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POTATO BLACK SCURF, PRODUCTION PRACTICES AND FUNGITOXIC EFFICACY OF *RHIZOCTONIA SOLANI* ISOLATES IN HILLY AREAS OF GILGIT-BALTISTAN PAKISTAN

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Abstract

Potato is the main cash crop of Gilgit-Baltistan in particular of buffer zone of Central Karakorum National Park (CKNP). There are many biotic and abiotic threats to its production. Amongst them the black scurf disease caused by *Rhizoctonia solani* is the major constraint. In order to assess black scurf of potato a comprehensive survey was carried out in four main valleys of the region consisting of 24 villages. The survey revealed that valley wise mean disease prevalence was high in Bagrote (63.85±9.06) and low in Haramosh (45.40±14.62). Maximum mean disease incidence and severity were recorded in Hoper valley while least disease incidence appeared in Haramosh and severity in Bagrote valley. Production practices as risk factor were assessed through a questionnaire. The majority of farmers performed activities in field that were supportive to the black scurf epidemics, such as, use of pits for storage of late harvested potato tuber for growing in next season, table potato, lack of general awareness about black scurf, not maintaining proper depth of sowing, irrigation, harvesting time and crop rotation. *In vitro* efficacy of three fungicides at different concentrations i.e. 50, 100, 150 and 200 ppm were tested against *Rhizoctonia solani* isolates. Results indicated that increase of fungicide concentration significantly inhibited the mycelial growth of respective isolates. However, sensitivity reaction of each of isolates against each fungicide was also differential. Fungicide ridomil gold and mencozeb showed more toxic effect against the fungus compared to vitavax.

Key words: Black scurf, Production practice, Fungicide, *Rhizoctonia solani*, Gilgit-Baltistan.

Introduction

Potato (*Solanum tuberosum* L.) occupies a distinct position among the major crops of the world. Today potato holds the fourth position in the world food production next to maize, rice and wheat (Anon., 2008).Its commercial cultivation is common in all climatic condition except tropical low lands (Hijmans, 2001). In Pakistan, it's ranked third after wheat and rice as well as its consumption increasing over the year. The agro climatic conditions of Pakistan specifically Gilgit-Baltistan is conducive for growing potato. The region was considered as favorable for good quality seed production but unfortunately the yield as well as quality of the produce has declined due to a variety of problems including insect pest, diseases, unavailability of certified seeds and imbalanced application of fertilizers. Diseases that affect the potato production include, early and late blight, black scurf and potato virus (X and Y) etc. Black scurf of potato caused by *Rhizoctonia solani* represent an economically important disease. Due to its complex nature it occurs all over the world where potato is grown (Powelson *et al*., 1993; Banville *et al*., 1996; Banville &Carling, 2001). It is one of the serious threats to potato crop that reduced the production (qualitatively and quantitatively). *Rhizoctonia solani* is a polyphagous fungus causing different types of disease symptoms including seed decay, damping off, stem canker, black scurf, root decay, and foliage disease. Among them black scurf is identified on the surface of progeny tubers, is a significant problem for the potato growers (Carling & Leiner 1986).It is highly destructive pathogen and lives as sclerotia or mycelium in infected plant residues or in the soil and remains there forever (Sneh *et al*., 1996). These infective propogules have potential to infect potato crop growing next season in the same soil (Agrios, 2005).The current study was therefore designed to assess black scurf epidemiology, existing growing practices that influence black scurf and *In vitro* fungitoxic effect against *Rhizoctonia solani* isolates.

Materials and Methods

Black scurf: Disease distribution **(**prevalence, incidence and severity) was carried out through extensive survey of four valleys of CKNP region Gilgit-Baltistan. These valleys comprised of 24 villages and 130 potato fields. Inspection of potato fields and collection of diseased tubers were subjected to the willingness of farmers. Disease distribution was calculated using formulas as described by Rauf *et al*. (2007). Formulas used for assessment of black scurf of potato were:

(i) $$P\,\% = \frac{F.S}{T.F} \times 100$$

(P % = Prevalence %; F.S = Farm showing black scurf symptom; T.F = Total farm)

(ii) $$I\,\% = \frac{P.T}{T.P.T} \times 100$$

(I % = Incidence %; P.T = Potato showing black scurf symptom; T.P.T = Total potato tuber)

(iii) $$DS = \frac{S.I}{T.R \times M.D\ (5)} \times 100$$

(DS = Disease severity; SI = Sum of all disease rating; TR = Total number of rating; MD= Maximum disease grade)

Production Practices as risk factors: This study was based on primary data collected from potato growers of Bagrote, Haramosh, Hoper and Shigar valley of CKNP region Gilgit-Baltistan, Pakistan during the year 2013-14 (Fig. 1). Before launching, the survey, questionnaire was developed and was improved accordingly. Sample survey was undertaken and personal interviews were conducted to collect the information about production practices of potato crop.

Sample size: The survey was based on one hundred randomly selected potato growers. Twenty five potato growers were selected from each valley. The analysis was carried out in relation to percentage analysis; including potato tuber used for sowing, depth of sowing, and awareness about black scurf disease, irrigation and harvesting time.

***Rhizoctonia solani* isolates:** Twenty *Rhizoctonia solani* isolates (RS_1 to RS_{20}) were collected from potato tuber during field survey. These isolates were cultured in laboratory and purified by sub culturing. The sub culture of each isolate was examined under microscope and identified as described by Burgess *et al*., 1994.From the twenty isolates only eight isolates (RS_2, RS_3, RS_4, RS_5, RS_{10}, RS_{12}, RS_{15} and RS_{16}) were selected on the basis of their rapid growth in PDA medium and used in the current study.

***In vitro* efficacy of fungicide:** In order to test the fungicide sensitivity of *R. solani* isolates, three fungicides viz; redomil gold, vitavax and mencozeb at four concentrations 50, 100, 150 and 200 ppm were studied using poison food technique (Nene & Thapliyal, 1982). Petri plates (9 cm-diameter) having 20 ml of fungicide amended potato dextrose agar (PDA) medium were used. In each Petri plates culture of *Rhizoctonia solani* isolate was cut into 5 mm diameter disc from actively growing margins using cork borer and placed it at the center of each treatment. Three replicates along with control were maintained. The inoculated Petri dishes were incubated at 27±2°C and data were recorded after one week of incubation. The data were recorded for mycelial growth reduction in millimeter while the mean inhibition percentage of the mycelial growth was recorded by using following formula:

(IMGR = Isolate mycelial growth reduction %; MGC = Mycelial growth in control; MGT = Mycelial growth in treatment).

Results

Black scurf prevalence, incidence and severity: The study was carried out during 2013-14 to investigate black scurf distribution. Results showed that black scurf intensity varied significantly ($p \leq 0.05$) in the study area. Valley wise mean disease prevalence was found higher in Bagrote (63.85±9.06) followed by Hoper (57.61±12.71), Shigar (54.95±16.81) and Haramosh (45.40±14.62) respectively. Results pertaining to disease incidence showed maximum in Hoper (14.47±3.45) followed by Shigar (14.28±5.74), Bagrote (13.99±3.26) and Haramosh (11.82±2.84), whereas, higher disease severity was recorded in Hoper (3.77±1.41) and Shigar (3.74±1.60), while least in Bagrote (3.57±1.20) and Haramosh (3.58±1.07) valleys respectively (Table 1).

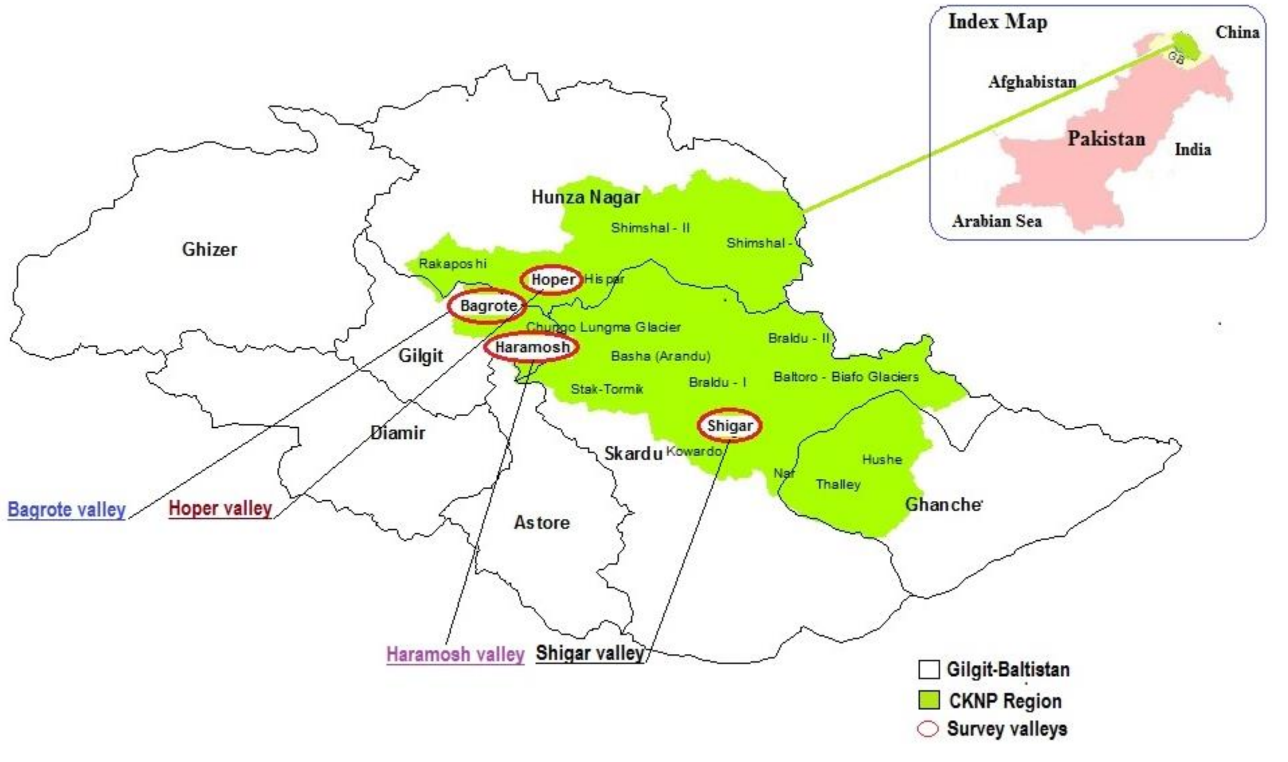

Fig. 1. Map of study area of CKNP valleys.

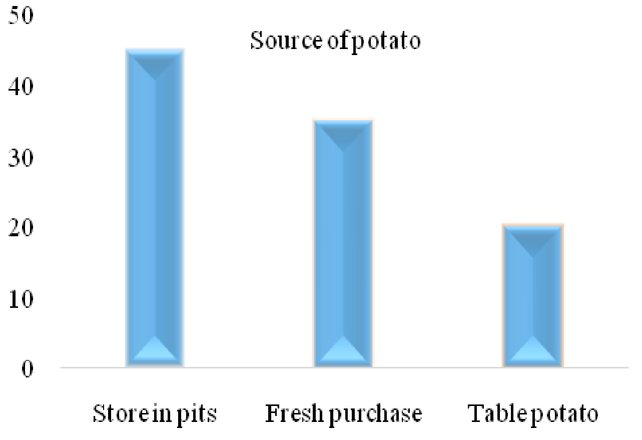

Fig. 2. Categories of potato used for sowing.

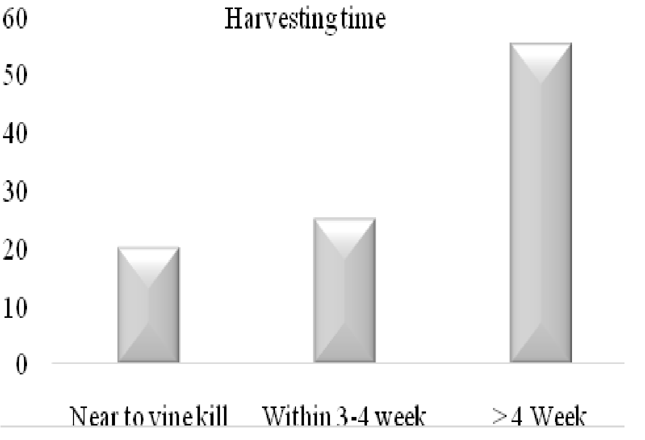

Fig. 5. Harvesting time of potato crop.

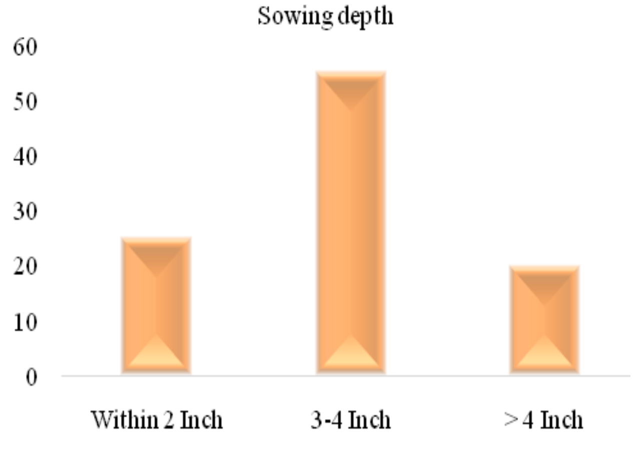

Fig. 3. Sowing of potato in different depth.

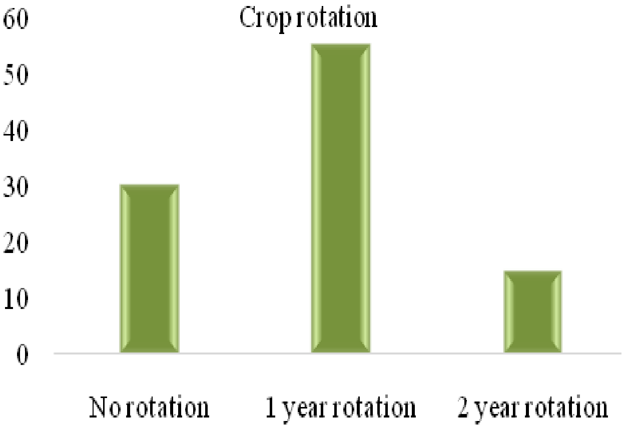

Fig. 6. Crop rotation practices adapted by potato growers.

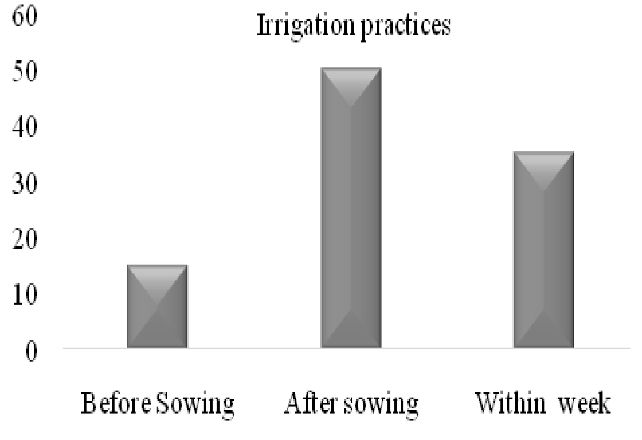

Fig. 4. Irrigation practices of potato crop.

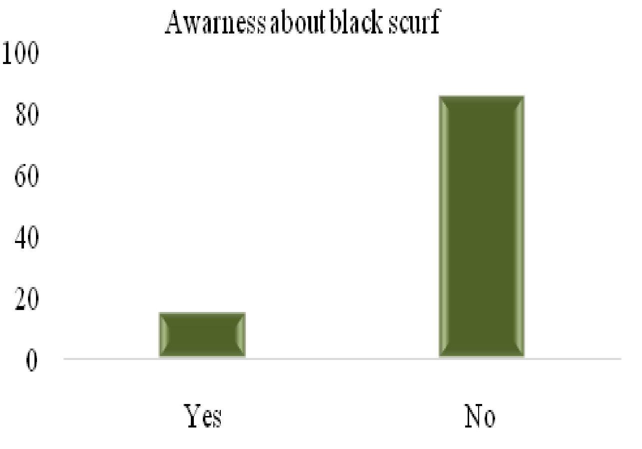

Fig. 7. Awarness about potato black scurf disease.

Assessment of production practices: Assessment of production practices as risk factors contributing to black scurf intensity was carried outthrough a questionnaire based survey, conducted among the potato growers of CKNP region GB. The information about sources of potato seed, depth of sowing, irrigation, time of harvesting, awareness about black scurf and crop rotation was ascertained. Result showed that 45% of farmers used pits to store potato, 35% obtained fresh seed from Agriculture Department GB and 10% used table potatoes obtained from market (Fig. 2). The depth of sowing of potato seed was recorded as 25, 65 and 20% farmer placed potato seed in soil within 2 inch, 3-4 inch and above 4 inch (Fig. 3). 15, 50 and 35% farmers irrigated potato before sowing, after sowing and within a week (Fig. 4). The percent of farmers harvesting potato crops was recorded as 20% (immediately after vine kill), 25% (within 1-2 week) and 55% (after 3-4 week) (Fig. 5). Majority of farmers adopted one year crop rotation in this region while 85% of farmers were not aware about black scurf of potato (Figs. 6-7). During the harvesting time the temperature of study area fell in the range of 20-30°C in August and 16-28°C in September (Fig. 8).

Table 1. Distribution of potato black scurf in the study area.

Disease	Valleys			
	Bagrote	Haramosh	Hoper	Shigar
% Prevalence	63.85 ± 9.06	45.40 ± 14.62	57.61 ± 12.71	54.95 ± 16.81
% Incidence	13.9 ± 3.26	11.82 ± 2.84	14.47 ± 3.45	14.28 ± 5.74
% Severity	03.57 ± 1.20	03.58 ± 1.07	03.70 ± 1.41	03.74 ± 1.60

The values represent mean ± St. Dev for the given parameters in different valleys

Table 2. *In vitro* effect of fungicide ridomil gold on the redial colony growth of sclerotial isolates of *Rhizoctonia solani*.

Isolates	Control	50 ppm		100 ppm		150 ppm		200 ppm	
		RCG (mm)	% I	RCG (mm)	% I	RCG (mm)	% I	RCG (mm)	% I
RS_2	84.43	60.93bc	27.83	50.60ab	40.06	33.62c	60.18	16.30e	80.69
RS_3	85.36	60.21cd	29.46	52.23a	38.81	34.12b	60.02	17.06d	80.01
RS_4	86.40	63.28a	26.75	49.37ab	42.85	35.21a	59.24	21.18a	75.48
RS_5	84.37	60.18cd	28.67	50.53ab	40.10	33.28d	60.55	18.80c	77.72
RS_{10}	84.37	61.38b	27.24	49.10a	41.80	34.31b	59.33	20.12b	76.15
RS_{12}	82.23	60.12cd	26.88	48.18b	41.40	32.22e	60.82	21.19a	74.23
RS_{15}	85.03	63.88a	24.87	42.86c	49.60	30.01f	64.70	16.74d	80.31
RS_{16}	84.30	60.00d	28.82	44.60c	47.09	29.58g	64.91	15.64f	81.44
Mean	84.56	61.24	27.56	48.43	42.75	32.79	61.21	18.38	78.25

Means in each column followed by the same letter are not significantly different at LSD test ($p \leq 0.05$); RCG: Colony growth rate and % I: % of inhibition

Table 3. *In vitro* effect of fungicide vitavax on the redial colony growth of sclerotial isolates of *Rhizoctonia solani.*

Isolates	Control	50 ppm		100 ppm		150 ppm		200 ppm	
		RCG (mm)	% I	RCG (mm)	% I	RCG (mm)	% I	RCG (mm)	% I
RS_2	84.43	62.29c	26.22	50.11d	40.64	34.00e	59.72	16.75e	80.16
RS_3	85.36	63.72b	25.35	56.30a	34.04	38.12b	55.34	23.18a	72.84
RS_4	86.40	63.30b	32.43	53.12b	45.08	38.06b	62.87	23.42a	79.06
RS_5	84.37	65.45a	22.42	51.16c	39.36	39.09a	53.66	22.24b	73.64
RS_{10}	84.37	63.28b	24.99	49.24e	41.64	38.11b	54.82	20.16c	76.10
RS_{12}	82.23	61.75c	24.90	48.32f	41.23	37.15c	54.82	19.64d	76.11
RS_{15}	85.03	60.06d	29.36	47.11g	44.60	38.24b	55.02	20.07c	76.39
RS_{16}	84.30	59.84d	28.67	45.30h	46.26	36.29d	56.95	16.61e	80.29
Mean	84.56	60.46	26.79	50.08	41.60	37.38	56.65	20.26	76.82

Means in each column followed by the same letter are not significantly different at LSD test ($P \leq 0.05$); RCG: Colony growth rate and % I: % of inhibition

Table 4. *In vitro* effect of fungicide mencozeb on the redial colony growth of sclerotial isolates of *Rhizoctonia solani.*

Isolates	Control	50 ppm		100 ppm		150 ppm		200 ppm	
		RCG (mm)	% I	RCG (mm)	% I	RCG (mm)	% I	RCG (mm)	% I
RS_2	84.43	58.77cd	30.39	48.46a	42.60	33.25a	60.61	17.74bc	78.98
RS_3	85.36	60.00ab	29.70	48.98a	42.62	33.26a	61.03	18.26a	78.60
RS_4	86.40	58.38d	32.43	47.45b	45.08	32.08bc	62.87	17.54c	79.69
RS_5	84.37	59.00cd	30.06	46.14c	45.31	33.20a	60.64	18.09ab	78.56
RS_{10}	84.37	59.34bc	29.66	44.94d	46.73	32.30b	61.72	17.41c	79.36
RS_{12}	82.23	59.92ab	27.13	45.75c	44.36	31.52c	61.66	16.26d	80.22
RS_{15}	85.03	60.71a	28.60	45.44cd	46.56	30.31d	64.35	17.56c	79.34
RS_{16}	84.30	60.08ab	28.73	44.17e	47.60	29.65e	64.82	16.39d	80.55
Mean	84.56	59.52	29.58	46.42	45.10	31.95	62.21	17.40	79.41

Means in each column followed by the same letter are not significantly different at LSD test ($p \leq 0.05$); RCG: Colony growth rate and % I: % of inhibition

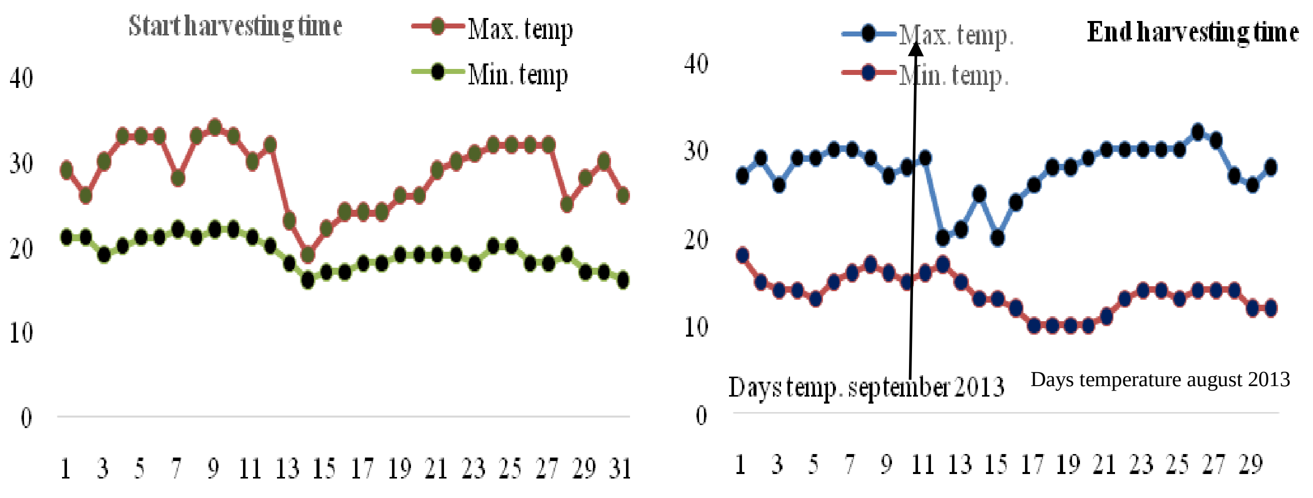

Fig. 8. Harvesting time of potato crop in Hilly areas of Gilgit-Baltistan.

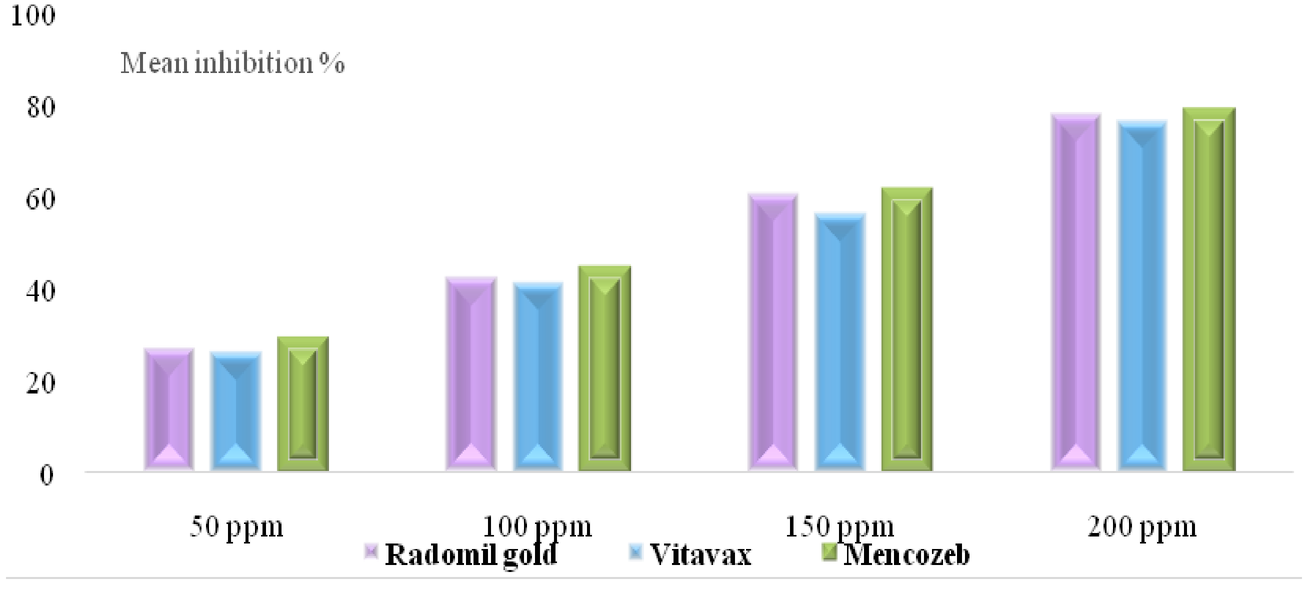

Fig. 9. Mean inhibition percentage of *Rhizoctonia solani* isolates at different concentration of fungicides.

***In vitro* efficacy of fungicide against *R. solani* isolates:** The data on *In vitro* efficacy of fungicides ridomilgold, mencozeb and vitavax against eight *R. solani* isolates was evaluated at 50, 100, 150 and 200 ppm using food poison techniques (Nene &Thapliyal, 1982). Results showed that the radial colony growth of all isolates significantly decreased with the increased in concentration of fungicide. However, fungicide ridomilgold and mencozeb sowed high toxic effect as compared to vitavax over control. Similarly sensitivity reaction of each isolates against fungicides was differential. Fungicide ridomilgold inhibited mycelial growth >80% in RS_2, RS_3, RS_{15} and RS_{16}, vitavax in RS_2 and RS_{16} and mencozeb in RS_{12} and Rs_{16}) at 200 ppm concentration (Tables 2, 3 and 4). Among the tested fungicide ridomilgold and mencozeb were more effective comparatively than vitavax (Fig. 9).

Discussion

The general trend of agriculture in Gilgit-Baltistan is shifting from subsistence level to commercial enterprise especially for potato production, which is key income generating activity. The agro climatic condition of GB is considered a rich basket of producing quality seed potato. Unfortunately, during the last twenty year potato crop faced different pathological problems. Amongst, black scurf is one of the major constraints in terms of qualitative and quantitative losses. There are few published reports on black scurf of potato in Gilgit-Baltistan especially the buffer zone of Central Karakoram National Park (CKNP). Keeping in view the above facts, a compressive study was carried out in the selected valleys. The survey results revealed that distribution of black scurf of potato varied from valley to valley. High disease prevalence of black scurf was found in Bagrote, incidence in Hoper and severity in Shigar valley was recorded. Some reports reveal that high incidence and severity of black scurf in GB is due to flow of susceptible potato seed, weak quarantine system, lack of crop rotation and poor crop husbandry (Bhutta *et al.*, 2004). It is further supported by the findings of Hooker (1981) that favorable climatic conditions, mono-cropping, and growing susceptible cause by high incidence and severity. Previous findings concluded that harvesting time of potato crop is another factor for high incidence of black scurf. The extended harvesting time results into greater incidence of black scurf (Daami-Remadi *et al*., 2008a; Rajpal *et al*., 2005). Potato varieties Desire and Cardinal are grown in GB followed by Ultimus and Diamant (Bhutta *et al*., 2004). Previous research reports showed that Cardinal, Desire, Diamant, Ultimus and Cleopatra were highly susceptible to this particular disease (Zanoni, 1991; Ahmad *et al*., 1995c; Rauf *et al*., 2007).

Production practices as risk factor for black scurf epidemiology was also assessed in the study area. The result revealed that majority of the framers of the study area heavily irrigate potato fields, did not follow sowing depth, delay harvesting time and one year crop rotation. It was further observed that they store late harvest in pits in the field or nearby their residence for next year growing and also lack of awareness about black scurf as well as low temperature during harvesting time. Previous research findings agree that all these production practices support the black surf epidemiology. It has been further reported that early harvesting, minimum two year rotation, maintaining moisture contents, good drainage practice are essential to minimize black scurf occurrence (Abdessamad &Maria 2006; Dijst *et al*., 1986; Peters *et al*., 2005). The findings of current study were further supported by Peters *et al.* (2005) that more than two year rotation practices reduce the severity of *Rhizoctonia solani*. Some previous studies also showed that good agriculture practices such as use of resistant varieties, crop rotation, irrigation and proper depth of sowing were instrumental in reducing the incidence of soil borne diseases (Olanya *et al*., 2006; Peters *et al*., 2004; Honeycutt *et al*., 1996). Rauf*et al*., 2007 reported that low soil temperature during harvesting, monocropping and high moisture contents support high black scurf of potato in Pakistan. Crop rotation, using disease free tuber, maintaining soil depth (2 inch or less), and harvest without delay after vine kill are the only possible ways to manage black scurf disease in field (Lacy & Hammerschmidt, 1994; Hill & Anderson, 1989). Increase of disease in soil has always been a problem for the growers and researchers (Gul *et al*., 2005). *Rhizoctonia solani*, *Fusarium solani* and *Pythium ultimum* are most important soil borne pathogens that cause numerous vegetable diseases (Nicolle *et al*., 2003). The most suitable, effective and viable methods to manage plant disease is use of resistant varieties but it takes lot of time (Wada 2003). Fungicide is another option to manage pathogens in short term. In current investigation three fungicides, namely, ridomilgold, vitavax and mencozeb were tested against eight isolates of *Rhizoctonia solani* through poisoned food technique. The results indicated that the increase of fungicide concentration suppressed the mycelial growth of respective isolates. However, the level of sensitivity of each isolate against fungicide was observed to be different. Our study is in agreement with Delp & Dekar (1985). He stated that resistance to fungicide is a stable, inheritable and adjusted by the fungus to a particular fungicide. Brent (1998) stated that for management of fungicide-resistant pathogens effectively, it is imperative to observe the occurrence and distribution of resistant isolates, as well as the degree of resistance to the fungicide applied. According to Virgen-Calleros *et al*. (2000), variation of sensitivity of fungicide against *R. solani* isolates means the capability of isolates to fight against active ingredient. This statement is in agreement with our current study. Use of fungicide against *R. solani* is the most common method to protect potato crop. Broad spectrum fungicides are used. However, no fungicides were recorded as effective against all species of *Rhizoctonia*. Variation in sensitivity to fungicides has been frequently reported within *R. solani* (Kataria *et al*., 1991; Csinos & Stephenson, 1999; Virgen-Calleros *et al*., 2000). Many researchers have used ridomil gold, vitavax and mencozeb against plant pathogen viz ridomil gold and mencozeb against *Sclerotium rolfsii* isolates and *Sclerotinia sclerotiorum* (Abida *et al*., 2007; Iqbal *et al*., 2003) and Vitavax against *R. solani* isolates (Maniruzzaman *et al*., 2010), while efficacy of ridomil gold was tested against *F. solani* (Zahoor *et al*., 2012; Naik *et al*., 2007). Nasreen & Gaffar (2010) used ridomilgold, vitavax and mencozeb against wide range of pathogen including *F. solani* that cause damping off, seed decay, seedling and infection of root.

Conclusion

The farming communities of the region can be empowered for improving production skill and management of black scurf disease. The result revealed that the area was severely infected with black scurf disease and percentage of prevalence, incidence and severity was varied within the valleys. Some of the leading factors like poor sowing practices, unavailability of resistant varieties, weak internal quarantine system, poor purchasing power of quality seed, lack of awareness about disease, delay in harvesting after vine killing and poor crop rotation are responsible for higher black scurf incidence. *In vitro* efficacy of fungicides showed that with the increase in their concentration mycelial growth of respective isolates was significantly reduced. However, fungicide ridomilgold and mencozeb showed high toxic effect compared to vitavax. Fungicide application is important tool for control of plant disease specially soil borne disease mainly when disease is prevalent in the field. In addition effective fungicides identification would help for combination of different components required for formulation of integrated disease management.

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