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RESEARCH PAPER

Effect of an introduced beneficial inocula of native and exotic bioagents on microbial and dominant fungal population in pigeonpea rhizosphere of calciorthent soil

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Abstract : The study was carried out to identify the impact of native and exotic bioagents on microbial and dominant fungal population under pigeonpea cropping system of calciorthent soil. The effect of *Rhizobium* alongwith native and exotic strains of PSB and PGPR during the harvest season 2008-2009 was observed. The response of biological treatment on microbial and dominant fungal populations was compared in a field experiment. The soil samples were collected from the rhizosphere of pigeonpea crop at monthly intervals throughout the growth period, microbes and dominant fungi were enumerated through dilution plate technique on their respective media. The result indicates that the change in fungal population was not following any definite trend. Variation in total bacterial population was much wider in treatments where exotic beneficial agents were used in comparison to native bioagents. The total PGPR population was much stabilized under different treatments in comparison to total fungal and bacterial populations. Higher combinations of biological agents had suppressed the *Penicillium* and *Cladosporium* population than those of individual combinations. Though, the population of *Aspergillus* remained higher in all the treatments but was suppressive in different bioagent's combinations to control. Exotically, incorporated biogents did not influence much the population of *Trichoderma* as their distributions was at par with control. Effect of native bioagents on *Geotrichum* population was negligible as their values were almost identical to those of untreated soil whereas it was differential in treatments with exotic bioagents.

Key Words : Bioagents, Dominant fungi, Pigeonpea, Rhizosphere

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INTRODUCTION

In the agriculture production system, the need of the hour is to maximise the efficiency of each external inputs by using the judicious combination of biological entities for sustainable agricultural production. Beneficial plant-microbes interactions in the rhizosphere are the determinants of plant health and soil fertility (Jefferies *et al*., 2003). In the calciorthent soil, the pH is above 8.0, and most of the mineral P is in the form of poorly soluble calcium mineral phosphate (CaP) due to their buffering capacity (Ae *et al*., 1991).

Pigeonpea [*Cajans cajan* (L.) Millspaugh] is a deep rooted and drought tolerant crop (Troedson *et al.,* 1990), can fix atmospheric nitrogen up to 40 kg^{-1}, and its root helps in releasing soil bound phosphorus to make it available for plant growth. Soil micro-organisms that mobilize phosphorus (P) are important in providing this nutrient to plants (Patel *et al*., 2008). Micro-organisms that dissolve poorly soluble CaPs are termed as mineral phosphate solublizer (MPS) (Dobbelaere *et al*., 2003 and Goldstein *et al*., 2003). A number of species of bacteria are able to solublize phosphorus *in-vitro* and some of them can moblize P in plants (Antoun *et al*., 1998 and Piex *et al*., 2000). Phosphate solublizing microorganisms (PSM) convert these insoluble phosphates into soluble forms through the process of acidification, chelation, exchange reactions and production of gluconic acid (Rodriguez *et al.*,

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2004 and Chung *et al*., 2005). Whereas, PGPR stimulate plant growth either by direct or indirect mechanism which is broadly based on production of enzymes and elicitors. *Pseudomonads* are the most widely used and studied group of gram negative bacteria that solublize phosphate and improve the growth of plant. Much attention has been given to both the phytopathogens inhibitory and plant growth promotion (PGP) activities of rhizobacteria from agricultural plants (Raupach and Kloepper, 2000 and Barka *et al.*, 2000), but scanty work has so far been conducted to assess the comparative impact of incorporation of native and exotic bioagents on shift in total population of resident soil microbes.

An understanding of population dynamics of soil biological parameters including fungal dominance under influence of native and exotic bioagents in pigeonpea cropping system is very critical, as microbial communities inhabiting rhizosphere soil can be affected by root architect, root age and plant age (Gomes *et al*., 2001; Kuske *et al.*, 2002; and Nicole *et al*., 2003) but the complex interaction between soil type, plant species and root zone location probably is the main factor (Marschener *et al*., 2001). Therefore, the aim of this study was to document the consequences of use of native and exotic bioagents in pigeonpea cropping system from area of the study. So as to explore the new possibility to select the native bioagents which are either at par or superior to exotic bioagents in promoting the soil microbiological parameter, and reduce the cost of transportation of nationally accepted bioagents along with uncertainty regarding their adaptation and performance.

MATERIALS AND METHODS

Site description and treatments:

The study was conducted at Research Farm, T.C.A., Dholi campus (25° – 39'N; 85° – 40' E) of Rajendra Agricultural University, Bihar in the north-eastern part of India during 2008-10. The soil is calciorthent (Free CaCo3 33 per cent), with pH (8.1), EC (0.14) and organic carbon (0.34 per cent). The experiment was laid out in Randomised Block Design (RBD) with plot size of 4.8 X 5 m² with five replications. The seed was treated with *Rhizobium* (R) alongwith phosphorus solublizing bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) of native (L) and exotic (E) isolates under following treatment combinations.

T_1 = *Rhizobium* + PSB (L)
T_2 = *Rhizobium* + PSB (L) + PGPR (L)
T_3 = *Rhizobium* + PSB(E) + PGPR (E)
T_4 = *Rhizobium* + PGPR (E)
T_5 = Control

Sample collection and isolation:

The soil samples were collected from 10 points along a diagonal transit from each block of different treatments pooled separately. The total soil samples may yield around 25 in number as one sample per block from 5 blocks of five treatments was taken into consideration.

Fungal isolation:

Dilution plate method was used to estimate total fungal count on two different media including MEA (Malt extract agar) and Rose Bengal agar. One gram of composite soil sample of different treatments was used separately by placing 9 ml sterilized distilled water in a sterilized universal tube. The tubes were capped tightly and shook for 30 min for the preparation of first ($=10^{-1}$) dilution. Similarly, 2nd dilution ($=10^{-2}$ dilution), 3rd and 4th dilutions were prepared by taking 1 ml from respective dilutions and added it to a fresh 9 ml of diluents. Plates with different media were added with 0.1 ml ($=10^{-4}$) of suspension and kept at 22 ± 2°C for 15 days. The colonies were transferred to test tubes with PDA. Macroscopic examination of fungal colonies that resembled *Aspergillus* and *Penicillium* species were subcultured on malt extract agar (MEA) for further identification. *Fusarium* was subcultured on dichloran- chloramphenicol-peptone agar (DCPA), *Cladosporium* on potato dextrose agar (PDA) and *Geotrichum* on potato dextrose-novobiocin-agar medium. The fungi were identified with the help of available literature (Thom and Raper, 1945; Ellis, 1971; Barnett and Hunter, 1972 and Nelson *et al.*, 1983).

Bacterial and PGPR isolation :

One gram of soil near the root surface was collected and transferred to a 250 ml conical flask containing 100 ml of sterile water followed by shaking for 15 min. in a shaker, different dilutions were prepared. One millilitre of each 10^{-5} and 10^{-6} dilution was pipetted into sterile Petri-dishes containing prepared (Hi-media Pvt. Ltd.) media *viz*., Pseudomonas agar (flourescein) and Nutrient agar in triplicate, respectively. These Petri-dishes were incubated in bacteriological incubator at 28± 1°C for 24 hrs.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Total fungal population:

The variation in total fungal population during different months of the crop growth, as well as among the different treatments was depicted in Fig 1. From August to November, there was an increase in fungal population and decrease was recorded thereafter. In *Rhizobium* + PSB (L) treatment, the trend in fungal population was increasing during vegetative phase and started decreasing with the onset of reproductive phase of the crop. After 30 days of crop growth, there was

sharp decline (3.09 x 10^4 cfug^{-1} soil) in fungal population which further resumed very soon and remained almost identical up to 60 days in *Rhizobium* + PGPR + PSB (L). Fungal population was highest (4.16 x 10^4 cfug^{-1} soil) in the month of August and lowest (2.51x10^4cfug^{-1}soil) in the month of March was recorded in *Rhizobium* + PGPR (E) + PSB (E). Fluctuation in total fungal population during the first four months of the crop growth was quite wide which became constant afterwards in *Rhizobium* + PGPR (E). The result indicates that the change in fungal population was not following any definite trend. It was probably due to the differential physical, chemical and biological properties of the rhizospheric soil. It is well established that the microbial life only occupies a minor volume of soil being localised in hot spots such as the rhizosphere soil (Nannipieri *et al*., 2003), where microflora has a continuous access to a flow of low and high molecular weight organic substrates derived from root. This flow, together with specific physical, chemical and biological factors, can markedly affect microbial activity and community structure of the rhizosphere soil (Sorensen, 1977 and Brimecombe *et al*., 2001).

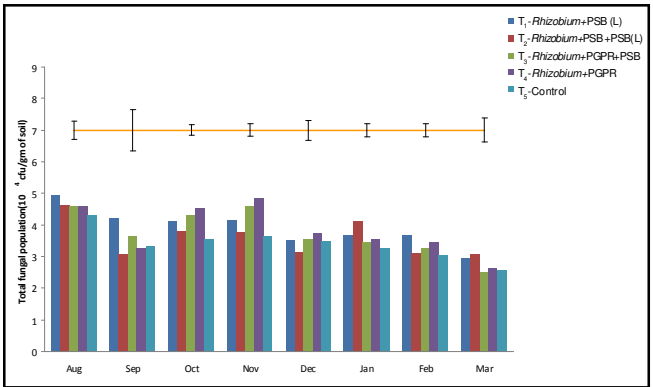

Fig. 1 : Variation in total fungal population under pigeonpea cropping system. Significant difference (C.D. at 5%) is indicated by bar line

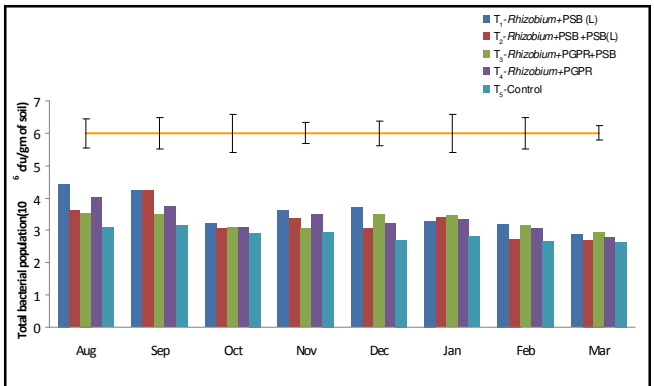

Fig. 2 : Total bacterial population (10^6 cfu/g of soil) under pigeonpea cropping system. Significant difference (C.D. at 5%) is indicated by bar line

Total bacterial population:

Bacterial population was highest (4.43 x 10^6 cfug^{-1} soil) in *Rhizobium* + PSB (L) during the entire growth period of the crop in comparison to other treatments, except in September and January. Marked variation in total bacterial population was observed under *Rhizobium* + PGPR (L) + PSB (L) during the first four months of the crop period followed by constant decline from January onwards. Under *Rhizobium* + PGPR + PSB (E), highest (3.55 x 10^6 cfug^{-1} soil) population was recorded in the month of August and lowest (2.93 x 10^6 cfug^{-1} soil) in March. In *Rhizobium* + PSB (L), the effect of biological treatment was found promotive on bacterial population during August and September while sharp decline was observed in October, which resumed their population thereafter with decreasing trends till the harvest of the crop. Total bacterial population was lowest under control in comparison to other treatments during the entire growth period of crop. In the month of October 2008, effect of treatments was negligible on bacterial population as the values of all the treatments were at par. Variation in total bacterial population was much wider in treatments where exotic beneficial agents were used in comparison to native bioagents, the reason ascribed to such changes may be the differential release of different carbon sources like glucose, oxalic acid and glutamic acid which are readily utilised by the native bioagents than the exotic ones. According to Fontaine *et al.* (2003) addition of easily available organic C can stimulate the growth of r-strategist and the successive growth of k- strategist is responsible for the degradation of recalcitrant organic matter. Another hypothesis explains the positive priming effect due to the increased turnover of native microbial biomass (Chander and Joergensen, 2001 and De Nobili *et al.,* 2001) whereas Kuzyakov *et al.* (2000) suggested that the activation of soil micro-organisms by the addition of the easily available organic C, increased enzyme synthesis with higher degradation of soil organic carbon.

Total PGPR population:

Results presented in Fig.3 showed the variation in total PGPR population under different biological treatments. Under *Rhizobium* + PGPR + PSB (L), the highest (2.51, 2.70 and 2.37x10^4 cfug^{-1} soil) PGPR population was noticed during August, September and November, respectively, compared to other treatments. The highest (2.37 x10^4cfug^{-1}soil) PGPR population was observed in the month of September and lowest (1.82 x10^4 cfug^{-1} soil) in November under *Rhizobium* + PSB (L) treatment. Continuous decrease in total PGPR population from the start of the crop growth was recorded, with exception in the month of November and December where fluctuations were marked in *Rhizobium* + PGPR (E) + PSB (E) treatment. There was not much variation in PGPR population throughout the growth period in *Rhizobium* + PSB (E). The population of PGPR remained lower in control compared to

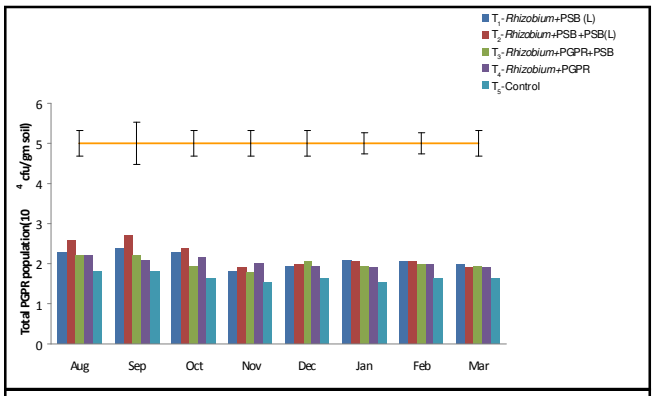

Fig. 3 : Variation in total PGPR population (10^4 cfu/g of soil) in pigeonpea cropping system. Significant difference (C.D. at 5%) is indicated by bar line

other treatments, but decline was much noticeable in November and January. The month of November showed detrimental effect on the PGPR population in all the treatments. Result indicates that the total PGPR population was much stabilized under different treatments in comparison to total fungal and bacterial populations. This might be due to the ability of the PGPR to get easily stabilized in soil ecosystem, as they are known to possess several activities like growth promotion, nutrient recycling as well as reducing the plant pathogen's population through releasing antimicrobial substances. Renella *et al*. (2005) reported that different root exudates were mineralised to different extents and had different stimulatory effect on microbial growth and on hydrolase activities, mostly localised in the rhizosphere zone. The rapid increase in the alkaline phosphatase activity could be considered as an indirect evidence of the important role of rhizobacteria in the synthesis of this enzyme in the rhizosphere (Tarafdar and Jungk, 1987).

Dominant mycoflora:

Fig. 4 shows that the most frequent genera isolated from pigeonpea rhizosphere were *Aspergillus, Cladosporium, Fusarium, Geotrichum, Penicillium* and *Trichoderma.* The genera of *Aspergillus* was isolated 29 per cent in the soil samples of *Rhizobium* + PGPR (L) + PSB (L), but its frequency increased up to 37 per cent in control. Frequency of *Aspergillus* was almost identical (33 per cent) in *Rhizobium* + PSB (E) and *Rhizobium* + PGPR (E) + PSB (E) treatments. The second most frequently isolated genus was *Penicillium*, with isolation frequency of 12 to 22 per cent under different treatments. Lowest frequency of 12 per cent and 13 per cent was observed in *Rhizobium* + PGPR (L) + PSB (L) and *Rhizobium* + PGPR (E) + PSB (E), respectively, whereas highest (22 per cent) in *Rhizobium* + PSB (L). The genus *Cladosporium* showed an isolation frequency between 2 to 6 per cent. The *Fusarium* genus was isolated in low (3 to8 per cent) frequency. Similarly, *Trichoderma* genus was not much affected by different treatment combinations as its isolation percentage remained identical (4 per cent) in control along with *Rhizobium* + PGPR (E) + PSB (E) and *Rhizobium* + PGPR (E). Frequency of *Geotrichum* genus was lowest (3 per cent) in *Rhizobium* + PGPR (E) + PSB (E) while highest (9 per cent) in *Rhizobium* + PGPR (E). The fungi imperfecti are the major group of fungi found in soil due to their ability to produce dormant structure like conidia, clamydospore, sclerotia etc. The genera isolated in this work were similar to an isolation pattern found in pre-harvest maize ecosystem (Nesci *et al.*, 2006).

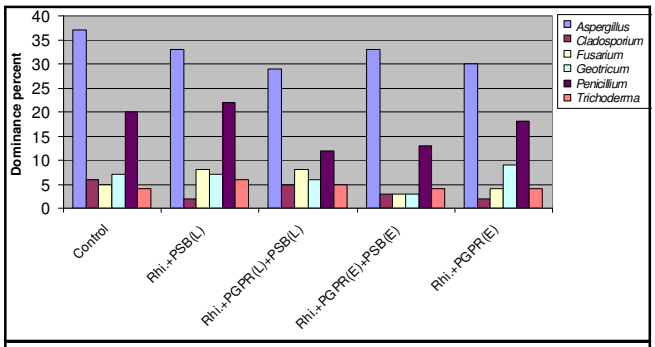

Fig. 4 : Dominant fungal genera under different biological treatments

It is evident from the Fig. 4 that more combinations of biological agents had suppressed the *Penicillium* and *Cladosporium* population than those of individual combinations. Though, the population of *Aspergillus* remained higher in all the treatments but was suppressive in different bioagents combinations to control. Possibly, higher interactions of enzymatically active genera of *Aspergillus, Penicillium* and *Cladosporium* with *Pseudomonas* spp. in soil may subject to decrease in their population. Naseby and Lynch (1998) observed that the genetically modified strain of *Pseudomonas fluorescens* increased the urease and chitobiosidase activity of rhizospheric soil and decrease alkaline phosphatase, which was attributed to a displacement of the rhizospheric community producing enzyme. However, exotically incorporated biogents did not influence much the population of *Trichoderma* as their distributions was at par with control, which signifies that *Trichoderma* genus has higher adaptability and get least affected by external disturbance, as they secrete various toxins. Harman *et al.*(2004) reviewed that the *Trichoderma* spp. are free-living fungi that are highly interactive in root and soil ecosystem, as they produce various antibiotic compounds and also compete with other soil organisms for space and nutrition. Effect of native biogents on *Geotrichum* population was negligible as their values were almost identical to those of untreated soil whereas, it was differential in treatments with exotic bioagents. It might be due to the differential enzyme and antifungal compounds secreted by the native and exotic bioagent. *P. fluorescens*

F113, which normally produce antifungal 2,4-diacetylphloroglucinol (DAPG), increased alkaline phosphatase, phosphodiesterase and arylsulfatase activities of pea rhizosphere whereas the other inocula reduced enzyme activities compared to the control (without bacterial inoculation) (Naseby and Lynch, 1998).

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