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Effect of bio-pesticides on mycelial growth of *Rhizoctonia solani* and management of black scurf of potato

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Abstract

In vitro evaluation of five different neem based bio-pesticides viz., neem oil, neem leaf difusate, replin, nimakil and nimboli for their efficacy against mycelial growth of *Rhizoctonia solani* AG 3 isolate SL-41 was assessed at 0.5, 1, 1.5 and 2% concentrations of the potato dextrose agar medium. Antifungal activity of bio-pesticides in inhibiting mycelial growth of the fungus differed and depended on bio-pesticides and their concentrations. Replin was found to be the most effective as it caused complete (100%) inhibition in mycelial growth of the fungus at 2% concentration followed by nimboli and nimakil. Neem leaf diffusate was least effective in reducing the mycelial growth of the fungus. Neem oil at 2% concentration was as effective as Nimakil at 1.5% concentration. All the tested bio-pesticides completely inhibited the induction of stem girdling and stem canker symptoms of the disease. Black scurf management by potato tuber treatment with three bio-pesticides, selected on the basis of their antifungal activity against mycelial growth of the isolate SL-41, differed in terms of number of eyes germinated, sprout killed and black scurf incidence & severity. Maximum eye germination was achieved through the application of Replin. Nimakil was the most effective in decreasing black scurf incidence and severity over non-treated inoculated control while nimboli resulted in the least number of sprouts killed but overall manifested the least effectiveness.
Keywords: Potato, black scurf, *Rhizoctonia solani*, bio-pesticides, disease management.

Introduction

Potato, *Solanum tuberosum* L. is the fourth major vegetable crop of Pakistan. It has played a remarkable role in human diet as a supplement to wheat and rice and has been found to produce more food per unit area than the cereals. In Pakistan, it is cultivated in eight different agro-ecological zones of potato production on an area of 101.5 thousand hectares with an annual production of 1666.1 thousand tonnes (Anonymous, 2002). Although, the area and production has increased manifold but the present per hectare yield of 13 tonnes is still too low as against 25-40 tonnes obtained in the major potato growing countries viz., USA, France, Japan, Peru, Turkey and Egypt. This may be due to various agronomic and environmental factors as well as attack of various diseases and pests.

Potato is commonly known as disease oriented problematic crop throughout the world. Therefore, potato health management requires continuous monitoring in the field and laboratory. Black scurf caused by *Rhizoctonia solani* Kuhn affects the marketability of table stocks fresh packed potatoes as it is an extremely important seed-borne phase of the pathogen that deserves attention. The most economical way of controlling this disease is the use of resistant varieties that are not available at present. Biological and cultural control of this disease are less explored, while the use of soil fungicides is a costly approach.

Different options are available for soil and seed-borne disease management. The problem of pathogen resistance to chemicals and of contamination of the bio-sphere associated with the large scale use of wide spectrum chemicals have necessitated the need for effective, bio-degradable chemicals with better selectivity (Sexena, 1983). This awareness has created a worldwide interest in the evaluation and use of the traditional botanical disease control agents. Neem (*Azadirachta indica* A. Juss.) and neem products are a good source of bio-pesticides that are being used against different soil and air-borne plant pathogens. However, these have not been tested so far, against black scurf pathogen in Pakistan. Due to systemic nature of neem products, they cause reduction in disease development either directly or by acting on the causal pathogen or by inducing resistance in the host plant (Bhowmick and Vardhan, 1981; Chaturvedi *et al.,* 1987; Datar, 1995; Devi *et al.*, 1982).

Neem derivatives such as oil, extracts and cakes have been found affective against many fungi under laboratory and field conditions. Singh (1968) reported lower *R. solani* incidence of 12, 16, 25 and 25% due to incorporation of mustard cake, peanut, neem and castor cake respectively against 31% incidence in the control. Singh *et al.* (1980) reported growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *S. sclerotiorum* by *Azadirachta indica* extracts of leaf, trunk, bark, fruit pulp and neem oil and the production of disease free seedlings by oil treated seeds. Plant oil emulsion of *A. indica* (5%) also inhibits the germination of *Sclerotium rolfsii*, *Rhizoctonia oryzae* and *Sclerotium hydrophyllum* (Banerjee *et al.,* 1989). Lakshman and Mohan (1989) reported significant inhibition of mycelial growth and sclerotial germination of *Thanatephorus cucumeris* by using water extract of *A. indica* under greenhouse conditions. Narasimhan *et al.* (1995) achieved 57% inhibition in spore germination and 35.8% inhibition in mycelial growth of *Alternaria solani* by neem oil. Sajid *et al.* (1995) reported that neem oil completely inhibited uredospore germination of *Puccinia recondita*. Ilyas *et al.* (1997) reported replin, neem oil and neem cake effective in reducing sclerotial formation of *Macrophomina phaseolina* in the descending order and complete inhibition of mycelial growth of *M. phaseolina* by replin at 0.8 %.

The neem products are based on various extracts of the seed kernels, leaves, bark, flowers and wood. Biological activities of these products are mainly due to the presence of alkaloids such as isoperenoids more specially 'Limonoids'. Management of *Rhizoctonia* must be integrated since no single approach gives effective and complete disease control. Comprehensive research work pertaining to management of black scurf of potato and its pathogens is lacking and needs to be addressed. Keeping in view the environment friendly nature and fungicidal properties of neem, its products were evaluated for their effectiveness against *Rhizoctonia solani*. The present studies were planned with the objective of developing disease management strategy against black scurf of potato.

Materials and Methods

The studies were carried out using *Rhizoctonia solani* anastomosis group 3 (AG 3) isolate SL-41.

Multiplication of *Rhizoctonia solani* AG 3 isolate SL-41: Potato dextrose agar (PDA) medium (potato starch: 20 gm, dextrose: 20 gm, agar: 20 gm and water: to make the volume 1 litre) was used for the multiplication of *R. solani* isolate SL-41. The medium was sterilized in a gas operated autoclave at 15 pounds per square inch (PSI) for 15 minutes. Petri-dishes containing PDA medium were inoculated with the hyphae of isolate SL-41 and incubated at 25°C for 5 days.

Mass multiplication of *R. solani* isolate SL-41: Inoculum of *R. solani* isolate SL-41 was grown in 500 ml flasks containing 100 g of barley and wheat grains (2:1) plus 120 ml double distilled, sterilized water autoclaved for 1 hour at 15-20 PSI on three consecutive days (Balali *et al.*, 1995). Flasks were inoculated with 5 plugs of 5 mm diameter (dia.) of isolate SL-41 taken from the margin of a week old culture grown on PDA medium and incubated at 25°C.

The sensitivity of *Rhizoctonia solani* AG 3 isolate SL-41 mycelium to different concentrations i.e. 0.5, 1.0, 1.5, and 2% of neem oil, neem leaf diffusate, replin, nimakil and nimboli was evaluated by poison food technique. A weighed quantity of each bio-pesticides was amended in PDA medium after autoclaving to obtain the required concentration. PDA medium without biopesticides served as control. Twenty ml of the amended and non-amended medium was poured in each of the four 9 cm diameter petridshes. After solidification, 5 mm dia. plugs cut by using a sterile cork-borer from 5 days old PDA culture of the isolate SL-41, were placed in the centre of Petri-dishes and incubated at 25°C. Mycelial growth was recorded after 5 days of incubation when the growth of isolate SL-41 was completed in the control treatment Petri-dishes. The data were analysed statistically after Steel and Torrie (1980). Three best biopesticides viz., replin, nimboli and nemakil were selected after this study for further investigation for black scurf disease management.

Management of disease by potato tuber treatment

Preparation and sterilization of potting mixture: The potting mixture (clay, sand and farmyard manure 1:1:1) was sterilized with 37% commercial formalin diluted to 1:9 ratio (formalin 1: water 9). The potting mixture was placed over a cemented floor in layers. The mixture was moistened with formalin solution and covered with a polyethylene sheet for 48 hours and then exposed to air until all the smell had vanished. Clay pots (8x11 inch) were filled with the sterilized potting mixture.

Sowing of potato tubers: Tubers of "*Desiree*", a commonly cultivated potato variety, were used for the determination of effective inoculum dose of isolate SL-41 for pathogenicity test. Sprouted tubers of similar size with three eyes

were sown, one tuber per pot, and watered weekly or as required. The pots were kept at 23±2°C. The data on shoot and root length and tuber number and weight were recorded.

Twelve potato germplasm and varieties were evaluated during this study. The trial was conducted in earthen pots (8 x 11 inch) in glasshouse during autumn season of year 2002. Fifteen to twenty days old culture of *R. solani* AG 3 isolate SL-41 @ 20 g was added to each pot of the treatments except control. There were two sets with three replications of the trial. The data regarding eye germination, number of sprouts and sprout killing were taken after 30 days of sowing by harvesting the first set of experiment, whereas, the data on stem girdling, stem canker, stolon canker, black scurf incidence and severity were recorded 90 days after sowing, by harvesting the second set, using the following methods /scales.

Eye germination, number of sprouts and sprouts killed: Number of eyes, sprouts and sprouts killed in each replication of a treatment were counted and the means were calculated.

Stem girdling: The data on stem girdling were recorded on the basis of the scale (Rauf, 2002) where 0= No girdling, 1= Slight girdling, 2= Moderate girdling, 3= Severe girdling and 4= Very severe girdling.

Stem canker: Stem cankers were recorded on the basis of scale (Rauf, 2002) where 0= No canker, 1= up to 25%, 2= 26-50%, 3= 51-75%, 4= 76-100%.

Stolon canker: The data on stolon canker were recorded on the basis of scale (Rauf, 2002) where 0= No girdling, 1= Slight girdling, 2= Moderate girdling, 3= Severe girdling and 4= Very severe girdling.

Black scurf disease rating: For the assessment of disease, two parameters, disease incidence and disease severity were taken into account. The incidence was based on percentage of tubers infected and severity was assessed on a visual disease rating scale based on percent tuber surface showing disease symptoms following the NIAB and ADAS keys (Anonymous, 1976a, 1976b, 1985). Visual disease rating was done by the scale described by Ahmad *et al.* (1995) where 0 = No symptoms on potato tubers, 1 = Less than 1% tuber area affected, 2= 1-10% tuber area affected, 3= 11-20% tuber area affected 4 = 21-50% tuber area affected and, 5 = 51% or more of tuber area affected.

Results and Discussion

Effect of bio-pesticides on mycelial growth of *R. solani* AG 3 isolate SL-41: Effect of different neem based bio-pesticides against mycelial growth of isolate SL-41 was assessed at 0.5, 1, 1.5 and 2% concentrations. The toxicity of bio-pesticides in inhibiting growth of the fungus varied and depended on bio-pesticides used and their concentrations. Replin was found to be the most effective causing complete (100%) inhibition of mycelial growth of the fungus at 2% concentration (Table 1) followed by nimboli and nimakil. Neem leaf diffusate was least effective in reducing the mycelial growth of the fungus. However, at 1.5 and 2% conc. it was as effective as neem oil at 0.5% conc. Similarly, neem oil at 2% conc. was as effective as nimakil at 1.5% conc. The differential sensitivity of mycelial growth of the fungus to various bio-pesticides may be due to their chemical configuration and active ingredient, slower or faster absorption and detoxification after absorption on account of metabolic activity of the fungus (Viyas, 1984).

Table 1: *In vitro* efficacy of bio-pesticides against mycelial growth of *Rhizoctonia solani* AG 3 isolate SL-41.

Concentrations (%)	Colony diameter (cm) in different treatments*					
	Neem oil	Neem leaf diffusate	Replin	Nimboli	Nimakil	Control
0.50	8.00 *bc*	9.00 *a*	8.40 *ab*	7.80 *bc*	7.50 *c*	9.00 *a*
1.00	6.90 *d*	9.00 *a*	4.90 *fg*	4.50 *gh*	6.00 *e*	9.00 *a*
1.50	6.40 *de*	8.00 *bc*	3.50 *ij*	4.00 *hi*	5.30 *f*	9.00 *a*
2.00	5.33 *f*	8.00 *bc*	0.00 *l*	1.80 *k*	3.20 *j*	9.00 *a*

* Any two means not sharing a common letter differ significantly at 0.05 probability level.

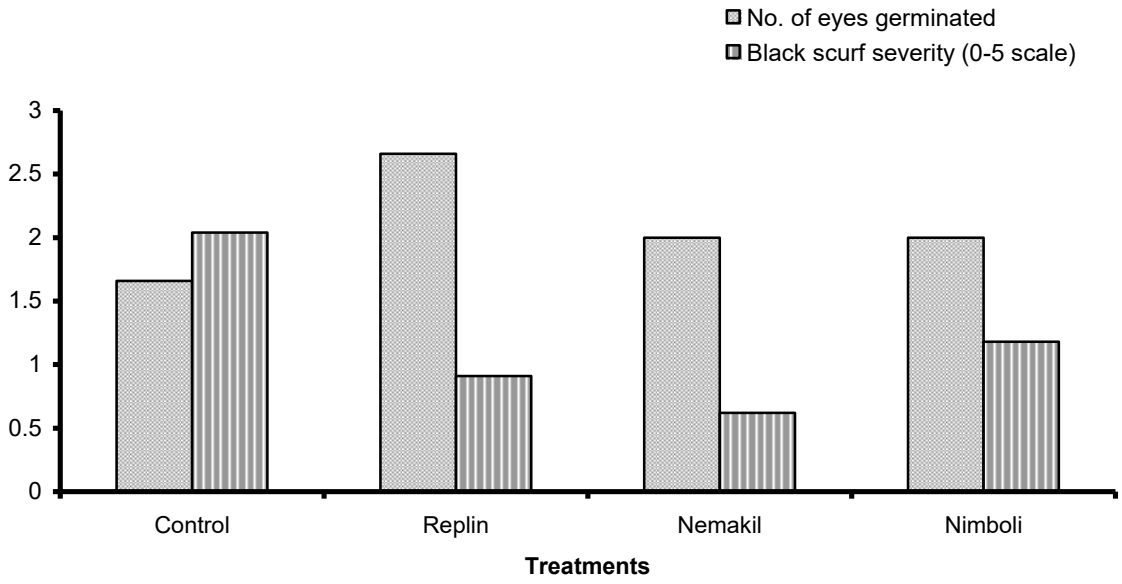

Fig. 1(a) Effect of bio-pesticides as potato tuber treatment on eye germination and black scurf severity (0-5) on cv. 'Desiree'.

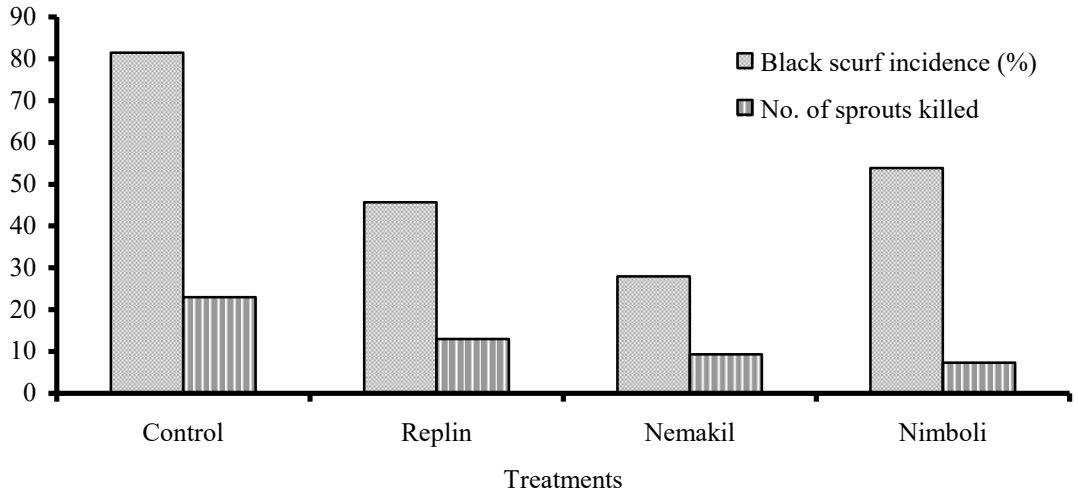

Fig. 1(b) Efficacy of bio-pesticides as potato tuber treatment on black scurf incidence and sprout killing on cv. 'Desiree'.

Management of the disease by potato tuber treatment

Black scurf management by tuber treatment with three bio-pesticides evaluated against *R. solani* isolate SL-41 varied in terms of number of eyes germinated, sprout killed, stem girdling, stem canker, black scurf incidence and black scurf severity. Replin was the most effective bio-pesticide in increasing eyes germination (Fig. 1-a) while nimboli in decreasing number of sprouts killed (Fig. 1-b), whereas, nimakil was found to be the most effective in decreasing BSI (Fig. 1-b) and BSS (Fig. 1-a) over non-treated inoculated control. However, overall, nimboli manifested the least effective performance. All the tested bio-pesticides

completely inhibited the induction of stem girdling and stem canker symptoms of disease.

Results of the present investigation suggest that the efficacy of various neem formulations is encouraging as regard mycelial growth of the fungus and disease management through potato tuber treatment. The study offers a new environment friendly approach for the management of black scurf disease of potato. The bio-pesticide formulations under investigation provide new avenues for further bio-chemical research for isolation, purification and characterization of active antifungal compounds. Evaluation of proper formulation and method of application could be the interesting aspect of further neem-based investigations. The commercialization of environment friendly neem formulation can be further enhanced (Mohan *et al.*, 1995) through extensive field trials.

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