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Bioefficacy of botanical extracts and bioagents against sclerotial isolates of *Rhizoctonia solani*

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Key words: Botanical extract, bioagent, potato, black scurf *Rhizoctonia solani*, isolates, poison and dual culture.

Abstract

The antifungal efficacy of six botanical extracts viz., *Cannabis sativa L.*, *Peganum harmala* L., *Datura starmonium* L., *Artemisia brevifolium* L., *Capparis spinosa* L., *Mentha royleana* L. and two bioagents viz., *Trichoderma harizanum* and *Trichoderma viride* were evaluated *in vitro* against sclerotial isolates of *Rhizoctonia solani* causing black scurf of potato through food poison and dual culture technique, respectively. The data revealed that increasing concentration form 5 to 15% of botanical extract suppressed the mycelial growth of all isolates. A highest antifungal property was found in *C. sativa* which was followed by *P.harmala* and *D. starmonium* while least in *Capparis spinosa*. Mycelial inhibition range in the concentrations of 5-15% were recorded in *C. sativa* (36.43-80.00%) *P.harmala* (26.71-69.20%), *D. starmonium* (26.44-70.28 %), *A.brevifolium* (24.04-66.67%) *C. spinosa* (21.70-50.16%) and *M. royleana* (24.14-61.94). Highest sensitivity against botanical extracts was observed in isolates RS_{10}, RS_4 and RS_5 while least in RS_{12} and RS_{16} at concentration of 5, 10 and 15% respectively. Among the tested bioagents mycelial growth inhibition of *R. solani* isolates was recorded in case of *T.harzianum* (48.32-72.72%) and *T.viride* (28.75-56.80%). *T. harzanium* caused highest mycelial inhibition in isolate RS_{10} and least effective in isolate RS_4 whereas *T.viride* was most effective in isolates RS_3 and least in isolate RS_{15}.

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Introduction

The agriculture and agri-food sector is expected to move towards environmentally sustainable development, while increasing its productivity and simultaneously protecting the valuable crops from disease is base for future generations. The increasing demand for a steady crop production to the growing world population requires controlling of plant diseases. Agriculture is the back bone of mountainous region of Gilgit-Baltistan (GB). Majority of population depends upon agriculture especially potato crop. Potato is the main cash crop and climate is ideally suited for the cultivation. For the last few years, soil and seed-borne diseases have become main threat to potato production. Among these diseases, *Rhizoctonia* canker commonly called black scurf, caused by the fungus *Rhizoctonia solani* Kuhn has been a severe problem in all potato production areas (Bhutta *et al*, 2004). So the ecofriendly management of this disease is very important to alleviate poverty through sustainable production of economic crops. To manage this disease, different options are used all over the world but the most suitable and effective are the botanical extract and bioagents. Management of plant disease through fungicides leads to soil residual problem and health hazards, as well as involving higher input cost. The only economical and environment friendly option for disease management of crops is utilization of plant extract (Samuel *et al*, 1995). Fungicides are commonly used to control *Rhizoctonia* disease. However field application always not desirable because continuous and injudicious use of fungicide can cause toxic effect to none target organisms as well as development of resistant strains of pathogen (Arcury and Quandt, 2003; Deising *et al*, 2008). The natural plant products derived from plants effectively meet this criterion and have enormous potential to influence modern agrochemical research. When extracted from plants, these chemicals are referred to as botanicals. The use of botanical pesticides is now emerging as one of the prime means to protect crops and their products and the environment from pesticides (Sanjay, 2009).

Soil and tuber-borne inoculum play key role for the development of *R.solani* disease on potato tuber (Demirci, 1995). Different chemicals and cultural methods were used to minimize inoculum level from soil and tuber, along these many biocontrol agents have been used against *R.solani* that cause disease on potato. A variety of bioagents were used to control pathogen *R.solani,* among these bioagent *Trichoderma* species is one of them (Beagle *et al*, 1985). The potential values of Trichoderma spp. as bioagents were reported previously by many researchers (Bankole and Adebanjo 2004; Howell, 2003).

The present study was therefore carried out to investigate bioefficacy of botanical extracts and bioagents against sclerotial isolates of *R.solani* to formulate effective management programme of the black scurf disease.

Materials and methods

Rhizoctonia solani isolates

Twenty potato sclerotial isolates of *Rhizoctonia solani* (RS_1-RS_{20}) were collected from different areas of mountainous region of Central Karakorum National Park of GB (Fig 1). The isolates were identified by hyphal tip method and stored in test tubes in agar slant at 10°C. Among the 20 isolates 8 isolates were selected on the basis of their rapid growth in PDA medium. These eight sclerotial isolates of *Rhizoctonia solani* (RS_2, RS_3, RS_4, RS_5, RS_{10}, RS_{12}, RS_{15} and RS_{16}) were used in the current study.

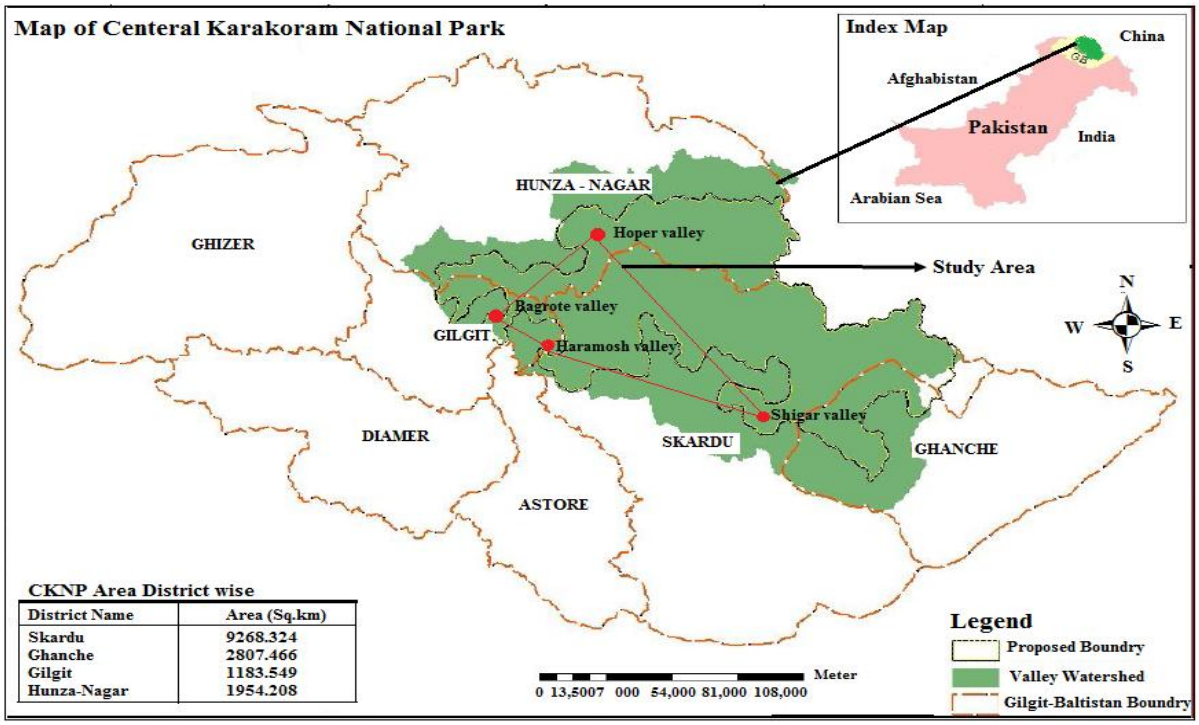

Fig 1. Isolates collection valley of CKNP region Gilgit-Baltistan

Collection of plant species

Fresh leaves of six plant species (*Cannabis sativa L*, *Peganum harmala L.*, *Datura starmonium* L., *Artemisia brevifolium* L., *Capparis spinosa* L. and *Mentha royleana* L.) were collected from different areas of GB. The plant material were washed with distilled water and dried in shade and made fine powder. Hundred grams of each plant powder was homogenized by laboratory blender in 200 ml of ethanol (80%) and distilled water (20: 80 v/v) for 10-15 min, and then put in dark glass bottles for three days for complete extraction. The plant extracts were then filtered through thin cheesecloth. The final extracts were placed in dark bottle and exposed to 60°C in water bath for half an hour for ethanol evaporation.

In vitro Screening of Botanical Extract by Food Poisoning Techniques

All botanical extract were subject to poisoned food technique as described by Manmohan and Govindaiah (2012) to study colony growth response of *R. solani* isolates against botanical extracts @ 5%, 10% and 15%. Potato dextrose agar was used as medium. Approximately 20 ml of poisoned medium were poured into 90 mm petri-dish and allowed for solidification. After solidification 5mm actively growing culture of each isolates were cut and put in center of petri dish. Three replicates and three treatments were maintained for each isolates and botanical extract. All the petriplates were incubated at 25±2°C for 4-5 days. Mycelial growth reduction was recorded in mm while % growth inhibition data were recorded by using the following formula (Vincent, 1947).

$$IMGR\ \% = \frac{MGC - MGT}{MGT} \times 100$$

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

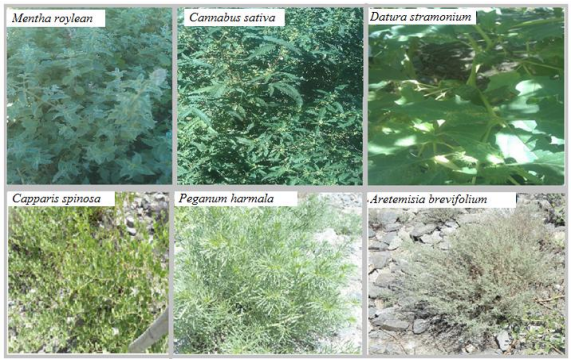

Fig 2. Botanical plant used in this current study

Antagonistic effect of bio-control agents

Two fungal bioagents *Trichoderma harzianum* and *Trichoderma viride* were used against the *Rhizoctonia solani isolates*. The antagonistic effects of tested bioagent against mycelial growth were tested *in vitro* using dual culture technique as described by Coskuntuna and Ozer (2008). Potato dextrose agar was used as medium and plates were equally divided into two portions (Karunanithi and Usman, 1999). In the first half, actively growing culture of bioagent (5 mm) was incubated while in the opposite side (5mm) of each *R. solani* isolates were placed (Fig 3). Three replicates were maintained for each isolates and experiments were repeated twice. All incubated petriplates were kept at 25±2°C until the growth of each isolates in the control to reach to the edge of Petri dish. Data were recorded by using formula as described earlier.

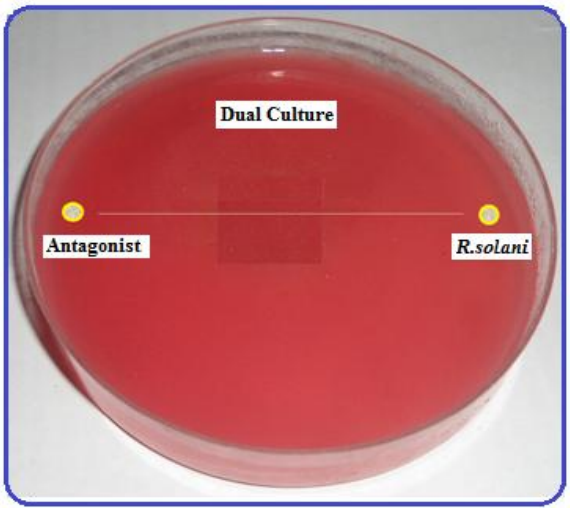

Fig 3. Diagrammatic illustration of dual culture technique**.**

Statistical Analysis

The *in vitro* experiment was laid out in a randomized complete block and analysis of variance (ANOVA) of the data was performed using the statistical package STATISTICA 8.1 while Least Significant Difference (LSD) was used to compare treatment means. Percentage of mycelial growth inhibition was determined by above mentioned formula.

Results

The bioefficacy of botanical extracts and bioagents against *R.solani* isolates were evaluated *in vitro* by food poison and dual culture techniques. The data on botanical extracts revealed that all tested botanical extracts showed varied degree of inhibition over control in the mycelia growth of *R. solani* isolates at different concentration. Colony growth decreased significantly (P 0.05) with the increase of concentrations. The mycelial growth reduction and inhibition %age of *R.solani* isolates against botanical extract were presented in the tables 1, 2 and 3. At 5% botanical extract mycelial growth inhibition %age of *R.solani* isolates against botanical extract were ranged as *C. sativa* (36.43-42.45%), *P. harmala* (26.71-33.34%), *D. starmonium* (26.26-28.15%), *A. brevifolium* (24.04-28.36.84%), *C. spinosa* (21.70-27.21) and *M. royleana* (24.14-27.50) (Table 1). At 10% extract *C. sativa* (52.14-62.41), *P. harmala* (45.56-53.91), *D. starmonium* (51.74-55.14), *A. brevifolium* (47.58-52.96), *C. spinosa* (39.59-44.44) and *M. royleana* (45.45-50.48) Table2). The growth inhibition %age of mycelia in 15% concentration of botanical extracts was recorded in the ranged of 76.26-80.39, 62.28-69.20, 66.92-70.28, 62.80-66.67, 50.16-54.53, (58.52-61.93) in *C. sativa P. harmala D. starmonium A. brevifolium C. spinosa M. royleana* respectively Table 3. Highest mean antifungal properties were found in *C. sativa* followed by *D. starmonium* and *P.harmala,* while least antifungal properties found in *C. spinosa* (Fig 4). As well as highest mean sensitivity of *R.solani* isolates against botanical extracts at different concentration were found in RS_{10}, RS_5 and RS_4, while the least in RS_{12} and RS_{16} (Fig 5). Regarding the antagonistic activity of bioagents the results showed that *T.harzianum* and *T. virid* reduced the mycelial growth of eight sclerotial isolates of *R. solani*. Fungal bioagent *T. harizanum* suppressed the mycelial growth of eight isolates of *R.solani* in the range of 48.32 to 72.72%, while, *T. viride* reduced the mycelial growth of the isolates by 28.75 to 56.80 %. *T.harzianum* showed more antagonistic effect on RS_{10} followed by RS_5 and RS_2, while the least in RS_4, whereas RS_3, RS_2 and RS_5 were

more sensitive to *T. viride* and RS_{15} was the least sensitive (Table 4).

Table 1. In vitro growth reduction (mm) and inhibition % of *R. solani isolates in* response to different botanicals extract @ 5 %.

Botanicals	RS_2	RS_3	RS_4	RS_5	RS_{10}	RS_{12}	RS_{15}	RS_{16}
C. sativa	51.37c (39.15)	49.12d (42.45)	51.94c (39.88)	48.68d (42.30)	50.69b (39.92)	52.40c (36.43)	53.07d (37.58)	52.50d (39.52)
P.harmala	59.56b (29.45)	58.86c (31.04)	57.71b (33.20)	56.23c (33.35)	58.85ab (30.24)	60.15b (27.02)	60.16c (29.24)	60.31c (26.71)
D.starmonium	61.00b (27.75)	61.33bc (28.15)	62.12a (28.10)	61.68b (26.89)	61.29ab (27.35)	60.63b (26.44)	62.50ab (28.84)	60.68bc (26.26)
A.brevifolium	60.77b (28.02)	61.67ab (27.75)	61.93a (28.32)	60.82b (27.91)	53.29ab (36.84)	60.98b (26.02)	61.94abc (27.15)	62.51ab (24.04)
C.spinosa	64.06a (24.12)	64.11a (24.89)	62.89a (27.21)	64.82a (23.17)	64.26a (23.83)	63.82a (22.58)	63.87a (24.88)	64.44a (21.70)
M.royleana	61.30b (27.39)	62.36ab (26.94)	63.36a (26.66)	62.60ab (25.80)	61.36ab (27.27)	62.53a (24.14)	61.65bc (27.50)	61.10bc (25.76)
Mean	59.67 (29.32)	59.57 (30.21)	59.99 (30.56)	59.13 (29.91)	58.29 (30.91)	60.08 (27.11)	60.53 (28.81)	60.25 (26.79)
Control	84.43	85.36	86.40	84.37	84.37	82.43	85.03	82.30

Means in each row followed by the same letter are not significantly different at LSD test (P = 0.05) while number in parenthesis indicate inhibition % over control.

Table 2. In vitro growth reduction (mm) inhibition % of *R. solani isolates in* response to different botanicals extract @ 10%.

Botanicals	RS_2	RS_3	RS_4	RS_5	RS_{10}	RS_{12}	RS_{15}	RS_{16}
C. sativa	35.81e (57.58)	34.40f (59.70)	32.47e (62.41)	34.69e (52.14)	32.29e (61.72)	34.40d (58.26)	36.18e (57.45)	35.58e (56.76)
P.harmala	40.50c (52.03)	41.19d (51.74)	39.82d (53.91)	40.71c (51.74)	42.29c (49.87)	44.05b (46.56)	43.18b (49.22)	44.80b (45.56)
D.starmonium	38.66d (54.21)	39.90e (53.26)	41.69c (51.74)	38.80d (54.04)	37.58d (55.14)	38.96c (52.73)	38.22d (55.05)	37.34d (54.62)
A.brevifolium	44.26b (47.58)	42.42c (50.30)	40.64cd (52.96)	40.68c (51.78)	41.29c (51.06)	42.57b (48.35)	40.79c (52.02)	40.57c (50.70)
C.spinosa	50.62a (40.04)	49.22a (42.33)	48.00a (44.44)	49.77a (41.00)	48.97a (41.96)	50.24a (39.05)	49.11a (42.24)	49.71a (39.59)
M.royleana	43.34b (48.66)	44.29b (48.11)	45.32b (47.54)	46.02b (45.45)	44.64b (47.09)	42.98b (47.86)	42.10bc (50.48)	41.51c (49.56)
Mean	42.20 (50.02)	41.90 (50.91)	41.32 (52.17)	41.77 (50.49)	41.17 (51.20)	42.20 (48.80)	41.59 (51.08)	41.58 (49.48)
Control	84.43	85.36	86.40	84.37	84.37	82.43	85.03	82.30

Means in each row followed by the same letter are not significantly different at LSD test (P = 0.05) while number in parenthesis indicate inhibition % over control. .

Table 3. In vitro growth reduction (mm) inhibition % of *R. solani isolates in* response to different botanicals extract @ 15%.

Botanicals	RS_2	RS_3	RS_4	RS_5	RS_{10}	RS_{12}	RS_{15}	RS_{16}
C. sativa	18.83e (80.00)	17.54f (80.39)	18.93e (76.93)	17.35e (79.43)	17.68e (79.04)	19.33f (76.54)	17.31e (79.64)	19.54e (76.26)
P.harmala	27.20d (67.78)	29.35d (65.62)	31.78c (63.22)	28.05c (66.75)	25.98d (69.20)	28.52d (65.40)	29.85c (64.89)	31.04b (62.28)
D.starmonium	26.26d (68.70)	26.06e (69.47)	28.58d (66.92)	25.64d (69.61)	25.28d (70.03)	26.99e (67.26)	25.27d (70.28)	26.22d (68.14)
A.brevifolium	30.62c (63.73)	30.97C (63.72)	29.01d (66.42)	28.12c (66.67)	30.33c (64.05)	30.66c (62.80)	31.89b (64.49)	29.16c (64.56)
C.spinosa	41.33a (51.04)	40.36a (52.72)	39.42a (54.46)	39.40a (53.30)	41.04a (51.36)	41.08a (50.16)	38.66a (54.53)	38.98a (52.64)
M.royleana	34.44b (59.20)	34.42b (59.68)	34.26b (60.34)	33.09b (60.78)	34.99b (58.52)	32.11b (61.04)	32.36b (61.94)	31.33b (61.93)
Mean	29.78 (64.72)	29.78 (65.11)	30.33 (64.89)	28.60 (66.10)	28.21 (66.56)	29.78 (63.87)	29.22 (65.63)	29.37 (64.31)
Control	84.43	85.36	86.40	84.37	84.37	82.43	85.03	82.30

Means in each row followed by the same letter are not significantly different at LSD test (P = 0.05) while number in parenthesis indicate inhibition % over control.

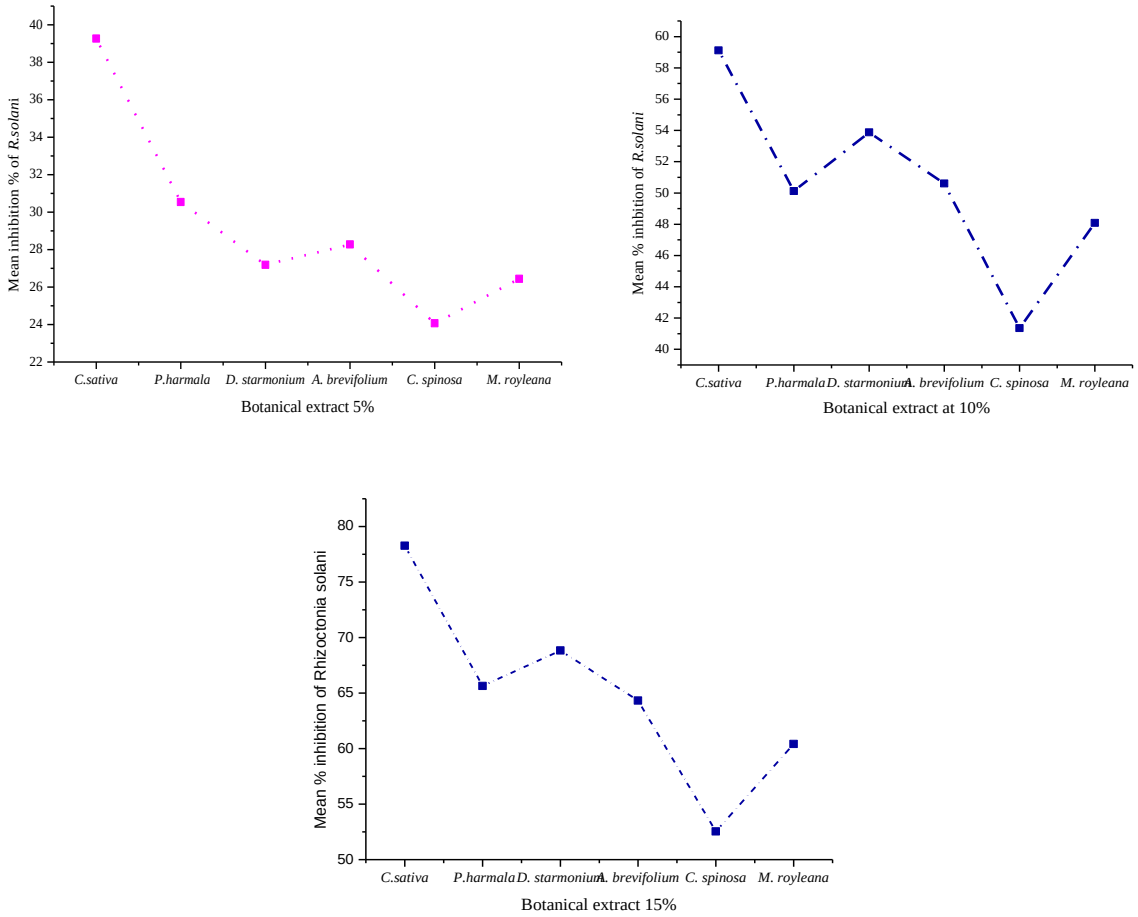

Fig 4. Mean inhibition % botanical extracts at 5, 10 and 15% against *R.solani*

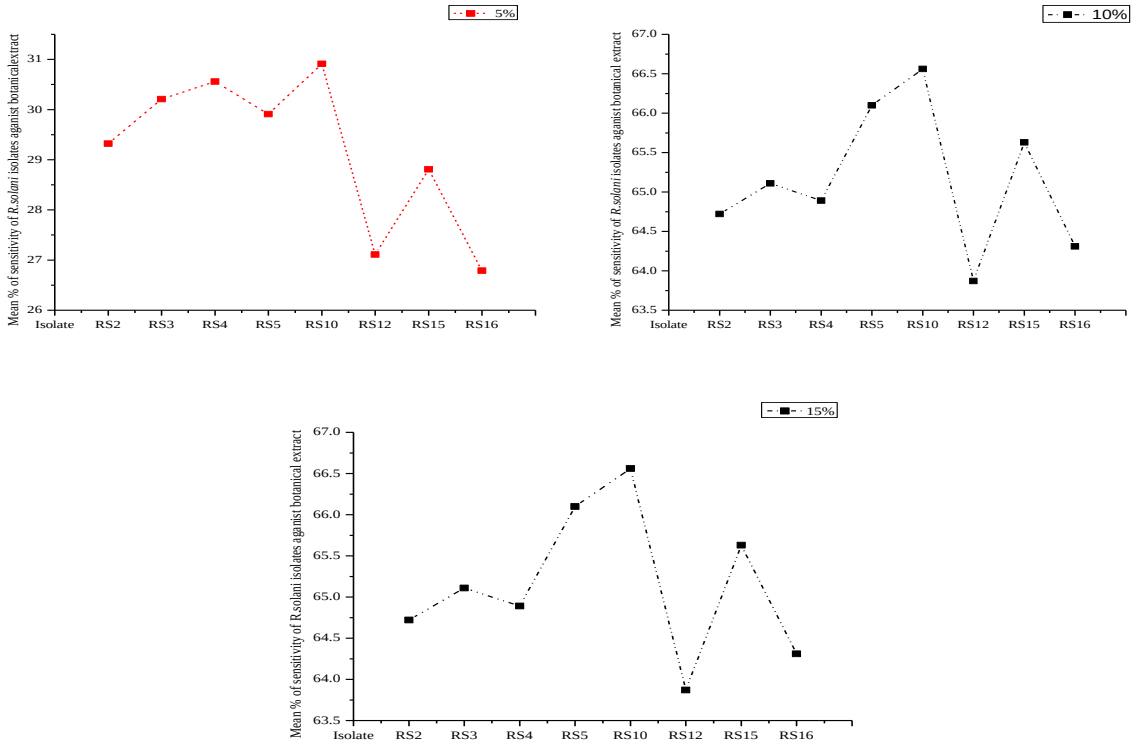

Fig 5. Mean % of sensitivity of *R.solani* isolates against botanical extracts

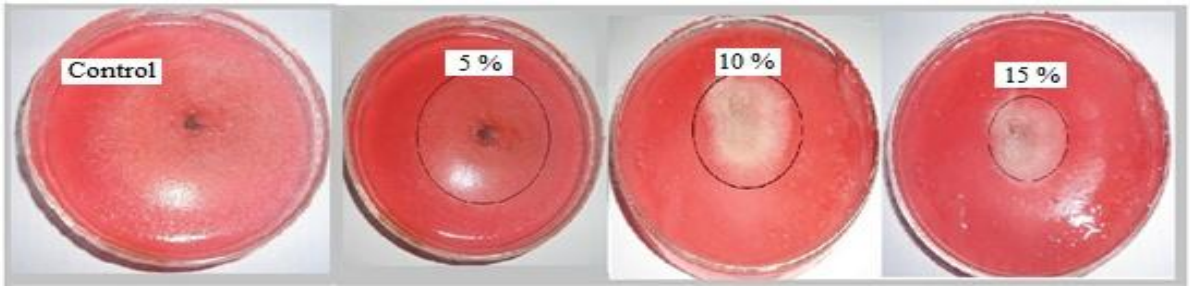

Fig 6. Effect of different concentration of botanical extract against Rhizoctonia solani

Table 4. Antagonistic effect of bio-control agents against mycelial growth of Rhizoctonia *solani* isolates

Fugal Isolates	*Trichoderma harzianum*	*Trichoderma viride*
	Mycelial Growth Reduction (%)	
RS_2	61.87b	54.40a
RS_3	54.76cd	56.80a
RS_4	48.32e	39.24c
RS_5	62.48b	46.49b
RS_{10}	72.72a	43.40bc
RS_{12}	58.37bc	32.50d
RS_{15}	49.70e	28.75d
RS_{16}	50.91de	48.11b
Mean	57.39	43.71
Minimum	48.32	28.75
Maximum	72.72	56.80
SD	8.22	9.74
CV %	14.32	22.28

Means in each column followed by the same letter are not significantly different at LSD test (P = 0.05). SD: Standard deviation, CV: Coefficient of variation.

Discussions

Black scurf is an important disease of potato all over the world. In GB it is one of the most noticeable diseases. This agent *Rhizoctonia solani* causes necrosis of stem and reduces yield (Keijer *et al*, 1997). Sclerotia present on potato skin are important features of *R. solani* (Ciampi *et al*, 2006). Control of this disease has been unpredictable, difficult and expensive; however, the search of a new control system by botanical extract and bioagent is becoming a new possibility. In the current study six botanical extracts and two bioagents were used for *in vitro* assessment against *R.solani* isolates. Result revealed that among the botanical extracts *C. sativa* was most effective followed by *P. harmala, D.starmonium, A.*

brevifolium, M. royleana while least antifungal activity was shown in *C. spinosa*. Our results of current study were in agreement with Gaurav and Berijesh, 2013, Shivpuri and Gupta, 2001 and Sarpeleh *et al*, 2009. Many researchers also reported inhibitory effect of *Cannabis sativa*, Datura specifies on *Alternaria solani, Fusarium oxysporum Macrophomina phaseolina* and *Rhizoctonia solani* (Jalander and Gachande, 2012; Shahnaz *et al,* 2010). The mycelial inhibition of plant extract possible due to the presence of antifungal compounds like phenols, phenolic acids, quinones, flavones, flavonols, tannins and coumarins (Baker, 1981; Kuc and Shain, 1977 Das *et al*, 2010; Cowan, 1999). Similarly C. sativa contains cannabinoids, flavanoid glycosides, carbohydrates, simple alcohols, aldehydes, ketones, acids, esters and lactones; non-cannabinoid phenols; nitrogenous compounds; and vitamins and pigments (Hillig and Paul, 2004; ElSohly, 2005; Flores-Sanchez and Verpoorte, 2008); *D. stramonium* (alkaloids, atropine, scopolamine, tannin (Aqib and Mohib, 2014) and *P. harmala* (Harmine, harmaline and their derivatives, harmal, harmalol, tetrahydroharmine and tetrahydroharmol) and have shown activity against bacteria, algae and fungi (Zaidi *et al*, 2004; Kumar *et al*, 2005). Biological management of pathogenic organisms considered as potential tools in the recent and coming decades. Searching of effective and highly potential bioagent is continuing. Currently *Trichoderma sp* is the most common and useful fungal bioagent and have been known as effective antagonists against plant pathogenic fungi (Chet, 1987; Papavizas, 1985). So in this study two species of *Trichoderma harzianum* and *Trichoderma viride* were used against the sclerotial isolates of *Rhizoctonia solani*. The results showed that the tested bioagents significantly suppressed the mycelial growth of R. solani isolates. Among the bioagent *T.harzianum* is most effective as compare to *T.viride*. *T.harzianum* produces protease enzyme that are capable of degrading the pathogen cell and reduce the capacity pathogen to grow or infect the plant (Elad and Kapat, 1999). Chet, 1987 stated that management of *R.solani* by *T.harzianum* may be affected through direct penetration of host hyphae. This bioagent grow towards hyphae of the other fungi coil them through lectin mediated infection and break the cell wall of the target pathogen. All these statement justified our current study. *Trichoderma* had a significant reducing effect on plant diseases caused by pathogens such as *Rhizoctonia solani, Pythium aphanidermatum, Sclerotium rolfsii, Fusarium oxysporum, F. culmorum* and *Gaemannomyces graminis* var. *tritici* under greenhouse and field conditions (Basin *et al*, 1999). The present research outcomes are also in agreement with that of Munshi and Dar, 2004, who reported that the formation of inhibition zone by *Gliocladium* sp. against *Fusarium pallidoroseum*. *T. viride* and *T. harzianum* have also been reported to be effective fungal bioagent against *A. solani* and *Sclerotium rolfsii* (Ganie *et al*, 2013; Alice *et al*, 1998) and *Rhizoctonia solani* (Elad *et al*, 1980; Shalini and Kotasthane, 2007).

Conclusion

The botanical extract of current study clearly showed that they contain antifungal property especially *C.sativa, D. starmonium and P. harmala*. These biofungicide can be used for management of plant diseases especially potato as well as they would be very much beneficial for the farmers, who unable to purchased expensive chemical fungicide. At the same time economic uses of these unwanted plants and formulating integrated disease management plan. Beside these two types of bioagents *T. harzanium* and *T.viride* was used. *T.harzianum* has higher antagonistic potential as compared to *T.viride*. Further investigations needed for isolation and utilization of other local isolates of *Trichoderma* species.

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