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RESEARCH ARTICLE

MANAGEMENT OF ROOT-ROT COMPLEX DISEASE AND ASSESSMENT OF PLANT GROWTH PROMOTING CHARACTERS IN VEGETABLE PEA WITH NATIVE AND COMMERCIAL ANTAGONISTICS THROUGH SEED BIOPRIMING

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ABSTRACT

This study was conducted to evaluate the seed treatment effects of native and commercial antagonistics for management of root rot complex disease and plant growth promoting activities in vegetable pea using individual and combination of bioagents. Initially, a survey was conducted in major vegetable growing areas in Uttarakhand, India to collect pathogen infected seedlings. Pathogenicity test showed that 50% samples were found infected with complex of *Rhizoctonia solani* and *Fusarium solani* pathogens, 33.0 % with *Fusarium solani* and 17.0% with *Rhizoctonia solani*a. Field experiments were conducted in experimental area for investigating disease management and Plant growth activity. A marked increase in shoot, root and seedling lengths, vigor index, number of pods, number of grains per pods, pod weight and yield were observed in combination seed treatment effect of *Trichoderma harzianum* and *Pseudomonas fluorescens.* Among the all, antagonistic combinations of *T.harzianum* and *P.fluorescens* were found to be most effective in promoting the plant growth activities as well as increasing yield parameters of pea crop in field conditions. The observations revealed that *T.harzianum* and *P.fluorescens* combination are quite effective against root rot complex pathogen (% disease incidence (PDI); 11.9 %), followed by *P. fluorescens* (PDI; 15.4%) and *Trichoderma harzianum + Pseudomonas fluorescens + Trichoderma virens* (PDI; 15.9%) compared to untreated control (PDI; 21.6%). Disease severity (0-9 scale) was also observed and seed treatment with *T.harzianum + P. fluorescens* was found to have least disease severity (20.0 %) followed by *T.harzianum+ P. fluorescens + T.virens*(30 %), *P. fluorescens*(31.1 %).

INTRODUCTION

Uttarakhand state of India extends between 28º44' and 31º25' North latitude, 77º35' and 81º02' East longitude and occupies a well-demarcated geographical and socio-cultural unit forming a part of central Himalaya. The total geographical area of Uttarkhand is 53,483 sq. km., of which 62.5 % is under forest and 14.0 % is under agriculture. About 75-85 % population of Uttarakhand predominantly depends on agriculture especially on off-season vegetables for their livelihood. The agricultural practice in the hill region is marked by small and fragmented land holdings, small-sized terraced fields with rainfed irrigation.

Vegetable pea (*Pisum sativum L.*) is an important crop in Uttarakhand hills and cultivated twice in a year (July and November).In India, this crop is cultivated over an area of about 5, 40,000 ha with total production of 5, 30,000 tons (Singh and Tuli (2002). In Uttarakhand, the crop is cultivated in an area of 11,646 ha with an annual production of 7, 80, 50 tonnes in 2011-12 (Source-Horticulture Directorate of Uttarakhand (Chaubatiya) Ranikhet).This crop contains good market potential in local market. In India this crop is grown in Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh, Sikkim states and South Indian hills.

The Vegetable pea (*Pisum sativum L.*) crop is often attacked by number of pathogens but root rot complex disease is considered as the most destructive among all as it affects and damages entire root system and initial crop stant. Root rot is almost occurring in all pea varieties cultivated area in Uttarakhand hills. The diseases like root rot, seed rot and damping-off collectively represent a detrimental disease complex (hence referred as root rot in this study). The major pathogens which cause root rot disease are *Rhizoctonia solani* and *fusarium solani* and its complexes in Uttarakhand hills (Negi et al. 2005).

Environmentally safe and eco-friendly methods of disease management is an urgent need in Uttarakhand agriculture and Horticulture because this state is declared as an organic state. In this context, biological control is an effective alternative for disease management. In Uttarakhand, Department of Agriculture and Horticulture provide many biopesticides (e.g. *Psedomonas fluorescens, Tricoderma viride, Trichoderma virens, Bacillus subtilis, Beauveria bassiana etc.*) for the disease and insect management but most of the time farmers report about their inefficiency against the specific diseases. Biological control agents for sustainable agriculture are now

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being used in many developed countries for combating the diseases with an aim of increasing crop yield and seed quality.

Soil application of biocontrol agent is not feasible, as large quantities are needed. Thus application of antagonist through seed treatment is a viable alternative for the introduction and establishment of a biocontrol agent.

Present study demonstrates the use of biocontrol agents (Individual and combined) for treatment of root rot complex disease in pea.

MATERIAL AND METHODS

Periodic surveys were carried out in various pea growing areas during kharif and rabi seasons of Uttarakhand hills during 2009. During the course of surveys, the plants with infected roots were collected in paper bags and brought to the laboratory for immediate isolation of the associated pathogens and kept at ambient for future use. The survey was carried out randomly in locations of different vegetable growing areas in hill district.

Field experiment on management of disease development and plant growth was carried out at the experimental area of plant pathology section, State Training & Research Centre for Organic Farming Majkhali, Ranikhet (Almora) India. This research centre is located at 29 ^{0} 40 ´ 45.36 ˝ N and 79^0 30 ´ 47.78 ˝ E (1600 mtr MSN). The field trail was conducted in 3 x 2 mtr plots under rain fed condition. Fields were manually prepared and appropriate organic fertilizers were applied as per recommended agronomical practices for vegetable pea. The experiments were laid out in Randomized block design (RBD) with three replications. 'Arkil' variety of vegetable pea was sowed in October. For conducting experiment, combination and individual bio-agents (*Trichoderma harzianum, Trichoderma virens and Pseudomonas fluorescens*) were tested for disease control.

Seeds of vegetable pea ('Arkil') were obtained from the department of Horticulture mobile unit Majkhali (Ranikhet). The antagonists *Trichoderama harzianum* (Strain Th-21) & *Pseudomonas fluorescens* (Strain Pf-173) used in present study were obtained from laboratory of plant pathology G.B.Pant Univ. of Ag. & Tech., Hill campus Ranichauri, Tehri Garhwal, Uttrakhand. *T. virens* was obtained from the NAFED Biofertilizer, Rudrapur, U.S Nagar. The colony forming unit (CFU) of *Trichoderam harzianum* & *Pseudomonas fluorescens* were 10^6 while CFU of *T. virens* was 10^7. The treatments details are: T1- Seed treatment by *Trichoderma harzianum*, T2- Seed treatment by *Pseudomonas fluorescens* , T3- Seed treatment by *Trichoderma virens* T4- Seed treatment by *Trichoderma harzianum* + *Pseudomonas fluorescens* ,T5- Seed treatment by *Trichoderma harzianum* + *Trichodermavirens*, T6- Seed treatment by *Trichoderma virens* + *Pseudomonas fluorescens*, T7-Seed treatment by *Trichodermaharzianum* + *Pseudomonas fluorescens* + *Trichoderma virens* and T8-Untreated control.

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Talc base formulation of *T.harzianum*, *P. fluorescens* and *T.virens* were used for seed treatment @ 10 gm/kg. Seeds to be treated were weighed and moistened with tap water. Any excess water was drained.

Field data was recorded on plant growth prompting parameters i.e., germination rate, shoot, root and seedling lengths, vigor index, pod weight, pod length, number of pods per plant, number of grains per pod, root rot incidence and disease severity. For estimating root rot incidence, the data was recorded throughout the cropping period at fix intervals; from date of appearance of disease till the termination of experiment. Data were analysed by (ANOVA) variance in RBD for calculating the significance by magnitude of the F value (P=0.05).

Disease severity in each plant was rated on a 0-9 scale in vegetable pea (Tu, 1987) (0 = < 10 % of roots with symptoms, 1 = 11-20% of root with symptoms, 2 = 21-30 %, 3= 31- 40 %, 4=41-50 %, 5= 51-60 %, 6= 61-70 %, 7= 71-80 %, 8= 81-90% 9= plant dead by visual scoring of roots after washing the soil off the roots on a screen with running water.

RESULTS AND DISCUSSION

Disease incidence and prevalence observed in vegetable pea

The disease incidence of vegetable pea in field was found as 8.8- 35.4 % in Uttarakhand hills. Maximum root rot disease occurrence of 35.4% was recorded in Ramgarh (District Nanital) while, lowest disease incidence of 8.8% were recorded in Lamgara location of Almora district (table 1). Augustmuni in Rudraparyag and Gopeswar in Chamoli district also showed second and third highest rank of percent disease occurrences (32.3 & 31.2%). A range between 20-30 % diseases incidences were observed in Majkhali (Almora),

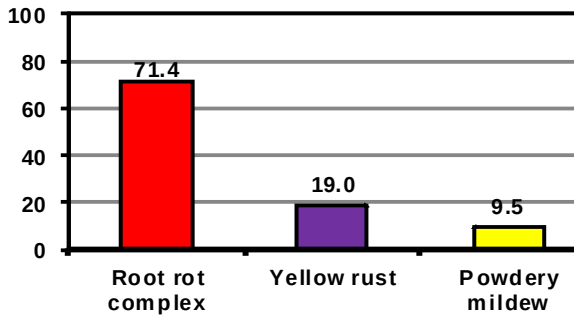

Fig. 1: Average percent disease incidence observe in vegetable pea

Chakrata (Dehradun), Chamba & Ranichouri in Tehri Garhwal

and Dunda of Uttarkashi districts respectively in randomly selected field. `Average percentage disease incidence (including root rot and collar rot) in vegetable pea is shown in figure 1.

samples of root rot and collar rot seedlings were collected from the different locations of farmer's field. After isolation from samples, 50% samples were identified as a complex of

Table 1 Major vegetable pea diseases of Uttarakhand hills observed in 2009.

S.No.	District	Locality	Major diseases in vegetable pea	Disease incidence (%)
1	Almora	Majkhali	Root rot	23.4
			Powdery mildew	33.2
		Hwalbag	Collar rot	12.4
		Lamgarha	Root rot	8.8
2	Chamoli	Gopeswar	Root rot	31.2
		Dewal	Yellow rust	10.3
		Gwaldam	Root rot	11.2
3	Dehradun	Chakrata	Collar rot	13.2
			Root rot	23.9
4	Nanital	Ramgarh	Root rot	35.4
5	Pouri	Dugadda	Root rot	11.2
6	Garhwal Rudraparyag	Bharsar	Yellow rust	5.1
		Augustmuni	Root rot	32.3
		Naryanbagarh	Collar rot	14.3
7	Tehri	Chamba	Root rot	27.2
			Powdery mildew	26.1.
		Thatyur	Root rot	15.3
		Ranichouri	Root rot	27.2
8	Uttarkashi	Gaja	Powdery mildew	20.1
		Dunda	Root rot	28.4
		Chinyalisour	Yellow rust	5.8
		Purola	Yellow rust	2.8

*At least 50-100 plants in field were examined for each site

Regarding disease prevalence, 100 % disease was observed in Dehradun, Nanital and Rudraparyag districts while, In Almora, Chamoli Tehri Pouri Garhwal and Uttarkashi 75.0, 66.6 ,60.0, 50.0 and 33.3 % disease prevalences were observed respectively.

Pathogen infection rate in vegetable pea

In vegetable pea total 19 locations were surveyed and 12

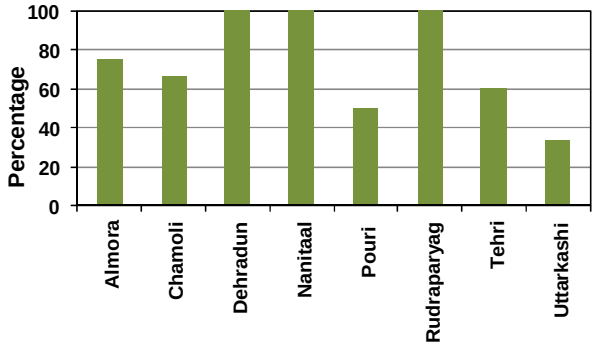

Fig. 2 : Root rot complex prevalence in vegetable pea

Pathogenicity

After pathogenicity test in poly houses, the fungus in cultures was white, brown, radiating growth in medium plate. It was identified only from mycelia characteristics. Its hyphal cells were multinucleate. It also produced white to deep brown mycelium when grown on potato dextrose agar medium. The hyphae were 4-15 µm wide and tend to branch at right angles. On the basis of morphological and cultural characteristics as described by Parameter and Whitney (1970) the fungus was identified as *Rhizoctonia solani*.

In PDA culture second white mycelia was also isolated. Culture produced white macroconidia, which were primarily Three septate and curved therefore this fungus was identified as *Fusarium solani. F. solani* infects the cotyledon, epicotyls, and hypocotyl and the disease progresses to the soil line and down the root system. Early symptoms are stunting and epinasty. Reddish brown streaks on the roots coalesee as the disease progresses, and the infected area later appears dark to stunted and chlorotic, and lower leaves die.

Table 2 Plant growth parameter of vegetable pea in field experiment.

Name of treatments (Seed treatment by)	Germination percentage	Shoot length (Cm)	Root length (Cm)	Seedling length (Cm)	Vigor index (Cm)
Trichoderma harzianum	87.2	25.4	13.5	38.9	4407
Pseudomonas fluorescens	89.7	27.2	14.6	41.8	4664
Trichoderma virens	81.4	25.8	12.7	38.4	3722
Trichoderma harzianum + Pseudomonas fluorescens	**93.9**	**28.5**	**16.4**	**44.9**	**5707**
Trichoderma harzianum + Trichoderma.virens	84.4	23.3	11.2	34.5	3235
Trichoderma virens + Pseudomonas fluorescens	84.4	22.8	10.9	33.9	3391
Trichoderma harzianum + Pseudomonas fluorescens + Trichoderma virens	88.3	26.9	14.6	41.5	4222
Untreated control	76.2	22.0	8.0	30.1	2473
S.E.m.	3.4	0.75	0.6	-	-
CD @ 5 %	10.2	2.3	1.85	-	-

*Mean of three replications of two year data

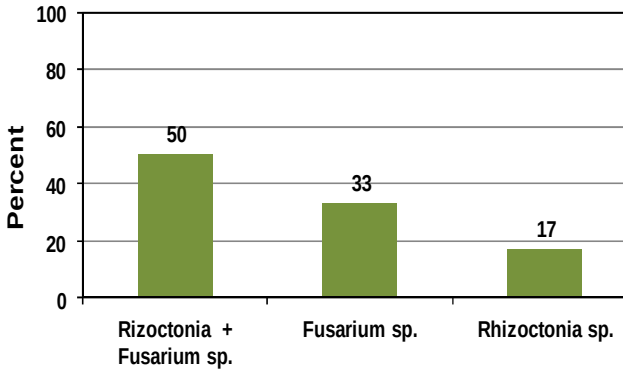

Fig 3: Pathogen isolate from infected vegetable pea

by *T.harzianum* + *P. fluorescens* indicated maximum shoot length (28.5 cm) followed by seed treatment by *P. fluorescens* (27.2 cm), seed treatment by *T.harzianum* + *P. fluorescens* + *T. virens* (26.9 cm) , while in control plot shoot length was recorded as 22.0 cm.

Seed treatments of native *T.harzianum* + *P. fluorescens* showed maximum root length (16.4 cm), seed treatment by *P. fluorescens* and seed treatment by *T.harzianum* + *P. fluorescens*+ *T. virens* both showed 14.6 cm root length, which was significantly higher compared to untreated control, which showed 8.0 cm root length.

T.harzianum + *P. fluorescens* treatment recorded 44.9 cm seedling length. This length was significantly higher than

Table 3 Yield increasing parameters of vegetable pea in field experiment.

Name of treatments (Seed treatment by)	Number of pods	Pods length	Pod weight	No. of grains /Pod	Yield (qtl ha^{-1})
Trichoderma harzianum	16.0	6.9	179.2	6.9	101.2
Pseudomonas fluorescens	17.8	7.4	187.2	7.7	103.8
Trichoderma virens	16.5	6.6	177.7	6.3	98.4
Trichoderma harzianum + Pseudomonas fluorescens	**20.1**	**7.6**	**198.1**	**8.8**	**108.9**
Trichoderma harzianum + Trichoderma.virens	16	6	173.3	6.3	98.9
Trichoderma virens + Pseudomonas fluorescens	15.9	6	169.7	6.4	95.9
Trichoderma harzianum + Pseudomonas fluorescens + Trichoderma virens	17.8	7.3	182.8	7.2	101.1
Untreated control	13.7	5.9	152.9	5.4	88.7
S.E.m.	0.3	0.25	-	0.16	0.83
CD @ 5 %	1.05	0.7	-	0.46	2.44

*Mean of three replications of two year data

chocolate brown (Tu 1992). Severely affected plants are
The causal organism of root rot complex was observed as *R. solani, F. solani* and their complex in our surveys result. Present survey results are in accordance with several studies that have been reported earlier. Negi et al. (2005) observed that root rot caused by *Fusarium solani* is a major threat to successful cultivation of pea in Garhwal Himalayas. Xue (2003) also reported that pea root rot complex caused by *R. solani, F. solani, F. oxysporum,* and *S. clerotiorum* was major pathogen isolated from pea infected seedling in Canada.

Data revealed that all the antagonistic seed treatment increased germination percentage compared to control. In experimental pea crop, maximum germination (93.9 %) was recorded in seed treatment by combination of *Trichoderma harzianum* + *Pseudomonas flurosence@* 10 gm/kg, followed by seed treatment by *P.flurosence*(89.8 %), *T.harzianum* +*P. flurosence* + *T. virens* (88.4 %), while, untreated control showed 76.2 % germination. Among the all antagonistics (individual and combination), *T. virens* indicated the least germination percentage of 81.4%. It has been observed that application of *T. harzianum* in combination with *P. fluorescens* as a seed coating greatly increased the percent germination within 7, 10 and 13 days respectively. The present results are also in agreement with those of Kumar and Dubey (2001) and Xue (2003), who have also reported significant increase in seed germination of pea by treatment with antagonistic but they used individual antagonistic for seed treatment. We used native antagonistic in combinations for seed treatments and found better seed germination.

Seed treatment with *T.harzianum* + *P. fluorescens* was found Significantly shoot length was recorded with all the antagonistic treatments compared to control. Seed treatment

control (30.1 cm) followed by seed treatment by *P. fluorescens* (41.8 cm), *T.harzianum* + *P. fluorescens*+ *T.*

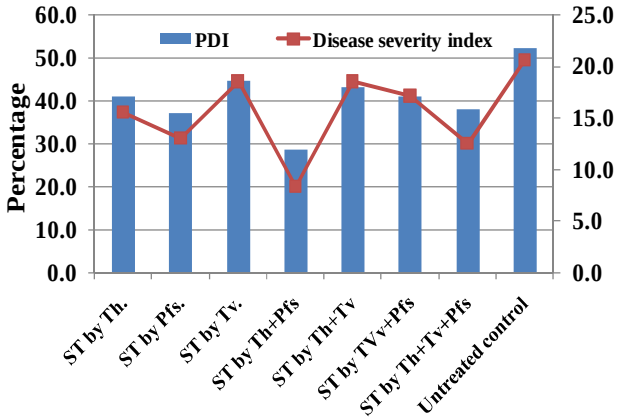

Fig.4: Relation between root rot disease and severity index

virens (41.5 cm).
Combination of *T.harzianum* + *P. fluorescens* also showed highest vigor index (Table 2) Number of pods, Pods length, pods weight and number of grains in pods also showed higher values compared to individual application of antagonistic and untreated control.

Earlier growth stimulatory effects of *Trichoderma* species has been reported by many researchers (Lynch et al. 1991); (Baker 1989); (Biswas and Sen 2000) while (Ahmadzadeh et al. 2006) reported growth stimulatory effects of *Pseudomonas flourescence*. Present study also investigated plant growth promoting activities of all antagonistic but we observed that combination effect of both native strains were more significant compared to untreated control.

to be most effective combination for the control of root rot complex disease (fig 4). Minimum disease incidence of 11.9% in the treatment *T.harzianum + P. fluorescens* was noticed followed by seed treatment by *P. fluorescens* (15.4 %), *T.harzianum + P. fluorescens + T.virens* (15.8%), while untreated control showed 21.7% root rot incidence. Regarding statistic of PDI, excluding *T. virens* all of the antagonistic were found superior to check while *T. virens* were at par with check. The critical difference calculated by ANNOVA at 5 % was observed as 3.5.

T. harzianum and *P.fluorescens* effectively controlled diseases in some crop plants in greenhouse as well as field conditions (Whipps and Lumsden 2001; Pan and Bhagat 2007; Joshi et al. 2007) and significant effects on growth promotion have also been reported by (Bhatiya et al. 2005). In present study we also observed that *T. harzianum* reduced root rot disease in vegetable pea but to the less extent compared to *T. harzianum* and *P. fluroescens* combination against *R. solani*, *F. solani* and its complex.

Seed treatments with bioagents were found to be superior over untreated control in disease severity parameter. Seed treatment with *T.harzianum + P. fluorescens*was was observed with least disease severity (20.0 %). This was followed by *T.harzianum+ P. fluorescens + T.virens* (30 %), *P. fluorescens*(31.1 %), while untreated control showed (49.4 %) disease severity.

The data of yield (qtl/ha) is presented in table 3. Seed treatments with *T. harzianum + P. fluorescens* showed significantly higher green pod yield for both years (108.9 qtl/ha.). This was followed by *P. fluorescens* (103.8 qtl/ha.), *T.harzianum*(101.2 qtl/ha),while untreated control showed 88.7 qtl/ha yield.

The yield results of this experiment with the T. *harzianum* and *P.fluroescens* (individual and combination) is in agreement with the observations by Raguchander et al. (1997); Das et al. (1998); Sharma et al. (1999) and Hyseik et al. (2002).

CONCLUSION

In this study, we observed that the native strains (isolated from the native soil and applied on native pathogen) has performed better than commercial (isolated from the areas which differ with respect to climate, soil and geography) strains. *T. harzianum* and *P.fluroescens* antagonistics are compatible to each other and were already tested in in vitro studies in G.B. P.U. Agriculture & Technology, Ranichouri, Uttarakhand, India. So it can be concluded that specific antagonistic if commercialized for the particular region pathogen can show effective results compared to other commercial antagonistics. Duffy and Weller (1995) also observed that application of consortium of native PGPR strains would more effectively mimic and might broaden the spectrum of action to enhance the efficacy and reliability of control.

Banyal et al. (2008) also observed that native strain of *T. viride* inhibit maximum mycelium growth of pathogen in tomato color rot. Butista et al. (2007) also showed significant result of native strain of *P.fluorescens* against the *R. solani* of Potato crop in Colombia.

Joshi et al. (2007) observed that indigenous *T. harzianum* strain Tr 34 and Tr 14 manage the root rot incidence 20.8 and 23.4 % of french bean in Uttarakhand (India) while, we observed minimum disease incidence of 11.9% in the treatment of *T.harzianum + P. fluorescens* by using native strain in pea root rot. After the compatibility test of antagonistic it can be applied in combination because the two antagonistics show different properties such as HCN production, siderophore production, Phosphate solbulization, antibiosis, and antifungal volatiles to antagonize pathogen (Gardener et al. 2000; Pal et al. 2000; Validov et al. 2005 and Singh et al. 2006). It is also recommend that mass production of commercial antagonistics should be given preferences as mixture of two or more antagonistics for application and would be easily applicable to give better performance in farmer's field.

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