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Review article

A review of *Annona* species in Sri Lanka

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ABSRACT

Sri Lankan is a country with an agrarian based economy, distinct number of annona species can be grown in Sri Lanka, as the country has different agro ecological zone. Among different species Annona muricata, Annona squamosa, Annona reticulate are the commercially available ones with prominent cultivars. Many countries considered it as an underutilized crop while it also considered as one of minor fruit species in Sri Lankan condition. It contains phytochemicals such as alkaloids, flavonoids, phenolic compounds, several pigments, essential oils, fatty acids and vitamins. Those chemicals demonstrate broad spectrum activities like anti-microbial, anti-fungal anti-oxidant, anti-inflammatory, anti-diabetic and the anti-tumour activity. Researchers should be focused on the medicinal value characterization and the genetic conservation of the available annona gene pool in the country. This review article deals about the morphological properties, physiological properties,phytochemicals,medicinal property and multiple uses of the different annona species in Sri Lanka. This review aimed to create effective awareness about different annona varieties in country, their medicinal, nutritional values and importance of conservation measures. Many of these species are significant for local food and nutrition security other than use as a traditional medicine.

***Keywords*:** Annona species, bioactive compound, medicinal plant, minor fruits, plant morphology, underutilised crops

INTRODUCTION

Contribution of the neglected and underutilized crop species are important for the economy of the country and ensure the food security and the nutrients requirements of population in the world. Sri Lanka has nearly more than 60 underutilized fruit crops (Dhanayaka, 2015) which are rarely found in market and commercial level cultivation. Other than direct consumption of these underutilized fruits, there are huge potential to prepare the different preserved and value added food with higher shelf life with huge medicinal value. Genus *Anonna* (belongs to Annonaceae family) consists of 109 species worldwide. Among them only a few species are used as the commercial cultivars, such as *A. muricata, A. squamosa*, *A. reticulata* and few hybrid species like *A. cherimola, A. diversifolia* (Abdulrahman and Kumar, 2015). Many counties *Anonna* spp are growing in small scale, as cottage cultivation. Because of the factors such as climatic condition, poor agronomic conditions, and limited knowledge about harvesting, post-harvest handling and processing techniques. Specified agro climatic requirements (altitude, temperature, RH and the soil characters) restricted *Annonaceae* family plants to grow in some regions of the world. Sri Lanka is a country with diverse climatic conditions covering 46 agro ecological zone, many *Annona* species can be grown. According to the Department of Agriculture, Sri Lanka, there are 6 *Anonna spp* are cultivated as a commercial scale (Dilrukshi and Abhayagunasekara, 2020).

Annona is a tree or shrub, evergreen or semi deciduous with a yearly producing pattern (Ross and Ross, 2003). In few countries *Annona* is grown for commercial purposes, even though it's considered as the underutilised and non-neglected fruit, with multiple uses. Fruit flesh is edible, rest of the plant parts can used for pharmaceutical purposes, health product, food supplement, cosmetic preparation etc. (Sandeep and Mittal, 2017). Since it contains number of naturally occurring phytochemicals with different biological properties and bio-active compounds responsible for the different treatments including muscular strength, cooling and reducing the burning sensation, enrich the blood supply. Leaves are important as anti-helminthic medicine (Sudip *et al*., 2014), which are important for the treatment of certain pathogen (Ross and Ross, 2003).

Table 1: Common names and botanical names of the different *Anonna* spp.

Vernacular name	Common name	Botanical names
Cherimoya	Cherimoya	*Anonna cherimoya* L.
Katuanoda	Sour sop	*Anonnamuricata* L.
Welianoda	Bullock's heart	*Anonnareticulata* L.
Sinianoda	Sugar apple, Sweet sop	*Anonnasquamosa* L.
Welatha	Pond apple	*Anonnaglabra* L.

(Pinto *et al.,* 2005)

Annona considered as one of the important minor fruits and possesses high nutritional properties same as other underutilized fruits as reported by Kalkame *et al.*(2018). It is considered as an excellent sources of vitamin C (Padmini *et al.,* 2017) while its flesh contain more than 58 % of sugar on dry weight basis (Sudip *et al*., 2014). Fresh fruit is used for preparation of many beverage types such as wine, jellies, jams, fruit-butter like preserved, value added fruit items with long shelf life. *Anonna* plant species can be adapted to the hard climatic conditions and poor soil condition,so it can be recognized as a plant that is adopted for the future food production under the harsh climatic conditions.

Seed consist of hard outer cover with wax coating. It creates great barrier to the water intake for initiate the germination (Ferreira *et al*., 2016) hence seeds take much time for the germination. Seeds show more dormancy period of 6-8 months with regards to its anatomical and physiology of the embryo. Even the environmental conditions are favoured, seed germination get delayed because of the anatomical and physiological dormancy. Other than the physical and chemical methods, the plant hormones are one of another effective method to speed up the germination process by overcoming the dormancy. According to Chagas *et al.* (2013) by soaking seeds in Gibberellic acid at 50 -70 mg L^{-1} for 12 hours is the more effective way to enhance the germination process. According to the Dresch *et al.* (2014), dormancy can break by applying GA_3. GA_1, GA_2, GA_7 which are biologically active compound,turn down the time spend for seed germination. Other than that cold scarification, storage times are effective to reduce the dormancy period (Dresch *et al*., 2014). According to the Dresch *et al.* (2014), seed germination percentage can be enhances by store seeds in a 5^0C (34% RH) for 30 days and immerse in Gibberellinacid is very effective comparative to the other seed treatments.

There are few researchers focused on leaves but seeds and roots are still poorly studied. Aim of this review is aware the peoples about the available *Anonna* spp in the Sri Lanka, nutritional and medicinal properties and pay the attention to researchers to study in poorly studied area.

Species in Sri Lanka

In Sri Lanka, major grown Annona species are *Annona muricata, Anona reticulata, Annona squamosa* and rarely available hybrid spp. called Cherimoya (Encina *et al*., 2014).

1. *Annona muricata* / katuanoda: Different names are used to introduce *A.muricata*, such as Soursop, Katuanoda, Graviola and Gunabana. It's a kind of small plant with 5-8 m height and has an extensive root system. Branches are formed with the shape of inverted cones (Moghadamtousi *et al.,* 2015). As this plant is a small and early-bearing habit which can be used as intercropping with larger fruit trees, like mango, avocado etc. Leathery featured leaves are present with ovate to elliptical shape. Flower is protandrous, large and light green in appearance. Petals are 6 in 2 whorls and outer whorls have three triangular sepals with ovate to acute shape. They are thick, fleshy and fitting together at the edges of petals. Narrow petals are present in the inner whorls, smaller and concave with the shape of finger nail. Numerous stamens are present with the shape of shield. Number of stigma present around the one ovule consists of a sticky stigma. As a result, pollinators are attracted

1.1

1.2

1.3

Figure 1.1: *A. muricata* leaf with shine leaf blade, Figure 1.2: *A. Muricata* plant, Figure 1.3:*Annona muricata* fruit,

to the plant and lead to pollination. Fruits are heart in shape, consisting of a number of carpel covered with fleshy, about 1.5mm length, pulp is white and juicy that is covered with its seeds. Fruit consists of 67.5% edible pulp, 20% peel, 8.5% seeds. Sugar constitute about 68% of the total solids. The most desirable feature is the extremely pleasing fragrance and flavour of the *A. muricata* fruit pulp (Ross, 2003).

A. muricata contains thirty seven phenolic compounds. Mainly seeds and pulp shows the more vitamins and carotenes and also thirty seven volatile compounds have been found other than eighteen essential oils. *A. muricata* is highly used for the treatment of cancer cells. It increases the existing ability of the non-cancer cells in the body. Higher amount flavonoids present and important to inhibit the cells proliferation and supress the migration of the cancer cells. Which induces the reaction with reactive oxygen species (ROS). *A. muricata* leaf extraction examined against the gastric injuries and demonstrated that toxicity provided against the worms in the human digestive system (Moghadamtousi *et al.,* 2015).

This is the widely available *anonna* spp in the Sri Lanka. Within the different agro climatic zones in the country, a number of *A. muricata* accession have been identified (Wahab *et al.,* 2011). Under the local condition many research focus on the nutritionals, medicinal and bio active compound of different plant parts of a muricata other than improvement on the germ plasma.

2. *Annona reticulate:* It is known as bullock's-heart, normally can be seen in the home garden of the low country wet zones. Plant height is around 6 - 7.5 m,with numerous lateral brunches. The plant comparatively less drought-tolerant than other *Annona* species. Upper leaves shine more than the lower leaf surfaces and have thin hair like structures. Leaves are deciduous, form an alternative pattern, shape is oblong or narrow-lanceolate and have a bad smell. Two to four flowers present with heart in shape, flowers rarely open, appear as clusters, have good fragrant and slender with 3 outer fleshy, consist with narrow petals, externally light-green and pale-yellow with a dark-red or purple inside at the base (Moghadamtousi *et al*., 2018). Fruits are heart shaped; turn to yellow when turning to the ripening stage. When fruit turn in to the well ripening stage, white colour powder is appear in outer appearances.The seeds contain a hard outer ring, oval shape with shine exocarp. Around 75 seeds can be found in a matured fruit. There is a thick, cream-white layer of flesh beneath the skin. In each segment there is a hard, single, dark-brown to black and oblong shape, smooth seed nearly half an inch long (Moghadamtousi *et al*., 2018 ; Yapwattanaphun *et al*., 2011).

Figure 2.1: A. reticulate fruit

Figure 2.2: A. reticulate leaves

Figure 2.3:*Anonna* reticulate plant

Figure 3.1: Plant

Figure 3.2: Leaf

Figure 3.3: Fruit and Figure 3.4: Seeds of *A. squamosa*

Plant contains numerous alkaloids. steroids, flavonoids, tannins, glycosides, phenolic compounds, amino acids, carbohydrates and proteins and the minerals like Ca, P, K, Mg, Na, Cl, S, Fe, Cu, Se, Co and Cr (Inkoto *et al.*, 2018). Nutritional content of the fruit is reported as nearly 17% sugar, 1.6% protein, and 0.26% fat. The fruit is a good sourceto enrich the blood, increase the muscular strength, traditionally which is used for the different aliment like diabetes, heart stokes, worm infection, internal parasite, constipation (Coria-Téllez *et al.,* 2018). Leaves are used as an anti-helminthic medicine.

3. *Anonna squamosa*: Small plant with 3-7 m height, and it's a kind of deciduous plant with irregular branching habits. Inner barks are lighter yellow than outer bark.This can be easily grown with minimum care and is easily grown in the hot and dry climatic condition under any soil condition. Leaves are light green colour with lanceolate in shape with invisible hairs. Top portion of the plants shows leaves with dull green to dark green colour appearances, while the bottom part of the plant leaves appears as pale blue green appearances. Flowers appear as clusters with 2-4 flowers with good fragrance and greenish white in colour during the January to May and fruits are produced during July to August. Sepals are about 16mm long and 6mm width, hairy and pointed. The shape of fruits normally round to heart. When fruit begins to ripe it turns into a greenish yellow colourand form white colour powdery sooty around the exocarp. Flesh is edible, sweet in taste, smooth and white in colour. Flesh is divided into carpel along the central axis. Each endocarp consist of seed with shiny outer appearance and oblong shape with 1.3- 1.6cm in size range (Orwa *et al.,* 2009).

Different plant parts are used for conventional medical purposes and the pharmaceuticals such as heart ailments, diabetes, hyperthyroidism. *A. squamosa* traditionally used for the treatment of epilepsy, diarrhea, worm infestation, constipation, hemorrhage, fever and thirst (Abdulrahman and Senthil Kumar, 2015).

4. *Annona senegalensis* (wild soursop): It's not highly domesticated spp that can be found in

the rural areas. Kind of small semi deciduous shrub or tree with rough bark with greyish, black in colour. Leaves are ovate to oblong in shape with rounded apex, lower surfaces of the leaf consist hair like brown colour structure, while upper surface is smooth.

Flowers have long stalk, green colour and have a specific fragrant. Fruit are globose or subglobose form, flesh is white to yellow with pleasant aroma and have many number of seeds in one fruit. Bark is used for preparation of different types of dye, and wood is prominently use for preparing different hand tools.

CONCLUSION

Annona spp is consider as a minor fruit with number of vitamins, minerals, bio active compounds, antioxidants and different plant base medicinal compounds. It have potential to develop the medicines and drugs for different auruvedic treatments with broad spectrum. As a Sri Lanka is a country, rich in different climate, contain higher number of *annona* accession with endemic values. But at the present condition, studies are limited to the few of selected accessions. *Annona* fruit consist with sweat pulp, which have greater potential to further process. Post-harvest management, value addition, research on mass propagation method and effective conservation measures are few of approaches that should be essential to further address.

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Antimitotic effect of *Verbascum sinuatum* L. extracts on meristematic cells

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ABSTRACT

Verbascum sinuatum L. is a medicinal plant of the Scorphuliaceae family, widespread in Algeria. It is rich in bioactive molecules, which gives it various biological effects. This study targeted the fraction of alkaloids. We studied the effect of this fraction on the mitosis of meristematic cells. Different indices of genetic alterations were calculated. These results were compared to the negative and positive control. An ANOVA type statistical study ($p< 0.05$) was performed. The amount of alkaloid found in the studied fraction is 50.17µg/g of plant powder. This targeted fraction presents a significant antimitotic effect the cells of onion. This mitodepressive effect generated several abnormalities in chromosomes, nuclei and cells represented mainly by agglutinations and chromosomal fragmentations.

Keywords: *Allium cepa* L., *Verbascum sinuatum* L., alkaloids, antimitotic, genotoxic.

INTRODUCTION

Verbascum sinuatum L.,is also called the sinuous mullein. This biennial herbaceous plant of the family Scorphulariaceae is widespread in the Mediterranean area. They are 80 to 200cm tall plants with sinuous basal leaves and yellow flowers (Qureshi and Bhatti, 2008). Phytochemical studies of the genus *Verbascum* have isolated and characterized a large number of bioactive molecules like (Sarralheiro *et al.,*2020) in their study on the identification of metabolites of *Verbascum betonicifolium* L. identified mainly iridoids, glycosides and flavonoids. This richness in bioactive molecules makes this genus a reservoir of medicinal plants widely used in phytotherapy in many treatments. Priyanka and Ghosh (2016) established the relationship between plants for therapeutic use with physicochemical properties of soils. The biological effects of different species of Verbascum have been of great interest. The work of (Yagmur *et al.,* 2019) on leaf extracts of *Verbascum exuberans* L. revealed an anti-inflammatory effect by suppression of TNF α and interleukin 1- beta production. Furthermore, leaf extracts of *Verbascum siniatucum* L. have an effect on the viability of *Trypanosoma congolense* and may present a solution in pest control (Mergia, 2016). Many other biological effects such as antioxidant and hepatoprotective effects have been reported (Grygor *et al.,* 2013).

Alkaloids are nitrogenous compounds of very variable structure and essentially of plant origin. There is an interest in the therapeutic use of these bioactive molecules. Thus, in the face of managing the spread of the corona virus, studies have targeted the antiviral action of alkaloids (Yejin *et al.,*2021) have highlighted the action of gemcitabine, oxysophoridine and lycorine on the proliferation of the corona virus. The alkaloids derived from spermine are the most frequently met in the genus *Verbascum.* They present, in general, a macrocyclic skeleton constituted most often, by a hydroxystrilamine group, a typical amino acid and a â hydroxyamino acid. The biological effects are closely related to the structure of these biomolecules. They have mechanisms of action and molecular targets closely related to their structure (Sarralheiro *et al.,*2020). Their main targets are the microtubules of the mitotic spindle and topoisomerase I and II. As such, they are potential candidates in the development of new molecules in chemotherapeutics in the treatment of various cancers (Imperatore *et al*., 2014).

In this context, we were interested in evaluating the effect of *Verbascum sinuatum* L. leaf alkaloids

on the division of root cells of *Alluim cepa* L. harvested in northern Algeria (Bejaia). To our knowledge, the mitotoxic and genotoxic effects of leaf alkaloids *of Verbascum sinuatum* L. isolated in Algeria have not been reported. Our study is based on the *Allium cepa* test. It allows, on the one hand evaluating chromosome abnormalities in cells of onion and on the other hand to highlight disturbances of mitosis (Ma *et al*., 2005). This assay is widely used assessment of genotoxicity of natural substances in the environment. It is based on the use of meristematic cells of *Allium cepa* L., to view the damage and disruption of cell division (Olorunfemi *et al.*, 2012).

MATERIALS AND METHODS

Sampling

Verbascum sinuatum L., leaves were collected in the region of Addekar in Sif El Hammam in the region of Bejaia (Algeria). They were dried in a dry place and then reduced to powder. The vegetable powder obtained was kept at 4°C until use.

Preparation of the alkaloid fraction

The extraction of the alkaloid fraction is based on their solubility difference according to the pH, according to the protocol of (Harbonne,1998) with some modifications. 5g of *Verbascum sinuatum* L. plant powder was defatted with 10ml petroleum ether for 24h at 25°C. The defatted plant powder was soaked as in methanol and then filtered. After drying we proceed to the solubilization in chloroform acidified at pH 3. After decantation, the acid phase is added with 10 ml of chloroform then adjusted to pH 9 with Na_2CO_3. After evaporation at 60° C., the dry residue obtained is stored at 4° C. until use. During its use, this fraction is dissolved in physiological water.

Antimitotic test

This test is based on the work of (Aashiq *et al*., 2016), onion bulbs (0.5 to 1 cm) are put in distilled water for 3 days at 37°C then root apices were contacted with the alkaloid fraction at 1mg/ml. After one day of incubation, roots apices are fixed with acetic acid and 95% methanol (1:3 V/V). The fixed roots are stained with acetic carmine. The samples are observed under an optical microscope at magnification (X400).We count the different stages of cell division. Aberrations are counted out of 350 cells

Alkaloid assay

Leaf alkaloid content is estimated according to the protocol of Patel et *al*. (2015). This assay is based on the ability of alkaloids to form a colored complex in the presence of ferric chloride ($FeCl_3$) with a maximum absorbance at 380nm. A standard curve of equation: y=0.007x; R2=0.988 is established using colchicine as a control. We express the results as µg quercitin equivalent/g vegetable powder (µg eq Q/g).

Cytogenetic analysis

The parameters evaluated are: Mitotic index (MI): dividing cells/total cells X100. Phase index (PI): cells in each phase/total cells X100. Cytotoxicity limit value (CLV): Mitotic index of treated cells/ Mitotic index of untreated cells X100. Aberration index (AI): cells with aberration/total observed aberrations X100.

Statistical analysis

An analysis of variance (ANOVA) was performed with SATISTICA software.

RESULTS AND DISCUSSION

Mitotic index is used to assess cell division. It allows estimating the number of cells actually able to divide (Siviková, 1996). *Allium cepa* L cells treated with distilled water (T-) showed the highest mitotic index reaching 92.20±1.34 (Table 1). These root cells exhibited all phases of cell division. Thick chromosomes located at the equatorial plate identify the prophase, characterized by a condensed nucleus and the metaphase. Anaphase can be recognized by the migration of the two groups of chromosomes towards the poles. Telophase represents the reconstitution of the nucleus in the two daughter cells (Figure 1). The analysis of Table 1 revealed a significant action of colchicine and the leaf alkaloid fraction of *Verbascum sinuatum* L on root cell division. They exhibited mitotic indices of 35.00±0.72 and 33.00±2.33 in contact with colchicine and alkaloid fraction, respectively.

Table 1: Mitotic parameters evaluated with our different samples.

	Negative control	Positive alkaloid control	Alkaloid fraction
Mitotic Index	92.20±1.34	35.00±0.72	33.00±2.33
Prophase Index	82.91±3.07	32.28±1.73	32.162.41
Metaphase Index	0.62±0.39	0.11±0.05	0.05±0.04
Anaphase Index	0.96±0.34	0.28±0.13	0.00±0.00
Telophase Index,	7.98±3.52	2.85±0.70	0.91±0.47
Chromosomal agglutinations	-	05.60±1.42	21.49±1.05
Binucleated cells	-	0.74±0.66	0.05±0.04
Chromosomal bridges	-	0.00±0.00	0.05±0.04
Chromosomal fragmentations	-	1.89±0.55	2.06±0.5
Disorganization of the equatorial plate	-	0.23±0.14	0.00±0.00
Cells without a nucleus	-	2.8±1.61	1.94±0.20
Cell elongations	-	0.86±0.37	0.29±0.25

Cell cycle checkpoints allow genome integrity. DNA damaging agents activate cell cycle checkpoints that block entry into mitosis. The mechanisms of checkpoint activation by alkaloids have been explored in the development of antiproliferative molecules to treat many cancers. Some alkaloids activate these checkpoints. Following oxidative stress, xylophene induces cycle arrest in G2/M leading to cell death. As for piperine, another alkaloid, induces apoptosis by blocking the p13K/Akt/Gsk3â signal transduction pathway in OVACAR-13 ovarian cancer cells (Chen *et al*., 2020).

The antimitotic effect of some alkaloids is related to their structural properties. This is the case for pyrizidine alkaloids. They contain functional groups capable of binding directly to the DNA molecule. The DNA-alkaloid complex leads to cross-linking of the DNA fragment and a blockage of mitosis. Alkaloids can also block mitosis by binding to DNA and induce inhibition of topoisomerase II (Sung *et al*., 1999).

Topoisomerases I are involved in the regulation of DNA supercoiling during its transcription and replication. They represent a privileged target of antimitotic agents. Some alkaloids by binding to the TOP I-DNA complex cause irreversible DNA breaks, which induce a slowing down of the replication fork progression. Recently, Pourquier and Lansiaux (2011) developed clinical derivatives of alkaloids to better target their binding Site.

Alkaloids can also block mitosis by preventing mitotic spindle formation. Colchicine is an alkaloid known for its high affinity to tubulin. It binds irreversibly to microtubules. Its binding domain is located between the two subunits of the same dimer to form a complex that is unfavorable to the polymerization of microtubules and thus interferes with the formation of the mitotic spindle. Also other alkaloids act as mitotic spindle poisons. They interact directly with tubulin, induce structural changes, and thus inhibit its depolymerization. This leads to a blockage of mitosis in prophase by preventing the formation of the mitotic spindle (DeLuca *et al*., 2020).

Alkaloids of plant origin are of growing interest for their antiproliferative effect. This biological effect is closely related to concentration. Recently, Israel *et al*. (2015) optimized the extraction conditions of alkaloids. We obtained in this study 50µg eq Q /g of plant powder. The rate of molecule that can be extracted is closely related to the extraction conditions (Chaudhary *et al*., 2008).

Microscopic observations showed that the alkaloid fraction of *Verbascum sinuatum* L leaves as well as colchicine at 1mg /ml (positive control) induced a large number of chromosomal abnormalities (Table1). Mostly, agglutinations were observed in the presence of the leaf alkaloid fraction with an aberration index of 21.49±1.05% against 05.60±1.42% for the positive controls. Binucleated cells, chromosomal fragmentations,

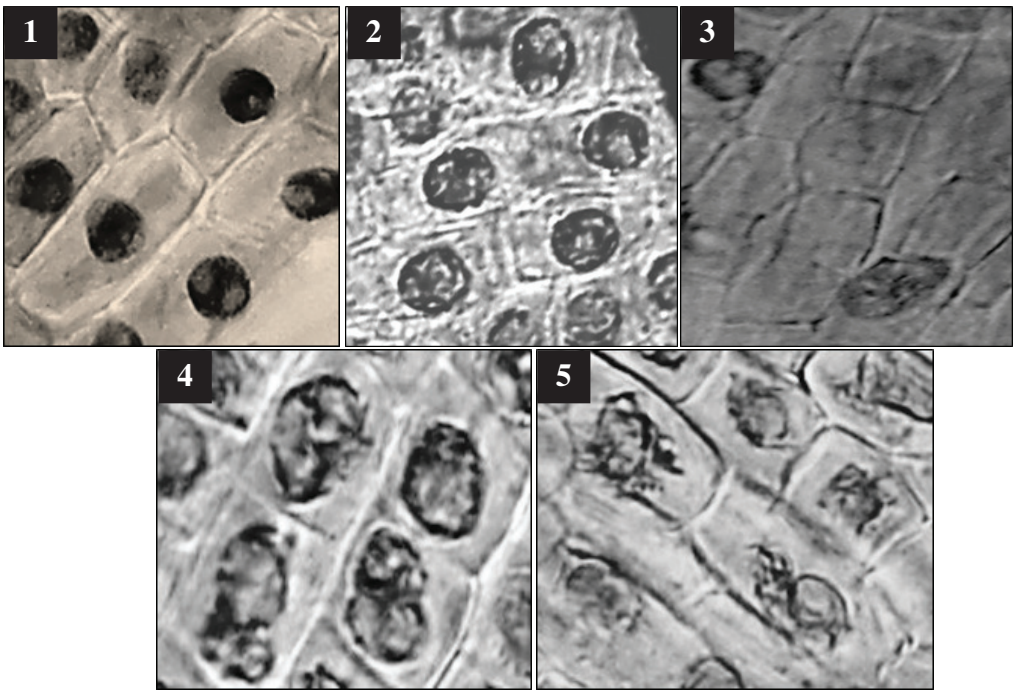

Fig.1 : **Abnormalities identified in onion cells (X400 magnification)**
1 : **Cell elongations**; **2**: **Chromosomal agglutinations; 3: Cells without a nucleus;**
4 : **Binucleated cells**; **5** : **Chromosomal fragmentations.**

and cells without nuclei were very weakly observed in contact with both samples. No disorganization of the equatorial plate was observed upon contact with the alkaloid fraction. However, chromosome bridges were observed only in contact with the latter (05±0.04%).

Disorganizations of the equatorial plate of *Allium cepa* meristematic cells upon contact with *Verbascum sinuatum* L. leaf alkaloids may be due to an aneugenic effect. This effect is called numerical damage of chromosomes. It is related to the ability to induce poor chromosome separation resulting from disruption of kinetochore and mitotic spindle regulation. Taxol is selected as a reference indicator of this effect in the development of software to determine the molecular mechanism of genotoxic agents. Nucleus-less cells and binucleated cells is an anagenic effect that may be related to the activity of Aurora kinases. These enzymes are key enzymes in the regulation of the centromere cycle and are therefore biomarkers in the determination of chromosome mis-segregation.

Structural damage of chromosomes is at the origin of the formation of chromosome bridges and fragmentations observed in contact with *Verbascum sinuatum* L. leaf alkaloids. This clastrogenic effect may be the result of DNA damage. Modeling genotoxic effects is the subject of a large number of studies (Dertinger *et al*., 2019).

CONCLUSION

Our work revealed a mitodepressive effect of the alkaloid fraction of *Verbascum sinuatum* L. leaves on cells of onion. The genotoxic action of this fraction causes a large number of cell division abnormalities. Agglutinations represent the majority of this dysfunction of mitosis. We also noted with variable frequencies, gigantic cells, binucleated cells, disorganization of the equatorial plate and chromosomal fragmentations. It would be very interesting to optimize the extraction conditions and to elucidate the molecular basis of the aneugenic and clastrogenic effect of *Verbascum sinuatum* L. leaf alkaloids.

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Intergeneric and interspecific crossing in *Vitaceae*: an attempt for disease resistant types

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ABSTRACT

Wild grapes species are conserved, characterized and utilized at MACS-Agharkar Research Institute, Pune, in a crop improvement programme. In the present study, 20 species/ cultivars belonging to the family Vitaceae collected from different sources within India and abroad were used for making intergeneric as well as interspecific crosses. Out of 263 seeds obtained from 21 cross combinations, four hybrids were successfully grown from V. rotundifolia as one of the parents showed resistance against downy and powdery mildew. The hybrids were evaluated for eleven different fruit characters. These hybrids could be utilized as pre-breeding material in future hybridization programmes.

***Keywords*:** Disease resistance, Interspecific hybridization, Vitaceae, *Vitis rotundifolia*

INTRODUCTION

India is one of the biodiversity rich countries of the world and fortunately harbors numerous wild relatives of grapes, which belong to the family *Vitaceae*. The grape is commercially important fruit crop of India.Grapes are considered as rich source of vitamins, minerals and unique natural products like resveratrol. Grapes are gaining lot of importance in daily diet due to its anti-oxidant, anti-carcinogenic, immunomodulatory, antidiabetic, antiatherogenic, neuroprotective, anti-obese and anti-aging properties (Yadav *et al*., 2009).

Out of total grapes produced in India, about 78.83 per cent are produced only in Maharashtra, that to in a narrow belt parallel to eastern side of Western Ghats comprising Nasik, Pune, Solapur and Sangli districts (Anon., 2018) . Though the primary centres of origin of cultivated grape is the region between Caspian and Black seas and secondary centres of origin as North America, about 70 species consisting of 8 genera have been reported from India (Tetali *et al.,* 2013). Indian species belonging to the family *Vitaceae* have been reported from different biogeographical regions, maximum number of species are reported from North-Eastern region of India covering Assam, Nagaland and the adjacent regions (Chadha and Shikhamany, 1999). Other than this, the Western Ghats also reported to have a high number of species belongs to family *Vitaceae*.

All varieties which are in commercial cultivation are susceptible to fungal diseases. Powdery and downy mildew are two most common fungal diseases that are considered as severe diseases of grapes. They can cause total loss if infected during the flowering stages in warm and wet climate. Grape growers spend lot of money for chemical control of these diseases, which eventually increases the cost of cultivation. Management of these diseases on traditional grapevine varieties requires regular application of fungicides. The intensive use of chemicals has its own limitations because of their cost, risk on human health and negative environmental impact. Furthermore, some fungicide-resistant strains of *Plasmopara viticola* are now observed in the vineyard, decreasing the efficiency of these sprays. Plant breeding for disease resistance the most effective way to avoid grapevine diseases.

Wild relatives of grapes are gene banks for heritable resistance to diseases and pests (Wan *et al*., 2007) and need to be conserved and evaluated as a valuable source for improvement of commercial grapes. Novel ways for genetic improvement using untapped genetic diversity available in crop wild relatives and closely related species must be explored (Tetali and Karkamkar, 2016). Keeping that in mind, grape improvement programme was initiated during 1970 at Maharashtra Association for cultivation of science later changed its name as MACS-Agharkar

Research Institute (an autonomous institute of DST, Govt. of India) in 1992 under All India Co-ordinated Research project- Fruits, of ICAR, Govt. of India, New Delhi. A large number of indigenous species of grapes were collected from different sources. These species were maintained and evaluated and being utilized in crop improvement programme. Many wild relatives of grapes belonging to the family *Vitaceae* are recorded to be resistant to most commonly observed fungal diseases of cultivated grapes. The use of wild relatives to improve crop performance is well established. There are many successful examples of using wild species as resistant rootstocks to control soil borne pathogens in various horticultural crops (Pereira *et al*., 2018; Panth *et al*., 2020). Hence, the collection was explored to harness their potential as rootstocks or as a parent in breeding program in grape improvement. Interspecific and intergeneric hybridization was carried out. The results are discussed in the present communication.

MATERIALS AND METHODS

Vitis species and wild species of family *Vitaceae* are collected from western Ghats of Maharashtra along with other regions of India as well as abroad and are being conserved at Institute's main premises and also the farm that is situated at Hol, Taluk Baramati, Dist. Pune (Maharashtra). Identification of species, locality and GPS data were recorded (Table 1). Some of the grape cultivars were collected from Ganesh Khind Fruit Research Station, Pune, in 1971, and some were acquired from Federal Research Institute for grapevine breeding, Germany, in 1978.

The present study was carried out during 2012 to 2019 at MACS-Agharkar Research Institute. The relationship between cultivated grapes and their wild relatives is given in Table 2. Pollen viability was worked out by the aceto-carmine test. Targeted hybridization programme was attempted at research farm of this institute situated at Hol, Taluk Baramati using cultivated varieties *Vitis vinifera* (Anab-e-Shahi, Bhokri, Cheema Sahebi, Gulabi, James and Ribier), *V. labrusca* (Catawba) and *V. rotundifolia* (James) and species of Ampelocissus, *Cayratia, Cissus* and *Leea species* in hybridization programme.

Emasculations and pollinations were carried out by conventional procedure using selected cultivars/ species in hybridization programme. Data on berry set, seeds per berry and germination percentage were recorded. To achieve maximum germination, cross seeds were subjected to chilling treatment for 90 days at 4^0C; followed by H_2O_2 (0.5M) and GA (1000 ppm) treatments for 24 h. each. The seeds were sown after treatment with fungicides in a mixture of soil, sand and FYM (1:1:1) in seedling trays. Germinated F_1 hybrids were transplanted into polythene bags, along with parents after one year for evaluation and further studies. The hybrids and parents were subjected to the same cultural practices. The hybrids started fruiting after 3-4 years, and observations on fruit characters and disease incidence were recorded in the field. The bunch and berry characters were also studied. Data on qualitative characters like bunch maturity, berry colour, berry shape, skin thickness, juice colour and quantitative characters like bunch weight, 100-berry weight, berry size, total soluble solids (TSS) and seeds per berry were recorded.

Observations for powdery mildew incidence were recorded when disease symptoms were fully developed in natural conditions. Twenty-five leaves were surveyed for each plant. Each leaf was graded as: 0, 1, 2, 3, 4, 5, 6 and 7 based on the estimated percentage of lesions over the whole leaf area: 0, 0.1-5, 5.1-15, 15.1-30, 30.1-45, 45.1-65, 65.1-85.0 and > 85.0 respectively. Results of grading were converted to the severity index (SI) by using following formula as described by Wang *et al.* (1995).

$$\text{Severity index (SI)} = \frac{\text{Sum of (Grade value} \times \text{number of leaves in that grade)}}{\text{(Total No. leaf number x Highest grade value)}} \times 100$$

The resistance level of each hybrid was rated based on its SI: R, Resistant, SI = 0 -10; MR, Moderately Resistant, SI= 10.1-25; MS, Moderately Susceptible, SI = 25.1-50; S, Susceptible, SI = 51.1-75; HS, Highly susceptible, SI. = 75.1-100.

RESULTS AND DISCUSSION

In the present study, out of 20 species/ cultivars, thirteen wild relatives of grapes were collected from Konkan and Western Ghats of Karnataka and Maharashtra. The list of species used in the present experiment, the place of collection including GPS data is given in the Table 1. They include 4 *Leea* species, 3 species of *Ampelocissus*, *Cayratia* and *Cissus* each and seven *Vitis* species.

The members of *Vitaceae* are characterized as small trees or climbing shrubs sometimes herbaceous and usually having tendrils on the opposite side of leaves. The family *Vitaceae* is closely associated with its sister family *Leeaceae* and can be distinguished by the presence of stipulate leaves, sunken ovary with 1-6 seeded berries. *Vitaceae* members are occurring in abundance in the Konkan region in the rainy season. Most of them have ephemeral habit. After sprouting in the rainy season, they complete their lifecycle and go into hibernation in the form of rhizome/tubers. *Ampelocissus* and *Cayratia* species are climbers. Members of family *Vitaceae* are mostly climbing shrubs, whereas *Leea* species are perennial shrubs or small trees in habit. Plant habit and distribution of species is given in Table 2a.

The variation in a leaf characters is shown in Table 2b. Leaves of *Vitis*, *Ampelocissus* and *Ampelopsis* species are simple lobed with cordate/ palmate in shape whereas *Leea* species have simple/pinnate leaves with sheathing stipule as a differentiating character. Flower characters recorded are presented in Table 2c. *Vitis* species show tendril bearing panicle with compound raceme. Flowers of *Ampelocissus* spp. and *Ampelopsis* are tendril bearing pedunculated cymes. Almost all species show hermaphrodite penta or tetramerous flowers.

Cytological observations of different genera and species are presented in Table 2d. The diploid chromosome number of commonly cultivated species of *Vitis, i.e. Vitis vinifera* is 2n=38 (Sax 1929) whereas it is 40 in American cultivated species of *Vitis rotundifolia* which is reported to be the source of resistance to downy and powdery mildew (Olmo, 1937). The chromosome number 2n=40 is also observed in the genus *Ampelocissus*, *Ampelopsis* and *Parthenocissus*. The largest chromosomal variation is observed in the genus *Cayratia* ranging from 2n=20 to 120. (Shetty, 1958; Shetty and Raman, 1960; Vatsala, 1960, Patil *et al.,*1980, Karkamkar *et al*., 2010). In *Cissus* the diploid chromosome number ranged from 2n =22,24,28,32 and 48 (Ghimpu, 1929) and Tetrastigma 2n= 22,44 and 52 (Eichhorn, 1938; Krishnaswamy *et al.,* 1954; Shetty 1958; Shetty and Raman 1960; Patil *et al* 1980). The diploid chromosome number of *Leea* was recorded as 2n= 24 and 48.

Average pollen length has been observed to be maximum in *Leea macrophylla* (50.5 µm) and minimum in *Vitis* species *var*. Cheema sahebi (22.0 µm) (Table 2e). These observations are in conformity with earlier reports (Patil, 1998). Pollen viability plays a vital role in the success of crossing programme. Higher pollen viability (95%) is recorded in var. Anab-e-Shahi in commercially cultivated varieties of *Vitis vinifera* whereas it was lowest in *V. labrusca* and *V. rotundifolia. Cissus* species also showed higher pollen viability. These observations are in conformity with earlier reports (Patil, 2001 and 2006).

Spherical, Oblate, 1-4 seeded berries are observed in *Vitis* spp., *Ampelocissus*, *Ampelopsis*, *Parthenocissus*, and *Cissus* species show spherical, round, 1-4 seeded berries whereas berries of *Cayratia* and *Tetrastigma* are ellipsoidal oblate 2-4 seeded. Depressed, globular 3-6 berries are recorded in *Leea* species as described in Table 2f.

Disease reaction in general for downy mildew, powdery mildew and anthracnose observed in different genera and species are presented in Table 2g. Most of the genera from the family *Vitaceae* and Leeaceae, *i.e. Ampelopsis*, *Parthenocissus*, *Cissus*, *Cayratia*, *Tetrastigma* and *Leea*, are resistant to mildews and anthracnose diseases. In contrast, wide diversity in reactions to the diseases mentioned above is observed in wild species of *Vitis* and *Ampelocissus.*

On the basis of available data on chromosomal constitution combined with taxonomical affinities, preliminary attempt to cross cultivars and wild relatives were made**.** Data on the number of flower bunches combinations attempted and number of flowers emasculated and pollinated is given in the Table 3. *Vitis rotundifolia* cv James used in crossing programme is a known source of resistance of

Table 1: Collection and identification of grape germplasm

Sl. No.	Name of species	Source of collection	GPS data		
			Altitude (m)	Latitude (N)	Longitude (E)
1	*Ampelocissus indica*	Karwar	0	14⁰ 49′22.8"	74⁰ 07′41.8"
2	*Ampelocissus latifolia*	Ambolisawantwadi road	573	16⁰ 29′45.1"	74⁰ 07′07.2"
3	*Ampelocissus tomentosa*	Saked before Gargoti	300	16⁰ 29′45.1"	74⁰ 09′07.0"
4	*Cayratia auriculata*	Shimoga-Agumbe road	567	12⁰ 51′42.8"	73⁰ 33′20.1"
5	*Cayratia elongata*	Honavar-Gokarna road	14	14⁰ 28′22.1"	74⁰ 26′21.1"
6	*Cayratia trifoliata*	Honavar-Gokarna road	14	14⁰ 28′22.1"	74⁰ 26′21.1"
7	*Cissus quadrangularis* (Round)	Pune	569	18⁰ 52′4.0"	73⁰ 85′0.0"
8	*Cissus quadrangularis* (Winged)	Pune	569	18⁰ 52′4.0"	73⁰ 85′0.0"
9	*Cissus woodtrowii*	Khambataki	120	16⁰ 30′00.1"	74⁰ 20′00.0"
10	*Leea crispa*	Ambolighat	215	15⁰ 56′20.4"	73⁰ 57′5.6"
11	*Leea indica*	Radhanagarighat	445	16⁰ 29′54.1"	74⁰ 03′09.9"
12	*Leea macrophylla*	Madoni Dadra Nagar Haveli	30	20⁰ 02′58.3"	73⁰ 13′47.2"
13	*Leea sambucina*	Ambolighat	215	15⁰ 56′20.4"	73⁰ 57′5.6"
14	*Vitis vinifera var.* Bhokri (IC-0616667)	GFRS, Pune	570	18⁰ 53′00"	73⁰ 87′00"
15	*V. vinifera var.* Anab-e-Shahi (IC-0616636)	GFRS, Pune	570	18⁰ 53′00"	73⁰ 87′00"
16	*V. vinifera var.* Cheemasahebi (IC-0620725)	GFRS, Pune	570	18⁰ 53′00"	73⁰ 87′00"
17	*V. labrusca var.* Catawba (IC-0612104)	FRIGVB, Germany	130	51⁰ 78′00"	11⁰15′00"
18	*V. labrusca var.* Concord (IC-0612112)	FRIGVB, Germany	130	51⁰ 78′00"	11⁰15′00"
19	*V. vinifera var.* Gulabi (IC-0612119)	GFRS, Pune	570	18⁰ 53′00"	73⁰ 87′00"
20	*V. rotundifolia var.* James (IC-0623206)	GFRS, Pune	570	18⁰ 53′00"	73⁰ 87′00"

FRIGVB, Germany: Federal Research Institute for Grape Vine Breeding, Germany; GFRS, Pune : Ganesh Khind Fruit Research Station, Pune

Table 2: Relationship between cultivated grapes and their wild relatives

a) Plant habit and distribution

Sr. No.	Genera	Habita	Distribution	
			World	India
1	*Vitis sp.*	Climbing shrub	America, Europe, Asia	Maharashtra, Karnataka, Telangana, Tamil Nadu, Punjab, Haryana
2	*Ampelocissus*	Weak climber/creeper	Europe, Asia	Western Ghats, Eastern Ghats
3	*Ampelopsis*	Ceriferous liana	Europe, Asia	Himalayan region
4	*Parthenocissus*	Ceriferous liana	Asia, Africa	South and North India
5	*Cissus*	Weak climber/ erect shrubs	Asia, Africa	Deciduous forest
6	*Cayratia*	Large/ weak climbers	Asia, Africa	Deciduous forest
7	*Tetrastigma*	Large woody evergreen climbers	Asia, Africa	Evergreen forest
8	*Leea*	Herb/shrub/small tree	Tropical Asia and Africa and rare in Australia	North east Western Ghats, Andaman

Contd.

b) Variation in leaf characters

Sr. No.	Genera	Nature	Shape
1	*Vitis* sp.	Simple lobed	Cordate/ palmate
2	*Ampelocissus*	Simple lobed digitate	Cordate/ lanceolate
3	*Ampelopsis*	Simple lobed pinnate	Cordate/Ovate
4	*Parthenocissus*	Usually trifoliate	Ovate lanceolate
5	*Cissus*	Simple rarely trifoliolate	Cordate, ovate, lanceolate
6	*Cayratia*	3,5,7or 9 foliolate, leaflet, digitate/pedate	Lanceolate, obovate, ovate
7	*Tetrastigma*	3,5,7 foliolate, pedate, rarely simple	Lanceolate, acuminate
8	*Leea*	Simple/pinnate with sheathing stipule	Lanceolate, ovate, oblong

c) Variation in flower characters

Sr. No.	Genera	Inflorescence	Flowers
1	*Vitis* sp.	Tendril bearing panicle, or compound raceme	Hermaphrodite, Pentamerous
2	*Ampelocissus*	Pedunculate cyme trendril bearing	Polygamo-monoecious, female pseudo hermaphrodite
3	*Ampelopsis*	Pedunculatecorymbose cyme	Hermaphrodite, 4-5 merous
4	*Parthenocissus*	Terminal or leaf opposed dichotomous cyme ending in umbellus	Hermaphrodite pentamerous
5	*Cissus*	Umbellately divided cyme	Hermaphrodite, Tetramerous
6	*Cayratia*	Axillary, corymbose, pseudoterminal or umbel	Hermaphrodite, Tetramerous
7	*Tetrastigma*	Axillary, corymbose, cyme,2-3 chotamous	Polygamous, dioecious, stigma 4 lobes, tetramerous
8	*Leea*	Pedunculatecorymbose or cyme	Hermaphrodite, staminoidal tube pentamerous

d) Cytological studies

Sr. No.	Genera	Chromosome number (2n)	Reference
1	*Vitis* sp.	38, 40	Sax 1929; Olmo 1937; Krishnaswamy *et al.* 1954, Shetty & Raman, 1960; Patil *et al* .1980
2	*Ampelocissus*	40	Vatsala 1960; Shetty & Raman 1960
3	*Ampelopsis*	40	Sax 1929
4	*Parthenocissus*	40	Sax 1929; Shetty 1958
5	*Cissus*	22,24,26,28,32,48	Ghimpu 1929; Krishnaswamy *et al*, 1954; Shetty 1958; Shetty & Raman 1960; Vatsala 1960; Patil *et al* 1980
6	*Cayratia*	20,22,24,30,40,60,80,120	Shetty, 1958; Shetty & Raman, 1960; Vatsala 1960; Patil *et al* ,1980
7	*Tetrastigma*	22,44,52	Krishnaswamy *et al* 1954; Shetty 1958; Shetty & Raman 1960; Patil *et al* 1980
8	*Leea*	24,48	Vatsala ,1960; Shetty & Raman, 1960; Patil *et al* , 1980

powdery and downy mildew (Tetali *et al.*, 2018). Inter-specific crosses involving *V. rotundifolia* were successful in the field. The cultivars of *Vitis vinifera* (Anab-e-Shahi, Bhokri, Cheema-sahebi, Gulabi) were used as male parents and *V. rotundifolia* cv. James were used in hybridization as female parent and vice-versa. Total 263 seeds were obtained but only four seeds were viable. Pre-zygotic barriers which might have prevented the growth of the pollen tube through the style, and possible post-zygotic barriers, which might have prevented fertilization resulting into failure to produce a

e) Variation in pollen characters

Sl. No.	Name of species	Pollen size (ìm)		Wall thickness (ìm)	Protoplasmic area (ìm)²	Pollen viability (%)
		Length	Diameter			
1	*Ampelocissus* spp.	30.7	30.5	2.16	559	88
2	*Cayratia* spp	41.6	39.9	2.70	963	77
3	*Cissusquadrangularis* (Round)	39.5	38.3	2.53	899	92
4	*Cissusquadrangularis* (Winged)	38.8	37.5	2.56	839	80
5	*Cissuswoodtrowii*	44.4	43.2	2.04	1238	96
6	*Leeacrispa*	44.2	40.1	3.77	942	96
7	*Leeaindica*	43.9	42	3.80	984	76
8	*Leeamacrophylla*	50.5	46.3	3.50	1347	93
9	*Leeasambucina*	46.1	42.9	3.60	1127	42
10	*Vitis vinifera var.* Bhokri	25.6	24.8	2.20	338	82.6
11	*Vitis vinifera var.* Anab-e-Shahi	25.0	23.7	2.40	298	95
12	*Vitis vinifera var.* Cheemasahebi	22.0	21.0	1.80	254	80
13	*Vitis labrusca var.* Catawba	24.4	23.4	2.00	310	41
14	*Vitis labrusca var.* Concord	24.3	23.6	1.90	319	45
15	*Vitis vinifera var.* Gulabi	22.2	21.2	1.70	265	93
16	*Vitis rotundifolia var.* James	24.3	23.7	1.91	319	40
	Mean	**34.09**	**32.63**	**2.54**	**687.56**	**76.04**
	SD+/-	**10.21**	**9.35**	**0.73**	**391.43**	**21.37**

f) Variation in fruit characters

Sr. No.	Genera	Berries	Seeds
1	*Vitis sp.*	Spherical, oblate, 1-4 seeded	Pyriform/obovoid furrowed
2	*Ampelocissus*	Spherical, round, 2-3 seeded	Oblong, Obovoid, convex on back
3	*Ampelopsis*	Spherical, round, 2-4 seeded	Obovoid, 2 grooved
4	*Parthenocissus*	Round spherical, 2-4 seeded	Obovoid, globose, convex on back
5	*Cissus*	Round oblate, usually 1 seeded	Ellipsoidal, pyriform, smooth
6	*Cayratia*	Ellipsoidal, oblate, 2-4 seeded	Obcordate, oblong, angular
7	*Tetrastigma*	Ellipsoidal, oblate, 1-4 seeded	Globose oblong, pyriform, smooth
8	*Leea*	Depressed, globular, 3-6 seeded	Wedge shape, with hard testa

g) Variation disease resistance (R Resistant, S Susceptible, Moderately resistant)

Sr. No.	Genera	Downy mildew	Powdery mildew	Anthracnose
1	*Vitis* sp.	S / MR	S / MR	R / S
2	*Ampelocissus*	R/S	R/S	R /S
3	*Ampelopsis*	RR	RR	RR
4	*Parthenocissus*	RR	RR	RR
5	*Cissus*	RR	RR	RR
6	*Cayratia*	RR	RR	RR
7	*Tetrastigma*	RR	RR	RR
8	*Leea*	RR	RR	RR

viable seed in hybrid. Berries might have been developed through parthenocarpy in most of the fruits (Royo *et al*., 2016). Variations in percent berry set on pollination, seeds per berry and seed germination indicated the genetic effect of male parent. The possible reasons for such differential seed set might be due to (i) relative compatibility among the species (ii) genetic behaviour of male parent and (iii) artificial pollination may not be as effective as natural pollination (Patil *et al*.,1992).

According to Olmo, reciprocal approach to introduce the better fruit quality of *vinifera* has been

Table 3: Hybridization in *V. vinifera* and wild relatives of grapes

Sr. no.	Parental combinations	Combinations attempted	Flowers pollinated	Berry set	Berry harvested	Seed extracted	Seed germination
1	*V. vinifera var. Bhokri x Ampelocissus latifolia*	1	32	10	6	4	0
2	*V. vinifera var. Anab-e-Shahi x Leea indica*	9	1002	202	109	70	0
3	*V. vinifera var. Gulabi x Cayratia elongata.*	5	247	75	58	17	0
4	*V. vinifera var. Bhokri x Cissus woodrowii*	14	2294	1633	232	107	0
5	*V. vinifera var. Gulabi V.* x *rotundifolia var. James*	2	95	10	10	24	1
6	*V. labrusca var. Catawba x Cissus woodrowii.*	5	140	1	1	1	0
7	*V. labrusca var. Concord x Cayratia trifoliata.*	3	243	50	37	46	0
8	*V. labrusca var. Concord x Leea crispa.*	1	249	64	42	18	0
9	*V. rotundifolia var. James x V. vinifera var.* Cheemasahebi	2	70	11	11	26	1
10	*V. rotundifolia var. James x V. vinifera var.* Gulabi	2	50	26	21	35	3
11	*Cayratia auriculata x V. vinifera var.* Gulabi.	4	578	0	0	0	0
12	*Cissus woodrowii* x *V. vinifera* var. Cheemasahebi.	6	170	0	0	0	0
13	*V. vinifera* Bhokri x *Ampelocissus latifolia*	1	28	4	4	0	0
15	*V. vinifera* Bhokri x *Cissus woodrowii*	2	43	0	0	0	0
16	*V. vinifera* var. Ribier x *Cissus elongata*	2	47	4	4	3	0
17	*V. vinifera* var. Ribier x *C. quadrangularis* (*winged*)	1	17	0	0	0	0
18	*V. vinifera* var. Cheemasahebi x *Cissus quadrangularis* (ornamental type)	2	150	0	0	0	0
19	*V. vinifera* var. Cheemasahebi x C. *quadrangularis*(round)	3	319	0	0	0	0
20	*V. vinifera* var. Cheemasahebi x *Leea sambucina*	1	17	0	0	0	0
21	*V. vinifera* Gulabi x *C. quadrangularis* (round)	1	62	0	0	0	0
	Total	**49**	**5055**	**2035**	**485**	**263**	**5**

Table 4: Evaluation of successful hybrids

Hybrid Characters	H 265 (James x Gulabi)	H 267 James x (Gulabi)	H 584 (James x Cheema sahebi)	H 297 (Gulabi x James)
Bunch Maturity	Uneven	Uneven	Even	Uneven
Berry colour	Brick Red	Green	Bluish black	Black
Berry shape	Ellipsoidal	spherical	Spherical	Obovoid
Bunch weight (g)	80.7	90.9	68.15	35
100 berry weight (g)	125	154	171	190
Berry length (cm)	14.3	14.7	10.94	13.8
Berry width (cm)	12.3	13.84	10.81	13.26
Seeds/berry	2.19	0.75	1.63	1.88
TSS (^{0}B)	22.81	17.25	21.5	21.5
Skin thickness	Medium	Thin	Thin	Medium
Juice colour	White	White	White	White
Disease severity index (PM)+/- SD	7.07+/- 4.54	8.00 +/-2.30	7.33+/- 1.46	17.22 +/- 2.66

hindered by the lack of cross compatibility when *rotundifolia* is used as the female parent(Olmo, 1937). In the present experiment cross compatibility was observed when we used *V. rotundifolia* as female parent. This was may be due to the use of different cultivar of *V. rotundifolia* on present study.

After planting these seedlings in the field, they started fruiting after 3-4 years. Hybrids were evaluated for eleven different fruit characters. Performance of hybrids based on resistance and quality of fruits are shown in Table 4. Three hybrids H 265, H 267 and H 584 recoded severity index less 10 hence considered as tolerant for powdery mildew under natural field conditions. H 297 showed moderately resistance (SI =17.22) based on severity index.

In Maharashtra, powdery mildew usually appears in December and peaks in January. Disease severity index was lowest in James, which reveals its importance as source of resistance to powdery mildew. It can also be noted from the table that all hybrids produced small bunches having seeded berries which may not be useful to be used as table purpose in present conditions. But it can be used as pre-breeding material for transferring the disease resistance. One or two more backcrosses may be required to attain the berry size and quality to be used for table purpose. Thus, modern tools like somatic hybridization, embryo rescue, gene expression experiments may be taken into consideration for successful utilization of wild germplasm to incorporate traits of interests in cultivated varieties of grapes.

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Evaluation of Cape gooseberry (*Physalis peruviana* L.) genotypes under Jammu Plains

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ABSTRACT

Evaluation of Cape gooseberry cultivars was carried out at Research Farm, Division of Fruit Science, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu. The performance of the ten Cape gooseberry genotypes in terms of vegetative, flowering, fruiting, yield, and biochemical parameters was evaluated. The maximum plant height (114.66 cm) and leaf size (62.02 cm^2) was observed in CITH CGB Sel-10 genotype followed by CITH CGB Sel-9 genotype with 105.33 cm and 61.28 cm^2. The maximum days for flowering, fruiting and harvesting was taken by CITH CGB Sel-10 genotype (62.20, 72.07 and 134.28 days respectively) followed by CITH CGB Sel-9 genotype (61.61, 70.90 and 131.57 days respectively). The CITH CGB Sel-10 genotype showed the highest fruit weight (13.37 g) and fruit yield (1.26 kg $plant^{-1}$) followed by CITH CGB Sel-9 genotype (13.13 g and 1.20 kg $plant^{-1}$). The maximum TSS (12.61^{0}B) was observed in CITH CGB Sel-9 followed by CITH CGB Sel-3 (12.42^{0}B) and CITH CGB Sel-10 (12.31^{0}B). The highest ascorbic acid (31.12 mg 100 g^{-1}), total sugar (10.75 %) were observed in CITH CGB Sel-10 genotype while lowest carotenoids (1.13 mg 100 g^{-1}) and total sugar (5.22%) were found in CITH CGB Sel-5.

***Keywords*:** Ascorbic acid, Cape gooseberry, carotenoids, genotypes, Jammu plains, TSS, Total sugar, yield

INTRODUCTION

Cape gooseberry is a herbaceous, semi-shrubby, erect plant which grows as an annual in temperate regions but as a perennial in tropical & subtropical areas. It is known by different names in different parts of the world like Rasbhari in India, Uchuva in Colombia, Cape gooseberry in South Africa, Goldenberry in English-speaking countries, Topotopo in Venezuela Aguaymanto in Peru and Uvilla in Ecuador. Per 100g of edible portion of berry contains 11.5 per cent carbohydrates, 1.8 per cent protein, 0.2 per cent fat, 3.2 per cent fibre, 0.6 per cent mineral matter and 49 milligrammes ascorbic acid, 13% TSS, 6.0% reducing sugars, 8.6% total sugars and 1.52% total titrable acidity. The fruit or berries are the most important and potential sources of Vitamin A (2380 IU), Vitamin C (49 mg) and produces 55 calories energy per 100 g of fruits. It is successfully grown in several regions including Uttar Pradesh, West Bengal, Madhya Pradesh, Haryana, Punjab, the Nilgiri Hills and other regions of the country but also has good potential to grow under the subtropics and plains of Jammu. It gains special attention because of its availability during lean period, wider adaptation, rapid growth in nature, high productivity, non-perennial land occupation and tasty fruit with a pleasant acetic flavour. Because it is a small fruit crop in India, there is a scarcity of scientific data on its area and productivity, as well as new enhanced production methods under various agroclimatic conditions. Introduction and evaluation is one of the important method for bringing improvement in any fruit crop and for the selection of parents in a viable hybridization programme. Insect-pest occurrence and fruit cracking during fruit growth, development, and maturity are major issues in cape gooseberry (Bisht *et al*., 2018). To give Cape gooseberry a commercial boost, it is critical to find the right genotype. The aim of this study is to evaluate the most elites genotypes for cultivation under the Jammu plains.

MATERIALS AND METHODS

The present investigation was carried out at the Research Farm, Division of Fruit Science, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu during the year 2020-2021. Ten genotypes of Cape

gooseberry were evaluated for various growth, flowering, yield and biochemical parameters. The experiment was carried out in Randomized Block Design with three replications. Ten genotypes of cape gooseberry viz. CITH CGB Sel-1, CITH CGB Sel-2, CITH CGB Sel-3, CITH CGB Sel-4, CITH CGB Sel-5, CITH CGB Sel-6, CITH CGB Sel-7, CITH CGB Sel-8, CITH CGB Sel-9 and CITH CGB Sel-10 which were procured from Central Institute of Temperate Horticulture (CITH) Srinagar as a basis of the plant material. The plants were tagged in each replication of the treatment for data collection. The height of plant was measured at maturity stage using a measuring tape and was recorded in centimeter (cm). The thickness of the stem was also measured at 3 cm above the ground level at maturity stage using vernier caliper and expressed in centimeter (cm). The average shoot number was recorded by counting the number of shoots when the plant was fully grown. The leaf size was calculated by measuring the area of the graph paper covered by the leaf in square centimeter. The days of opening of flower bud in all the treatments was considered as the time of initiation of flowering. The days of start of fruit set in all the treatments was considered as the days for first fruit set. The days when calyx changed its colour from green to papery brown in maximum number of fruits was considered as the days for harvest of each treatment. With the use of an electronic balance, the weight of 10 randomly selected fruits from each replication of all the treatments was weighed. The average weight of fruit was computed and expressed in gram (g). The mean fruit length and breadth were measured using vernier caliper. The fruit volume was measured using water displacement method. The average yield plant^{-1} was calculated as product of average fruit weight and the total number of fruits produced/ plant and expressed in kilogram. The TSS was found using ERMA hand refractometer. The total acidity, reducing sugars, non reducing sugars, total sugars, pectin content, ascorbic acid were measured using A.O.A.C. (1995) method. The total carotenoids was recorded using the method described by (Carvalho *et al*., 2012). Resistance to viral infection and fruit cracking were determined by counting the number of infected fruits in each replication of all the treatment and computed in percent (%).

RESULTS AND DISCUSSION

Growth parameters of plant

Data with respect to different growth parameters of Cape gooseberry *viz.,* plant height, stem thickness, shoot number and leaf size is described in Table 1 which clearly indicates the significant variations. The differences in growth parameters might be due to inherent character acquired by strains from prevailing climatic conditions. The highest height of plant (114.66 cm) was found in CITH CGB Sel-10 which was statistically at par with CITH CGB Sel-9 (105.33 cm) while, lowest height of plant (90.33 cm) was recorded in CITH CGB Sel-2. These results are in accordance with the findings of Dwivedi *et al*. (2015); Ali and Singh (2016) and Panayotov (2016) in Cape gooseberry. The highest stem thickness (3.15 cm) was observed in CITH CGB Sel-10 whereas, minimum stem thickness (2.95 cm) was observed in CITH CGB Sel-2. Dwivedi *et al.* (2015) stated that stem diameter varied from 4.5 cm to 5.65 cm at different sowing time and spacing in Cape gooseberry. Singh *et al*. (2014) also reported that in Cape gooseberry maximum stem diameter (1.28 mm) was observed at thirty days after germination. Maximum number of shoot (16.07) was found in CITH CGB Sel-10, which was statistically at par with CITH CGB Sel-5 (15.60) and CITH CGB Sel-9 (16.00) whereas, minimum values of shoot number (13.61) was observed in CITH CGB Sel-2. Diversity in the synthesis of endogenous hormones like auxin and gibberellins as a result of genetic variability and inherent character in different genotypes might be the possible reason for difference in shoot number among various genotypes. Kour and Bakshi (2006) stated that highest number of shoots per plant (13.90) was found in the FRB strain of cape gooseberry. The leaf size ranged from 62.02 cm^2 to 51.40 cm^2 among the genotypes. Similar results were also reported by Kour and Bakshi (2006) that leaf area ranged from 51.40 cm^2 to 56.50 cm^2 among the different strains of cape gooseberry.

Flowering and fruiting behavior

The flowering and fruiting parameters were significantly differed among different genotypes (Table 2). The data mentioned in Table 2 revealed

Table 1: Growth characteristics of selected genotypes of Cape gooseberry under Jammu plains

Treatments	Average plant height (cm)	Average stem thickness (cm)	Average shoot number	Average leaf size (cm^2)
T_1 - CITH CGB Sel-1	92.73	2.98	13.91	53.49
T_2 - CITH CGB Sel-2	90.33	2.95	13.61	51.40
T_3 - CITH CGB Sel-3	104.00	3.10	15.83	61.12
T_4 - CITH CGB Sel-4	95.16	3.00	14.45	59.97
T_5 - CITH CGB Sel-5	99.79	3.07	15.60	60.73
T_6 - CITH CGB Sel-6	97.47	3.03	14.32	55.83
T_7 - CITH CGB Sel-7	94.14	2.99	14.08	55.76
T_8 - CITH CGB Sel-8	91.47	2.96	13.81	51.58
T_9 - CITH CGB Sel-9	105.33	3.09	16.00	61.28
T_{10} - CITH CGB Sel-10	114.66	3.15	16.07	62.02
C.D. (0.05) **(S.E.±m)**	**10.17** **3.42**	**0.12** **0.04**	**1.45** **0.49**	**7.65** **2.58**

Table 2: Flowering and fruiting behavior of selected genotypes of Cape gooseberry under Jammu plains

Treatments	Days for initiation of flowering (days)	Days for initial fruit set (days)	Days for fruit harvest (days)
T1- CITH CGB Sel-1	31.69	38.48	86.50
T2- CITH CGB Sel-2	45.80	51.55	101.42
T3 - CITH CGB Sel-3	56.36	64.17	123.11
T4- CITH CGB Sel-4	54.49	61.81	113.72
T5 - CITH CGB Sel-5	55.70	63.56	117.73
T6- CITH CGB Sel-6	58.34	66.96	127.79
T7- CITH CGB Sel-7	57.20	65.53	125.55
T8 - CITH CGB Sel-8	59.02	68.17	129.10
T9- CITH CGB Sel-9	61.61	70.90	131.57
T10 - CITH CGB Sel-10	62.20	72.07	134.28
C.D. (0.05) **(S.E.±m)**	**6.87** **2.31**	**9.43** **3.18**	**14.61** **4.92**

that maximum time to initiate flowering (62.20 days) was taken by CITH CGB Sel-10 which was statistically at par with CITH CGB Sel-3 (55.70 days), CITH CGB Sel-5 (56.36 days), CITH CGB Sel-7 (57.20 days) and CITH CGB Sel-9 (61.61 days) while minimum time to initiate flowering (31.69 days) was taken by CITH CGB Sel-1. The variation in the time of flowering might be based on the differences in various physiological phenomenon in different genotypes. Maximum time of fruit set (72.07 days) was also observed CITH CGB Sel-10 whereas, minimum time of fruit set (38.48 days) was observed in CITH CGB Sel-1. Dwivedi *et al*. (2014) reported that first flower opening and fruit set were observed at 24.50 and 32.00 DAT respectively in cape gooseberry**.** The

Table 3: Physical characteristics of fruits of selected genotypes of Cape gooseberry under Jammu plains

Treatments	Average weight of fruit(g)	Average length of fruit (cm)	Average breadth of fruit(cm)	Average volume of fruit (cc)	Average yield per plant (kg)
T_1- CITH CGB Sel-1	11.55	2.08	2.41	11.72	1.02
T_2- CITH CGB Sel-2	11.10	2.00	2.31	11.50	0.88
T_3 - CITH CGB Sel-3	12.87	2.97	3.20	12.17	1.18
T_4 - CITH CGB Sel-4	12.44	2.45	2.80	12.20	1.15
T_5- CITH CGB Sel-5	12.74	2.51	3.11	12.54	1.17
T_6- CITH CGB Sel-6	12.15	2.21	2.50	13.50	1.11
T_7- CITH CGB Sel-7	12.13	2.14	2.48	12.10	1.08
T_8 - CITH CGB Sel-8	11.45	2.11	2.45	11.15	0.98
T_9 - CITH CGB Sel-9	13.13	2.72	3.44	13.72	1.20
T_{10} - CITH CGB Sel-10	13.37	3.04	3.47	14.71	1.26
C.D. (0.05) **(S.E.±m)**	**1.29** **0.44**	**0.41** **0.14**	**0.42** **0.14**	**1.64** **0.55**	**0.17** **0.06**

Table 4: Biochemical parameters of fruits of (selected) genotypes of cape gooseberry under Jammu plains

Treatments	T.S.S (°Brix)	Acidity (Per cent)	Carotenoids (mg/ 100g)	Pectin (Per cent)	Ascorbic acid (mg/ 100g)	Reducing sugar (Per cent)	Non reducing sugar (Per cent)	Total sugar (Per cent)
T_1 - CITH CGB Sel-1	10.40	0.89	1.40	0.53	24.28	5.47	5.26	10.73
T_2- CITH CGB Sel-2	9.63	0.95	1.20	0.99	22.41	3.08	2.93	6.00
T_3 - CITH CGB Sel-3	12.42	0.46	1.45	1.01	22.70	3.26	3.13	6.40
T_4 - CITH CGB Sel-4	11.50	0.61	1.55	0.98	23.17	3.55	3.46	7.02
T_5- CITH CGB Sel-5	11.92	0.52	1.13	1.02	24.22	2.68	2.55	5.22
T_6- CITH CGB Sel-6	10.73	0.78	1.60	0.97	26.10	4.03	3.84	7.87
T_7- CITH CGB Sel-7	10.52	0.88	1.42	0.95	29.66	4.85	3.68	8.50
T_8 - CITH CGB Sel-8	9.48	1.10	1.49	0.67	29.79	4.50	4.31	8.80
T_9 - CITH CGB Sel-9	12.61	0.41	1.28	0.61	30.57	5.24	4.95	10.22
T_{10} - CITH CGB Sel-10	12.31	0.50	1.39	0.57	31.12	5.48	5.27	10.75
C.D. (0.05) **S.E. (±m)**	**1.18** **0.40**	**0.09** **0.03**	**0.18** **0.06**	**0.10** **0.03**	**3.24** **1.09**	**0.41** **0.14**	**0.35** **0.12**	**1.06** **0.36**

time of harvesting ranged from 134.28 days to 86.50 days among the various genotypes. These results are in agreement with Kaur and Bakshi, (2006); Panayotov and Popova, (2014), Singh *et al*. (2014) and Gond *et al*. (2018) in cape gooseberry.

Physical parameters of fruit

The data mentioned in Table 3 revealed that maximum value of fruit weight (13.37 g) was observed in CITH CGB Sel-10 whereas, minimum fruit weight (11.10 g) was observed in CITH CGB Sel-2. Kour and Bakshi, (2006) observed that

weight of fruit ranged from 9.80 grammes to 12.20 grammes in various strains of Cape gooseberry. Similarly maximum fruit length (3.04 cm) was observed in CITH CGB Sel-10 which was statistically at par with CITH CGB Sel-9 (2.72 cm) and CITH CGB Sel-3 (2.97 cm) while minimum fruit length (2.00 cm) was observed in CITH CGB Sel-2. The diameter of fruit ranged from 3.47 cm to 2.31 cm. Genotype CITH CGB Sel-10 recorded maximum fruit volume (14.71 cc) which was statistically at par with CITH CGB Sel-6 (13.50 cc) and CITH CGB Sel-9 (13.72 cc) whereas, minimum fruit volume (11.15 cc) was observed in CITH CGB Sel-8. Highest yield per plant (1.26 kg) was observed in CITH CGB Sel-10 whereas, lowest yield per plant (0.88 kg) was observed in CITH CGB Sel-2 (T_2). Sharma *et al.* (2019) reported that the yield per plant varies between 1.20 kg/plant to 3.64 kg/plant in various genotypes of cape gooseberry.

Biochemical parameters

Mean performance of different Cape gooseberry genotypes for the chemical parameters clearly showed the significant difference (Table 4). TSS ranged from 12.61^0 Brix in (CITH CGB Sel-9) to 9.48^0 Brix in (CITH CGB Sel-8). The variation in TSS among various genotypes could be attributed due to their genetic constitution as well as it depends on the nature of the variety which governs the chemical composition of the fruits. Silva, (2013) stated that average TSS (6.52^0 Brix) was observed in *Physalis pubescens*. The maximum titratable acidity (1.10 %) was observed in CITH CGB Sel-8 whereas, the minimum titratable acidity (0.41 %) was observed in CITH CGB Sel-9. The variation in acidity among various genotypes could be attributed to environmental condition during the peak growth of the fruits as well as the genotype differences. Ersoy and Bagci (2011) recorded that mean titratable acidity value ranged from 0.78 % to1.83 % in golden berries. Maximum reducing sugar (5.48 %) was observed in CITH CGB Sel-10 while minimum value of reducing sugar (2.68 %) was observed in CITH CGB Sel-5. Rodrigues *et al.* (2014) recorded 6.4 % reducing sugar in *Physalis peruviana* L. Highest magnitude of non reducing sugar (5.27 %) was reported in CITH CGB Sel-10 which showed non significant difference with CITH CGB Sel-10 (4.95 %) while, minimum value of non reducing sugar (2.55 %) was recorded in CITH CGB Sel-5. The total sugar ranged from 10.75% to 5.22%. Kumar *et al.* (2021) reported that total sugar ranged from 2.05 % to 10.86 %. Maximum carotenoids content (1.60 mg/ 100 g) was observed in CITH CGB Sel-6 whereas, minimum carotenoids content (1.13 mg/ 100 g) was observed in CITH CGB Sel-5. Patidar *et al.* (2018) reported that in different fertilizers treatments carotenoid content ranges from 1.13 to 1.63 mg/ 100 g. Ascorbic acid varied from 31.12 mg to 22.41 mg/ 100 g. Sharma *et al.* (2017) reported that ascorbic acid value varied from 22.69 mg/100 g to 32.24 mg/100 g among different genotypes. Maximum value of pectin content (1.02 %) was observed in CITH CGB Sel-5 which was statistically at par with CITH CGB Sel-2 (0.99 %) and CITH CGB Sel-3 (1.01 %) while minimum value of pectin content (0.53 %) was observed in CITH CGB Sel-1. Such variations might be due to varietal differences and environmental conditions during ripening. Mazova *et al.* (2020) reported that *Physalis peruviana* L. contains 1.03 % pectin.

CONCLUSION

In this study the growth, yield and biochemical parameters were assessed in different genotypes of Cape gooseberry. Significant variations were observed among the various parameters. Based on the overall study of all the parameters i.e earliness physical and chemical characters of fruits in various genotypes, CITH Sel-10, CITH Sel-9 and CITH Sel-3 were found to be superior with respect to plant height, stem thickness, shoot number, leaf size, fruit weight, length of fruit, breadth of fruit, volume of fruit, yield, TSS, reducing sugar, non reducing sugar, total sugar, carotenoids and pectin content.

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A comparative study of maturity indices (heat unit) for indigenous and exotic date palm germplasm at Kachchh-India

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ABSTRACT

Date palm (Phoenix dactylifera L.) is an important fruit crop in many gulf countries and in recent years it is gaining popularity for cultivation in India. To understand the maturity period for six date palm germplasm a study was conducted at Date palm Research Station, Mundra-Kachchh during 2016-2020. The data were pooled for five years and an average heat unit and number of days required for maturity was identified. The lowest duration and heat unit was required for ADP-1 while highest was found in Barhee.

***Keywords*:** Date palm, heat unit, *Khalal* stage, maturity indices

INTRODUCTION

Date fruits are known for their sweet, succulent and exotic flavor and regarded as a fruit with high calorific value and nutrients. It is a good source of antioxidants and polyphenolics (Sharma *et al*., 2021). Date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated crops in the world having its estimated origin dating back to 4000 BC in Mesopotamia (Current Iraq) (Johnson *et al.*, 2013), while in India, it is estimated to be around 450 years old with its major share of presence in the states like Gujarat, Rajasthan and Punjab where Kachchh district of Gujarat accounts for the largest share (Baidiyavadra *et al.*, 2019). Although the crop has its presence for almost five centuries in Kachchh, the majority of the plantation are still of seedling origin resulting in a huge variability in shape, size, colour as well as their maturity period (Sharma *et al*., 2019). Unlike the most of the major date palm producing countries where the fruits are harvested at *Dang* (*Rutab*) or *Pindkhajoor* (*Tamar*) stage, in India, majority of the fruits are harvested in their *Doka* or *Khalal* stage due to the climatic compulsions. In India, generally the dates reach its *Khalal* stage during June-end to mid-July which is also the peak rainfall period in this area, making it mandatory for the growers to harvest the crop or may result up to cent per cent crop loss. Fruits harvested at *Khalal* stage are done based on its colour, sweetness and astringency level. An early rainfall may cause a major damage to the crop due to fruit cracking and spoilage. Thus, it is important to identify early maturating cultivars, or short duration cultivars. However, for effective planning of the labour, financial requirements and sales, computed method can be a better alternative. One of the most used computed methods of date palm is using "heat units", also called as "degree days". The idea behind the absorption of certain heat unit is the growth of plant or plant part (Chandra *et al.,* 1992). It helps to calculate the time required to reach that particular stage. A few of the earlier studies were made using exotic varieties in India; however, indigenous varieties were not examined (Kalra and Bajwa, 1976; Chandra *et al.*, 1990). The current experiment was conducted to understand the heat unit required for fruit maturity at *Khalal* stage.

MATERIALS AND METHODS

The experiment was conducted at Date palm Research Station, Sardarkrushinagar Dantiwada Agricultural University, Mundra-Kachchh during 2016 to 2020. Six germplasm were evaluated, where Barhee and Halawy are exotic varieties introduced in India propagated through offshoots and recommended for cultivation in Gujarat and Rajasthan under ICAR-All India Co-ordinated Research Project on Arid Zone Fruits in 2003 (Sharma *et al.*, 2019), while ADP-1, MDP 20, MDP 21 and MDP 22 were tissue culture raised plants of Indian origin seedling plants. The flowering and fruiting data were recorded from five plants as five

replications with two bunches from each plant. The date of pollination of each bunch was noted and their respective date of harvesting maturity was noted for all the five years of experimentation. Temperature parameters of the fruiting season was observed using automatic weather station present at the research station. The data of maximum temperature and minimum temperature was used to calculate the degree days where 10°C was used as base temperature as per the earlier experiments (Chandra *et al*., 1990). Statistical analysis and graphical representation of the data was done using R (ver 4.1.2) based on the methods described by Panse and Sukhatme (1978).

$$\text{Heat Unit} = \frac{\text{Minimum Temperature} + \text{Maximum Temperature}}{2} - \text{Base Temperature}$$

RESULTS AND DISCUSSION

Date palm cultivation in India, especially in the western border districts of Gujarat and Rajasthan are mainly dependent on the rainfall pattern. The data presented in Figure 1, Figure 2 and Table 1 represents the heat unit and number of days required for maturity of date palm upto *Khalal* stage. Based on the Figure 1 and 2, it can be understood that every year the maturity period does not remain same and variation on the maturity days

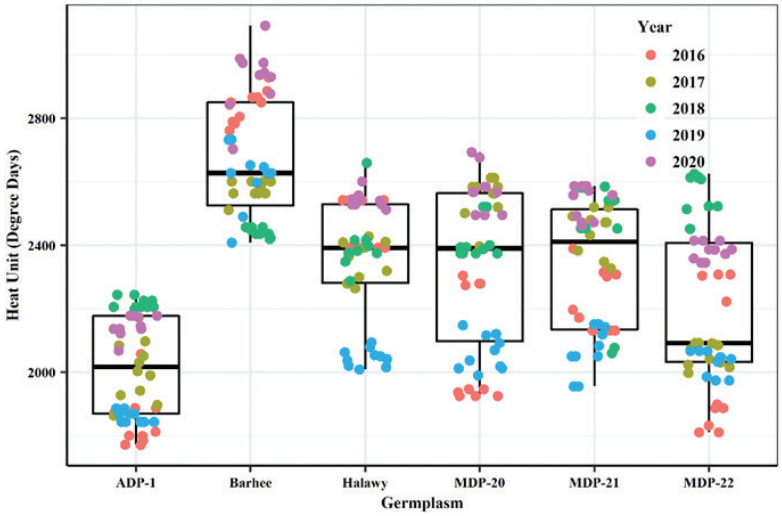

Figure 1: Heat unit needed for different date palm germplasm at *Khalal* stage

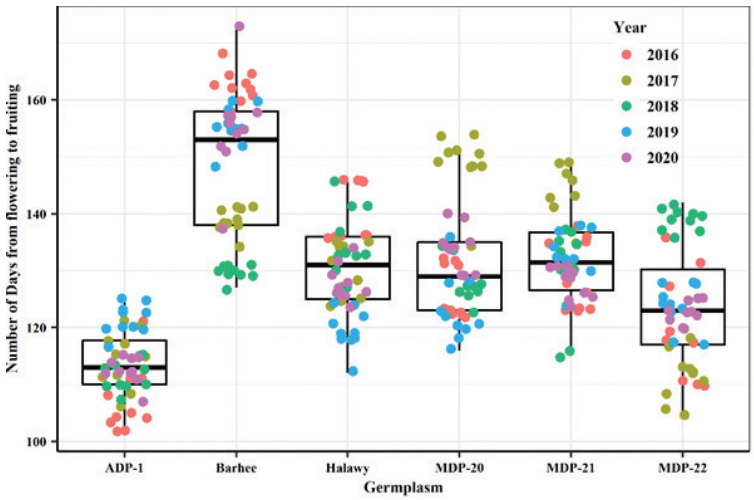

Figure 2: Number of days needed for maturity of different date palm germplasm at *Khalal* stage

Table 1: Heat units required for maturation of date palm cultivars (Pooled for 2016-2020)

S. No.	Germplasm	Maturity period	Heat units (base 10°C) for *Khalal* Stage*	Number of days from flowering to fruiting
1.	ADP-1	June Mid- June End	46.42(2011)	113.42
2.	Barhee	July End- August First	62.51(2679)	148.32
3.	Halawy	June End-July First	54.72(2360)	130.52
4.	MDP-20	June End-July First	54.95(2334)	131.94
5.	MDP-21	June End-July First	54.65(2341)	132.12
6.	MDP-22	June End-July First	50.40(2209)	123.70
		C. D. @ 5 %	6.74	16.58

* Data are sq. root transformed value. Value in the parenthesis are original value.

were observed. The pooled data showed that the lowest duration (113.42 days) and heat unit (2011 degree days) required for ADP-1 while the highest duration (148.32 days) and heat unit (2679 degree days) required for Barhee. It was observed that ADP-1 matures earliest among all the germplasm and often reaches *tamar stage* or *pind khajoor* stage before the Barhee reaches its *Khalal* stage. Next to the maturity, was MDP-22 which required 123.70 days and 2209 degree days for maturity. Chandra *et al.* (1990) reported that 1951 and 2323 heat units were required to reach colour turning stage for Halawy and Barhee respectively, while Kalra and Bajwa (1976) suggested the need of 1800°C to 2000°C HSU above 18°C to reach their ripening stage, which suggests similarity with our present study.

CONCLUSION

Date palm is now getting popularity in Tamil Nadu, Maharashtra, Andhra Pradesh *etc.* and for further expansion, early maturating cultivars will be needed. Evaluation of germplasm based on heat unit can be a better tool for identification of potential germplasm.

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Effect of pre-treatments on germination of Latka (*Baccaurea sapida* Muell. Arg.)

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ABSTRACT

Latka (Baccaurea sapida)is an under-utilized fruit crop, native to South-east Asia, belongs to family Euphorbiaceae, having ethno-medicinal, social, religious and nutritional values. The plant is mostly propagated by seeds, which are recalcitrant in nature. In the present study various pre-sowing treatments were experimented for the propagation of the plants through seeds to determine the germination per cent, germination value and mean germination time. Seeds soaked in2% NaH_2PO_4 for 12 hours showed the highest germination per cent (87.00%); maximum germination value (10.18) and minimum mean germination time (21.11 days)followed by1% NaH_2PO_4 and 6% thiourea for 12 hours, that was exhibited equal germination per cent (85.00%) in both, with germination value was 9.69 and 9.55and mean germination time was 21.78 and 22.25 days, respectively. Hence, the above pre-treatments are recommended for the commercial propagation of this species with taking adequate care and handling operation because seeds are recalcitrant in nature.

***Keywords*:** Germination percent, germination value, latka, pre-sowing.

INTRODUCTION

Baccaurea sapida is an under-utilized fruit crop, native to South-east Asia, belongs to family Euphorbiaceae (Rahman *et al*., 2014) which is known as Burmese grape (in English), Latka (in Bengli), Khattaphal (in Hindi), Kusum (in Nepali), Amda (in Odia) and Sohramdieng (in Khasi).The generic name 'baccaurea' is derived from a Latin term referring to the golden-yellow fruit colour (Chakrabarty and Gangopadhyay, 1997). It is widely distributed in tropical moist forests and homestead gardens in entire Terai region of West Bengal (Bhowmick *et al*., 2016), eastern sub-Himalayan tract (Rymbai *et al*., 2016) from plains of Bihar to high lands of Arunachal Pradesh and in lower hilly areas of North-eastern states, Odisha and Andaman and Nicobar islands. The tree is tall, decorative, dioecious, shade-bearer and evergreen in nature. Flowering occurs in summer and fruits are matured during monsoon period and fruit bearing is cauliflory in nature (Bhowmick, 2011). The plant also shows mild biennial fruiting (Pal *et al*., 2008).

The whole plant including leaves, roots, fruits and seeds have ethno-medicinal importance and health benefits. The fruit is traditionally offered by locals in the Holy Chariot Procession of Lord Madan Mohanin Cooch Behar, West Bengal. The fruit rich in vitamin C, protein and Fe (Peter, 2007).The seeds of the plant are also used to shade orange colour in silk and cotton textiles, as an alternative to "annatto" dye (Raghavan and Ramjan, 2018).The fruit is used as an anti-inflammatory and pain killer and used for the treatment of injuries, rheumatoid arthritis, cellulitis, abscesses *etc.* (Lin *et al.*, 2003).The bark is also reported to be used for curing skin diseases in Manipur and Meghalaya (Singh *et al*., 2014; Momin *et al.,* 2016).

Application of various seed treatments, *viz.* scarification, stratification, soaking in cold water, application of growth regulators and other chemicals, are beneficial to break the dormancy and enhance the seed germination. Several experiments conducted by different researchers on various minor fruit crops by application of certain chemicals to improve the germination, such as *Mimusops elengi* (Dey *et al*., 2021); *Spondia*

spinnata (Dey *et. al*., 2016); *and Syzygium cumini* (Barman *et al*., 2015) respectively. But no standard methodology and practices are carried out on *Baccaurea sapida* for enhancing germination potential of seeds because of its short period of viability. In this regard, the current experiment was conducted to study the effect on the germination of *Baccaurea sapida* using different pre-treatments.

MATERIALS AND METHODS

The experiment was carried out in the Central nursery of Department of Forestry under Uttar Banga Krishi Viswavidyalaya, Cooch Behar in 2018-19. The experimental site is located at 43m above msl at $26^0$23' 45.8" N latitude and 89^0 23' 16.7" E longitude. Climate is subtropical humid with wider seasonal and diurnal temperature variation. The mean annual temperature varied, 21.84^0-33.51^0C with relative humidity, 64-98%. The mean annual rainfall of this location is 2300-2500mm which is concentrated in pre-monsoon and monsoon period.

After observing the physiological maturity visually, ripened fruits were collected from the nearby areas of the university. Seeds were de-pulped manually to remove seeds and dried under shade at room temperature for one day. The experiment was conducted with two factorial complete randomized block design.The primary factor was the chemicals used for enhancing the seed germination, having 11 levels including control, namely:T_1: control; T_2: cold water; T_3: 1% KNO_3; T_4: 2% KNO_3; T_5: 3% KNO_3; T_6: 1% NaH_2PO_4; T_7: 2% NaH_2PO_4; T_8: 3% NaH_2PO_4; T_9: 2% thiourea; T_{10}: 4% thiourea and T_{11}: 6% thiourea, comprising three replications with 100 seeds per replication. In other hand, soaking period was the secondary factor having 02 levels, namely: S_1: soaking for 12 hours and S_2: soaking for 24 hours. A total of 6,300 seeds were sown in polybags of 5''x7'' comprising well pulverized sand,soil and FYM (1:1:1 v/v) with adequate drainage facility. Weeding and watering was regularly done or as per need.

Observations on germination of seeds were recorded from the date of sowing up to one month and the parameters were documented as follows: Germination per cent (%) = (Number of seed germinated x Total number of seed $sown^{-1}$) x 100. Germination value (GV) was evaluated as per the method given by Czabator (1962). Mean Germination Time (MGT) was determined by MGT= "(Daily Germination x Days) x Number of seed $sown^{-1}$ (Un-germinated seeds at the end of the test were given values of n+1, where n is the number of days in the test) by following Bonner (1983) and Dey *et al.* (2021). One way ANOVA for each parameter was carried out by MS Excel 2019 and mean difference between the treatments was encountered by following Critical difference $(CD)_{P\leq 0.05}$ test. Angular transformation was carried out following the procedure as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The effect of various pre-treatments with regards to germination percentage, germination value and mean germination time was recorded and a significant germination potential of seeds was found due to significant interaction between the chemicals and period of soaking (Table 1). A greater germination response was recorded in all the pre-treatments with germination per cent varied, 46.67-87.00%. Seeds soaked in 2% NaH_2PO_4 for 12 hours had significantly highest germination (87.00%) which was at par with 1% NaH_2PO_4and6% thiourea for 12 hours recording 85.00% germination in both.

It was observed that irrespective of chemical applied, seeds soaked for 12 hours had higher germination percentage (73.52%) than that soaked for 24 hours (67.03%). It might be due to the recalcitrant nature of the seeds (King and Roberts, 1979) and higher embryo-respiration (Zaghdani *et al*., 2000). Seeds soaked with cold water had 64.67% and 64.00% for 12 and 24 hours, respectively. Irrespective of soaking period, the germination percentage was showed decreasing trend from 81.67 to 60.00% with increasing concentration of KNO_3; whereas an increasing trend from 60.67 to 85.00% % with increasing concentration of thiourea while germination percentage showed increasing trend with increasing concentration and then declined in higher concentration of NaH_2PO_4. Seed size within species has also been shown to affect the percentage of seed germination.The present findings are in close alinement with the results of Kumar *et al*. (2003) and Dey (2011) in *Gmelina arborea.*

Table 1: Effects of pre-treatments on germination percentage (GP), germination value (GV) and mean germination time (MGT) in seeds of *Baccaurea sapida*

Pre- treatments	GP (%)	GV	MGT (Days)
Soaking period			
S_1:12 hours	73.52 (59.04)	7.36	23.27
S_2:24 hours	67.03 (54.97)	6.03	24.10
SEm±	**0.41**	**0.13**	**0.08**
C.D. (p=0.05)	**1.18**	**0.38**	**0.24**
Chemicals			
T_1: Control	46.67 (43.10)	2.84	26.06
T_2: Cold water	64.33 (53.34)	5.36	24.91
T_3: 1% KNO_3	79.33 (62.97)	8.42	22.71
T_4: 2% KNO_3	73.83 (59.24)	7.21	23.71
T_5: 3% KNO_3	63.50 (52.84)	5.33	24.43
T_6: 1% NaH_2PO_4	79.00 (62.74)	8.34	22.87
T_7: 2% NaH_2PO_4	81.00 (64.17)	8.88	21.80
T_8: 3% NaH_2PO_4	69.50 (56.49)	6.50	23.32
T_9: 2% thiourea	66.33 (54.54)	5.81	24.66
T_{10}: 4% thiourea	70.67 (57.22)	6.67	23.35
T_{11}: 6% thiourea	78.83 (62.62)	8.28	22.71
SEm±	**0.97**	**0.31**	**0.20**
C.D. (p=0.05)	**2.77**	**0.89**	**0.56**
Interaction			
T_1 S_1: Control	46.67 (43.10)	2.84	26.06
T_1 S_2: Control	46.67 (43.10)	2.84	26.06
T_2 S_1: soaking in cold water for 12 hours	64.67 (53.54)	5.43	25.24
T_2 S_2: soaking in cold water for 24 hours	64.00 (53.14)	5.28	24.58
T_3 S_1: soaking in 1% KNO_3 for 12 hours	81.67 (64.66)	8.96	22.55
T_3 S_2: soaking in 1% KNO_3 for 24 hours	77.00 (61.35)	7.87	22.86
T_4 S_1: soaking in 2% KNO_3 for 12 hours	74.67 (59.79)	7.44	23.09
T_4 S_2: soaking in 2% KNO_3 for 24 hours	73.00 (58.70)	6.97	24.34
T_5 S_1: soaking in 3% KNO_3 for 12 hours	67.00 (54.95)	5.91	24.12
T_5 S_2: soaking in 3% KNO_3 for 24 hours	60.00 (50.78)	4.76	24.74
T_6 S_1: soaking in 1% NaH_2PO_4 for 12 hours	85.00 (67.23)	9.69	21.78
T_6 S_2: soaking in 1% NaH_2PO_4 for 24 hours	73.00 (58.70)	6.99	23.96
T_7 S_1: soaking in 2% NaH_2PO_4 for 12 hours	87.00 (68.88)	10.18	21.11
T_7 S_2: soaking in 2% NaH_2PO_4 for 24 hours	75.00 (60.01)	7.57	22.49
T_8 S_1: soaking in 3% NaH_2PO_4 for 12 hours	72.00 (58.06)	7.00	22.85
T_8 S_2: soaking in 3% NaH_2PO_4 for 24 hours	67.00 (54.95)	6.00	23.79
T_9S_1: soaking in 2% thiourea for 12 hours	72.00 (58.06)	6.78	24.01
T_9 S_2: soaking in 2% thiourea for 24 hours	60.67 (51.17)	4.84	25.30
T_{10} S_1: soaking in 4% thiourea for 12 hours	73.00 (58.70)	7.16	22.90
T_{10} S_2: soaking in 4% thiourea for 24 hours	68.33 (55.76)	6.17	23.80
T_{11} S_1: soaking in 6% thioureafor 12 hours	85.00 (67.23)	9.55	22.25
T_{11} S_2: soaking in 6% thiourea for 24 hours	72.67 (58.49)	7.00	23.17
SEm±	**1.37**	**0.44**	**0.28**
C.D. (p=0.05)	**3.91**	**1.26**	**0.79**

Values in parentheses are arc-sine values.

Irrespective of the different chemical applied and period of soaking, the germination value was varied from 2.84 to 10.18. The mean maximum germination value (10.18) was noticed in seeds treated with 2% NaH_2PO_4 for 12 hours followed by 1% NaH_2PO_4 and 6% thiourea for 12 hours recording the value of 9.69 and 9.55, respectively while the minimum (2.84) was observed in control. The germination value was followed the same trend as germination percentage. Germination value indicates the seedling vigour that produced by the seed under study (Willan, 1985). The present experiment rigidly supports the statement that, seed sources having more germination of seed had more germination value. This finding is well in line with the results of Dey (2011) and Mutha *et al*. (2004).

The significant effects of various pre-treatments on mean germination time (days) were observed. The highest average (26.06 days) was observed in control whereas the lowest (21.11 days) was exhibited in 2% NaH_2PO_4 for 12 hours followed by 21.78 and 22.25 days was noticed when seeds soaked with1% NaH_2PO_4 and 6% thiourea for 12 hours, respectively. The present findings also support the statement that the better germination will evident with lesser time (MGT) for germination. This experiment could be elaborated that soaking seeds with 2% NaH_2PO_4 for 12 hours plays a great role in breaking of dormancy and greater physiological activity at a faster rate. The findings were similar with Roy *et al*. (2004) and Sherpa (2021) in *Pinus roxburghii* and *Michelia champaca,* respectively.

In the present study, seeds soaked with 2% NaH_2PO_4 for 12 hours showed higher germination per cent, germination value and lowest mean germination time followed by 1% NaH_2PO_4 for 12 hours and 6% thiourea for 12 hours, respectively. Moreover, seeds soaked for 12 hours had higher germination percentage and germination value than that soaked for 24 hours. Hence, the above said pre-treatments are recommended for the commercial seedling propagation of this species.

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Effect of nutrient feeding on controlling leaf chlorosis, yield and physico-chemical composition of sweet orange (*Citrus sinensis* L. Osbeck) cv. Mosambi

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ABSTRACT

The present investigation was conducted in sub-tropical weather in farmer's field at Jhargram, West Bengal during 2018, to know the effect of nutrient feeding on controlling leaf chlorosis, physico-chemical composition of sweet orange (Citrus sinensis L.Osbeck) *cv. Mosambi. The investigation wascompleted in a randomized complete block design(RCBD) with seven treatments (T_1-Vermiwash @6ml/litre, T_2-Multiplex- @ 3ml/litre, T_3-Humar @ 3ml/litre, T_4-$ZnSO_4$ @ 0.4% + borax @ 0.2% +$FeSO_4$ @ 0.2% + $MnSO_4$ @ 0.2%, T_5-Nitrophoska @8g/litre, T_6-T_4 +after 07 days T_5 and T_7- Control) with four replica. Based on the results, it was concluded that the minimum leaf chlorosis (5.1%)with maximum vitamin C (41.8 mg/ 100g) were observed with treatment T_2, while the maximum number of fruits per plant (65.0), yield per plant(6.63 kg), fruit juice (43 %) and TSS content (9.4 %) were observed with treatment T_4 followed by T_6 and maximum fruit weight (108 g) and minimum acidity (0.29 %) were observed with treatment T_6. The vermiwash, an organic sources of different nutrients, hormones, etc. also showed a good results with regard to reducing leaf chlorisis, increasing fruit yield and quality fruits and is recommended for organic farming.*

***Keyword*:** Foliar feeding, fruit yield and quality, major and micro-nutrients, Mosambi-sweet orange, organic and inorganic sources.

INTRODUCTION

Sweet orange (*Citrus sinensis*) is considered as most important fruit crop of citrus group which belonging to the family Rutaceae and is a native of Southern China. In India, it is widely grown in the subtropical zone of Andhra Pradesh, West Bengal, Karnataka, Maharashtra, Punjab, Rajasthan and Haryana. In West Bengal, the western part of the state is suitable for growing Mosambi-sweet orange and quality fruits in higher quantity have been reported (Nandi and Ghosh, 2016). Sweet orange occupies an important place in the economy of the world on the basis nutritional and antioxidant value. Fruits are a good source of vitamin C (65.69 mg/ 100g), and their daily consumption helps to prevent scurvy, a disease caused by a lack of vitamin C in the diet. Apart from this, 100 g fruit also contains protein 0.8-1.4 g, fat 0.2-0.4 g, vitamin-A 198 I.U, 0.113 mg vitamin B_1, 0.046 mg riboflavin, fiber 0.8 g, 0.2- 0.8 mg iron, potassium 192-201 mg, 0.16 mg calcium (Thorat *et al.* , 2018). As a result of growing awareness of nutritional security and the rapid development of processing companies around the world, demand of this crop has been increased tremendously.

With the rising demand of mosambi-sweet orange fruits, the area of this fruit has been increasing at a faster rate in India, even in the non-traditional citrus growing regions. Despite massive area expansion, crop production and fruit quality, but the situation in non-traditional areas has not improved to a satisfactory level. Out of many factors,the nature of the soil *i.e.*, calcareous and alkaline, which hinders the smooth uptake of micronutrients to the plants from soil, is one of the key reasons for the low yield and poor fruit quality of mosambi under non-traditional citrus growing conditions (Nandita *et al.*, 2020). Recently, foliar spraying of nutrients, has acquired a lot of attention particularly in perennial crops because of its well-recognized beneficial effect on fruit quality and crop yield (Bhanukar *et al*., 2018; Singh *et al*.,

2017). Foliar application gives a quick effect as nutrients can be absorbed rapidly through the stomata of the leaf and in some instances through the cuticles (Fernandez *et al.*, 2013). Considering the beneficial effect of foliar feeding of nutrients, a study was made with different products, containing nutrients of organic and inorganic sources, with the view to improve the plant health and to increase production of quality fruits of sweet orange cv. Mosambi in red and laterite soils.

MATERIALS AND METHODS

This experiment was conducted in a sub-tropical weather on farmer's field at Jhargram, West Bengal during 2018. Eight years old plants of sweet orange cv. Mosambi of uniform vigour were selected for the study. There was seven treatments *viz.*,T_1-Vermiwash @6ml/litre, T_2-Multiplex- @ 3m/litre, T_3-Humaur @ 3ml/litre, T_4-$ZnSO_4$ (0.4%) + borax (0.2%) +$FeSO_4$ (0.2%) + $MnSO_4$ (0.2%), T_5-Nitrophoska (19:19:19 :: N:P:K) @8g/litre, T_6-T_4+ after 07 days T_5, T_7- Control(water spray). Each treatment was replicated four times with two plants in each replication. Humaur is a bioorganic foliar nutrient which content various nutrients, vitamins and enzyme manufactured by Hindusthan Antibiotic Limited, Pune, Maharashtra, India. Vermiwash is the leached water, collected during the preparation of vermi-compost; nitrophoska- is the water soluble fertilizer, containing N, P and K at 19:19:19 ratio and multiplex- is a water soluble foliar micro-nutrient prepared by Karnataka Agrochemicals Pvt. Ltd. and reported to have different micronutrients in soluble form. Plants were sprayed as per treatment after sunset, three times *i.e.,* on 10th March (after fruit set), 10th June and 10th August. Water soluble sticker was added for increasing the efficiency of sprayed particles on the leaves. The investigation was completed in a randomized complete block design (RCBD).

For management of the plants, each plant was fertilized three times with 40kg of FYM + urea 300 g + di-ammonium phosphate 300g +dolomite 200 g/plant on 15th March; on 15th May with urea 300 g +SSP 300 g+MOP 200g/plant and on15th July with urea 300 g +MOP 500g +mustard cake 1.0 kg. To check the fruit drops, the plants were sprayed with different bio-regulators four times *viz.,* 2,4-D at 10ppm at just after fruit set (March); GA_3 at 25 ppm on 15th May; 2,4-D at 10ppm on 15th June and GA_3 at 25ppm on 15th July. Irrigation was provided through drip during dry period (March to middle of June). Insecticides and fungicides were sprayed as per need of the plant.

For measuring leaf chlorosis, 4-shoots/ plant were tagged in four directions. Then total numbers of green and yellow leaves were counted in March, April, May, June, July, August and September, to know the plant's need of time of micro-nutrients. Before counting yellow leaves, all dry shoots from every plant were removed in February.The number of fruits/plant was counted before harvesting (20th September). Fruit weight (g) was taken with the help of a balance (average of 10 fruits/plant) and fruit yield/plant was calculated by multiplying the number of fruits per plant with average fruit weight. For juice content (%) first fruits are weighed and recorded then the percent juice contents were calculated by using the following formula; % juice contents = (juice weight ÷ fruit weight) x 100 (Jamil *et al*., 2015). Total soluble solid content (TSS) of fruits was estimated with the help of a refractometer and calibrated at 0°Brix. The acidity (%) and ascorbic acid ((mg/100g),) content were estimated as per the methods suggested by A.O.A.C. (1990).

RESULTS AND DISCUSSION

Leaf chlorosis

It is clearly seen from the data in Table 1 that the effect of nutrient feeding on minimizing the leaf chlorosiswas significantly varied. In the different months, all the products of micronutrients were found effective in reducing the chlorosis in the leaves and they were significantly at par among themselves. Among the different sources or products of micro-nutrients, multiplex at 3ml/l was the most effective as it resulted in lowest chlorosis in June (13.2%), July (10.1%), August (7.3%) and September (5.1%) followed by T_4 [$ZnSO_4$ (0.4%) + borax (0.2%) +$FeSO_4$ (0.2%) + $MnSO_4$ (0.2%)]. The treatment T_5 which content NPK (Nitrophoska (19:19:19 :: N:P:K) @8g/litre) was ineffective in this regard which indicated that leaf chlorosis was mainly due to deficiency of micro-nutrients during May to June (fruit growth and development period). Another interesting observation was noted that the demand of micro-nutrients was more during June to August as because the control plants showed the

Table 1: Effect of nutrient feeding on leaf chlorosis (%) in Mosambi

	May	June	July	August	September
Treatment	**Yellow leaf**	**Yellow leaf**	**Yellow leaf**	**Yellow leaf**	**Yellow leaf**
T_1 Varmiwash	4.2	17.7	23.2	17.3	16.3
T_2 Multiplex	4.7	13.2	10.1	7.3	5.1
T_3 Humaur	5.7	15.9	22.4	18.1	13.9
T_4 (Zn, B, Fe, Mn)	7.2	13.8	14.3	8.4	5.4
T_5 Nitrophoska(NPK)	32.6	30.4	41.0	25.1	24.6
T_6 (T4, after 07 days T5)	9.5	17.5	15.6	8.7	6.7
T_7 (Control)	12.8	29.4	40.4	35.9	25.3
SE(m) ± C.D. at 5%	**2.502 7.434**	**1.803 5.358**	**3.103 9.220**	**2.631 7.819**	**2.175 6.461**

T_1-Vermiwash @6ml/litre, T_2-Multiplex- @ 3m/litre, T_3-Humaur @ 3ml/litre, T_4-$ZnSO_4$ (0.4%) + borax (0.2%) +$FeSO_4$ (0.2%) + $MnSO_4$ (0.2%), T_5-Nitrophoska (19:19:19 :: N:P:K) @8g/litre, T_6-T_4 + after 07 days T_5, T_7- Control (water spray)

Table 2: Effect of nutrient feeding on fruit yield and physico-chemical composition of Mosambi.

Treatment	Fruits plant^{-1}	Weight of fruit (g)	Yield plant^{-1}(kg)	Juice (%)	TSS (%)	Acidity (%)	Vitamin C (mg/ 100g)
T_1	39.3	105	4.13	41	8.3	0.30	36.2
T_2	38.8	103	4.00	40	9.3	0.32	41.8
T_3	36.7	101	3.71	42	9.0	0.35	37.4
T_4	65.0	102	6.63	43	9.4	0.32	41.7
T_5	33.0	104	3.43	42	9.3	0.30	40.6
T_6	47.5	108	5.13	42	8.9	0.29	41.0
T_7	32.5	99	3.22	40	8.1	0.38	36.0
SE(m) ± C.D. at 5%	**2.8892 8.5842**	**1.2263 3.6436**	**0.3023 0.8981**	**0.4914 1.4602**	**0.1543 0.4585**	**0.0086 0.0256**	**0.7541 2.2406**

T_1-Vermiwash @6ml/litre, T_2-Multiplex- @ 3m/litre, T_3-Humaur @ 3ml/litre, T_4-$ZnSO_4$ (0.4%) + borax (0.2%) +$FeSO_4$ (0.2%) + $MnSO_4$ (0.2%), T_5-Nitrophoska (19:19:19 :: N:P:K) @8g/litre, T_6-T_4 + after 07 days T_5, T_7- Control (water spray)

maximum chlorosis in these months. The reason of high demand of micronutrients during these months was due to fruit growth and development. It was noted that varmiwash, organic source of micronutrients, was also effective in reducing leaf chlorosis in different months as compared to the control plants. In sweet oranges leaf chlorosis due to deficiency of major and minor nutrients is well known but the study indicated that the demand of micro-nutrients is more during fruit growth period as compared to other periods. The result is near to Devi *et al*.(1997) in Sathqudi orange in their experiment where lowest chlorosis (2.5%) was observed with soil application of 50 g/plant each of $FeSO_4$, $ZnSO_4$ and $MnSO_4$ combined with foliar application of 0.5 % each of the above three micronutrients.

Yield and physico-chemical composition

Number of fruits/ plant and yield/ plant (kg) varied significantly due to various nutrient feeding (Table 2). Highest number of fruits/plant (65.0) and yield/plant (6.63 kg) was noted in treatment of $ZnSO_4$ @0.4% + borax @ 0.2% +$FeSO_4$ @ 0.2%

+ $MnSO_4$ @ 0.2% (T_4) wherein lowest fruit number and yield/plant (32.5 and 3.22 kg respectively) was observed in control plants (T_7). The combined use of Fe, Zn, B and Mn prolonged the photosynthetic activities which resulted in more production of carbohydrates that resulted in fruit production in mosambi sweet orange. The findings are supported with the results of Singh *et al.* (2018) and Nandita *et al*. (2020) in sweet orange. Fruit production in terms of number of fruits (47.5) and yield/plant (5.13kg) was also higher in T_6 (combination of micronutrients and NPK).

Foliar application of nutrients helped to improve fruit weight (Table 2) significantly and highest fruit weight (108g) was recorded fromT_6 (combination of micronutrients and NPK) as compared to control (99 g). Highest fruit weight in T_6 may be explained from the fact NPK (readily available form) in combination with micronutrients, helped to increase more synthesis of carbohydrate and other photosynthates. Similar type result was found by Reetika *et al.* (2018) in Kinnow Mandarin when the plants were sprayed with a combination of urea 1.0% + $ZnSO_4$ 0.5% + K_2SO_4 1.0% + H_3BO_3 0.2% + $FeSO_4$ 0.5%. In another research, Kazi *et al*. (2012) found a similar result in sweet orange and they recorded that fruit weight/tree improved with the application of NPK and multi micronutrients.

Table 2 cue that the application of nutrients significantly increased juice content and TSS. The highest juice content (43 %) was found in T_4 whereas the lowest (40 %) was in control. The study made by Rama and Bose (2000) and Kaur *et al*. (2015) in mandarin orange indicated that the highest juice content was associated with the application of Zn, B, Fe. TSS was highest (9.4° brix) in T_4 and the lowest (8.1° brix) in control. This finding was confirmed by Ghosh and Basra (2000), who observed that the application of zinc +boron in sweet orange increase TSS.

The acidity content was maximum in control fruits (0.38%) and lowest in T_6 (o.29%) (Table 2). Lower fruit acidity in nutrients treated plants may be due to the transformation of organic acid into sugar. These results are supported by Kazi *et al.* (2012) who found the application of NPK and micronutrients minimize acidity in sweet orange.

The ascorbic acid content in the fruits was significantly increased with the application of nutrients (Table 2). The highest vitamin C (41.8 mg/100ml) was measured from the plants in T_2 closely followed by T_4 (41.7mg/100ml) and the lowest (36.0 mg/100ml) in T_7 (control). Application of micronutrient increased the sugar level and vitamin C synthesized from sugar which may be a possible reason and zinc also plays a major role in the creation of auxin, which is also boost vitamin C content (Alloway, 2008; Nawaz *et al.,* 2008). Similar observations were also documented by Tariq *et al.* (2007) in Sweet orange.

CONCLUSION

The conclusion of the experiment that, the application of micronutrients $ZnSO_4$ (0.4%) + borax (0.2%) +$FeSO_4$ (0.2%) + $MnSO_4$ (0.2%) in soluble form during fruit set, growth and development period (March to August) helped to minimize the leaf chlorosis, fruit drop and increased fruit yield and physico-chemical composition in Mosambi. Foliar feeding with soluble N, P and K like nitrophoska may have synergistic effect on increasing more sizeable fruits of quality fruits. Vermiwash, an organic sources of different nutrients, hormones, etc. showed good results with regard to reducing leaf chlorisis, increasing fruit yield and quality fruits as compared to control plants. Multiplex, commercial products of micronutrients, is also helpful in reducing leaf chlorosis and improving production of quality Mosambi fruits.

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Effect of gamma irradiation on seed germination, survivability and growth performances of *Calotropis gigantea* (Vara)

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ABSTRACT

Physical mutagenesis is an effective mutational breeding method for improving various morphological characteristics of agricultural and medicinal crops. The objective of the conducted experiment was determining the effective dose of gamma radiation to induce mutations in Calotropis gigantea. Well matured seeds of C. gigantea were exposed to Gamma irradiation using "Gamma chamber 1200 Cobalt-60" research irradiator and these treatments were carried out at the Horticultural Crop Research and Developmental Institute at Gannoruwa, Sri Lanka. Mature seeds were subjected to seven treatments as 150Gy, 300Gy, 450Gy, 600Gy, 750Gy, 900Gy and non-irradiation (0 Gy) as a control to study their effect on germination percentage, survival rate and growth performances of C. gigantea. Treated seeds were arranged under shade house condition in Completely Randomized Design with four replications and each replication contained twenty-four seeds. The mutagenic treatments were tested for lethal dose of 50% and the dose at which 50% of the survival at four months was considered as LD50 value. Data were analyzed by ANOVA using Minitab 17 software and compared the treatment means using Dunnett's test at 0.05 significant level. The study revealed that the higher dosages of gamma irradiation was significantly decreased the germination percentage, survivability and growth performances ($P<0.05$). Plant survivability, number of leaves and plant height were significantly ($P<0.05$) reduced with the higher dosages of 450 Gy to 900 Gy. Lethality level of gamma irradiation for Calotropis gigantea was found as 395 Gy at maturity stage and it could be concluded that the radiation below 395 Gy should be imposed to induce mutations in Calotropis gigantea.

***Keywords*:** *Calotropis gigantea*, dosage, gamma irradiation, germination, survival

INTRODUCTION

Calotropis gigantea is a shrub which belongs to family Apocynaceae, commonly known as "milk weed" or "crown flower" in English, *Wara* or *Helawara* in Sinhala and *Mannakkovi* in Tamil (Abeysinghe, 2018). The shrub has a branched woody stem covered with a corky bark (Sharma *et al.*, 2011). Thick, waxy and grey green leaves show ovate shaped and simple, opposite leaf arrangement. Flower colour is white or lavender and having waxy appearance (Ganeshan *et al*., 2018). The fruit is green in color, spongy simple and follicle. It is identified as a diploid (2n=22) outcrossing plant and reproduction success mostly depends on hymenopteran insect pollination. This plant is most diverse in Asian and South East Asian countries such as Sri Lanka, India, Cambodia, Bangladesh, Indonesia, Malaysia, Pakistan and Philippines where tropical and subtropical climate prevails (Ganeshan *et al*., 2018). There are several medicinal values in *C. gigantea*. Indonesians use the roots of *C. gigantea* as an antidote for snake bite (Kitagawa *et al*., 1992). Leaves of *C. gigantea* are used to treat ailments such as skin and liver diseases, leprosy, earaches and worms (Rajakaruna *et al*., 2002). Its latex is reported to possess wound healing ability due to the presence of proteases such as Calotropins DI (Urs *et al*., 2017). Several studies reported that antimalarial and anticancer effects of *C. gigantea* (Wong *et al*., 2011).

Changes in the plant's genetic structure can result in more physiologically and chemically efficient plant varieties with higher secondary metabolite synthesis. Mutation is a genetic difference caused by a rapid change in the gene. Gamma radiation is a mutagenic agent that is commonly used to induce mutations (Rifnas *et al*., 2020). *C. gigantea* is still an undomesticated plant

with uncertain economic returns. There is a need of facilitating for breeding purpose and improvement of growth characters of this plant to domesticate as miniature plant due to its medicinal and aesthetic value. Therefore, this experiment was carried out to find out the effect of gamma irradiation on survivability and growth performances of *C. gigantea.* Further, this will provide a baseline for exploration of physical mutagenesis by gamma irradiation on germination, survival and growth performances of *C. gigantea* for researchers.

MATERIALS AND METHODS

The experiment was performed at the University of Colombo's Institute for Agro Technology and Rural Sciences at Weligatta, which is located in Sri Lanka's Dry Zone, where the average annual rainfall is 1250 mm-1500 mm and the average annual temperature is 29 - 33^{0}C. Soil types of the area are reddish brown earth and low humic gley soil. Healthy and mature *C. gigantea* seeds collected from different areas in the dry zone were used as experimental material. At the Horticultural Crops Research and Development Institute in Gannoruwa, Sri Lanka, seeds of *C. gigantea* were irradiated with gamma rays from a Cobalt 60 research irradiator. The seedlings were treated with seven different treatments: 150 Gy, 300 Gy, 450 Gy, 600 Gy, 750 Gy, 900 Gy, and no irradiation (0 Gy). The treatment dosages were determined based on the previous experiments conducted on the related plant families. Treated seeds were planted in poly bag containers (250 gauge, 12.5 cm height ×10 cm width) at 0.5 cm depth in sand: compost 1:1 media. The poly bags were kept in a net house and watered daily to maintain adequate moisture. The experimental design consisted of four replicates and 24 seeds per replicate under the Completely Randomized Design (CRD). Radical emergence was taken as the indicator for seed germination. Plants were transferred to 30 cm diameter pots containing sand: compost: top soil 1:1:1 media, 8 weeks after germination.

Measurement of traits

Data on seed germination were recorded starting from the first day of emergence of the radicals of the plant from the growth medium. Cumulative number of germinated seeds was recorded until eleventh day from germination where plant germination was observed to be constant. The germination percentage was calculated as follows:

$$\text{Cumulative germination percentage } (\%) = \frac{No.of\ seeds\ germinated\ at\ final\ count}{No.of\ seeds\ planted} \times 100$$

Plant growth parameters such as cumulative leaf number and height of seedlings (measured from the surface of the planting medium to the tip of the longest branch in cm) were recorded from plants growing in the net house at 2 weeks after planting by using ruler and steel tape. Survival rate of seedling plants was calculated after 6 months from planting as the ratio of number of living (survived) plants to the total number of seedlings planted as follows;

$$Survival\ rate\ of\ seedling\ plant\ (\%) = \frac{No.\ of\ survived\ plants}{No.\ of\ seedlings\ plant} \times 100$$

The percentage inhibition or stimulation over control which is lethality over control (LOC) was calculated as follows;

$$LOC = \frac{(Survival\ in\ control\text{-}Survival\ in\ treatments)}{Survival\ in\ control} \times 100$$

Data analysis

Minitab was used to statistically analyse the collected data using Analysis of Variance (ANOVA), and Dunnets's test was used to separate the means at the 0.05 significant level.

RESULTS AND DISCUSSION

Effects of gamma irradiation on seed germination

The cumulative germination (CG) percentages of *C. gigantea* seeds treated with 6 doses of gamma rays and the control are shows the increasing pattern of their germination (Figure 1). According to the results, it was found that, there were significant (P>0.05) differences between the treatments over control on seed germination. The

highest cumulative germination was observed in control (96.25%) where seeds were not exposed to any irradiation doses and the lowest (32.5%) was observed where seeds treated with the highest dosage (900 Gy). As in many other studies an inverse relationship between the gamma dosages and germination percentage was observed. Several studies of gamma radiation on different crops are on par with present results. According to Aynehband *et al*. (2012), gamma radiation ranging from 0 Gy-250 Gy was significantly decreased the percentage of Amaranth seeds germination. The highest percentage of germination (54.4%) was reported at zero and the lowest germination percentage (51.1%) was reported at the highest dose of gamma radiation at 250 Gy in Amaranth seeds. As mentioned by Rifnas *et al.* (2019), there was a progressive reduction in seed germination of sunflower was observed while exposing the seeds to increasing gamma radiation doses ranging 0-125 Gy. According to Kon *et al.* (2007), when maize seeds were exposed to gamma irradiation at dosages ranging from 0.1-1 kGy, the highest inhibition of the germination process (51%) was reported at 1 kGy. Therefore, it is clear that when increasing the radiation dosage, seed germination is decreased as experienced in present experiment as well. When increased the radiation dosage, seeds may have been damaged and this may have prevented them from germinating well.

Seedling survival

Survival of seedlings, 1- 4 months after germination of seeds treated with different gamma radiation dosages are given in Table 1. Seedling survival rate has shown significant difference when compared to the control and the other treatments in each month. Survival rate of seedlings was 100% up to 450 Gy and a significant reduction of survival rate of seedlings (>46%) was observed in treatments over 600 Gy after one month from germination. Seedling survival percentage was significantly reduced over 450 Gy and no plants were able to survive in treatments of 750 Gy and 900 Gy after two months from germination. The result shows that the survivability of all plants gradually declined after four months from germination, even 0 Gy – 300 Gy treatments had 100% survival rate at 2 months after germination. Further, survival of plants was poor in higher dosages of this study such as 600Gy- 900Gy treatments throughout, and after 4 months more than 90% of the plants died. The plants germinated in 750 Gy and 900 Gy treatments were weak and they appeared with pale yellow color cotyledons. After 1 month from germination, all the plants germinated were died in above treatments. There are many studies on different plants reported the effects of higher irradiation doses on seedling survival.

In *Alamanda cathartica*, Rifnas *et al*. (2020) found that as the dose of gamma radiation increased (0 Gy to 21 Gy), survival of plants dropped. Regardless of the method of irradiation, the effect of gamma rays on plant viability was gradual, depending on the degree of exposure (Sawangmee *et al.*, 2011). The rationale for the decreased survival may be due to the damage to cells and cellular components while another factor behind mortality is the rupture of cellular organelles (Navabi *et al*., 2016). Plants were started to die slowly during the early months of treatment and later they recovered. However, the survival rate has been significantly reduced over time. Therefore, it could be stated that gamma ray doses below 450 Gy should be applied to recover useful induced mutations in *C. gigantea*. The LD50 (50% lethal dose) value obtained by plotting survival data after 4th months against the applied dose rate is shown in Figure 2. The half lethal dose obtained from the figure is 395 Gy. Therefore, exposing below 395 Gy gamma ray dosages are suitable for mutation induction of *C. gigantea* with minimizing the lethality effect.

Effect of gamma radiation on different growth parameters

Plant height

Plant height of *C. gigantea* as affected by different doses of gamma radiation is given in Table 2. Different treatments showed differences in plant height, and height of plants in higher doses (>600 Gy) was significantly different to height in lower doses. When the lower doses of gamma radiations were considered (<450 Gy) it was found that there was no significant difference in the height of the seedlings compared to the control in every week. Extreme reduction in the plant height was observed with the higher dosages of 450 Gy to 900 Gy. It is evident from the data presented in Table 2 that the

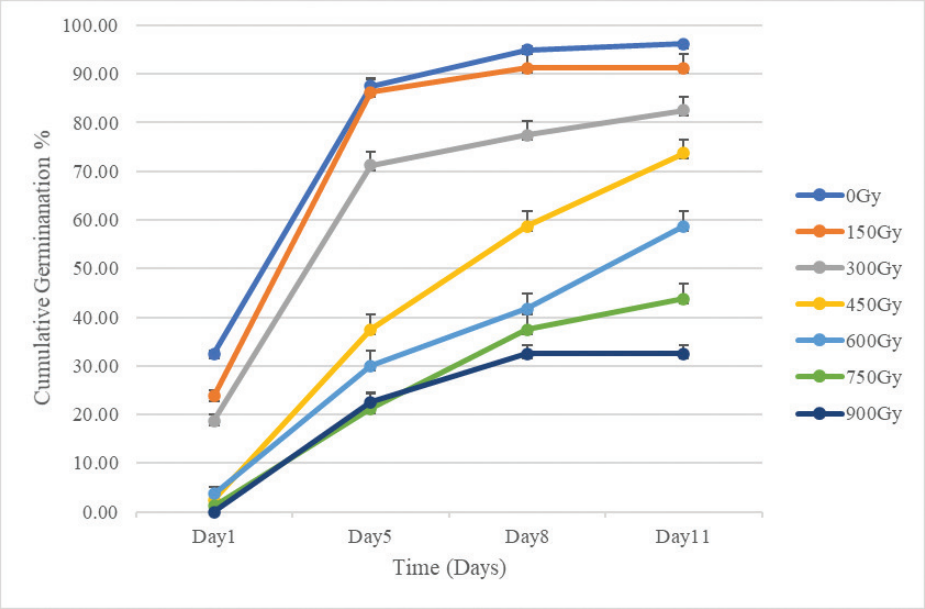

Figure 1: Cumulative Germination percentage of *C. gigantea* as affected by various dosages of gamma rays

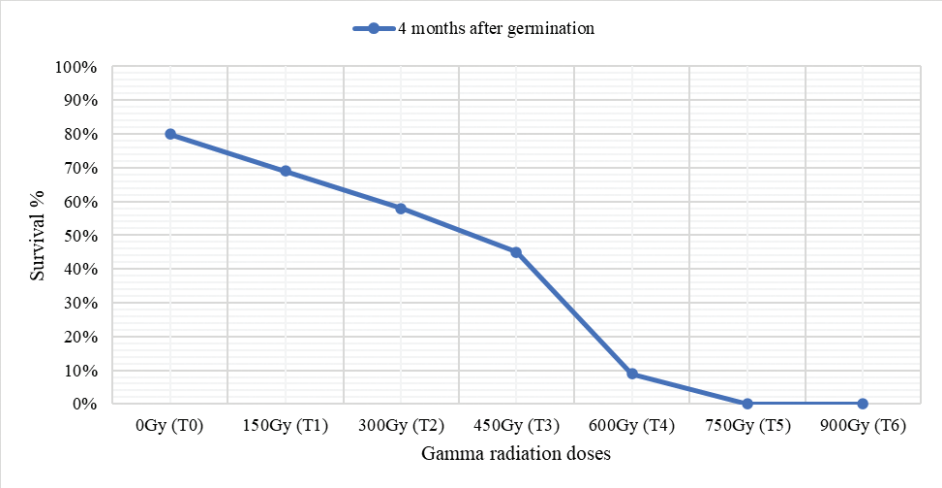

Figure 2: Survival percentage of *C. gigantea* seedlings with different doses of gamma radiation and the LD50 level

highest plant height was recorded from plants grown from non- irradiated seeds followed by seeds from 150 Gy of gamma dosage at every week after planting when compared to other treatments. A similar correlation *i.e.* "low dose-high growth" was observed in *Molluccella lavis* by Minisi *et al.* (2013), in chick pea by Hameed *et al.* (2008) and in amaranth by Aynehband *et al.* (2012). A reducing trend in plant height of *Allamanda cathartica* was observed by Rifnas *et al.* (2020) when exposing the plants to increasing doses of gamma radiation.

In this study, plant height of all the plants above 450 Gy gamma dosages was showing a significant reduction in their height. In mutagenic treatments, this may result in auxin degradation, suppression of auxin production (Gordon, 1954), and chromosomal abnormality (Gunckel and Sparrow, 1961). An experiment in *Moluccella laevis* by Minisi *et al.* (2013) proved that increase in gamma radiation dose decreased the height of *Moluccella laevis* plant. They also stated that higher dosage irradiation induced growth retardation, which they attributed to cell cycle arrest at G2/M phase during

Table 1: Survival percentage of *C. gigantea* seedlings after gamma irradiation treatments

Seedling Survival						
Treatments	**1 month after germination (%)**	**SE±**	**2 months after germination (%)**	**SE±**	**4 months after germination (%)**	**SE±**
T_0 - 0Gy	100[a]	±0.0	100[a]	±0.0	80[a]	±0.82
T_1 - 150Gy	100[a]	±0.0	100[a]	±0.0	69[b]	±1.68
T_2 - 300Gy	100[a]	±0.0	100[a]	±0.0	58[b]	±1.08
T_3 - 450Gy	100[a]	±0.0	86[b]	±0.91	45[b]	±1.08
T_4 - 600Gy	30[b]	±1.41	12.7[b]	±1.04	9[b]	±0.41
T_5 - 750Gy	46[b]	±0.91	0[b]	±0.0	0[b]	±0.0
T_6 - 900Gy	27[b]	±0.58	0[b]	±0.0	0[b]	±0.0

Means labelled with the same letter are not significantly different at $p< 0.05$

Table 2: Plant height of *C. gigantea* with different doses of gamma irradiation

Plant height (cm)						
Treatments	**2nd Week**	**4th week**	**6th week**	**8th week**	**12th week**	**16th week**
0 Gy	3.72[a]	4.59[a]	10.03[a]	11.5[a]	25.9[a]	69.75[a]
150 Gy	3.62[a]	4.58[a]	8.37[a]	10.15[a]	21.5[a]	64.60[a]
300 Gy	2.85[a]	3.73[a]	7.87[a]	10.12[a]	14.17[b]	52.60[b]
450 Gy	1.20[b]	2.02[b]	4.50[b]	7.37[a]	9.80[b]	41.30[b]
600 Gy	0.70[b]	1.18[b]	1.37[b]	2.47 [b]	7.00[b]	26.40[b]
750 Gy	0.68[b]	0.70[b]	-	-	-	-
900 Gy	0.50[b]	0.70[b]	-	-	-	-

Means labelled with the same letter are not significantly different at $p< 0.05$.

Table 3: Number of leaves of *C. gigantea* as affected by different dosages of gamma irradiation

Mean No. of leaves					
Treatments	**4th Week**	**6th Week**	**8th Week**	**10th Week**	**12th Week**
0 Gy	5.04[a]	5.41[a]	6.54[a]	7.60[a]	12.57[a]
150 Gy	5.08[a]	6.00[a]	6.58[a]	7.41[a]	9.70[a]
300 Gy	5.25[a]	6.62[a]	6.10[a]	6.83[a]	9.40[a]
450 Gy	3.68[b]	5.00[a]	5.75[a]	5.85[a]	6.01[b]
600 Gy	1.83[b]	2.06[b]	2.90[b]	4.20[b]	6.05[b]
750 Gy	1.75[b]	-	-	-	-
900 Gy	0.72[b]	-	-	-	-

Means labelled with the same letter are not significantly different at $p< 0.05$.

Table 4: Germination % and LOC % of *C. gigantea* with different dosages of gamma rays

Treatments	Germination % (After 11 days)	Lethality Over Control (LOC)%
0 Gy	96.25	0
150 Gy	91.25	5.44
300 Gy	82.5	14.51
450 Gy	73.75	23.58
600 Gy	58.75	39.12
750 Gy	43.75	54.66
900 Gy	32.5	66.32

somatic cell division and/or different genomic damages.

Number of leaves

Number of leaves of *C. gigantea* as affected by different doses of gamma radiation is given in Table 3. Every treatment showed a significant difference on mean number of leaves when compared to the control ($P<0.05$). The results revealed that there is a significant reduction of number of leaves always above 450 Gy dosages in every week. When the gamma irradiation dosage is higher, it was significantly affected for initiation of number of leaves per plant and it drastically reduced. According to Yadav (2016), larger doses of gamma radiation reduced the number of leaves in *Canscora decurrens* Dalz. According to Asare *et al.* (2017), the average number of leaves produced by okra reduced dramatically as gamma irradiation dosages were raised. Plants exposed to 400 Gy had the largest average number of leaves, whereas plants exposed to 1000 Gy had the lowest. The production of growth regulators and kinetin may have been blocked in this study, which may have resulted in an increase in the number of leaves.

Lethality Over Control (LOC%)

Mortality of the seedlings becomes evident with increased dosage of gamma treatment in *C. gigantea*. The plant survival to which the controls were adjusted 100 percent as LOC (Table 4). Seedlings from irradiated seeds have not been kept their survival capacity compared to the control. Germinability, plant development, and ultimately survival are all dependent on increased chromosomal damage when the radiation dose is increased. The results of previous work are aligned with our findings where *Centella asiatica* plantlets survival kept decreasing with the increased dose for three weeks after the irradiation (Moghaddam *et al.,* 2011).

CONCLUSION

Calotropis gigantea seeds exposed to greater doses of gamma rays (^{60}CO) revealed a declining trend of seed germination, survival rate, plant height, and leaf number as the irradiation dose was increased. Plants developed from seeds exposed to a dosage of more than 750 Gy were died. Further, the plants showed better results with the optimum survival rate below 395 Gy of gamma irradiation dose. Hence it could be concluded that the gamma irradiation below 395 Gy can be suggested to induce mutations in *Calotropis gigantea* with a minimum mortality rate.

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Antibacterial and Antioxidant Activities of Various Extracts and Essential Oil from Dried Leaves of *Artemisia herba-alba* Asso of Tamanrasset (South Algeria)

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ABSTRACT

The objective of this study was to evaluate the antibacterial and antioxidant activities of two extracts (aqueous and ethanolic) and essential oil from dried sheets of Artemisia herba alba collected in southern Algeria. The extracts were prepared separately with different polarity solvents (water and ethanol). Total phenolics, flavonoids and tanins contents were evaluated. The essential oil was isolated using hydrodistillation. Two tests were established to assess the antioxidant activity (DPPH and FRAP), agar-well diffusion method was used to evaluate the antibacterial effect: Escherichia coli, Enterococcus faecalis, Staphylococcus aureus and Bacillus cereus. The yield of the aqueous extract is higher than that of the ethanolic extract. The phytochemical study revealed the presence of phenolic compounds, flavonoids and tannins. The aqueous extract contains higher amounts of total phenolics (97.17 ± 1.06 mg/g DM), flavonoids (35.61 ± 0.39 mg/g DM) and tannins (46.58 ± 0.91 mg/g DM) compared to the ethanolic extract, 28.69 ± 0.99, 10.98 ± 0.64 and 15.11 ± 0.49 mg/g DM respectively. Antioxidant activity (IC50) of aqueous, ethanolic extracts and essential oil were 2.02, 0.753 and 1.088 mg/ml, respectively. Analysis of the antibacterial activity showed that aqueous extract exhibited much higher activity that the ethanolic extract and essential oil. RP HPLC analysis of aqueous extract show the presence of certain compounds that belong to flavonoids (cathecine and apigenin) and others to phenolic acids (caffeic acid and ferulic acid). The results of this study demonstrated that the essential oil and extracts can be used as antioxidant and antimicrobial agents.

***Keywords*:** Antibacterial activity, antioxidant activity, *Artemisia herba alba*, phytochemical screening

INTRODUCTION

Nowadays, herbal medicine is booming with a growing interest in the use of medicinal plants as palliative treatments to conventional medicine. Indeed, synthetic drugs are very expensive with undeniable adverse effects on human health (Nair and Chandra, 2006; Rahman and Fakir, 2015; Mirihagalla and Fernando, 2021). Several studies have demonstrated that medicinal plants are the core of many bioactive phyto-chemicals that possess the antimicrobial potential and have the ability to protect the human body from stress arises due to free radicals that might cause heart and neurodegenerative disorders, joints inflammation, cancer and several malfunctions.

Artemisia (A) herba alba is widespread in the semi-arid and arid steppes of North Africa, including Algeria. It is a medicinal and aromatic plant, rich in phytochemicals (phenolic compounds, flavonoids, sterols, tannins and essential oil) which are known for their antioxidant activities (Yoon *et al*., 2011; Dif *et al*., 2016; Laouini *et al*., 2018). Many works have demonstrated that it has several pharmacological properties: Antidiabetic (Al-Khazraji *et al.*, 1993), antispasmodic (Goze *et al*., 2009), antimicrobial (Zouari *et al*., 2010), antimalarial and antioxidant (Bourgou *et al*., 2016).

The aim of this work is based on the quantification and phyto-chemical screening (total phenols, total flavonoids and condensed tannins), as well as the study of antioxidant activities (DPPH radical scavenging and power iron-reducing) and antibacterial (*Escherichia coli* ATCC 25922, *Enterococcus faecalis WDCM 009*, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579) of the extracts (aqueous and ethanolic) and essential oil of *A. herba alba* collected in southern Algeria.

MATERIALS AND METHODS

Plant materiel

The plant, *A. herba alba*, was collected in December 2015 and comes from the Assekrem area of the southern Algerian town of Tamanrasset (the Ahaggar Mountains) (Figure 1). This is the main town of the Tuaregs and is located at an altitude of 1,320 meters.

Preparation of the extracts

The leaves were dehydrated in the dark at room temperature, crushed and stored in glass bottles, protected from light and moisture. Aqueous and ethanolic extracts are prepared by maceration of 10 % (w/v) leaf powder in distilled water and ethanol, respectively. The mixtures were then filtered and the filtrates were lyophilized (Ingel *et al*., 2017).

Essential oil extraction

The essential oil was extracted from leaves powder (0.05%) by hydrodistillation for 3h using a Clevenger apparatus type. The oil was stored at + 4 °C (Bruneton, 1999).

Phytochemical Screening

Secondary metabolites were revealed following the methods described by Ayoola *et al*. (2008) and Ravalison *et al*. (2015).

Estimation of total phenolics content

The amount of total phenolics was determined using the Folin-Ciocalteu (Singleton and Rossi, 1965). A calibration curve of gallic acid was prepared and the results were expressed as mg gallic acid equivalents per gram of the sample (mg EAG/g). In this method, 0.2 mL of sample was mixed with 1 ml of Folin-Ciocalteu reagent. Then, 800 µL of 7.5% sodium carbonate solution is added. The mixture is allowed to stand for 30 min and absorbance was measured at 700 nm using a spectrophotometer.

Estimation of flavonoids content

The concentration of flavonoids was quantified according to the colorimetric assay of Kosalec *et al*. (2004). One milliliter of sample was made up to 3 ml of methanol (95 %), mixed with 5.6 ml of distilled water and then 0.2 ml of potassium acetate (1 M), 0.2 ml of 10% $AlCl_3$ solution was added. The mixture was allowed to stand for a further 30 min, and absorbance was measured at 420 nm. The total flavonoids content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight.

Estimation of tannins content

Tannin contents were determined by spectrophotometry (λ = 700 nm) as described by Makkar *et al*. (1993). The results were expressed in terms of mg tannic acid equivalents (TAE) per gram of dry matter (mg TAE/g) using the tannic acid calibration curve (0.5-1 mg/ml).

Evaluation antioxidant activities

Antioxidant activity by DPPH assay

This activity measures the free radical scavenging capacity by reduction of the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) as described by Brand Williams *et al*. (1995). Absorbance at 517 nm was used to calculate radical scavenging activity (% of inhibition) with the formula:

$$DPPH\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Abs control: Absorbance of DPPH solution mixtures without extract. Abs sample: Absorbance of DPPH mixtures containing extract.

Ascorbic acid was used as a reference. The results obtained allow us to calculate the IC_{50} (concentration in which the 50 % of the free radical DPPH is reduced).

Ferric reducing assay

The antioxidant capacity was also evaluated using ferric reducing antioxidant power (FRAP) assay. FRAP assay was performed based on the methods of Oyaizu, 1986. The reducing power was expressed by the increase in optical density of the sample measured at 700 nm, using ascorbic acid as a standard.

Antibacterial test

Antimicrobial testing and minimum inhibitory concentration were performed by agar well diffusion (Nalawade *et al*., 2016) against *E. coli* ATTCC 25922, *S. aureus* ATTCC 25923, *B. cereus* ATCC 14579, and *E. faecalis* WDCM 009. 1 ml of

each microbial suspension to be tested was incorporated into Mueller Hinton agar. Then, 6-mm-diameter wells were perforated in the medium, filled with 20 µl (1.5 mg/ml) of sample and incubated at 37°C/24 hours. DMSO (10 %) and standard antibiotics were used as negative and positive controls, respectively. After incubation, activity was assessed by measuring the diameter of the growth inhibition zone.

Chromatographic analysis

RP-HPLC was performed using AGILENT Ultimate 1100 system equipped with degasser, quaternary pump, auto sampler, column oven and ultraviolet (UV) detector. Separation was done using Hypersil BDS C18 column (250 mm×4.6 mm). Column temperature was maintained at + 30 °C, injection volume was 5 ìl, and a gradient mobile phase was used which comprised of acetonitrile (A), 0.2 % acetic acid in water (B). Flow rate was maintained at 1.5 ml/min. The retention times of the different standards were used to identify the composition of the aqueous extract.

Statistical Study

The data are presented as the means ± standard deviations from the three replicates. Calculations were carried using the SAS v. 9.1.3 program.

RESULTS AND DISCUSSION

Extraction

The yield of the ethanolic extract is higher than that of the aqueous extract (Table 1). The solvents used for extraction influence the composition and quantity of secondary metabolites (phenolic and flavonoid) and/or their biological activity (Rebey *et al*., 2012; Ngo *et al*., 2017). Dirar *et al*. (2019) reported that the aqueous and ethanolic extracts showed high contents of phenolic compounds. The essential oil yield obtained is lower than the results found by Esam *et al*. (2016) and Ezzoubi *et al*. (2018) for the aerial part of the same plant collected in Jordan and Morocco.

Phytochemical screening

The results of phytochemical screening are mentioned in Table 2. These results show the presence of flavonoids, terpenoids, phenols, tannins and reducing compounds. Moreover, the tests performed do not detect any presence of alkaloids, free quinines, glycosides and saponins. The same results were reported by Dif *et al*. (2016).

RP HPLC

Table 3 shows the results of the chromatography analysis. RP HPLC analysis of aqueous extract show the presence of certain compounds that belong to flavonoids (cathecine, apigenin, lutelin) and others to phenolic acids. In addition, the extract was characterized by the presence of caffeic acid in higher amounts. In concordance of our results, apigenin was also detected in the aerial parts of *A. herba alba* collected from Egypt and Tunisia (Bourgou *et al*., 2016).

Total polyphenols, flavonoids and tannins contents

Total polyphenols, flavonoïds and condensed tannins were estimated in two extracts. The results are given in Table 4. Significantly different contents of phenolic compounds, flavonoids and tannins were found in the two extracts. The highest amounts of these compounds are shown in aqueous extract. The results obtained in this study are similar to those reported by Shahid *et al*. (2012), who showed that water is more efficient than ethanol in the extraction of polyphenols. The influence of the solvent used on the extraction yield of polyphenols shows a correlation with its polarity (Dixon *et al*., 2011). Compared to our results, Sekiou *et al*. (2018) reported lower total phenolics, flavonoids and condensed tannins amount in Algerian *A. herba alba* aqueous extract. The quantitative and qualitative composition of polyphenols depends on the origin of the plant, the nature of the extraction solvent and the method used (Zhao *et al*., 2006; Ashraf *et al*., 2015; Mohammed *et al*., 2021). In addition, adverse environmental conditions such as water deficit cause physiological stress in the plant, which leads to the synthesis and accumulation of phenolic compounds (Ashraf *et al*., 2017).

Antioxidant activities (DPPH radical scavenging activity and FRAP)

Table 5 shows the results of the antiradical activity (DPPH). The antiradical activity is more important when the IC_{50} or EC_{50} is lower. The ethanolic extract had the highest antioxidant activity, followed by essential oil and aqueous ex-

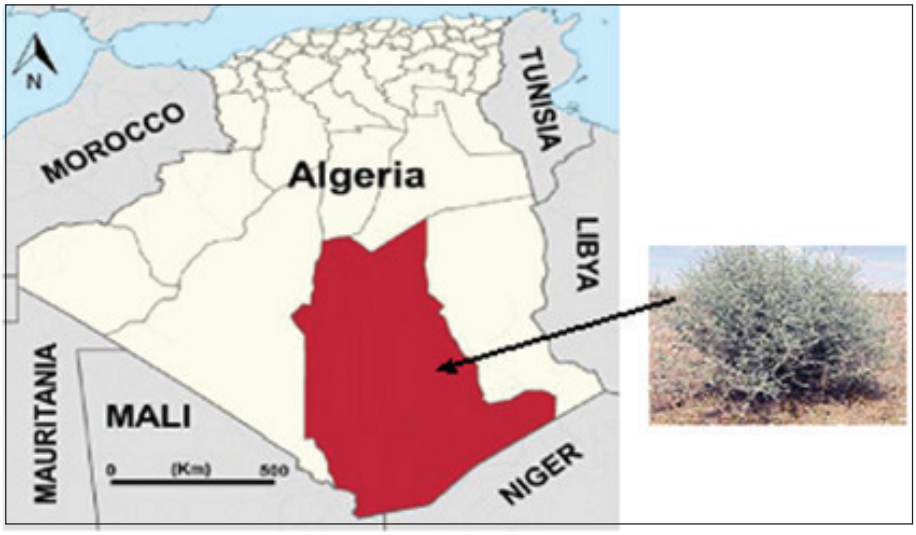

Figure 1: Location of the Wilaya of Tamanrasset in south Algeria.

tract. Ethanol extract has the highest scavenging activity (DPPH) with the lowest IC_{50} value of 0.735 mg/ml. The comparative study of the antiradical activity of the tested samples is weaker than that of ascorbic acid. On the other hand, the antioxidant power of the essential oil is higher than the extracts. These results correlate with those reported by Dirar *et al*. (2019) and El-Massry *et al*. (2002), who showed that the nature of the solvents used significantly influences the composition of extracted polyphenols as well as their antioxidant effect. However, the antioxidant power of the aqueous extract is higher than that reported by Khilifi *et al*. (2013).

The results in Table 5 revealed that the essential oil of the studied plant was characterized by slightly lower EC_{50} values than those of the positive control (EC_{50} = 0.029 mg/ml). In our study both extracts (aqueous and ethanolic) have a low reducing power compared to the standard. However, the antioxidant activity of the essential oil of the studied plant could be associated with its high concentration of oxygenated monoterpenes (Griep *et al*., 2007; Lou *et al*., 2011).

The release of electrons or hydrogen atoms from polyphenols was considered to be the most important element of the structure-antioxidant activity relationship. The redox characteristics of these compounds can explain the different antioxidant mechanisms (*viz*., adsorption, neutralization of free radicals, chelation of metal ions, termination of autoxidative chain reactions, decomposition of peroxides, and quenching of singlet or triplet oxygen) (Dorma and Deans, 2000). So, the antioxidant activity of phenolic compounds is related to their ability to release electrons. The antioxidant power of flavonoids depends mainly on their ability to eliminate free radicals. Phenolic compounds were reported to be very strong antioxidants. The chemical structure and polarity of the antioxidant are crucial to its ability to trap free radicals. Synergistic but also antagonistic effects are observed in model solutions that contain several functional compounds with antiradical activity (Zaouali *et al*., 2010).

Antimicrobial Activity

The *in vitro* activity of extracts and essential oil from leaves of *A. herba alba* is determined. The antibacterial activity of the essential oil was very low against all bacteria tested. The results obtained indicated that Gram-positive *Staphylococcus aureus* was the most resistant strain tested to the essential oil and extracts, with a low zone of inhibition. An inhibition zone of 6.5 ± 0.7mm for *Enterococcus feacalis* and 7 ± 0.41mm for *Escherichia coli* and *Staphylococcus aureus* then 9 ± 0.7mm for *Bacillus cereus.* The aqueous extract has an interesting antibacterial activity against all strains tested. The aerial parts aqueous extract exhibited the highest activity (20.5 ± 3.53 mm) against *Enterococcus faecalis* followed by

Table 1: Percentage yield of extracts and essential oil from *A. herba alba*

Extraction	Yield extract (%)
Water extraction (Aqueous extract)	15.68 ± 0.4
Solvent extraction (Ethanolic extract)	30.03 ± 2.07
Essential oil	0.18

Table 2: Phytochemical screening of extracts from of *A. herba alba*

Compounds	Aqueous Extract	Ethanolic extract
Flavonoids	+	+
Saponins	-	-
Reducing compounds	+	+
Phenols	+	+
Terpenoids	+	+
Glycosides	-	-
Catechic tannins	+	+
Tannins	+	+
Alkaloids	-	-
Free quinones	-	-

(+): Present; (-): Absent

Table 3: Compounds identified in the *Artemisia herba alba* extract by RP HPLC

Compounds	T_R (min)	Area of the peak (%)
Catherine	5.865	1.302
caffeic acid	7.155	13.268
p-OH benzoic acid	6.423	3.846
Rutin	8.284	6.209
ferulic acid	9.360	3.754
3-Hydroxy-4-methoxycinamic acid	9.738	7.295
Luteolin	13.087	2.662
Apigenin	14.714	1.183
Isoramenitin	15.064	3.011

Table 4: Total polyphenols, flavonoids and condensed tannins contents (mgE/gDW) from *A. herba alba*

	Aqueous extract	Ethanolic extract
Polyphenols	97.17 ± 1.06	28.69 ± 0.99
Flavonoids	35.61 ± 0.39	10.98 ± 0.64
Condensed tannins	46.58 ± 0.91	15.11 ± 0.49

E: Equivalent; DW: Dry Weight

Table 5: DPPH test (IC_{50} mg/mL) and reducing power (EC_{50} mg/mL)

	Ascorbic acid	Essential oil	Aqueous extract	Ethanolic extract
IC_{50} (mg/ml)	0.123	1.088	2.02	0.735
EC_{50} (mg/ml)	0.029	0.030	0.508	0.416

Table 6: The Minimal inhibitory concentration (MIC) values (mg/ml) of the extracts and essential oil

Bacteria species	Aqueous extract	Ethanolic extract	Essential oil
S. aureus	0.29 ± 0.13	0.75 ± 0	0.46 ± 0
E. coli	0.38 ± 0	0.46 ± 0	0.75 ± 0
E. faecalis	0.12 ± 0	0.12 ± 0	0.46 ± 0
B. cereus	0.05 ± 0	0.29 ± 0.13	0.05 ± 0.02

Bacillus cereus (17 ± 2.82 mm), *Escherichia coli* (12 ± 1.41 mm) and *Staphylococcus aureus* (10 mm). The ethanol extract exhibited 15±0.7 mm zone for *Bacillus cereus*, which is the maximum followed by 11.5 ± 0.7 mm zone for *Escherichia coli* and *Enterococcus faecalis* then10 ± 1.41mm for *Staphylococcus aureus*. Overall, the ethanolic extract of *A. herba alba* leaves was strongly bactericidal with larger inhibition zones and low MIC values (0.05-0.038 mg/ml) (Table 6). The richness of the essential oil of A. herba alba in oxygenated monoterpene compounds could explain the antimicrobial effect of this oil (Bertella *et al*., 2018; Amor *et al*., 2019).

Phenolic acids and flavonoids are known to be responsible for strong antimicrobial activity against a wide spectrum of microorganisms, through different processes such as substrate reduction, binding with polypeptides exposed on the microbial cell surface, formation of complexes with the microbial wall, disruption of the microbial cell membrane (Cox *et al*., 2000). Antibacterial activities of flavonoids were described previously. According to Cowan (1999), the antimicrobial activity of flavonoids without free hydroxyl groups is greater because the chemical affinity for membrane lipids is increased.

Various terpenoïds or phenolic components may be primarily responsible for this activity (Nedorostova and Kloucek, 2009; Pavela, 2014). Thus, to better understand the biological function of phenolics and terpenoids, it is therefore necessary to test these compounds separately and subsequently evaluate the structure-activity relationship. The antimicrobial power of the aqueous extract of the leaves of this plant could be attributed to its high content of phenolic compounds and flavonoids. In addition, the synergistic effect of various compounds could be an important factor of its antimicrobial activity.

The monoterpenes of essential oils can diffuse into cell membranes and damage them. These compounds are also capable of destroying cell integrity, inhibiting respiration and thus altering their permeability (Bertella *et al*., 2018). In fact, the variability in chemical components of essential oils can explain the biological effects (*viz*., antibacterial, antioxidant, anti-inflammatory, anticarcinogenic, antifungal) of these oils.

CONCLUSION

Phytochemical screening of leaves of *A. herba alba* Asso reveals the richness of the aqueous extract in polyphenols well known for their antioxidant property. The aqueous extract and essential oil of *Artemisia herba alba* exhibited interesting biological activities. The results of this study suggest that *Artemisia herba-alba* may be a potential source of biomolecules that can be used for applications such as food, cosmetics, pharmaceuticals and other related fields.

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Biological valorization and characterization of essential oil of Algerian *Mentha spicata* L.

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ABSTRACT

Main purpose of our work is to valorise Mentha spicata from the arid zone in Algeria by the study of it biological activities and chemical profile; antibacterial activity and antioxidant activity. Essential oil is characterized by GC-MS. Forty- four constituents, accounting for 98.41% of the total oil contents identified were carbon (42.23%) followed respectively by limonene (29.57%), 1,8-cineole (5.31%), β-pinene (3.54%). The antioxidant activity of the hydro distilled oil was studied using DPPH to determined IC50. The antibacterial activity of essential oils was tested against five microorganisms with the diffusion disc method on bacteria: Staphylococcus aureus ATCC 25923,Candida Albicans ATCC 10231, Escherichia coli ATCC 25922, Bacillus ATCC 11778, Pseudomonas aeruginosa ATCC 27853), diameters vary between 6 and 18 mm.

***Keywords*:** Antibacterial activity, antioxidant activity, chemical composition, essential oil, GC-MS, *Mentha spicata* L.

INTRODUCTION

Aromatics are the origin of metabolites, in particular secondary metabolites with biological effect. The heed on bioactive potential of essential oils and their compounds have been quickly expanded throw last few years (Premathilake *et al.*, 2018). *Our species* is one probably one of the best known and used mint throughout the world (Shahbazi, 2015). It is cultivated in Algeria for its medicinal and culinary application (Brahmi *et al*., 2016). Their leaves are used in the tea making and in some dishes like flavouring (Snoussi *et al*., 2015). *M. spicata* has largly placed on treat numerous pathologies such as some gastrointestinal disorder and also introduced in dental and oral hygiene products (Shahbazi, 2015). The aim of this work is to determinate the phytochemical profile of the essential oil of *Mentha spicata (EOMs)*, growing in the region of the El Bayadh positioned in the south-west of Algeria and to judge their antimicrobial and antioxidant effects.

MATERIALS AND METHODS

Extraction and characterization of essential oil

M. spicata was collected in the month of March 2020 from Brezina (El Bayadh) located in the south-west of Algeria. The aerial parts collected were dried at room temperature. *EOMs* was extracted by hydro distillation utilizing an apparatus of Clevenger type for 4H by mixing 200g of *mint* in1500 ml of distilled water. The evaluation of the chemical profile was carried out by GC-MS analysis performed with a Varian CP-3800 gas chromatograph built with a DB-5 capillary column (30 m × 0.25 mm, 0.25 ìm coating thickness) and a Varian Saturn 2000 ions. The analytical conditions were as follows: injector and transfer line temperatures 220°C and 240°C respectively; programmed over temperature from 60°C to 240°C; helium carrier gas at 1 ml / min; 0.2 µL injection (10% hexane solution); 1:30 division ratio. The identification of the constituents was on the basis of the comparison of retention times with those of authentic samples, comparing their linear retention indices with regards to the hydrocarbon series, and in computer with mass spectra of commercial and household libraries made out of pure substances and the different parts of known oils and data from MS literature data (Adams, 2007; Davies, 1990).

Antioxidant study by DPPH assay

2ml of different concentrations of *M. Spicata* essential was put into 0.4ml of DPPH solution in

ethanol (Blois, 1958). The mixture was kept in the dark for 30 min and was measured at 517 nm. We uses Ethanol as a get a grip on while, Gallic and ascorbic acids were as standards to compare the result.

Free scavenging convenience of DPPH radical was calculated utilizing the following equation: DPPH scavenging effect (%) = [(Absorbance of Control – Absorbance of Sample)/ Absorbance of Control] X 100. The outcomes were recorded as 50% inhibition concentration (IC50).

Antimicrobial study

Microorganisms

The antimicrobial activity of the *EOMs* was evaluated against five strains bacteria, three with Gram negative (*Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922**,** *Pseudomonas aeruginosa* ATCC 27853), two stains of Gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus* ATCC 11778)

Test of antibiotics

The antibiotics tested and their corresponding disc concentrations were the following: Trimethoprim 1.25µg + Sulfamethoxazole (**SXT)** 23.75µg, Chloramphenicol (C 30) 30µg, Clindanycin (CMN2) 2µg, Penicillin (PEN) 6µg. The diameter of zone of inhibition was determined using a standard method (Moard, 2008); (Moard, 2011). Plates were incubated for 24h at 37°C

RESULTS AND DISCUSSION

Chemical profile

The chemical composition of the *EOMs* is explained in Table 1. The study of the essential oil resulted in identification of 44 compounds, representing 98.41% of the total, with carbon (42.23%) followed respectively by limonene (29.57%), 1,8-cineole(5.31%), β-pinene (3.54%) β-caryophyllene(2.18%), α-Pinene (1.85%) and germacrene D (1.66%). A similar study of the chemical composition of *EOMs* from Saharan Atlas (Algeria) was made by Sanaa *et al*. (2018). And the same study of *EOMs* from Bejaia (located in Algeria) by Brahmi *et al.*(2016). The composition of this volatile oil is variable according to geographical location, depending on the changing of the climate, and soil, too. The chemical composition can also be modified by the time of the collect and the method of extraction (Laggoune *et al*., 2016).

Antioxidant Activity

The concentration providing 50% effect of DPPHÿ was calculated from the graph of the percentage of scavenging effect of DPPHÿ versus the concentration of the positive control. The results are summarizing in Table 2. In comparison to the conventional compound, the IC50 value for *EOMs* was 24.16 ± 2.12ìg/ml. The IC50 founded through this evaluation was more advanced than those showed by Hussain *et al.* (2010). In an identical study, the IC50 of *EOMs* underneath the IC50 of standards. Snoussi *et al*.(2015) which explain the important antiradical power of the spearmint's essential oil.

Antibacterial activity

Details sensitivity and resistance of the bacteria tested to the different antibiotics which are inside and indicates that the different antibiotics have a more or less similar to that of the Mint essential oils tested after twenty-four hours (Table 3).

Essential oil of spearmint tested against all microorganisms is demonstrated in Table 4. The outcomes of the current survey reveal that the *EOMs* shows an adequate antimicrobial effect from the tested microorganisms. Most Gram-negative bacteria were vulnerable to *EOMs* in addition to Gram-positive bacteria (Table 4). The inhibition zone diameter for *staphylococcus aureus* ATCC 25923 was from 18mm to 5 mm, *Escherichia coli* ATCC 25922 scored growth inhibition of 17mm to 5 mm, for *Candida Albicans* ATCC 10231 18mm to 7mm, for *Bacillus* 18mm to 6mm ATCC 11778 , and *Pseudomonas aeruginosa* ATCC 27853 17 mm to 0mm.

Kindred analysis turned up by Dhifi that the *EOMs* was reactive against Gram- - (*E.coli*) , Gram+ (*s.aureus*) and *Candida Albicans* , with growth diameter about 18 mm against *Escherichia coli,* and 26 mm against *Candida albicans* (Dhifi *et al.,* 2013). At exactly the same path; Rolden *et al.* (2010) reported against *E.coli* ATCC 25922. Mahboubi and Hagi (2008) determinate that the *EOMs* had a top antibacterial activity against *Escherichia coli Bacillus cereus*, and

Table 1: Chemical profile of the *EOMs* aerial parts

	Chemical composition	RI calculated (DB5)	RI Liturature % (Adams 2017)
1	Myrcene	990	0.38
2	α-Terpinene	1013	0.22
3	γ-Terpinene	1063	0.53
4	Limonene	1026	29.57
5	p-Cymene	1022	0.17
6	cis-β-Ocimene	1033	0.57
7	cis-Sabinene hydrate	1100	0.10
8	P-menth-2-en-1-Ol	1120	0.15
9	Terpinen-4-ol	1172	0.99
10	α-Thujone	1101	0.33
11	Eucalyptol = 1,8-cineole	1028	5.31
12	Borneol	1167	0.22
13	Linalool	1093	0.66
14	α-Terpineol	1185	0.15
15	Pulegone	1232	0.32
16	β-Pinene	975	3.54
17	Carvone	1242	42.23
18	Sabinene	967	0.1
19	cis-Carvone oxide	1260	0.10
20	Perillaldehyde	1269	0.11
21	Camphene	945	0.23
22	trans-Carvone oxide	1275	0.13
23	α-Copaene	1372	0.27
24	Bornyle acetate	1286	0.12
25	Camphor	1143	0.27
26	β-Bourbonene	1385	1.53
27	Dihydrocareol acetate	1305	0.17
28	trans-Carvyl acetate	1338	0.10
29	β-Elemene	1387	0.52
30	Eugenol	1355	0.11
31	3-octanol	993	0.65
32	cis-Carvyl acetate	1367	0.10
33	cis-Jasmone	1393	0.38
34	α-Caryophyllene	1455	0.11
35	β-Copaene	1433	0.17
36	Germacrene D	1483	1.66
37	Bicyclogermacrene	1497	0.52
38	α-Pinene	930	1.85
39	Dihydrocarveol	1195	0.44
40	Elemol	1545	0.33
41	β-Caryophyllene	1419	2.18
42	Caryophyllene oxide	1584	0.50
43	α-Thujene	923	0.19
44	β-Thujone	1114	0.13
	Total		98.41

Table 2: Antioxidant activity of *EOMs* by reducing power and DPPH essays

Compound	DPPH (IC50) µg/ml
Quercetin	14.09 ± 1.3a
Gallic acid	6.35 ± 0.41a
Ascorbic acid	7.24 ± 0.97a
Essential oil of M. Spicata	24.16 ± 2.12a

Table 3: Sensitivitiy of pathogen tested on antibiotics

	Zone of inhibition antibiotics (mm)			
	CMN	**SXT 25**	**C 30**	**PEN**
Candida Albicans	29	30	22	35
Staphylococcus aureus	29	21	22	43
Escherichia coli	30	27	25	35
Bacillus	19	30	20	23
Pseudomonas aeruginosa	25	33	28	44

Table 4: Sensitivity tests of bacterial pathogens to mint essential oil.

Pathogen	Zone of inhibition (mm)			
	Mint			
Concentration of *EOMs*				
Candida Albicans	18	15	14	7
Staphylococcus aureus	18	16	13	10
Escherichia coli	17	17	15	6
Bacillus	18	17	7	6
Pseudomonas aeruginosa	17	13	9	-

Staphylococcus aureus, and extended diameter between 8-21mm.

M. spicata acrylic Serbia was examined in the disc diffusion method and demonstrated that its effect against Gram + bacteria was a lot better than Gram- the antibacterial activity. The diameter of the inhibition zone ranges from 10mm to 25mm (Sokovic *et al.,* 2005)**.** Odds-on the antibacterial activity of *EOMs* were recorded to the percentage of oxygenated monoterpenes and monoterpene hydrocarbons (Dahiya and Manglik, 2013)**.** Subsequently, the rich present of carbon, limonene, cineole, can explain the good antibacterial effect that *EOMs* had.

CONCLUSION

The existing research has disclosed the composition of the hydro-distilled *EOMs*, cultivated in the region of El Bayadh (south-west of Algeria), which can be revealed by GC and GC/MS. 44 molecule constituting 98.41% of the *EOMs* and were identified with mains components were carbon, and limonene which can be well matching with the results obtained in other Algerian studies. The *EOMs* showed a higher antioxidant and a moderate to good antimicrobial activities. These biological effects are largely owing to the elevated content of carbon, in this mint.

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SHORT COMMUNICATION

Effects of Cinnamon (*Cinnamomum zeylanicum*) powder extract against the pest of Radish (*Raphanus raphanistrum* Subsp. Sativus)

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ABSTRACT

Cinnamon (Cinnamomum zeylanicum Blume) is an important spice and medicinal plant in Sri Lanka. It comprises essential oils with antibacterial, antifungal, antioxidant, and insecticidal properties. Radish (Raphanus sativus L.,) is an important and popular vegetable crop in Sri Lanka. The crop is attacked by many pests and farmers are using chemical insecticides to control pests. However use of chemical insecticides has bad impact on health and environment. Considering this important issue, a study was conducted at the Institute for Agro-technology and Rural Sciences of the University of Colombo in Weligaththa, Hambanthota to examine the insecticidal effect of different types of cinnamon preparations (bio-control) and their optimum dosage in radish cultivation. The treatments consist of 1% cinnamon powder water filtrate (CPWF), 1% cinnamon powder suspension (CPS), 0.6% abamectin and control using randomized complete block design. The results indicated that there was no significant different between chemical insecticide (abamectin) and the cinnamon powder applications for pest control. One percent cinnamon powder water filtrate significantly reduced the number of damaged leaves in radish plant with lowest number of pests. Therefore, cinnamon powder can be used to control pest damages of Radish (Raphanus raphanistrum) without significant effect on growth performance in radish cultivation. The best form of application was cinnamon power water filtrate and the optimum concentration was 1.0% for controlling pests in radish cultivation.

***Keywords*:** Cinnamon powder, insect repellents, pests, pesticide, radish

Cinnamon (*Cinnamomum zeylanicum* Blume) is a spice considered to be endemic to Sri Lanka (Liyanage *et al*., 2021; Ribeiro-Santos *et al*., 2017), which contains essential oils with antibacterial, antifungal, antioxidant and insecticidal properties ((Bandusekara *et al*., 2020; Ranasinghe *et al*., 2002). Cinnamaldehyde is the main constituent of cinnamon bark, but it also contains b-caryophyllene, linalool, and other terpenes. Further, Brari and Thakur (2015) reported that cinnamaldehyde and linalool exhibit contact and fumigant toxicity against the adults of *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.). Radish (*Raphanus sativus*) is a commonly consumed vegetable in the Brassicaceae family. Generally, people eat radish raw as salad and also as vegetables while the leaves used as vegetable. Radish possesses high medicinal and nutritional value. Which contains dietary fibers, sugar, protein, and even fat and fluoride. Additionally, it contains a number of water-soluble vitamins (B1, B2, B3, B5, B6, B9, and C) as well as minerals. Furthermore, radish has been discovered to have a variety of bioactive chemicals that may have human health advantages. Glucosinolates and isothiocyanates are the two most important bioactive chemicals found in radish. In Unani, Greeko-Arab, and Indian folk medicine, radish is used as a household remedy for a variety of ailments including jaundice, gallstones, liver illnesses, rectal prolapse, indigestion, and other gastrointestinal aches. Recently, several research studies reported that anti-inflammatory, anti-cancerous and antidiabetic activities of radish (Banihani, 2017; Zhao-liang *et al*., 2008).

One of the constrains in radish cultivation is the pest attack, which resulted in considerable loss of yield. The major pests associated with radish in Sri Lanka are vegetable leaf miner (*Liriomyza huidobrensis*), aphids, cut worms (*Agrotis* spp.) and flea beetles (*Phyllotreta cruciferae*) (DOA, 2021, Jayathilaka *et al*., 2016). To control these pests, farmers are used different chemical pesticides. Indiscriminate application of synthetic pesticides

in crop production leads to detrimental consequences in the ecosystem such as ground and surface water contamination, soil contamination, loss of non-target species and pesticide use can also result in resistance evolution in pest populations (Mahmood *et al*., 2016). Hence, attention has been paid towards organic farming by using appropriate cropping techniques, biological control, and bio-pesticides (Dar *et al*., 2021).

In comparison to synthetic pesticides, plant-based insecticides are safer for the environment, are generally less expensive, are easier to handle, and are employed by small industries and farms. Moreover, these pesticides are often active against variety of species, are often biodegradable, nontoxic and appropriate for use in integrated pest management. Essential oils are a possible alternative to botanical extracts used as pesticides because of their widespread availability and relatively low cost. These are secondary metabolites synthesized by plants, and play vital roles in plant defence (both against biotic and abiotic stresses) and signalling processes. Further, they including the attraction of pollinators and beneficial insects (Campolo *et al*., 2018).

Therefore, an experiment was conducted to evaluate the effectiveness of pesticides developed from cinnamon powder on the status of pest attack in radish cultivations in dry zone field conditions of Sri Lanka with the objectives of to identify best form of cinnamon pesticides and their effective doses as compared to synthetic one.

The experiment was carried out at the Institute for Agro-technology and Rural Sciences of the University of Colombo in Weligaththa, Hambanthota (DL_5 agro-ecological zone of Sri Lanka) during January – March of 2021. The investigation was consisted with two experiments; first experiment was to identify the best form of cinnamon pesticide spray on radish plants while the second experiment was to identify the optimum dosage of the cinnamon preparation as a pesticide.

Initially, radish seeds were placed in nursery trays and let to grow under shade house till seedling grew up to 4-5 leaf stage. Then, three weeks old radish (var. local) seedlings were transplanted in 1 m × 3 m size raised beds with 25 cm × 10 cm spacing (120 plans per plot) during the end of January 2021. Sprinkler irrigation was practiced during the crop season (1 ½ month period).

Initially, 1% cinnamon powder water filtrate (CPWF) was prepared by dissolving 10 g of commercially available cinnamon powder in 1 litre of distilled water. Then the mixture was filtered through muslin cloth (Raju *et al*., 2020). Likewise, 1% cinnamon powder suspension (CPS) was prepared without filtering.

The field planted radish plants were treated by spraying of 1% cinnamon powder water filtrate,1% cinnamon powder suspension, Abamectin 0.6% and control plants were maintained without application of any substances. Abamectin (commercially available chemical pesticide) was used to compare the effectiveness of prepared bio-pesticides. Different pesticides were sprayed evenly during the evening time of the day in every five days starting from ten days after transplanting to harvest. Plant growth data (Number of leaves per plant and leaf length) and pest incidence (Number of pests attacked plants and number of damaged leaves) were recorded. Data recording was started two weeks after the planting of radish and was performed every 4 days. Randomized Complete Block Design (RCBD) with four replicates was used for the experiment.

The best form of cinnamon pesticide selected from the first experiment was used for this experiment. The procedure followed for this experiment was precisely similar as described above. Three weeks old seedlings were transplanted in 1 m × 3 m size raised beds with 25 cm × 10 cm spacing during mid of February 2021. Field planted radish plants were treated with 1%, 1.5% and 2% cinnamon powder water filtrates. Treatments were allocated according to RCBD design with three replicates. Plant growth data (Number of leaves per plant and length of leaves) and pest incidence (number of pests attacked plants, number of damaged leaves and number of pests identified in experimental unit) were recorded from two weeks after spraying with four days intervals. Data were analyzed by ANOVA using MINITAB version 17 statistical software.

Application of different pesticides were not significantly ($p<0.05$) affected on plant growth of radish in respect of leaf number per plant and leaf

length as compared to the untreated control (Table 1). However, Different types of pesticides significantly ($p<0.05$) affected on pest incidence of radish plants (Table 1). During the study period, the major pests identified in experiment plots were leaf miner (*Liriomyza huidobrensis*) and leaf eating caterpillars (*Spodoptera litura* and *Crosidolomia pavonana*). Significantly highest number of pest attack plant and average number of damaged leaves were recorded in control plants compared to other treatments. Furthermore, between two forms of cinnamon powder preparations, 1% cinnamon powder water filtrate showed lowest pest attack on radish as compared to cinnamon powder suspension (Table 1). Therefore, cinnamon powder water filtrate was used as the best form of application for the second experiment. The result clearly indicated that bio-pesticide could replace the use of synthetic pesticide in radish cultivation.

Table 1: Effect of different pesticides on growth and pest attack of radish plant

Treatment	No. of leaves plant^{-1}	Average leaf length (cm)	No. of pest attacked leaves plot^{-1}	No. of pest attacked plant plot^{-1}
1% CPWF	13.9^{a}	28.5^{a}	61.0^{b}	21.3^{b}
1% CPS	14.1^{a}	28.6^{a}	63.8^{b}	21.5^{b}
0.6% Abamectin	13.9^{a}	28.5^{a}	57.8^{b}	21.0^{b}
Control	14.2^{a}	28.6^{a}	164.3^{a}	26.0^{a}

Note: 1% cinnamon powder water filtrate (CPWF), 1% cinnamon powder suspension (CPS), Means with the same superscript in a column are not significantly different from each other, however, means with different superscript are significantly different at $p<0.05$.

Different concentrations of cinnamon powder water filtrate were not significantly affected of plant growth of radish in respect of leaf number per plant and leaf length. Similarly, there was no significant effect of different concentrations of extract on number of pest attacked plant /plot of radish. However, different concentrations of cinnamon extract significantly ($p<0.05$) affected the number of pest attacked leaves /plot of radish. The lowest number of pest attacked leaves/plot (61.0) was recorded in 1% cinnamon powder water filtrate (CPWF) as against highest (80.7) in 1.5% CPWF (Table 2).

Table 2: Effect of different concentrations of cinnamon powder water filtrate on growth and pest attacked to the radish plant

Treatment	No. of leaves / plant	Average leaf length (cm)	No. of pest attacked leaves/plot	No. of pest attacked plant/plot
1% CPWF	12.7^{a}	27.1^{a}	61.0^{c}	21.7^{a}
1.5%CPWF	14.0^{a}	28.5^{a}	80.7^{a}	23.0^{a}
2% CPWF	13.6^{a}	28.3^{a}	71.0^{b}	22.7^{a}

Note: 1% cinnamon powder water filtrate (CPWF), 1% cinnamon powder suspension (CPS), Means with the same superscript in a column are not significantly different from each other, however means with different superscript are significantly different at $p<0.05$.

Several studies discussed insecticidal properties of cinnamon plant parts. For example, Samarasekera *et al.* (2006) investigated insecticidal activity of cinnamon bark and leaf oil against housefly (*Musca domestica*), where bark oil showed the better knock down effect and mortality against *M. domestica*. Further, Kowalska *et al.* (2020) investigated that tomato varieties Agro and Hamlet showed a positive reaction to cinnamon water filtrate spray and increased number of branches while controlling pest attacks. The result of the present study also agreed with this statement where cinnamon water filtrates were identified as the best form of application as compared to water suspensions.

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SHORT COMMUNICATION

Ethnobotanical study of medicinal plants in Telagh region (North-western Algeria)

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ABSTRACT

Medicinal plants are known for their uses because of their therapeutic virtues. A study was carried out to establish the medicinal plants to collect all the information concerning the therapeutic uses practiced by the local population in Telagh region (Wilaya of Sidi Bel Abbes. A survey was carried out among people of all ages, pharmacies, and herbalists in the Telagh region using 200 questionnaire cards for four months. From the results it was possible to identify medicinal plants divided into 23-families, among which Lamiaceae and Asteraceae families are the most dominant. Results showed that decoction and infusion are the most used part of remade forms of all cited pathologies. Our results make up a very valuable source of information for the region studied and for the national medicinal flora. The results could be a database for further research in the phytochemistry fields and scientific study of medical drugs.

***Keywords*:** Sidi Bel Abbes, medicinal plants, ethnobotanical, Telagh

Herbal medicine is a discipline that offers natural remedies accepted by the human body and is usually connected with conventional treatments. Nowadays, it is experiencing a great revival in the West, especially in chronic diseases treatment. Herbal treatments are coming back to the foreground because the potency of drugs such as antibiotics (considered the almost universal means to fix severe infections decreases (Goetz and Ghédira, 2012; Tripathi, 2021). Several authors have studied Algerian medicinal plants based on ethnobotanical surveys (Kechar *et al*., 2016; Dif *et al*., 2015). The multiplication of these ethnobotanical studies on a national scale enables: to gather more details on medicinal plants, enhance them, and save some knowledge acquired by the local population. The village of Telagh is located where; the population has a crucial ethnobotanical knowledge (Benabdeli,1983). This work consists of making an inventory of the plants employed by the neighborhood population of the Telagh region for ethnobotanical reasons.

The Telagh Forest Massif (Figure 1) is a combined succession of mountains that enclose the Dhaya Mountains that follow the Tlemcen Mountains to the west and the Saida Mountains from the east. It is bordered to the north by Kounteida and Bouettas forests, to the southwest by Touazizinz and Takrouma forests, to the south Zegla and Beni Matharet forests and to the east by the territory of the Wilaya of Saida. According to Emberger's coefficient, the forest of Telagh would be affected by the cold semi-arid climate, which affects almost the totality forest. The fresh semi-arid is located in some enclaves of the northern and northeastern slopes of the Khodida and Redaida mountains. January is the coldest month with an average temperature of around 0°C to 2°C and the hottest in July with an average of 34°C to 36°C. Near this forest, the village of Telagh is located and represents the object of our study, including the following forests: Bouettas, ZidelMoumen and Khodida in the North. Zegla, Ain El H'djar, and Beni Mathare in the South.

The survey is carried out on a sample of 200 individuals composed of men and women using random sampling in the city of Telagh during the period November 2016-March 2017; these individuals are between 20 and 80 years old. The questionnaire was made with individuals using plants. The taxonomic identification of the species was carried out at the eco-development of spaces

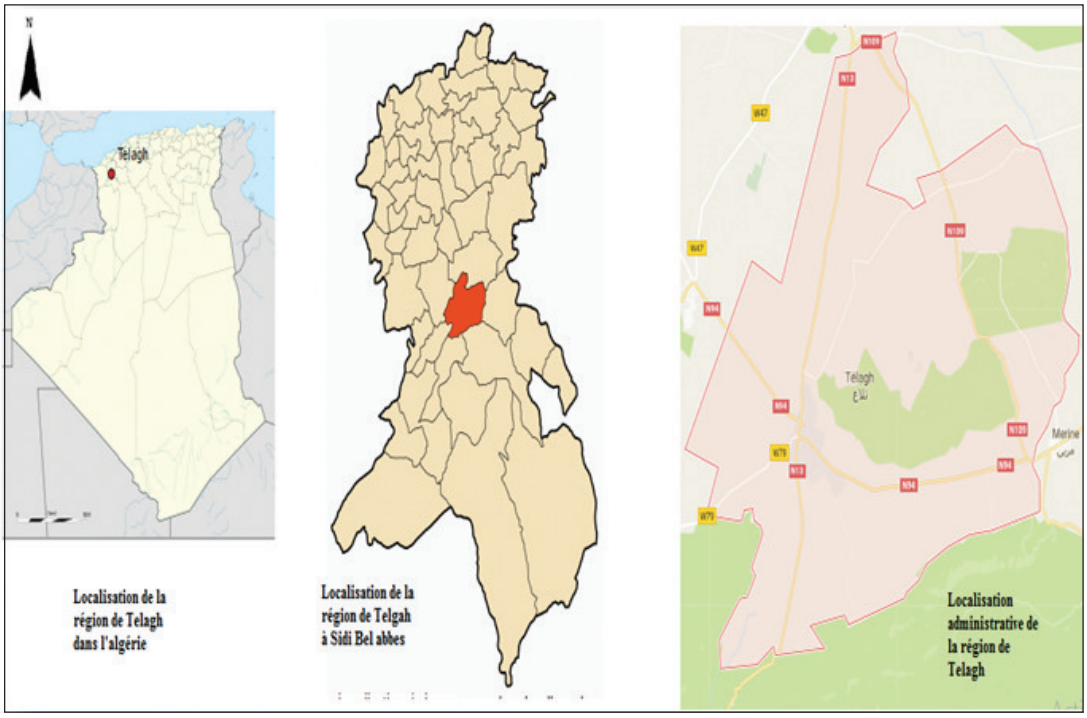

Figure 1: location of the Telagh region in Algeria and the Wilaya of Sidi Bel Abbes

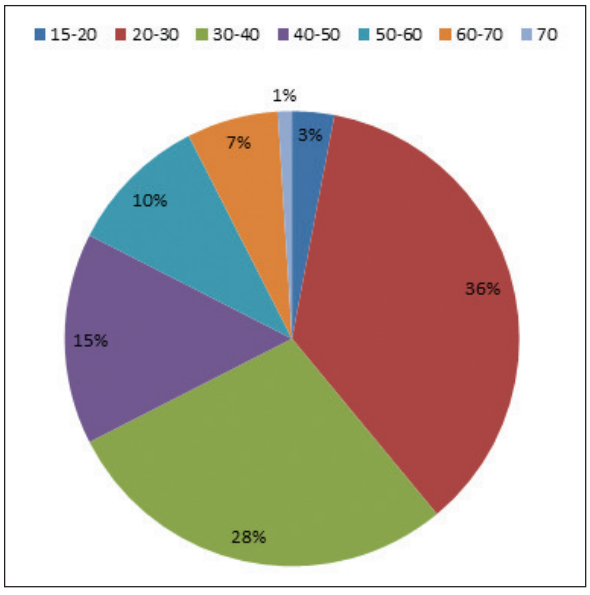

Figure 2: The frequency of the different age groups of the population questioned in the Telagh region

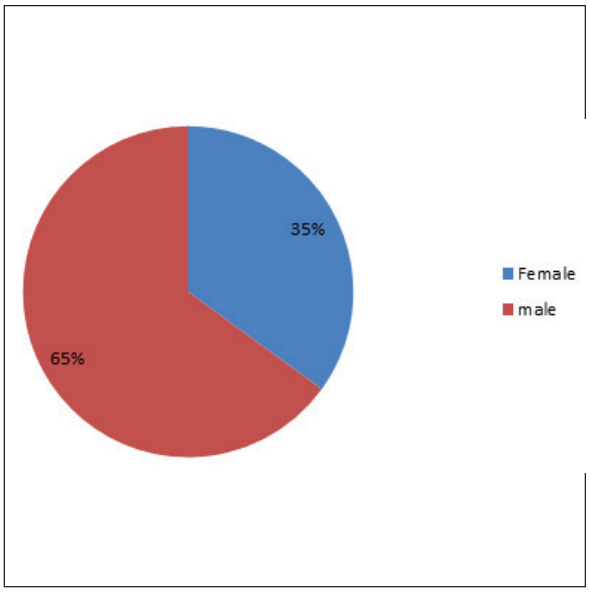

Figure 3: Sex frequency of the population questioned in the Telagh region

research laboratory using books and plant catalogs. Our results were processed by computer software Microsoft Excel 2010. It was able to distinguish groups of families and medicinal species used in the region of Telagh.

The results showed that the use of herbal remedies in Telgah is prevailing among all age groups (Figure 2), with predominance among people aged between 20-30 (36%). However, people aged between 30 and 40 years; there is a rate of (28%); for

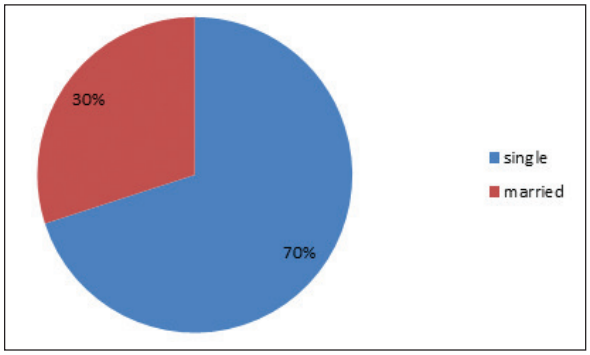

Figure 4: Frequency of the family situation of the questioned population the questioned population of the Telagh region

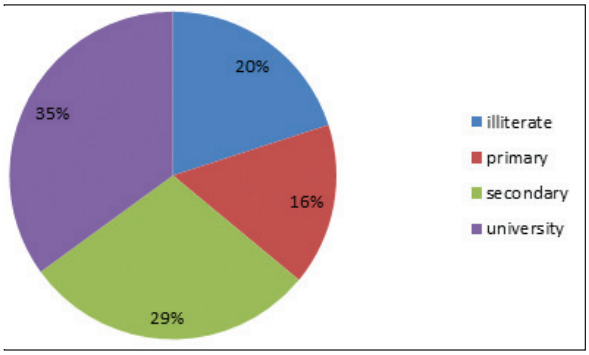

Figure 5: Frequency of university level of the population questioned in the Telagh region

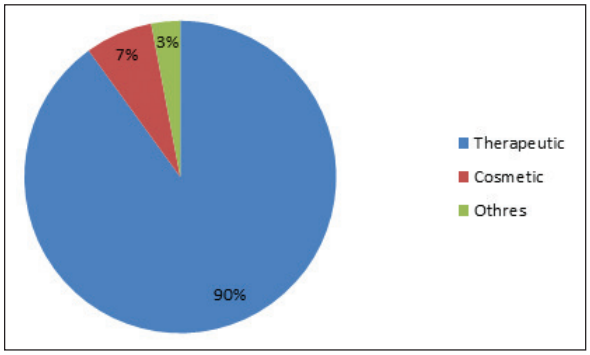

Figure 6: Frequency of uses of medicinal plants utilized by the populace questioned in Telagh region

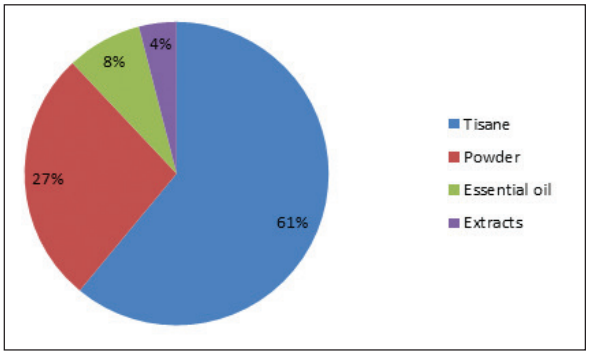

Figure 7: Frequency of method of preparation of medicinal plants used by the questioned population of the region of Telagh

the 40 to 50 age group (15%); for the 50 to 60 years group (10%); for the 60 to 70 y group (7%); and for the oldest people, the usage of medicinal plants (1%) does not represent a substantial therapeutic interest (Figure 2)**.** Familiarity with the properties and benefits of medicinal plants are often acquired following along experience accumulated and transmitted from one generation to the next. The transmission of this knowledge is currently in danger because it is not always assured (Saidi *et al*., 2015). The results obtained revealed that, in contrast to other age ranges, people who fit in with the age bracket of 20 to 30 years have more familiarity with medicinal plants.

Used herbal remedies vary by sex. Men take medicinal plants much more than women. Among the users, 65% are men, and 35% are women (Figure 3). It can be explained by; the utilization of herbal remedies by men because most of the survey were herbalists. As well as, most of the populations surveyed are men (Figure 3).

Medicinal plants are used much more by single people (70%) than by married people (30%) due to minimize the costs required by the doctor and the pharmacist (Figure 4).

In our study, the mass of medicinal plants users of those at a university level with a percentage of (35%). This rate is a sign that this part of the intellectual society is well aware of the importance of herbal medicine. However, people with secondary education have a significant percentage of medicinal plants use (29%), while those with illiterates and a primary school level use fewer medicinal plants (20% and 16% respectively) because the majority of surveys were with the level of university studies (Figure 5).

Information collected from the surveyed population shows that plants are often used for therapeutic purposes with a rate of 90%, while the use of plants for cosmetic purposes represents only 07% (Figure 6).

Table 1: Utilization of different medicinal plants in Telagh region for treatment of diseases

Local vernacular name	Vernacular name	Scientiûc name	Families	Dr	G	R	C	O	N	DD
Araar	Juniper	*Juniperus phoenicea L*	Cupressacea	+		+	-	-	-	-
Zataar	Thyme	*Thymus vulgaris L*	Lamiaceae	+	+		+	+	+	-
Nukha	Ammi	*Ammi Visnaga L*	Apiaceae	+		-	-	-	-	-
Kalitousse	Eucalyptus	*Eucalyptus globulus L*	Myrtaceae	+		-	-	-	-	-
Iklil l djabal	Rosemary	*Rosmarinus ocinalis L*	Lamiaceae	+	+		-	-	-	+
khozama	Lavender	*Lavandula dentata L*	Lamiaceae	+	+		+	-	+	+
Mrimia	Ocinal sage	*Salvia ocinalis L*	Lamiaceae	+	+		+	-	+	+
Naanaa	Green mint	*Mentha spicata L*	Lamiaceae	-	+		+	-	-	+
Marouioua	White horehound	*Marrubium vulgaris*	Lamiaceae	+	+		-	-	+	
Chihe	White mugwort	*Grass-alba Artemisia L*	Asteraceae	+	+		-	-	-	+
Roman	Grenadier	*Punicagranatum L*	Punicaceae	+	+		-	+	+	+
Babounag	Chamomile	*Matricaria chamomilla L*	Asteraceae	+	+		+	-	-	+

Dr: Dermatological; G: Genitourinary; R:Respiratory; C: Cardiovascular ; O: Osteo-articular; N: Neurological; DD: Disorder of the digestive tract

To liberate the active principle, numerous modes are used: namely decoction, infusion, raw, maceration, and poultice (Figure 7). This figure shows that infusion and decoction are the two most usable preparation methods with respective rates of 61% and 27 %.

Results of the plants' survey used by the local population indicated that they use these plants to treat many diseases like respiratory related diseases in the first place, secondly digestive tract diseases, Genito-urinary, in third place dermatological diseases, lastly come neurological and cardiovascular diseases (Table 1). The survey study has shown that traditional herbal medicine persists and is in demand by the inhabitants of Telgah. These results confirm with the findings obtained by other authors in other regions of Sidi Bel Abbes city (Dif *et al.*, 2015).

In the light of the results obtained, we notice a wide heterogeneity and diversity of species. Moreover, there are 24 families. We can see that the Lamiaceae dominates with 09 species with a rate of 20%, followed by The Asteraceae family which is represented by five (05) species, with a rate of 16% (Table 1). Concerning the species used by the inhabitants of Telgah, they are grouped into 12 families although the most cited is that of Lamiaceae. Many properties are attributed to Lamiaceae specifically; anti-inflammatory, antiviral, antibacterial, antiallergic, and antioxidant properties (Sijelmassi , 2011 ; Campanella *et al*., 2003; Dragland *et al*., 2003). These different properties are because of their chemically interesting constituents from a pharmacological point of view. These are tannins, coumarins, mucilages, flavonoids, and phenolic acids such as for instance rosmarinic acid (Exarchou *et al*., 2002; Lamiri *et al*., 2001). Additionally, this family is characterized by the clear presence of essential oils which have discovered a great invest therapy thanks for their broad spectral range of biological activities. Additionally, it represents an important supply of essential oils, infusions, and natural antibiotics for aromatherapy, perfumery even though synthetic fragrances tend to displace these essences. The cosmetics industry also uses different herbal plants of Lamiaceae family for his or her moisturizing and often antiseptic properties (Lamiri *et al*., 2001; Cimanga *et al*., 2002).

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