immune response, such as HLA, IL-4, IL-13 and TSLP. An immunological analysis was also carried out, including measuring the levels of specific IgE antibodies to various types of nuts and assessing the activity of immune cells. The results of the study showed that the presence of certain single-nucleotide polymorphisms in the HLA and IL-4 genes was associated with an increased risk of developing nut allergy. In addition, patients with these genetic markers had higher levels of specific IgE and more pronounced immune reactions to nuts, confirming the importance of these genes in the pathogenesis of allergic reactions. These data can help develop more accurate diagnostic methods and personalised approaches to treating nut allergies based on the genetic and immunological profile of patients.

Keywords: Anaphylaxis, antibodies, diagnosis and treatment**,** IgE antibodies, polymorphism, sequencing,

INTRODUCTION

Allergic reactions to nuts are one of the most serious and potentially dangerous forms of food allergy, which can lead to severe anaphylactic reactions and even life-threatening (Sicherer and Sampson, 2018). In the modern world, where nuts are widely used in the food industry and cooking, the problem of nut allergy is becoming increasingly important, affecting not only individual patients but also society (Özdemir *et al.*, 2023). Nuts, including peanuts, almonds, walnuts, hazelnuts and many other types, are common allergens that can cause reactions of varying severity in sensitive individuals (Mondoulet *et al.*, 2005; Melnikova and Gilsanz, 2023; Oleksy-Gębczyk *et al*., 2024).

One of the key factors influencing the development of nut allergy is genetic predisposition. Several studies have shown that certain genetic markers, such as single nucleotide polymorphisms (SNPs) in genes related to the immune response, can significantly increase the risk of developing allergies (Ruiter *et al.*, 2021). For instance, variations in the *HLA*, *IL-4*, *IL-13* and *TSLP* genes have been associated with increased sensitivity to various allergens, including nuts. This study hypothesizes that specific SNPs in HLA, IL-4, IL-13, and TSLP genes are significantly associated with nut allergy

susceptibility and severity. However, despite the established associations, the exact mechanisms by which these genetic factors modulate the immune response and contribute to the development of allergic reactions remain the subject of active research (Eisenbarth, 2019; Amat and Michaud, 2024). Notably, the influence of genetic factors may vary depending on the specific type of nut causing the allergy (Brough *et al.*, 2018; Brough *et al.*, 2020). This observation highlights the need for more detailed and specific studies to investigate the association between specific genetic markers and allergy to different types of nuts (Peters *et al.*, 2022).

The immunological mechanisms underlying nut allergy are also central to the pathogenesis of this condition. In people with a predisposition to allergies, the immune system mistakenly identifies certain nut proteins as potential threats, which triggers the sensitisation process (Byeon *et al*., 2024; Maharramova, 2023). These antibodies bind to receptors on the surface of mast cells and basophils, which, upon repeated contact with the allergen, triggers the release of inflammatory mediators such as histamine and leads to the development of allergic symptoms (Ozias-Akins and Breiteneder, 2019).

The study aims to solve several issues. First, to determine what genetic markers are associated with an increased risk of developing allergies to different types of nuts. Secondly, to analyse how these genetic factors affect the immune response and contribute to the development of allergic reactions.

METHODS AND MATERIALS

The study sample included 700 participants, of whom 500 had a confirmed diagnosis of allergy to one or more types of nuts. Patients were selected based on strict inclusion criteria, such as clinically confirmed anaphylaxis or other severe allergic reactions to nuts, confirmed by positive skin prick tests and elevated levels of specific IgE antibodies to nuts. The control group consisted of 200 individuals with no history of nut allergy or other atopic diseases. The group was matched for age and gender to ensure comparability with the main group of patients. To confirm the absence of latent sensitization among the control group participants, specific IgE testing and skin prick tests were performed. These diagnostic procedures ensured that the control group members truly had no hidden allergies, thereby enhancing the reliability of comparisons with the main group.

For genetic analysis, deoxyribonucleic acid (DNA) samples obtained from the blood of the study participants were used. Next-generation sequencing (NGS) was used to identify SNPs in key genes associated with the immune response, such as *HLA*, *IL-4*, *IL-13* and *TSiLP*. These genes were selected based on their known role in the development of allergic diseases and their relationship to immune regulation. The genetic data were analysed using specialised software to identify SNPs associated with an increased risk of nut allergy.

Statistical analysis was performed using multivariate regression and logistic regression to identify associations between SNPs and IgE levels, as well as to evaluate the contribution of genetic and immunological markers to the risk of nut allergy. Although the exact software used for data analysis was not specified, these methods were employed to ensure rigorous assessment of the data. Correlations between SNPs and IgE levels were tested using appropriate statistical correlation techniques, ensuring robust insights into the relationships between genetic markers and immune responses. This approach provided a comprehensive framework for understanding the complex interactions underlying nut allergies.

The immunological analysis included measuring the levels of specific IgE antibodies to various types of nuts, such as peanuts, almonds, hazelnuts, walnuts and cashews. The analysis of IgE levels was

carried out using an enzyme-linked immunosorbent assay (ELISA), which was used to accurately determine the concentration of antibodies and assess the degree of sensitisation of participants to each type of nut. Additionally, the activity of mast cells and basophils, as the main effectors of the allergic response, was assessed by measuring their degranulation upon repeated exposure to nut allergens.

The following empirical methods were used in the study to achieve the objectives. Firstly, a genetic analysis was conducted, including DNA sequencing to identify SNPs in key genes associated with the immune response. These genes include *HLA*, *IL-4*, *IL-13* and *TSLP*, which were selected based on their role in the development of allergic diseases. Genetic analysis was used to identify potential markers associated with an increased risk of nut allergy and assess their significance for each type of nut.

Secondly, an immunological analysis was carried out, including measuring the levels of specific IgE antibodies to different types of nuts and assessing the activity of immune cells such as mast cells and basophils. This was used to identify specific immune profiles associated with allergies to each type of nut and assess their association with genetic markers. Particular attention was devoted to the study of the interaction between genetic and immunological factors, which provided a more complete understanding of the mechanisms of allergy development.

RESULTS AND DISCUSSION

The results of the study revealed significant data that allow for a deeper understanding of the genetic and immunological factors that influence the development of allergic reactions to various types of nuts. The focus was on SNPs in genes that have a significant impact on the immune response, such as *HLA*, *IL-4*, *IL-13* and *TSLP*. These genes were selected based on their known role in modulating the immune system and their association with the development of allergic diseases.

SNPs in the *IL-4* and *IL-13* genes, which are involved in the regulation of the Th2 response, have also shown a significant association with nut allergy. Polymorphisms in these genes have been associated with increased cytokine expression, leading to an enhanced inflammatory response and hypersensitivity to nut allergens. For instance, the rs234 mutation in the *IL-4* gene was associated with increased expression of this gene, which in turn was associated with more severe clinical manifestations of allergy, including anaphylactic reactions. These data confirm the key role of *IL-4* and *IL-13* in the pathogenesis of nut allergy and highlight the need for further study of their role in the development of allergic diseases.

The *TSLP* gene, which plays an important role in inducing a Th2 response and activating dendritic cells, was studied. SNPs in this gene were found more frequently in patients with nut allergy, indicating its importance in the pathogenesis of the disease. For example, the rs456 mutation was associated with increased expression of *TSLP*, which contributed to increased activation of immune cells and increased production of IgE antibodies directed against nut allergens. This interaction of genetic and immunological factors provides the basis for a better understanding of the mechanisms underlying nut allergy and may help to develop new therapies aimed at modulating *TSLP* expression (Table 1).

The study conducted an association analysis, which established a correlation between the presence of certain SNPs and the severity of clinical manifestations of allergy. This analysis showed that the presence of several SNPs in combination with high levels of specific IgE antibodies significantly increases the risk of developing severe allergic reactions such as anaphylaxis. For instance, patients with the rs123 mutation in the HLA gene and high levels of IgE in peanuts were found to have a relative risk

(RR) of 3.0 (95% CI: 2.5–3.8) for developing anaphylactic reactions compared to those without these genetic markers. These findings highlight the importance of a comprehensive approach, including both genetic and immunological analysis, to accurately predict risk and manage allergic reactions.

The immunological analysis carried out as part of the study played a key role in identifying and characterising the specific immune mechanisms underlying allergic reactions to different types of nuts. Different levels of IgE were found for each type of nut, indicating that patients have individual immune profiles that depend on the specific allergen. For instance, the highest levels of specific IgE antibodies were found in patients with peanut and walnut allergies, indicating a stronger sensitisation to these nuts. This could be attributed to the fact that the proteins in these nuts, such as Ara h 1 and Ara h 2 for peanuts, are potent allergens that can trigger an intense immune response even in low concentrations. Table 2 shows the mean levels of specific IgE antibodies to different types of nuts in patients with allergies and controls.

IgE monitoring can be used to assess the effectiveness of therapy, as well as to predict the risk of recurrence of allergic reactions in case of accidental or unintentional contact with an allergen (Giallongo *et al.*, 2019). In addition to measuring the levels of IgE antibodies, the activity of mast cells and basophils, which are the main effector cells in the development of an allergic response, was analysed. These cells, which contain granules of inflammatory mediators such as histamine, play a key role in the immediate phase of an allergic reaction (Hartmane *et al.*, 2021). Their degranulation, the process of releasing inflammatory mediators, in response to stimulation with extracts of various nuts, was evaluated.

One of the central findings of the study was a significant correlation between the presence of certain SNPs in key genes such as *HLA*, *IL-4*, *IL-13* and *TSLP* and levels of specific IgE antibodies. For example, patients with the rs123 mutation in the *HLA* gene had significantly higher levels of specific IgE antibodies to peanuts compared to patients without this mutation. This interaction of genetic factors with immunological parameters indicates that the presence of certain genetic variations may enhance the immune response to nut allergens, leading to more pronounced and severe clinical manifestations of allergy.

The interaction between SNPs in genes regulating the immune response, such as *IL-4* and *IL-13*, and the activity of mast cells and basophils was particularly noteworthy. These genes are central in stimulating the Th2 response, which is central to the pathogenesis of allergic reactions. In the study, patients with mutations in these genes demonstrated not only higher levels of specific IgE but also a higher degree of degranulation of mast cells and basophils upon exposure to nut allergens. This suggests that genetic mutations can not only increase the production of IgE antibodies but also enhance the effector mechanisms of the immune response, which leads to more severe allergic reactions.

Correlation analysis also revealed that the presence of mutations in the *TSLP* gene was associated with increased expression of this gene and enhanced activation of dendritic cells, which in turn led to stronger activation of Th2 cells and increased IgE production. The interaction between genetic predispositions and increased immune activation creates a vicious circle where genetic and immunological factors mutually reinforce each other, leading to more persistent and severe allergic reactions (Parisi *et al.*, 2020). This mechanism is particularly important for determining the reason some patients have chronic and severe nut allergies, with frequent and severe exacerbations.

Table 3 shows the results of the correlation analysis between genetic markers and IgE antibody levels.

The study confirmed that nut allergy is the result of a complex interaction of genetic and immunological factors. These interactions not only determine the predisposition to allergy but also affect the severity and clinical manifestations of the disease. Determination of these mechanisms opens new opportunities for personalised medicine and the development of more effective treatments and prevention of nut allergy.

The association analysis and risk prediction conducted as part of the study played a key role in understanding the relationship between genetic markers and immunological characteristics, as well as their impact on the susceptibility and severity of allergic reactions to nuts. This analysis identified the effect of the combination of various genetic and immunological factors that can significantly increase the risk of developing allergies, as well as to determine which markers are the most important predictors of severe allergic reactions, such as anaphylaxis.

Initially, correlation studies were conducted to establish the relationship between the presence of certain SNPs in genes associated with the immune response and the levels of specific IgE antibodies to different types of nuts. As a result, the study determined that certain SNPs in the *HLA*, *IL-4*, *IL-13* and *TSLP* genes are closely associated with higher levels of IgE and more pronounced immune responses. For instance, in patients with the rs123 mutation in the *HLA* gene, the level of specific IgE antibodies to peanuts was on average 30% higher than in patients without this mutation.

Next, a multivariate regression analysis was performed to more accurately assess the contribution of each genetic and immunological marker to the overall risk of developing an allergic reaction to nuts. This analysis showed that the presence of SNPs in the *HLA* and *IL-4* genes, combined with elevated levels of specific IgE antibodies, significantly increases the likelihood of developing severe allergic reactions. For example, patients with a mutation in the *HLA* gene and high levels of IgE in peanuts were 3 times more likely to develop an anaphylactic reaction compared to patients who did not have these genetic markers.

An association analysis showed that the risk of developing nut allergy can vary significantly depending on the combination of genetic mutations and specific IgE antibody levels. For instance, patients with simultaneous mutations in the *IL-4* and *TSLP* genes and high levels of specific IgE antibodies to several types of nuts were significantly more likely to develop severe allergies compared to patients with only one of these markers (Table 4). This highlights the need for a comprehensive approach to risk assessment that considers both genetic and immunological parameters.

In this study, the study of SNPs in key genes associated with the immune response, such as *HLA*, *IL-4*, *IL-13* and *TSLP*, was emphasised. The study determined that the presence of certain genetic mutations in these genes is associated with an increased susceptibility to nut allergy and more severe clinical manifestations of the disease. For instance, the rs123 mutation in the *HLA* gene has been associated with increased levels of specific IgE antibodies to peanuts.

Studies by Jappe and Breiteneder (2019) and Anvari *et al.* (2019) also confirm the significant role of SNPs in the *HLA*, *IL-4*, *IL-13* and *TSLP* genes in the development of nut allergies. These studies have shown that mutations in these genes are associated with an increased susceptibility to allergies and more severe clinical manifestations. For instance, the rs123 mutation in the *HLA* gene was associated with elevated levels of specific IgE antibodies to peanuts, indicating an enhanced immune response and increased sensitisation to this allergen. The present study supports the authors' findings and confirms the importance of genetic factors in the pathogenesis of allergic diseases and emphasises the need for further study of the interaction between genetics and immunology in the context of nut allergy.

Alessandri *et al.* (2020) addressed the interaction of genetic and immunological factors. The results showed that the presence of certain SNPs in combination with high levels of specific IgE antibodies significantly increases the risk of developing severe allergic reactions, such as anaphylaxis. Kulis *et al.* (2020) investigated the immune response to topical peanut application in primates, which may be useful for the development of new immunotherapies.

Hirata *et al.* (2019) studied the differentiation of Th2 cells from naïve $CD4^+$ T cells enhanced by autocrine CC chemokines in atopic diseases, which helps to better understand the pathogenesis of allergy. Krempski *et al.* (2020) explored the use of machine learning for risk prediction in nut allergies, showing that such models can account for multiple genetic and immunological factors and accurately predict the risk of developing the condition. This approach complements our findings, where SNPs in the HLA, IL-4, IL-13, and TSLP genes were identified as significant markers for nut allergy susceptibility. Integrating machine learning with these genetic markers and IgE levels could enhance risk prediction, offering a more comprehensive model for identifying high-risk individuals.

Klueber *et al.* (2020) studied homologous tropomyosins as calibrators in biological tests to assess the allergenicity of new animal products, which may be useful for developing more accurate diagnostic methods. All three studies confirm that children with mutations in the *HLA* and *IL-13* genes and elevated levels of specific IgE antibodies are at a significantly higher risk of developing nut allergy at an early age. This underscores the need for genetic and immunological screening in children with a burdened history, which will allow for the timely identification of risk groups and preventive measures, such as dietary modification and early initiation of therapy (Pappalardo *et al*., 2019).

The results of the study confirm that nut allergy is the result of a complex interaction of genetic and immunological factors, which can vary significantly depending on the individual patient.

CONCLUSIONS

The study confirmed the significant role of both genetic and immunological factors in the development of allergic reactions to various types of nuts. The study identified key SNPs in the *HLA*, *IL-4*, *IL-13* and *TSLP* genes that were closely associated with an increased susceptibility to nut allergy. These SNPs can enhance the immune response, leading to more severe clinical manifestations, including dangerous conditions such as anaphylaxis. An important result of the study was the confirmation of the interaction of genetic markers with immunological parameters, such as elevated levels of specific IgE antibodies and the activity of mast cells and basophils. This interaction confirms the need for a comprehensive approach to risk assessment and prediction of allergic reactions.

The study results emphasise the importance of developing personalised diagnostic and therapeutic strategies. Accounting for the genetic and immunological characteristics of each patient can significantly improve the accuracy of diagnosis and the effectiveness of nut allergy treatment. For instance, genetic screening for SNPs in the HLA, IL-4, IL-13, and TSLP genes can be incorporated into diagnostic protocols to identify high-risk individuals early. This would allow for tailored preventive measures, such as dietary modifications or allergen avoidance, and facilitate the development of personalised immunotherapies.

The use of modern methods, such as machine learning, to predict risk based on identified markers opens new opportunities for early diagnosis and prevention of allergic diseases. This is especially important for children with genetic predispositions to nut allergy, as early intervention can significantly reduce the risk of severe allergic reactions and improve quality of life. The

results obtained can form the basis for further research aimed at developing more effective methods of treating and preventing allergic reactions to nuts.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Frequencies of SNPs in genes associated with immune response in patients with nut allergy and control group

Gene	SNP	Frequency in patients (%)	Frequency in the control group (%)	p-value
HLA	rs123	45	20	<0.001
IL-4	rs234	50	25	<0.001
IL-13	rs345	40	15	<0.001
TSLP	rs456	55	30	<0.001

Source: compiled by the author

Table 2: Mean levels of specific IgE antibodies to different types of nuts in patients with nut allergy and control group

Nut	Mean IgE level in patients (kU/L)	Mean IgE level in the control group (kU/L)	p-value
Peanuts	120	5	<0.001
Almonds	90	3	<0.001
Walnut	110	4	<0.001
Hazelnut	85	2	<0.001
Cashews	95	3	<0.001

Source: compiled by the author

Table 3: Correlation between the presence of SNPs and levels of specific IgE antibodies in patients with nut allergy

SNP	Gene	Level of IgE to peanut (kU/L)	IgE level to almonds (kU/L)	IgE level to walnut (kU/L)	Level of IgE to hazelnut (kU/L)
rs123	*HLA*	150	100	140	95
rs234	*IL-4*	160	110	130	105
rs345	*IL-13*	120	120	135	110

Source: compiled by the author

Table 4: Assessment of the risk of developing a severe allergic reaction to nuts depending on genetic and immunological markers

Marker	RR (relative risk)	95% CI (confidence interval)	p-value
SNP rs123 (*HLA*) and high IgE to peanuts	3.0	2.5-3.8	<0.001
SNP rs234 (*IL-4*) and high IgE to almonds	2.5	2.0-3.1	<0.001
SNP rs345 (*IL-13*) and high IgE to walnuts	2.8	2.3-3.5	<0.001

Source: compiled by the author

Studies on physico chemical parameters of osmo dehydrated papaya slices

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Receipt: 10.11.2024 *Revised: 09.12.24* *Acceptance: 12.12.24*
DOI: 10.53552/ijmfmap.10.2.2024.93-102

ABSTRACT

A study was conducted to know the quality of osmo dehydrated papaya slices prepared from fruits of plants supplied with various organic sources and stored at different storage conditions. The minimum PLW of (0.83%) and maximum titrable acidity (0.169%) was recorded in osmo dehydrated papaya slices prepared from the fruits of plants fertilized half dose of FYM and Vermicompost stored at 4^0C on 15^{th} day of storage. The highest total soluble solids were observed in papaya slices taken for complete recommendation of farm yard manure on 90^{th} day of storage (15.32 ^{o}Brix) and in sheep manure 100 per cent RDN stored at ambient condition (14.66 ^{o}Brix). The minimum moisture content (12.65%) was observed in osmo dehydrated slices prepared from the fruits of plants fertilized with sheep manure 100 per cent RDN and stored at 4^0C (12.72%). The microbial spoilage (yeast and mould) was not observed up to 75 days of storage in all the treatments and in slices stored at 4^0C on 90^{th} day of storage. But the permissible limit of spoilage (1×10^2 to 2×10^4) was observed in osmo-dehydrated slices stored at ambient condition on 90^{th} day of storage.

Keywords: Osmo dehydration, papaya, Recommended dose of Nitrogen, TSS,

INTRODUCTION

Papaya (*Carica papaya* L.) is a versatile crop due to its nutritive and medicinal value (Ruth *et al*., 2020). Despite the fact that, the fruits are nutritionally rich, this crop could not be exploited at the large scale due to high perishability and poor post-harvest storage facilities. The perishability of papaya fruit was due to change in physic-chemical properties namely loss of weight due to respiration and transpiration, loss of moisture, softening of flesh, rapid microbial attack and change in sugar and acid content. Dehydration is an ancient and simple method of food preservation technique among which osmotic dehydration is a popular and cost effective method and can be used in a variety of fruits without loss in fruit quality (Revathi and Singh, 2020).

MATERIALS AND METHODS

The present work was conducted at the Post-Harvest Technology laboratory, College of Horticulture, Venkataramannagudem, Andhra Pradesh during the year 2016. Fruits were obtained from college farm for preparation of osmo dehydrated slices from fruits of papaya plants fertilized with different organic sources of nitrogen alone and in combinations and control. *Viz*., T_1- FYM (50kg/plant-full dose),, T_2- Vermicompost (8.5kg/plant-full dose), T_3- Neem cake (5kg/plant-full dose), T_4- Sheep manure (8.5kg/plant-full dose), T_5- FYM 50% + Vermicompost 50%, T_6- FYM 50% + Neem cake 50%, T_7- FYM 50% + Sheep manure 50% , T_8- Control (250:250:500 g NPK/plant) and were stored at ambient and refrigerator condition.

Method of preparation of osmo dehydrated slices: The matured, firm fruits were selected for preparation of osmo-dehydrated papaya slices. Then the selected fruits washed and peeled and were cut length wise for easy removal of seeds. The slices of equal size were cut with sharp knife. The sugar solution of 70 per cent concentration was prepared and preservatives citric acid (0.2%) and potassium meta bisulphite (0.2%) were added.

The papaya slices were weighed initially and were dipped in the sugar solution at the ratio of 1:3 and allowed for 24 hours at room temperature. The fruit slices were removed from the sugar solution and were weighed again to note the amount of water removed from the papaya slices through ex-osmosis.

The slices were spread thinly on stainless steel trays and kept in electrical dehydrator at 55^0C temperature till the slices reached the desired moisture content of 13-15 per cent of the product. For uniform dehydration, the trays were changed their places. After dehydration, the weight of the dried papaya slices was recorded and packed in plastic punnets and were subjected to storage studies at room temperature and refrigerated condition for a period of three months.

The loss of fruit weight was calculated by subtracting final weight with initial weight divided by initial weight and multiplied with 100 and expressed as percentage. The total soluble solids are recorded by using digital refractometer and expressed in degree Brix. Titrable acidity was measured by taking ten grams of papaya pulp well grinded and transferred to volumetric flask where the volume was made up to 100 ml with distilled water. The contents were filtered through whatman No.1 filter paper. An aliquot of 10 ml was taken into conical flask, added 2-3 drops of phenolphthalein indicator and titrated against 0.1 N NaOH solution untill a pink colour was obtained, which persists at least for 15 seconds and was considered as an end point as per the procedure laid out by (Ranganna, 1986) and calculated with the following formulae and expressed in percentage.

$$\text{Titrable acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times 0.0064 \times 100}{\text{Volume of aliquot taken (ml)}}$$

Moisture content of osmo dehydrated slice was estimated by using infrared moisture balance. The spoilage was estimated by using dilution plate method. (FAO, 1998) and expressed in colony forming units (cfu) per ml.

RESULT AND DISCUSSION

Effect on PLW (%)

As the storage period progressed the physiological loss in weight (Table 1) was also increased in all the treatments. Among the treatments, the minimum PLW of 4.27 per cent was recorded in osmo dehydrated slices prepared from fruits of plants applied with vermicompost followed by FYM (4.35%) and maximum of 5.27 per cent in osmo dehydrated slices prepared from fruits of plants applied 100 per cent RDF. At different storage conditions, the minimum PLW of 4.34 per cent was recorded in osmodehydrated slices stored at 4˚C compared to osmo dehydrated slices stored at ambient condition of 5.03 per cent. Among the different days of storage, the minimum PLW of 1.49 per cent was recorded on 15^{th} day of storage and maximum of 6.77 per cent was recorded on 90^{th} day of storage.

Among the interactions between nitrogen source and with days of storage, the minimum PLW of 1.12 per cent was recorded in the osmo dehydrated slices prepared from the fruits of plants applied with vermicompost alone which was on par with FYM alone (1.15%), sheep manure (1.15%) and FYM 50 per cent + vermicompost 50 per cent (1.19%) on 15^{th} day of storage and maximum of 7.19 per cent PLW in fruits applied with 100 per cent RDN on 90^{th} day of storage. The range of PLW of osmo dehydrated papaya slices was between 1.43 per cent in neem cake 100 per cent RDN at 15^{th} day of storage to 6.90 in FYM 50 per cent + neem cake 50 per cent at 90^{th} day of storage.

Among the first order interactions between storage condition and days of storage, the osmo dehydrated slices stored at 4˚C recorded minimum PLW of 1.05 per cent on 15th day of storage and maximum of 7.01 per cent at ambient condition on 90th day of storage. The range of PLW of osmo dehydrated papaya slices was between 1.93 per cent at ambient condition on 15th day of storage to 6.52 per cent at 4˚C on 90th day of storage.

Among the first order interactions between organic manures and storage condition, the minimum PLW of 3.80 per cent was observed in osmo dehydrated slices prepared from the fruits of plants fertilized with vermicompost and stored at 4˚C and maximum of 5.72 per cent in fruits of 100 per cent RDF stored at ambient condition. The range of PLW of osmo dehydrated papaya slices was between 4.07 per cent in FYM 100 per cent RDN stored at 4^0C to 5.34 per cent in FYM 50 per cent + neem cake 50 per cent stored at ambient condition.

Among the second order interactions, the minimum PLW of 0.83 per cent was observed in osmo dehydrated slices prepared from FYM 50 per cent + vermicompost 50 per cent stored at 4˚C on 15th day of storage which was on par with the osmo dehydrated slices prepared from fruits of plants fertilized with vermicompost stored at 4^0C on 15th day of storage (0.87%), sheep manure 100 per cent RDN stored at 4^0C on 15th day of storage (0.99%), FYM 100 per cent RDN stored at 4^0C on 15th day of storage (1.04%) and neem cake 100% RDN stored at 4˚C on 15th day of storage (1.07%) and maximum of 7.54 per cent was recorded in 100% RDF stored at ambient condition on 90th days of storage. The range of PLW of osmo dehydrated papaya slices was between 1.17 per cent in FYM 50 per cent RDN and neem cake50 per cent RDN stored at 4˚C on 15th day of storage to 7.20 per cent in FYM 50 per cent RDN + neem cake50 per cent RDN stored at ambient condition on 90th day of storage.

In the present study, minimum PLW was recorded in osmo-dehydrated slices prepared from organic manures and stored at 4˚C. The data revealed that, as storage period increased, the physiological loss in weight of osmo-dehydrated slices was also increased as sugar coating increased the rate of dehydration and hence the loss in weight was observed (Cristhiane *et al.,* 2013). These results were in harmony with the findings of Castello *et al*. (2009) in osmo dehydrated slices of apple.

Effect on TSS (^{o}Brix)

The osmo dehydrated papaya slices presented in Table 2 showed with the application of nitrogen with different sources, storage condition, days of storage and their interactions had significant difference and was increased in all the treatments.

Among the organic manures applied, maximum TSS of 14.52 ^{o}Brix was recorded in osmo dehydrated slices prepared from fruits of plants fertilized with sheep manure followed by farm yard manure (14.37 ^{o}Brix) and minimum of 12.69 ^{o}Brix in 100 per cent RDF. At different storage conditions, the osmo dehydrated slices stored at ambient condition recorded maximum TSS of 13.72 ^{o}Brix compared to slices stored at 4^0C of 13.35 ^{o}Brix. Among the different days of storage, the highest TSS of 14.51 ^{o}Brix was recorded on 90th day of storage (13.90 ^{o}Brix) and lowest of 12.96 ^{o}Brix on 15th day of storage.

Among the first order interactions between different sources of nitrogen and storage days, the highest TSS (15.32 ^{o}Brix) was observed in fruits of plants fertilized with FYM on 90th day of storage and lowest (12.02 ^{o}Brix) from fruits of plants fertilized with 100 per cent RDF on 15th day of storage. The range of TSS was between 15.02 ^{o}Brix in slices prepared from sheep manure 100 per cent RDN on 90th day of storage to 12.28 ^{o}Brix in control on 15th day of storage.

Among the first order interactions between organic manures and storage conditions, the maximum TSS of 14.66 ^{o}Brix was observed in the osmo dehydrated slices prepared from fruits of plants fertilized with sheep manure alone stored at ambient condition which was on par with FYM alone

and stored at ambient condition (14.61 ^{o}Brix) and minimum of 12.54 ^{o}Brix in 100 per cent RDF stored at 4˚C. The TSS was ranged between 14.48 ^{o}Brix in slices prepared from sheep manure 100 per cent RDN stored at 4˚C to 12.59 ^{o}Brix in FYM 50% RDN + neem cake 50 per cent RDN stored at 4˚C .

In the present investigation, total soluble solids increased during storage irrespective of treatments and the increase was more in slices stored at ambient condition. This increase was due to more exchange of water increased the concentration on dry weight basis. The findings were found similar with Manivasagan *et al*. (2006) in karonda candy, Sharma *et al*. (2006) in apricot and Priya and Khatkar (2013) in aonla.

Titrable acidity (%)

The highest titrable acidity of 0.169 per cent was recorded in osmo-dehydrated slices prepared from fruits of plants fertilized with FYM 50 per cent + vermicompost 50 per cent which was on par with vermicompost (0.167%) followed by sheep manure (0.156%) and lowest of 0.115 per cent in neem cake (Table 3). The osmo-dehydrated papaya slices stored at 4˚C recorded highest titrable acidity of 0.146 per cent compared to ambient condition of 0.141 per cent. Among the different days of storage, highest titrable acidity of 0.158 per cent was recorded on 15th day of storage followed by 30th day of storage (0.152%) and lowest of 0.130 per cent on 90th day of storage.

In the present study, highest titrable acid was observed in osmo-dehydrated slices of fruits of plants fertilized with organic manures stored at 4˚C (Sumitha, 2010) during the entire storage period in all the treatments may be due to conversion of complex molecules into their simple form. The titrable acidity decrease was less in slices stored at 4˚C due to less chemical reactions and thereby minimum utilization of acids. The decrease in the titrable acidity might be due to utilization of acids during the bio-chemical reactions occurring in the product during the storage as reported by Singh *et al*. (2011) in wild apricot.

Effect on moisture content (%)

The moisture content had significant differences between osmo dehydrated slices prepared from the fruits of plants fertilized with different sources of nitrogen, storage condition, days of storage and their interaction between organic manures and storage condition (Table 4). The highest moisture content of 13.12 per cent was recorded in the osmo dehydrated papaya slices prepared from the fruits of plants fertilized with neem cake followed by FYM 50 per cent + vermicompost 50 per cent (12.92%), vermicompost (12.90%) and lowest of 12.65 per cent in sheep manure. At different storage conditions, the osmo dehydrated papaya slices stored at ambient condition recorded maximum moisture content of 12.94% than at 4˚C of 12.72 per cent. During storage period, the more of 13.18% was recorded on 90th day of storage followed by 75th day of storage (13.06%) and less of 12.45 per cent on 15th day of storage.

Among the interactions between manure supplied and storage condition, the maximum moisture content was observed in slices prepared from fruits of plants supplied with neem cake and stored at ambient condition (13.21%) and minimum of 12.60% slices prepared from vermicompost 100 per cent RDN stored at 4˚C and sheep manure 100 per cent RDN stored at 4˚C. The similar change in moisture content during storage was in accordance with findings of Jesulin Aronika and Manimehalai (2014) in papaya, and Yadav and Singh 2014.

Spoilage (cfu)

The results on spoilage in terms of microbial count in dehydrated papaya slices were given in Table 5. The spoilage was not observed in osmo dehydrated slices stored at refrigerator condition in all the treatments of storage. The slices of different treatments stored at ambient conditions recorded microbial count ranged from 1×10^{2} to 1×10^{4} cfu. The highest of 2×10^{4} cfu was observed

in osmo dehydrated slices prepared from fruits of plants taken from control stored at ambient condition.

The osmotic dehydration of papaya slices decreased the spoilage by microorganisms by removing water from the product with increased osmotic pressure where the microorganisms cannot grow and multiply. Jorge and Favetto (1992) also reported lower microbial load after drying due to reduction in water activity. The similar results were also recorded in Kesar mango by Sakhale and Pawar (2011), Rahman *et al*. (2012) in jackfruit Vega *et al*. (2021) in papaya.

CONCLUSION

Osmotic dehydration is one of the most qualities improving food preservation technique. Papaya osmo dehydrated slices had maintained good quality in terms of both physical and chemical parameters during the storage period. There was no spoilage of osmo dehydrated papaya slices even at 90 days of storage and hence papaya fruits can be processed to osmodehydrated slices

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect on physiological loss in weight (%) of osmo-dehydrated papaya slices during storage period

Manures (M)	Days after storage (D)						Mean
	15	30	45	60	75	90	
T_1- FYM	1.15	3.32	4.03	5.15	5.94	6.52	**4.35**
T_2 - Vermicompost	1.12	3.06	4.24	5.01	5.72	6.48	**4.27**
T_3 - Neem cake	1.43	4.19	4.99	5.58	6.19	6.83	**4.87**
T_4 - Sheep manure	1.15	3.11	4.66	5.61	6.07	6.72	**4.55**
T_5 - FYM 50% + Vermicompost 50%	1.19	3.37	3.85	5.51	6.07	6.73	**4.45**
T_6 - FYM 50% + Neem cake 50%	1.97	3.51	5.49	5.93	6.36	6.90	**5.03**
T_7 - FYM 50% + Sheep manure 50%	1.68	3.57	4.59	5.33	6.18	6.76	**4.68**
T_8 -Control 100% (RDF)	2.22	4.32	5.25	6.01	6.65	7.19	**5.27**
Storage condition (S)							
S_1- 4^0C refrigerator condition	1.05	3.22	4.26	5.18	5.82	6.52	**4.34**
S_2- Ambient condition	1.93	3.89	5.02	5.85	6.46	7.01	**5.03**
Mean	**1.49**	**3.55**	**4.64**	**5.51**	**6.15**	**6.77**	

Interaction effects (M x S):

Factor	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	Mean
S_1	4.07	3.80	4.60	4.27	4.14	4.72	4.32	4.81	**4.34**
S_2	4.64	4.74	5.14	4.83	4.76	5.34	5.05	5.72	**5.03**
Mean	**4.35**	**4.27**	**4.87**	**4.55**	**4.45**	**5.03**	**4.68**	**5.27**	

Interaction effects (M x S x D):

Treatments	Days after storage (D)											
	15		30		45		60		75		90	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
T_1	1.04	1.26	3.15	3.49	3.48	4.58	4.60	5.70	5.84	6.05	6.27	6.78
T_2	0.87	1.38	2.45	3.67	3.75	4.72	4.30	5.72	5.30	6.15	6.16	6.81
T_3	1.07	1.80	3.70	4.67	4.71	5.28	5.31	5.86	6.11	6.28	6.72	6.94
T_4	0.99	1.31	2.88	3.35	4.08	5.24	5.51	5.71	5.81	6.33	6.37	7.07
T_5	0.83	1.55	2.93	3.81	3.35	4.35	5.24	5.77	5.89	6.24	6.63	6.83
T_6	1.17	2.77	3.67	3.35	5.19	5.80	5.77	6.10	5.89	6.83	6.61	7.20
T_7	1.09	2.28	3.15	3.99	4.37	4.81	5.20	5.47	5.55	6.89	6.57	6.94
T_8	1.37	3.07	3.82	4.81	5.11	5.39	5.54	6.47	6.22	7.08	6.84	7.54

Factor	T	S	D	T*S	T*D	S*D	T*S*D
S.Em±	0.03	0.01	0.02	0.04	0.06	0.03	0.09
C.D (0.05)	0.07	0.04	0.06	0.11	0.18	0.09	0.26

Table 2: Effect on total soluble solids (^{o}Brix) of osmo-dehydrated papaya slices during storage period

Manures (M)	Days after storage (D)						Mean
	15	30	45	60	75	90	
T_1- FYM	13.50	13.89	14.12	14.52	14.88	15.32	**14.37**
T_2 - Vermicompost	13.01	13.23	13.42	13.61	13.86	14.09	**13.53**
T_3 - Neem cake	12.85	13.05	13.21	13.45	13.72	13.99	**13.38**
T_4 - Sheep manure	13.94	14.14	14.53	14.64	14.85	15.02	**14.52**
T_5 - FYM 50% + Vermicompost 50%	13.33	13.56	13.79	13.95	14.20	14.41	**13.87**
T_6 - FYM 50% + Neem cake 50%	12.29	12.45	12.68	12.88	13.13	13.34	**12.79**
T_7 - FYM 50% + Sheep manure 50%	12.76	12.77	12.98	13.24	13.46	13.70	**13.15**
M_8 -100% RDF	12.02	12.28	12.54	12.88	13.12	13.31	**12.69**
Storage condition (S)							
S_1- 4^0C	12.91	13.03	13.23	13.41	13.66	13.89	**13.35**
S_2- Ambient condition	13.01	13.31	13.59	13.88	14.14	14.40	**13.72**
Mean	**12.96**	**13.17**	**13.41**	**13.65**	**13.90**	**14.51**	

Interaction effects (M x S):

Factor	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	Mean
S_1	14.13	13.36	13.20	14.38	13.69	12.59	12.96	12.54	**13.35**
S_2	14.61	13.71	13.55	14.66	14.06	13.00	13.35	12.85	**13.72**
Mean	**14.37**	**13.53**	**13.38**	**14.52**	**13.87**	**12.79**	**13.15**	**12.69**	

Interaction effects (M x S x D):

Treatments	Days after storage (D)											
	15 Day		30 Day		45 Day		60 Day		75 Day		90 Day	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
T_1	13.38	13.62	13.72	14.05	13.96	14.30	14.10	14.94	14.58	15.17	15.05	15.59
T_2	12.90	13.07	13.04	13.42	13.18	13.66	13.41	13.81	13.63	14.08	13.94	14.24
T_3	12.80	12.90	12.91	13.19	13.08	13.34	13.24	13.67	13.49	13.96	13.71	14.27
T_4	13.92	13.97	14.06	14.22	14.40	14.67	14.52	14.76	14.62	15.08	14.76	15.29
T_5	13.24	13.42	13.42	13.69	13.57	14.00	13.72	14.18	13.99	14.42	14.20	14.63
T_6	12.20	12.39	12.34	12.57	12.43	12.93	12.62	13.14	12.89	13.37	13.05	13.63
T_7	12.87	12.66	12.60	12.93	12.83	13.13	12.97	13.52	13.14	13.78	13.33	14.07
T_8	11.94	12.10	12.15	12.42	12.38	12.71	12.74	13.03	12.94	13.31	13.08	13.54

Factor	T	S	D	T*S	T*D	S*D	T*S*D
S.Em±	0.03	0.01	0.03	0.04	0.07	0.04	0.11
C.D (0.05)	0.09	0.04	0.07	0.11	0.22	N.S	N.S

Table 3: Effect on titrable acidity (%) of osmo-dehydrated papaya slices during storage

Manures (M)	Days after storage (D)						Mean
	15	30	45	60	75	90	
T_1- FYM	0.148	0.142	0.137	0.133	0.127	0.123	**0.135**
T_2 - Vermicompost	0.182	0.175	0.171	0.164	0.159	0.153	**0.167**
T_3 - Neem cake	0.128	0.126	0.116	0.113	0.108	0.104	**0.115**
T_4 - Sheep manure	0.169	0.164	0.157	0.152	0.148	0.144	**0.156**
T_5 . FYM 50% + Vermicompost 50%	0.183	0.176	0.172	0.167	0.161	0.154	**0.169**
T_6 - FYM 50% + Neem cake 50%	0.150	0.141	0.134	0.129	0.124	0.118	**0.133**
T_7 - FYM 50% + Sheep manure 50%	0.171	0.165	0.158	0.151	0.145	0.139	**0.155**
T_8 -Control 100% (RDF)	0.135	0.123	0.122	0.116	0.112	0.106	**0.119**
Storage condition							
S_1- 4^0C	0.159	0.153	0.149	0.144	0.138	0.132	**0.146**
S_2- Ambient condition	0.157	0.150	0.143	0.137	0.133	0.127	**0.141**
Mean	**0.158**	**0.152**	**0.146**	**0.140**	**0.135**	**0.130**	

Interaction effects (M x S):

Factor	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	Mean
S_1	0.137	0.169	0.117	0.158	0.171	0.136	0.157	0.122	**0.146**
S_2	0.133	0.166	0.114	0.153	0.167	0.129	0.152	0.117	**0.141**
Mean	**0.135**	**0.167**	**0.115**	**0.156**	**0.169**	**0.133**	**0.155**	**0.119**	

Interaction effects (M x S x D):

Treatments	Days after storage (D)											
	15 Day		30 Day		45 Day		60 Day		75 Day		90 Day	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
T_1	0.151	0.145	0.143	0.141	0.140	0.135	0.135	0.131	0.130	0.125	0.126	0.120
T_2	0.183	0.181	0.177	0.174	0.172	0.170	0.167	0.161	0.163	0.156	0.156	0.151
T_3	0.129	0.127	0.130	0.126	0.119	0.114	0.116	0.111	0.109	0.106	0.105	0.103
T_4	0.171	0.167	0.166	0.164	0.160	0.154	0.155	0.148	0.152	0.145	0.146	0.142
T_5	0.185	0.181	0.179	0.176	0.175	0.170	0.169	0.165	0.163	0.160	0.155	0.152
T_6	0.151	0.150	0.145	0.141	0.140	0.129	0.134	0.124	0.127	0.121	0.122	0.114
T_7	0.172	0.170	0.166	0.165	0.161	0.156	0.155	0.147	0.149	0.141	0.143	0.135
T_8	0.135	0.135	0.124	0.123	0.125	0.119	0.120	0.113	0.114	0.110	0.108	0.104

Factor	T	S	D	T*S	T*D	S*D	T*S*D
S.Em±	0.001	0.000	0.001	0.001	0.002	0.001	0.003
C.D (0.05)	0.003	0.001	0.002	N.S	N.S	N.S	N.S

Table 4: Effect on moisture content (%) of osmo-dehydrated papaya slices during storage

Manures (M)	Days after storage (D)						Mean
	15	30	45	60	75	90	
T_1- FYM	12.33	12.48	12.63	12.72	12.82	12.99	**12.66**
T_2 - Vermicompost	12.51	12.65	12.77	13.00	13.18	13.26	**12.90**
T_3 - Neem cake	12.57	12.79	12.97	13.20	13.45	13.72	**13.12**
T_4 - Sheep manure	12.29	12.50	12.68	12.80	12.90	12.75	**12.65**
T_5 . FYM 50% + Vermicompost 50%	12.46	12.62	12.74	12.88	12.98	13.06	**12.79**
T_6 - FYM 50% + Neem cake 50%	12.59	12.76	12.87	12.96	13.09	13.23	**12.92**
T_7 - FYM 50% + Sheep manure 50%	12.38	12.55	12.65	12.81	12.99	13.24	**12.77**
T_8 -Control 100% (Recommended dose of fertilizer)	12.46	12.63	12.79	12.91	13.06	13.17	**12.84**
Storage condition (S)							
S_1- 4^0C	12.39	12.53	12.66	12.77	12.92	13.05	**12.72**
S_2- Ambient condition	12.51	12.71	12.87	13.05	13.20	13.31	**12.94**
Mean	**12.45**	**12.62**	**12.76**	**12.91**	**13.06**	**13.18**	

Interaction effects (M x S):

Factor	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	Mean
S_1	12.49	12.60	13.02	12.60	12.70	12.87	12.70	12.79	**12.72**
S_2	12.83	13.19	13.21	12.70	12.89	12.96	12.83	12.89	**12.94**
Mean	**12.66**	**12.90**	**13.12**	**12.65**	**12.79**	**12.92**	**12.77**	**12.84**	

Interaction effects (M x S x D):

Treatments	Days after storage (D)											
	15 Day		30 Day		45 Day		60 Day		75 Day		90 Day	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
T_1	12.26	12.40	12.34	12.62	12.44	12.82	12.50	12.94	12.60	13.00	12.82	13.17
T_2	12.32	12.71	12.44	12.86	12.54	13.00	12.68	13.32	12.79	13.57	12.84	13.66
T_3	12.53	12.61	12.72	12.87	12.89	13.00	13.06	13.33	13.34	13.56	13.57	13.88
T_4	12.25	12.33	12.41	12.59	12.61	12.76	12.69	12.92	12.81	12.99	12.87	12.63
T_5	12.43	12.49	12.53	12.72	12.63	12.88	12.79	12.97	12.87	13.10	12.92	13.19
T_6	12.55	12.64	12.69	12.82	12.83	12.90	12.93	13.00	13.06	13.12	13.16	13.31
T_7	12.37	12.40	12.54	12.56	12.60	12.70	12.69	12.94	12.91	13.07	13.14	13.35
T_8	12.44	12.48	12.60	12.66	12.77	12.82	12.86	12.97	12.98	13.13	13.08	13.26
	12.39	12.51	12.53	12.71	12.66	12.87	12.77	13.05	12.92	13.20	13.05	13.31

Factor	T	S	D	T*S	T*D	S*D	T*S*D
S.Em±	0.03	0.01	0.02	0.04	0.07	0.04	0.11
C.D (0.05)	0.08	0.04	0.07	0.12	N.S	N.S	N.S

Table 5: Spoilage (cfu) of osmo-dehydrated papaya slices during storage

Treatments	Days after storage (days)						
	1(Initial)	15	30	45	60	75	90
$T_1(T_1S_1)$	0	0	0	0	0	0	0
$T_2(T_1S_2)$	0	0	0	0	0	0	$1x10^2$
$T_3(T_2S_1)$	0	0	0	0	0	0	0
$T_4(T_2S_2)$	0	0	0	0	0	0	$1x10^4$
$T_5(T_3S_1)$	0	0	0	0	0	0	0
$T_6(T_3S_2)$	0	0	0	0	0	0	$1x10^2$
$T_7(T_4S_1)$	0	0	0	0	0	0	0
$T_8(T_4S_2)$	0	0	0	0	0	0	$1x10^4$
$T_9(T_5S_1)$	0	0	0	0	0	0	0
$T_{10}(T_5S_2)$	0	0	0	0	0	0	$1x10^4$
$T_{11}(T_6S_1)$	0	0	0	0	0	0	0
$T_{12}(T_6S_2)$	0	0	0	0	0	0	$1x10^4$
$T_{13}(T_7S_1)$	0	0	0	0	0	0	0
$T_{14}(T_7S_2)$	0	0	0	0	0	0	$1x10^4$
$T_{15}(T_8S_1)$	0	0	0	0	0	0	0
$T_{16}(T_8S_2)$	0	0	0	0	0	0	$2x10^4$

Responses of dragon fruit (*Selenicereus undatus*) to NaCl-Induced salinity stress

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Receipt: 30.10.2024 *Revised: 02.12.24* *Acceptance: 04.12.24*

DOI: 10.53552/ijmfmap.10.2.2024.103-112

ABSTRACT

Dragon fruit, an obligate CAM species, demonstrates high water efficiency and drought resistance. A pot experiment assessed its response to saline conditions using water with 0, 25, 50, 75, and 100 mM salt concentrations. Under salinity stress, dragon fruit showed changes in shoot and root growth. New shoot production was stable up to 75 mM salinity, with a slight reduction at 100 mM. Compared to the control, shoot production decreased by 13.33% at 75 mM and 33.33% at 100 mM. Increasing salt stress reduced total plant fresh weight, with the highest and lowest values at 0 mM and 100 mM, respectively. A similar trend was observed for plant dry weight, peaking at 0 mM and lowest at 75 mM. Salinity stress significantly decreased chlorophyll content and NDVI in dragon fruit. Plant mortality varied with salinity, reaching 40% at 100 mM and 20% at 50 mM. Salt stress also delayed cutting sprouting by 4-10 days. Higher salinity levels reduced shoot and root biomass, though new shoot formation persisted up to 75 mM, and shoot girth remained unaffected. Notably, root elongation occurred under saline conditions. While salt stress negatively impacted some growth aspects, other indicators showed positive responses. Therefore, investigating genetic variability within dragon fruit populations to identify salt-resistant genotypes is essential.

Keywords: Chlorophyll, mortality, root biomass, salinity, stress tolerance,

INTRODUCTION

Salinity, a significant abiotic stress factor, profoundly affects agriculture and human development globally. Rising salinity threatens arable land, agricultural productivity, plant growth, biodiversity, freshwater wetlands, land and water resources, and ecosystem functions, potentially leading to desertification (Herbert *et al*., 2015; Canedo-Arguelles *et al*., 2013). Climate change, characterized by global warming and altered precipitation patterns, exacerbates salinization, expanding saline soil areas due to population growth and development. Increased temperatures enhance evaporation, raising soil salt concentrations, while changes in rainfall patterns may prevent adequate salt leaching or cause waterlogging, further contributing to soil salinization (Bannari and Al-Ali, 2020; Hassani *et al*., 2020). Population growth and continuous development exacerbate the problem. Intensive farming methods, such as irrigation without proper drainage, lead to waterlogging and increased saline groundwater reaching the soil surface. Irrigating with saline water worsens this issue. Urban development disrupts natural water flow and drainage, causing soil salt

build-up. Deforestation and land use changes reduce natural salt leaching, heightening salinization risks. Salinization is categorized as natural or anthropogenic. In arid and semi-arid regions, natural or primary salinization occurs due to low precipitation and high evaporation rates. Anthropogenic or secondary salinization results from human activities like excessive irrigation, poor drainage, and using low-quality water for irrigation (Bannari and Al-Ali, 2020; Ahamad *et al.,* 2023). Consequently, soil salinity, saline groundwater, and water scarcity due to limited resources or declining quality from salinization pose serious challenges in these areas. These issues significantly affect water quantity and quality globally and in India, impacting plant growth and development (Pandey *et al*., 2014; Sarkar *et al.*, 2024).

Salt stress negatively impacts plant growth and development through multiple mechanisms, including osmotic stress reducing water availability, ionic stress from ion toxicity, nutritional imbalances, and oxidative stress from reactive oxygen species (ROS) (Carillo *et al*., 2011; Zhou *et al*., 2024). Specifically, salinity stress causes water deficits and harmful Na^+ and Cl^- ion accumulation in cells, leading to physiological and biochemical changes such as reduced chlorophyll, leaf water content, photosynthesis, respiration rates, and carbohydrates, while sometimes increasing proline and polyamines. These changes collectively impair plant growth and development (Shafieizargar *et al*., 2015; Alam *et al*., 2020). High salt concentrations near the roots can severely hinder plant growth and development.

Dragon fruit, an obligate CAM plant, demonstrates exceptional water use efficiency and drought resistance (Wang *et al*., 2019). Some studies highlight its ability to thrive in high-salinity environments, while others note sensitivity to salt stress. Dragon fruit shows potential for growth in saline environments, but its sensitivity to salinity in certain scenarios necessitates further research and careful cultivation practices. The inconsistent results regarding its performance under salt stress highlight the need for comprehensive studies to clarify these findings. Despite its drought resistance reputation, limited research has explored its ability to thrive in high-salinity conditions, resulting in a lack of data on saline water irrigation or cultivation in salt-affected soils within arid and semi-arid regions. The exact salinity tolerance thresholds for dragon fruit remain unclear or poorly documented, with divergent claims from previous studies emphasizing the need for further investigation, particularly in regions facing salinity challenges. Earlier studies classifying it as salt-sensitive were based on seedling observations, which are generally considered less robust than commercial cuttings (Kakade *et al*., 2019; Kakade *et al*., 2024), underscoring the importance of conducting additional research on dragon fruit plants propagated through stem cuttings. Although some genotypes have shown favourable responses to salinity (Barcenas-Abogado *et al*., 2002; Ortiz *et al*., 2014), comprehensive examinations of responses under salt stress are crucial for more successful cultivation. This study was conducted to evaluate the morphological alterations and assess the physiochemical changes in dragon fruit cuttings exposed to various salinity levels.

MATERIALS AND METHODOLOGY

A pot experiment was conducted from January 2023 to May 2024 at ICAR-National Institute of Abiotic Stress Management, Baramati, India. During the experiment, monthly mean temperatures ranged from 21.8 to 30.2 °C, with the maximum temperature peaking at 38.6 °C in April and the minimum temperature ranging from 13.8 (January) to 22.3 °C (May). Morning relative humidity varied between 59 (April) and 85% (January). Local white dragon fruit cuttings, representing *S. undatus* are subjected to NaCl-induced salinity stress. Mature, disease-free cuttings (25 ± 4 cm long, 15 ± 2 cm girth) were taken from the mother block

orchard, kept in shade for ten days, and treated with 0.25% copper oxychloride. Cuttings were potted in a mixture of black soil and well-decomposed farmyard manure, with each pot receiving fertilizers biweekly. The potted cuttings were irrigated with water containing different salt concentrations (0, 25, 50, 75, and 100mM) prepared using laboratory-grade NaCl. From January to March, cuttings were irrigated weekly with 500 ml of water per pot, which increased to 750 ml per pot per week in April and May. New sprouts, the girth of new cladodes (cm), total shoot length (cm), total plant biomass (g), primary root length (cm), shoot-to-root ratio, plant mortality (%), and days to sprout cuttings were measured. Chlorophyll content (µg ml^{-1}) was extracted using N, N-dimethylformamide (Inskeep and Bloom, 1985) and absorbance was measured at 647 and 664 nm. Normalized difference vegetation index (NDVI) was determined using a green seeker. Cladode moisture content (%) by oven drying the cladode samples. The membrane stability index (MSI) was measured using the protocol developed by Sairam (1994). Water use efficiency (WUE) was also assessed by dividing biomass produced per litre of water applied. Soil EC and pH were measured by mixing 10 g of soil with 25 ml of distilled water, stirring, and using a pH meter and EC meter. Soil moisture was determined gravimetrically. The study employed a completely randomized block design with five salinity levels, replicated four times with four cuttings per replication. Morphological, physiological-biochemical, and soil chemical properties, data were collected. Two-way ANOVA and least significant difference tests were conducted using 'R' studio (Versions 4.1.1 and 1.4.1417; $P < 0.05$).

RESULTS AND DISCUSSION

Dragon fruit exhibited notable alterations in shoot and root development when exposed to salinity stress. New shoot production remained stable up to 75 mM salinity, with a slight decrease at 100 mM. Compared to the control, shoot production declined by 13.33% at 75 mM and 33.33% at 100 mM (Fig. 1A). Salinity stress also impacted shoot length, with the longest shoots observed at lower salinity levels. At 75 mM and 100 mM, shoot length decreased by 28.74 % and 17.96 %, respectively, relative to the control (Fig. 1B). However, shoot girth was not significantly affected, ranging from 10.43-11.47 cm across treatments (Fig. 1A). As salinity stress intensified, total plant fresh weight decreased, with the highest and lowest values recorded at 0 mM and 100 mM, respectively. A similar pattern was observed for total plant dry weight, with the maximum at 0 mM and minimum at 75 mM (Fig. 1B). The shoot-to-root ratio was highest at 0 mM salinity, comparable at 25 and 100 mM, and reduced at 50 mM and 75 mM. Plant mortality varied under different salinity conditions, with 40% mortality at 100 mM and 20% at 50 mM (Fig. 1A). Salinity stress also delayed sprouting in cuttings by 4-10 days (Fig. 1A). Salinity stress adversely affects crop growth and development (Anjum, 2008; Kakade *et al*., 2014). Research on the salinity response of dragon fruit is sparse, with some studies labeling it as salt-sensitive and others identifying it as salt-tolerant. This inconsistency necessitates further investigation considering drought tolerance, clonal propagation robustness, and genotypic diversity (Wang *et al*., 2019; Tomaz de Oliveira *et al*., 2020), potentially revealing genotypes with salinity tolerance. The findings of this study are vital for promoting dragon fruit cultivation in saline environments. In this study, salinity stress reduced the shoot and root biomass of dragon fruit but promoted root elongation. Previous studies by Cavalcante *et al*. (2007) and De Sousa *et al*. (2021) reported adverse effects of salinity on the growth metrics and biomass of dragon fruit seedlings. Pandey *et al*. (2014) have observed that salinity has resulted in to decrease in plant growth, leaf production and leaf area in mango grown under salinity. Furthermore, they also observed a decrease in the fresh and dry

weights of plants with increasing salinity. Irrigation with 75, 100, and 150 mM salt resulted in shorter plants, decreased stem width, lower plant dry weight, fewer flowers, smaller leaf area, and reduced fruit yield in cherry tomatoes (El-Mogy *et al*., 2018). These observations are consistent with our findings. Salinity stress alters water relations, causing osmotic stress and water deficits, and affecting cell turgor and water use efficiency (Munns, 2002). Water stress affects growth during short-term exposure and induces stomatal closure, reducing CO_2 availability and photosynthetic efficiency (Chauhan *et al*., 2023). Combined osmotic and ionic stress leads to excessive reactive oxygen species (ROS) production, causing oxidative damage to macromolecules and redox imbalance (Kesawat *et al*., 2023). Therefore, the combination of these stresses affects physiological and biochemical processes, such as photosynthesis and protein synthesis, ultimately impairing plant growth and development (Dexana *et al*., 2022). In the present study, salinity stress affected growth parameters, but shoot length and shoot girth remained unaffected, indicating some positive responses of dragon fruit to salt stress. Dragon fruit is known for its drought tolerance and ability to grow in degraded lands, and has mechanisms to combat various stresses (Jinger *et al*., 2024). Identifying specific genotypes and responses is crucial for assessing the performance of dragon fruit under salinity stress. Interestingly, root length increased under salinity stress compared to the control.

The greatest root length was observed at 75 mM salinity, which was comparable to other treatments, while the control showed the shortest roots. Root length increased by 18.19 % to 30.06 % across treatments compared to the control (Fig. 2A). The highest below-ground fresh biomass was recorded at 50mM salinity, similar to other treatments except for 100mM (Fig. 2A). No significant differences were found in below-ground dry biomass. Roots, the primary organs encountering salt stress, undergo changes similar to shoots, but play a more crucial role in adaptation. The effects of salt stress on roots include decreased quantity, length, and biomass, as reported in previous studies (Cavalcante *et al*., 2007; Alam *et al*., 2020; De Sousa *et al*., 2021). Our study noted reduced root biomass under salt stress, although root elongation increased with increasing stress levels. Enhanced root biomass and elongation may improve water uptake and performance under stress conditions (Zou *et al*., 2022). Thus, dragon fruit plants attempt to grow deeper roots to mitigate salt stress. However, another hypothesis suggests that increased root mass might lead to greater absorption of harmful ions, intensifying salt stress (Fernández-Ballester *et al*., 2003). Glycophytes show reduced growth and yield, and halophytes thrive and reproduce under saline conditions. In the present study, although plants showed potential salt tolerance, plant mortality questioned their tolerance capacity. Plant survival is an important indicator of salt tolerance (Munns, 2002). Goodman *et al*. (2012) found soil salinity 1.5 times higher where tree cactus plants were dead compared to where they were alive. Consequently, white-fleshed dragon fruit plants failed to sustain growth as stress severity increased.

The chlorophyll content in dragon fruit significantly decreased with salinity stress. The highest chlorophyll content (4.68 to 5.66 µg/ml) was observed at lower salinity concentrations, followed by the control. It decreased to 3.64 µg/ml at 75 mM and 3.49 µg/ml at 100 mM salinity stress (Fig. 2B). Similar trends were noted for chlorophyll b and total chlorophyll content. The highest NDVI (0.55) was reported in the control, decreasing under various salinity stresses, with the lowest NDVI (0.32) observed at 100 mM (Fig. 2B). Water use efficiency (WUE) was found highest in the control treatment, showing minor reductions under various salinity stress conditions. Salinity stress led to a decrease in WUE by 22.00 to 37.03% across different treatments (Fig. 2B). Under salinity stress, cladode moisture content

remained relatively stable, ranging from 87.48 to 89.84 %, with a slight decrease to 84.4 0% at 100mM salinity stress (Fig. 3A). Membrane stability index (MSI) peaked at lower salinity stress levels and in the control, while reaching its lowest point in the 75 and 100 mM salinity stress treatments (Fig. 3A). Previous studies have reported that salinity decreases plant growth, net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency and chlorophyll concentration in avocado (Musyimi *et al*., 2007). Al-Gaadi *et al*. (2024) also reported reduced stomatal conductance, photosynthesis, leaf chlorophyll content under salinity stress. They observed variations of 5%, 9%, and 5% in photosynthesis, stomatal conductance, and chlorophyll content of leaves, respectively, between medium- and high-salinity trials. This decrease might be attributed to the inhibition of enzymes, impairment of pigment protein complexes, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} deficiency, and chlorophyll pigment destruction under salt stress (Gao *et al*., 2024). High salt concentrations in soil disrupt the water extraction capacity of roots from soil because of reduced soil water potential, which causes difficulties in the uptake of water by plants, eventually leading to physiological drought conditions (Lu and Fricke, 2023), resulting in reduced cladode moisture. However, this reduction was marginal, which may be because dragon fruit is a cactus, have succulent and waxy stems, and keeps stomata closed during day time, which helps in storing water for longer periods under both normal and stressful periods (Nobel and De la Barrera, 2002). Degradation of chlorophyll content and deficit water-induced stomatal closure might have resulted in the overall reduction in photosynthesis and thus reduced NDVI under salinity stress in the present experiment.

Salinity stress significantly affected soil pH, with higher salinity levels corresponding to increased soil pH. The control group exhibited the lowest soil pH at 7.49, while the 100mM salinity stress treatment resulted in the highest pH of 8.05 (Fig. 3B). Soil electrical conductivity (EC) followed a similar pattern, with the control group showing the lowest EC at 1.50 dS/m and the 100 mM salinity stress treatment producing the highest EC at 9.49 dS/m (Fig. 3B). Irrigation with higher salt concentrations enhanced the soil pH and EC compared to the control because the presence of NaCl increases the soil pH and dissociation of NaCl into Na^+ or Cl^- when dissolved in water, enhancing the ability to conduct electricity, which enhances the EC of the soil and makes the soil saline. Thus, increased soil pH and EC could be associated with Na^+ and Cl^- ion exclusion mechanisms and the retention of these ions in the soil. Salinization due to greater accumulation of either Na^+, Cl^-, or both, in the root zone or plant cells leads to deficiencies or toxicities of nutrients and disturbances in ion homeostasis, ultimately affecting plant growth and development (Munns, 2002; Munns and Tester, 2008).

CONCLUSION

The current research revealed significant changes in dragon fruit's shoot and root development when subjected to salt stress. As salinity levels increased, the plants demonstrated decreased shoot and root biomass production. Nevertheless, new shoot formation persisted up to 75 mM salt concentration, and shoot girth remained unaffected by salt stress. Interestingly, root elongation was observed under saline conditions. While salt stress negatively impacted certain growth and developmental traits of dragon fruit plants, some growth parameters showed positive responses. Consequently, there is a need to explore the genetic diversity within dragon fruit populations to identify salt-tolerant genotypes or varieties. This approach could potentially enable the expansion of dragon fruit cultivation into saline environments.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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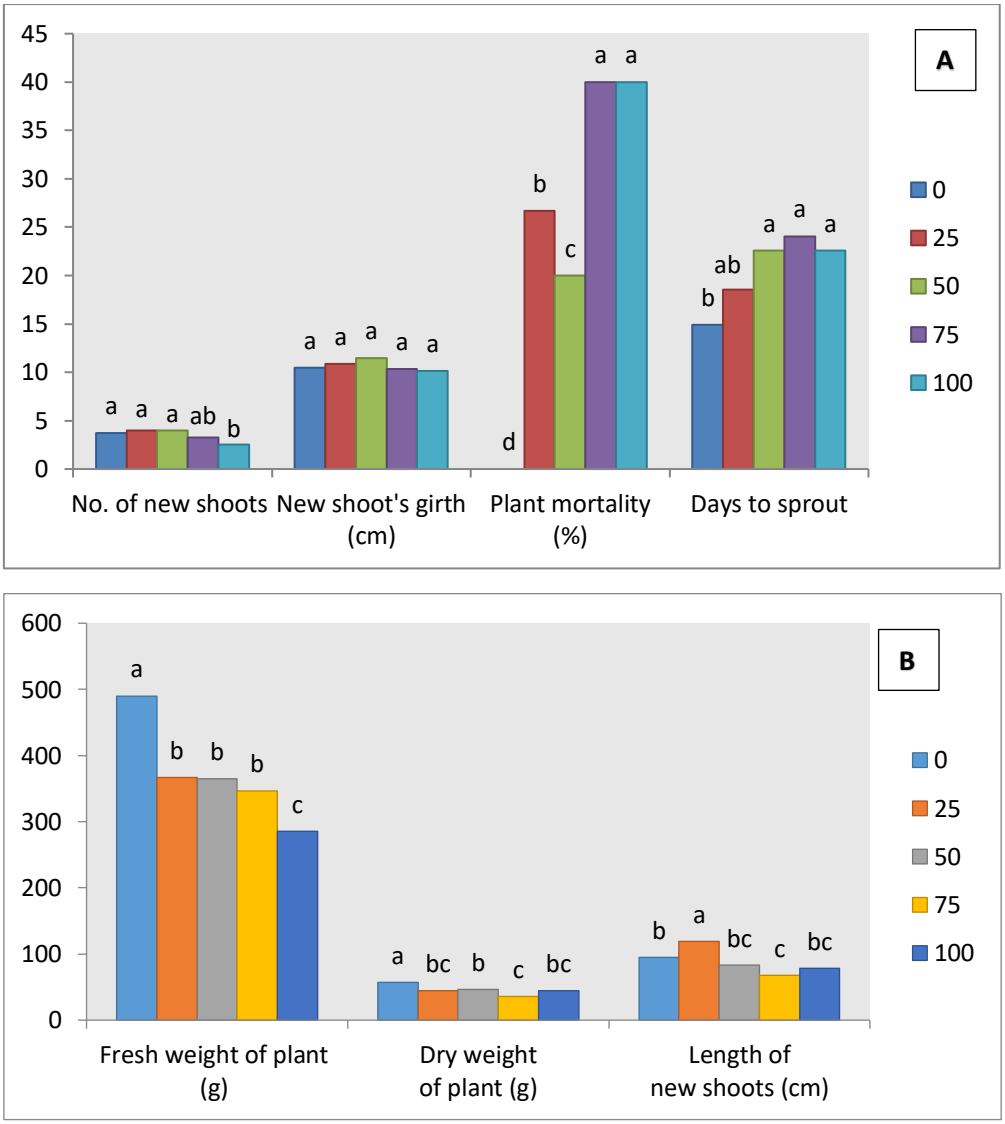
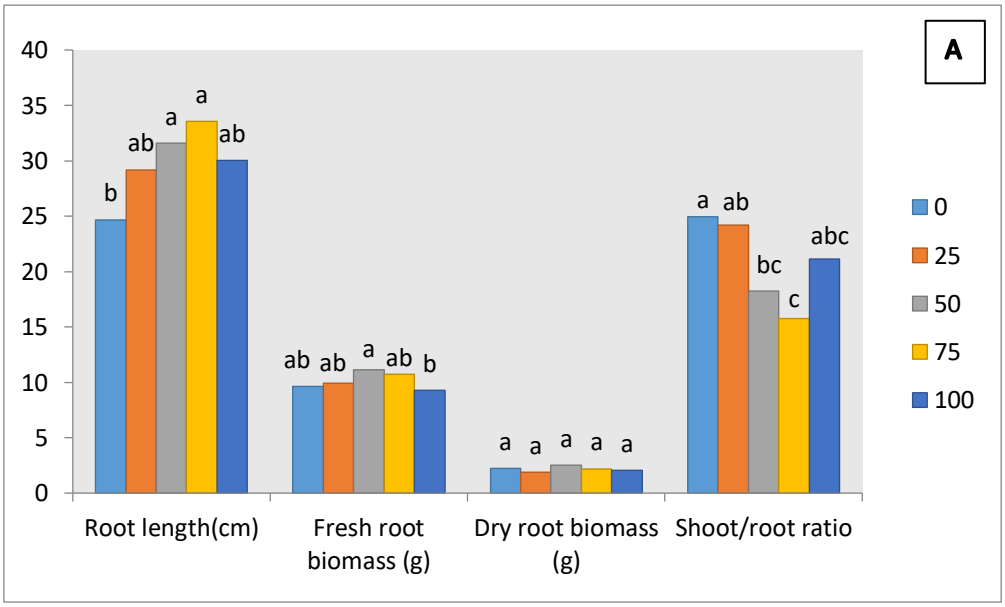


Fig. 1: Effect of different saline water levels on different plant growth traits (A & B) of dragon fruit



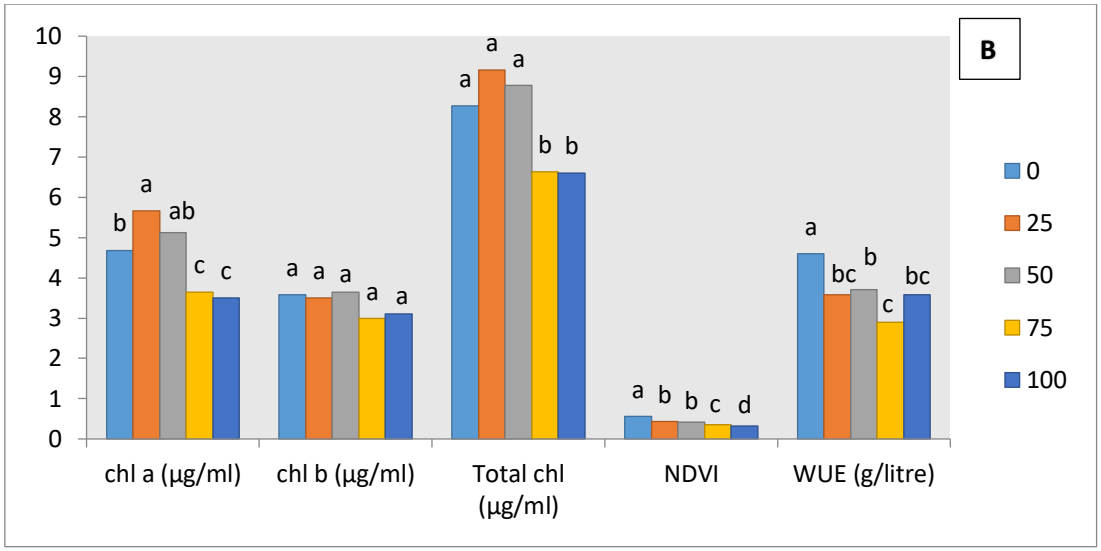


Fig. 2: Effect of different saline water levels on root growth (A) and physiological traits (B) of dragon fruit

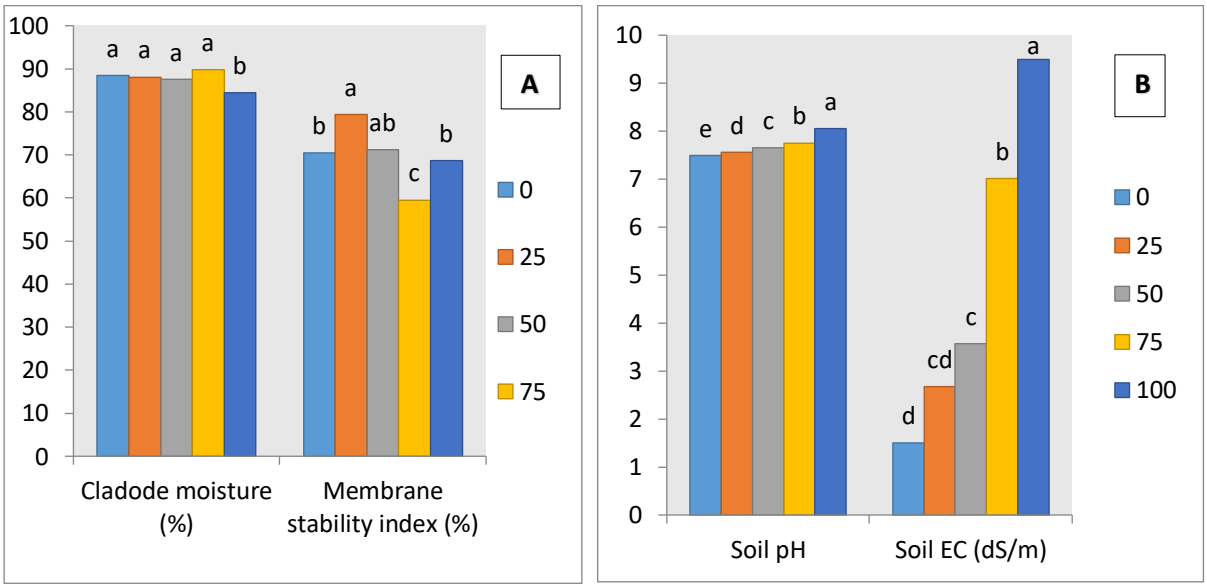


Fig. 3: Effect of different saline water levels on cladode moisture and membrane stability index (A), and soil pH and EC (B) of dragon fruit

Variation in fruit morpho-biochemical characters and bioactive compounds of bilimbi (*Averrhoa bilimbi* L.) genotypes under semi-arid lateritic belt of West Bengal

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Receipt: 01.11.2024 *Revised: 08.12.24* *Acceptance: 10.12.24*
DOI: 10.53552/ijmfmap.10.2.2024.113-122

ABSTRACT

Bilimbi is one of the underutilized fruits and abundantly found under semi-arid lateritic belt of West Bengal. As there is no such report of scientific study on this fruit from this region, the study has been conductedduring the year 2022 and 2023 for morpho-biochemical characterization, determination of bioactive compounds and genetic diversity analysis of different bilimbi genotypes selected from natural vegetation. A wide array of distinctness with respect to fruit length (3.56 to 5.78 cm), fruit diameter (1.51 to 2.43 cm), fruit weight (9.52 to 14.11g), number of seeds (6.55 to 17.23), TSS (3.5 to 4.6ºBrix), acidity (1.26 to 1.41%) and ascorbic acid content (33.5 to 57.2 mg/100g) has been found. Additionally, the fruits are found to be rich in antioxidants (70.5 to 87.0% of DPPH inhibition), total phenols (38.4 to 30.2µgGAE/g) and flavonoids (29.7 to 41.2mgQE/g). Statistical analysis of observations gave out significantly positive correlation between number of seeds, fruit length, fruit diameter, ascorbic acid, antioxidant activity. Ascorbic acid content of bilimbi fruits has exhibited very high positive correlation with antioxidant activity, fruit weight, fruit length, fruit diameter, and total sugar. Noteworthy negative correlation was also noted between TSS and number of fruits per cluster, acidity in addition tobetween fruit diameter and number of fruits per clusterand acidity. The population of bilimbi genotypes fall under three clusters comprising 5, 7 and 4 different genotypes while five different parameter clusters have been noted to create variation among the bilimbi genotypes. With respect to fruit size and quality parameters bilimbi genotype BG-16 and BG-10 was found best under the present study.

Key words: Bilimbi, bioactive compounds, fruit morphology, genetic diversity, quality parameters,

INTRODUCTION

The bilimbi (*Averrhoa bilimbi* L.) is also commonly known as *Chemmeenpuli* in Malayalam, *Irumbanpuli* in Tamil and *Bilimbi Tenga* in Assamese (Billah *et al*., 2015). Bilimbi is also commonly referred as cucumber tree. It is hardy, long-lived tropical plant closely related to *Averrhoa carambola* (commonly known as carambola or starfruit). Bilimbi belongs to a category of minor fruits, a diverse group of plants often found growing naturally in unmanaged locations like roadsides, homesteads, and wastelands while their cultivation is generally limited. It has originated in the Southeast Asia and is claimed as a native of the West Malaysia and the Indonesian Moluccas (Veldkamp, 2004), mostly found in tropics and subtropics. Bilimbi is a nutrient-dense, medicinally valuable fruit. In addition to carambola, bilimbi is another woody plant comes under the family Oxalidaceae and only fruit-

bearing species valued for their edible fruit rich in oxalic acid (Hazmi *et al*., 2024).

Bilimbi fruits are eaten raw, as well used for preparation of pickle, vinegar, wine and as a substitute for tamarind in dishes and flavouring agent (Dewi and Juwithninglyas, 2024). Mature and ripened fruits are commonly used for preservation in sugar or are cut, sun dried and preserved for future use (Ho *et al*., 2020). Fruit juice is very sour and is utilized in beverages preparation (Nilugin and Mahendran, 2016).The fruit is high in Vitamin C, and it also contains fibers, protein, anthocyanin, tannins, and minerals (Jayawardane *et al*., 2022; Dangat *et al*., 2024). Fruits are reported to be used to cure fever, rectum inflammation, diabetes, rheumatism, whooping cough, stomach ache, ulcer, and other conditions (Iwansyah *et al*., 2021; Aparna *et al*., 2022). Different plant parts of bilimbi like leaves, seeds, bark, fruits, flowers and roots as well as the entire plant as a whole are reported touse for the treatment of many diseases, mainly utilized as antidiabetic agents (Kumar *et al*., 2013).

The semi-arid lateritic belt of West Bengal comprises the districts Birbhum, Bankura, West Burdwan and parts of Purulia. The scorching heat with heat wave during summer followed by high humid rainy season and cold winter are the climatic features of this region. Most of the soils are red-lateritic in nature and resulted from incomplete weathering of rocks. Soils are deficient in carbon and other major nutrients. Natural vegetation and discrete forest area are also the reservoir of flora diversity including a considerable number of underutilized fruits. Distribution of bilimbi plants in natural greeneries, backyard gardens and household vegetations are common in the districts of Birbhum, West Burdwan and Bankura district but no scientific reports are there with respect to the genetic diversity of the fruit. Despite the lack of scientific research findings with respect to morpho-bichemical diversity, bilimbi is popular among local peoples for its unique sweet and sour flavour, rich nutritional profile and medicinal properties like as other minor fruits (Pradhan *et al*., 2015). Therefore, the present study has been aimed to determine the diversity analysis of available bilimbi genotypes under semi-arid lateritic belt of West Bengal with respect to morpho-biochemical characters and bioactive compounds.

MATERIALS AND METHODS

Present investigation has been performed selecting sixteen different bilimbi genotypes, all aged between 15 to 20 years, from various locations of Birbhum, West Burdwan and Bankura Districts of West Bengal which come under semi-arid lateritic zone, during the year 2022 and 2023. The GPS coordinates of each bilimbi plant were recorded using a handheld Garmin GPS 12H device (Table 1). The fully mature and ripe bunch of bilimbi was brought to Department of Horticulture and Post-harvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, West Bengal, India. Physical parameters of the fruit were recorded on-site, and samples of both the bunch and fruit were collected for further physical and biochemical analysis.The specifics of the experiment, including the materials used and the techniques employed, are detailed as follows.

Fruit physical characters: The fruit morphological characteristics of selected bilimbi plants were recordedas per the need of the study. Measurements included fruit length, diameter, weight, seed count, number fruits in a single bunch etc. Fruit length and diameterwere measured using a vernier caliper. Fruit weight, seed weights weremeasured using a digital balance. **Fruit biochemical Characters:** Total Soluble Solids (TSS) content was measured by digital refractometer (0-65°B, Konika Minolta, Japan) and expressed in °Brix. Acidity of bilimbi fruit juice was measured by titration method (Rangana, 1986). Total sugarwas measured following the method as described by AOAC (1990).Ascorbic acid was quantified using the indophenol dye

method (Rangana, 1986). Antioxidant activity has been determinedusing the DPPH assay (Brand-Williams *et al*., 1995) with the help of a double beam UV-Visible spectrophotometer (LABMAN, LUV2000T, India). Total phenolic content was measured by Folin Ciocalteu's method (Dewanto *et al*., 2002) using UV visible spectrophotometer (LABMAN, Model LMS PUV 1200)and expressed as mg of quercetin equivalent weight (mgQE/100 g).

Statistical analysis of data: The data on the observations were subjected to descriptive statistics and analysis of variance, following the method proposed by Ronald A. Fisher (Gomez and Gomez, 1984).The fruit morphological and biochemical data were investigatedwith thestatistical softwareSPSS (Statistical Package for Social Sciences, IBM SPSS Version 27)for correlation and cluster analysis.

RESULTS AND DISCUSSION

The present study focused on diversity of morphological, biochemical parameters of sixteen different bilimbi genotypes and their bioactive compounds. The mean variance analysis of morphological characters, biochemical characters and bioactive compounds of sixteen bilimbi (*Averrhoa bilimbi* L.) genotypes are discussed below:

Morphological characters: All the statistically analyzed observations on fruit morphological characters are cited in Table 2. **The number of fruits per cluster** of different bilimbi genotypes under the present study has been ranged from 4.21 to 8.71. The bilimbi genotype BG-3(8.71) produced the maximum average number of fruits per cluster, followed by BG-5 (8.44), BG-7 (7.17) and BG-11 (6.81) and it was lowest in BG-16 (4.21) and preceded by BG-13 (4.67), BG-14 (4.99). The meanvalue of number of fruits per cluster of bilibmi genotypes was 6.15. **The length of fruits** across sixteen bilimbi genotypes varied between 3.56 cm to 5.78 cm. The highest fruit length was observed in BG-16 (5.78 cm) followed by BG-10 (5.65 cm), BG-14 (5.37 cm) and BG-6 (5.22 cm). The lowest reading was noted in BG-5 with a fruit length of 3.56 cm preceded by BG-3 (3.99 cm) and BG-1 (4.06 cm).**The diameter of bilimbi fruits** in the present study ranged from 1.51 to 2.43 cm. The lowest fruit diameter was observed in BG-3 (1.51 cm) preceded by BG-9 (1.62 cm), BG-2 (1.63 cm) and BG-5 (1.63 cm). The maximum fruit diameter was found in the genotype BG-16 (2.43 cm) followed by BG - 6 (2.20 cm).**The fruit weight** of different bilimbi genotypes varied between 14.11 g in BG-16 to 9.52 g in BG-5. Higher fruit weight was also recorded in BG-10 (13.25 g), BG-14 (12.83 g) and BG-13 (12.57 g). Lowest fruit weight was also recorded in BG-3 (10.06 g) followed by BG-7 (10.53 g) and BG-1 (10.85 g).**The number of seeds per fruit** of bilimbi genotypes under the study ranged from6.55 to 17.23. The lowest number of seeds was observed in BG-3 (6.55) and followed by BG-1 and BG-5 (7.78 and 8.56 respectively). The highest number of seeds per fruit was noted from BG- 10, BG-16 and BG-14 (17.23 16.63, 15.56).**The 10 seed weight** of the bilimbi genotypes varied between 1.52 to 1.91 g under the present study. The highest reading was observed in BG-1 (1.91 g) followed by BG-12 (1.90 g), BG-3 (1.88 g) and BG-5 (1.85 g). The lowest reading was observed in BG-10 (1.52 g), BG-13 (1.57 g) and BG-14 (1.61 g).

The result of the current investigation with respect to fruit length, fruit diameter and fruit weight has the similarity with the findings of Dangat *et al*. (2014). The average numbers of seeds of bilimbi fruits in the present study are in line with the findings of Bhaskar and Shantaram (2013). The difference in the findings are might be due to the genotypic variation of bilimbi plants.

Biochemical characters: All the statistically analyzed observations on fruit biochemical characters are furnished in Table 3. **The total soluble solids** content of bilimbi fruits significantly varied from 5.5º to 6.6º Brix, with 5.8º Brix in general (Table 2). In which

maximum TSS of bilimbi fruits was noted in genotype BG-16 and lowest in genotype BG-3 and BG-5. Elevated TSS was also found in BG-14 and BG-6 (6.5and 6.4º Brix respectively). The percentage of **total acidity** in bilimbi genotypes have been ranged from 1.26% to 1.41%. BG-14, BG-16, and BG-13 were categorized under the low-acid group, with acidity levels of 1.26%, 1.28%, and 1.29%, respectively. Conversely, BG-2, BG-4, and BG-1 were classified under the high-acid group, with acidity levels of 1.41%, 1.40%, and 1.39%, respectively.The percentage of **total sugar** also revealed considerable variation from 4.02 to 5.80 % with an average of 4.60 %.The total sugar percentage was extremely low in BG-1(4.02%) preceded by BG-8 (4.09%) and BG-2 (4.17%). On contrary BG-16 (highest), BG-14, BG-10 and BG-6 were scored higher total sugar content. The Bilimbi genotypes in the present experiment exhibited an extensive array of **ascorbic acid content** (33.5 to 57.2 mg/100g). The truncated ascorbic acid content was observed in BG-9 (33.5mg/100g),gone advanced by BG-5 (33.8 mg/100g). The maximum ascorbic acid content was noted in accession BG-14 (57.2mg/100gm), closely followed by BG-10 (55.2mg/100gm).

Ferreira *et al*. (2022) and Arroxelas *et al*. (2001) have found the TSS range of 3.2 to 4.3º Brix which has the consonance with the findings of present experiment. More or less closer result with respect to acidity of bilimbi fruits has been reported by Nilugin and Mahendran (2016) and Ferreira *et al*. (2022). Nilugin and Mahendran (2016) has found average 4.2% total sugar in bilimbi fruits. The range of ascorbic acid content in bilimbi has been reported as 26.5 to 51.2 mg/100g in the studies by other scientists like Ho *et al*. (2020), Nilugin and Mahendran (2016), Dewi and Juwithninglyas (2024) and Arroxelas *et al*. (2001) which support the similarity of the result of present experiment.

Bioactive compounds: All the statistically analyzed observations on fruit bioactive compounds are presented in Table 3. **Antioxidant activity (DPPH radical scavenging activity %)**of sixteen different bilimbi accessions has been ranged from 70.5 to 87.0%. The maximum total antioxidants percentage was expressed by the germplasm BG-14 (87%)on the other hand BG-1(70.5%) possessed lowest content of antioxidant, preceded by BG-9 (71.3%) and BG-5 (72.7%). Some other potential bilimbi accessions with respect to higher antioxidant content are BG-13 (83.2%) and BG-16 (82.1%).**Total Phenolic compound:** Total phenolics are also considered as important bioactive compound. The bilimbi fruits of sixteen different genotype possessed considerable high range of total phenolics (30.2 to 38.4 µgGAE/g) in the bilimbi fruit (BG-7 and BG-5) followed by higher phenolics content recorded under genotype BG-4 (37.9µgGAE/g) and BG-6 (35.3µgGAE/g). **Flavonoid content (mgQE/g):**The different genotypes of bilimbi fruits have exhibited a greater variation in flavonoid content. Highest flavonoid content (41.2 mgQE/g) was recorded in BG-6 and lowest in BG-16 (29.7 mgQE/g). The bilimbi accessions namely BG-5 and BG-3 also possessed higher flavonoid content of 39.0 and 38.4 mgQE/g.

Very high antioxidant activity of bilimbi fruit juice and the pulp have been noted by Asna and Noriham (2014), Iwansyah *et al*. (2021) and Sreedharan *et al*. (2020) and they have found the range of DPPH inhibition range of 69.2 to 87.4% which is closely supports the result of the current study. Iwansyah *et al*. (2021) and Abraham (2016) found considerable phenol content in bilimbi fruits which have ranged from 28.6 to 38.7mgQE/g. Thus the phenol content of bilimbi genotypes under present study has the consonance with the report of Iwansyah *et al*. (2021) and Abraham (2016). Supporting to the result of flavonoid content of bilimbi fruits of present study, Iwansyah *et al*. (2021), Ferreira *et al*. (2022), Abraham (2016) and Asna and Noriham (2014) have reported similar result. All the variations of results on bioactive compounds of bilimbi

fruits conceivably be due to genetic makeup and environmental variation of growing conditions.

Correlation analysis: The analysis of correlation between different parameters (level of significance =0.05) is exhibited in Table 4. Average number of seeds present in single bilimbi fruit under the present study has shown high positive Correlation with fruit length (+0.86) fruit diameter (+0.81) ascorbic acid (+0.84) antioxidant activity (+0.82)and T.S.S. (+0.78). While a great extent of negative correlation between number of seeds per fruit and number of fruits per cluster (-0.76), acidity(-0.72),10 seed weight (- 0.71) and flavonoid content (-0.67) have been noticed. Amount of ascorbic acid in bilimbi fruits has exhibited very high positive correlation with antioxidant activity (+0.91), fruit weight (+0.88), fruit length (+0.84), fruit diameter (+0.84) and total sugar (+0.82). Moderate negative correlation between amount of ascorbic acid in bilimbi fruits and number of fruit per clusters (-.76), 10 seed weight (- 0.66), flavonoid content (-0.69) and acidity (-0.64) were recorded. Positive correlation of antioxidant activity with fruit length (0.79), fruit weight (+0.78), total sugar (+0.77) and T.S.S. (+0.68) have been found. On contradictory,negative correlation have been found between antioxidant activity with acidity (-0.72), number of fruits per cluster (-0.68), flavonoid content (-0.58) and 10 seed weight (-0.58). TSS of bilimbi fruits has shown high positive correlation with fruit weight (+0.83), total sugar (+0.78), fruit length (+0.76) and fruit diameter +0.76). Notable negative correlation between T.S.S. and number of fruits per cluster (-0.79), acidity (-0.76) were also observed in the present experiment. Total sugar was greatly positively correlated with fruit weight (+0.81), fruit diameter (+0.80) and fruit length (+0.79), while resolutely negative correlation has been found with acidity (-0.75). Fruit diameter was under positive correlation with fruit length (+0.86) and fruit weight (+0.89) and notable negative correlation noticed with number of fruits per cluster (-0.75) and acidity (-0.65).Although the fruit length has shown high positive correlation with fruit weight (0.92),however it resembledraised negative correlation with number of fruits per cluster (-0.77) and flavonoid content (-0.66).Fruit weight possessed high degree of negative correlation with number of fruits per cluster (-0.87), flavonoid content (-0.74) and acidity (-0.63). Acidity of bilimbi fruits in the present research has shown moderate positive correlation with total phenolics (+0.57), flavonoid content (+0.53) have been observed in bilimbi fruits.

Till date there is no report of correlation studies of fruit morphological and biochemical characters of bilimbi fruit. However, Pawar *et al*. (2014) have reported the strong positive correlation of fruit weight with fruit length, fruit diameter, volume of fruits, and number of seeds in carambola. This report of Pawar *et al*. (2014) has similarity with the findings of present experiment. The dissimilarity of the results might be attributed to the variation in genotypes and difference of growing conditions.

Two-way hierarchical clustering: Two-way hierarchical clustering of sixteen bilimbi genotypes has shown in Figure 1 with clear picture of three clusters in the present population. Five bilimbi genotypes (BG-16, BG-10, BG-13, BG-14 and BG-6) have been placed in a far most cluster (Cluster I), the second cluster (cluster II) comprised of seven bilimbi genotypes (BG-15, BG-11, BG-2, BG-12, BG-8, BG-4 and BG-7). The smallest cluster (cluster III) was populated with four bilimbi genotypes (BG-3, BG-1,BG-5 and BG-9). Out of these three clusters, cluster I was most distant and farthest from other two clusters (cluster II and cluster III). The genotypes under third cluster where most close to each other.Clustering of parameters shows five distinct divisions out of which antioxidant activity was in the nearest distinction (parameter cluster I) followed by fruited and number of seeds per fruit (parameter cluster

II). Acidity, fruit diameter and ten seed weight has created little bit greater variation among the bilimbi genotypes under present experiment (parameter cluster III). While fruits per cluster, TSS,fruit length and total sugar has also contributed a good variation among bilimbi genotypes (parameter cluster IV). Ascorbic acid content, total phenolic and flavonoid content have created most distinct variation among the bilimbi genotypes (parameter cluster V).

Padun and Singh (2018) have found four clusters of carambola genotypes under Arunachal condition out of which three major clusters were prominent and this finding has consonance with the findings of present experiment.

CONCLUSION

Present investigation revealed a wide array of variation on fruit morpho-biochemical characters and bioactive compounds of bilimbi genotypes grown under semi-arid lateritic belt of West Bengal. Determination of bioactive compounds of bilimbi genotypes also shown away the fruits to be rich in antioxidants (70.5 to 87.0% of DPPH inhibition), total phenols (38.4 to 30.2µgGAE/g) and flavonoids (29.7 to 41.2mgQE/g). Positive correlation between number of seeds, fruit length, fruit diameter, ascorbic acid, antioxidant activity and TSSwas noted. Additionally, ascorbic acid content has exhibited very high positive correlation with antioxidant activity, fruit weight, fruit length, fruit diameter, and total sugar. Noteworthy negative correlation was also noted between TSS and number of fruits per cluster, acidity in addition to between fruit diameter and number of fruits per clusterand acidity. The population of bilimbi genotypes fall under three clusters comprising 5, 7 and 4 different genotypes while five different parameter clusters have been noted to create variation among the bilimbi genotypes. With respect to fruit size and quality parameters bilimbi genotype BG-16 and BG-10 was found best under the present study.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: GPS locations of bilimbi genotypes selected under present study:

Genotypes (BG)	Address	GPS location
BG-1	Ruppur village, Bolpur, Birbhum, West Bengal	23.66ºN, 87.60ºE
BG-2	Kasthagara, Mallarpur, Birbhum, West Bengal	24.11ºN, 87.70ºE
BG-3	Gunutia, Birbhum, West Bengal	23.86ºN, 87.83ºE
BG-4	Gangarampur, Khoirashole, Birbhum, West Bengal	23.81ºN, 87.20ºE
BG-5	Goalmal, Murarai, Birbhum, West Bengal	24.40ºN, 87.90ºE
BG-6	Barshal, Rampurhat, Birbhum, West Bengal	24.15ºN, 87.78ºE
BG-7	Dhundabad, Kulti, West Burdwan, West Bengal	23.77ºN, 86.88ºE
BG-8	Nimsa, Pandabeshwar, West Burdwan, West Bengal	23.72ºN, 87.19ºE
BG-9	Shokna, Panagarh, West Burdwan, West Bengal	23.44ºN, 87.42ºE
BG-10	Bhagabanpur, Mankar, West Burdwan, West Bengal	23.40ºN, 87.57ºE
BG-11	Senara, Raghunathpur, Bankura, West Bengal	23.57ºN, 86.74ºE
BG-12	Nidhirampur, Gangajalghati, Bankura, West Bengal	23.47ºN, 87.11ºE
BG-13	Kuludihi, Chhatna, Bankura, West Bengal	23.32ºN, 86.96ºE
BG-14	Chechurya, Taldangra, Bankura, West Bengal	23.05ºN, 87.08ºE
BG-15	Rajganja, Joypur, Bankura, West Bengal	23.03ºN, 87.44ºE
BG-16	Sihas, Kotulpur, Bankura, West Bengal	23.00ºN, 87.63ºE

Table 2: Fruit morphological diversity of different bilimbi genotypes under semi-arid lateritic part of West Bengal

Bilimbi genotypes (BG)	No. of fruits/ cluster	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	No. of seeds/ fruit	10 seed weight
BG-1	6.55	4.06	1.80	10.85	7.78	1.91
BG-2	6.34	4.11	1.63	11.21	8.92	1.65
BG-3	8.71	3.99	1.51	10.06	6.55	1.88
BG-4	5.93	4.88	1.90	11.50	10.31	1.62
BG-5	8.44	3.56	1.63	9.52	8.56	1.85
BG-6	5.82	5.22	2.20	12.46	14.82	1.70
BG-7	7.17	4.72	1.71	10.53	12.40	1.66
BG-8	5.08	5.01	1.99	11.92	12.72	1.70
BG-9	6.12	4.59	1.62	11.35	10.86	1.78
BG-10	5.74	5.65	2.11	13.25	17.23	1.52
BG-11	6.81	4.73	1.91	11.84	12.35	1.81
BG-12	6.22	4.92	1.82	11.96	10.29	1.90
BG-13	4.67	5.11	1.88	12.57	15.14	1.57
BG-14	4.99	5.37	2.11	12.83	15.56	1.61
BG-15	5.72	4.14	1.77	11.44	13.25	1.69
BG-16	4.21	5.78	2.43	14.11	16.63	1.72
SD	1.18	0.61	0.23	1.14	3.13	0.11
Mean	6.15	4.74	1.87	11.71	12.08	1.72

Table 3: Biochemical characters and bioactive compound diversity of different bilimbi genotypes under semi-arid lateritic part of West Bengal

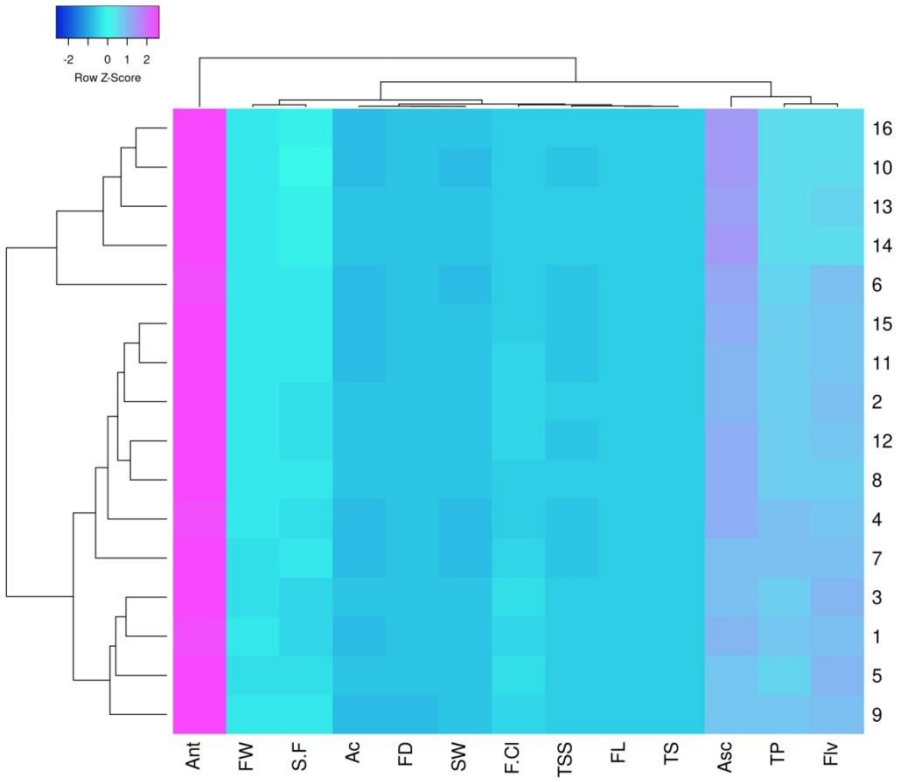
Bilimbi genotypes (BG)	TSS (ºBrix)	Acidity (%)	Total sugar (%)	Ascorbic acid (%)	Antioxidant activity (DPPH % inhibition)	Total phenolics (mg GAE/g)	Flavonoid content (mgQE/g)
BG-1	5.8	1.39	4.02	38.3	70.5	33.7	37.1
BG-2	5.8	1.41	4.17	40.6	75.2	32.8	36.8
BG-3	5.5	1.37	4.30	37.1	73.6	32.5	38.4
BG-4	5.7	1.40	4.56	44.2	74.3	37.9	36.6
BG-5	5.5	1.32	4.35	33.8	72.7	30.2	39.0
BG-6	6.4	1.30	5.11	49.5	81.8	35.3	41.2
BG-7	5.7	1.40	4.20	38.0	76.0	38.4	37.3
BG-8	6.0	1.31	4.09	45.6	79.9	33.7	33.8
BG-9	6.3	1.33	4.38	33.5	71.3	33.2	35.9
BG-10	6.1	1.30	5.25	55.2	80.7	31.6	30.4
BG-11	5.9	1.34	4.28	41.3	76.4	32.9	36.0
BG-12	5.7	1.38	4.44	43.8	78.6	33.5	35.9
BG-13	6.2	1.29	5.03	50.8	83.2	31.4	32.3
BG-14	6.5	1.26	5.39	57.2	87.0	31.3	31.5
BG-15	5.8	1.37	4.24	43.7	75.8	33.4	35.2
BG-16	6.6	1.28	5.80	52.9	82.1	32.0	29.7
SD	0.33	0.04	0.52	7.10	4.52	2.15	3.07
Mean	5.96	1.34	4.60	44.09	77.44	33.36	35.44

Table 4: Correlation analysis of different morphological, biochemical parameters and bioactive compounds of sixteen bilimbi genotypes under semi-arid lateritic belt of West Bengal

	No. of seeds/ fruit	Ascorbic acid	Antioxidant activity	TSS	Total sugar	Fruit diameter	Fruit length	Fruit weight	Acidity	Total phenolics	Fruit length	No. of fruits/ cluster	Flavonoid content
No. of seeds/ fruit	1												
Ascorbic acid	+0.84	1											
Antioxidant activity	+0.82	+0.91	1										
TSS	+0.78	+0.68	+0.68	1									
Total sugar	+0.78	+0.82	+0.77	+0.78	1								
Fruit diameter	+0.81	+0.84	+0.74	+0.76	+0.80	1							
Fruit length	+0.86	+0.84	+0.79	+0.76	+0.79	+0.86	1						
Fruit weight	+0.86	+0.88	+0.78	+0.83	+0.81	+0.89	+0.92	1					
Acidity	-0.72	-0.64	-0.72	-0.76	-0.75	-0.65	-0.60	-0.63	1				
Total phenolics	-0.12	-0.20	-0.23	-0.22	-0.30	-0.06	0.05	-0.18	+0.57	1			
10 seed weight	-0.71	-0.66	-0.58	-0.43	-0.49	-0.41	-0.57	-0.53	+0.33	-0.1	1		
No. of fruits/ cluster	-0.76	-0.76	-0.68	-0.79	-0.60	-0.75	-0.77	-0.87	+0.50	+0.05	+0.58	1	
Flavonoid content	-0.67	-0.69	-0.58	-0.55	-0.60	-0.55	-0.66	-0.74	+0.53	+0.40	+0.51	+0.70	1

(Level of significance P=0.05)

Figure 1: Two-way hierarchical clustering of sixteen bilimbi genotypes grown under semi-arid lateritic belt of West Bengal



Effect of different concentration of IBA on the success of hardwood and softwood cuttings of water apple

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Receipt: 04.11.2024 *Revised: 06.12.24* *Acceptance:08.12.24*
DOI: 10.53552/ijmfmap.10.2.2024.123-128

ABSTRACT

Considering the nutritional, commercial potentiality and popularity there is an increasing tendency to grow water apple in different parts of West Bengal. However, due to the unavailability of genuine quality planting material, area under the cultivation of this crop is limited. Till date growers are raising this crop mostly through seed propagation. Asexual means of propagation reduces the long gestation period. Considering the above facts, an experiment was conducted to assess the effect of various concentrations of IBA on different types of cuttings of water apple. According to the results obtained, IBA concentration at 3000 ppm showed better response in terms of days taken for sprouting (8.66 days), number of leaves (54.23), sprouting (88.25 %), success (86.75) and survival rate (93.23 %) with least mortality rate (17.75 %) IBA at 3000 ppm performed better in terms of number of roots (23.70) and rooting per cent (91.75 %).

Keywords: Cuttings, IBA, propagation, water apple,

INTRODUCTION

Water apple [*Syzygium aqueum* (Brum. F. Alston)] is a tropical minor fruit crop under the Myrtaceace family. It is considered as potential minor fruit crop of India has believed to be primary centers of origin in Southern India and Malaysia. With passage of time, this crop successfully expanded its geographical presence to the whole Indian subcontinent, South America, the warmer regions of North America as well as in sub-Saharan Africa (Djipa *et al*., 2000). The fruit crop is commonly known as *Jamrul* in West Bengal and basically popular for its refreshing thirst-quenching property. Mainly the fruit is consumed as fresh although various post-harvest preparation like jam, jellies etc can be made through it. This minor fruit is getting attention for its medicinal values (Tripathi, 2021). Due to its high-water content it is regarded to as low-calorie fruit. Besides its unique property, water apple is also considered as one of the rich sources of essential minerals and antioxidants (Hartati, 2022; Lim *et al*., 2007).

Understanding the future potential of nutritive crops, the farmers are now a days interested in the commercial cultivation of nutrient rich fruits. Water apple is a genuine candidate to meet the farmers' interest specific to this purpose. Its cultivation has already been accelerated in various parts of India. It fetches good price during summer months and due to the increasing demands in day by day and the off-season cultivation has been started in the southern parts of West Bengal particularly in South-24 Parganas district. However, the lack of genuine, quality planting materials is one of the main constraints regarding area expansion of this

crop. Traditionally, water apple is propagated by seed, but its recalcitrant seeds quickly lose viability. Besides, seed propagation leads to the significant genetic variation in fruit colour, shape, and size. Therefore, asexual means of propagation are required to produce uniform plants as well as to preserve genetic purity. In light of these considerations, the present study was designed to standardize propagation techniques through cuttings by using different concentrations of IBA.

MATERIALS AND METHODS

The present study was conducted at the Instructional Farm, Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India during 2021 under poly-house condition. Geographically the district lies in the foothills of eastern Himalayas and is located at 28º58'86'' N latitude, 81º66''73'' E longitude at an elevation of 42 m above mean sea level. The experiment was laid out in Complete Randomized Design with six treatments consisting of T_1 (control), T_2 (IBA @1000 ppm), T_3 (IBA @ 2000 ppm), T_4 (IBA @ 3000 ppm), T_5 (IBA @ 4000 ppm) and T_6 (IBA @ 5000 ppm). A total of sixty stem cuttings were taken in each treatment and replicated four times. Softwood and hardwood cuttings (15 cm long) of water apple were taken from 8-year-old tree with a 3-4 nodes in each during the month of June. The small portions from both the ends of the cuttings slightly above and below the nodes were removed in order to separate the new shoots from the cuttings and trimmed up to necessary length. A slant cut was made at the basal end of the cutting to promote the most absorbent area possible for successful rooting. Cuttings were treated by dissolving on required IBA solution through rapid dip method as mentioned above. After that the cuttings were planted in poly bags containing 1:1:1 FYM+ Soil+ Sand as the rooting media and kept in poly-house condition for 120 days. Five cuttings form each replication were selected randomly for recording observations under this experiment. For statistical interpretation, analysis of variance for each parameter was performed using Proc Glm of Statistical Analysis System (SAS) Software (Version 9.3). Means separations for different accessions under different parameter were performed using Least Significant Difference (LSD) test ($P \leq 0.05$). Normality of residuals under the assumptions of ANOVA was tested using Kolmogrov-Smirnov test using Proc-Univariate procedure of SAS (Version 9.3).

RESULTS AND DISCUSSION

The perusal of data from the experiment showed several growth benefits across both softwood and hardwood cuttings of water apple when treated in different concentration of IBA than control. Both hardwood and softwood cuttings of the plant showed maximum sprouting percentage (Table 1) when they were treated with 3000ppm of IBA (79.75% and 88.00% respectively). The result was statistically similar with the treatments where 2000 ppm IBA was used. 3000 ppm IBA also showed the quickest sprouting (13.77 and 9.21 days respectively) and also produce highest sprout length (31.97 and 31.65 cm respectively) followed by highest number of leaves (42.64 and 31.75 respectively) after 120 days of the treatment in the respective type of cuttings (Table 1 and 2). The number of leaves produced by *Acalypha hispida* were also high in hardwood cuttings (Rifnas *et al*., 2021). The significant effect of various concentrations of IBA on cuttings for sprouting might be due to a high concentration of stored carbohydrates and low to moderate concentrations of nitrogen were used to develop shoot systems by hydrolyzing, mobilizing, and using nutritional reserves in the area of shoot development. Adequate concentration of IBA enhanced the nutrient uptake and resulted in more photosynthetic provide required energy for cell division and cell elongation which ultimately helps to improve the sprouting on the cuttings (Singh *et al*., 2020; Almedia *et al*., 2010; Henrique *et al*.,

2006). The use of different concentrations of IBA may have increased leaf count due to induced robust root, allowing cuttings to absorb more nutrients in order to produce more leaves as well as the activation shoot growth which probably increased the number of nodes that lead to development of a greater number of leaves (Srihari *et al.*, 2018).

The treatment with 3000 ppm IBA also results in maximum number of roots (19.03 and 23.70 cm respectively) with fastest root induction behavior (24.20 and 19.61 days respectively) over the other treatments in both softwood and hardwood cuttings respectively (Table 2). The probable cause behind least number of days taken for rooting might due to the use of IBA which promotes the development and division of the first root initial cells. The mechanisms by which IBA stimulates root formation in stem cuttings are through its conversion to IAA, enhancement of endogenous IAA synthesis, or the action of IAA synergistically, preventing IAA degradation and increasing its activity (Hartmann *et al*., 2010). Induction of maximum numbers of roots is due to stimulation of cambial activity involved in root initiation by growth regulators. IBA promote adventitious root formation by their ability to promote the initiation of lateral roots and also enhanced the transport of carbohydrates to basal portion of cuttings and due to its cell wall plasticity, which accelerates cell division stimulates callus development and root growth (Shao *et al*., 2018; Malik *et al*., 2013).

Observation recorded on rooting percentage showed that the cuttings when treated with IBA 3000 ppm exhibited highest rooting percentage in softwood cuttings (85.50%) and hardwood cuttings (91.75 %). The maximum fresh (30.55 and 27.65g) and dry weight (12.68 and 13.64g) of roots were recorded also with the same treatment in softwood and hardwood cuttings respectively (Table 3). The application of IBA might had an indirect influence by enhancing the speed of transformation of rooting primordia and movement of sugars to the base of cuttings and consequently formation of young active root and increase in rooting percentage (Srihari *et al*., 2018; Paul and Aditi 2009). The increase in weight of roots might be due to of increased root number, length of roots, photosynthesis, relative growth rate, and lateral branching of the shoots brought about by the IBA applied cuttings. The increment might be due to the cuttings contained higher amount of stored carbohydrate, when comes to contact with IBA increased the number of roots resulting a higher root dry matter accumulation the promoting effect of IBA on shoot parameters can be attributed to the reason that the better rooting coupled with better leaf growth and at the same time sustained the root strength to continue vigour and vitality in taking up nutrients as well as moisture as similar results were observed in the cuttings treated with IBA 3000 ppm (Abdullah *et al*., 2006).

Among different concentration of IBA, 3000ppm IBA resulted in maximum success (77.25% and 86.00% respectively) in both softwood and hardwood cuttings followed by with IBA 2000ppm treatment with statistically similar results (Table 4). The same treatments also showed statistically similar results for survival across the type of cuttings. Among the different methods of propagation, hardwood cuttings performed well (80.60% survival rate) in *Acalypha hispida* (Rifnas *et al*., 2021). Root and shoot formation as well as growth, which is well recorded to be influenced by a number of factors, including genotypes, physiological and ontogenetic age of cuttings, endogenous hormone contents, type of wood, carbohydrate contents, preconditioning treatment of cuttings, and external factors like cuttings micro-environment and the use of root-promoting substances may be the possible reasons for the present experimental results (Sharma *et al*., 2009).

CONCLUSION

The study reflects that the application of IBA significantly influences the propagation potential of water apple through stem cuttings. Hardwood cuttings treated with IBA at 3000 ppm exhibited superior performance across all parameters, including sprouting percentage, rooting percentage, number of leaves, number of roots, and success rate. These cuttings also showed reduced time to sprouting and rooting, with enhanced root and shoot growth, ensuring a higher survival rate and lower mortality. Softwood cuttings also responded better with 3000 ppm IBA, although hardwood cuttings showed overall superior results.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Effect of IBA on sprouting percentage, sprouting days and sprout length of water apple cuttings

Treatments	Sprouting percentage of cuttings		Days taken for sprouting		Sprout length (cm)	
	SWC	**HWC**	**SWC**	**HWC**	**SWC**	**HWC**
T_1 (Control)	50.25^{d} (45.13)	56.25^{d} (48.60)	17.38 ^{a}	13.49 ^{a}	15.62^{e}	16.73^{e}
T_2 (IBA@1000 ppm)	65.25^{bc} (53.88)	78.25^{bc} (62.19)	14.35 ^{e}	10.44 ^{c}	26.76^{b}	26.68^{c}
T_3 (IBA @2000 ppm)	73.50^{ab} (59.45)	84.75 ^{a} (67.26)	14.69 ^{d}	9.84 ^{e}	27.04^{b}	28.01^{b}
T_4 (IBA @3000 ppm)	79.75^{a} (63.35)	88.00 ^{a} (70.05)	13.77 ^{f}	9.21^{f}	31.97^{a}	31.65^{a}
T_5 (IBA @4000 ppm)	59.50^{cd} (50.54)	70.50 ^{c} (57.48)	15.85 ^{c}	12.79 ^{b}	24.08^{c}	21.94^{d}
T_6 (IBA @1000 ppm)	53.00^{d} (46.71)	66.50 ^{c} (54.72)	16.67 ^{b}	12.81^{b}	21.74^{d}	20.94^{d}
S.Em.(±)	2.22	2.56	0.02	0.02	0.51	0.43
CD at ≤ 5%	6.65	7.66	0.07	0.05	1.53	1.29

* SWC- Softwood cutting; HWC; Hardwood cutting; DAC- Days after Cutting. ** Means with same letter are not significantly different, Values in the parenthesis are the angular transform values

Table 2. Effect of IBA on number of leaves, days taken for rooting and root numbers of water apple cuttings

Treatments	Number of leaves		Days taken for rooting		Number of roots	
	SWC	HWC	SWC	HWC	SWC	HWC
T_1 (Control)	21.93^{e}	16.73^{e}	28.04^{a}	23.21^{a}	11.13^{e}	11.60^{e}
T_2 (IBA @1000 ppm)	29.35^{c}	26.68^{c}	25.32^{d}	20.35^{d}	13.88^{bc}	17.88^{c}
T_3 (IBA @2000 ppm)	38.18^{b}	28.01^{b}	24.79^{e}	19.84^{e}	15.98^{b}	20.90^{b}
T_4 (IBA @3000 ppm)	42.75^{a}	31.65^{a}	24.20^{f}	19.61^{e}	19.03^{a}	23.70^{a}
T_5 (IBA @4000 ppm)	27.68^{cd}	21.94^{d}	25.50^{c}	22.26^{c}	13.35^{cd}	14.65^{d}
T_6 (IBA @5000 ppm)	25.13^{d}	20.94^{d}	27.04^{b}	22.78^{b}	11.73^{de}	13.68^{d}
S.Em.(±)	0.90	0.43	0.08	0.11	0.71	0.70
CD at ≤ 5%	2.71	1.29	0.03	0.32	2.14	2.09

* SWC- Softwood cutting; HWC; Hardwood cutting; DAC- Days after Cutting. ** Means with same letter are not significantly different

Table 3. Effect of IBA on rooting percentage, fresh and dry weight roots of water apple cuttings

Treatments	Rooting percentage at 120 DAC		Fresh weight of root (g)		Dry weight of root (g)	
	SWC	HWC	SWC	HWC	SWC	HWC
T_1 (Control)	48.00 ^{e} (43.83)	60.75 ^{d} (51.20)	19.24^{f}	12.77^{f}	5.99^{d}	6.74^{b}
T_2 (IBA @ 1000 ppm)	68.25 ^{c} (55.74)	78.75 ^{c} (62.54)	25.79^{c}	20.13^{c}	10.80^{b}	11.98^{a}
T_3 (IBA @ 2000 ppm)	79.25 ^{b} (62.94)	87.00 ^{b} (68.88)	27.24^{b}	26.04^{b}	10.94^{b}	13.52^{a}
T_4 (IBA @ 3000 ppm)	85.50 ^{a} (67.67)	91.75 ^{a} (73.28)	30.55^{a}	27.65^{a}	12.68^{a}	13.64^{a}
T_5 (IBA @ 4000 ppm)	67.00 ^{c} (54.94)	83.25 ^{bc}(65.83)	23.42^{d}	17.37^{d}	9.35^{c}	8.37^{b}
T_6 (IBA @5000 ppm)	57.75 ^{d} (49.44)	82.50 ^{bc}(65.51)	22.61^{e}	13.87^{e}	9.33^{c}	7.24^{b}
S.Em.(±)	1.21	1.19	0.22	1.65	0.46	0.63
CD at ≤ 5%	3.61	3.57	0.66	0.55	1.38	1.88

* SWC- Softwood cutting; HWC; Hardwood cutting; DAC- Days after Cutting. ** Means with same letter are not significantly different, Values in the parenthesis are the angular transform values

Table 4. Effect of IBA on success rate and survival percentage of water apple cuttings

Treatments	Success rate (%)		Survival percentage	
	SWC	**HWC**	**SWC**	**HWC**
T_1 (Control)	45.75^{d} (42.53)	54.00^{c} (47.30)	73.56^{c} (59.21)	71.22^{a} (57.62)
T_2 (IBA @1000 ppm)	63.75^{bc} (52.98)	76.25^{ab} (60.840)	84.52^{ab} (66.93)	80.66^{abc} (64.04)
T_3 (IBA @2000 ppm)	73.00^{ab} (59.08)	82.50^{a} (65.48)	87.35^{a} (69.20)	82.26 ^{ab} (65.21)
T_4 (IBA @3000 ppm)	77.25 ^{a} (61.65)	86.00^{a} (68.27)	90.24^{a} (72.04)	87.41^{a} (69.28)
T_5 (IBA @4000 ppm)	55.50^{cd} (48.21)	68.50^{b} (56.21)	80.16^{bc} (63.60)	77.39^{bcd} (61.69)
T_6 (IBA @5000 ppm)	51.50^{cd} (45.84)	65.25^{bc} (53.94)	76.44^{c} (61.11)	74.27^{cd} (59.58)
S.Em.(±)	2.43	2.57	1.85	1.77
CD at ≤ 5%	7.28	7.70	5.53	5.30

* SWC- Softwood cutting; HWC; Hardwood cutting; ** Means with same letter are not significantly different, Values in the parenthesis are the angular transform values

Foliar application of micronutrients to reduce fruit drop and enhance quality and yield attributes in ber (*Ziziphus mauritiana* Lamk.) cv. Gola

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Receipt: 11.11.2024 *Revised: 10.12.24* *Acceptance:12.12.24*
DOI: 10.53552/ijmfmap.10.2.2024.129-135

ABSTRACT

A field experiment was conducted in Narendra Deva University of Agriculture and Technology Kumarganj, Faizabad (U.P.) during the years 2023-24 to evaluate the performance of micro-nutrient on fruit setting, retention, physical characteristics, and yield of ber fruit cv. Gola. Seven treatment of micronutrient and their combination were used with included a control (water spray).Results indicated that treatment T_5(Zinc sulphate @ 0.5% + Borax @ 0.3%) was most effective in achieving the highest fruit retention (38.42 %), and minimal fruit drop (61.58 %) leading to the maximum yield (131.00 q/ha).Additionally, Treatment T_7 with the combination of $ZnSO_4$ at 0.5%, $FeSO_4$ at 0.5%, and Borax at 0.3% resulted in highest fruit weight (18.59g), largest fruit dimensions (length-3.34cm and width-2.29cm), volume (19.83cc), and specific gravity (1.11) over control. Treatment- T_7 also enhanced fruit quality parameters, including higher percentages of total soluble solids (TSS-15.44 ^{0}B), ascorbic acid (84.85 mg/100g), reducing sugar (5.43%), non-reducing sugar (5.87%) and total sugar (11.30%), and the lowest acidity (0.31%).Thus the investigation concluded that treatment T_5- Zinc sulphate @ 0.5% + Borax @ 0.3% is most favourable to reduce fruit drop, enhance retention and yield attributes while treatment T_7- Zinc sulphate @ 0.5% + ferrous sulphate @ 0.5% + Borax @ 0.3% produced most favourable result among all treatment in physico-chemical parameters.

Keywords: Ber, borax, fruit drop, yield, zinc sulphate,

INTRODUCTION

The Indian jujube, also referred as ber (*Zizyphus mauritiana* Lamk), holds significant importance as a fruit tree cultivated across a wide range of tropical, subtropical, and dry land areas. It belongs to the Rhamnaceae family, which includes approximately 50 species, with 18-20 of them originating from India. Ber thrives both in wild habitats and cultivated environments across warmer regions, reaching as high as 1500 feet above sea level (Yadav *et al*., 2021). Its ability to withstand various conditions has made it a commercially viable option in several countries, including South Africa, the Indo Malayan region, the Middle East, Australia, Iran, the USA, Syria, as well as specific regions of Spain and Italy. In India, commercial cultivation of this crop is widespread across states like Haryana, Punjab, Maharashtra, Uttar Pradesh, Rajasthan, Madhya Pradesh, Bihar, Andhra Pradesh, and Tamil Nadu. In India, ber cultivation spans across an area of 54,000 hectares, yielding approximately 596,000 MT (Anonymous, 2021-22). Ber yields high-quality produce, demonstrating remarkable productivity even in constrained environments. Its resilience allows it to thrive in challenging conditions such as extreme heat and drought. Ber fruit contain elevated levels of key nutrients per 100 grams of pulp, including ascorbic acid (69 mg), protein (1.2 g), energy (79 kcal),

carbohydrates (20.2 g), and fats (0.2 g). Typically consumed fresh, ber is renowned for its rich content of ascorbic acid, carbohydrates, and vital minerals (Pareek *et al.*, 2009). Nutrients play a crucial role in various physiological processes such as vegetative propagation, induction of seedlessness, increasing fruit set, preventing pre-harvest fruit drop, regulating flowering, and managing fruit size and thinning and Nutrients like iron (Fe), potassium (K), borax, zinc (Zn), and calcium (Ca) are essential for enhancing flowering, fruit set, size, quality, and yield in many tree crops (Singh *et al.*, 2016). Borax in particular, is widely used to manipulate physiological events and improve fruit quality. Given the increasing importance of micronutrients in modern agriculture, their foliar application has emerged as a crucial strategy. This method allows for the direct delivery of nutrients to the leaves, ensuring timely availability during critical growth phases. Thus, it is vital to assess the effects of various micronutrients and their combinations on plant growth, yield, and fruit quality in ber cultivation. This research is essential not only to enhance the productivity and quality of ber but also to ensure its sustainable cultivation, thereby supporting local economies and food security.

MATERIALS AND METHODS

The present experiment was conducted at main experiment station, Department of Horticulture, ANDUA&T, Kumarganj, Ayodhya during the year 2023–2024 on Gola variety of ber. Its geographic co-ordinates are 26.470 N latitude, 82.120 E longitude, and 113 m above mean sea level. This location is in the Eastern Uttar Pradesh Indo-Gangetic Plains, a typical saline-alkaline zone. The experiment was laid out in randomized block design with three replications. The treatment consisted two foliar applications of Zinc sulphate, Iron sulphate and borax which were consisted T_0 (Control- water spray), T_1 (Zinc sulphate @ 0.5%), T_2 (Ferrous sulphate @ 0.5%), T_3 (Borax @ 0.3%), T_4 (Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5%), T_5 (Zinc sulphate @ 0.5% + Borax @ 0.3%), T_6 (Ferrous sulphate @ 0.5% + Borax @ 0.3%) and T_7 (Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5% + Borax @ 0.3%). The foliar sprays of micro-nutrient were applied two times. The first foliar spray was applied in the third week of October at the fruit set stage, followed by a second spray in the third week of November during the active growth phase, using a foot sprayer. Physical parameters like Fruit size (length and width) measured by Vernier calliper, Fruit Weight (g) is measured by Digital weighing machine and Biochemical parameters like Total soluble solids (TSS ^{0}Brix), Acidity (%), Ascorbic acid (mg/100g pulp), Reducing sugar (%), Non reducing sugar (%), Total sugars (%) and Total invert sugar (%) were analyzed by AOAC(1995) method in the Post Graduate Laboratory, Department of Fruit Science CHF, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (U.P.) during 2023-24. The data is subjected to two way ANOVA and analyzed as per method suggested by Punse and Sukhatme (1995).

RESULTS AND DISCUSSION

Fruit drop and fruit retention

The micro-nutrients in general were effective in increasing fruit setting significantly in comparison to control. Data collection occurred at seven specific intervals (Table 1). The combined application of $ZnSO_4$ @ 0.5% and $FeSO_4$ @ 0.5% which is treatment (T_5) resulted in the lowest fruit drop with only 61.58% followed by Treatment T_7 (64.67%) with foliar application of $ZnSO_4$ @ 0.5% + $FeSO_4$ @ 0.5% + Borax @ 0.3% over control and at par with all the mentioned treatments. Adhikary *et al.* (2019) in reported in ber that a very heavy fruit drop occurred immediately after fruit set and fruit drop was reduced as fruit development advanced. It was observed that maximum fruit retention (38.42%) was observed in T_5 (Zinc sulphate @ 0.5% + Borax @ 0.3%). Involvement of Zn in auxin synthesis and B in translocation of starch to fruit resulted into better photosynthesis. Presence of borax and zinc stimulates auxin production and postponing the development of

the abscission layer in the initial phases of fruit growth. The increase in fruit retention seen with borax sprays implies that these treatments may have influenced the balance of auxin, thereby preventing fruit drop (Kumar and Shukla (2010) in ber) and Yadav *et. al.*, (2017) in guava cv. Lalit.

Physical parameter

The maximum fruit size in terms of maximum fruit length (3.34 cm) and width (2.29 cm) was revealed with combined spray of Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5% + Borax @ 0.3% (T_7) expressed in Table 2. Treatment T_7 shows significant difference over control in fruit length but in fruit width there is no significant difference between T_7 and T_5. This effect is might be due to Zinc improves quality in many fruit crops as well as Borax helps in cell wall synthesis and elongation of so it's application can influence fruit diameter (Tripathi *et al.*, 2018). Result is closely related with the Mishra *et al*. (2017) in aonla, Meena *et al.* (2008) worked in ber trees. The improvement in volume of fruit (Table 2) and specific gravity (19.83 cc and 1.11) was recorded respectively with the combined spray of Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5% + Borax @ 0.3% (T_7) followed by treatment (T_5) whereas minimum recorded in control plants. Specific gravity (Table 2) shows significant difference among treatment and also at par with the rest of treatments. Meena *et al.* (2008) also reported favourable effects of potassium, boron and ferrous sulphate on various constituents of ber fruits and these findings also closely align with Majumder *et al*. (2017) in ber.

Biochemical parameters

Data on biochemical parameters like TSS, ascorbic acid, acidity, ascorbic acid, reducing sugar, non-reducing sugar and total sugars are presented in Table 2. Increased TSS and minimum acidity content was estimated in treatment T_7 ($ZnSO_4$ @ 0.5% + $FeSO_4$ @ 0.5% + Borax @ 0.3%) (15.44 ^{0}B and 0.31% respectively) followed by T_5-$ZnSO_4$ @ 0.5% + Borax @ 0.3% (13.80 ^{0}B and 0.32%) and decreased TSS and increased acidity were seen over control (11.44 ^{0}B and 0.38%). All the treatment were showed significance difference over control but in acidity there is no significance difference among all the treatments. Borax stimulates the functioning of number of enzymes in the physiological process which probably cause an increase in T.S.S. content. Pandey and Kumar (2022), Pal *et al*. (2021) reported highest TSS in ber cv. Gola and Kumar *et al*. (2024) in papaya. Likewise similar results were obtained by Singh *et al.* (2012) with the spray of zinc sulphate to reduce the acidity of aonla fruit, cv. Banarasi due to transformation of organic acids into sugars during ripening and their derivative by the reaction involving reversal of glycolytic path way. The highest levels of ascorbic acid (84.85 mg/100g,) reducing (5.43%), non-reducing (5.87%), and total sugars (11.30%) were recorded by the treatment T_7 with foliar application of Zinc sulphate 0.5% + Ferrous sulphate 0.5% + Borax 0.3% followed by treatment T_5 (81.35 mg/100g, 4.98%, 5.56% and 10.54% respectively) with foliar application of Zinc sulphate 0.5% + Borax 0.3 and least content were observed in control. Treatment T_7 was found significant and at par with rest of the treatment. Borax also stimulates the functioning of number of enzymes in the physiological process resulting increased ascorbic acid content and iron also act as catalyst in oxidation process. These finding has close conformity with by Pal *et al*. (2021) and Pandey and Kumar (2022) in ber cv. Gola and Majumdar *et al.* (2017) in ber. Increased in sugar level in fruits may be due to effectiveness of boron which facilitating sugar translocation within the fruits and boost the sugar level to increase (Pandey and Kumar, 2022 and Pal *et al*., 2021 in ber).

Yield parameter

The results revealed that the effect of foliar spray of different micronutrients alone and in combination influenced the overall yield (Table 3). The application of treatment T_7-$ZnSO_4$@ 0.5% + $FeSO_4$ @ 0.5% + Borax @ 0.3% gained maximum weight (18.59 g per fruit) followed by T_5 (18.52g per fruit) while

T_5 ($ZnSO_4$ @ 0.5% + Borax @ 0.3%) treatment gave the maximum yield 83.97 kg/per tree and 131.00 q/ha as compared to other treatments. Cumulative effect of zinc and boron help to increase the fruit retention per centage as well as minimise fruit drop per centage and thereby increasing the fruit yield. Kumar and Shukla (2010) noted impacts of zinc and borax on fruit yield, Bhatt *et al.* (2012) in mango, Gurjar *et al*. (2015) in mango, Chaudhary *et al*. (2018) in aonla, Kumar *et al*. (2024) in papaya and Yadav *et al.* (2017) reported in guava cv. Lalit.

Conclusion

The current study's findings, which involved a 24-year-old ber cv. Gola plant, indicated that the treatment T_5, which received $ZnSO_4$ @ 0.5% + Borax @ 0.3%, was the most suitable nutrient dose for sodic soil conditions in order to maximize fruiting, yield and minimize fruit drop. Whereas after analysing the impacts of each treatment on different parameters, it was determined that treatment T_7 (receiving $ZnSO_4$ @ 0.5% + $FeSO_4$ @ 0.5% + Borax @ 0.3%) found the best in terms of physical and chemical attributes of ber.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of specific micro nutrient on periodical fruit drop (%), total fruit drop (%) and fruit retention (%) of ber cv. Gola.

Treatments	1st week of Nov.	2nd week of Nov.	3rd week of Nov.	3rd week of Dec.	3rd week of Jan.	3rd week of Feb.	At Harvest	Total Fruit drop (%)	Fruit retention (%)
T_0	8.59	9.17	11.42	12.73	20.59	7.23	8.94	78.67	21.33
T_1	8.92	6.89	11.18	10.08	20.16	7.05	8.20	72.48	27.52
T_2	7.27	8.28	10.63	14.75	19.72	6.73	7.89	75.27	24.73
T_3	7.81	10.31	11.24	11.25	17.56	5.37	7.29	70.83	29.17
T_4	9.30	5.09	10.28	11.22	16.19	5.06	9.21	66.35	33.65
T_5	7.03	5.13	7.02	10.64	19.68	5.04	7.04	61.58	38.42
T_6	7.29	6.44	9.41	12.38	18.50	6.12	8.36	68.50	31.50
T_7	6.39	6.26	11.08	13.09	17.40	4.34	6.11	64.67	35.33
S. Em±	**0.14**	**0.14**	**0.17**	**0.21**	**0.31**	**0.10**	**0.16**	**0.61**	**0.49**
CD @ 5%	**0.41**	**0.43**	**0.51**	**0.64**	**0.94**	**0.30**	**0.48**	**1.86**	**1.48**

T_0-Control- water spray, T_1-Zinc sulphate @ 0.5%, T_2- Ferrous sulphate @ 0.5%, T_3- Borax @ 0.3%, T_4- Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5%, T_5- Zinc sulphate @ 0.5% + Borax @ 0.3%, T_6- Ferrous sulphate @ 0.5% + Borax @ 0.3% and T_7-Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5% + Borax @ 0.3%

Table 2: Effect of specific micro nutrient on physical and biochemical characters of ber cv. Gola.

Treatments	Fruit length (cm)	Fruit width (cm)	Fruit volume (cc)	Specific gravity	TSS (^{0}Brix)	Acidity (%)	Ascorbic acid (mg/100g)	Reducing sugar (%)	Non-reducing sugar (%)	Total sugar (%)
T_0	2.23	1.91	16.70	0.82	11.44	0.38	68.01	3.71	3.78	7.49
T_1	2.80	2.13	18.10	0.93	13.44	0.36	73.82	4.17	4.23	8.40
T_2	2.56	2.03	17.60	0.90	12.91	0.37	72.55	3.93	4.11	8.04
T_3	2.61	2.07	18.10	0.93	13.56	0.35	75.00	4.32	4.53	8.85
T_4	3.17	2.19	18.30	1.00	13.77	0.33	79.16	4.77	5.34	10.11
T_5	3.19	2.25	18.40	1.05	13.80	0.32	81.35	4.98	5.56	10.54
T_6	3.06	2.17	18.20	0.95	13.61	0.34	77.27	4.56	5.02	9.58
T_7	3.34	2.29	19.83	1.11	15.44	0.31	84.85	5.43	5.87	11.30
S. Em±	**0.03**	**0.04**	**0.24**	**0.03**	**0.20**	**0.01**	**1.34**	**0.07**	**0.07**	**0.08**
C.D. at 5%	**0.10**	**0.11**	**0.73**	**0.10**	**0.60**	**0.02**	**4.06**	**0.22**	**0.22**	**0.23**

T_0-Control- water spray, T_1-Zinc sulphate @ 0.5%, T_2- Ferrous sulphate @ 0.5%, T_3- Borax @ 0.3%, T_4- Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5%, T_5- Zinc sulphate @ 0.5% + Borax @ 0.3%, T_6- Ferrous sulphate @ 0.5% + Borax @ 0.3% and T_7-Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5% + Borax @ 0.3%

Table 3: Effect of specific micro nutrient on yield attributes of ber cv. Gola.

Treatments	Fruit weight (g)	yield per (kg/plant)	yield (q/ha)
T_0	16.27	65.74	102.56
T_1	16.80	71.61	111.71
T_2	16.47	68.24	106.46
T_3	16.95	73.77	115.08
T_4	18.01	80.85	126.12
T_5	18.52	83.97	131.00
T_6	17.25	76.02	118.58
T_7	18.59	83.80	130.73
S. Em±	**0.29**	**0.91**	**0.20**
C.D. at 5%	**0.87**	**2.76**	**0.60**

T_0-Control- water spray, T_1-Zinc sulphate @ 0.5%, T_2- Ferrous sulphate @ 0.5%, T_3- Borax @ 0.3%, T_4- Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5%, T_5- Zinc sulphate @ 0.5% + Borax @ 0.3%, T_6- Ferrous sulphate @ 0.5% + Borax @ 0.3% and T_7-Zinc sulphate @ 0.5% + Ferrous sulphate @ 0.5% + Borax @ 0.3%

SHORT COMMUNICATION

Popularization of raised bed method of turmeric cultivation for rhizome rots control

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Receipt: 04.09.2024 Revised: 03.10.2024 Acceptance: 04.10.24
DOI: 10.53552/ijmfmap.10.2.2024.136-139

ABSTRACT

Krishi Vigyan Kendra, Vonipenta had conducted Front Line Demonstration on Turmeric from 2020 to 2022 in Adireddypalli, Settivaripalli, Chapadu, kesalingayapalli, Gangavaram, Viswanathapuram and Kasinayana villages to study the potential yield reduction factors that are mainly due to the diseases and yield difference between the farmers' practice and demonstration. To control rhizome rot seed treatment with metalaxyl + mancozeb @ 2 g/l + monocrotophos @ 1.5ml/l of water followed by soaking in Trichoderma viride @ 5g/l of water. The net returns were higher in raised bed method of cultivation followed by seed treatment and suggested the large area also to undergo this practice.

INTRODUCTION

Turmeric is the major crop which is spread in an area of 6000 acres in the year 2020 to 2022 and it is one of the most important commercial crops being cultivated in the major mandals with an area of 2144 acres in the year 2023 (Report of State Department of Horticulture, Andhra Pradesh). We have observed turmeric package of practices, yield and marketing aspects from the year 2017, but the farmers are facing major problem like Rhizome rot infestation and the yield was reduced with a major loss of more than 60 per cent. So, in order to overcome this problem, we have initiated FLD (Front Line Demonstration) on Raised bed method of turmeric cultivation in the year 2020-2022 and selected villages were Adireddypalli, Settivaripalli, Chapadu, kesalingayapalli, Gangavaram, Viswanathapuram and Kasinayana

Farmers generally grow turmeric by adopting traditional method of sowing in the flat beds. This method results in increased incidence of rhizome rot which increases cost of cultivation, decreases yield and economic returns. Planting method of rhizome influence growth and yield of turmeric. Ridge and furrow method, raised bed method of turmeric cultivation decreases rhizome rot incidence. However, these methods were adopted previously in relatively lesser area of YSR district. So, ICAR, KVK, Vonipenta have conducted trails and demonstrations of turmeric cultivation on raised bed method with drip irrigation in farmers' field to facilitate larger adoption of the practices and for better yield.

Rhizomes rot of turmeric in flat bed method of cultivation

The disease causes root rot and rhizome rot caused by *Pythium aphanidermatum*, resulting in typical rot of rhizomes from October onwards. The affected rhizomes appear soft and shrunken to start with, later dry up and become hard. Foliar yellowing

and drying up of foliage which are the normal symptoms of maturity of the crop during October - November would be indistinguishable from the symptoms of the disease affected clumps. When infected rhizomes are cut open, the infected zones typically appear as dull brown and dark (Choudhary *et al.*,2009). The disease development occurs as the pathogen is facultative parasites and lives as a saprophyte on the organic matter in the soil for several years. It spreads from vulnerable plants and the disease is favored by 35 °C soil temperature, 15-20 percent soil moisture in alluvial or sandy soils. In order to control this rhizome rot infestation raised bed method of planting is practiced. Non adoption of seed treatment, inadequate use of recommended fertilizers and lack of awareness on plant protection measures are the major cause under plant pathology aspects.

Background of improved method of cultivation

Under Horticulture aspects -KVK, Vonipenta has popularized turmeric cultivation on raised bed with drip irrigation system for efficient use of natural resources and prevention of rhizome rot in order to get higher yields and net returns. Each year, the Front line demonstration was conducted in 10 locations covering these four villages. The demonstration on cultivation of turmeric by raised bed method with drip irrigation comprised of cultural, biological and chemical methods. Apart from showcasing the viability of raised bed method, farmers were also sensitized on the relevance of these technologies (Hiremath and Hilli, 2012) by organizing awareness programmes on seed treatment in the farmers' fields during sowing of turmeric, focused group discussions on how important the seed treatment is in reducing the rhizome rot in turmeric, conducting method demonstrations by involving RHWEP (Rural Horticultural Work Experience Programme) students in adopted villages, training programmes on package of practices in turmeric crop and sending timely messages through different Information and Communication Technologies and also through kisan mobile advisories.

Good practices

The FLD was conducted to study the potential yield reduction factors that are mainly due to the diseases and yield difference between the farmers' practice and demonstration. To control rhizome rot seed treatment with metalaxyl + mancozeb @ 2 g/l + monocrotophos @ 1.5ml/l of water followed by soaking in *Trichoderma viride* @ 5g/l of water. Cultivation of Turmeric by raised bed (Paired row with drip) method (convenient length, 20-25cm height, 90cm width, 30cm between two beds for drainage and 45cm between the paired rows) (Nagarjuna *et al*.,2021). Horticulture department officials, Agriculture department officials and ATMA staff are conducting programmes through trainings for VHAs (Village Horticultural Assistants) and VAAs (Village Agricultural Assistants) and also through RBKs (Rythu Bharosa Kendras) they are conducting the programmes.

Challenges

Initially the farmers in Mydukur and Turmeric growing regions, the farmers were not practicing seed treatment as they were thinking it is time taking and increased the cost of cultivation, but after practicing the farmers realized the importance of seed treatment which had a great impact on reduction of rhizome rot by reducing the chemical sprays through FLDs and training programmes.

Benefit and impact

1. Rhizome rot infestation had been reduced
2. Seed treatment also reduced the incidence of rhizome rot
3. Obtaining good yields due to less incidence of rot
4. Cost of cultivation had been reduced as the seed treatment reduced the chemical sprays
5. Fetching higher net returns and the impact nearly reached to 35 to 40 villages directly and indirectly to almost 28 villages through magazine readings etc.

The benefit and impact of raised method of cultivation have been detailed in the following Tables and graph.

Sustainability and scaling up

Use of this raised bed method of turmeric cultivation reduced the incidence of rhizome rot and cost of cultivation which is the need of the hour to be incorporated into the farmers of its importance through awareness, method demonstrations, on and off campus training programs in the adopted villages of KVK (Sunil Kumar *et al.*,2021). This can happen even without KVKs through the line departments of agriculture and horticulture as the KVKs can't reach the entire district.

Lessons learned

- Farmers need more attention after harvesting of the fresh rhizomes for advanced storage facilities next crop and post- harvest management of fresh rhizome.
- They need facilities like turmeric boilers at-least one or two per each village on rent basis through KVK system and this can be provided through other KVKs where turmeric cultivation is mainly promoted.
- Farmers-producer organizations in KVK operational areas are providing services to the farmers through KVKs on trainings and marketing aspects on cultivation and post- harvest management techniques in turmeric.
- NGOs, Coromandel and Grow more companies interacted with the KVK regarding success in turmeric cultivation and they are also taking into other villages also.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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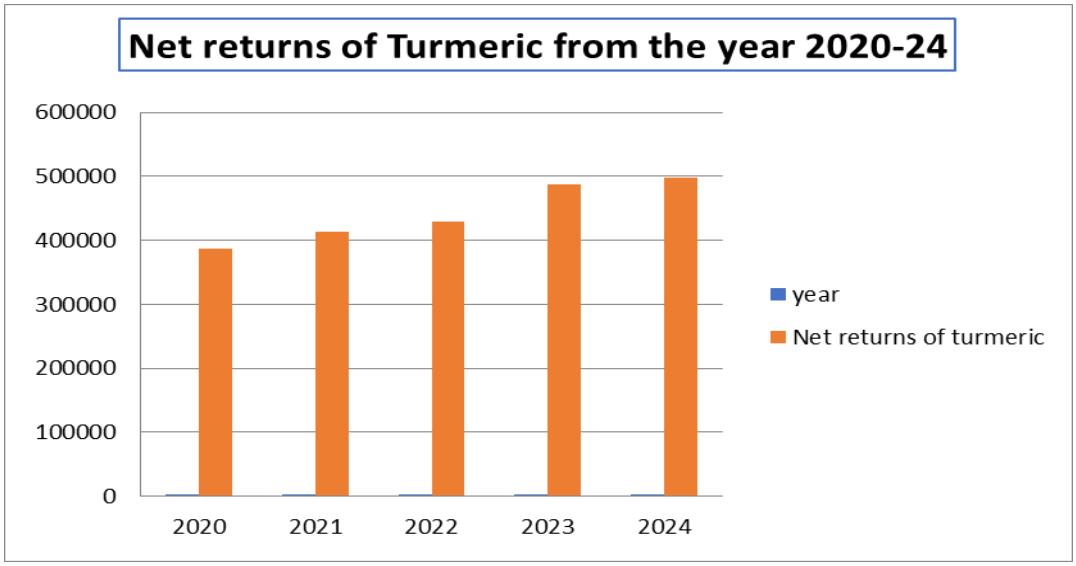
Table 1: Average yield aspects from 2020 to 2022 of the Turmeric crop under FLD

Crop(Turmeric)	Yield (t/ha.)	Cost of Cultivation (Rs./ha)	Gross returns (Rs./ha)	Net returns (Rs./ha)	B:C Ratio
Demonstration	39.6	1,70,500	5,80,800	4,10,300	3.41
Farmer practice	34	1,76,400	5,19,734	3,43,334	2.94

Table 2: Through awareness programmes in the year 2023 and 2024 the yield aspects are as follows

Crop (Turmeric)	Yield (t/ha.)	Cost of Cultivation (Rs./ha)	Gross returns (Rs./ha)	Net returns (Rs./ha)	B:C Ratio
Demonstration	42.3	1,50,000	6,43,500	4,93,500	4.29
Farmer practice	35	1,67,400	5,25,000	3,57,600	3.13



SHORT COMMUNICATION

Influence of ecology factors on the walnut forests of Kyrgyzstan

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Receipt: 11.10.2024 Revised: 29.10.24 Acceptance:31.10.24

DOI: 10.53552/ijmfmap.10.2.2024.140-147

ABSTRACT

The article presents data on the importance of walnut-fruit forests in Kyrgyzstan, the protective and ecological role of natural walnut forests. It describes in detail the influence of abiotic factors and the ecology of walnut. In general, the climatic conditions of the walnut-fruit forest belt are favorable for the growth and development of walnut. However, a number of factors such as spring frosts, summer droughts, abnormally high temperatures in summer due to climate change negatively affect the condition and fruiting of walnut. Information is given on the ecological confinement of walnut and the types of walnut forests. Information is given on the influence of biotic and anthropogenic factors on natural walnut forests. Information is given on the influence of major pests and diseases on walnut forests. Due to pests and diseases of the forest, productivity decreases and the condition of walnut forests worsens. Effective biological measures to combat forest pests and diseases are needed. The solution to the issues of preserving natural walnut forests and their biodiversity is of great scientific and practical importance.

***Keywords**:* Abiotic and biotic factors, natural walnut forests, walnut,

INTRODUCTION

In the belt of walnut-fruit forests of Kyrgyzstan, the main forest-forming species is the walnut (*Juglans Regia* L.). Walnut fruits have high nutritional properties, and the wood is of particular value in the production of furniture and other products. Natural walnut forests growing on slopes of various exposures and steepness, perform soil-protecting functions and prevent erosion processes, play a water-protecting and water-regulating role. It is of great importance for the local population as a source of nuts. In the belt of walnut-fruit forests the great species and form diversity of tree and shrub species attracts many researchers and can serve as a gene pool of world significance (Gan *et al*., 1997). The current state of walnut forests is influenced by a number of factors, in particular anthropogenic factors and the influence of other biotic factors such as pests and forest diseases. Due to anthropogenic factors, there is a decrease in forest biodiversity. Besides, damage by pests and diseases worsens the sanitary condition of walnut forests, the productivity of forests and the quality of fruits decrease. Threats to biodiversity are associated with human anthropogenic activity and include habitat change, fragmentation of natural communities due to overuse, overharvesting, environmental pollution and climate change.

In addition to anthropogenic and other biotic factors, the condition of walnut forests is also affected by abiotic factors such as high summer temperatures, drought,

lack of moisture and low temperatures in winter and spring. Due to climate change, in some years there are abnormally high temperatures in summer and abnormally low temperatures in winter, which affect the growth, development and productivity of walnut and other wild trees. Therefore, a strategy to address the complex problem of preserving Kyrgyzstan's walnut forests will have to restore the ecological functioning and productivity of forests while meeting the socio-economic needs of the population dependent on these resources. Restoring walnut forests on non-forest and degraded lands could combine ecological and socio-economic objectives while reducing the pressure on natural walnut-fruit forests.

Influence of abiotic factors on walnut forests

Walnut growing in different microclimatic conditions of the walnut-fruit forest belt has its own ecological features. Walnut is demanding of light, moisture, warmth and richness of the soil. Due to their light requirements, walnut trees in dense plantings occupy the first tier and form narrow crowns (Fig. 1). In natural conditions on slopes of northern exposures in walnut forests, trees have a high trunk and narrow crowns, as they strive for sunlight and perform photosynthesis functions. Walnut trees growing in the wild, where they are illuminated from all sides, form a powerful crown and bear fruit well. The demand for light is confirmed by the condition of walnut crops created under the canopy of old forests, where they have low survival rates (Nikitinsky, 1970).

Walnut grows and develops well with sufficient soil moisture. This is confirmed by the best growth of walnut trees on northern and north-eastern slopes, since moisture is retained in the soil longer at these exposures than at southern ones. We saw that the demand for richness of walnut soils in the fact that they grow better on black-brown soils, which are very fertile. Individual trees and groups of trees can be found on exposed layers of red sandstone. Walnut trees do not tolerate heavy clay and poorly drained soils.

Walnut is a heat-loving species. The optimum temperature for its development is 26-30^0 C (Kolov, 1985). Resistance of adult walnut trees to low temperatures (-30-32 ^{0} C) and slightly lower is possible only during the period of deep dormancy, *i.e.*, the greatest readiness of the protective and adaptive system to withstand cold. In the autumn-winter period, walnut trees are characterized by varying winter hardiness. In early autumn, trees do not have sufficient resistance to low temperatures, as they have not yet completed hardening. By the end of autumn, the hardening of plants is complete, and they can withstand temperatures below -17 ^{0} C without damage. The critical temperature for stamen buds during the dormant period is an air temperature of -21.5^0 C, for apical buds, growth and pistillate growth buds the critical temperature is -22-23^{0}C. After leaving the dormant period, the resistance of trees, and especially the reproductive organs of the walnut, decreases as the activity of physiological and biochemical processes corresponding to this biological rhythm increases (Richter, 1985). A decrease in frost resistance of plants is observed in March, with a significant decrease in April and May. Walnut trees have the least frost resistance during the growth period. When the temperature drops from -1 to -3 ^{0} C, flowers die, and from -4 to -5 ^{0} C, tender spring shoots die. In natural walnut forests and in plantations there are late-flowering and early-flowering forms of walnut, which are distinguished by a shorter

growing season and are less affected by spring and autumn frosts. Depending on the biological characteristics of the walnut, the sum of active average daily positive temperatures preceding the beginning of the phenological phase of opening of bud scales, or the beginning of bud growth, ranges from 900 to 1200^0 C .

The influence of abiotic factors has both positive and negative character. Favorable climatic conditions of the walnut-fruit forest belt, especially not hot summer, relatively mild winter, sufficient amount of precipitation in winter and spring and accumulation of moisture in the soil have a favorable effect on the growth and development of walnut. However, a number of factors have a negative effect on the condition and fruiting of walnut, these are frequent spring frosts, many cases of severe winter frosts affecting the condition of vegetative and generative organs of trees, even shoots and branches of trees are damaged. In the zone of walnut-fruit forests in spring the air temperature often drops to -1-2 ^{0} C, and in some years the air temperature in the second and third ten days of April drops much lower. For example, in 1999, the air temperature dropped to -6.9 ^{0} C in April and these late spring frosts damaged not only young shoots, but also two-three-year-old branches of walnut. Cases of spring frosts occurred in 2003 and 2005, when in mid-April the air temperature dropped to -3, and the frost damaged the blossoming generative and vegetative buds on walnut trees. These late spring frosts are destructive for fruit trees, especially walnuts, the condition worsens and leads to the absence of a nut harvest.

Summer droughts also affect the growth and fruiting of walnuts. In recent years, the maximum air temperature in summer often rises to 40-43^0 C, which causes a lack of moisture in the soil and leads to premature yellowing and drying of leaves, and unsatisfactory fruiting of walnuts. According to long-term data from the Ak-Terek meteorological station (1750 m above sea level), in the belt of walnut-fruit forests, the average amount of precipitation per year is about 1100 mm. From the presented figure 2 it is clear that the distribution of precipitation has a pronounced seasonal character. The maximum amount of precipitation falls in the winter-spring period (November, March-April), and the minimum - in the summer-autumn (August-September).

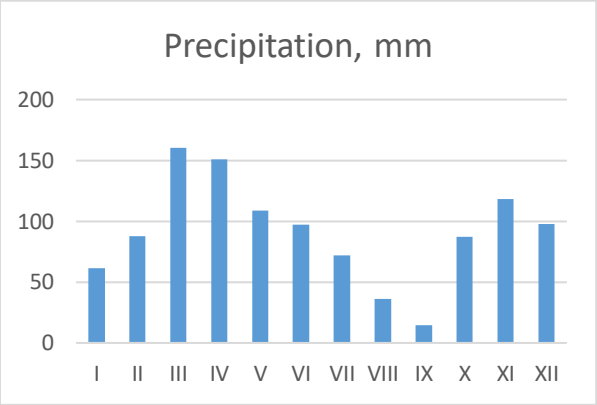

Fig. 2. Average monthly precipitation in the walnut-fruit forest belt (according to Ak-Terek weather station)

The small amount of precipitation in the hot months leads to a lack of moisture in the soil and unsatisfactory growth and fruiting of trees. In some years, there is insufficient precipitation or less than 1000 mm per year and because of this, less moisture accumulates in the soil, which is necessary for plants during the growing season.

Another factor is soil conditions. Walnut grows well on fertile black-brown soils, on slopes with northern exposures, where there is a sufficient humus layer. On other soils, brown and gray soils, where there is less humus and less moisture is retained, there is poor growth and development of walnut trees. On heavy and stony soils, trees need regular watering until late autumn and often become unstable to winter frosts. A decrease in the biodiversity of trees and shrubs in nut plantations leads to a change in the soil structure and the accumulation of moisture in the soil, which has a detrimental effect on the condition of the trees and a decrease in the ecological role of forests.

In early spring, wet snowfalls often cause snowbreaks of trees in old-growth walnut stands, branches and trunks break, and severe winter frosts cause frost cracks (Nurmanbaev, 2008). In snowy years in February and March, snow avalanches are observed on steep slopes, which causes snowdrifts of trees. In the valleys of mountain rivers in spring and summer, mudflows often wash away the banks along with tree and shrub vegetation.

The ecological confinement of walnut allowed researchers to divide natural walnut plantations into different types. Thus, according to previous studies, walnut forests are divided into 14 types (Niktitinsky, 1970). Among all types of walnut forests, the most productive nut plantations are the short-stemmed hazel of gentle slopes. These types of walnut forests have good soil conditions, slopes of northern exposure.

According to research on forest typology (Griza, Venglovsky *et al*., 2008), walnut forests, depending on the different combinations of accompanying species and location, growing conditions, are represented by several types: six clearly distinct types have been identified and their names are presented below.

1. Walnut with short-stemmed grass – type 1
2. Walnut with extra moisture – type 2
3. Walnut with spruce-fir – type 3
4. Walnut with hawthorn – type 4
5. Walnut with maple-apple – type 5
6. Park type walnut forest – type 6

Walnut forests are distributed within the altitude range from 1100 to 2000 (2200) m above sea level and occupy mainly gentle and steep slopes of northern orientation. The distribution of walnut forests up and down the absolute altitude is limited by climatic conditions. The upper limit of natural growth is limited by low air temperatures, the lower limit by insufficient moisture.

At the lower boundary of its growth (1100-1200 м), walnut plantations have an island distribution character and grow in areas with additional moisture. Associated species are usually hawthorn, hackberry, apple; of the shrubs - species of rose hips, honeysuckle, cotoneaster. In the middle part of the belt (1400- 1800 м), walnut forests are dense, highly productive, both pure and mixed. At the upper limit of its distribution (1800- 2000 м), walnut grows with maple, hawthorn, in the shrub layer - Sogdian cherry plum, species of honeysuckle, etc.

Researchers noted that the main object of economic use for obtaining fruits should be short-stemmed hazels of gentle and steep slopes, short-stemmed with additional moisture. In the plantations of these forests, all efforts should be directed at forming well-developed crowns of trees, which will allow obtaining high yields of fruits in the future. Maple-apple, spruce-fir, poplar-ash hazels should be used mainly as protective plantations (Nikitinsky, 1970). However, at present there is widespread use of walnut forests by local populations and all types of walnut plantations are used for economic purposes.

Therefore, taking into account, the forest types and their ecological confinement, it is necessary to take into account the fruit productivity of nut plantations. For example, according to research, the productivity of the short-stemmed walnut on gentle slopes is 174 kg per 1 ha, and the short-stemmed walnut on steep slopes is 4-3 kg per 1 ha (Vinogradov, 1970). According to research on forest typology, the yield of walnut forests has been presented in Fig. 3.

Figure 3 shows that satisfactory yields are observed in Type 1 of gentle slopes and in the Type 2 with additional moisture. In the remaining plantations mixed with spruce, hawthorn, maple and apple, the yield is comparatively low.

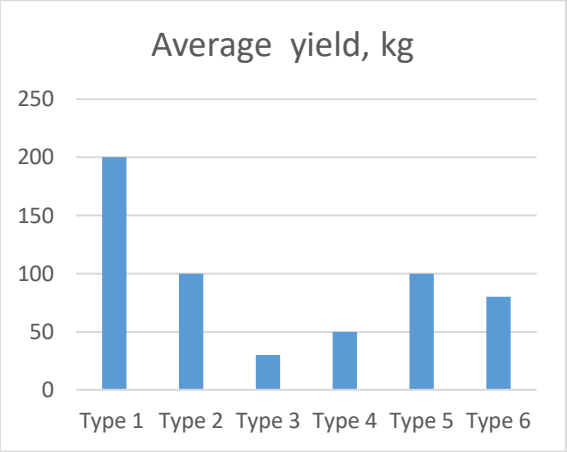

Fig. 3: yield of walnut in different types

It should be noted that the yield fluctuates greatly due to the weather conditions of the year and when determining fruit productivity it is necessary to take into account long-term yield data.

Biotic factors affecting walnut forests

Biotic factors include damage to walnuts by pests and diseases. Walnuts are often damaged by gypsy moths, walnut moths, aphids and other pests. Among the diseases, walnuts are affected by fungal diseases - marsonia , bristly- haired tinder fungus and others. Fungal diseases dramatically reduce the quality of wood. Certain types of stem rot, especially when affecting the sapwood of living trees, lead to their weakening and drying out. Mass development of rot in plantations gradually worsens their sanitary condition. The rate of spread of rot depends on the physical and technical properties of the wood and, mainly, on the biological characteristics of the pathogen, the location of fruiting bodies on the trunk and the number of infection penetration sites. Forest growth conditions also greatly affect the development and spread of fungal pathogens. Climatic factors affect the growth, development, reproduction and spread of fungi. The latter are very demanding of environmental conditions and are usually confined to a certain set of environmental factors.

The main reason for walnut damage by stem rot is mechanical damage to the bark. Intensive temperature increases in the area of walnut-fruit forests in early spring contribute to sunburn of the bark and deterioration of the physiological condition of the tree. Bark damage often occurs at an early age, since the walnut has very thin and delicate bark at this time.

Marssoniasis of walnut (*Marssonina juglandis.* Magn or brown spot): The disease affects leaves, green shoots and fruits. In early or mid-May, small round spots of brown or light brown (later grayish) color with a wide brown border appear on young leaves. The spots often merge. Affected leaves fall off prematurely. Small, sunken, reddish-brown spots form on the ovaries. In damaged areas, the tissue lags in growth, the fruits dry out, crack and often fall off prematurely. Sometimes they rot, the kernel spoils (turns black, dries out) and becomes inedible. Green, non-woody shoots are affected, especially in the nursery.

Bacteriosis of walnut (Xanthomonas juglandis): It affects all varieties of walnut. The disease manifests itself in the form of various kinds of spots on leaves, branches, fruits and inflorescences. On leaves, the spots are small, reddish-brown, often angular. Similar spots are on young branches and fruits. On fruits, the spots gradually increase in size and become brown; later they become depressed and turn black. On young fruits, while the nut shell has not hardened, bacteria can penetrate the kernel, causing rotting. In ripe fruits, the kernel is not damaged. In places of damage, a liquid is released in which bacteria accumulate in large quantities. The pathogen overwinters mainly in the kidneys. The most severe damage Bacterial disease occurs in years with damp and warm springs.

Codling moth (*Sarrothripus muscle* Ersch.). It belongs to the family (Cymbidae). It is widespread in Central Asia and has a great negative economic

significance. The damage to fruits by it reaches 40-50%, and in some areas 80% and more. Nuts damaged by the caterpillars of the first generation fall off completely, the caterpillars of the second generation feed on the pericarp. The fruits have dark spots on the pericarp. To combat the walnut codling moth, use the method of cleaning the peeling bark of trees, removing dried branches.

Gypsy moth (Lymantria dispar L.): The gypsy moth is characterized by pronounced sexual dimorphism: the male and female are very different in appearance. The male has a wingspan of up to 45 mm, with a thin abdomen and feathery antennae; the wings are dark gray or brownish -gray with intermittent dark transverse stripes. The female is almost twice as large as the male (wingspan up to 75 mm) and is lighter in color, with thread-like antennae and a thick abdomen. Life cycle: In the nut-fruit forests of southern Kyrgyzstan and throughout its vast range, the gypsy moth has a one-year generation (Ashimov, 2010). One of the biological control measures against the gypsy moth is the use of the drug Virinensh.

In the Kyrgyz Republic, in works on forest pests, including the gypsy moth, only K.E. Romanenko (according to Ashimov, 2010) has a list of parasites and predators trophically associated with it. A total of 11 species were noted, including the wasps – *Anastatus disparis* Rusch., *Telenomus phalaenarum* Mayr, *Brachimeria intermedia* (Nees), *Dibrachus cavus* Walk., *Pimpla instigator* F., *P. turionellae* L.; *Dermestes lardarius* L., *Malachius bipustulatus* L., *Calosoma sycophanta* L., *Exorista flies larvarum* L. and *Pseudosarcophaga affinity* Fall . The available information is limited to brief data on the biology of parasites and predators, without specifying the degree of infection and destruction of the host at different stages of its development. There is no information on the biology and ecology of most entomophagy, their distribution and numbers in different habitats.

Aphids are a typical common pest that feed on the juice of walnut leaves and buds. Because of this diet, aphids weaken plants and minimize yields. They harm seedlings the most. But they can also have an extremely negative effect on an adult tree, attacking in whole colonies, especially if it has rained. If we do not quickly eliminate aphids, we can lose a significant part of the harvest. We can determine whether a walnut is damaged by aphids by visually inspecting the plant. Such pests look like small, round bodies on leaves and shoots, especially on the back. They can be yellow, light green or black. The presence of aphids is also indicated by a viscous surface of the green mass (insects produce a specific liquid), a change in its usual shape and twisting.

Influence of anthropogenic factors

The condition of walnut forests is also greatly influenced by anthropogenic factors. Currently, the rapid increase in population, expanding the anthropogenic territory due to the growth of settlements in these relict forests, thereby increases the pressure on the forests. And children widespread grazing of livestock due to lack of pastures, haymaking is carried out in the forest (under the forest canopy), high demand for firewood leads to the gradual cutting down of the undergrowth, i.e. shrubs and associated second-tier trees. Research results show that natural regeneration of walnut is very low, young trees do not find enough space for vegetation. These unique forests will have a future if the people living there comply with the standards of their use and protection, and if the political limits of conditions open up a long-term development perspective for the regions.

Various ways of using the forest - collecting nuts and berries, forest pastures, firewood, haymaking and many others - are

integral components of local land use systems. Moreover, these forests are of great importance due to their natural and ecological functions, such as the function of protecting the soil from erosion or the natural water balance. Research has shown that walnut-fruit forests and their products perform a variety of functions for different population groups at different levels. Subsistence concerns and urgent economic interests are currently causing irreversible damage to the forest. For this reason, it is urgently necessary to develop criteria for the restoration, protection and use of unique forests. In doing so, such requirements must be met that are consistent with concern for forest protection. And forms of forest use that are harmful to the forest must be effectively prevented or at least alternatives to them must be identified.

According to forest fund accounting data for 2015 (Anon., 2015), the area of mature and over mature walnut forests is more than 60%, which indicates an increase in old-growth trees in the forest and a deterioration in the condition of natural walnut stands. Effective methods and mechanisms are needed to restore and preserve walnut forests. Failure to comply with and the absence of sanitary and health measures and the difficulties of their implementation in mountainous conditions contribute to the spread of stem and other pests. To a large extent, this is facilitated by the prohibition of sanitary felling and the failure to carry out complex forestry felling of forest care. According to researchers, currently the walnut-fruit forests still have a good chance of receiving the status of a territory recognized by UNESCO as a world heritage site, but if the forest continues to suffer losses, then in a few years or decades these changes will be lost (http://nabu.kg).

SUGGESTIONS

- Taking into account the current state, the prevalence of over mature walnut forests and the lack of natural regeneration of walnut in many areas, it is necessary to pay attention to forest restoration activities and improving the efficiency of silvi-cultural work in the walnut-fruit forest belt.
- When planning forest restoration activities, the ecology of walnut and the ecological confinement of walnut forests should be taken into account.
- The use of more effective biological methods of combating walnut diseases and pests will help preserve the natural environment and obtain organic products that meet international standards.
- Needed to analyze the current state and determine the fruit productivity of walnut forests and plantations in different types and environmental conditions and the impact of climate change.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SHORT COMMUNICATION

The effect of PGRs on growth and yield attributes of sapota cv. Cricket Ball in Chhattisgarh Plains Zone

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Receipt: 13.11.2024 *Revised: 10.12.24* *Acceptance: 12.12.24*

DOI: 10.53552/ijmfmap.10.2.2024.148-155

ABSTRACT

The current study was conducted at the Horticulture Farm, Department of Fruit Science, CoA, IGKV, Raipur (C.G.) in the years 2020–21 and 2021–22.Twenty-year-old sapota cv. Cricket Ball trees were sprayed with varying PGRs doses during two stages, such as 50% flowering and the pea stage of fruit development. This investigation was arranged by utilisingRandomised Block Design, replicated thrice along with twenty five treatments. The findings revealed that plant growth parameters such as length (11.64 cm), girth of new shoots (1.13 cm) and number of leaves shoot^{-1} (28.33 cm), were highest in T_{12} (GA_3 @ 150 ppm at 50% flowering + pea stage *) among all treatments, while the maximum yield (qt-ha^{-1}) was recorded in T_6 (*NAA @ 200 ppm at 50% flowering + pea stage) *27.72 qt-ha^{-1}. The gross return and net return (in rupees ha^{-1}) of Sapota were also recorded highest in T_6 (*NAA @ 200 ppm at 50% flowering + pea stage*) Rs 159045.90 and Rs 114977.89 ha^{-1} but the benefit-cost ratio was highest in T_3 (*NAA @ 100 ppm at 50% flowering + pea stage*) 2.81.*

Keywords: Benefit: cost, CCC, GA_3, gross realisation, growth parameters**,** NAA,

One of the delectable fruits of humid tropical and subtropical regions is the sapota [*Manilkaraachras*(Mill.) Forsberg], is commonly known as chiku in Indian parlance. Nowadays, fruit growers are very much attracted towards the cultivation of sapota; the main reason for this is that it can be grown in different soil and climatic conditions. Sapotaplant areflowers and fruits throughout the year. It gives a single crop in April and May after flowering in summer. Both flowers and fruits suffer from high temperatures. Many civilizations have utilized sapodilla fruit as a traditional indigenous medicine (Lim 2013). When boiled, the unripe fruits, which are rich in tannins, can be used to cure diarrhea. Young fruit extracts have also been shown to alleviate pulmonary issues (Kulkarni*et al.*, 2007). Minerals like potassium, calcium, iron, copper, and zinc, as well as phenolic components, are abundant in the fruits (Kulkarni*et al.* 2007; Mund*et al.* 2016; Sumati and Sivasankar 2017). In India, the cultivation of Sapota is 163.90 thousand hectares and production is 1495 thousand metric tons, with a productivity of 9.1 metric tons per hectare (Anonymous, 2019). In Chhattisgarh, the total area under Sapota is 340 hectares, with an annual production of 1578 metric tons (Anonymous, 2019).

The remarkable expansion in area reveals grower's readiness to embrace this fruit in exchange for significant financial rewards. However, in the plains of Chhattisgarh sapota cultivation faces twin problem of poor agro-climate and self-incompatibility leading to low production. The use of plant growth regulators is an effective method to increase fruit set and reduces dropping of fruit in sapota to increase production. PGR$_S$ (Plant Growth Regulators) are used to control all stages of crop development including plant growth, flowering, fruit set, fruit growth and development and are used at particular stages to have maximum effect. Fruit length, fruit diameter, fruit volume, specific gravity, average pulp weight, average peel weight, number of seeds per fruit, weight of seeds per fruit, and fruit weight in sapota have all been found to increase with varying concentrations of plant growth regulators such as synthetic auxins, gibberellins, and CCC (Bhujbal*et al.,* 2013; Kavyashree*et al.,* 2018; Akshay*et al.,* 2020). Amongst different synthetic auxins, NAA seems to be most useful in terms of fruit setting and fruit retention in some fruits crops (Siriwardana*et al.,* 2019; Godi*et al.,* 2020; Kour*et al.,*2019; Singh *et al.* 2018). Likewise, CCC and GA_3were found to enhance the number of flowers and number of fruits per tree (Agarwal and Dikshit, 2008). Considering beneficial role of PGRs, an experiment was conducted to standardize appropriate stage of application and optimum doses of plant growth regulators on growth and fruit yield of sapota cv. Cricket Ball and To estimate the economics and B: C ratio of different plant growth regulators treatments.

The experiment was carried out at Horticulture Instructional Farm, Department of Fruit Science, College of Agriculture, Indira Gandhi KrishiVishwavidyalaya, Raipur, Chhattisgarh, India, during years 2020–21 and 2021–22. The experimental site is located in the plains zone of Chhattisgarh at 21.25^0 N latitude and 81.63^0 E longitude with an altitude of 289.15 meters above the mean sea level. The South-West monsoon is the source of rainfall. It receives an annual average rainfall of 1200–1400 mm. The maximum temperature goes as high as 42.50°C during summer and the minimum as below 7.0°C during winter months. The soil of the experimental field was clay-loam, which is locally known as Dorsa in the region.Twenty years old sapota plants cv. Cricket Ball was taken for the study. There were twenty-five treatments along with a control and each treatment was replicated thrice in a complete Randomized Block Design. Different concentrations of plant growth regulators were used in the treatments at 50% flowering and pea stage which are shown in Table 1.

Table 1: Different treatment of Plant Growth Regulators

Treatment	Notation	Treatment	Notation
T_0	Control (water spray)	T_{13}	Ethrel @ 500 ppm at 50% flowering + pea stage
T_1	NAA @ 100 ppm at 50% flowering stage	T_{14}	Ethrel @ 500 ppm at pea stage
T_2	NAA @ 100 ppm at pea stage	T_{15}	Ethrel @ 500 ppm at 50% flowering + pea stage
T_3	NAA @ 100 ppm at 50% flowering + pea stage	T_{16}	Ethrel @ 1000 ppm at 50% flowering stage
T_4	NAA @ 200 ppm at 50% flowering stage	T_{17}	Ethrel @ 1000 ppm at pea stage
T_5	NAA @ 200 ppm at pea stage	T_{18}	Ethrel @ 1000 ppm at 50% flowering + pea stage
T_6	NAA @ 200 ppm at 50% flowering + pea stage	T_{19}	Cycocel @ 200 ppm at 50% flowering stage
T_7	GA_3 @ 100 ppm at 50% flowering stage	T_{20}	Cycocel @ 200 ppm at pea stage
T_8	GA_3 @ 100 ppm at pea stage	T_{21}	Cycocel @ 200 ppm at 50% flowering+ pea stage
T_9	GA_3 @ 100 ppm at 50% flowering + pea stage	T_{22}	Cycocel @ 400 ppm at 50% flowering stage
T_{10}	GA_3 @ 150 ppm at 50% flowering stage	T_{23}	Cycocel @ 400 ppm at pea stage
T_{11}	GA_3 @ 150 ppm at pea stage	T_{24}	Cycocel @ 400 ppm at 50% flowering + pea stage
T_{12}	GA_3 @ 150 ppm at 50% flowering + pea stage		

During both the years (2020-21 and 22), the spraying took place during the third week of August when the flowers were 50% fully bloomed and during the last week of September when the pea stage was just started. Statistical analysis of different data was carried out using MS-Excel and OPSTAT (online statistical analysis software) for each observed character under study. Data investigation was analysed using randomised block design (RBD), with each treatment replicated three times with the help of the book by Gomez and Gomez (1984).

Length of new shoots (cm): The length of new shoots in sapota obtained from different treatments was significantly different among the treatments (Table 2). New shoots in T_{12} (11.64 cm) had the highest length, which was prominent among all treatments, while T_0 (8.13 cm) recorded the lowest new shoot length. Increased shoot length in GA_3 treatment may be due to faster elongation, increased cell division, and growth. Similar findings have been obtained in Sapota in this regard (Sahu*et al.*, 2022; Patil*et al.*, 2011; Mishra *et al*. 2023; Datta*et al.*2024).

Girth of new shoots (cm): The girth of new shoots measured was varied among the treatments (Table 2). Maximum (1.13 cm) was recorded from T_{12} GA_3 @ 150 ppm treatment at 50% flowering + pea stage, followed by T_9, T10, T11, T_6& T_7 (1.08, 1.05, 1.04, 1.01 & 0.97 cm), respectively. However, minimum girth (0.26 cm) was observed under T_0 (control). This may be due to the effect of GA_3, which induces cell division, cell elongation, and cell growth and also helps in the synthesis of proteins, including various enzymes, thereby increasing the rate of cell growth and photosynthetic capacity. Sahu*et al. (2023)*; Bhujbal*et al.* (2013); and Akshay*et al.* (2020) have also obtained similar findings in Sapota.

Number of leaves shoot^{-1}: The effect of PGR_S in the experiments also showed a positive and significant difference in the number of leaves shoot^{-1} of sapota trees treated with different treatments, as shown in Table 2. At 50% flowering + pea stage, the significantly highest number of leaves shoot^{-1} was observed under GA_3 at 150 ppm (28.33 cm), which was found to be a non-significant difference when compared between treatments T_9, T_{10}, and T_{11} and T_8 and T_7, and the number of leaves shoot^{-1} was (27.83, 27.50, and 27.16), respectively. The lowest number of leaves shoot^{-1} was observed to be 21.66 under control. This is possibly due to the association of the apical meristem with leaf initiation, which is affected by growth inhibitors. The results are consistent with earlier work obtained in Sapota by Sahu *et al.* (2023); Kour *et al.* (2019); Singh *et al.* (2018); and Chavan *et al.* (2009).

Yield (qt-ha^{-1}): The variation in fruit yield qt-ha^{-1} of twenty-five different treatments in Sapota trees cv. Cricket Ball was shown in the Table 3. Significantly, the highest fruit yield of 22.72 kg was observed under NAA treatment at 200 ppm at 50% flowering + pea stage (T6), followed by T_{12}, T9, and T_3 (21.55, 21.40, and 21.01 qt ha^{-1}). While the lowest yield (13.91 qt-ha^{-1}) was recorded under untreated control. This may be due to the fact that NAA have reduced the flower drop and promoted fruit set and fruit retention, which ultimately resulted in higher yield. These findings are aligned in the line of Rehman*et al. (2018);* Bagul*et al. (2021);*Siriwardana*et al.* (2019); Kour*et al.* (2019) and Patil*et al.* (2011) in fruits crops.

Gross realization (Rs.-ha^{-1}): Significantly higher gross realization of Rs. 159045.90 ha^{-1} was noted under the NAA treatment at 200 ppm at 50% flowering + pea stage (T_6) while the lowest gross realisation of Rs 97424.61 ha^{-1} was recorded under untreated control (Table 3). The above findings are largely in

agreement with the results obtained in Sapota (Singh *et al.* (2020) and Joshi *et al.* 2016).

Net realization (Rs. /ha): The net realization of different treatments that are applied in sapota treatments in the present study has shown significant variation (Table 3). The NAA treatment at 200 ppm at 50% flowering + pea stage (T_6) had higher net realization of 114977.89 Rs-ha^{-1} during both the years. The minimum net realization of 64396.60 Rs-ha^{-1} was found under control. Similar findings obtained by Desai *et al.(2017),* Jain *et al. (*2020), and Kavyashree *et al. (*2018) in Sapota.

Benefit: cost ratio: Maximum benefit cost ratio 2.81 was noticed under the treatment NAA @ 100 ppm at pea stage, which was found at par with the treatments T_1, T_7, T_5 &T_2 and T_7, T_3, T_5& T_{23} and T_7, T_8& T_5 having benefit cost ratio 2.87, 2.87, 2.86 & 2.81 and 2.71, 2.68, 2.68 & 2.67 and 2.79, 2.78 & 2.77, respectively. However, the minimum benefit: cost ratio of 1.95 was noticed under the control. The above findings are in close conformity with the results obtained by Sahu*et al*. (2018); Singh *et al*. (2018); Mishra *et al.* (2023); Kumar *et al.* (2024); Kaur and Singh (2024) in different fruit crops.**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2: Effect of foliar spray of different concentrations of plant growth regulators on length of new shoots (cm), girth of new shoots (cm) and numbers of leaves shoot^{-1} of sapota cv. Cricket Ball (pooled data of 2020–21 and 2021–22)

Treatments	Length of new shoots (cm)	Girth of new shoots (cm)	Number of leaves shoot^{-1}
T_0 - Control (water spray)	8.13^{k}	0.267^{g}	21.66^{l}
T_1 - NAA @ 100 ppm at 50% flowering stage	9.75^{ghij}	$0.67^{abcdefg}$	25.33^{defgh}
T_2 - NAA @ 100 ppm at pea stage	9.65^{hij}	0.59^{bcdefg}	24.16^{hij}
T_3 -NAA @ 100 ppm at 50% flowering + pea stage	10.31^{defgh}	0.89^{abcd}	25.66^{defg}
T_4 -NAA @ 200 ppm at 50% flowering stage	10.03^{efghij}	$0.75^{abcdefg}$	24.16^{hij}
T_5 -NAA @ 200 ppm at pea stage	10.44^{cdefg}	0.86^{abcdef}	24.83^{efghij}
T_6 -NAA @ 200 ppm at 50% flowering + pea stage	11.05^{abc}	1.01^{ab}	26.16^{cde}
T_7 - GA_3 @ 100 ppm at 50% flowering stage	10.65^{bcde}	0.97^{ab}	27.16^{abc}
T_8 -GA_3 @ 100 ppm at pea stage	10.39^{cdefg}	0.95^{abc}	27.16^{abc}
T_9 -GA_3 @ 100 ppm at 50% flowering + pea stage	11.15^{ab}	1.08^{ab}	27.83^{a}
T_{10} -GA_3 @ 150 ppm at 50% flowering stage	10.89^{bcd}	1.05^{ab}	27.50^{ab}
T_{11} -GA_3 @ 150 ppm at pea stage	10.89^{bcd}	1.04^{ab}	27.50^{ab}
T_{12} -GA_3 @ 150 ppm at 50% flowering + pea stage	11.64^{a}	1.13^{a}	28.33^{a}
T_{13} -Ethrel @ 500 ppm at 50% flowering + pea stage	8.64^{k}	0.44^{defg}	23.66^{ijk}
T_{14} -Ethrel @ 500 ppm at pea stage	8.71^{k}	0.46^{cdefg}	22.50^{kl}
T_{15} -Ethrel @ 500 ppm at 50% flowering + pea stage	9.49^{j}	0.60^{bcdefg}	24.50^{ghij}
T_{16} -Ethrel @ 1000 ppm at 50% flowering stage	9.69^{hij}	$0.64^{abcdefg}$	23.83^{ij}
T_{17} -Ethrel @ 1000 ppm at pea stage	9.53^{j}	$0.78^{abcdefg}$	23.50^{jk}
T_{18} -Ethrel @ 1000 ppm at 50% flowering + pea stage	10.04^{efghij}	0.88^{abcde}	24.66^{fghij}
T_{19} -Cycocel @ 200 ppm at 50% flowering stage	9.58^{ij}	0.38^{efg}	25.00^{defghi}
T_{20} -Cycocel @ 200 ppm at pea stage	9.68^{hij}	0.31^{g}	25.33^{defgh}
T_{21} -Cycocel @ 200 ppm at 50% flowering+ pea stage	10.26^{defghi}	0.35^{g}	26.00^{cdef}
T_{22} -Cycocel @ 400 ppm at 50% flowering stage	10.23^{defghi}	0.31^{g}	25.83^{defg}
T_{23} -Cycocel @ 400 ppm at pea stage	9.89^{fghij}	0.36^{fg}	25.33^{defgh}
T_{24} -Cycocel @ 400 ppm at 50% flowering + pea stage	10.44^{cdef}	0.34^{g}	26.33^{bcd}
SE(m)±	**0.126**	**0.06**	**0.48**
CD at 5%	**0.360**	**0.18**	**1.37**

Table 3: Effect of foliar feeding of different concentrations of plant growth regulators on yield (kg-tree^{-1}), gross realization (Rs./ha), net realization (Rs./ha) and benefit: cost ratio of sapota cv. Cricket Ball (pooled data of 2020–21 and 2021–22)

Treatments	Gross realization (Rs./ha)	Net realization (Rs./ha)	Benefit:cost ratio	Yield (qt-ha^{-1})
T_0 - Control (water spray)	97424.61^{x}	64396.60^{x}	1.95^{d}	13.91^{m}
T_1 - NAA @ 100 ppm at 50% flowering stage	133353.79^{l}	97565.74^{l}	2.72^{ab}	19.05^{efgh}
T_2 - NAA @ 100 ppm at pea stage	133602.70^{k}	97814.72^{k}	2.73^{ab}	19.08^{efg}
T_3 -NAA @ 100 ppm at 50% flowering + pea stage	147113.79^{d}	108565.79^{b}	2.81^{a}	21.01^{bcd}
T_4 -NAA @ 200 ppm at 50% flowering stage	138808.29^{j}	100260.29^{j}	2.60^{abc}	19.83^{def}
T_5 -NAA @ 200 ppm at pea stage	145564.90^{e}	107016.89^{d}	2.77^{a}	20.79^{bcd}
T_6 -NAA @ 200 ppm at 50% flowering + pea stage	159045.90^{a}	114977.89^{a}	2.60^{abc}	22.72^{a}
T_7 - GA_3 @ 100 ppm at 50% flowering stage	141569.09^{f}	104281.10^{f}	2.79^{a}	20.22^{cde}
T_8 -GA_3 @ 100 ppm at pea stage	141062.40^{g}	103774.39^{g}	2.78^{a}	20.15^{cde}
T_9 -GA_3 @ 100 ppm at 50% flowering + pea stage	149840.70^{c}	108292.70^{c}	2.60^{abc}	21.4^{bc}
T_{10} -GA_3 @ 150 ppm at 50% flowering stage	140895.29^{h}	101477.29^{i}	2.57^{abc}	20.12^{cde}
T_{11} -GA_3 @ 150 ppm at pea stage	141085.90^{g}	101667.89^{h}	2.57^{abc}	20.15^{cde}
T_{12} -GA_3 @ 150 ppm at 50% flowering + pea stage	150861.40^{b}	105053.39^{e}	2.29^{abcd}	21.55^{b}
T_{13} -Ethrel @ 500 ppm at 50% flowering + pea stage	106106.39^{w}	72103.38^{w}	2.12^{cd}	15.15^{lm}
T_{14} -Ethrel @ 500 ppm at pea stage	108053.89^{v}	74050.90^{v}	2.17^{bcd}	15.43^{l}
T_{15} -Ethrel @ 500 ppm at 50% flowering + pea stage	114606.20^{t}	79628.18^{t}	2.27^{abcd}	16.37^{kl}
T_{16} -Ethrel @ 1000 ppm at 50% flowering stage	114026.70^{u}	79048.71^{u}	2.26^{abcd}	16.29^{kl}
T_{17} -Ethrel @ 1000 ppm at pea stage	117183.10^{r}	82205.11^{s}	2.35^{abcd}	16.74^{jk}
T_{18} -Ethrel @ 1000 ppm at 50% flowering + pea stage	130812.39^{m}	93884.38^{n}	2.54^{abc}	18.68^{fgh}
T_{19} -Cycocel @ 200 ppm at 50% flowering stage	119724.79^{q}	85316.81^{q}	2.48^{abcd}	17.1^{ijk}
T_{20} -Cycocel @ 200 ppm at pea stage	116870.89^{s}	82462.88^{r}	2.39^{abcd}	16.69^{jk}
T_{21} -Cycocel @ 200 ppm at 50% flowering+ pea stage	127412.89^{o}	91624.86^{o}	2.56^{abc}	18.2^{ghi}
T_{22} -Cycocel @ 400 ppm at 50% flowering stage	124563.50^{p}	88775.50^{p}	2.48^{abcd}	17.79^{hij}
T_{23} -Cycocel @ 400 ppm at pea stage	130497.60^{n}	94709.59^{m}	2.64^{abc}	18.64^{fgh}
T_{24} -Cycocel @ 400 ppm at 50% flowering + pea stage	139941.59^{i}	101393.60^{i}	2.63^{abc}	19.99^{de}
SE(m)±	**3,274.298**	**3274.612**	**0.087**	**0.46**
CD at 5%	**9,339.180**	**9340.076**	**0.250**	**1.33**

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Place of Publication	Green Tower, 2nd Floor, Flat No C/6, 3 No. Priyanath Chatterjee Street, Belghoria,Kolkata-700 056, West Bengal, India.
Periodicity of publication	Two issue per year (June and December)
Printer's name	Shri Dipankar Sarkar
Whether citizen of India	Yes
Address	B-17/35 (S), Kalyani, Dist. Nadia, West Bengal, India, PIN 741235
Publisher's name	Satyanarayan Ghosh
Nationality	Indian
Address	Green Tower, 2nd Floor, Flat No C/6, 3 No. Priyanath Chatterjee Street, Belghoria,Kolkata-700 056, West Bengal, India.
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