Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

ANTIFUNGAL POTENTIAL OF PLANT EXTRACTS AND CHEMICALS FOR THE MANAGEMENT OF BLACK SCURF DISEASE OF POTATO

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ABSTRACT

Five plant extracts and five chemicals were evaluated against *Rhzioctonia solani* in lab conditions under completely randomized design (CRD). In plant extracts maximum inhibition against the pathogen expressed by *Azadirachta indica* (71%) followed by *Allium sativum* (56%), *Eucalyptus camaldulensis* (48%), *Allium cepa* (41%) and *Peganum harmala* (34%) while in chemicals Moncerene (75.8%) showed significant results followed by Topsin-M (62%), Copper oxychloride (53%), Score (45%) and Shankar (41%) as compared to control. In greenhouse different concentrations of Moncerene and *Azadirachta indica* alone and their combination were evaluated against the black scurf disease of potato. Combine treatment of Moncerene x *A. indica* showed highest eyes germination (78%) followed by Moncerene (58%) and *Azadirachta indica* (62%). Minimum disease incidence % and severity was recorded on combine treatment of Moncerene X *Azadirachta indica* (8%, 1%) followed by Moncerene (19%, 1.2%) and *Azadirachta indica* (20%, 1.4%) respectively. Under field conditions same combine treatment of Moncerence X *Azadirachta indica* showed highest eyes germination (78%) with minimum disease incidence (8%) and severity (1%) as compared to Moncerene (56.6%, 12.4%, 2.4%0) and *Azadirachta indica* (51%, 12.2%, 2.4%) respectively. Randomized complete block design (RCBD) was applied in field conditions. All experiments were conducted in Research Area of Department of Plant pathology, University of Agriculture, Faisalabad..

Keywords: *Rhizoctonia solani*, Black scurf of Potato, Plant extracts, Chemicals.

INTRODUCTION

With the world wide population expected to grow at a rate of 100 million people each year for the next twenty years, mainly in developing countries, issues surrounding food security and availability will gain an extraordinary importance (Suvillan, 2010). Potato (*Solanum tuberosum* L.) is one of the most important crops in the world (FAO, 2008). The potato plant produces more nutritious food in a shorter period of time on less land and in colder climates than any other food crop. Plant scientists suggest the potato originated as a food crop around Lake Titicaca in the Peruvian Andes about 8,000 years ago (Roy 2008). Approximately 15% of the total area under potato cultivation around the world is used for the production of seed tubers. However, with the conventional methods of vegetative propagation, potatoes are often prone to pathogens such as fungi, bacteria and viruses; thereby resulting in poor quality and yield (FAO, 2008).

Unluckily potato crop suffers from plentiful fungal, viral, bacterial and nematode diseases (Malik, 1995 and Bhutta, 2009). Thirty fungal, ten viral and three bacterial pathogens attacked on the potato plant (Crous *et al*., 2000 and Millard, 2003). Among fungal diseases black scurf disease of potato caused by *Rhizoctonia solani* become a major problem to potato crop all over the world (Ahmed *et al*., 1995). Erampalli and Johston, 2001 revealed that *Rhizoctonia solani* decrease the progeny, tuber quality as well as quantity. In world, this disease

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was first described by Khun (1858) on potato plant while in Pakistan reported by Geddes (1989) and is found in eight ecological zones of Pakistan (Khan *et al*., 1995 and Rauf, 2002).

Black scurf of potato can manage through different approaches. Seed dressing with suitable fungicides is very good method to combat the disease (Powelson *et al*., 2008; Sullivan, 2010). Management through fungicides includes two methods: 1) dusting or spraying of fungicides on freshly cut seeds. 2) Spraying of liquid fungicides in furrows (Atkinson *et al*., 2011). Use of plant extracts for the management of black scurf disease of potato is a good alternative to chemicals due to their less negative impact on environment (Sneh *et al*., 1996). Keeping in view the above facts the present study was conducted to manage the disease through different chemicals and plant extracts.

MATERIALS AND METHODS

***In-vitro* evaluation of different plant extracts against black scurf disease:** Fresh leaves of mentioned plants were collected from Ayub Agriculture Research Institute, Faisalabad. For the preparation of plant extracts 75 gm leaves were crushed in 25 ml sterilized distill water with pestle and mortar. After this these crushed leaves were passed through four layered muslin cloth and then filtered through Whatmans filter paper No. 41. This extract considered as standard dose (Hiltunen *et al*., 1996) and stored in refrigerator at 4 ^{0}C. These plant extracts were added in potato dextrose medium (PDA) at 200 gm/L and poured into petri-dishes (Aqsa *et al*., 2010). Petri-dish with ethanol and water considered as control treatment. Five mm plug of pure culture was taken and transferred into every petri-plate. Then all petri-plates were transferred in incubator at 25 $\pm$ 2 for seven days (Aqsa *et al*., 2010). Data was recorded on the basis of food poison technique under Complete randomized design (CRD) with 6 treatments and every treatment has five replications. Mean radial mycelial growth was recorded by using the following formula:

$$\% \text{ inhibition of growth} = \frac{\text{Pathogen growth without plant extract} - \text{Pathogen growth with plant extract}}{\text{Pathogen growth without plant extract}} \times 100$$

(Naz *et al*., 2006)

T_1= *Allium cepa*
T_2= *Azadirachta indica*
T_3= *Allium sativum*
T_4= *Eucalyptus camaldulensis*
T_5= *Peganum harmala*
T_6= Control

***In-vitro* evaluation of different chemicals against black scurf disease of potato:** For lab evaluation of chemicals five different chemicals (Moncerene, Score, Topsin-M, Copper oxychloride and Shankar) were used at 0.5%, mixed in potato dextrose medium (PDA) and transferred to petri-plates. Then petri-plates were inoculated with 5 mm diameter mycelial blocks of pathogen obtained from pure culture of *Rhizoctonia solani* (Khandaker *et al*., 2010). Petri-plates with no chemical application served as control. There were six treatments including one control treatment and for every treatment 5 replications were used. Food poison technique was applied with complete randomized design (CRD) (Khandaker *et al*., 2010). Percent inhibition in pathogen (*Rhizoctonia solani*) growth was recorded by using the following formula:

$$\% \text{ inhibition of growth} = \frac{\text{Pathogen growth without chemical} - \text{Pathogen growth with chemical}}{\text{Pathogen growth without chemical}} \times 100$$

(Naz, 2006)

T_1= Moncerene (0.5%)
T_2= Score (0.5%)
T_3= Topsin-M (0.5%)
T_4= Copper oxychloride (0.5%)
T_5= Shankar (0.5%)
T_6= Control

Evaluation of Moncerene and *Azadirachta indica* L. against black scurf disease under greenhouse conditions: *Azadirachta indica* L. (S, S/5, and S/10) and Moncerene (0.5%, 1%, and 1.5%) alone and in combination were used for greenhouse experiment. Soil was sterilized with 37 % formalin and fill in 13 cm pots (Idrees *et al*., 2009). Seed treatment was applied. Data was recorded on eyes germination percentage, Disease incidence and Disease severity (Ahmed *et al*., 1995). Eyes germination was recorded after 30 days of sowing. Ten treatments including one control treatment were used and each treatment was replicated five times under complete randomized design (CRD).

T_1= Moncerene (0.5%)
T_2= Moncerene (1%)
T_3= Moncerene (1.5%)

T_4= *A. indica* (S)
T_5= *A. indica* (S/5)
T_6= *A. indica* (S/10)
T_7= Moncerene (1.5%) + *A. indica* (S)
T_8= Moncerene (1.5%) + *A. indica* (S/5)
T_9= Moncerene (1.5%) + *A. indica* (S/10)
T_{10}= Control

Evaluation of Moncerene and *Azadirachta indica* against black scurf disease under field conditions: *Azadirachta indica* L. (S/20), Moncerene (1.5%) alone and in combination were applied under randomized complete block design (RCBD) and evaluated against black scurf disease of potato. Treated seed tubers were sown in sick field. Four treatments and three blocks were used with five replications. Data was recorded on eyes germination percentage, disease incidence and severity by following Ahmed *et al*., 1995.

T_1= Moncerene (2%)
T_2= *Azadirachta indica* (S/20)
T_3= Moncerene (2%) + *Azadirachta indica* (S/20)
T_4= Control

Statistical Analysis: SAS/STAT statistical software was used for the analysis of data of all experiments. Mean of the results was separated by using the least significant difference method. Resistant and highly susceptible varieties were found by applying this software while all significant treatment and the comparison of these treatments with control treatment were found. In case of co-relation experiment their means were co-related with soil factors with the help of SAS/STAT package (SAS Institute, 1990).

RESULTS AND DISCUSSION

In-vitro* evaluation of different plant extracts and chemicals against *Rhizoctonia solani: Out of five different plant extracts maximum inhibition of the pathogen was observed in *Azadirachta indica* extract (79 %) followed by *Allium sativum* (56%), *Eucalyptus camaldulensis* (48%) and *Allium cepa* (41%) and *Peganum harmala* extract (34%) as compared to control (Table. 1) while in case of chemicals, maximum pathogen inhibition (%) was recorded in Moncerene (75.8%) followed by Topsin-M (62%), Copper oxychloride (53%), Score (45%) and Shankar (41%) as compared to control treatment (Table.2).

Table 1. *In vitro* evaluation of plant extracts on growth of *Rhizoctonia solani.*

Sr.	Treatments	*Rhizoctonia solani* inhibition (%)
T_1	*Azadirachta indica*	71 a
T_2	*Allium cepa*	41 d
T_3	*Peganum harmala*	34 e
T_4	*Allium sativum*	56 b
T_5	*Eucalyptus camaldulensis*	48 c
T_6	Control	0 f
	LSD	1.96

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table 2. *In-vitro* evaluation of different chemicals against growth of *Rhizoctonia solani.*

Sr.	Treatments	Inhibition of fungal growth (%)
T_1	Moncerene (0.5%)	75.8 a
T_2	Topsin-M (0.5%)	62 b
T_3	Copper oxychloride (0.5%)	53 c
T_4	Score (0.5%)	45 d
T_5	Shankar (0.5%)	41 e
T_6	Control	0.00 f
	LSD	1.82

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Evaluation of Moncerene and *A. indica* against black scurf disease of potato under greenhouse conditions: Three concentrations of Moncerene and *Azadirachta indica* alone and their combination were evaluated Under green house conditions. Table 4.3 showed that maximum eye germination percentage (78.8%) was

recorded in Moncerene (1.5%) + *Azadirachta indica* L. (S/10) with least disease severity (1) and incidence (8%) while minimum percentage in eyes germination was observed in Moncerene (40%) with high disease incidence (34%) and severity (2.6%). Regarding disease incidence and severity combination of Moncerene and *Azadirachta indica* (1.5% X S/10) revealed minimum disease susceptibility (8%, 1%) followed by Moncerene (1.5%) (19%, 1.2%) and 20% and 1.4 % on *Azadirachta indica* respectively.

Table.3. Effect of Moncerene and *Azadirachta indica* L. against black scurf disease under greenhouse conditions.

Sr.	Treatments	Eyes germination (%)	Disease incidence (%)	Disease severity	Reaction type
T_1	Moncerene (0.5%)	40 h	34 c	2.6 b	S
T_2	Moncerene (1%)	48 g	25 e	2 bc	S
T_3	Moncerene (1.5%)	58 e	19 fg	1.2 d	MR
T_4	*A.indica* (S)	49 g	39 b	2.6 b	S
T_5	*A. indica*(S/5)	54 f	28 d	2.2 b	MS
T_6	*A. indica* (S/10)	62 d	20 f	1.4 cd	MR
T_7	Moncerene x *A. indica* (1.5%+S)	65 c	18 g	2.2 b	MS
T_8	Moncerene x *A. indica* (1.5%+S/5)	73 b	13 h	2 bc	MR
T_9	Moncerene x *A. indica* (1.5%+S/10)	78 a	8 i	1.0 d	R
T_{10}	Control	31 i	68 a	4.8 a	HS
	LSD	2.0372	2.0611	0.6884	-

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$)

Evaluation of Moncerene and *Azadirachta indica* against black scurf disease under field conditions: Maximum eyes germination percentage (70.6 %) was recorded in Moncerene (1.5%) + *A. indica* (S/20) treatment with minimum disease incidence (8%) and severity (1) followed by Monceren (1.5%) expressed 56.6% eyes germination with 12.4% disease incidence and 2.4 disease severity while 51% eyes germination percentage was observed on *A. indica* (S/20) with 12.2% disease incidence and 2.4 % disease severity as compared to control as revealed in table 4.

Table 4. Evaluation of Moncerene and *Azadirachta indica* (L.) against black scurf disease under field conditions.

Sr.	Treatment	Eyes germination(%)	Disease incidence(%)	Disease severity	Reaction type
T_1	Moncerene (1.5%)	56.6 b	12.4 b	2.4 b	MS
T_2	*Azadirachta indica* (S/20)	51 c	12.2 b	2.4 b	MS
T_3	Moncerene x *A. indica* (1.5%+S/20)	70.6 a	8 c	1.0 c	R
T_4	Control	37 d	56.37 a	4.4 a	HS
	LSD	1.96	2.53	0.70	-

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

DISCUSSION

Use of different plant extracts has good antimicrobial properties against the *Rhizoctonia solani* (Marshall, 2005; Baljeet *et al*., 2005; Weinhold *et al*., 1982). The foremost features of plant extracts are that they have least toxic effect on environment and humans, quick degradation and have narrow range of activity (Kirkgaard *et al*., 1993). From the results it was cleared that combination of plant extract *Azadiracta indica* with Moncerene gave better results as compared to their single treatment.

Identical results were found by Naz, (2006) in which twenty six palnt diffusates were evaluated against *Rhizoctonia solani*. Out of all these diffusates Neem extract showed maximum pathogen inhibition while Eucalyptus and Onion also gave good results. Similar results were found by Aqsa *et al*., 2010 in an experiment in which five different plant extracts were evaluated against *Rhizoctonia solani*. Out of these *Azadirachta indica* showed maximum inhibition in pathogen growth. Anderson *et al*., 1993 evaluated sixteen naturally available plant extracts against *Rhizoctonia solani,* out of Neem extract showed significant results against the pathogen. Matching results were found by an

experiment in which five different plant extracts (*Adhatoda zeylanica, Azadirachta indica, Capparis, Dodonaea viscosa and Salvadora oleoides*) were evaluated. Results show that *Azadirachta indica* L. showed best response against black scurf disease while *Adhatoda zelynica* also express significant inhibition of the pathogen. In this experiment as the concentration of plant extracts increases the pathogen growth decreases (Aqsa *et al*., 2010). Matching results were found in an experiment in which twenty two plant extracts were evaluated against *Rhizoctonia solani* under lab conditions. Out of these extracts chilli, Lantana, Lemon grass and onion seeds highly reduced the pathogen activity while basil, castor beans, chamomile and peppermint showed least significant results (Kataria and Sunder, 1988). Equivalent results were found when sixteen naturally available plant extracts were tested against *Rhizoctonia solani* in lab conditions. *Azadirachtar indica* L. extract showed significant results against the pathogen growth. In another experiment different plant extracts were evaluated in which clove and Hermal was present but from results clove shows maximum inhibition of the pathogen (Khandaker, 2010). These results also showed the minimum effect of Hermal on *Rhizoctonia solani.* The reason behind the significant results is that *Azadirachtin* present in Neem extract which suppress the growth of pathogen (Kumar *et al*., 1999).

Chemical control is a very effective treatment against *Rhizoctonia solani* especially in black scurf disease of potato and it is widely used all over the world (Powelson *et al*., 1993; Loria *et al*., 1997). Moncerene showed significant results against *Rhizoctonia solani* and inhibits the maximum pathogen growth. Similar results were found by Khandaker *et al*., (2010) in which they used eight different fungicides against *Rhizoctonia solani* under lab conditions. Out of these fungicedes vitavax showed maximum pathogen inhibition followed by Moncerene. Kataria and Gisi (1996) evaluated various fungicides against *Rhizoctonia solani* out of which Pencycuron (Moncerene) and Azoxystrobin showed maximum inhibition of the pathogen. Active ingredient of Moncerene is Pencycuron which acts primarily on *Rhizoctonia solani* and inhibits the growth of pathogen. Pencycuron attack on the metabolic activity of *Rhizoctonia solani* and inhibits its further growth.

From the results Moncerene (1.5%) expressed significant results. Similar results was found by an experiment in which dusting of tolclofos-methyl and spray with finpiclonil and pencycuron showed same results to control the black scurf disease (Stack *et al*., 1999). In Kaghan valley in Pakistan, different chemicals like Dithane M-45, bleaching powder, boric acid and elemental sulphur was used against black scurf disease. Out of all these chemicals at 35 concentration boric acid was found the most effective chemical against black scurf disease of potato while treatment with bleaching powder and elemental sulphur was not effective against this disease (Jan *et al*., 2003). Topsin-M, derosol and copper oxychloride were evaluated in an experiment to control the black scurf disease. Out of the results derosol and Topsin-M showed statistically significant control against the pathogen as compared to other chemicals. These chemicals enhance the eyes germination by inhibiting the pathogen growth (Idress *et al*., 2009). Same results were found by Kataria and Gissi, 1996. They used different fungicides that can be used on *R. solani* but a careful selection should be performed to gain effective control against this pathogen. Only pencycuron and tolcolophos methyl was effective against *R. solani* in an *in-vitro* experiment.

Results revealed that Moncerene along with combination of *Azadirachta indica* L. gave best results under field conditions. Similar results regarding Moncerene were found by Calleros *et al*., (2000). In their experiment azoxystrobin and pencycuron (Moncerene) showed best results against *Rhizoctonia solani*. These fungicides showed highest efficacy against the sclerotial development of the pathogen. These results were similar to the findings of Kataria and Gisi, 1996. Pencycuron and Azoxystrobin was found were effective against the pathogen in their experiment. Results of Lootsma and Scholte (1996) used revealed that pencycuron was found the most efficient chemical which suppress the growth of the pathogen. Another experiment revealed that application of pencycuron with the combination of Captan can significantly reduce the disease severity and incidence of pathogen. This combination enhances the yield of potato crop. If we use this combination in dip treatment then there is less effect of chemicals towards pathogen. Mode of action of Pencycuron (Moncerene) was that this active ingredient attack on spore development stage of the *Rhizoctonia solani* and suppresses its further growth (Rauf *et al*., 2002).

CONCLUSION

Combined application of plant extracts and chemical gave better results as compared to their single application. By using the combine treatment of Moncerene and *Azadirachta indica* black scurf disease of potato can easily control.

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