



Occurrence and bio-control of stem canker and black scurf (*Rhizoctonia solani*) of potato (*Solanum tuberosum*) in subtropical plains of India

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Potato (*Solanum tuberosum* L.) is one of the major tuber crops in the hills and plains of India and is grown in 2 m ha with an annual production of 45 mt (Indian Hort. Database 2013). Black scurf caused by *Rhizoctonia solani* is one of the major diseases of potato wherever cultivated (Ahmad *et al.* 1995) and is reported to occur at a moderate to high levels in the country (Khurana *et al.* 1998). Black sclerotia on the surface of the tuber and sunken necrotic lesions on roots, stems and underground parts of potato plants are common symptoms of *Rhizoctonia* disease that often go unnoticed (Carling and Leiner 1986). Although black scurf is the most noticeable sign, stem canker is the most damaging component of the disease. Partial or complete girdling of stems result in rusetting of plant tops, purple pigmentation, upward chlorosis and rolling of leaves. In India, black scurf phase is common over stem canker. During the survey for late blight in 2006-07 in Kanpur and surrounding areas, stem canker was also noticed (Narayana Bhat *et al.* 2010). Although economically important, efforts on systematic study on the incidence of stem canker in potato and its management have not been attempted fully. Effective management of black scurf is possible through chemicals and bio-agents (Arora and Somani 2001). However, reports on testing of bio-agents against stem canker and black scurf simultaneously is scanty. Hence, in the present study, survey on the occurrence of stem canker in major potato growing areas of Kanpur and Fatehpur districts of Uttar Pradesh and its management through eco-friendly bio-control agents were attempted.

A roving survey was conducted in Fatehpur and Kanpur districts of Uttar Pradesh for three successive years of

2009-10, 2010-11 and 2011-12 for stem canker of potato in farmers' field. In each district, 10 villages belonging to various tehsils and a minimum of 10 fields per village were selected for the survey. In each field, 10 spots of 15 plants were observed for prevalence of stem canker and based on this observation, per cent incidence was calculated.

Fourteen *Trichoderma* spp. isolated from the rhizosphere of potato, chilli and bell pepper by serial dilution (Aneja 2003) and five each isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* (Division of Plant Pathology, IARI, New Delhi) were evaluated for their efficacy against *Rhizoctonia solani*, by dual culture technique. Five mm bits of fresh cultures of both *R. solani* and *Trichoderma* were placed at the opposite ends of Petri dish and allowed to grow at 27 °C in BOD incubator till such time when the growth of *R. solani* in untreated control reached the periphery of the other end. Test fungi were also observed for overgrowth on *R. solani* mycelium. Fresh 48 hr old cultures of five each isolates of *B. subtilis* and *P. fluorescens* were streaked at both ends, equidistant point along the perimeter of the plate and simultaneously 5 mm plug for the leading edge of 7 day old culture of *R. solani* was placed at the centre of the plate. Plates without bacteria served as control. Three replications were maintained for each treatment. Plates were incubated at 27°C till such time when the growth in the control covered the entire Petridish. Inhibition % was calculated based on the radial growth of *R. solani* and bio-control agents (Dennis and Webster 1971).

Formulations of *P. fluorescens*, *B. subtilis* and *Trichoderma* were prepared by following the methods of (Nandakumar *et al.* 2001, Jeyarajan 2006 and Ramanujam *et al.* 2010). Loopful of freshly growing *P. fluorescens* and *B. subtilis* were transferred to King's B broth and nutrient broth, respectively and incubated in a rotary shaker at 150 rpm for 48 hr. Four hundred ml of the bacterial cultures were thoroughly mixed with sterilised talcum powder. Fifteen g calcium carbonate and 10 g carboxy methyl cellulose were added and air dried under shade to reduce the moisture content to 20%. Formulation was packed in a polypropylene

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bag and sealed. At the time of application, the population of bacteria was 3.1×10^8 cfu/g. *Trichoderma* was multiplied for 10 days in pre-soaked sterile sorghum seeds incubated at 28°C in a BOD incubator. Sorghum seeds containing green *Trichoderma* mass were air dried overnight and ground to a fine powder in a blender. Sterilised mixture of talcum powder and carboxy methyl cellulose (10 g/kg) were added to the powder so as to get a desired concentration of 2.5×10^8 cfu/g formulation.

A field experiment was conducted during 2011-12 and 2012-13 *rabi* seasons on the efficacy of nine selected bio-agents against stem cancker and black scurf of potato at Mahuagaon of Kanpur tehsil of Kanpur district, Uttar Pradesh. The treatments consisted of five *Trichoderma* isolates, two each of *P. fluorescens* and *B. subtilis*. Pencycuron, which was reported to be highly effective against black scurf *in vitro* and field conditions, was used as standard control @ 250 g/acre seed treatment (Thind *et al.* 2002).

Seed tuber treatment with formulations was applied @ 12.5 g/kg to heavily infected tubers (> 4 black scurf index). Each treatment was replicated thrice in a RBD design with a plot size of 3 m × 2 m with a spacing of 60 cm × 20 cm. Crop was sown in second week of November and raised as per recommended package of practices. Recommended dosage of fertiliser 150N:80P:100K with 50% N and full dose of P and K were applied at the time of planting and 50% N at the time of earthing up.

Observations on incidence and severity of stem cancker and black scurf were made at the time of harvesting during first week of March. Ten randomly selected plants were harvested, washed and rated for stem cancker on a 0-5 scale (Brewer and Larkin 2005) where 0= no disease symptoms; 1= brown discoloration of stems; 2= canckers covering < 25% of stem circumference; 3= 25-75% stems covered by canckers; 4= 75% coverage by stem canckers; and 5= stem completely nipped off or death of the plants. Incidence of stem cancker was measured as the percentage of plants with a severity rating of 2 or greater. Black scurf was assessed based on a scale of 0-5 (Brewer and Larkin 2005) where 0= no visible sclerotia; 1= sclerotia covering 1% of the skin covered tuber surface; 2= 2-5% covered; 3= 5-10% covered; 4= 10-15% and 5= > 15% tuber covered by sclerotia. Incidence of black scurf was calculated as the percentage of tubers with a severity rating of 1 or greater. Yield data was also recorded in each plot and converted into tonnes/ha. Treatment means were compared using Duncan's Multiple Range test (DMRT).

Survey carried out during 2009-10 at Kanpur and

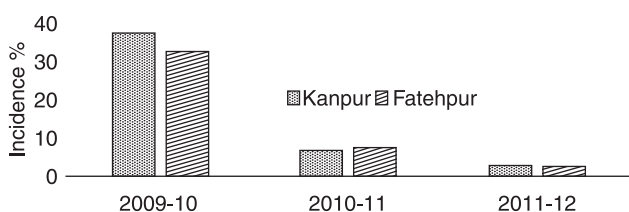


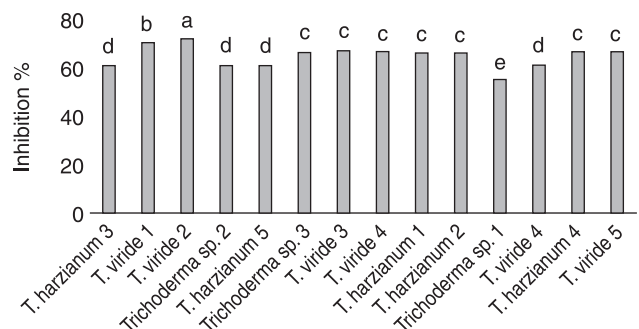
Fig 1 Incidence of stem cancker of potato in UP

Fatehpur districts of Uttar Pradesh revealed higher incidence of 32.6% in Fatehpur and 38.5% in Kanpur. Incidence ranged from 28.2-57.5% and 19.9 to 41.6% in these two districts respectively (Data not shown). During 2010-11 and 2011-12, the incidence was drastically low with 6.8-7.5% during 2010-11 and 2.5-2.8% during 2011-12 in these two districts. Cool temperature and high soil moisture favour *Rhizoctonia* development in potato (Wharton *et al.* 2007). During the present study, these factors might have contributed for the variation in disease severity. Though report on incidence of stem cancker is scanty in India, incidence in the neighbouring country of Bangladesh is reported to be around 10% (Khandaker *et al.* 2006).

Highest inhibition (72.2%) was recorded by *T. viride* 2 followed by *T. viride* 1 (Fig 2). Two isolates recorded 67.4%, and five isolates recorded 66.7% inhibition of *R. solani*. Two isolates of *T. harzianum* (1 and 2) and three isolates of *T. viride* (1, 2 and 3) that completely overgrew *R. solani* apart from showing significant inhibition were selected for further study under field conditions. Among the bacterial isolates, two each of *B. subtilis* and *P. fluorescens* were selected based on inhibitory activities *in vitro*. *B. subtilis* DTBS 5 and DTBS 4 inhibited *R. solani* to an extent of 73.7% and 51.4% respectively and were superior to other isolates (Fig 3). Similarly, *P. fluorescens* pf3 and pf80 isolates were highly effective with inhibition of 68.7 and 49.7% respectively. Destructive mycoparasitism of *R. solani* by *Trichoderma* and disintegration of mycelium by *P. fluorescens* have been well established (Pandey and Pundhir 2013)

Effect of bio-agents against R. solani under field conditions

During 2011-12 and 2012-13, bio-agents were significantly superior to untreated control in reducing stem cancker and black scurf and improving the crop yield (Table 1). During 2011-12, *B. subtilis* DTBS-5 was highly effective against stem cancker followed by *P. fluorescens* pf3. Incidence and severity in these treatments ranged 23.3-26.6% and 0.87-0.93% respectively. Almost a similar trend was observed with these two isolates during 2012-13. *B. subtilis* DTBS-4 and *P. fluorescens* pf80 were also effective. *T. viride* 1 showed moderate effect followed by *T. viride* 2



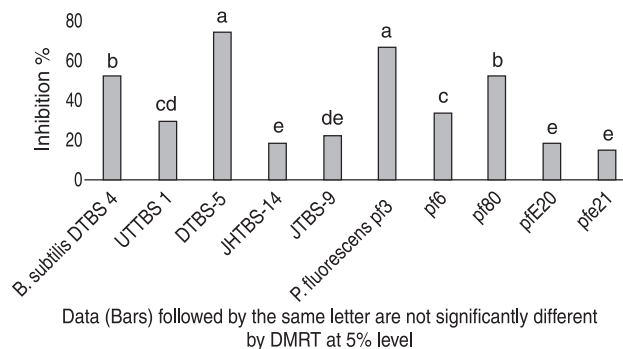
Data (Bars) followed by the same letter are not significantly different by DMRT at 5% level

Fig 2 In vitro inhibition of R. solani by Trichoderma

Table 1 Effect of bio-agents against stem canker and black scurf of potato

Treatment	Stem canker			Black scurf			Yield tonnes/ha								
	Incidence (%)			Severity (%)			Incidence (%)			Severity (%)					
	2011-12	2012-13	Pooled	11-12	12-13	Pooled	2011-12	2012-13	Pooled	11-12	12-13	Pooled			
<i>Trichoderma viride</i> (1)	50.0 (45.0) ^{cd}	33.2 (35.2) ^{bc}	41.6 (40.1) ^{cd}	1.43 ^{cd}	1.13 ^d	1.28 ^{cd}	62.0 (51.9) ^d	62.0 (51.9) ^{bc}	62.0 (51.9) ^{cd}	1.65 ^e	1.34 ^{de}	1.5 ^e	27.1 ^{cd}	30.2 ^e	28.3 ^c
<i>Trichoderma harzianum</i> (1)	53.3 (46.9) ^e	40.0 (39.1) ^b	46.7 (43.0) ^{bc}	1.5 ^{bc}	1.4 ^{bc}	1.45 ^{bc}	75.3 (60.3) ^e	66.0 (54.5) ^{bc}	70.6 (57.4) ^e	2.32 ^{bc}	1.81 ^{bc}	2.1 ^c	24.5 ^{de}	29.2 ^{ef}	26.9 ^d
<i>T. harzianum</i> (2)	66.7 (54.8) ^b	40.0 (39.2) ^b	53.3 (47.0) ^b	1.8 ^b	1.43 ^b	1.61 ^b	87.3 (69.1) ^b	74.7 (59.8) ^{ab}	81 (64.5) ^b	2.53 ^b	2.01 ^b	2.3 ^b	24.1 ^e	29.8 ^e	27.0 ^{cd}
<i>T. viride</i> (2)	53.3 (46.9) ^e	46.7 (41.1) ^{ab}	50 (44.0) ^{bc}	1.53 ^{bc}	1.23 ^{bc}	1.38 ^c	81.3 (64.7) ^{bc}	58.7 (50.0) ^c	70.0 (57.4) ^c	2.10 ^{cd}	1.65 ^{cd}	1.9 ^d	26.7 ^{cd}	30.7 ^{de}	28.7 ^c
<i>T. viride</i> (3)	56.7 (48.9) ^{bc}	36.7 (37.2) ^{bc}	46.7 (43.0) ^{bc}	1.5 ^{bc}	1.27 ^{bc}	1.38 ^c	75.3 (60.2) ^e	62.0 (52.0) ^{bc}	68.6 (56.1) ^e	2.11 ^{cd}	1.57 ^{cd}	1.8 ^d	26.2 ^{de}	31.0 ^{cd}	28.6 ^c
<i>Pseudomonas fluorescens</i> pf3	26.6 (31.0) ^f	23.3 (28.8) ^{cd}	25.0 (29.9) ^{ef}	0.93 ^e	0.83 ^e	0.88 ^f	51.3 (45.7) ^{de}	40.0 (39.1) ^d	45.6 (42.5) ^e	1.23 ^g	0.62 ^g	0.9 ^f	30.9 ^{ab}	35.3 ^a	33.1 ^a
<i>P. fluorescens</i> pf80	40.0 (39.1) ^{de}	36.7 (37.2) ^{bc}	38.8 (38.2) ^d	1.17 ^{de}	1.17 ^{cd}	1.16 ^{de}	77.3 (61.5) ^{bc}	52.0 (46.1) ^{cd}	64.6 (53.9) ^{cd}	1.89 ^d	1.10 ^{ef}	1.5 ^e	29.2 ^{bc}	32.5 ^{bc}	30.9 ^b
<i>Bacillus subtilis</i> DTBS-5	23.3 (28.2) ^{fg}	16.7 (23.3) ^d	20.0 (26.1) ^{fg}	0.87 ^{ef}	0.77 ^e	0.81 ^f	44.0 (41.5) ^e	41.3 (39.9) ^d	42.6 (40.7) ^f	0.86 ^h	0.83 ^{fg}	0.8 ^g	32.5 ^a	33.5 ^{ab}	33.0 ^a
<i>B. subtilis</i> DTBS-4	33.3 (35.2) ^{ef}	23.3 (28.8) ^{cd}	28.3 (32.0) ^e	1.1 ^e	0.87 ^e	0.98 ^{ef}	60.7 (51.1) ^d	52.0 (46.1) ^{cd}	56.3 (48.6) ^d	1.47 ^f	1.0 ^f	1.2 ^f	30.7 ^{ab}	33.1 ^{bc}	32.3 ^a
Un-treated control	83.3 (66.13) ^a	56.7 (48.9) ^a	70.0 (57.5) ^a	2.6 ^a	1.77 ^{ag}	2.18 ^a	94.7 (78.8) ^a	84.7 (67.1) ^a	89.3 (73.0) ^a	4.29 ^a	2.41 ^a	3.4 ^a	21.0 ^f	27.5 ^f	24.3 ^e
Pencycuron (std. check)	16.7 (23.3) ^g	13.3 (21.1) ^d	15.0 (22.5) ^g	0.6 ^f	0.4 ^f	0.5 ^g	22.0 (27.9) ^f	19.3 (25.8) ^e	20.6 (26.9) ^g	0.41 ⁱ	0.3 ^h	0.4 ^h	30.8 ^{ab}	34.2 ^{ab}	32.5 ^a

Figures in parenthesis are arc sine transformed values. Data followed by the same letter in column are not significantly different by DMRT at 5% level

Fig 3 In vitro inhibition of *R. solani* by bacterial antagonists

and 3. Two isolates of *T. harzianum* showed lesser effect, though found significantly superior to untreated control. Pooled data for both the years indicated that *B. subtilis* DTBS 5 was superior to other bio-agents, which recorded only 20.0% incidence, followed by *P. fluorescens* pf3 that recorded 25.0%. Effectiveness of *B. subtilis* against black scurf was reported earlier by Somani and Arora (2010). Brewer *et al.* (2005) reported that *B. subtilis* is one of the most consistent bio-agent for control of stem canker.

During 2012-13, among the bio-agents, lowest incidence (44.0%) of black scurf was noticed in *B. subtilis* DTBS-5 treatment, which was at par with *P. fluorescens* pf3 (51.3%). However, severity in *P. fluorescens* pf3 treatment was high (1.23) as against only 0.86 in *B. subtilis* DTBS 5, which was significantly superior to other bio-agents. Incidence in *Trichoderma* treated plots varied from 62.0% in *T. viride* 1 to 87.3% in *T. harzianum* 2. Severity in these treatments ranged from 1.65 to 2.53 as against 4.29 in untreated control. Almost similar trend of less incidence and severity of black scurf was noticed during 2012-13 compared to 2011-12 in majority of the treatments. Pooled data of two years indicated that *B. subtilis* DTBS 5 and *P. fluorescens* pf3 were at par and significantly superior to other bio-agents in managing black scurf. *B. subtilis* DTBS 4 was next in superiority followed by *P. fluorescens* pf80 which showed average severity of 1.5 which was at par with *T. viride* 1. In rest of the *Trichoderma* spp., severity ranged from 1.8 to 2.3 with corresponding incidence of 68.6% to 81.0%.

Highest yield of 32.5 tonnes/ha was recorded in *B. subtilis* DTBS 5, which was at par with pencycuron, *B. subtilis* DTBS 4 and *P. fluorescens* pf3 treatments. *Trichoderma* treated plots, yielded 24.1 to 27.1 tonnes/ha as against 21.0 tonnes/ha in untreated control. However, during 2012-13, highest yield (35.3 tonnes/ha) was recorded in *P. fluorescens* pf3 closely followed by *B. subtilis* DTBS 5, which was at par with *B. subtilis* DTBS 4 and pencycuron. Pooled analysis for yield showed *P. fluorescens* pf3 to be highly effective (33.1 tonnes/ha) followed by *B. subtilis* DTBS 5 and *B. subtilis* DTBS 4 and pencycuron. *Pseudomonads* are reported to be involved in growth promotion by the production of phytohormones, nitrogen fixation, phosphate solubilisation (Park *et al.* 2005). *Trichoderma* treated plots yielded 26.9 to 28.7 tonnes/ha as against 24.3 tonnes/ha in untreated control. *Trichoderma*

spp. have positive effect root morphology enabling roots to explore large volume of soli (Maral *et al.* 2012).

SUMMARY

High incidence (32.6 to 37.5%) of stem canker during 2009-10 followed by a moderate (6.8 to 7.5%) and low (2.5 to 2.8%) level during 2010-11 and 2011-12 respectively were recorded in Kanpur and Fatehpur districts of Uttar Pradesh. *Trichoderma harzianum* isolates, 1 and 2 and *Trichoderma viride* isolates, 1, 2 and 3 completely overgrew *Rhizoctonia solani* mycelial growth by > 65%. *Bacillus subtilis* DTBS 5 and DTBS 4 and *Pseudomonas fluorescens* pf-3 and pf80 inhibited *R. solani* by > 50% and were significantly superior to other isolates. Selected *B. subtilis* and *P. fluorescens* reduced stem canker by 45.5 to 63.6% and black scurf by 55.8 to 76.4% under field conditions. Highest tuber yield (33.1 tonnes/ha) in *P. fluorescens* pf3 treatment on par with *B. subtilis* DTBS 5 (33.0 tonnes/ha) and pencycuron (32.5 tonnes/ha) was recorded. *Trichoderma* spp were moderate in reducing the disease and increasing the tuber yield.

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REFERENCES

- Ahmad I, M H Soomro, S Khalid, S Iftikhar, A Munir, K Burney and A Hussain. 1995. Recent distributional trends of potato diseases in Pakistan. (In) *Proceedings of the National Seminar* held at NARC, Islamabad, Pakistan, 23-25 April 1995, pp 117-25.
- Aneja K R. 2003. *Experiments in Microbiology, Plant Pathology and Biotechnology*. New Age International (pvt.) Ltd, New Delhi.
- Arora R K and Somani A K. 2001. A bio-formulation for control of black scurf disease of potato. *Journal of the Indian Potato Association* **28**: 88-9.
- Brewer M T and Larkin R P. 2005. Efficacy of several bio-control organisms against *Rhizoctonia solani* on potato. *Crop Protection* **24**: 939-50.
- Carling D E and R H Leiner. 1986. Isolation and characterization of *Rhizoctonia solani* and binucleate. *Rhizoctonia solani*-like fungi from aerial stems and subterranean organs of potato plants. *Phytopathology* **76**: 725-9.
- Dennis C and Webster J. 1971. Antagonistic properties of species of *Trichoderma*—III. Hyphal interactions. *Transaction of British Mycological Society* **57**: 363-9
- Indian Horticulture Database 2013. National Horticultural Base (NHB), Gurgaon. Ministry of Agriculture, Government of India, p 289.
- Jeyarajan R. 2006. Prospects of indigenous mass production and formulation of *Trichoderma*, pp 74-80. (In) *Current Status of Biological Control of Plant Diseases Using Antagonistic Organisms in India*. Rabindra R J and Ramanujam B (Eds). Project Directorate of Biological Control, Bangalore, p 445.
- Khandaker M M, Khurshed, Alam Bhuiyan and Abul Khair. 2006. Prevalence of stem canker and black scurf disease of potato in Bangladesh. *Bangladesh Journal of Botany* **35**: 87-90.
- Khurana S M P, Pandey S K, Patel R L, Singh R B, Pundir V S, Pathak S P and Pani A K. 1998. Surveillance for potato diseases in India over last five years. *Journal of the Indian Potato Association* **25**: 16-20.
- Maral S, Sozer Y, Gezgin C, Kara S, Sargin R, Eltem, F and Vardar-Sukan F. 2012. Evaluation of properties of *Trichoderma* spp. as bio-control agents and bio-fertilizer. *Environmental Engineering and Management Journal*, 11, supplement, S 154.
- Nandakumar R, Babu S, Viswanathan R, Sheela J, Raguchander T, Samiyappan R. 2001. A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. *Biological Control* **46**: 493-510.
- Narayana Bhat M, Singh B P, Arora R K, Rai R P, Gard I D and Trehan S P. 2010. Assessment of crop losses in potato due to late blight disease during 2006-2007. *Potato Journal* **37**: 37-43.
- Pandey S and Pundhir V S. 2013. Mycoparasitism of potato black scurf pathogen (*Rhizoctonia solani* Kuhn) by biological control agents to sustain production. *Indian Journal of Horticulture* **70**: 71-5.
- Park M S, Jung S R, Lee M S, Kim K O, Do J O, Lee K H, Kim S B and Bae K S. 2005. Isolation and characterization of bacteria associated with two sand dune plant species, *Calystegia soldanella* and *Elymus mollis*. *Journal of Microbiology* **43**: 219-27.
- Ramanujam B, R D Prasad, S Sriram and R Rangeswaran. 2010. Mass production, formulation, quality control and delivery of *Trichoderma* for plant disease management. *Journal of Plant Protection Sciences* **2**: 1-8.
- Somani A K and Arora R K. 2010. Field efficacy of *Trichoderma viride*, *Bacillus subtilis* and *Bacillus cereus* in consortium for control of *Rhizoctonia solani* causing black scurf disease of potato. *Indian Phytopathology* **63**: 23-5.
- Thind T S, Chander Mohan and Kaur S. 2002. Promising activity of pencycuron, a henylurea-based fungicide for effective management of black scurf of potato. *Indian Phytopathology* **55**: 39-44.
- Wharton P, Kirk W, Berry D and Snapp S. 2007. *Rhizoctonia* stem canker and black scurf of potato. Extension Bulletin E 2994, Michigan State University, USA.