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Efficacy of rhizospheric bacteria and mycoflora against *Fusarium udum* under *in vitro*

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Abstract

Pigeon pea is an important pulse crop of the world which is an important source of protein and other nutrients. Redgram wilt is a major disease of pigeon pea which is caused by *Fusarium udum*. *In vitro* evaluation of different isolates of biocontrol agents against *F. udum* was carried out to test the efficacy of antagonists against the test pathogen. Percent inhibition in the mycelia growth of the pathogen was determined. Potential isolates showed significant inhibitory effect on the pathogen. Under dual culture method maximum percent inhibition of *F. udum* was shown in RB-4 (*Bacillus subtilis*) 73.31 percent and RF-15 (*Penicillium citrinum*) 75.03 percent. The study suggests that the *Bacillus subtilis* and *Penicillium citrinum* has a potential to be used as biocontrol agents against *Fusarium udum* of pigeon pea.

Keywords: Fusarium wilt, redgram, biocontrol, *Fusarium oxysporum* f. sp. *udum*

Introduction

Pigeon pea, *Cajanus cajan* (L.) Millsp, is an important pulse crop in India and is a major source of protein for most of the vegetarian population. It has a protein content of 22 percent, which is almost three times that of cereals. In India, pigeonpea is cultivated in an area of 3.90 mha with a production of 3.17 Mt and productivity of 813 kg ha-1 (IIPR, 2014 and FAO, 2016). The wilt caused by *Fusarium udum* (Butler) is one of the most important disease of pigeonpea in India resulting in yield losses up to 67 percent at maturity and 100 percent in case of infection at pre pod stage (Kannaiyan and Nene, 1981) [8]. The pathogen is a soil and seed borne. The genus Fusarium has wide host range and survives for long time in field in the absence of host plant. The disease may appear during early stages of plant growth (4-6-week-old plant) period and essentially affects yield. F. udum, the causal organism of redgram wilt is soil borne and is capable of saprophytic survival on crop residues in the soil for up to eight years (Nene, 1980) [13]. Although several measures including chemical treatments are used by growers to control the disease, the results are not satisfactory. Hence, there is an urgent requirement to develop alternative control measures and biological control is a promising alternative. Various workers stated that use of biocontrol agents (BCAs) is an effective method to manage soil borne diseases of crops without resistant sources (Moradi *et al*. 2012, Mukherjee *et al*. 2012, Saxena *et al*. 2015) [9, 10, 15]. BCAs like Trichoderma, Bacillus and Pseudomonas are known as effective antagonists against many soil borne pathogens (Saxena *et al*. 2016) [16]. Microbial antagonism is an important factor for biological control of soil-borne plant pathogens (Baker and Cook 1974; Garrett, 1965) [1, 6] and has a large bearing on the establishment of an organism in the rhizosphere. Therefore, it has drawn considerable attention of soil microbiologists and plant pathologists in recent years. In the present investigation an attempt has been made to study in the antagonism between F. udum and the rhizosphere microflora of pigeon-pea.

Materials and Methods

Isolation of the pathogen

Samples of plants (red gram) with disease symptoms were collected in a paper envelope and brought to the laboratory. The pathogen is isolated by direct culturing of infected parts according to Chopada *et al*. (2015) [3] with minor modifications. Briefly, the infected roots were washed with running tap water to remove all adhering soil particles, and then cut into small pieces prior to surface sterilization using 96 percent ethanol for 30s. All the sterilized pieces were placed onto Potato Dextrose Agar (PDA) plates (Nash and Snyder, 1962) [11]. Plates were incubated under the standard incubation conditions (Chehri *et al*. 2010) [2] for 48 h and the resulting single-spore of *Fusarium* colonies were transferred to fresh Potato Dextrose Agar (PDA) plates for further studies. The species were identified on the basis of macroscopic and microscopic characteristics such as growth rates, pigmentations of colony, types of conidiogenous cells, shape and size of conidia, and presence or absence of sporodochia and chlamydospore.

Isolation of bioagents from rhizosphere

Bioagents were isolated by following serial dilution technique (Nazir, 2007) [12]. Composite soil sample was collected from rhizosphere of healthy redgram plants. The soil was dried under shade and then used for serial dilution. To get 10-1 dilution, 10 g of soil was dissolved in 100 ml of sterile distilled water and one ml of soil suspension was taken and added to 9 ml of sterile distilled water to get 10-2 dilution. This step was repeated until a dilution of 10-4 for the isolation of fungi and 10-6 for bacteria.

Screening of fungal isolates for their antagonistic activity against *Fusarium udum* in dual plate assay

Antagonistic activity of isolated rhizosphere mycoflora of redgram against *Fusarium udum* was studied by following the dual culture technique (Finney, 1971) [5]. For this, the five-day-old culture of both rhizosphere mycoflora and *F. udum* was used. Five mm disc of rhizosphere mycoflora and *F. udum* were placed 4 cm apart on the PDA medium in opposite direction and 2 cm away from the periphery of petriplate. Control plates with only pathogen 5 mm disc *F. udum* without antagonist were also maintained. The inoculated plates were incubated at 25 ± 2 ºC for 7 days and the observations on the growth and ability of biocontrol agents to restrict and colonize *F. udum* were recorded on the 7th day, observations on radial growth of the test pathogens in the presence or absence of antagonist were recorded. The percent growth inhibition over control was calculated by using the formula

$$\text{Percent Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Screening of bacterial isolates for their antagonistic activity against *Fusarium udum* in dual plate assay

The isolated rhizospheric bacterial isolates were tested for their antagonistic properties against the test pathogen *viz Fusarium udum* under *in vitro.*

Antagonism test was conducted *in vitro* by dual culture method (Finney, 1971) [5]. Potato dextrose agar was prepared and 20ml poured into Petri plates allowed for the solidification. Isolated bacterial strains were inoculated on one side of the plate and pathogen on the other side. The assay plates were incubated at 28 ± 1 °C for four days and observations will be made on inhibition of the mycelial growth of the test pathogen. Three replicates with suitable controls were maintained for each test and the percent inhibition over control was calculated by adopting the following formula,

$$\text{Percent Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Results

Testing efficacy of rhizobacteria

Antagonistic activity of fifteen isolates of rhizobacteria against *Fusarium udum* was studied by adopting a dual culture technique. The study showed that the highest percent inhibition of radial growth over control was recorded with RB-4 *Bacillus subtilis* (73.31 percent) (Plate-1, A)followed by RB-13 (70.35 percent), RB-8 (70.15 percent), RB-2 (59.76 percent), RB-5 (57.54 percent), RB-1 (52.65 percent), RB-11 (46.38 percent), RB-7 (46.38 percent), RB-3 (44.28 percent), RB-12 (41.97 percent), RB-15 (41.97 percent), RB-9 (41.93 percent), RB-6 (41.08 percent), RB-10 (34.20 percent), RB-14 (33.08 percent) (Table 1, Fig 1).

Testing efficacy of rhizosphere mycoflora

Efficacy of fifteen isolated rhizosphere mycoflora of redgram plant tested against *Fusarium udum* results showed that out of the rhizosphere mycoflora evaluated against all the isolates *Penicillium citrinum* (RF-15) showed maximum inhibition (75.03 percent) (Plate-1, B) followed by RF-2 (71.43 percent), RF-14 (70.81 percent), RF-8 (68.55 percent), RF-4 (53.18 percent), RF-11 (46.92 percent), RF-13 (45.37 percent), RF-10 (43.11 percent), RF-12 (42.07 percent), RF-9 (39.81 percent), RF-5 (34.70 percent), RF-7 (29.03 percent), RF-1 (28.44 percent) while least inhibition percentage was seen in (RF-6) *Aspergillus* spp. having (21.40 percent) (Table 2, Fig 2).

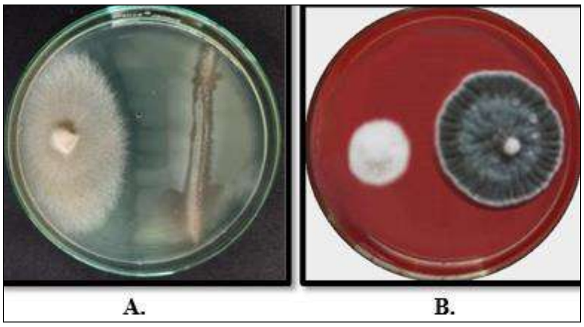

Plate 1: Dual plate assay indicating the antagonistic activity of fungi and bacterial isolates A: *Bacillus* isolate B: *Penicillium* isolate

Table 1: Efficacy of bacterial biocontrol agents on radial growth of *F. udum* under dual culture method

Isolate No.	Radial growth (mm)	Percent inhibition over control
RB-1	32.70	52.65 (46.52)
RB-2	29.82	59.76 (50.63)
RB-3	40.66	44.28 (41.72)
RB-4	15.28	73.31 (58.89)
RB-5	27.57	57.54 (49.33)
RB-6	43.50	41.08 (39.87)
RB-7	39.22	46.38 (42.93)
RB-8	17.70	70.15 (52.34)
RB-9	43.21	41.93 (40.35)
RB-10	50.28	34.20 (35.79)
RB-11	39.29	46.38 (42.93)
RB-12	42.91	41.97 (40.38)
RB-13	16.96	70.35 (56.44)
RB-14	50.54	33.08 (35.12)
RB-15	43.19	41.97 (40.38)
Control	90.00	90.00
C.D.	2.878	0.035
SE(m)±	0.992	0.012
SE(d)	1.403	0.017

Table 2: Efficacy of fungal biocontrol agents on radial growth of *F. udum* under dual culture method

Isolate No.	Radial growth (mm)	Percent inhibition over control
RF-1	60.37	28.44 (32.13)
RF-2	10.71	71.43 (57.59)
RF-3	43.09	39.81 (39.03)
RF-4	31.98	53.18 (46.74)
RF-5	48.02	34.70 (36.00)
RF-6	65.31	21.40 (27.46)
RF-7	55.43	29.03 (32.51)
RF-8	12.09	68.55 (55.79)
RF-9	45.56	39.81 (39.03)
RF-10	41.85	43.11 (40.95)
RF-11	38.15	46.92 (43.15)
RF-12	44.81	42.07 (40.35)
RF-13	33.21	45.37 (42.25)
RF-14	10.99	70.81 (57.19)
RF-15	6.05	75.03 (59.92)
Control	90.00	90.00
C.D	0.044	0.325
SE(m)±	0.015	0.112
SE(d)	0.022	0.159

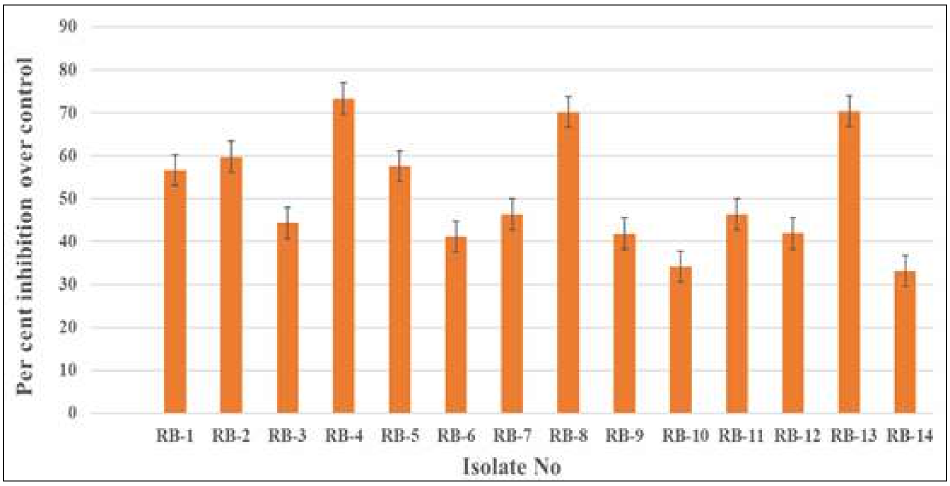

Fig 1: Biocontrol activity of rhizospheric bacterial isolates Inhibition of fungal plant pathogen

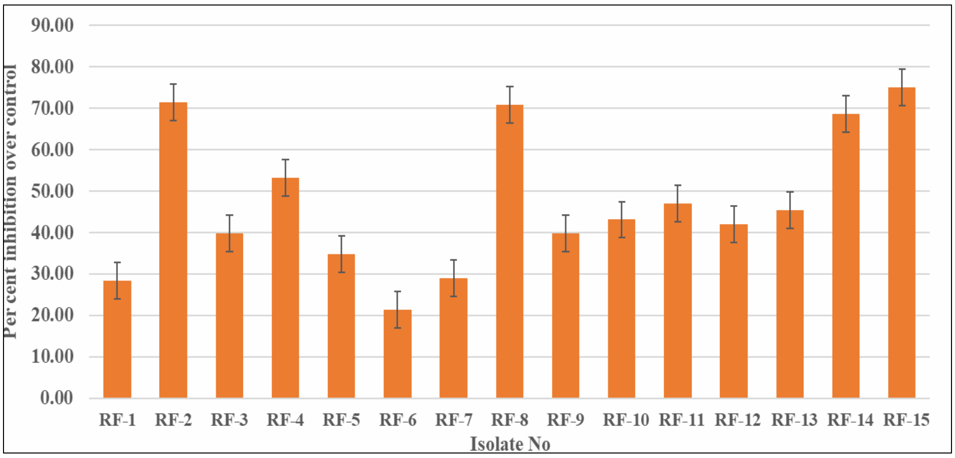

Fig 2: Biocontrol activity of rhizospheric fungal isolates Inhibition of fungal plant pathogen

Discussion

Rhizospheric isolates of bacteria and fungi were isolated from rhizosphere soil of Redgram. Morphological and biochemical characterization studies revealed that isolates showing highest inhibition percentage belongs to *Penicillium citrinum* and *Bacillus subtilis*. These isolates were used for their biocontrol activity against the *Fusarium* wilt of Redgram.

Sumitha and Gaikwad (1995) [17] studied the inhibition effect of antagonists on *F. udum* and found that *Bacillus subtilis* produced a wide zone of inhibition against *Fusarium udum* and inhibited spore germination completely. Ramezani *et al*. (2000) [14] evaluated bacterial bioagents *Pseudomonas fluorescens* and *Bacillus subtilis* against the chickpea vascular wilt pathogen. *Frasarium oxysporum* f.sp. *ciceri in vitro* condition using dual culture technique. The zone of inhibition produced by *P. fluorescens* and *Bacillus subtilis* against fungal pathogen was 13 and 12 mm, respectively.

The results are also in conformity with Devi *et al*. (2012) studied the antagonistic activity of isolated rhizospheric fungi which 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%). Goudar and Kulkarni (2000) [18] studied the antagonistic effect of *Trichoderma viride*, *T. harzianum*, *Aspergillus niger*, *A. flavus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Penicillium* spp. and *Streptomyces* spp. against pigeonpea wilt pathogen, *F. udum* showed that *T. harzianum* and *Penicillium* spp completely suppressed the growth of *F. udum* even though no inhibition zone was observed.

Summary and Conclusion

In this experiment, fifteen bacterial and fifteen fungal cultures were isolated and screened against the wilt causing pathogen i.e., *Fusarium* spp., through their biocontrol mechanisms. They were identified based on the morphological and biochemical characterization. The isolates *Bacillus subtilis* (RB-4) and *Penicillium citrinum* (RF-15) from fungi and bacteria respectively recorded maximum inhibition against *Fusarium udum* in dual plate assay. So these isolates can be further screened at field level and can be used as biocontrol agents against redgram wilt disease as an ecofriendly control measure of disease.

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