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Ranjani SN
Department of Agricultural Microbiology, UAS, G.K.V.K., Bengaluru, Karnataka, India

L Krishna Naik
Department of Agricultural Microbiology, UAS, G.K.V.K., Bengaluru, Karnataka, India

Kushala G
Department of Horticulture, UAS, G.K.V.K., Bengaluru, Karnataka, India

Correspondence
Ranjani SN
Department of Agricultural Microbiology, UAS, G.K.V.K., Bengaluru, Karnataka, India

Effect of inoculation of PGPRM on population density of inoculated organisms in the cashew rhizosphere under poly house condition

Ranjani SN, L Krishna Naik and Kushala G

Abstract
Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens, Trichoderma viride and *Glomus fasciculatum* were efficient PGPRM. Hence, they were subjected to compatibility test by dual culture method. All the four PGPR microorganisms (*A. chroococcum, B. megaterium, P. fluorescens,* and *T. viride*) were found to be compatible under *in vitro* condition both on solid and in liquid media. Population density of inoculated PGPR microorganisms in the cashew rhizosphere at different intervals was found to be maximum in the treatments receiving that organisms alone or with other PGPR microorganism. Population density of inoculated organisms increased gradually up to grafting and there after slight decline was noticed.

Keywords: Effect, inoculation, population density, inoculated organisms, *Pseudomonas fluorescens, Trichoderma viride*

Introduction
Cashew (*Anacardium occidentale* L.) a tropical plant of commercial importance, belongs to the family Anacardiaceae. It is one of the export oriented cash crops of our country. It is believed to be the native of tropical America, from where it was introduced to the Malabar Coast of India by early Portuguese settlers more than 500 years ago. It is a perennial, low spreading tree and can reach to the height of about 15 meters with number of primary and secondary branches. Earlier, it was grown mainly to check the soil erosion, but gradually it has gained commercial importance as a plantation crop and has assumed a prominent position in Indian economy, as an export oriented crop as it earns lot of foreign exchange.

Cashew is a cross pollinated crop and as such highly heterozygous plants are produced if seed propagation is resorted. The most common and the simplest method of raising cashew trees is from seeds, with the advancement in propagation techniques, it is now possible to propagate vegetatively in large numbers. Approximate methods of propagation like rooting of cuttings, budding, layering and grafting were standardized in various horticultural crops.

Early studies on vegetative propagation of cashew were mainly confined to air layering (Rao, 1957) [11]. Though this is the simplest, cheapest and successful method, poor field establishment is the main draw back. In case of epicotyl grafting although an initial success of about 80 percent could be obtained (Aravindakshan *et al.,* 1984) [2], it was observed that these epicotyl grafts were more susceptible to collar rot resulting in high percentage of field mortality. Methods like side grafting, veneergrafting and patch budding, though found feasible in cashew, the success rate recorded by them were not encouraging for large scale multiplication other of clonal planting materials. It is now possible to multiply commercial cashew varieties by soft wood grafting which is the cheapest and easiest method of vegetative propagation. This method also proved to be the best under 'maidan parts' of Karnataka (Anon., 1994) [1].

Soft wood grafting depends on the type of media used to raise the root stocks and the quality of root stocks and scions. In the eastern dry zone of Karnataka, it has been observed that poor quality of root stocks is one of the major causes for low success of soft wood grafts even under conducive environment. It has been observed that stocks raised using potting mixture (red earth, sand and FYM – 1:1:1) have minimum roots, thus making the stock growth less vigorous and more lanky. It has been suspected that the less environment of beneficial soil microorganisms might be the reasons for such results.

Application of biofertilizers is known to improve the soil fertility and crop productivity in several crops through atmospheric nitrogen fixation, solubilization of inorganic and organic phosphorus and other nutrients and synthesis of growth regulators. They also play an important role in improving germination, root proliferation and suppress plant diseases (Verma, 1993; Subba Rao, 1995) [14, 12]. The beneficial effect of *Azotobacter* treatment has been reported in establishment of healthy and sturdy seedlings (Sundara Rao *et al*., 1963a) [13].

Material and methods

This experiment was carried out during the year 2007- 08 in collaboration with All India Coordinated Research Project on cashew, nursery section at Agricultural Research Station (ARS), Chintamani, Kolar (Dist.), Karnataka.

Five grams of lignite based inoculum of plant growth promoting rhizomicroorganism was applied in the poly bag containing soil (2 – 3 cm below the soil surface), according to the treatment details.

Treatment details

T_1: control
T_2: *Azotobacter chroococcum*
T_3: *Bacillus megaterium*
T_4: *Glomus fasciculatum*
T_5: *Pseudomonas fluorescens*
T_6: *Trichoderma viride*
T_7: *Azotobacter chroococcum* + *Bacillus megaterium*
T_8: *Azotobacter chroococcum* + *Glomus fasciculatum*
T_9: *Azotobacter chroococcum* + *Pseudomonas fluorescens*
T_{10}: *Azotobacter chroococcum* + *Trichoderma viride*
T_{11}: *Bacillus megaterium* + *Glomus fasciculatum*
T_{12}: *Bacillus megaterium* + *Pseudomonas fluorescens*
T_{13}: *Bacillus megaterium* + *Trichoderma viride*
T_{14}: *Pseudomonas fluorescens* + *Trichoderma viride*
T_{15}: *Glomus fasciculatum* + *Trichoderma viride*

For enumeration of PGPR microbial population, soil sample was collected carefully from the rhizosphere of cashew seedlings at different intervals and population density was determined by soil dilution plate method. Ten grams of the pooled soil sample of each treatment was collected from different replications. Each ten grams of soil was mixed in 90 ml sterilized water blank and mixed thoroughly to give 10^{-1} dilution. Subsequent dilutions up to 10^{-6} were made by transferring 1ml to 9 ml water blanks. For enumeration of *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas fluorescens* and *Trichoderma viride*, different dilutions were selected. One ml of suspension from each dilution was transferred aseptically into sterile petriplates. Fifteen ml. of appropriate medium was poured into plates and gently rotated in clockwise and anticlock wise direction to let the suspension distribute uniformly in the medium. Three replications were maintained for each dilution. The plates were incubated at 27±1°C for 5 days and colonies were counted on a colony counter and population was estimated and expressed as CFU per gram dry weight of soil (Johnson and Curl, 1972) [5].

The population of above said organisms was estimated at 30, 60 days after sowing and 30, 60 day after grafting (Note: During the estimation of *Bacillus* spp., the soil suspension was subjected to simmering for 10 minutes).

Procedure for enumeration of VAM spores

Fifty grams of rhizosphere soil was suspended in 500 ml of water and shaken thoroughly. After the sedimentation of course sand, the suspension was decanted over a series of test sieves (a sieve set with 1mm pore size 350µm, 250 µm, 180 µm and 45µm arrange in the descending order). Then the content of 108µm and 45µm sieve were carefully washed into a conical flask it was shaken and allowed to settle for 30 sec and spores were trapped on a nylon mesh of 40 µm placed on a sieve. The nylon mesh was then transferred on to a glass petri plate the number of spores was then counted under stereomicroscope and expressed as spore number/ 50g dry soil.

Results and discussion

Maximum population of *Azotobacter* spp. per gram soil at 30, 60 DAS and 30, 60 DAG was obtained in the treatments receiving *Azotobacter chroococcum* alone or with other PGPR microorganisms respectively as compared to *Azotobacter* spp. uninoculated treatments. Similar findings were reported by Lakshmipathy *et al*., (2000) [7] in cashew by inoculation of *Glomus fasciculatum*. Maximum population of *Azotobacter* in the inoculated treatments was obtained by Oblisami *et al*., (1985) [8]. With respect to population density of *Bacillus* spp also, the treatment which received *Bacillus megaterium* alone followed by the treatments which received *Azotobacter chroococcum* with *Bacillus megaterium* and *Bacillus megaterium* with *Psuedomonas fluorescens* recorded maximum population. The treatments which received *Bacillus megaterium* alone or *Bacillus megaterium* with other PGPR microorganisms gave higher population density of *Bacillus* spp. than *Bacillus megaterium* uninoculated treatments. In case of *Psuedomonas* spp also, similar trend was noticed. Maximum population density of *Psuedomonas* spp. was observed in the treatment receiving *Psuedomonas fluorescens* alone at all the intervals followed by the treatment receiving *Azotobacter chroococcum* with *Psuedomonas fluorescens* and *Psuedomonas fluorescens* with *Trichoderma viride*. All the treatments which received *Psuedomonas fluorescens* alone or *Psuedomonas fluorescens* with other PGPR microorganisms recorded higher population density of *Psuedomonas* spp than *Psuedomonas fluorescens* uninoculated treatments. Calvet *et al*., (1989) [3] obtained higher population density of bacteria and N_2 fixers in the plants colonized by *Glomus fasciculatum*. This is in accordance with the results of the present study. Maximum population density of *Trichoderma* spp. at different intervals of estimation was obtained in the treatment receiving *Trichoderma viride* alone. Treatments receiving *Trichoderma viride* alone or *Trichoderma viride* with other PGPR microorganisms recorded maximum *Trichoderma* spp. population as compared to *Trichoderma viride* uninoculated treatments. Prasad and Rangeswaran (2000) [9] obtained higher population of *Trichoderma* spp. in chickpea rhizosphere by inoculation of granular formulation of *Trichoderma harzianum*. In the present study also, maximum population of PGPR microorganism was obtained in the inoculated treatment. This suggests better survival of PGPR microorganisms through growth and multiplication in cashew rhizosphere. Another observation made in the present study was the increase in the population density of inoculated PGPR microorganisms upto 75 DAS and a decrease thereafter. Similar finding was noticed by Kerni and Gupta, (1986) [6] in mango seedlings by application of *Azotobacter*.

The highest VAM spore number per 50g soil was obtained in the treatment receiving *Bacillus megaterium* with *Glomus fasciculatum*. Treatment which received *Glomus fasciculatum* alone or *Glomus fasciculatum* with PGPR microorganism recorded higher VAM spore density than *Glomus fasciculatum* uninoculated control. These results clearly indicated the proliferation of VAM fungi, when it was coinoculated with other PGPR microorganisms. It might be due to synergistic effect. These findings uphold the views of Praveen Kumar (2003) [10] who reported such increased population of VAM in the rhizosphere of Gherkin due to introduced microorganisms and Govind Rao *et al*., (1989) [4] who found 100 percent root colonization in *Mentha revensis* by coinoculation.

Table 1: Effect of application of PGPRM on population density of *Azotobacter* spp. in the rhizosphere of cashew seedlings under poly house condition.

Treatments	*Azotobacter* spp. (No. of CFU x 10^4 / g soil)			
	30 Das	60 Das	30 Dag	60 Dag
T1 – Control	15.50 ^{d}	17.50 ^{b}	20.00 ^{cde}	20.50 ^{bcd}
T2 - *Azotobacter chroococcum*	29.00 ^{a}	30.00 ^{a}	31.00 ^{a}	28.50 ^{a}
T3 - *Bacillus megaterium*	19.50 ^{bcd}	20.00 ^{b}	16.00 ^{e}	22.00 ^{abcd}
T4- *Glomus fasciculatum*	17.50 ^{cd}	19.50 ^{b}	18.00 ^{de}	20.50 ^{bcd}
T5 - *Pseudomonas fluorescens*	19.50 ^{bcd}	20.00 ^{b}	19.50 ^{de}	20.50 ^{bcd}
T6 - *Trichoderma viride*	19.50 ^{bcd}	20.50 ^{b}	19.00 ^{bcd}	17.50 ^{d}
T7 - *Azotobacter chroococcum* + *Bacillus megaterium*	26.00 ^{abc}	29.50 ^{a}	24.00 ^{ab}	26.50 ^{ab}
T8 - *Azotobacter chroococcum* + *Glomus fasciculatum*	27.50 ^{ab}	29.50 ^{a}	30.00 ^{ab}	25.50 ^{abc}
T9 - *Azotobacter chroococcum* + *Pseudomonas fluorescens*	28.50 ^{a}	30.00 ^{a}	30.00 ^{ab}	28.00 ^{a}
T10 - *Azotobacter chroococcum* + *Trichoderma viride*	28.50 ^{a}	29.50 ^{a}	26.50 ^{abc}	27.00 ^{ab}
T11 - *Bacillus megaterium* + *Glomus fasciculatum*	19.50 ^{bcd}	22.00 ^{b}	19.00 ^{de}	20.50 ^{bcd}
T12 - *Bacillus megaterium* + *Pseudomonas fluorescens*	17.00 ^{d}	22.50 ^{b}	22.50 ^{cde}	16.00 ^{d}
T13 - *Bacillus megaterium* + *Trichoderma viride*	18.50 ^{cd}	19.00 ^{b}	22.50 ^{cde}	17.00 ^{d}
T14 - *Pseudomonas fluorescens* + *Trichoderma viride*	17.50 ^{cd}	18.50 ^{b}	21.00 ^{cde}	18.50 ^{cd}
T15 - *Glomus fasciculatum* + *Trichoderma viride*	18.00 ^{cd}	18.50 ^{b}	19.00 ^{cde}	15.50 ^{d}
CD (P = 0.05)	2.36	1.80	1.83	2.00

Note: PGPRM = Plant Growth Promoting Rhizo microorganisms.
DAS = days after sowing. DAG = Days After Grafting.

Table 2: Effect of inoculation of PGPRM on population density of *Bacillus* spp. in the rhizosphere of cashew seedlings under poly house condition.

Treatments	*Bacillus* spp. (No. of CFU x 10^6/ g soil)			
	30 Das	60 Das	30 Dag	60 Dag
T1 – Control	16.00 ^{c}	17.50 ^{b}	17.50 ^{b}	15.00 ^{d}
T2 - *Azotobacter chroococcum*	18.00 ^{abc}	18.50 ^{b}	18.50 ^{ab}	17.50 ^{cd}
T3 - *Bacillus megaterium*	28.50 ^{a}	30.00 ^{a}	31.50 ^{a}	31.00 ^{a}
T4- *Glomus fasciculatum*	18.50 ^{abc}	19.00 ^{b}	18.50 ^{ab}	18.50 ^{bcd}
T5 - *Pseudomonas fluorescens*	17.00 ^{bc}	19.00 ^{b}	20.00 ^{ab}	20.00 ^{abcd}
T6 - *Trichoderma viride*	19.50 ^{abc}	21.00 ^{ab}	21.00 ^{ab}	21.00 ^{abcd}
T7 - *Azotobacter chroococcum* + *Bacillus megaterium*	27.00 ^{abc}	29.50 ^{a}	31.00 ^{a}	31.00 ^{a}
T8 - *Azotobacter chroococcum* + *Glomus fasciculatum*	20.00 ^{abc}	21.00 ^{ab}	22.00 ^{ab}	21.50 ^{abcd}
T9 - *Azotobacter chroococcum* + *Pseudomonas fluorescens*	20.00 ^{abc}	21.50 ^{ab}	20.50 ^{ab}	18.50 ^{bcd}
T10 - *Azotobacter chroococcum* + *Trichoderma viride*	19.50 ^{abc}	20.50 ^{ab}	21.00 ^{ab}	21.00 ^{abcd}
T11 - *Bacillus megaterium* + *Glomus fasciculatum*	26.50 ^{abc}	28.50 ^{a}	30.00 ^{ab}	29.00 ^{abc}
T12 - *Bacillus megaterium* + *Pseudomonas fluorescens*	27.00 ^{abc}	29.50 ^{a}	30.00 ^{ab}	30.00 ^{ab}
T13 - *Bacillus megaterium* + *Trichoderma viride*	27.50 ^{ab}	29.50 ^{a}	27.00 ^{ab}	22.50 ^{abcd}
T14 - *Pseudomonas fluorescens* + *Trichoderma viride*	20.00 ^{abc}	21.00 ^{ab}	20.50 ^{ab}	20.50 ^{abcd}
T15 - *Glomus fasciculatum* + *Trichoderma viride*	19.50 ^{abc}	20.50 ^{ab}	18.50 ^{ab}	18.50 ^{bcd}
CD (P = 0.05)	2.89	2.50	3.46	3.08

Note: PGPRM = Plant Growth Promoting Rhizo microorganisms.
DAS = days after sowing. DAG = Days After Grafting.

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