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Effect of microbial inoculants on germination of papaya (*Carica papaya* L.) *Cv*. red lady seedlings under Polyhouse conditions

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Abstract
A study was conducted to find out the effect of microbial inoculants on growth of papaya seedlings under polyhouse condition at UAS, Bengaluru during the year 2017-18. Papaya *(Carica papaya* L.) is an important fruit crop of tropical world and has been known as wonder fruit of the tropics. It belongs to the family Caricaceae. To improve the seedling quality, a nursery study was conducted to select the suitable bio-inoculants for growth of papaya seedlings. The bio-inoculants such as *Glomus fasciculatum*, *Pseudomonas fluorescence*, *Azoto bacter chroococcum*, *Azospirillum lipoferum* were isolated and inoculated individually and in combinations. Seedlings were kept at 120 days under polyhouse conditions. Among them, early seed germination (5 days) in treatment with *Glomus fasciculatum* + *Pseudomonas fluorescence* + *Azotobacter chroococcum* + *Azospirillum lipoferum* (T_{11}) was recorded. This treatment also influenced most of the growth parameters positively, *Viz*. higher plant height (63.21 cm at 120 DAS), higher number of leaves (15.35 at 120 DAS), more leaf area (72.37 cm^2 at 120 DAS), more number of roots per plant (16.33), higher root length (15.67 cm), higher fresh (73.83 g) and dry weight (3.83 g) of roots per plant and highest chlorophyll content(22.4 mg). Thus, it can be concluded that, the combined inoculation of bio-inoculants are beneficial for increasing growth and quality seedling production in papaya *Cv*. Red Lady.

Keywords: Bio-inoculants, *Pseudomonas fluorescence*, *Azotobacter*, *Glomus fasciculatum*, *Azospirillum lipoferum*, *Azotobacter chroococcum* and quality

Introduction
Papaya (*Carica papaya* L.) is an important fruit crop, belonging to family *Caricaceae* and native of tropical America (Singh, 1990) [12]. *Carica*is the largest of the four genera with 48 species, among which *Carica papaya* L. is most important and cultivated all over the world (Badillo, 1971) [1]. Papaya is believed to have originated in Mexico. In India, papaya was introduced in early part of 16th century from Philippines through Malaysia. The popularity of papaya fruit has made it ubiquitous in tropical and sub-tropical regions of the world.
Bio-inoculants are carrier based preparations containing live micro-organisms in a viable form. They can be used as soil application or seed treatment and designed to improve the soil fertility and help the plant growth by increasing their number and biological activity in the rhizosphere (Subba Rao, 1998) [15]. Utilization of bio-inoculants improve the growth and yield of papaya and sustain soil fertility resulting in incremented crop yield without causing any enivornmental hazards. Out of many micro-organisms, which are indentified as bio-inoculants, *Azotobacter, Azospirillum*, Phosphate solubilizing bacteria, Fungi and Vesicular Arbuscular Mycorrhiza fungi (VAM) have a consequential role in horticulture crops.
Role of bio-inoculants in reducing the utilization of chemical fertilizers for amending the growth and development of many plants, information on the utilization of microbial-inoculants in nursery management of papaya is very limited. Keeping these points in view, the present study has been undertaken to asses the suitable microbial- inoculants for better seed germination and seedling survival.

Material & Methods
Experiment "Effect of microbial inoculants on growth of Papaya (*Carica papaya* L.) *Cv.* Red lady seedlings under Polyhouse Conditions" was carried out at Department of Horticulture, College of Agriculture, University of Agricultural Science, G.K.V.K., Bengaluru, during

2017-18. The experiment was tested in Complete Randomized Design (CRD) with three replications and consisted of twelve treatments namely T1 (*Glomus fasciculatum*), T2 (*Pseudomonas fluorescens*), T3 (*Azotobacter chroococcum*), T4 (*Azospirillum lipoferum*), T5 (*Glomus fasciculatum* + *Psuedomonas fluorescens*), T6 (*Glomus fasciculatum* + *Azotobacter chroococcum*), T7 (*Glomus fasciculatum* + *Azospirillum lipoferum*), T8 (*Psuedomonas fluorescens* + *Azotobacter chroococcum*), T9 (*Psuedomonas fluorescens* + *Azospirillum lipoferum*) T10 (*Azotobacter chroococcum* + *Azospirillum lipoferum*) T11(*Glomus fasciculatum* + *Psuedomonas fluorescens* + *Azotobacter chroococcum* + *Azospirillum lipoferum* and T12 (Control). Observations are recorded on seed germination, per cent of seed germination and mortality rate after sowing.

Plate 1: Different bio-inoculants used

Plate 2: Sowing of seed

Observation on seed germination in papaya from each treatment were recorded separately for each day after seed sowing and expressed in number of days. The mean per cent of seed germination for each treatment were estimated and expressed as per centage. Mortality rate was measured by using formula

$$\text{Mortality rate} = \frac{\text{Number of seedlings dead}}{\text{Total number of seedlings germinated}} \times 100$$

Statistical analysis was estimated by analysis of variance as per the technique given by Fisher (1963) [2]. Data has been suitably presented in the form of tables and graphs.

Results and Discussion

Days taken for seed germination

The data on days taken for seed germination as influenced by different sources of bio-inoculants are presented in Table 1.

Significant differences were observed among the treatments with respect to days taken for seed germination, in treatment containing different combination of bio-inoculants *viz*. *Glomus fasciculatum* + *Psuedomonas fluorescen* + *Azotobacter chroococcum* + *Azospirillum lipoferum* (T_{11}) recorded early seed germination (5 days) which was followed by treatment received with *Glomus fasciculatum* + *Azospirillum lipoferum* (T_7). The longest period (10 days) was perceived the seed germination in control treatment where, no bio-inoculants were added to it.

The growing condtion prevailed for the seed germination is under controlled enivornment condition. Therefore, the less number of days taken for seed germination may be due to the bio-inoculants attributed to creating favourable conditions such as optimum moisture retention, temperature, secreation of vitamins, growth promoting substances and water absorption.

Similarlly, early germination was also noticed with bio-inoculant *Azotobacter* attributed to creating favourable conditions like optimum moisture retention, temperature and secretion of plant growth regulators like gibberellins, vitamins and water absorption. Similar results were supported by Rakesh Kumar Yadav *et al*., (2012) [10] in acid lime, and Vasu *et al*., (2010) [16] in *Lens culinaris* Medic.

Per cent of seed germination

Non significant differences were observed among the treatments with respect to per centage seed germination. (Table 1)

However, the different combination of bio-inoculants added to the media that enhanced the seed germination per centage in all treatments. This may be due to the application in combination of bio- inoculants helps in nutrient and water uptake and also maintenance of good physical and chemical properties of media. The results were in conformity with the observation made by Geetha *et al*., (2007) [3] in mango and Parmeswari *et al*., (2001) [8] in tamrind.

The similar results are in line with Jayant Raman (2012) [4] where, increased germination due to application of biofertilizers i.e. *Azotobacter* in apple. The same was also reported by Sinish *et al*., (2005) [13] in cashew and Pathak *et al.*, (2013) [9] in guava.

Mortality rate of seedlings after sowing (%)

The influence of bio-inoculants showed non significant differences among the treatments with respect to mortality rate after sowing. The minimum mortality rate (4.33%) was recorded in the treatment receiving *Psuedomonas fluorescens* (T_2) and most of the other treatments were on par with each other (Table 1).

Early seed germination and reduced mortality rate could be attributed to efficiency of microbial inoculants in supplying the required crop nutrients, suppression of harmful organism as well as moisture absorption.

The minimum mortality rate can be attributed application of bio-inoculants, these bio-inoculants fixes the nitrogen or solublization of insoluble phosphate or secreting hormones and vitamins.

Similar trend was shown by Rani and Sathimoorty (1997) [11] in papya seedlings inoculated with *Glomus mosseae* which increased germination and decreases the mortality rate of seedlings.

Plate 3: Seed germination

Table 1: Effect of microbial inoculants on days taken for seed germination, per cent of seed germination and mortality rate after sowing in papaya *Cv.* Red Lady

Treatment combinations	Days after imposing treatments		
	Days taken for seed germination	Per cent of seed germination	Mortality rate after sowing (%)
T_1 = *Glomus fasciculatum*	8^b	99.66(9.97)	4.66
T_2 = *Pseudomonas fluorescens*	9^a	100.0(10.0)	4.33
T_3 = *Azotobacter chroococcum*	9^a	99.66(9.97)	4.66
T_4 = *Azospirillum lipoferum*	7^c	100.0(10.0)	5.00
T_5 = *Glomus fasciculatum+Psuedomonas fluorescens*	9^a	100.0(10.0)	5.00
T_6 = *Glomus fasciculatum+Azotobacter chroococcum*	8^b	100.0(10.0)	5.00
T_7 = *Glomus fasciculatum+Azospirillum lipoferum*	6^d	99.66(9.97)	4.66
T_8 = *Psuedomonas fluorescens+Azotobacter chroococcum*	9^a	100.0(10.0)	5.00
T_9 = *Psuedomonas fluorescens+ Azospirillum lipoferum*	9^a	99.66(9.97)	4.66
T_{10} = *Azotobacter chroococcum+Azospirillum lipoferum*	9^a	99.66(9.97)	4.66
T_{11} = *Glomus fasciculatum + Psuedomon fluorescens + Azotobacter chroococcum + Azospirillum lipoferum*	5^e	100.0(10.0)	5.00
T_{12} = Control	10^a	100.0(10.0)	4.66
F-test	*	NS	NS
S.Em ±	0.16	0.19	0.22
C.D. @ 5%	0.48	0.78	0.85

significant at 5% NS- Non significant
Azotobacter - (*Azotobacter chroococcum*) (2g/polybag) GF - *Glomus fasciculatum* (2 g/polybag)
Azospirillum - *Azospirillum lipoferum* (2 g/polybag)*Psuedomonas* - *Pseudomonas fluorescens (2*g/polybag)
1.5g/polybag for combination treatment

Table 2: Effect of bio-inoculants on bacteria population and VAM spore in rhizosphere soils of papaya at 60 DAS and 120 DAS

Treatment combinations	Total bacteria No × 104 CFU/g of soil		VAM spore count No/50 g of soil	
	60 DAS	120DAS	60 DAS	120DAS
T1 = *Glomus fasciculatum*	41.33b	85.00d	55.33c	127.6c
T2 = *Pseudomonas fluorescens*	28.33c	51.00f	08.33g	24.33f
T3 = *Azotobacter chroococcum*	26.66d	44.00g	14.66f	33.00e
T4 = *Azospirillum lipoferum*	44.00b	93.33c	65.00b	198.6b
T5 = *Glomus fasciculatum + Psuedomonas fluorescens*	30.33c	57.00e	50.00d	115.0d
T6 = *Glomus fasciculatum + Azotobacter chroococcum*	24.66d	47.33f	14.33f	25.00f
T7 = *Glomus fasciculatum + Azospirilluml ipoferum*	45.66b	105.3b	68.00b	199.0b
T= *Psuedomonas fluorescens+Azotobacter chroococcum*	24.00d	54.33e	15.66f	20.00g
T9 = *Psuedomonas fluorescens+Azospirillum lipoferum*	22.00d	45.00g	20.33e	25.00f
T10 = *Azotobacter chroococcum+Azospirillum ipoferum*	30.00c	33.33h	14.66f	22.66g
T11 = *Glomus fasciculatum + Psuedomonas fluorescens + Azotobacter chroococcum +Azospirillum lipoferum*	54.66a	111.3a	78.33a	203.3a
T12 = Control	13.33f	28.33i	11.00g	21.66g
F-test	*	*	*	*
S.Em ±	1.54	1.40	1.17	0.94
C.D. @ 5%	4.52	4.13	3.44	2.76

Bacteria and spore population at 60 DAS and 120 DAS in rhizosphere soil of papaya

The data on population per g of soil in rhizosphere of papaya as influenced by different bio-inoculants at 60 DAS and 120 DAS was given in Table 2.

At 60 and 120 DAS, the highest bacterial population (54.66 x 104 cfu g-1soil and 111.3 x 104 cfu g-1 soil) was found in the rhizosphere soils of treatment containing the combination of *Glomus fasciculatum* + *Psuedomonas fluorescens* + *Azotobacter chroococcum* + *Azospirillum lipoferum*(T11) followed by T7 (*Glomus fasciculatum* + *Azospirillum lipoferum*) 45.66x 106 cfu g-1 and 105.33 x 104 cfu g-1soil and it was found to be on par with treatment *Azospirillum lipoferum* (T4). The lowest bacterial population was recorded in the rhizosphere soil of uninoculated control (T12) 13.33 $x10^4$ cfu g^{-1}soil and 28.33 x104 cfu g-1soil.

There was a significant increase in mycorrhizal spore count (78.33 and 203.33 spores per 50 g of soil) at 60 DAS and 120 DAS, the highest spore count was observed in the treatment containing the combination of *Glomus fasciculatum* + *Psuedomonas fluorescens* + *Azotobacter chroococcum* + *Azospirillum lipoferum* (T11) followed by T7 (*Glomus fasciculatum* + *Azospirillum lipoferum*) 68 and 199 spores per 50 g of soil and it was found to be on par with treatment with *Azospirillum lipoferum* (T4). The lowest spore count was recorded in the control treatment (T12) 11 and 21.66 spores per 50 g of soil.

Results of present investigation are in confirmation with the findings by Kale *et al*. (1992) [6] and Maheshwarapa *et al*. (1999) [7]. These results have close conformity with findings of Joolka *et al*. (2004) [5].

Yao *et al*. (2009) [17] also reported that, significant difference was found in root colonization of trifoliate orange seedling inoculated with AM fungi varied from 31.7 to

49.5 per cent. Sharma and Sharma (2009) [14] studied, spore population per cent root colonization and *Azotobacter* count of citrus orchards. They recorded maximum number of AM fungi from Jach (3947 per Kg of soil), maximum root colonization (10.50%) samples collected from Daad and maximum *Azotobacter* count (4.3×106 CFU per 100 g soil) from Jhamrari.

Conclusion

The conclusions drawn from the experiment are combined inoculation of bio-inoculants and dual inoculation treatment was found to be effective on early seed germination, increased seed germination per centage and mortality rate after sowing of papaya seedlings. Bio-inoculants act as perpetually renewable inputs helping better nutrient management and maintenance of soil health.

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