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Screening of Botanicals and Bio-agents to Manage Potato Black Scurf, Caused by *Rhizoctonia solani* Kühn

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ABSTRACT

Potato (*Solanum tuberosum*) black scurf is caused by *Rhizoctonia solani* is a seed and soil born disease making severe problem in many potato growing regions in the world since age, resulting huge economic losses. An experiment was conducted by using ecologically sound products, botanicals (Neem, onion, ginger, turmeric and garlic) in laboratory condition as well as in pot condition and bio-agents (*Trichoderma viride, Trichoderma asperellu, T. koningii, T. harzianum* and *T. longibrachiatum*) in pot condition to find out the more effective approaches for disease management during two consecutive years. In laboratory, botanicals were amended with Potato Dextrose Agar for growing the fungus and for pot condition potato seed tubers were treated with different concentration of aqueous extract of botanicals and spore suspensions of bio-agents. Among all 15ml, 20ml and 25ml of garlic extract successfully 100% restrict the growth of *Rhizoctonia solani.* All concentrations of ginger extract were found to be less effective. 25% garlic extract registered 79.22% of eye germination of seed tuber and 5% of ginger extract registered the least 11.84%. Minimum disease incidence (5.68%) and disease severity (0.16%) were recorded in 25% of garlic extract and maximum (disease incidence 78.17% and disease severity 12.95%) were recorded in 5% of ginger extract. *T. harzianum* did the best at eye germination %, reduction of disease severity and incidence over control during 2 years (89.74%, 83.30% and 57.60% respectively). Oppositely, *T. longibrachiatum* was not enough efficacious for controlling the disease compare to others. These tested selective treatments can shift effective, profitable and ecologically sound management of this potato disease.

***Key words*:** Black scurf, Bio-agent, Bio pesticide, Phyto-extract, *Rhizoctonia solani*

Potato (*Solanum tuberosum*) is an annual herbaceous dicotyledonous crop, which belongs to the Solanaceae family. In the world, potato ranked 4th all after rice, wheat and maize [1]. Except potato Indian vegetable basket is incomplete. It provides the energy, protein and other edible products. It is nutritionally superior vegetable and also staple food in India as well as worldwide. Unfortunately, potato crop suffers from immense fungal, viral, bacterial and nematode diseases. Potato plant suffers thirty fungal, three bacterial and ten viral pathogens [2-3]. Within fungal diseases potato black scurf disease caused by *Rhizoctonia solani* (*Thenatephorus cucumeris* Frank) become a major threat to potato crop worldwide and present severe to moderate form in different region [4-5]. *Rhizoctonia* is an ancient Greek word (*rhiza*, "root" + *ktonos*, "murder") generally called black scurf. It is one of the oldest (in 1858, Julius Kuhn first observed on potato tuber) and the most ordinary infection of the potato plant at different time during growing. From planting to harvest, it attacks the potato plants but early stage is the most serious one because eye germination inhibition and sprout death occur in this stage. Due to eye germination inhibition and sprout killing, it can cause up to 100% yield loss. Malformation, cracking, pitting and necrosis on stem end are other symptoms of this disease which results in poor quality tubers. The root cause of reduction of quality and market value is, formation of black irregular lumpy encrustations on the surface of potato tubers. Alotibi *et al.* [6] studied the antifungal activity of the aqueous botanical extracts against some fungal species. Onarag and Sanlam [7] used methanol extracts of different parts (leave, flower, root, fruit and shoots) of *Smilax excels*, *Trachystemon orientalis*, *Phytolacca americana*, *Rhododendron ponticum* and *Prunus laurocerasus* against three economically important plant diseases *Botrytis cinerea*, *Alternaria solani* and *Rhizoctonia solani*. Kumar *et al.* [8] found that, this fungus causes significant yield losses up to 34% in few years. For controlling diseases random pesticides application has done a huge harm to the animals, human beings, vegetation as well as environment as a whole. Estimation of the world health organizations (WHO), every year approximately 7.5 lakhs people are getting ill

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because of pesticides poisoning and among them 14,000 die in agony as these enter the food chains [9]. Undesirable impact of chemical like, long degradation periods, drastic and acute toxicity, accumulation in food chain and destroy both harmful and useful pests [10]. Day by day increasing awareness among the consumers has drawn the attention of the farmers for shifting chemicals to eco-friendly bio control agents and botanicals. These days' bioactive plant origin products as well as bio control agents become the focus of attention for being less persistent in environment, safe to mammals as well as non-target organisms. Many researchers have been confirmed the dynamic activity of many kinds of plant extracts against different phyto-pathogenic fungi. However still it is not enough. Keeping all these points in mind, effort for the present studies have been made to manage black scurf disease with ecologically sound and easily available natural products viz., phyto-extracts (Neem, onion, ginger, turmeric and garlic) and bio-agents (*Trichoderma asperellum, T. koningii, T. viride, T. longibrachiatum* and *T. harzianum*), which are inexpensive, non-hazardous and more ecofriendly to the potato farmers.

MATERIALS AND METHODS

Isolation of Rhizoctonia solani from infected potato tubers

From a local market the potato tubers were collected which was infected by black scurf disease. Sclerotia were separated and collected from those potatoes and washed those properly by using tap water. After that surface sterilization was done to eliminate the contaminants with 0.1 percent solution of mercuric chloride for 30 seconds, then sclerotia were washed three times in sterile distilled water. On sterile filter papers washed sclerotia were allowed to dry. Those sclerotia pieces were then aseptically plated on the Potato Dextrose Agar (PDA) medium. At 27 ± 2°C the inoculated plates were incubated for 7 days [11].

Mass multiplication of inoculums

Sorghum grains were used mass multiplied of the test fungi [12]. Grains were presoaked in 5% sucrose solution overnight and next day drained excess water. Soaked grain were transferred into heat tolerant plastic bag @100 gm and autoclaved (at 15 lb psi, 121.6° C) for 20 minutes. At room temperature the autoclaved bags were allowed to cool and with five mm discs of 4 days old culture of *Rhizoctonia solani* which was grown on PDA inoculated aseptically. 5 disc per bag were added and bags were incubated for 3 weeks at 27±2°C. Bags were shake regularly and uniformly at 24 hrs interval.

Preparation of phyto-extracts

For preparation of phyto-extracts, 100 gram of plant products (neem leaves, garlic clove, onion bulb, rhizomes of ginger and turmeric) were collected, thoroughly wash in normal tap water, air dried at room temperature and homogenized with equal amount of distilled water (100mL) by crashing them separately with electric grinder. The extract was strained through double-layered muslin clot. The supernatant was collected consequently filtered through Whatman No.1 filter paper which was considered as standard solution [13].

Preparation of spore suspension

Antagonistic fungi were isolated from experimental fields of Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan and identified on the basis of their morphological and cultural characteristics. Culture suspension of bio agents were prepared by mixing of 3 petri plates of (90 mm dia.) in 1 L of sterilized water with electric blender [14]. Later, the suspension of bio agents was adjusted to 1×10^{-5} to see the colony forming units (CFU)/ml employing serial dilution method, and it was per L 2.3×10^{8}.

In-vitro study

Poison food technique was followed [15] for *in-vitro* study of using botanicals. Five different concentrations (5ml, 10ml, 15ml, 20ml, and 25ml) were used for the study. Each level concentrations were mixed separately per 100ml of PDA. PDA without aqueous extract served as control. Radial growth was taken at 10 days after inoculation. Calculation of per cent inhibition in radial growth over control (check) was done by using the formula which was given by Vincent [16].

Preparation of the planters

8" plastic bags filled with sterilized potting mixture, were inoculated with previously weighed inoculum of *Rhizoctonia solani*, mixed to the depth of 5-cm, 30 days prior to sowing for better establishment of pathogen inoculum in soil and watered them 2-3 times in a week. Two whole sprouted tuber (35-50 gm) with 3-4 eyes/bag were planted. For the study of botanicals, seed tubers were dipped into aqueous phyto-extract solution for half an hour at different concentrations (5%, 10%, 15%, 20% and 25%), for biocontrol study seed tubers were dipped into biocontrol spore suspension for 30 minutes separately. Each seed tuber was placed in a 4-5 cm depth hole and covered with sterilized potting mixture and watered weekly as required. Potato variety, K. Jyoti was used for this study.

Each study was done during two consecutive years. Eyes germination (EG), disease incidence and black scurf disease severity these three parameters were assessed on each plant. Disease incidence was calculated using methods described previously by [17-18]. Based on percent tuber surface showing disease symptoms, disease severity was calculated by using 0-5 disease severity grades (Table 1) [1].

One-way ANOVA was performed for assessing the significance of quantitative changes in mycelia growth due to different botanicals and eye germination, disease incidence and severity due to various kind of botanicals and bio-control agents.

Table 1 Disease severity scale for the assessment of black scruf of potato

Disease severity grades	Percentage of disease
0	No disease symptoms
1	< 1% tuber surface affected
2	1 to 10% tuber surface affected
3	11 to 20% tuber surface affected
4	21 - 50% tuber surface affected
5	> 50% tuber surface affected

RESULTS AND DISCUSSION

In laboratory condition

In this study, the extent of mycelium growth inhibition of *Rhizoctonia solani* varied considerably with different botanicals over control (Table 2). Significant

differences were noticed among all the treatments. After ten days of inoculation, garlic was found to be the most effective, allowing 0mm radial growth of *Rhizoctonia solani* at 15ml, 20ml, 25ml and only 11.65mm at 5ml and 8mm at 10ml concentration (Table 2). Followed by neem (28.05mm at 25ml, 30.30 mm at 20ml), turmeric (35.75mm at 25ml). However, no significant difference of colony diameter of *Rhizoctonia solani* was observed on the ginger amended Potato Dextrose Agar (PDA) when compared with control. Among five botanicals, garlic showed the maximum inhibitory effect (86.67 to 100%) and least efficacy was obtained (16.67 to 33.34%) from ginger against the growth of black scurf fungus of potato at all taken concentrations (Table 2).

Table 2 Efficacy of botanicals on the radial growth of *Rhizoctonia solani*

Botanical	5ml		10ml		15ml		20ml		25ml	
	mm	% of GI	mm	% of GI	mm	% of GI	mm	% of GI	mm	% of GI
Neem	34.48 (35.95)	61.69	30.98 (33.81)	65.58	30.88 (33.75)	65.69	30.30 (33.39)	66.33	28.05 (31.97)	68.83
Garlic	11.65 (19.85)	87.06	8.00 (16.34)	91.11	0.00 (0.00)	100	0.00 (0.00)	100	0.00 (0.00)	100
Ginger	74.88 (59.92)	16.80	67.42 (55.20)	25.09	65.03 (53.75)	27.74	63.30 (52.72)	29.67	60.33 (50.96)	32.97
Turmeric	50.98 (45.56)	43.36	40.36 (39.44)	55.16	38.53 (38.37)	57.19	36.02 (36.87)	59.98	35.75 (36.71)	60.28
Onion	59.93 (50.73)	33.41	53.86 (47.21)	40.16	52.45 (46.41)	41.72	50.72 (45.42)	43.64	45.90 (42.65)	49.00
Control	90 (71.57)	0.00	90 (71.57)	0.00	90 (71.57)	0.00	90 (71.57)	0.00	90 (71.57)	0.00
SEm	0.73		0.59		0.49		0.63		0.55	
CD	2.18		1.75		1.45		1.86		1.64	

Figures in parentheses are the arcsine transformed values
Each value is an average of four replicates
GI referred as growth inhibition

Efficacy of plant extract against R. solani in pot condition

It is proved from the (Table 3) that at 25% of all botanical concentration except onion and ginger, gave the significant result for eye germination during two years over control. All concentrations of garlic and neem were found to be more potential when compare with other botanicals. However, garlic at 25% concentration showed the maximum eye germination (79.22%), followed by 20% concentration of garlic (72.42%), which was more efficient than highest concentration of neem, 25% (69.83%). But moistest concentrations of ginger could not show any effective result (Table 3), thereof 5% (11.84%) and 15% (16.05%) concentrations could not make any noticeable effect on eye germination.

All concentrations of garlic potentially control the black scurf disease. It is evident from the Table 4 that except ginger all botanicals seed treatment significantly reduced black scurf disease incidence during 2 years. Of all the treatments, tuber seed treatment with garlic extract at 25% concentration was found to be most effective for reducing disease incidence and resulted minimum disease incidence during two years (5.68%) followed by again garlic at 20% concentration (11.06%). Highest concentration of neem and turmeric also restricted the disease incidence 13.17% and 28.92 % respectively. But in case of ginger, it showed maximum disease incidence 56.50% to 78.17% depending on different taken concentrations.

Table 5 for disease severity show that, again 25 % of garlic extract successfully suppress, even rest of all concentration of garlic also remarkably reduce the disease severity of potato black scurf, followed by the neem extract. Minimum disease severity was recorded at 25% of garlic (0.16%), and maximum at 5% of ginger (12.95%). Ginger was found the least effective for controlling the disease severity, followed by onion.

As compared to all treatments, *T. harzianum* did the best at eye germination %, reduction of disease severity and incidence over control during 2 years (89.74%, 83.30% and 57.60% respectively). The next significantly effective treatment *T. viride* also successfully reduce the disease incidence and severity more than 50% over control (55.7% and 70.37% respectively). Oppositely, *T. longibrachiatum* was not enough efficacious for controlling the disease compare to others.

Table 3 Evaluation of botanicals on eye germination percentage of seed potato tubers

Botanicals	5%	10%	15%	20%	25%
Neem	40.35 (39.31)	49.97 (44.98)	55.91 (48.39)	62.74 (52.39)	69.83 (56.69)
Garlic	53.37 (46.87)	61.85 (51.86)	67.22 (55.09)	72.42 (58.33)	79.22 (62.90)
Ginger	11.84 (21.40)	16.05 (23.52)	20.40 (26.81)	28.29 (32.13)	37.12 (37.53)
Turmeric	28.10 (31.71)	34.98 (36.26)	42.27 (40.55)	49.42 (44.67)	60.85 (51.27)
Onion	19.14 (25.34)	26.91 (31.24)	31.16 (33.91)	38.97 (38.62)	49.67 (44.81)
Control	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
SEm	0.34	0.59	0.66	0.56	0.45
CD	1.00	1.72	1.92	1.64	1.31

Figures in parentheses are the arcsine transformed values
Each value is an average of five replicates

Table 4 Evaluation of botanicals on black scurf disease incidence percentage of potato caused by *Rhizoctonia solani*

Botanicals	5%	10%	15%	20%	25%
Neem	39.02 (38.66)	32.72 (34.88)	27.91 (31.86)	21.17 (27.37)	13.17 (21.22)
Garlic	34.77 (36.12)	30.07 (33.25)	24.55 (29.66)	11.06 (19.38)	5.68 (13.71)
Ginger	78.17 (62.15)	71.73 (57.89)	69.24 (56.33)	61.05 (51.39)	56.50 (48.74)
Turmeric	49.22 (44.15)	43.38 (41.19)	39.15 (38.72)	34.06 (35.70)	28.92 (32.52)
Onion	56.13 (48.52)	50.67 (45.38)	46.65 (43.08)	39.61 (39.00)	34.21 (35.79)
SEm	0.63	0.57	0.78	0.64	0.63
CD	1.87	1.68	2.30	1.89	1.85

Figures in parentheses are the arcsine transformed values
Each value is an average of five replicates

Table 5 Evaluation of botanicals on black scurf disease severity percentage of potato caused by *Rhizoctonia solani*

Botanicals	5%	10%	15%	20%	25%
Neem	5.94 (2.54)	4.86 (2.31)	3.70 (2.04)	1.71 (1.48)	1.06 (1.24)
Garlic	4.48 (2.23)	2.96 (1.86)	2.01 (1.58)	0.90 (1.18)	0.16 (0.81)
Ginger	12.95 (3.67)	12.03 (3.54)	11.21 (3.42)	9.49 (3.16)	7.50 (2.83)
Turmeric	8.71 (3.03)	7.39 (2.81)	6.00 (2.55)	5.00 (2.34)	3.73 (2.05)
Onion	10.26 (3.28)	9.04 (3.09)	7.01 (2.74)	6.01 (2.55)	4.88 (2.32)
SEm	0.05	0.04	0.05	0.05	0.04
CD	0.16	0.13	0.16	0.14	0.12

Figures in parentheses are the arcsine transformed values
Each value is an average of five replicates

Table 6 Evaluation of biocontrol agents on black scurf of potato caused by *Rhizoctonia solani*

Bio-control agents	Eye germination (%)	Disease incidence (%)	% Reduction over control	Disease severity (%)	% Reduction over control
Trichoderma asperellum	70.35 (57.01)	53.72 (47.13)	44.06	4.1 (2.17)	51.42
Trichoderma koningii	79.17 (62.85)	51.67 (45.96)	46.20	3.34 (1.96)	60.42
Trichoderma Viride	85.35 (67.51)	45.89 (42.64)	55.70	2.50 (1.73)	70.37
Trichoderma harzianum	89.74 (71.33)	40.72(39.65)	57.60	1.41 (1.38)	83.30
Trichoderma longibrachiatum	62.15 (52.03)	57.98 (49.59)	35.60	5.51 (2.45)	34.72
Control	20.89 (27.19)	96.04 (78.81)	0.00	8.44 (2.99)	0.00
SEm	0.38	0.63	-	0.03	-
CD	1.10	1.83	-	0.09	-

Figures in parentheses are the square root transformed values-
Each value is an average of five replicates

Various kind of plant extracts have good antimicrobial properties against *Rhizoctonia solani* [19-20]. The application of phyto-biocides is a cheap, sustainable and environmentally sound approach to plant disease control. Many researchers have reported fungicidal and bactericidal effects of plant extract on specific soil borne pathogen [21-22]. In case of bio-agent, it can control many fungi by antagonistic effect. Side by side it can improve soil and plant health as well. Nowadays availability of formulation of bio-agents has increased because of very cheap, eco-friendly in nature and easily manageable.

This study reveals that, among all phyto-extracts, all concentration of garlic mainly comparative higher concentration remarkably reduces the radial growth of the black scurf fungus *in-vitro*, as well as in pot condition, it increase eye germination % and reduce the disease incidence and severity successfully. Other than garlic neem, turmeric and onion gave the satisfactory result for three parameters. But ginger extract could not show any noticeable inhibitory effect on *R. solani*. For inhibitory effect on the growth of *R. solani*, these phyto-biocides have some chemical metabolites. Garlic has sulphur content metabolic compound like allicin and ajoene which have strong suppressing effect on fungus. Like-wise neem has quercetin and ß-sitosterol, which is the first polyphenolic flavonoids purified from neem fresh leaves and were known to have antibacterial and antifungal properties [23]. Turmeric contain curcumin which is responsible for fungus suppression. May be ginger and onion don't have any strong antimicrobial metabolic compounds for suppressing the disease [10]. Neem and garlic are more effective than others on redial growth inhibition of potato black scurf fungi [24]. Due to antagonistic effect, biocontrol agents can beautifully dominate pathogenic fungi. *T. harzianum* was the most effective antagonist for black scurf fungus, followed by *T. viride*, *T. koningii* and *T. asperellum*. *T. harzianum* was most effective for making infection free tubers followed by *T. viride* in reducing disease [25]. During this time, because of excessive use of agrochemicals, soil productivity is remarkably decreased. Therefore, this method may restore the soil health as well as human health. Farmers also will be motivated to take up this practice to reduce the costs of chemicals and earn more money.

CONCLUSION

The plant extracts which used in this study was showed a different level of antifungal activities in a dose depend manner. The extract determined activities showed that can be used as bio-pesticides. The comparison among five different phyto-extract indicated the superiority of garlic and neem extracts over turmeric, onion and ginger extract in reducing the growth of *Rhizoctonia solani in-vitro.* The study, efficacy of the aqueous extracts of botanicals and

biocontrol agents at pot condition explore the possibilities to control the fungal pathogenesis and provoking the possible applications in agriculture after *in-vivo* field investigations as bio-pesticide. Phyto-biocides such as Garlic and Neem and biocontrol *Trichodrerma harzianum* and *Trichodrerma viride* should also be used against other soil borne plant pathogenic fungi. Combination with plant extracts and bio-agents should be tested *in-vivo* to find their efficacy under field conditions. Nature itself is its solution for every problem.

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