



Full Length Article

Management of Black Scurf of Potato with Effective Microbes (EM), Biological Potassium Fertilizer (BPF) and *Trichoderma harzianum*

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Abstract

The efficacy of soil application with microbial preparations viz. *Trichoderma harzianum*, effective microbe (EM) culture and biological potassium fertilizer (BPF) was evaluated for the management of soil-borne inoculum of *Rhizoctonia solani* the cause of black scurf of potato cv. Desiree. Soil application with three dosages of culture suspension of *T. harzianum*, effective microbe (EM) culture and biological potassium fertilizer (BPF) were applied in the soil to know the efficacy of these treatments in reducing the disease. Soil application with *T. harzianum* at the time of sowing followed by two and three dosages at 20 days intervals gave significant protection to eyes with EGI of 30.55%, SK 24.07%, SCI 36.10%, StCI 30.60%, BSDI 26.43% and YR of 35.09% against the fungus which ultimately contributed to better crop stand and increased yield as compared to inoculated control and rest of the treatments. © 2015 Friends Science Publishers

Keywords: *Rhizoctonia solani*; *Solanum tuberosum*; *Biomangement*; *Beneficial microbes*

Introduction

Potato (*Solanum tuberosum* L.) is an annual, herbaceous, dicotyledonous plant of family *Solanaceae*. Potato is commonly known as disease oriented problematic crop throughout the world. The management of *R. solani* is difficult due to its soil-borne nature. The fungus is present in most of the soils (Banville *et al.*, 1996). Once it becomes established in a field, it remains viable there indefinitely (Agrios, 2005). Dry sclerotia of the pathogen are reported to survive up to six years when stored at room temperature (Kumar, 1976). Soil-borne inoculum of *R. solani* is the main cause of black scurf on potato tubers and also contributes to eyes germination inhibition, sprouts killing, stem, stolon and root damage (Hide *et al.*, 1973; Frank and Leach, 1980). Pioneer studies on the prevalence, incidence, severity and biology of black scurf disease pathogen in Pakistan have been reported by Rauf *et al.* (2007a). He reported that *R. solani* anastomosis group 3 is the primary cause of black scurf in Pakistan, like in most parts of the world (Banville *et al.*, 1996). The disease was found prevalent in all the eight potato production agro-ecological zones, and was the most wide spread and prevalent in zone 2, a major potato production area with 70-80% of the potatoes production in Pakistan. It comprises of Sahiwal Pakpattan, Okara, Sialkot, Jhang and Faisalabad districts of thr Punjab Province. This zone is also an important source of seed potatoes distribution to almost all the potato production areas of Pakistan. The imported and basic seed is also multiplied in

this zone (Zanoni, 1991). As black scurf is soil and seed-borne disease, so the inoculum of *R. solani* is carried through seed tubers from this zone to all potato production zones of Pakistan, thus adding more to existing inoculum load. This continuous addition in inoculum may ultimately lead to epidemics of disease in future.

Seed treatment with bio-formulations has been found better than the chemical treatments in integrated management of most of soil-borne disease of potato (Shternshis, 2002). Among antagonistic microorganisms, *Trichoderma* sp. was found the most capable of parasitizing *R. solani* *in vitro*. Reduced black scurf disease index compared to control together with maximum germination and yield of potato has been reported by seed treatment with *T. viridae* (Arora *et al.*, 2001). However, when *T. viridae* was compared with *T. harzianum* causing black scurf of potato, *T. harzianum* was found the most effective in inhibiting mycelial growth (Srivastava and Singh-Kavi, 2001). It was also found the most effective in reducing disease incidence and increase in yield over control in green house studies (Hazarika *et al.*, 2000). Mixed cultures of beneficial microorganisms named EM4 have been developed by Higa (1988; Unpublished). EM4 is a mixed solution cultures of beneficial microorganisms. The effect of EM culture has also been studied on fungal populations in soil which significantly increased the population of *Trichoderma* that has an antagonistic effect on *R. solani*, and suppressed the plant pathogenic fungi in soil. Physical properties of the soil including cultivation depth and

porosity were also improved by EM treatment. So integrating EM in black scurf management, not only help in increasing the population of *Trichoderma* (the antagonist of *R. solani*), but can also reduce the population of *R. solani* in soil, thus it can help in reducing the threats of black scurf epidemics.

Disease potential is generally increased by deficiency of potassium, sodium and. It therefore, stands to reason that disease can be reduced by fertilization, although the survival of *R. solani* in artificially infested soil was shown to be little affected by soil fertility (Das and Western, 1959). Other procedures have also been employed to alter the nutrient and microbial status of soils. Nitrogen and phosphoric fertilizers are greatly applied in soil and the straw returning to field is inadequate, as a result, soil nutrients are in disequilibrium, and potassium (K) deficiency is increasing day by day (Davey and Papavizas, 1959). K deficiency has become a restrictive factor to increase yield. As a result of K deficiency, plants mechanical tissues become weak and cell wall becomes thin, the stem becomes soft and weak and can be easily invaded by diseases and insects (Das and Western, 1959). Biological potassium fertilizer (BPF) is a pure microbial preparation and there is no chemical in it. It increase yields and quality of crops, because silicate bacteria can activate the soil nutrients of K, P, Mg, Si and Mo and secrete cytokinins and gibberellins, which promote plant growth, suppress soil borne fungal pathogens and strengthens resistance to drought and diseases. So by strengthening the host plant, disease can be escaped. Moreover, the application of BPF along with normal recommended NPK, supports higher yield of potato as compared to control (Mahendran and Chandramani, 1998). Accordingly, the objective of the present studies was; to explore the efficacy of *Trichoderma harzianum*, effective microbe (EM) culture and biological potassium fertilizer (BPF) in the management of soil-borne inoculum of *R. solani*.

Materials and Methods

Maintenance and Multiplication of Inoculum

Rhizoctonia solani AG-3 isolate CL-58, the most virulent isolate ascertained by Rauf *et al.* (2007b) in a previous study, was multiplied on potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g per liter of distilled water. PDA was sterilized in a gas operated autoclave at 121°C temperature or 15 PSI for 20 min. Mass inoculum of isolate CL-58 was prepared following Naz *et al.* (2008).

Preparation of the Pots

The procedure for the preparation of potting mixture was trailed from Naz *et al.* (2008). Clay pots (8"x11") filled with sterilized potting mixture, were inoculated with weighed inoculum of isolate CL-58, mixed to the depth of 5-cm, four

days prior to sowing of one whole sprouted tuber (35-50 mm diameter.) with 3-4 eyes/pot. Each tuber was placed in a hole, to the depth of 4-5 cm and covered with potting mixture and watered weekly as required. Pots were kept in greenhouse at 25°C under natural light.

Parameters Studied

The following parameters were assessed on each plant; inhibition in eyes germination (EGI), sprouts killed (SK), stem canker index (SCI), stolon canker index (STCI), black scurf disease index (BSDI) and reduction in yield (compared to non inoculated control). Data regarding all six parameters in percentage was recorded after Naz *et al.* (2008).

Two sets of experiment were made. Data regarding percent eyes germination and sprouts killed was recorded 30 days after sowing, by harvesting first set of experiment, whereas the data regarding rest of the parameters was observed after 90 days of sowing by harvesting second set of experiment. The experiment was repeated once.

Black Scurf Management by the Use of *T. harzianum* Effective Microbe Culture and Biological Potassium Fertilizer

Following ten treatments were employed including control. In each treatment sterilized potting mixture (SPM) added with 20 g of mass inoculum (Naz *et al.*, 2008), sown with one sprouted potato tuber (S.T) was followed by the treatments viz. (T₁) control, (T₂) soil application with *T. harzianum** 20 days after sowing (T₃) soil application of *T. harzianum* at 1st, 20 and 40, days after sowing, (T₄) soil application of *T. harzianum* at the time 20, 40 and 60 days after sowing, (T₅) soil application with EM**20 days after sowing, (T₆) soil application with EM at the time, 20 and 40 days after sowing, (T₇) soil application with EM at the time, 20, 40 and 60 days after sowing, (T₈) soil application with BPF*** 20 days after sowing, (T₉) soil application with BPF at the time, 20 and 40 days after sowing, (T₁₀) soil application with BPF at the time, 20, 40 and 60 days after sowing of sprouted tubers.

Culture of *T. harzianum* an antagonistic micro-organism was obtained from Crop Disease Research Programme (CDRP), NARC and grown on (PDA) medium. Culture suspension was prepared by mixing of 3 petri plates of (90 mm. dia.) in 1 L of water with electric blender (Saleem *et al.*, 2000). Later, the conidial suspension of *T. harzianum* was adjusted to 1 × 10⁷ colony forming units (CFU)/mL employing serial dilution method.

EM4 is commercially available as EM culture, which was diluted to concentration 0.1% from liquid stock media and watered into the soil. Biological potassium fertilizer (BPF) was obtained from China through personal resources. It is registered with the trade mark "JU-WEI". It was applied as suspension @ 50 g L⁻¹ of water and 100

mL suspension per pot was applied near the roots, because BPF/silicate bacteria can function around the roots.

Data Analysis

All treatments were replicated three times. Data regarding parameters were recorded as described earlier and analyzed statistically following Steel *et al.* (1997).

Results

Soil application of three dosages of culture suspension of *T. harzianum*, effective microbe (EM) culture and biological potassium fertilizer (BPF) were evaluated by sowing cv. Desiree. Objective was to manage the soil-borne inoculum of *R. solani* and to determine the efficacy of these treatments in reducing the disease.

Eyes Germination Inhibition

All the soil applications tested nevertheless, significantly, conferred protection to eyes relative to inoculated control (Fig. 1). Treatment 4 (soil application of *T. harzianum* at the time of sowing, 20 and 40 days after sowing) with 29.98% and treatment 3 (soil application at the time of sowing, 20, 40 and 60 days after sowing) with 30.55% eyes germination inhibition gave the maximum and statistically similar protection to eyes against the inoculum followed by treatment 6 (soil application with EM at the time of sowing, 20 and 40 days after sowing) and treatment 7 (soil application with EM at the time of sowing, 20, 40 and 60 days after sowing), which in turn were at par with rest of the treatments. As a group, *T. harzianum* soil application treatments afforded the lowest inhibition in eyes germination.

Sprouts Killing

The lowest mean sprout killing was observed in treatment 3 (24.07%), 4 (23.28%) and were found statistically similar and the most effective bio-formulation in protecting the sprouts against inoculum of black scurf followed by T₆ and T₇ in which EM were applied (Fig. 2). Soil application with *T. harzianum*, 20 days after sowing (T₂) and all the three treatments with BPF were statistically similar and at par with inoculated control and rendered the lowest protection to the sprouts against *R. solani*.

Stem Canker Index

Soil application of *T. harzianum*, at the time, 20 and 40 days after sowing revealed the lowest stem canker index of 36.10% followed by treatment 4, in which an additional dose of *T. harzianum* was also applied after 60 days of sowing, but statistically no difference was observed between the two treatments (Fig. 3). Treatment 9 and T₁₀ were next

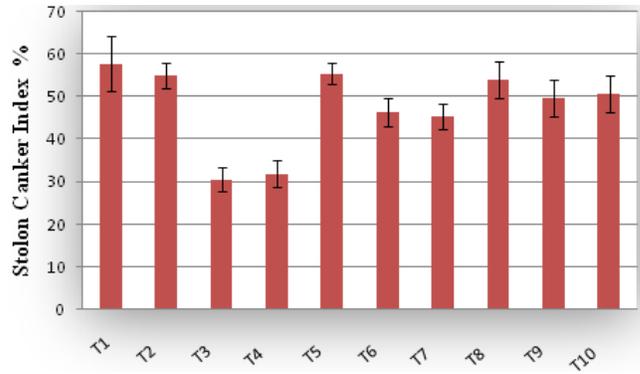


Fig. 4: Response of soil application of *T. harzianum* (T₂, T₃ and T₄), effective microbes (EM) (T₅, T₆ and T₇), biological potassium fertilizer (BPF) (T₈, T₉ and T₁₀) in inhibiting stolon canker on cv. Desiree caused by soil-borne inoculum of *R. solani* AG 3 isolate CL-58

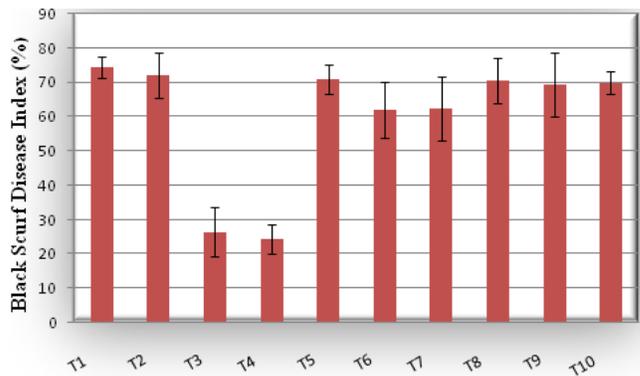


Fig. 5: Response of soil application of *T. harzianum* (T₂, T₃ and T₄), effective microbes (EM) (T₅, T₆ and T₇), biological potassium fertilizer (BPF) (T₈, T₉ and T₁₀) in black scurf production on progeny tubers of cv. Desiree against soil-borne inoculum of *R. solani*

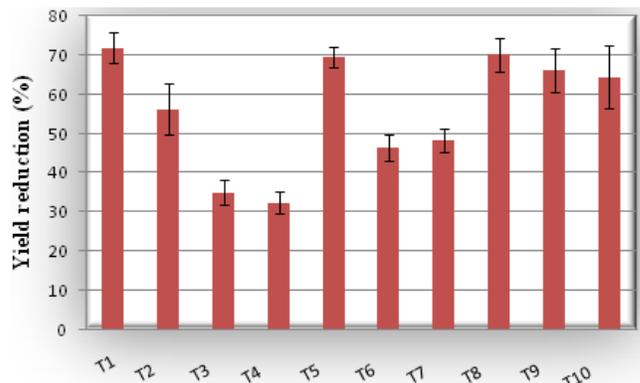


Fig. 6: Response of soil application of *T. harzianum* (T₂, T₃ and T₄), effective microbes (EM) (T₅, T₆ and T₇), biological potassium fertilizer (BPF) (T₈, T₉ and T₁₀) on yield reduction of cv. Desiree elicited by soil-borne inoculum of *R. solani*

Table 1: Response of soil application of *T. harzianum*, effective microbes (EM), biological potassium fertilizer (BPF) on cv. Desiree against soil-borne inoculum of *R. solani* AG 3 isolate CL-58 towards six disease producing symptoms of black scurf

Treatments	Disease Parameters (%)					
	Eyes germination inhibition	Sprouts killed	Stem canker index	Stolon canker index	BSDI*	Yield reduction
T ₁ Control (20 g mass inoculum + sowing of 1 sprouted tuber)	76.67 a**	55.55 a**	73.33 a**	57.70 a**	74.44 a**	71.93 a**
T ₂ (20 g mass inoculum + soil application with <i>T. harzianum</i> 20 days after sowing)	47.78 b	43.45 ab	70.00 a	55.00 ab	72.00 ab	56.26 bc
T ₃ (20 g mass inoculum + soil application of <i>T. harzianum</i> at the time of sowing, 20 and 40, days after sowing)	30.55 c	24.07 c	36.10 c	30.60 c	26.43 c	35.09 d
T ₄ (20 g mass inoculum + soil application of <i>T. harzianum</i> at the time of sowing, 20, 40 and 60 days after sowing)	27.78 d	23.28 c	37.78 c	31.90 c	24.40 c	32.41 d
T ₅ (20 g mass inoculum + soil application with EM 20 days after sowing)	58.89 b	41.91 ab	69.55 a	55.55 ab	71.00 ab	69.52 a
T ₆ (20 g mass inoculum + soil application with EM at the time, 20 and 40 days after sowing)	51.11 b	40.28 b	49.67 bc	46.33 ab	62.00 b	46.52 c
T ₇ (20 g mass inoculum + soil application with EM at the time, 20, 40 and 60 days after sowing)	52.22 b	41.11 b	48.89 bc	45.33 b	62.44 b	48.37 c
T ₈ (20 g mass inoculum + soil application with BPF 20 days after sowing)	55.55 b	46.82 ab	73.89 a	54.00 ab	70.44 ab	70.19 a
T ₉ (20 g mass inoculum + soil application with BPF at the time 20 and 40 days after sowing)	46.67 bc	43.98 ab	52.67 b	49.67 ab	69.33 ab	66.29 ab
T ₁₀ (20 g mass inoculum + soil application with BPF at the time, 20, 40 and 60 days after sowing)	46.67 bc	41.91 ab	53.33 b	50.67 ab	69.78 ab	64.41 ab
LSD (5%)	16.90	13.92	12.73	12.01	11.35	10.78

*Black scurf disease index

**Means within column followed by the same letters are not significantly different according to LSD test ($P=0.05$)

to T₃ and T₄ but statistically were at par with T₆ and T₇ where EM was applied.

Stolon Canker Index

Treatment 3 and 4 manifested the best and statistically similar control of stolon canker i.e., 30.60 and 31.95%, respectively followed by T₇, 45.33% (Fig. 4). Efficacy of the rest of treatments in reducing stolon canker index was statistically at par with the inoculated control.

Black Scurf Disease Index

T. harzianum gave significant protection against inoculum of *R. solani* and significantly less amount of black scurf was accumulated on progeny tubers harvested from the plants in which culture suspension of the antagonist were applied i.e., T₃ and T₄ (Fig. 5) followed by T₆ and T₇, similar statistically. Whereas, soil application of all three bio-formulation tested and T₂ and T₅ rendered no reduction in black scurf disease index on progeny tubers and were at par with the control.

Yield Reduction

The lowest reduction in yield is evident by treatment 3 and 4 (Fig. 6). Treatment 6 and 7 were rated the second best in inhibiting yield reduction. Soil application with *T. harzianum* 20 days after sowing (T₂) was found statistically at par with T₆ and T₇.

Soil application with *T. harzianum* at the time of sowing followed by two (T₃) and three (T₄) dosages at 20 days intervals gave significant protection against soil-borne inoculum of the fungus and significantly less inhibition in eyes germination, reduction in sprouts killing, stem canker index, stolon canker index and black scurf disease index were observed, which ultimately contributed to better crop stand and increased yield as compared to inoculated control and rest of the treatments (Table 1). An additional dose (60 days after sowing) of culture suspension of *T. harzianum* in the soil did not have supplementary effect in inhibiting the disease in question.

Discussion

T. harzianum is one of the oldest and the most frequently evaluated biocontrol agents against *Rhizoctonia* on various crops (Chet et al., 1982; Beagle-Ristaino and Papavizas, 1985; Tsror et al., 2001). Antagonism by *T. harzianum* depends on mycoparasitism rather than antibiosis, as evident from the relatively slight inhibition of mycelial growth of *R. solani*, particularly AG-3, in dual culture (Shternshis, 2002). Mycoparasitism by *T. harzianum* is a complex process, involving recognition of the host, attachment to the mycelium, coiling around the hyphae, partial degradation of the cell wall and penetration of the host mycelium (Elad et al., 1983a, b; Benhamou and Chet, 1993). Cell wall degradation is achieved by six chitin-induced chitinolytic enzymes (comprising two B-1, 4-N- acetylglucosaminidases and four endochitinases),

all of which are required for effective parasitism (Haran *et al.*, 1995). Once, the host mycelium has been penetrated, additional extracellular enzymes such as lipases and proteases are produced to induce degradation of the cell contents (Elad *et al.*, 1982). When attacked, hyphae of the susceptible host respond with rapid vacuolation, collapse and disintegration (Chet and Baker, 1981). Besides being an aggressive mycoparasite, *T. harzianum* is also known to enhance plant growth in the absence of any pathogens, probably by producing plant growth promoting metabolites in the rhizosphere (Chang *et al.*, 1986; Kleifeld and Chet, 1992). It has, however, been observed to parasitize endomycorrhizal fungi (Rousseau *et al.*, 1996), on which potato seems to be particularly dependent for optimal growth (Gerdemann, 1968). *T. harzianum* effectively controlled the disease when applied as a soil drench (Jager *et al.*, 1991) and also found the most effective in reducing disease incidence and increase in yield over control in greenhouse (Hazarika *et al.*, 2000). According to Adams (1990), *T. harzianum* has potential for broad-spectrum control of fungal pathogens, but cannot be applied cost-effectively, because of the excessive amounts of antagonist/its formulated products required to obtain disease control. In the present study, *T. harzianum* at the concentration of 1×10^7 cfu mL⁻¹ applied @ 300 mL per pot in three split dosages was found the best in inhibiting the disease in artificially infested sterilized soil. Further investigations of the dosage requirement, range of the pathogens affected, soil colonization capacity and effect on beneficial organisms of this antagonist in different soils is certainly required.

T. harzianum is one of the oldest and most frequently evaluated biocontrol agents against *Rhizoctonia* on various crops (Chet *et al.*, 1982; Beagle-Ristaino and Papavizas, 1985; Tsror *et al.*, 2001). Antagonism by *T. harzianum* depends on mycoparasitism rather than antibiosis, as evident from the relatively slight inhibition of mycelial growth of *R. solani*, particularly AG-3; in dual culture (Shternshis, 2002). Besides, being an aggressive mycoparasite (Elad *et al.*, 1983b; Benhamou and Chet, 1993; Haran *et al.*, 1995; Elad *et al.*, 1982; Chet and Baker, 1981), *T. harzianum* is also known to enhance plant growth in the absence of any pathogens, probably by producing plant growth promoting metabolites in the rhizosphere (Chang *et al.*, 1986; Kleifeld and Chet, 1992).

Conclusion

Soil application with *T. harzianum* at sowing followed by two and three dosages at 20 days intervals significantly protected the eyes from the fungal attack, leading to better crop stand and increased yield as compared to inoculated control and other treatments.

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