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Evaluation of Rhizobium and Biopesticides against Fusarium Wilt of Pigeonpea

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Abstract:
Rhizobium isolates from root nodules of pigeonpea and biopesticides viz. margosom (Azadirachtin 0.03%) and mixed formulations of Trichodermaviride, T. harzianum, Paecilomyceslilacinus (10^8 spores/g) were evaluated under in vitro and field conditions for testing their effectiveness against wilt disease of pigeonpea caused by Fusariumudum. Highest inhibition (76.92%) of mycelial growth was observed in plates cultured with mixed formulation of T.viride, T. harzianum, Paecilomyceslilacinus(10^8 spores/g). Inoculation of pigeonpea seeds with rhizobial isolates and biopesticides showed higher nodulation, increase in plant height and dry weight (biomass) over control. Plants raised from inoculated seeds showed reduced disease incidence and severity over control.

Keywords*: Biopesticides, Fusarium, Rhizobium, Pigeonpea, Wilt*

1. Introduction

Pigeonpea (*Cajanuscajan* (L.) Millsp.), belonging to the family Fabaceae has the highest yield potential among the pulses. In Manipur the commercial production of pigeonpea has been declined due to various factors. Among them, wilt disease caused by *Fusariumudum* Butler is one of the major constraints posing serious threats to its cultivation. Being a leguminous crop pigeonpea fixes atmospheric nitrogen through legume-rhizobium symbiosis which has been considered as one of the cheapest inputs of nitrogen in soil. The rhizobia infecting pigeonpea is taxonomically grouped with the cowpea miscellany group (Dahiya*etal*., 1980). The farmers cultivated the crop without input of any fertilizers and pesticides as a result the yield is very low. There is a need to develop suitable disease management practices that too economically feasible as well as ecofriendly for sustainable agriculture. The use of effective strains of Rhizobia and biopesticides against various diseases has been gaining interest in recent times due to health hazards and environmental pollution caused by fertilizers and agrochemicals. Not much work has been done on the management of wilt disease of pigeonpea in Manipur agroclimatic conditions. The effort has been made to manage the disease using Rhizobial isolates and biopesticides.

2. Materials and Methods

2.1. Isolation of Rhizobium

Rhizobium were isolated from the root nodules of pigeonpea following the method described by Saha and Haque (2005). A healthy, unbroken nodule wascut from the root of pigeonpea and washed repeatedly with tap water and surfaced sterilized for 2 minutes in 0.1% sodium hypochlorite solution and then 95% alcohol for 3 minutes. Each nodule was crushed in a small aliquot of sterile water with the help of a sterile glass rod and the milky fluid was streaked on yeast extract mannitol agar (YEMA) containing congo red. The plates were incubated at 28 ± 1^0c for five days. The mucoid or gummy colonies appearing on the plates were selected and plated on YEMA medium for purification. Isolates were sub cultured on YEMA medium for checking purity as done by Khan and Zaidi., (2002). The pure cultures were sent to IMTECH, Chandigarh for phenotypic characterization of the bacterium. The *Rhizobium* sp. was further tested *in vitro* conditions for testing their efficacy against *Fusariumudum* along with certain other biopesticides (viz. margosom& mixed formulation of *Trichodermaviride, T. harzianum, Paecilomyceslilacinus*) and also in field conditions to see their effect on nodulation, plant height ,biomass and wilt disease.

2.2. Fungus :Fusariumudum

Fusariumudum was isolated from the rhizosphere of wilted pigeonpea plants and pure cultures were maintained on potato dextrose agar (PDA) medium.

2.3.Rhizobium- Fusarium interaction *in vitro*

Antifungal activity of *Rhizobium* sp. against *F.udum* was done by spot plate method (Kaur and Seema, 2002). *Rhizobium* sp. was spread over the plate containing modified Martin medium. Fungal disc of *F.udum* in 4mm diameter was then placed in the center of the plate already inoculated with the *Rhizobium.* Fungal disc without *Rhizobium* serve as control. The plates were replicated thrice. Plates were then incubated at 28 ± 1^0c for 96 hours and observed the mycelial growth of tested pathogen.

2.4. Fusarium-biopesticides interaction *in vitro*

The growth inhibitory potential of biopesticides namely Margosom (Azadirachtin 0.03%) (Agri Life, Andhra Pradesh) and mixed formulation of *Trichodermaviride, T. harzianum, Paecilomyceslilacinus* (10^8 spores/g) (Biocon Pvt. Limited, Bangalore) on *F. udum*were determined following the method described by Dhingra and Sinclair (1995) . With the help of cork borer (4mm), agar discs were removed from each petriplate containing solidified potato dextrose agar media, one from the center and three equidistantly on its periphery. Two drops of margosom 5 ppm and mixed formulation of *T. viride, T.harzianum, P. lilacinus*(10^8 spores/g) were placed in each well. Test fungus was placed in the center. In control, the wells were filed with sterile distilled water and test fungus was similarly inoculated as above. Each plate was replicated thrice. The plates were incubated at 28 ± 1^0c for 6 days and mycelial growth of the tested pathogen was observed.

2.5. In vivo application of biofertilizer&biopesticides

A field trial was conducted with two local varieries of pigeonpea V_1 (early maturing variety) and V_2 (late maturing variety) at SenjamChirang 16 km from Imphal in a randomized block design with three replications for two consecutive years (2010 to 2011). Plot size use was 2×2 m^2. Pigeonpea seeds were treated with *Rhizobium*sp. and biopesticides were applied following Jayraj*et al*. (1999). *Rhizobium* cell suspension was prepared by culturing *Rhizobium* sp. in conical flask containing yeast extract mannitol broth and kept at incubator for three days at 28 ± 1^0c. The number of *Rhizobium* population was maintained through serial dilution and plating in YEMA. Treatments consist of:

- T_1: Margosom 5 ppm @ 100 ml/kg of seed
- T_2: *Rhizobium* cell suspension (1×10^8cfu/ml) @ 100 ml/kg of seed
- T_3: mixed formulation of *T. viride, T. harzianum, P. lilacinus* (10^8 spores/g) @ 4g/kg of seed
- T_4: dual application of margossom and *Rhizobium* sp.
- T_5: dual application of Rhizobium and commercial formulation of *T. viride, T. harzianum, P. lilacinus*(10^8 spores/g)
- control

For dual application, seeds were first soaked in Rhizobial cell suspension followed by biopesticides. The seeds were air dried for 30 minutes before sowing. Plots without any treatment were maintained as control plots. The mean no. of nodule per plant, plant height, dry weight g/plant after 60 days were recorded. Assessment of the disease parameters were made as mentioned above.

3. Statistical Analysis

The mean no. of nodule/plant, plant height, dry weight g/plant after 60 days were analyzed using one way ANOVA for testing statistical significance between the treatments.

The data obtained from *in vitro* test of *Rhizobium* was analyzed using t-test to test if there is any significant effect of *Rhizobium* sp. against the test pathogen. One way ANOVA was used for analysis of data obtained from *in vitro* test to test the effectiveness of biopesticides against test pathogen.

Two years data obtained from *in vivo* test were pooled for statistical analysis and subjected to two ways ANOVA for determining any significant differences among the treatments. Microsoft excel was used for the purpose.

4. Results and Discussion

4.1. In vitro interaction of Fusariumudum and biofertilizer and biopesticides

Rhizobium isolates are found to restrict the mycelial growth of test pathogen when compared with control. The percent inhibition of mycelial growth was 46.8% (table-1). The inhibition of mycelial growth could be due to the production of secondary metabolites having antimicrobial properties. The same observation has been reported (Kaur and Seema, 2002). Inhibition of mycelial growth of *Sclerotiumrolfsii* by *Rhizobium* isolates L-11was also reported by Gupta *et al.*, (2005). As such, our findings corroborates with their results.

The biopesticides namely margosom and mixed formulation of*Trichodermaviride, T. harzianum, Paecilomyceslilacinus* also restricted the growth of test pathogen. Among them, mixed formulation of *Trichodermaviride, T. harzianum, P. lilacinus* showed higher inhibition of mycelial growth (76.92%) (table-2). The inhibition activities towards the growth of mycelia could be for competition, antibiosis and lysis. The antagonistic potential of *Trichoderma* spp. and microorganisms were also reported earlier (Elad*et al*.,1980;

Radhakrishnan*et al*., 1995). *Trichoderma* spp. *Gliocladium* spp. and *Bacillus subtilis* were antagonistic to *Botryodiplodiatheobromae*by reducing the mycelial growth and conidial production *in vitro* due to production of fungitoxicmeta bolites (Radhakrishnan*et al*., 1995). The effectiveness of neem based formulations against several plant pathogens were also reported. Bunker and Mathur (2008) reported maximum growth inhibition and sporulation of *Exserohilumturcicum*, the leaf blight pathogen of sorghum with*neem* seed extract (NF1). As such, these biopesticides could be of use in controlling *F. udum* wilt in pigeonpea.

Effect of bofertilizer and biopesticides on plant height, nodulation and dry weight accumulation of pigeonpea

Data presented in (table-3) showed that plant height after 60 days of showing significantly higher over control when the seeds were inoculated with both *Rhizobium* and mixed formulation of *Trichodermaviride, T.harzianum, P. lilacinus* (V_1: 37.2cm ; V_2: 91.2cm). Number of nodules per plant were higher in seeds inoculated with combined treatment of *Rhizobium*and Margosom (V_1: 14 ; V_2 : 16) in comparison with control. Higher dry weight (biomass) was observed with combined inoculation of *Rhizobium* and *Trichodermaviride, T. harzianum, P. lilacinus* (V_1 : 2.7g/plant ; V_2 : 6.5g/plant) over control. The present observation indicated that pigeonpea plants raised from inoculated seeds with biofertilizer and biopesticides showed higher plant height, dry weight and nodulation over control. In a similar experiment on chickpea, Gupta *et al*., (2005) observed that plant height and number of nodules per plants were significantly increased when the plants were raised from seeds inoculated with *Trichodermaviride* (Tv_2, Tv_1 and Tv TNAU) and Rhizobium. However, Jayraj and Ramabadran (1999) found non-significant increase in nodulation of blackgram between*Rhizobium* alone and *Rhizobium +Trichoderma* treatments in pot culture and field experiment.

4.2. In vivotest of biofertilizerand biopesticides

Pooled data presented in (table-4) showed that minimum incidence (47.21%) and severity (43.5%) of wilt disease in early maturing local variety (V_1) of pigeonpea with *Trichodermaviride, T.harzianum, P. lilacinus* treated plots. In late maturing local variety (V_2), minimum disease incidence (55.17%) and severity (53.11%) was associated with plots treated with margosom (table-5). The present investigation clearly indicated the role of *Rhizobium* isolate and biopesticides in suppressing the incidence and severity of wilt of pigeonpea as against maximum disease incidence and severity in control plots. Bhatnagar (1995) demonstrated that commercial formulation of *Trichoderma* MTR-35 can effectively control pigeonpea wilt if amended in soil at 2.0 and 2.5% (w/w) concentrations. They also suggested that use of*Trichoderma* MTR-35 either alone or in combination with other components like host plant resistance or cultural practices may be an alternative and sustainable ways of disease control. Jayaraj*et al*., (1999) also reported significant reduction in the incidence of root rot of black gram with combined treatment of *Rhizobium* and *Trichoderma*. Similar findings were reported by Gupta *et al*., (2005) against wilt complex of chickpea using *Trichodermaviride* and *Rhizobium.*

The use of commercial formulations of neem for control of various plant diseases have been reported by many workers. Chandel and Tomar (2007) tested the commercial formulations of neem namely, Neemazal, Nimbicidine and Achook under field condition against gladiolus wilt caused by *Fusariumoxysporum* f. sp. *gladioli* Snyder and Hansen. They observed lowest disease incidence in Achook . Chandel and Tomar (2008) again tested the effectiveness of bioagents and neem formulations against wilt of carnation in *in vivo* condition. Among the neem formulations, they observed maximum disease control in Achook. Among bio-control agents tested, *Trichodermaharzianum* proved to be superior over *T. viride* and *Gliocladiumvirens* in controlling wilt.

Treatments	***Colony diameter (cm)**	**Percent growth inhibition**
Rhizobium and *F. udum*	1.33	46.80
Control	2.50	
$t_{0.05}$	**8.03	

Table 1.Quantificaation of in vitro interaction of Rhizobium and Fusariumudum
**mean of three replications; ** significant (p=0.05)*

Treatments	***Colony diameter (cm)**	**Percent growth inhibition**
Margosom and *F. udum*	2.33	64.15
T. viride, T.harzianum, P. lilacinus + F. udum	1.50	76.92
Control	6.50	
CD (p=0.05)	0.42	

Table 2. Quantification of in vitro interaction of biopesticides and Fusariumudum
**mean of three replications*

**Treatments	Plant height (cm)		No. of nodules/ plant		Dry weight (g/plant)	
	*V_1	*V_2	*V_1	*V_2	*V_1	*V_2
T_1	32.2	60.8	4.66	13.00	1.8	3.0
T_2	26.8	80.0	10.33	13.66	1.0	5.2
T_3	31.0	71.5	7.33	11.00	2.3	3.5
T_4	30.2	48.4	14.00	16.00	2.1	2.5
T_5	37.2	91.2	6.00	12.66	2.7	6.5
T_6	26.7	24.1	1.60	2.00	0.5	1.4
CD (p=0.05)	7.21		3.31		1.86	

Table 3. Effect of different biofertilizer and biopesticides on various attributes in pigeonpea varieties cultivated in wilt affected field after 60 days

V_1: early maturing variety; V_2: late maturing variety; data are mean of three replications

***T_1:Margosom (Azadirachtin 0.03%);T_2: Rhizobium (1×10^8 CFU/ml); T_3: mixed formulation of T. viride, T. harzianum, P. lilacinus (10^8 spores/g); T_4: T_1+T_2; T_5: T_2+T_3; T_6: Control*

**Treatments	*Disease incidence (%)		Mean value	*Disease severity (%)		Mean value	(%) disease control
	2009	2010		2009	2010		
T_1	55.00	55.00	55.00	50.80	50.19	50.50	43.27
T_2	68.33	71.67	70.00	63.64	67.35	65.50	27.80
T_3	45.56	48.86	47.21	41.31	45.68	43.50	51.30
T_4	60.00	62.22	61.11	57.64	59.14	58.39	36.97
T_5	52.78	54.45	53.62	49.57	52.57	51.02	44.69
Control	96.11	97.78	96.95	95.99	97.65	96.82	
CD (p=0.05)	19.34			17.28			

Table 4. Effect of biofertilizer and biopesticides on disease parameters of wilt disease in pigeonpea (V_1)

**average value of occurrence of disease for three months (i.e. starting from the month of August each year)*

***T_1:Margosom (Azadirachtin 0.03%);T_2: Rhizobium (1×10^8 CFU/ml); T_3: mixed formulation of T. viride, T. harzianum, P. lilacinus (10^8 spores/g); T_4: T_1+T_2; T_5: T_2+T_3*

**Treatments	*Disease incidence (%)		Mean value	*Disease severity (%)		Mean value	Percent disease control
	2009	**2010**		**2009**	**2010**		
T_1	52.67	57.67	55.17	51.50	54.71	53.11	43.03
T_2	65.67	69.33	67.50	62.70	67.11	64.91	30.30
T_3	50.67	62.67	56.67	47.81	60.34	54.08	41.48
T_4	62.00	64.67	63.34	60.52	61.48	61.00	34.59
T_5	61.00	63.67	62.34	58.92	61.82	60.37	35.63
Control	97.00	96.67	96.84	97.44	96.41	96.25	
CD ((p=0.05)	11.13			10.04			

Table 5 . Effect of biofertilizer and biopesticides on wilt disease parameters of wilt disease in pigeonpea (V_2)

**average value of occurrence of disease for five months (i.e. starting from the month of August each year)*

***T_1:Margosom (Azadirachtin 0.03%);T_2: Rhizobium (1×10^8 CFU/ml); T_3: mixed formulation of T. viride, T. harzianum, P. lilacinus (10^8 spores/g); T_4: T_1+T_2; T_5: T_2+T_3*

5. References

1. Bhatnagar, H. (1995). Use of commercial biofungicide against wilt of pigeonpea (Cajanuscajan (L.) Millsp.)Indian J. Plant Prot, 23 , 224-225.
2. Bunker. R. N. andMathur, K. (2008). Evaluation of neem based formulations and chemical fungicides for the management of sorghum leaf blight. Indian Phytopath, 61 (2) , 192-196.
3. Chandel, S. and Tomar, M. (2007). Evaluation of fungicides and biopesticides against Fusarium wilt of gladiolus. Indian Phytopath, 60 (1) , 115-117.
4. Chandel, S. and Tomar, M. (2008).Effectiveness of bioagents and neem formulations against Fusarium wilt of carnation.Indian Phytopath, 61 (2) , 152-154.
5. Dahiya, J. S. Khurana, A. L. and Dudeja, S. S. (1981). Evaluation of Cajanuscajan (L.) Rhizobia.In Proceedings of the International Workshop on Pigeonpeas, 15-19 Dec. 1980, ICRISAT Center, India, vol. 2.Patancheru, A. P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. pp. 373-379.

6. Gupta, S. B. Chowdhary, T. Thakur, K. P. and Thakur, M. P. (2005). Exploiting Rhizobium isolates from Lathyrus as biocontrol agent against legume rot pathogen Sclerotiumrolfsii. J.Mycol. Pl. Pathol, 35 (2) , 224-227.
7. Dhingra,O. D. and Sinclair, J. B. (1995). Basic Plant Pathology Methods.2nd Edition, CRC Press, Florida, USA.p.292.
8. Elad, Y. Chet, I. and Katan, J. (1980).Trichodermaharzianum : A biocontrol agent effective against Sclerotiumrolfsii and Rhizoctoniasolani. Phytopathology,70 , 119-121.
9. Jayaraj, J. and Ramabadran, R.(1999). Rhizobium- Trichoderma interaction in vitro. IndianPhytopath, 52 (2), 190-192.
10. Kaur, M. and Seema, (2002). Antagonistic activity of Pseudomonas strains isolated from the rhizosphere of medicinal plants, In Proc. Natl. Sem. Role of antimicrobials for sustainable horticulture. I.G.A.U. 20 Jan. pp. 6-12.
11. Khan, S. Md. and Zaidi, A. (2002).Determination of antagonistic potentials of plant growth promoting rhizobacteria and nitrogen fixing microorganisms.Environmental Biology and Conservation, 7 , 1-6.
12. Radhakrishnan, N. V. Ramabadran, R. and Jayaraj, Jayaraj, J. (1995). Botryodiplodia rot in Mulberry control through bio-control agents. Indian J. Mycol. Pl. Pathol,25 (1&2) , 146.
13. Saha, A.K. and Haque, Md. F. (2005).Effect of inoculation with Rhizobium on nodulation and growth of bean, Dolichos lablab. J. Life Science, 1 (1) , 71-74.