

Original Research Article

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Management of Alternaria Leaf Spot of Blond Psyllium (*Plantago ovata* Forssk.) Through Plant Extracts and Bio-Agents

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ABSTRACT

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Studies were conducted to know the efficacy plant extracts and bio-agents against Alternaria leaf spot of blond psyllium/isabgol (*Plantago ovata* Forssk.) caused by *Alternaria alternata* which has become an severe problem in isabgol growing areas of Rajasthan. Among five plant extracts tested, garlic (10%) was recorded most effective in inhibiting mycelia growth (74.30%) as well as in reducing disease intensity (53.26%) by applying two sprays at 7 days interval. Among four bio-agents, *Trichoderma viride* was observed highly effective in inhibiting mycelial growth (79.20%) and in reducing disease intensity (41.84%).

Introduction

Blond psyllium (*Plantago ovata* Forssk.) also known as “isabgol”, is an annual herb with narrow linear rosette like leaves belonging to the family *Plantaginaceae*. Among 200 species of blond psyllium, *Plantago ovata* Forssk is known for superior quality of husk. Isabgol seeds and husk is used in medicines especially for relieving constipation. Isabgol is an important cash crop cultivated for its export and being of important medicinal value is reported to have larger demands and is traded in major medicinal drug markets of the world. India commands nearly monopoly in the production and export of the seed and husk to the world market. India is earning about Rs. 1600 million as foreign exchange

from the export of blond psyllium products to countries like USA, Germany, France, England, Spain and Belgium (Maiti, 2000). In India, the isabgol crop is mainly grown as commercial crop in Gujarat, Rajasthan and Madhya Pradesh. However, the crop is spreading to other non-traditional parts of the country such as Haryana, Uttar Pradesh and Karnataka. In Rajasthan, it is being cultivated in 304430 hectare area with a total production of 144177 tonnes of seeds with an average productivity of 474 kg/ha (Anonymous, 2015-16). In Rajasthan, isabgol mainly cultivated in Barmer, Jalore, Nagaur, Jodhpur, Pali, Sirohi, Chittorgarh, Udaipur and Jaisalmer districts. Alternaria leaf spot of isabgol (*Plantago*

ovata Forssk.) caused by *Alternaria alternata*, has become a severe problem in Isabgol growing areas of Rajasthan. Shekhawat and Prasad (1971) have tested nine plant extracts against *Alternaria alternata*, out of them five viz., *Allium cepa*, *A. sativum*, *Ocimum sanctum*, *Mentha piperita* and *Beta vulgaris* showed strong inhibitory action. Parveen and Kumar (2004) proved *Trichoderma viride* as most effective antagonist in inhibiting the mycelial growth of *Alternaria triticina*.

Material and Methods

Efficacy of plant extracts (*in vitro*)

The effect of plant extracts was tested at two concentrations i.e. 5 and 10 per cent. For preparing plant extracts of plant parts including leaves, rhizomes and cloves to be tested were first washed with tap water followed by sterilized water and then air dried. Plant parts then thoroughly grind separately in grinder using equal amount of sterilized distilled water to get stock solution. The mixture was squeezed with double-layered sterilized cheese cloth.

The extract thus obtained was considered as of 100 per cent concentration. It was further diluted to get 5 and 10 per cent of concentrations using sterilize distilled water. The effect against mycelial growth was tested using Poisoned Food Technique. Required quantity of each plant extract was mixed thoroughly in sterilized melted PDA aseptically under laminar flow and thoroughly mixed to get desired concentrations; medium amended with desired quantity of plant extract was poured aseptically in sterilized Petri dishes and was allowed to solidify. Each plate was inoculated with 2 mm disc of mycelial bit taken from the periphery of 10-day-old culture *A. alternata* growing on PDA. The inoculated Petri dishes were then incubated at 25 ± 1 °C. Four Petri dishes were

used for each treatment serving as four replications. Petri dishes without plant extract served as control. Experiment was conducted in Completely Randomized Design (CRD) with four replications. Colony diameter was measured after 7 days of incubation. Per cent growth inhibition was calculated as per formula of Vincent (1947).

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where,

C = Diameter of the colony in check (average of both diagonals)

T = Diameter of the colony in treatment (average of both diagonals)

Efficacy of plant extract (*in vivo*)

An experiment was conducted during *rabi* 2016-17 with local susceptible cultivar of isabgol with four replications. Artificial inoculation was done with spore suspension (1×10^5 spore/ml) of *Alternaria alternata* and after four days of inoculation of the culture, two sprays (10 %) of each plant extract was applied at an interval of seven days.

Observations on per cent disease intensity (PDI) was recorded by using 0-5 disease rating scale of Rathore and Pathak (2001) where, 0 = plant completely free from disease symptoms; 1=20% leaf area of a plant covered with leaf spot; 2=21-40% leaf area of a plant covered with leaf spot; 3=41-60% leaf area of a plant covered with leaf spot; 4=61-80% leaf area of a plant covered with leaf spot; 5=More than 80% leaf area of a plant covered with leaf spot and per cent disease intensity (PDI) was calculated as per formula given by McKinney (1923) as follows:

Sum of all individual ratings
Per cent
Disease intensity = ----- x 100
Number of leaves observed X
Maximum disease rating

Efficacy of bio-agent (*In vitro*)

Four bio-agents *i.e.* *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated for their antagonistic efficacy against *Alternaria alternata* by dual culture technique. Twenty ml of PDA was poured into sterile Petri plates. Fungal bio-agents were evaluated by inoculating the pathogen at one side of the Petri plate and the bio-agent inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used. In case of bacterial bio-agent evaluation, two mycelial discs of pathogen were inoculated and bacterial bio-agent was streaked in the center of the plate.

One control was maintained wherein only test fungus was grown. Such treatments replicated four times. The plates were incubated for seven days at 25 ±1 °C. After incubation, the colony diameter of *Alternaria alternata* was recorded. Per cent inhibition was calculated by using the formula of Vincent (1947).

Efficacy of bio-agent (*In vivo*)

An experiment was conducted during *rabi* 2016-17 with local susceptible cultivar of isabgol with four replications. Artificial inoculation was done with spore suspension (1X10⁵ spore/ml) of *Alternaria alternata* and after four days of inoculation of the culture, each bio-agent was sprayed and repeated after seven days of first spray. The observations on disease intensity were recorded after 10 days of last spray.

Result and Discussion

Five plant extracts *i.e.* datura (*Datura stramonium*), garlic (*Allium sativum*), giloy (*Tinospora cordifolia*), karanj (*Pongamia pinnata*) and ginger (*Zingiber officinale*) were tested at two concentrations *viz.* 5 and 10 per cent *in vitro* against *Alternaria alternata* on PDA by poisoned food techniques. Results of mean analysis (Table 1) revealed that maximum mycelial growth inhibition was observed in garlic clove extract (62.40%) followed by datura (56.25%), giloy (44.26 %) and ginger (40.15 %) and minimum 36.35 per cent in karanj extract. Minimum disease intensity (30.10%) was recorded (Table 2) in garlic clove extracts with decreased intensity by after applying two foliar sprays. This was followed by datura (32.40%), giloy (41.80%) and ginger (47.26%). Karanj extract was found least effective as it gave higher intensity (48.00%). The results obtained in the present study are in accordance with the results obtained by Barros *et al.*, (1995), Singh and Majumdar (2001), Jadeja and Pipliya (2008), Panchal and Patil (2009) and Bochliya *et al.*, (2012) and Shekhawat and Prasad (1971).

In the present study, four bio-agents were tested *in vitro* as well as *in vivo* against *A. alternata* causing Alternaria leaf spot of isabgol. All bio-agents tested reduced mycelial growth and disease intensity of *A. alternata* as compared to control. Among these, *T. viride* was observed to be significantly superior and recorded highest mycelial growth inhibition (79.20 %) and thereby proved most effective antagonist (Table 3) against the test fungus, followed by *T. harzianum* (74.40 %) and *Pseudomonas fluorescens* (60.40 %). Minimum inhibition was observed with *Bacillus subtilis* (44.20 %).

Table.1 Efficacy of plant extracts against *Alternaria alternata* by poisoned food technique at 25+1 0C for 7 days

Plant extracts	Per cent growth inhibition at different concentration		
	5%	10%	Mean
Datura	47.00 (43.28)	65.50 (54.03)	56.25
Garlic	50.50 (45.29)	74.30 (59.54)	62.40
Giloy	40.12 (39.30)	48.40 (44.08)	44.26
Karanj	30.40 (33.46)	42.30 (40.57)	36.35
Ginger	35.20 (36.39)	45.10 (42.19)	40.15
Control	0.00	0.00	-
	SEm±	CD (p= 0.05%)	
P	0.62	1.73	
C	0.88	2.45	
P x C	1.53	4.24	

*Average of four replications

Figures given in parentheses are angular transformed values

Table.2 Efficacy of plant extracts against *Alternaria alternata* of isabgol

Treatments	Concentration	Per cent disease intensity	Per cent disease reduction over control
Datura	10	32.40 (34.70)	49.69
Garlic	10	30.10 (33.27)	53.26
Giloy	10	41.80 (40.28)	35.09
Karanj	10	48.00 (43.85)	25.47
Ginger	10	47.26 (43.43)	26.61
Control	0.0	64.40 (53.37)	0.00
SEm±		1.53	
CD (p=0.05)		4.72	

*Average of four replications

Figures given in parentheses are angular transformed values

Table.3 Effect of bio-agents against mycelial growth of *A. alternata* after 7 days of incubation at 25 + 10C

Bio-agents	Per cent mycelia growth* inhibition
<i>Trichoderma harzianum</i>	74.40 (59.60)
<i>Trichoderma viride</i>	79.20 (62.87)
<i>Pseudomonas fluorescens</i>	60.40 (51.00)
<i>Bacillus subtilis</i>	44.20 (41.67)
Control	0.00
SEm+	1.84
CD (p=0.05)	5.12

* Average of four replication

Figures given in parentheses are angular transformed value

Table.4 Efficacy of bio-control agents against *Alternaria alternata* of isabgol

Bio-agents	Dose (g/lit)	Per cent disease intensity	Per cent disease reduction over control
<i>Trichoderma harzianum</i>	4	36.50 (37.17)	39.69
<i>Trichoderma viride</i>	4	35.20 (36.39)	41.84
<i>Pseudomonas fluorescens</i>	6	39.50 (38.94)	34.73
<i>Bacillus subtilis</i>	6	45.90 (42.65)	24.16
Control	0.00	60.52 (51.07)	0.00
SEm+		0.96	
CD (p=0.05)		2.95	

* Average of four replication

Figures given in parentheses are angular transformed value

Foliar sprays of bio-agents indicated that minimum disease intensity (35.20%) was recorded (Table 4) with *T. viride* and it was significantly superior over control followed by *T. harzianum* (36.50%). In case of bacterial bio-agents, *Pseudomonas fluorescens* was observed to be effective in decreasing (39.50%) disease intensity as compared to *Bacillus subtilis* (45.90%). Kumar (2004) have reported the effectiveness

of *Trichoderma viride* in inhibiting the mycelial growth of *Alternaria triticina* causing leaf blight of wheat *in vitro*. Jadeja and Pipliya (2008) have also been proved *T. harzianum* and *T. viride* as most effective antagonist by recording 100 per cent inhibition of *A. burnsii*, the causal organism of blight of cumin. Deepak *et al.*, (2008) have also been recorded maximum reduction with *T. harzianum* under *in vitro* and field

conditions against *A. burnsii*. The results are also in close agreement with the findings of Bochalya *et al.*, (2012) who reported that *T. viride* was most superior against *Alternaria alternata* over other treatment *in vitro* conditions.

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