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# Effect of microbial inoculants on physiological and biochemical characteristics in jamun (*Syzygium cumini* L. Skeels) under different propagation substrates

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## ABSTRACT

**The seeds of jamun (*Syzygium cuminii* L. Skeels) were sown in different propagation substrates pre-treated with different doses of microbial inoculants under shade house condition at Experimental Block of Division of Fruit Crops, Indian Institute of Horticultural Research, Bengaluru, Karnataka to obtain healthy vigorous seedlings suitable for grafting and subsequent successful establishment in field. The Arka fermented coco-peat was found better than the mixture of sand, soil and FYM as propagation substrate in terms of most of the parameters like speed of seed germination as indicated by germination vigour index (29.18% higher), polyembryony (203.06% higher), leaf chlorophyll content (13.79% higher chlorophyll a, 7.14% higher chlorophyll b and 12.03% higher total chlorophyll) and leaf total carbohydrate content (50.82% higher). Among different microbial inoculants, Arka microbial consortia (mixture of *Azotobacter tropicalis* strain PAN MC1, *Bacillus aryabhattai* strain Bel 6 and *Pseudomonas taiwanensis* Mpf2) @ 2.0% significantly improved speed of germination by 73.94% and seedling vigour by 179.70% and altered physiological and biochemical attributes in leaf such as chlorophyll a (69.23% higher), chlorophyll b (166.67% higher), total chlorophyll (87.50% higher), total carbohydrates (109.90% higher), total phenols (63.71% higher) and total antioxidants (82.95% higher), as compared to control. Thus Arka fermented coco-peat treated with 2.0% Arka microbial consortia prior to seed sowing can be used for quick raising of superior and healthy seedlings in jamun under shade house condition.**

**Key words:** Arka fermented coco-peat, Arka microbial consortia, jamun, total phenols, total antioxidants

## INTRODUCTION

Jamun (*Syzygium cumini* L. Skeels, family-Myrtaceae) is an evergreen tropical tree of many parts of Asia and Eastern Africa. It has been used worldwide in treatment of diabetes and has proven good anti-oxidant, anti-bacterial, antigenotoxic, anti-inflammatory and anti-HIV properties (Sagrawat *et al*., 2006). Seedlings are most vulnerable to mortality in their life cycle and germination determines when and where seedling growth begins (Llanes *et al*., 2005), thereby involving much cost and risk for obtaining seedlings and their subsequent maintenance till graftable stage. Nursery potting substrates influence the quality of seedlings produced (Agbo and Omaliko 2006), thereby influencing establishment in field (Baiyeri, 2006). The interaction between microbial inoculants and plant root system pave way to harness maximum benefits from microbial inoculants for improving plant growth (Raja *et al*., 2006). *Pseudomonas fluorescence* and *Trichoderma harzianum* have the potential to enhance seed germination as well as seedling vigour (Iqbal and Hasnain 2013; Priyarani *et al*., 1999). The microbial consortium, which is a group of different species of microorganisms that act together as a community, can complement functionally for plant growth promotion (Pandey and Maheshwari, 2007). The changes in total phenols and antioxidant activity induced in the host that are activated by microbial inoculation also develop resistance capacity in host against pathogens (Chakraborty *et al*., 2013). As seed germination is the first and most critical stage of plant development and the relative performance of individual plants during the early growth stage, including germination and plant establishment, can have more effects on growth and fitness (Houssard and Escarré 1991), the present study aimed to assess the potential of microbial inoculation for advancing seed germination and improving growth and antioxidant activity of jamun under different propagation substrates.

## MATERIALS AND METHODS

The experiment was conducted at the Experimental Block of Division of Fruit Crops, Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India, in a shade house where availed 70% light, during May to August, 2013. The seeds were obtained from an open-pollinated seedling progeny maintained at the Experimental Farm of IIHR. The expected age of the tree was 20 years, having medium sized canopy and average fruit yield of 80 kg/tree/annum. The average fruit weight was 9.71 g with 3.87 cm length and 2.70 cm diameter, with total

soluble solids of 13.41˚B. The average weight of seed was 1.39 g. To improve seed germination and seedling growth, two factors were studied. The first one was the propagation substrates such as Arka fermented coco-peat (CP) and the mixture of sand, soil and farm yard manure (SS) in 1: 1: 1 (v/v) proportion. The preparation of CP was done at the nursery of the institute in 30 days by solid-state fermentation of raw coir pith using a consortium of the fungus *Aspergillus* having inoculum size of 20−50% (v/v) based on the volume of the mineral medium and a substrate average particle size of 375 µm. The second factor was microbial inoculants including control ($T_0$) such as Arka Microbial consortia 1.0% (10 g/ kg media) ($T_1$), Arka Microbial consortia 2.0% (20 g/ kg media) ($T_2$), *P. fluorescence* 1.0% (10 g/ kg media) ($T_3$), *P. fluorescence* 2.0% (20 g/ kg media) ($T_4$), *T. harzianum* 0.5% (5 g/ kg media) ($T_5$) and *T. harzianum* 1.0% (10 g/ kg media) ($T_6$). Arka Microbial consortium was a lignite based microbial product developed by IIHR that contains N fixing *(Azotobacter tropicalis* strain PAN MC1 – 2.1 x $10^9$ CFU/ g), P & Zn solubilizing (*Bacillus aryabhattai* strain Bel 6 – 1.8 x $10^9$ CFU/ g) and plant growth promoting microbes (*P. taiwanensis* Mpf2 – 3.2 x $10^9$ CFU/ g) in single carrier. The culture of *P. fluorescence* was made in Kings B media and then raised in talc powder with spore count of 2.0 x $10^8$ CFU/ g. The culture of *T. harzianum* was made in potato dextrose agar media and then raised in chalk powder having the spore count of 2.0 x $10^6$ CFU/ g. Prior to seed sowing, the propagation substrates were separately treated with different doses of microbial inoculants and then filled in pro trays. Seeds were sown in those pro trays and then covered by wire net until the end of germination so as to protect the seeds from rodent attack. The experiment was laid out in a factorial completely randomized design. Each microbial treatment including control was replicated thrice with 10 seeds per replication. Watering was done with hand as when required. The seedlings were transferred after 30 days of sowing of seeds from pro trays to the poly bags of 26 x 10 cm (250 gauge) with two punch holes for drainage, filled with soil: sand: FYM (1: 1: 1) v/v. Data on seed germination was recorded regularly until no further germination upto 30 days after sowing. The germination percentage was calculated as the percent of germinating seeds in relation to the total number of seeds sown per replication per treatment. The polyembrony percentage was calculated as per cent of seeds producing multiple seedlings in relation to the total number of seeds germinated. Germination vigour index (GVI) was computed using the method as described by Hassanein (2010). Seedling vigour was calculated using the formula as given by Bewly and Black (1982). The leaf chlorophyll was estimated by the method suggested by Hiscox and Israelstam (1979). The different procedures were followed for estimation of biochemicals in leaf such as Anthrone method for total carbohydrates (Yemm and Willis 1954), Folin Ciocalteu method for total phenols (Singleton and Rossi 1965) and Ferric Reducing ability of Plasma (FRAP) assay for total antioxidants (Benzie and Strain 1996). The data obtained from the experiment were analyzed using Web Agri Stat Package version WASP2.0 (ICAR Research Complex for Goa, Ela, Goa- 403 402, India). The visual indication of data dispersion on bar and line graphs was achieved by means of standard error of the mean. Treatment difference was evaluated using least significant difference (LSD) at $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Germination and growth behaviour

The microbial inoculants significantly influence the seed germination of jamun under different propagation substrates. The germination was significantly initiated by 3.29 days earlier and completed by 4.00 days earlier in CP than SS. The speed of germination was significantly higher in CP, as indicated by 29.18% higher GVI over SS, although there was no significant difference for germination percentage (Figure 1A & 1B). The earliness in seed germination might be due to the use of fermented coco-peat, which is considered as a good growing media component with acceptable pH, electrical conductivity and other chemical attributes (Awang *et al*., 2009). Among different microbial treatments, $T_6$ significantly took minimum days to initiate and complete germination (7.00 and 8.33 days earlier, respectively) and also recorded 73.94% higher GVI, as compared to control ($T_0$), though germination percentage was non-significant among the treatments. The results indicated the beneficial effects of microbial consortia over single inoculation (Raja *et al*., 2006). The interaction study revealed minimum days for initiation and completion of germination in $T_6$ treated seeds sown in CP, which were statistically at par with $T_5$ and $T_3$ treated seeds sown in CP for completion of germination. The GVI was also significantly higher in $T_6$ treated seeds sown in CP. Thus the improvement in seed germination rate due to interaction effect of $T_6$ and CP depicted higher magnitude of plant growth promoting activities in the case of consortia or mixed microbial cultures than single strain under CP.

**Table 1. Response of jamun to microbial inoculants on chlorophyll content under different propagation media**

| Treatment | Chlorophyll a (mg $g^{-1}$ f.w.) | | | Chlorophyll b (mg $g^{-1}$ f.w.) | | | Total Chlorophyll (mg $g^{-1}$ f.w.) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Different propagation media | | | | | | | | |
| | CP | SS | Mean | CP | SS | Mean | CP | SS | Mean |
| $T_0$ | 1.18 | 0.64 | 0.91 | 0.17 | 0.25 | 0.21 | 1.34 | 0.89 | 1.12 |
| $T_1$ | 1.13 | 1.20 | 1.16 | 0.41 | 0.44 | 0.42 | 1.53 | 1.64 | 1.59 |
| $T_2$ | 1.25 | 1.07 | 1.16 | 0.47 | 0.41 | 0.44 | 1.72 | 1.48 | 1.60 |
| $T_3$ | 1.29 | 1.30 | 1.29 | 0.43 | 0.46 | 0.45 | 1.72 | 1.76 | 1.74 |
| $T_4$ | 1.18 | 1.42 | 1.30 | 0.49 | 0.48 | 0.49 | 1.67 | 1.90 | 1.79 |
| $T_5$ | 1.46 | 1.15 | 1.31 | 0.55 | 0.42 | 0.48 | 2.01 | 1.57 | 1.79 |
| $T_6$ | 1.75* | 1.33 | 1.54* | 0.63* | 0.49 | 0.56* | 2.38* | 1.82 | 2.10* |
| Mean | 1.32* | 1.16 | | 0.45* | 0.42 | | 1.77* | 1.58 | |
| For comparing the means of | S.Em± | LSD at 5% | | S.Em± | LSD at 5% | | S.Em± | LSD at 5% | |
| Propagation substrate (P) | 0.06 | 0.02 | | 0.01 | 0.02 | | 0.09 | 0.05 | |
| Treatment (T) | 0.07 | 0.04 | | 0.04 | 0.02 | | 0.11 | 0.05 | |
| Interaction (P × T) | 0.07 | 0.05 | | 0.03 | 0.04 | | 0.09 | 0.08 | |

'*' indicates significance at LSD (0.05), n= 3.

**Table 2. Response of jamun to microbial inoculants on biochemical changes under different propagation media**

| Treatment | Total Carbohydrates (mg $g^{-1}$ f.w.) | | | Total Phenols (mg GA equivalent $g^{-1}$ f.w.) | | | Total Antioxidants (mg ascorbic acid equivalent $g^{-1}$ f.w.) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Different propagation media | | | | | | | | |
| | CP | S | Mean | CP | S | Mean | CP | S | Mean |
| $T_0$ | 30.024 | 21.244 | 25.634 | 13.14 | 23.78 | 18.46 | 3.39 | 4.47 | 3.93 |
| $T_1$ | 42.463 | 24.902 | 33.683 | 19.58 | 25.29 | 22.44 | 4.63 | 7.68 | 6.16 |
| $T_2$ | 44.659 | 37.098 | 40.878 | 16.94 | 23.67 | 20.30 | 3.38 | 6.01 | 4.69 |
| $T_3$ | 58.073 | 45.878 | 51.976 | 13.33 | 30.01 | 21.67 | 4.42 | 5.69 | 5.05 |
| $T_4$ | 54.902 | 37.829 | 46.366 | 17.71 | 31.01 | 24.36 | 4.62 | 8.44 | 6.53 |
| $T_5$ | 68.317* | 32.463 | 50.390 | 25.76 | 25.66 | 25.71 | 5.93 | 7.48 | 6.71 |
| $T_6$ | 65.634 | 41.976 | 53.805* | 26.26 | 34.18* | 30.22* | 5.87 | 8.51* | 7.19* |
| Mean | 52.010* | 34.484 | | 18.96 | 27.66* | | 4.61 | 6.90* | |
| For comparing the means of | S.Em± | LSD at 5% | | S.Em± | LSD at 5% | | S.Em± | LSD at 5% | |
| Propagation Media (P) | 8.763 | 0.003 | | 4.35 | 0.05 | | 1.15 | 0.02 | |
| Treatment (T) | 3.951 | 0.004 | | 1.47 | 0.09 | | 0.45 | 0.03 | |
| Interaction (P × T) | 3.834 | 0.005 | | 1.72 | 0.14 | | 0.46 | 0.03 | |

'*' indicates significance at LSD (0.05), n= 5.

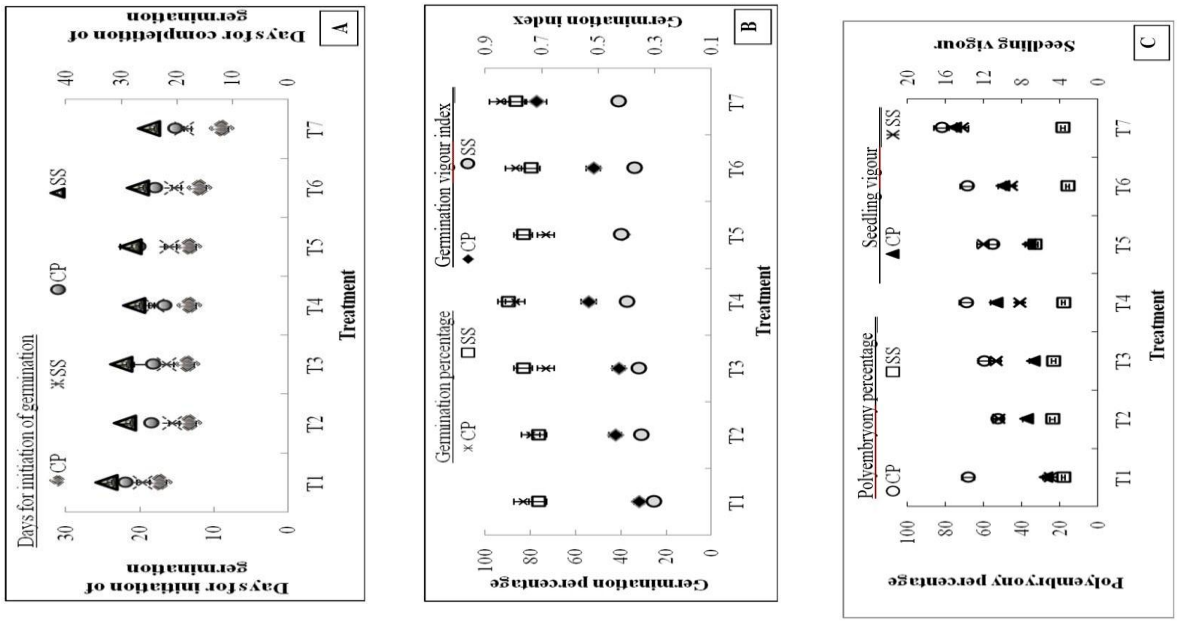


**Figure 1. Response of jamun to microbial inoculants on (A) days taken for seedling emergence and completion of germination; (B) germination percentage and germination index; and (C) polyembryony percentage and seedling vigour under different propagation media.**

The emergence of more than one seedling from a single seed due to polyembryony has been reported in jamun (Sivasubramaniam and Selvarani 2012). However, to our knowledge, this is the first report where we found significant effect of propagation substrate on emergence of multiple seedlings from a single seed (Figure 1C). The polyembryony was increased by 203.06% in CP than SS. Thus fermented coco-peat might have created favourable micro-climate for the germination of multiple embryos, resulting in emergence of multiple seedlings from single seed. However, application of microbial culture in propagation substrate did not have any significant influence on polyembryony.

The seedling vigour was non-significant among the seedlings raised in different propagation substrates (Figure 1C). However, among different treatments, it was significantly higher in $T_6$ over $T_0$ by 179.70%. The results indicated that microbial consortia prepared by mixing of different rhizobacteria produced a more pronounced influence while inoculation alone showed a lower effect on seedling vigour, as suggested by Stefan *et al.* (2013). Thus different bacteria present in consortia can act synergistically to stimulate the growth of host plant *via* production of plant growth promoters like auxin, gibberellins and cytokinins (Glick, 1995).

**Physiological changes**

The leaf chlorophylls (a, b and total) were significantly increased in CP over SS by 13.79, 7.14 and 12.03%, respectively (Table 1). Irrespective of propagation substrate, the leaf chlorophylls significantly recorded more in $T_6$ than $T_0$ by 69.23, 166.67 and 87.50%, respectively. The interaction effect revealed that CP and $T_6$ significantly interact to enhance the formation of leaf chlorophyll which might be attributed to their action on increasing availability of water and minerals due to improvement in plant growth mediated by microbial inoculants and coco-peat (Berg, 2009; Evans and Iles 1997).

**Biochemical changes**

The accumulation of total carbohydrates in jamun leaves was recorded 50.82% more in CP than SS (Table 2), which might be attributed to improved photosynthetic rate of seedlings under fermented coco-peat, due to improvement in hydraulic conductivity, porosity, water holding capacity, nutrient retention capacity and formation of humic substances in the rhizosphere having hormone-like activity (Prabhu and Thomas 2002). This parameter was also enhanced by microbial inoculation, regardless of propagation substrate, and the increase was 109.90% more in $T_6$ over $T_0$, which might be attributed to enhanced photosynthetic activity by microbial consortia (Stefan *et al.*, 2013). The interaction effect revealed significantly higher content of total carbohydrate in $T_5$ followed by $T_6$ under CP substrate.

The production of secondary metabolites like phenolics in plant is related to its growing condition (Saikia and Upadhyaya 2011). The accumulation of phenolic compounds in seedlings raised in CP than SS was lesser by 45.89% (Table 2), which might be due to better growing condition of plant as a result of better water maintenance ability of fermented coco-peat. The $T_6$ had significant effect on level of total phenols in leaf cytoplasm, regardless of any propagation substrate, and it was 63.71% more than that of $T_0$. The result is in agreement with that of Thiruveni *et al*., 2012). Thus increased level of total phenols among plants with more dose of microbial consortia inoculation could be due to reaction of host plants against microbial colonization, which thereby induced systemic resistance against pathogens (Chakraborty *et al*., 2013).

Total antioxidants in leaf was increased by 82.95% in SS than CP, as observed in Table 2, which might be associated with enhanced phenolics content in seedlings grown in SS (Vignesh *et al*., 2012). The $T_6$ had significant effect on production of total antioxidants, as compared to other treatments including $T_0$, regardless of propagation substrate. The elevated antioxidant activity due to microbial consortia could be correlated with increased stress tolerance (Stefan *et al.*, 2013). Similar results concerning antioxidant protective effects of microbial culture were previously reported by other authors (Vignesh *et al*., 2012). The interaction study revealed highest content of total antioxidants in $T_6$ under SS.

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**REFERENCES**

Agbo, C.U. and Omaliko, C.M. 2006. Initiation and growth of shoots of *Gongronema latifolia* Beuth stem cuttings in different rooting media. *African J. Biotechnol*., **5**: 425-428.

Awang, Y., Shaharomm, A.S., Mohamad, R.B. and Selamat, A. 2009. Chemical and physical characteristics of coco-peat-based media mixtures and their effects on the growth and development of *Celosia cristata*. *Am. J. Ag. Biol. Sci*., **4**: (1): 63-71.

Baiyeri, K.P. 2006. Seedling emergence and growth of pawpaw (*Carica papaya*) grown under different coloured shade polyethylene. *International Agrophysics*, **20**: 113-117.

Benzie, I.F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem.*, **239**: 70-76.

Berg, G. 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.*, **84**: (1): 11-18.

Bewly, J.D. and Black, B.M. 1982. Germination of seeds. *In. Physiology and Biochemistry of Seed Germination*. AA Khan (ed). Springer-Verlag, New York pp 40-80.

Chakraborty, U., Chakraborty, B.N., Chakraborty, A.P., Sunar, K. and Dey, P.L. 2013. Plant growth promoting rhizobacteria mediated improvement of health status of tea plants. *Indian Journal of Biotechnology*, **12**: 20-31.

Evans, M.R. and Iles, J.K. 1997. Growth of *Viburnum dentatum* and *Syrbrga* X *prestoniane* 'Donald Wyman' in sphagnum peat and coir dust-based substrates. *J. Environ. Hort.*, **15**: 156-159.

Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Can. J. Microbiol.*, **41**: 109-114.

Hassanein, A.M.A. 2010. Improving seed germination and seedling growth of some economically important trees by seed treatments and growing media. *J. Hort. Sci. Ornament Plants*, **2**: (1): 24-31.

Hiscox, J.D. and Israelstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.*, **57**: 1332-1334.

Houssard, C. and Escarré, J. 1991. The effects of seed weight on growth and competitive ability of *Rumex acetosella* from two successional old-fields. *Oecologia*, **86**: 236-242.

Iqbal, A. and Hasnain, S. 2013. Auxin producing *Pseudomonas* strains: biological candidates to modulate the growth of *Triticum aestivum* beneficially. *American Journal of Plant Sciences*, **4**: 1693-1700.

Llanes, A., Reinoso, H. and Luna, V. 2005. Germination and early growth of *Prosopis strombulifera* seedlings in different saline solutions. *World J. Agr. Sci.*, **1**: (2): 120-128.

Pandey, P. and Maheshwari, D.K. 2007. Two-species microbial consortium for growth promotion of *Cajanus cajan*. *Current Science*, **92**: (8): 1137-1142.

Prabhu, S.R. and Thomas, G.V. 2002. Biological conversion of coir pith into a value-added organic resource and its application in Agri-Horticulture: current status, prospects and perspective. *Journal of Plantation Crops*, **30**: (1): 1-17.

Priyarani, Aggarwal, A. and Malhotra, R.S. 1999. Growth responses in *Acacia nilokca* inoculated with VAM fungi (*Glomus mosseae*), *Rhizobium* sp. and *Trichoderma harzianum*. *Indian Phytopathology*, **52**: (2): 151-153.

Raja, P., Uma, S., Gopal, H. and Govindarajan, K. 2006. Impact of bio-inoculants consortium on rice exudates, biological nitrogen fixation and plant growth. *J. Biol. Sci.*, **6**: (5): 815-823.

Sagrawat, H., Mann, A.S. and Kharya, M.D. 2006. Pharmocological potential of *Eugenia Jambolana*: A review. *Phcog. Mag.*, **2**: 96-105.

Saikia, L.R. and Upadhyaya, S. 2011. Antioxidant activity, phenol and flavonoid content of *Asparagus racemosus* Willd, a medicinal plant grown using different organic manures. *Res. J. Pharm. Biol. Chem. Sci.*, **2**: (2): 457-463.

Singleton, V.L. and Rossi, J.A. 1965. A colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticul.*, **16**: 144-158.

Sivasubramaniam, K. and Selvarani, K. 2012. Viability and vigor of jamun (*Syzygium cumini*) seeds. *Braz. J. Bot.*, **35**: (4): 397-400.

Stefan, M., Munteanu, N., Stoleru, V. and Mihasan, M. 2013. Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean. *Romanian Biotechnol. Lett.*, **8**: (2): 8132-8143.

Thiruveni, T., Shanthi, M., Murali baskaran, R.K., Amutha, R. and Raguchander, T. 2012. Microbial and herbivore induced phytochemical changes in okra against shoot and fruit borer, *Earias vittella* (Fab.). *J. Biopest.*, **5**: 223-227.

Vignesh, R., Venkatesh, N.R., Meenakshisundaram, B. and Jayapradha, R. 2012. Novel instant organic fertiliser and analysis of its growth effects on spinach. *J. Biol. Sci.*, **12**: (2): 105-110.

Yemm, E.W. and Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.*, **57**: 508–514.