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Rhizosphere and non-rhizosphere microbial population dynamics and their effect on wilt causing pathogen of pigeonpea

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Abstract- Rhizosphere of healthy pigeonpea was heavily colonized by a number of microbes of which *Gliocladium virens* and *Penicillium* sp. were dominant. In contrast *Fusarium udum* dominated in the rhizosphere of diseased plant but there were mixed population of *G.virens* and *Penicillium* sp. in non-rhizosphere soil. Interestingly the fungi known for antimicrobial or antagonistic properties was high in the rhizosphere of healthy pigeonpea plants. Resident microorganisms were studied against *F. udum* causing wilt disease of pigeonpea *in vitro*, as well as *in vivo*. *Gliocladium virens, Trichoderma viride, Aspergillus niger, Penicillium citrinum,* which were found to be most potent ones in inhibiting the radial growth of the test pathogen were used in field. Minimum incidence of the wilt disease was observed in seeds treated with *G. virens*. Details of microbial population of both rhizosphere and non-rhizosphere and their interaction is presented in this paper.

Index Terms- Antagonist , *Fusarium udum,* Microbial population, Non- Rhizosphere, Rhizosphere

I. INTRODUCTION

Rhizosphere is characterized by greater microbial activity where many micro flora along with their harmful and beneficial activities are present. It is the site for harmful and beneficial activities where many key interactions take place between microbes and plant. Rhizosphere of disease as well as healthy plants harbours several fungi and bacteria [7]. Among the soil micro flora few of them may be beneficial antagonist. *Trichoderma viride* and *Aspergillus niger* as a part of micro flora of wilt resistant cultivar while susceptible cultivar showed a predominance of *Fusarium udum* and other *Fusarium* spp. during all the stages of plant growth [18]. This disease can attack at any stage of the crop. The disease causes complete yield loss when it occurs at pre-pod stage. Wilt disease cause an estimated loss of US$36 million in India and $5million in eastern Africa [5]. The present investigation was undertaken to find out the microbial population dynamic of rhizosphere and non- rhizosphere soil of pigeonpea in agro climatic conditions of Manipur and their effect against *F. udum*, the pathogen causing wilt disease of pigeonpea.

II. MATERIALS AND METHODS

Soil samples were collected from rhizosphere and non-rhizosphere of healthy and diseased plants of locally cultivated pigeonpea during the cropping season (May to Nov.,2009) from farmer's field at Kanglatongbi, Senapati District, Manipur, located at 23.83^0N and 25.68^0N latitude and 93.03^0E and 94.78^0E longitude . Five plants were pulled out and soil attached with the complete intact roots was collected and kept separately as healthy and diseased samples. Non- rhizosphere soil was collected from in between space of two rows of the pigeonpea field. Thus, five samples were collected together for composite sample. Finally one part of soil was taken out from composite sample for analysis [7].

Total fungi and total bacteria were isolated by dilution plate technique from 1g dried soil of rhizosphere and non- rhizosphere. Serial dilution of $1:10^3$ was prepared in sterilized water and plated on peptone dextrose rose Bengal agar medium for fungi. Serial dilution of $1:10^6$ was prepared and plated on soil extract agar medium for bacteria. One ml of soil suspension placed in each sterilized petriplate and 20 ml of cooled melted medium was poured in the same plate and gently rotated horizontally to get uniform distribution of the suspension in medium.These plates were incubated at 28± 1^oC for four days in three replications. Identification of the fungal cultures was done by using relevant literature and keys. Bacterial cultures were sent to IMTECH,Chandigarh for identification.Total number of fungal and bacterial colonies were counted and calculated in colony forming units per gram of soil (cfu g^{-1}).

Screening for antagonism between the microorganisms isolated from the rhizosphere viz, *Trichoderma viride, Aspergillus niger, Gliocladium virens Trichoderma harziamum, Penicillium citrinum* were done by dual culture technique [15]. The potato dextrose agar (PDA) medium in culture plates was simultaneously seeded with actively growing 3mm mycelial blocks of test pathogen and the antagonist isolates. Four days old *F. udum* block was seeded in the centre, where as three blocks of individuals antagonists were seeded at 4 cm equidistant point near the periphery from the centre and incubated at 28± 1^oC. Three replications of each isolates including a control i.e., without inoculation of the antagonists were maintained. For bacteria screening was done by filter paper disc method [13]. The plates were seeded and incubated as described above.

After 6 days incubations the percent inhibition in growth of pathogen was calculated by the formula:

$$\% \text{ inhibition} = \frac{r1 - r2}{r1} \times 100$$

Where, r_1= radial growth of *Fusarium* in control

r_2 = radial growth of *Fusarium* in dual inoculation

Interaction of antagonist fungi were noted every 24 hrs for 10 consecutive days. The type of reaction was noted and scored on [1].

Field application: Field experiment in randomized block design was carried out for two years (2010 and 2011) in wilt sick farmer's field located at Imphal West District, Manipur on a wilt susceptible local variety of pigeonpea. The sub treatments included different seed treatments and untreated seeds as control. Seed treatment was done following [19]. The seeds were treated with conidial suspension (1×10^{11} conidia per ml) for 24 hours of four antagonists which were found potent against the test pathogen. Untreated seeds were soaked for 12 hours in sterilized distilled water. The treated seeds were air dried for 7 days under ambient conditions before sowing. Plot size of 1×4 m^2 was prepared for each treatment and replicated thrice. The seeds of pigeonpea were sown in each plot at the rate of 40 seeds per plot. However, after germination, only 10 plants per plot were allowed to grow. The percent disease incidence was calculated by using the formula mentioned below after 60 days of sowing when the plants showed complete symptom of wilting.

Percent Disease incidence, (DI%)

$$= \frac{\text{Number of plants infected by the disease}}{\text{Total number of plants observed}} \times 100$$

Table 1a. Microbial population of rhizosphere soil of healthy pigeonpea plant

Microbial population*(fungi:cfug^{-1} soil×10^3 bacteria: cfug^{-1}soil×10^6)					
Microorganism Fungi	June	July	August	September	October
Aspergillus niger	-	-	6	2	-
Trichoderma viride	1	4	1	1	-
Gliocladium virens	9	14	13	3	-
Fusarium udum	-	-	2	3	1
Penicillium sp.	1	3	5	6	1
Curvularia sp.	1	1	-	-	-
Phoma sp.	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-
Rhizopus nigricans	-	1	1	-	1
Nigrospora sp.	-	-	-	2	-
Verticillium sp.	-	-	-	-	1
Trichoderma harzianum	-	-	-	2	2
Cladosporium sp	-	-	-	-	-
White sterile mycelium	2	-	2	1	1
Total Bacteria	14	23	30	20	7
Bacillus sp.MTCC 10514	-	1	1	1	2
B. licheniformis MTCC 10516	-	-	-	-	4
Pseudomonas montelli MTCC 10517	10	20	2	3	4
Total	10	21	3	4	10

*Each figure is a mean of three replications

Table 1b. Microbial population of rhizosphere soil of diseased pigeonpea plant

Microbial population*(fungi:cfug^{-1} soil×10^3 bacteria: cfug^{-1}soil×10^6)					
Microorganism Fungi	June	July	August	September	October
Aspergillus niger	-	3	-	-	-
Trichoderma viride	3	3	1	-	-
Gliocladium virens	1	2	-	-	-
Fusarium udum	6	9	12	4	2
Penicillium sp.	1	3	5	2	1
Curvularia sp.	1	3	-	-	-
Phoma sp.	-	-	-	3	4
Aspergillus flavus	-	-	3	-	-
Rhizopus nigricans	-	-	3	-	-
Nigrospora sp.	-	-	-	-	-
Verticillium sp.	-	-	-	-	1
Trichoderma harzianum	-	-	-	-	-
Cladosporium sp	-	1	-	-	-
White sterile mycelium	-	-	-	1	1
Total Bacteria	12	24	24	10	9
Bacillus sp.MTCC 10514	2	-	1	-	1
B. licheniformis MTCC 10516	-	-	1	1	1
Pseudomonas montelli MTCC 10517	5	15	2	-	2
Total	7	15	4	1	4

*Each figure is a mean of three replications

Table 1c. Microbial population of non- rhizosphere soil of pigeonpea

Microbial population*(fungi:cfug^{-1} soil×10^{3} bacteria: cfug^{-1}soil×10^{6})					
Microorganism	June	July	August	September	October
Fungi					
Aspergillus niger	-	-	5	-	-
Trichoderma viride	-	-	-	-	1
Gliocladium virens	9	10	4	4	1
Fusarium udum	-	-	-	2	1
Penicillium sp.	2	4	9	-	3
Curvularia sp.	-	-	5	-	-
Phoma sp.	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-
Rhizopus nigricans	-	1	1	-	1
Nigrospora sp.	-	-	-	-	1
Verticillium sp.	-	-	1	2	-
Trichoderma harzianum	-	-	-	-	-
Cladosporium sp	-	-	-	-	-
White sterile mycelium	-	-	-	-	-
Total	11	15	25	8	8
Bacteria					
Bacillus sp.MTCC 10514	2	-	-	-	-
B. licheniformis MTCC 10516	-	2	1	3	-
Pseudomonas montelli MTCC 10517	2	4	3	-	2
Total	4	6	4	3	2

*Each figure is a mean of three replications

Table 2. *In vitro* screening of microorganisms for antagonistic activity towards *Fusarium udum*

T1	*T2	T3	T4
Trichoderma viride + *F. udum*	1.3	78.3	2
Aspregillus niger + *F. udum*	1.9	68.3	1
Gliocladium virens + *F. udum*	3.9	35.0	1
Penicillium citrinum + *F. udum*	2.3	61.7	2
Trichoderma harzianum + *F. udum*	2.0	66.7	2
Bacillus sp MTCC 10514 . + *F. udum*	4.6	23.3	5
Bacillus licheniformis	3.9	35.0	4
Pseudomonas montelli MTCC 10517+ *F. udum*	4.7	21.7	5
CD@5%	0.27		

*Each figure is a mean of three replications

T1=Treatments, T2=Radial growth of *F. udum*,(cm) T3=Percent growth inhibition of *F. udum*, T4= Ranking on Bell's scale

Bell's scale (1-5)

1= Antagonist completely overgrew the pathogen and covered the entire medium surface.

2= Antagonist overgrew at least two-third of the medium surface.

3= Antagonist and the pathogen each colonized one-half of the medium surface (more than one-third and less than two thirds) and neither organism appeared to dominate the others.

4= The pathogen colonized at least two thirds of the medium surface and appeared with stand encroachment.

5= The pathogen completely overgrew the antagonist and occupy the entire medium-surface.

Table 3. Effect of bioagents on wilt disease of pigeonpea under field condition

Antagoists	Disease Incidence %		
	2010	2011	Mean
Gliocladium virens	67.8	69.9	68.9
Trichoderma viride	76.7	77.8	77.3
Aspergillus niger	72.2	73.3	72.8
Penicillium citrinum	84.4	96.7	90.5
Control	95.7	97.8	96.8

- Disease incidence presented above is the average values of occurrence of the disease

III. RESULT AND DISCUSSIONS

Total fungi : The analysis of rhizosphere and non-rhizosphere soil of pigeonpea showed that the colonies of *G. virens* were dominating in the rhizosphere of healthy pigeonpea plants (Table 1a). Colonies of *F. udum* appeared on all the soil samples. *F. udum* in the rhizosphere of diseased plant was 12×10^{3} cfug^{-1} which was higher than healthy plants and non- rhizosphere. The presence of *F. udum* in the healthy and non- rhizosphere soil is due to the planting of the crops in wilt sick field. The dominance of *F. udum* in the rhizosphere of diseased plant and presence of *F. udum* in healthy and non- rhizosphere soil was also earlier reported by [7]. Result presented herein (table 1a, 1b, 1c) indicated more microbial activity during early part of the growth of the plant. In general the fungal population was higher in rhizosphere than non- rhizosphere irrespective of healthy and diseased plant due to availability of nutrients released by the root exudates around the vicinity of root zone of pigeonpea. The fungi known for antagonistic activity such as *T. viride, A.niger, G. virens* was higher in the rhizosphere of healthy plants as compared to diseased and non- rhizosphere.

Total bacteria : Three types of bacterial colonies *Bacillus* sp.(MTCC 10514), (*Bacillus licheniformis*)(MTCC 10516),

Pseudomonas montelli (MTCC 10517) appeared on soil extract agar medium (table 1a, 1b,1c). The population of *P. montelli* was maximum in the rhizosphere of healthy, diseased and non-rhizosphere soil. However, maximum colonies (20×10^6 $cfug^{-1}$) were associated with healthy plant (table 1a). There is no clear trend of increasing or decreasing population of bacteria. However, the total number of bacteria was also high in rhizosphere of healthy plant as compared to diseased and non-rhizosphere as reported by [16].

Screening for antagonism

All the five fungal isolates tested were found to inhibit the growth of *F. udum*. Findings presented in(table 2) showed that the maximum inhibition of radial growth of *F. udum* was observed with the treatment of *T. viride* (78.3 %) followed by *A. niger* (68.3 %) and *T. harzianum* (66.6 %), *P. citrinum* (61.7%) and *G. virens* (35.0%). The percent growth inhibition of *G. virens* after 6 days was low but the interaction studies on Bell's scale (table 2) showed that *G. virens* and *A. niger* belonged to type 1 antagonist. Both of them completely overcolonized the pathogen after 10 days. Pathogen growth is completely restricted. The interaction of *Trichoderma* spp. With *F. udum* showed yellow pigmentation beneath overcolonized colony of the pathogen. The mycelium of both cultures comes in contact with each other at 3 days. They belonged to type 2 antagonists. Among the bacterial isolates *B. licheniformis* showed 35.0% inhibition of radial growth but belonged to type 4 antagonist. Others showed no antagonism. Potential antagonism of *Trichoderma* as evidenced by the results is due to competition, antibiosis and mycoparasitism [11] . Moreover these fungi produce antibiotics such as gliotoxin, viridin and cell wall degrading enzymes and also biologically active heat stable metabolites such as ethyl acetate. These substances are known to be involved in disease incidents suppression [10]. *A. niger, G. virens, P. citrinum, T. harzianum,* and species of *Bacillus* control soil- borne diseases [6]. *A. flavus, A. niger, and T. viride* amended in soil suppressed the growth of *F. oxysporum* f. sp. *ciceri* and exhibited strong fungistatic activity against germination of conidia of test pathogen [9].

Under field conditions, minimum disease incidence were observed with *G. virens* (68.9%) followed by *A. niger* (72.8%), *T. viride* (77.3%). *P. citrinum* (90.5%) (table 3). *G. virens* and *T. viride* have been recognized as the most effective antagonist for biological control of several plant pathogens by many investigators [2,3,4,17].

IV. CONCLUSION

The beneficial effects of the application of antagonistic microbes are well narrated. Our investigation also reported potentiality of the antagonistic fungus *G.virens* to incorporate in the integrated disease management for pigeonpea wilt. However further work is necessary to enhance the disease control capability of *G. virens*.

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