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*Role of Bio-agents against wilt of Isabgol (*Plantago Ovata Forsk*) and their Influence on its* Growth and Yield

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Abstract

Plantago ovata is one of the major medicinal crops having international acceptance and industrial significance. *Rhizoctonia* wilt caused by *Rhizoctonia solani* is the major limiting factor in the production of isabgol. An effort was made to develop an eco-friendly approach to control *Rhizoctonia* wilt in isabgol using *Trichoderma* virens, Trichoderma harzianum and Pseudomonas fluorescence. Besides direct interaction with plant pathogens, bioagents have been reported to induce systemic resistance in plants. In the present study *Trichoderma* virens, Trichoderma harzianum and Pseudomonas fluorescence were evaluated for their efficacy to control *Rhizoctonia* wilt. All of the three bio-agents used in this study significantly reduces the incidence of wilt and percent reduction in wilt incidence was recorded as 35.44%, 49.32% and 76.25% in the treatment of *Trichoderma* virens, Trichoderma harzianum and Pseudomonas fluorescence respectively. Seed treatment with Trichoderma virens, Trichoderma harzianum and Pseudomonas fluorescence significantly increases the seed germination, plant height, size of head, number of head per plant and root strength. From the post hoc tukey HSD, it is clear that all treatments are significantly different to each other and Pseudomonas fluorescens proved to be the best effective treatment.

Keywords: wilt incidence, Biocontrol agent.

1. Introduction

Plantago ovata (Isabgol) is an important medicinal plant which has been used in health care for many centuries in South Asia, but nowadays it is widely used all over the world (Malik et al 2011). India is the largest producer and exporter of this crop in the world and it is grown as a cash crop in Gujarat, Punjab and Uttar Pradesh. This plant can be grown under a wide range of agro-climatic conditions, but it is mostly confined to the arid areas of the world due to its low water requirements (Zahoor, *et al*., 2004). Among various factors responsible for low productivity of isabgol is the damage caused by various plant disease. Damping-off of seedlings, Rhizoctonia wilt, downy mildew and powdery mildew are the major fungal diseases among these, Rhizoctonia wilt is the important and widely spread disease (Farooqui and Sreeramu 2001). However, the indiscriminate use of fungicides has resulted in many problems, such as toxic residues in food, water, soil and disruption of the ecosystem so biological control is potentially a sustainable solution to manage plant disease (Ashraf & Zuhaib 2013). Antagonistic fungi especially *Trichoderma* spp. and the bacteria, *fluorescent pseudomonads* have been widely used against a number of phytopathogens (Rini and Sulochana 2007) Keeping this in view and the growing importance of biological control agents, the present study was carried out. The main objective was to evaluate the efficient biocontrol agent among *Trichoderma virens, Trichoderma harzianum* and *Pseudomonas fluorescence* in controlling Rhizoctonia wilt. Effect of Seed treatment with *Trichoderma virens, Trichoderma harzianum and Pseudomonas fluorescence* on the seed germination, plant height, size of head, number of head per plant.

2. Materials and Methods

2.1. Isolation and maintenance of Biocontrol agents

Trichoderma virens, Trichoderma harzianum and *Pseudomonas fluorescence* were isolated from the rhizosphere of healthy isabgol plants collected from isabgol field in the Faculty of Agricultural Sciences AMU, Aligarh. For *Trichoderma*, the fungal colonies with white mycelium, which later changed into different shades of green on the culture medium, were examined, purified, and transferred to potato dextrose agar (PDA) slants. Bacterial colonies showing the characteristic fluorescence in King's medium B (KMB) were picked up, purified, and maintained on KMB slants.

2.2. Collection and maintenance of Rhizoctonia solani

R. solani was isolated from wilt infected isabgol plants. Pathogenicity was tested following the Kochs postulate i.e Isabgol plants grown in sterilized soil in pots were inoculated by *Rhizoctonia solani* which was mass cultured on presoaked sorghum seeds and the symptoms of wilting appears . The pathogen cultures were purified and maintained on PDA slants and recultured at 15 days time interval during the study

2.3. Evaluation of Biocontrol agent (BCA) against wilt

Pot experiment was conducted in completely randomized block design with four replications to evaluate the performance of the most efficient BCA *Trichoderma virens, Trichoderma harzianum* and *Pseudomonas fluorescence* against wilt. Ten seeds of isabgol were sown in 12cm diameter pots containing 1 kg sterilized soil (sterilized at 1.1 kg^{-1} cm^{-2} for 1 hour) inoculated with 20 days old culture of the mass multiplied pathogen on sand maize meal water medium (90 g sand, 10 g maize meal, 20 ml distilled water) at 50 g kg^{-1} soil one week before sowing (Nene et al., 1981). The whole set of experiment was replicated 5 times.

The seeds were treated with *Trichoderma virens, Trichoderma harzianum* and *Pseudomonas fluorescence*, which showed good antagonistic activity against Rhizoctonia solani *in vitro*. The control was also main- tained without seed treatment. Wilt incidence was recorded at 15 days interval up to maturity of crop plants. Observations on seed germination, plant height, size of head, number of head per plant and were taken. All the observations recorded are

2.4 Statistical analysis

The data obtained from all treatments were subjected to analysis of variance (ANOVA) using Minitab-15 for Windows. Transformed means were analyzed across sample periods using repeated measures analysis of the protected Fisher's least significant difference (Tukey's HSD) test at $P<0.05$. All percentage data were analyzed using generalized linear mixed model (GLMM) prior to further analysis by using the language program R developed by Core Team ''R 2.10.1''unless stated otherwise.

3. Result and Discussion

3.1. Evaluation of Biocontrol agent against wilt

A significant difference was recorded in the efficacy of different biocontrol agents against wilt incidence caused by Rhizoctonia solani Table (1). The minimum wilt incidence was recorded with the seed treatment of Pseudomonas fluorescens and maximum wilt incidence was recorded with seed treatment of *Trichoderma virens* ($P<0.05$; $F = 2.271$; Pearson chi = 0.1503). It was also observed that in checking the wilt incidence the results shown by Pseudomonas fluorescens was almost nearer to seed treated with Thiram.

Table 1: Effect of seed treatments with *Trichoderma* virens, T. harzianum and Pseudomonas fluorescence on wilt incidence caused by Rhizoctonia solani in pots.

Treatments	Mean Wilt incidence %	Mean Wilt reduction%
Trichoderma virens	44.10	35.44
Trichoderma harzianum	30.22	49.32

Pseudomonas flourescens	3.29	76.25
Traidemifon (0.01%)	8.32	71.22
Thiram (0.01%)	1.09	78.45
Control (without seed treatment)	79.54	

3.2. Evaluation of Biocontrol agent in enhancing the plant growth parameters

The results revealed that the treatment effect was significant in respect of growth parameters viz. Plant height, head size and number of heads/ plant Table (2). Plant height (above ground) was maximum with Pseudomonas fluorescens (28.23) (df=3,11; F=119.3 at P=0.02) followed by *Trichoderma virens* (22.56) and the minimum in *Trichoderma harzianum* (21.32). Similarly the head size was maximum with *Pseudomonas fluorescens* (4.33) (df=3,11; F=3.96 at P=0.01) followed by *Trichoderma virens* (2.63) and the minimum in *Trichoderma harzianum* (2.00).

Number of heads/plant was maximum with Pseudomonas fluorescens (21) (df=3,11; F=19.23 at P=0.02) followed by *Trichoderma virens (*15) and the minimum in *Trichoderma harzianum* (12).

Table 2: Effect of seed treatments with *Trichoderma* virens, T. harzianum and Pseudomonas fluorescence on growth parameters.

Treatments	**Plant height(cm) (above ground)**	**Head size (cm)**	**No. of Head/ Plant**
Trichoderma virens	22.56 b	2.63b	15
T. harzianum	21.32 c	2.00c	12
Pseudomonas Fluorescens	28.23 a	4.33a	21
Control	20.14 d	1.50d	11

In case of percent mean seed germination the results revealed that treatment effect was significant table (3). Percent seed germination in sterilized soil inoculated with the pathogen was found to be maximum when the seeds were treated with *Pseudomonas fluorescens* (95%) followed by *Trichoderma virens* (86%) and then minimum in *T. harzianum* (84%).

Table 3: Effect of seed treatments with *Trichoderma* virens, T. harzianum and Pseudomonas fluorescence on seed germination of Isabgol in pot soil inoculated by Rhizoctonia solani.

Treatments	**Mean seed germination (%)**
Trichoderma virens	86
T. harzianum	84

Pseudomonas fluorescens	95
Control Without seed treatment	80

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