

Original Research Article

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In vitro Appraisal of Botanicals and Bioagents against *Alternaria brassicicola* Inciting Alternaria Leaf Spot of Cabbage

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ABSTRACT

Keywords

Cabbage, *Alternaria brassicicola*, Botanicals, Neem, Bioagents and *T. asperellum*

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The efficacy of botanicals and bioagents against *Alternaria* leaf spot of cabbage is caused by *Alternaria brassicicola*. Among the botanicals tested, the maximum per cent mycelium growth inhibition was found in neem (35.67, 38.14, 40.95, 47.17 and 57.64%) followed by garlic (26.88, 28.94, 32.61, 35.14 and 41.83%) and Tulsi, respectively. Whereas, minimum growth inhibition was found in camphor at (100, 500, 1000, 1500 and 2000 ppm). In bioagents, *T. asperellum* (73.55%) was most effective followed by *T. harzianum* (67.22%), *P. fluorescens* (64.45%), whereas, *B. cereus* was least effective.

Introduction

Cabbage is most preferable crops among the consumers for Indian and continental cuisine. The production of cabbage is mostly affected by various biotic factors at different stages of plant. The *Alternaria* causes of the heavy yield losses of different vegetable family viz. cucurbitaceae, brassicaceae and solanaceae. The three major species of *Alternaria* that causes serious damage to brassicas: *A. brassicicola*, *A. brassicae* and *A. raphani* infect cabbage, cauliflower, kale, brussels

sprouts, Chinese cabbage and turnip. There are two species of *Alternaria* which cause serious damage in cabbage: *Alternaria brassicae* and *A. brassicicola*, they can survive saprophytically outside of the host and diseased crop debris (Yadav *et al.*, 2014). *Alternaria* black leaf spot is the most destructive disease of cabbage and brassicas worldwide (Meah *et al.*, 2002). A complex of *Alternaria* species (*A. brassicicola* (Schw.) Wiltsh., *A. brassicae* (Berk.) Sacc., *A. alternata* (Fr.) Kreissler and *A. raphani* Groves and Skolko) are responsible for

important yield losses (Verma and Saharan, 1994). Mycelium of necrotrophic fungus *A. brassicicola* is septate, olive grey to grayish black in colour. The conidiophores are olivaceous, septate, branched measuring 35-45 μm in length and 5-8 μm in width. Conidia are dark, cylindrical to oblong, muriform without beak measuring 44-55 μm in length and 11-16 μm in width with 5-8 transverse and 0-4 transverse septa. The fungus grows faster in media with high sporulation and appears as well developed black sooty colour with distinct zonations (Kolte, 1985). Plants inoculated with *A. brassicicola* develop symptoms most quickly at 25°C, while seedlings from infected seeds develop symptoms most quickly at 30°C. No germination occurs at 3°C for all three pathogens namely *A. brassicicola*, *A. brassicae* and *A. raphani* (Bassey and Gabrielson, 1983). Among fungal diseases, Alternaria leaf spot of cabbage caused by *A. brassicicola* is seriously damage the production of cabbage around the world. Alternaria leaf spot is the most destructive diseases on cabbage. Mostly 20% of agricultural breakdown is caused by *Alternaria* spp. The major serious losses may reach upto 80% of yield and 59% loss of cabbage seed yield may causes due to Alternaria blight (Hossain and Mian, 2004). Alternaria leaf spot decreasing yield upto 50% without using any control (Cline, 2002). The present study was aimed for determining an effective management with botanicals and bio-agents on Alternaria leaf spot of cabbage.

Materials and Methods

Evaluation of botanicals against *A. brassicicola*

The experiment was conducted at Department of Plant Pathology, College of Horticulture, VCSG UUFH Bharsar (Pauri Garhwal) Uttarakhand, during 2018-19. The eight

botanicals *i.e.* neem (*Azadirachta indica*), garlic (*Allium sativum*), nettle (*Urtica dioica*), lemongrass (*Cymbopogon citratus*), mint (*Mentha piperita*), vach (*Acorus calamus*), tulsi (*Ocimum tenuiflorum*) and camphor (*Cinnamomum camphora*) at 100, 500, 1000, 1500 and 2000 ppm.

Preparation of botanicals

Plant extracts were prepared by grinding the required quantity of leaves (100g) before grinding equal quantity of water added in the respective plant leaves (1:1 w/v). The crude extract of botanicals was filtered different leaves was sieved through double layered muslin cloth. Each of the filtrate obtained was further filtered through Whatman No.1 filter paper using funnel and volumetric flasks (100 mL cap.) The final clear extracts obtained formed the standard plant extracts of 100 per cent concentration and the extract thus obtained were kept in a refrigerator at 4±1°C by using poisoned food techniques (Nene and Thapliyal, 1993). The flasks were shaken gently to ensure the proper mixing of botanicals in PDA, 20 mL of molten and cooled PDA was poured in each Petri plate. After solidification of media, mycelial disc (5 mm) were cut from the edges of seven days old culture of test pathogen with the help of sterilized cork borer. The fungus was incubated at 25 ± 2°C for five days with four times replicated. The observation on colony diameter in (mm) was recorded and per cent inhibition over control, calculated by using formula given.

Evaluation of bioagents against *A. brassicicola*

Four bioagents *i.e.* *Trichoderma harzianum*, *Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus cereus* were evaluated for their antagonistic properties against *A. brassicicola* following dual culture

technique (Faheem *et al.*, 2010). The bioagents and the test fungus were inoculated both sides on a single Petri plates containing solidified PDA with four replications for each treatment. Control was also run along with the other treatments. Inoculated plates were incubated at $25\pm 2^{\circ}\text{C}$ for five days. The radial growth of the colony of bioagents and the pathogen measured in two directions and average radial mycelia growth was recorded. Per cent mycelia growth inhibition was calculated by using the formula of Vincent (1947).

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

Where,

C= Colony mycelia in control

T= Colony mycelia in treatment

The data noticed was analyzed by using standard statistical procedure in the simple completely randomized design (CRD) with the help of OPSTAT and Graph Pad (3.05).

Results and Discussion

Effect of botanicals on mycelial growth of *A. brassicicola*

The observations were recorded in (Table 1) clearly indicates that all the test botanicals and bioagents were significantly superior over control on the growth of *A. brassicicola*. Among botanicals, the maximum per cent mycelium inhibition of *A. brassicicola* was recorded in neem (35.67, 38.14, 40.95, 47.17 and 57.64%) followed by garlic (26.88, 28.94, 32.61, 35.14 and 41.83%), tulsi and lemongrass, whereas the minimum per cent mycelium inhibition of *A. brassicicola* was recorded in camphor followed by vach, nettle and mint, respectively. Raza *et al.*, (2015) also observed the similar result, among all

five tested plant extracts, *Azadirachta indica* was significantly superior over other treatments followed by *Allium sativum*, *Parthenium hysterophorus* and *Datura stramonium*. Least inhibition was observed in *Eucalyptus camaldulensis* (49.31%). Overall results demonstrated that all the tested concentrations of *Azadirachta indica* were found significantly effective for controlling early blight of tomato. Gupta *et al.*, (2019) also found that the neem plant extract was found highly effective against pathogen at both concentrations (15 % and 25 %) and mycelial inhibition was recorded (65.55 at 15% and 68.88 at 25%). Sasode *et al.*, (2012) found related result like among the crude extract 10 per cent the minimum fungus growth was recorded in neem followed by eucalyptus, tulsi, lantana, datura and pudina. Neem was significantly superior over tulsi, lantana, datura and pudina but at par with eucalyptus. Mesta *et al.*, (2009) found that neem leaf extract with 38.49 per cent inhibition of spore germination and 43.90 per cent inhibition of mycelial growth was effective than all other plant extracts. Ravi *et al.*, (2014) also tested different botanicals against *R. solani* inciting leaf blight of Kalmegh (*Andrographis paniculata*). Garlic extract at 15% was found to be most effective with 78.89 % inhibition followed by thuja extract at 15.0 % concentration with 75.56 % inhibition. Vivekanand *et al.*, (2018) found that neem extract at 600 ppm resulted in maximum (57.20%) mycelial growth inhibition followed by garlic extract (53.83%) against *C. capsici*.

Effect of bioagents on mycelial growth of *A. brassicicola*

Evaluation of bio-agents per cent inhibition of mycelia over control against *A. brassicicola* in (Table 2) revealed that maximum per cent inhibition was recorded in *Trichoderma asperellum* (73.55%) followed by *T.*

harzianum (67.22%), *Pseudomonas fluorescens* (64.45%) whereas, *Bacillus cereus* was least effective. Kuzmanovska *et al.*, (2018) reported that the both bioagents, antagonists inhibited the mycelial growth (*T. asperellum* from 74.24% and *T. harzianum* from 71.07%). These bio-control agents used for control of gray mold disease in tomato. Patil and Prajapati (2017) also found that the highest growth inhibition was recorded in *T.*

asperellum (74.72 %) followed by *T. viride* (69.86 %) and *T. harzianum* (66.80 %) to control the *Rhizopus* soft rot of tomato. Khalse *et al.*, (2017) also observed that the maximum mycelial growth was recorded in T₁- *Trichoderma harzianum* (65.21%) followed by T₂- *Pseudomonas fluorescens* (62.41%). Similar, findings were also reported by Chavan *et al.*, (2015) and Maheshwari and Krishna (2013).

Table.1 Effect of botanicals on per cent mycelium inhibition of *A. brassicicola*

Treatments	Average per cent mycelium growth inhibition									
	100ppm		500ppm		1000ppm		1500ppm		2000 ppm	
	G	I	G	I	G	I	G	I	G	I
Control	67.10	0.00	67.10	0.00	67.10	0.00	67.10	0.00	67.10	0.00
Neem	43.15	35.67	41.50	38.14	39.41	40.95	35.44	47.17	28.39	57.64
Garlic	49.05	26.88	47.67	28.94	45.22	32.61	43.51	35.14	39.02	41.83
Nettle	59.09	11.88	57.43	14.40	55.04	17.96	53.22	20.67	50.57	24.63
Lemongrass	55.53	17.23	53.37	20.45	51.51	23.21	48.07	28.35	46.86	30.15
Mint	57.52	14.27	55.87	16.72	53.92	19.64	51.97	22.53	48.72	27.38
Vach	60.22	10.24	58.15	13.32	56.33	16.03	54.43	18.86	51.35	23.45
Tulsi	53.61	20.09	51.42	23.35	49.17	26.71	47.28	29.52	43.44	35.24
Camphor	61.06	8.98	59.47	11.36	57.55	14.21	55.34	18.26	53.39	20.75
S.E.(d)	0.40	-	0.33	-	0.28	-	0.25	-	0.38	-
C.D.(0.05)	0.83	-	0.68	-	0.59	-	0.52	-	0.78	-

G=Average mycelia growth in (mm); I= Average mycelia growth inhibition in (%)

Table.2 Effect of bioagents on per cent mycelia growth inhibition of *A. brassicicola*

Treatments	Mycelial growth(mm)± SE(m)	Percent inhibition of mycelia growth
Control	67.10 ±0.04	0.00
<i>Trichoderma harzianum</i>	21.55±0.10	67.22
<i>Trichoderma asperellum</i>	17.39±0.20	73.55
<i>Bacillus cereus</i>	30.09±0.09	54.25
<i>Pseudomonas fluorescens</i>	23.38±0.14	64.45
S.E.(d)	0.18	-
C.D.(0.05)	0.39	-

In bioagents *Bacillus cereus*, *Pseudomonas fluorescens* and *Trichoderma harzianum* were tested for antagonistic properties against *A. alternata* under *in vitro* (Tekiner *et al.*, 2019).

Raj Hans and Sharma (2017) found that among the bio-agents *T. harzianum* exhibited mycelial growth inhibition (46.15%) to evaluate their inhibitory effects against the *A.*

alternata causing mouldy core, core rot of apple. Ravi *et al.*, (2014) also found that *Trichoderma harzianum* (Th-4) caused maximum 88.71 % (*in vitro*) inhibition followed by Th-12 with 78.63% whereas, *Pseudomonas fluorescens* was least effective against *R. solani*. Vivekanand *et al.*, (2018) found that *in vitro* assessment of bio-control agents *i.e.* *T. harzianum* was found more effective with 50.89% inhibition than *P. fluorescens* against *C. capsici*.

In conclusion the tested eight botanicals against *A. brassicicola* at five different concentrations (100, 500, 1000, 1500 and 2000 ppm) in which maximum mycelium growth inhibition was obtained in neem followed garlic and Tulsi, respectively; while minimum inhibition was observed in camphor. Among four bioagents tested, maximum growth inhibition was found in *T. asperellum*, while *Bacillus cereus* was least effective.

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