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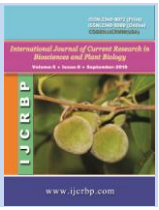

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In Vitro Assessment of Fungicide and Biocontrol Agent against Alternaria Leaf Spot of Cauliflower (*Brassica oleraceaea* var. *botrytis*)

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ABSTRACT

Cauliflowers (*Brassica oleraceaea, var. botrytis*) are annual or biennial and frost tolerant vegetable plants bearing large rounded leaves that resemble collards. It is often considered one of the healthiest foods on earth- owing to its rich supply of health-promoting phytochemicals, high level of anti-inflammatory compounds. Cruciferous plants (*Brassicaceae*) worldwide are severely affected by the *Alternaria* fungi. The leaf spot and seed disease caused by various species of *Alternaria* occur abundantly in some region of Garhwal Himalayas. This disease is highly prevalent and of late becomes so severe that growth and yield of the crop decreased considerably. The present work deals with the management of Alternaria leaf spot on cauliflower. The five isolates of *A. brassicicola* were collected and disease symptomatology was defined. *In vitro* efficacy of a fungicide, namely, Hexaconozole (a systemic fungicide) was tested against the isolates of *Alternaria* sp. Based on the results, it can be concluded that Hexaconozole is effective in suppressing the growth of *Alternaria* sp. In addition these isolates were interacted with two important biocontrol agents, *T. harzianum* and *T. viride.* Both the biocontrol agents were unable to control the growth of *A. brassicicola* as they did not touch the magical mark of 50 percent. Only one isolate (A5) was suppressed more than 50 percent by *T. harzianum* and *T. viride*.

Introduction

Brassica oleraceaea, var. botrytis, commonly known as cauliflower, belongs to family Brassicaceae. Brassicaceae or Cruciferae is a medium sized and economically important family of flowering plants commonly known as the mustards, the crucifers, or the cabbage family. The family contains the cruciferous vegetables, including the species such as *Brassica oleracea*

(e.g. broccoli, cabbage, cauliflower, kale, and collard). Cauliflower (*Brassica oleraceaea, var. botrytis*) is the most popular vegetable among Cole crops. It has small, thick stem bearing whorl of leaves and branched tap root system. The main growing point develops into shortened shoot system whose apices make up the convex surface of curd, so the curd is a prefloral fleshy apical meristem. The eatable part i.e. curd is generally white in colour and may be enclosed by inner leaves before its exposure. The curd colour varies with variety and environment. It may be white, creamy white, yellow, green or red (Chadha, 2013). China is the leading producer of cauliflower with 8,067,917 metric tons per year from 459 hectare area while India produces 7887 mT per year from 402m/ha area (Gupta et al., 2017). Major producer states are Punjab, Haryana, Uttar Pradesh, Bihar, Jharkhand, West Bengal, Assam, Maharashtra, Gujarat and Karnataka. Pudalov and Liang (2008) assessed the nutritive value of cauliflower suggesting that besides Vitamins B1, B2, B3, C & K, omega 3 fatty acid, phosphorus and magnesium are also present.

Foliar diseases are one of the most important limiting factors for cultivation of Brassicaceae in tropical and sub-tropical areas. Fungal species of the genus *Alternaria* are among the most important causal agents of leaf spot diseases on members of the Brassicaceae family (Humpherson-Jones, 1992; Cucuzza et al., 1994; Verma and Saharan, 1994; Peruch et al., 2006). Alternaria brassicicola was first recorded in 1924 from Holland on Cabbage (Bolle, 1924). The pathogens also occur on many weed species, which may serve as inoculum reservoirs (Farr et al., 1989; Cucuzza et al., 1994; Maringoni, 1997). They cause rounded, well-defined, dark brown leaf spots with pale centre, which are usually delimited by a chlorotic halo (Humpherson-Jones, 1992). The two most prevalent fungi associated with dark leaf spot diseases of Brassicaceae around the world are *Alternaria brassicae* (Berk.) Sacc. Ex Rape and *Alternariabrassicicola* (Schwein.) Wiltshire (Nakata and Takimoto, 1928; Rangel, 1945; Ellis, 1971; Degenhardt et al., 1982; Strandberg, 1992; Verma and Saharan, 1994). These fungi are causal agents of a complex foliar disease, which may have both pathogens in the same field, infecting a single host and/or host tissue at the same time (Verma and Saharan, 1994; Maringoni, 1997).The present work deals with the collection of pathogen and working out a strategy to control its incidence in field.

Materials and methods

Collection of fungal isolates

The survey was conducted at Alpine agricultural farm, Premnagar, Nanda Ki Chowki Dehradun, India (Located latitude: 30° 31' N and longitude: 77° 57' E). Dehradun city is just 640 meter above sea level. The survey was conducted from last week of Feb to March 2017.Different species of Brassicaceae have been investigated but mostly affected leaves are present in cauliflower (*Brassica oleracea*, var. *botrytis*). The disease indicates leaf spot of brassica and mostly black spots are present in lower leaves than upper ones. Leaves affected with Alternaria like symptoms were collected from infected plant and put in paper bags and marked for the date, place and plant source. Thus, in all 05 isolates of *A. brassicicola* were collected.

Isolation of the pathogen

Leaf section was cut from margin of lesions along with healthy portion of the leaf blade. Then, it was surface sterilized with a disinfectant (0.1% NaOCl) for 15 -12 sec. The leaf section was washed 4-5 times with sterilized distilled water. The excess water was blotted with sterilized paper towel. The leaf section was placed on Potato Dextrose Agar (PDA) in a Petri plate with the help of forceps. The Petri plate was incubated in a BOD incubator at $26^{o} \pm 1^{o}C$ for 3-4 days. Finally, the leaf section was observed for fungal growth.

Maintenance of pure culture

The apparently pure growth of the fungus was, then, transferred from the Petri plate to slants after a week or so. The isolates were maintained by sub culturing after every two months. These tubes were used as starter cultures (Aneja, 2005).

Growth

A small disk (0.5cm) of 7- day- old culture of the fungus was cut with sterilized cork borer. It was, then, transferred inversely and aseptically with the help of inoculating needle at the center of the Petri dish containing PDA. This is used as starter culture for experiments.

Fungicidal sensitivity test

The isolates of *A.brassicicola* were tested against one fungicides, Hexaconazole (systemic) using poisoned food technique (Nene and Thapliyal, 1979).The experiment was conducted using 4 concentrations of Hexaconazole, i.e., 25, 50, 75 and 100 ppm respectively. Inhibition percentage was calculated by using the formula given by Vincet (1947):

Percentage inhibition = Control – treatment / Control x 100

Biogenic sensitivity test

All the five isolates of *A. brassicicola* were tested against two *Trichoderma* species, *viz.*, *T. harzianum* and *T. viridae* (antagonists) by Dual Culture Method given by Dhingra and Sinclair (1995). The cultures of antagonists were obtained from Microbiology lab of Alpine Institute of Management and Technology, Dehradun.

Statistical analysis

Data for different parameters were analyzed with the help of GENSTAT 5 Release 3.22. Two way analysis was followed for biogenic and fungicidal sensitivity data. Treatments means were compared at 5 per cent level of significance.

Results and discussion

Cauliflower, *Brassica oleracea* var. *botrytis*, is an annual crop. The leaves surrounding the curd are ribbed, coarse green in colour. They protect it from sunlight, impeding the development of chlorophyll. The flowers are attached to a central stalk. It is an annual plant growing in moist areas having moderate temperature.

Symptomatology

The leaf spots caused by *Alternaria brassicicola* appeared on older leaves of the plant and progress to the new younger leaves. Initially, the spots were scattered on entire lamina. These lesions expand and develop concentric rings with chlorotic halos. Spots on leaf blades vary in size from pinpoint-sized dark circular spots, when young, to black, brown or tan spots 2 to 3 inches when older. Then these spots get bigger in size with a prominent bulls eye or target like appearance. These spots further coalesce and ultimately cause leaf to turn yellowish brown. Further, the halo deepens. The halo indicates the presence of toxin. Consequently, as the disease progress, whole leaf blade turns yellow the leaf dries up and droops down (Fig.1).

Fig.1: Symptoms of *A. brassicicola* on leaves of cauliflower.

The leaf spots can be identified due to black colour of spots and their regular shapes are some features that matched the symptoms of *A. brassicicola* present on Brassicaceae. However presence of halos around the black spot and conspicuously yellowing of leaf occurs. *A. brassicicola* causes leaf spot which are generally dark brown to black in colour, sparsely distributed throughout the lamina.

Bioagent sensitivity test

All the isolates were tested against the potent bio control agent *viz., Trichoderma harzianum* (T1) and *T. viride* (T2), both the antagonists showed differential activity against the isolates of *A.brassicicola* (T1- 441.94%& T2- 0.43%, Table 1 and Fig. 2). Irrespective of antagonists, maximum and significantly high growth inhibition was observed for isolate no A5 (58.85%) while minimum and significantly least value was observed for isolate no A2 (15.78%). Two isolates have at par growth inhibition (A4-44.78% and A1-45.72%). The interactions between pathogens and antagonists revealed that significantly high growth suppression was observed for isolate no A5 by *T. harzianum* (59.73%) which was at par with isolates no A5 by *T. viride* (57.96%) and A4 by *T. harzianum* (57.67%). In contrast, significantly less and minimum growth suppression was seen for isolate no A2 by *T. harzianum* i.e. 14.06%. Isolate no. A3 has at par growth inhibition by both the antagonists tested (40.80% and 40.78 %) respectively. Isolate no. A4 is suppressed maximally by *T. harzianum* (57.67%) where as its inhibition was less by *T. viride* (13.9%).

While testing the biological sensitivity against two common antagonistic fungi, namely, *T. harzianum* and *T. viridae.* It was seen that isolates of Alternaria species are not highly sensitive to these biocontrol agents as only one isolate (A5) in case of *T. harzianum* was suppressed by more than 50 percent (59.73%) which was at par in case of *T.viride* in isolate no. (A5) (57.96%). The observations are contrary to the findings of Mandare et al. (2008), Gveroska and Ziberoski (2012) and Taragam et.al, (2015) who assessed the biocontrol efficacy of *Alternaria* spp. isolates against *Trichoderma* species. The growth suppression was above the magical mark of 50 percent. However, the observations of the present study may be attributed to the different ecological niche of the pathogen and biocontrol agent.

Table 1. Growth inhibition of *A. brassicicola* by biocontrol agents (*T. harzianum* and *T. viride*)

Isolate no.	Treatments		Mean
	T. harzianum	***T. viride***	
A1	37.45	54.00	45.72
A2	14.06	17.50	15.78
A3	40.80	40.78	40.79
A4	57.67	31.90	44.78
A5	59.73	57.97	58.85
Mean	41.94	40.43	
	Isolate (I)	**Treatment (T)**	**Interactions (I X T)**
SEM	0.57	0.36	0.80
CD (5%)	1.7	1.2	2.4

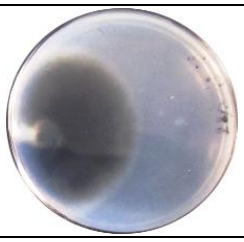
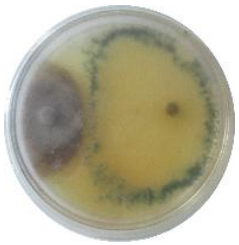
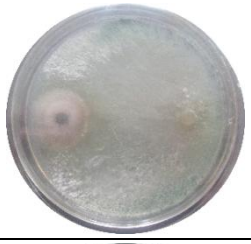
Isolate no.	Control	*T. harzianum*	*T. viride*
A1			
A2			
A3			
A4			
A5			

Fig. 2: Growth inhibition of *A. brassicicola* by biocontrol agents (*T. harzianum* and *T. viride*).

Fungicidal sensitivity test

The isolates of *A.brassocicola* were tested for their fungicidal sensitivity at different ppm ranging from the lowest concentration of 25ppm to the highest concentration of 100ppm. Among these isolates, isolate no. A4 registered the cent percent inhibition while the minimum inhibition was recorded for isolate no. A5 (93.13 %). Irrespective of the isolates a linear trend of growth inhibition over concentration of the fungicides was observed, as minimum growth inhibition of 91.22 percent was recorded at 25 ppm and highest of 100 percent at 75 and 100 ppm of Hexaconozole respectively (Fig.3 and Table 2).

When the interaction between isolates and concentration (IXC) was studied, the growth suppression of isolate no. A4 was cent percent right from the lowest concentration of 25 ppm to

highest concentration of 100 ppm. Growth of all the isolates was suppressed completely at concentration of 75 ppm and 100ppm respectively. Minimum growth suppression was observed for isolate no. A5 at 25 ppm. A graph was plotted for the observed data which depicts the overall effect of different concentrations of fungicides on each isolates. It shows that isolate no. A3 and A4 are completely suppressed (100 percent) at all the concentrations tested. While a linear growth suppression was observed for isolates no. A1, A2 and A5 (Fig. 4).

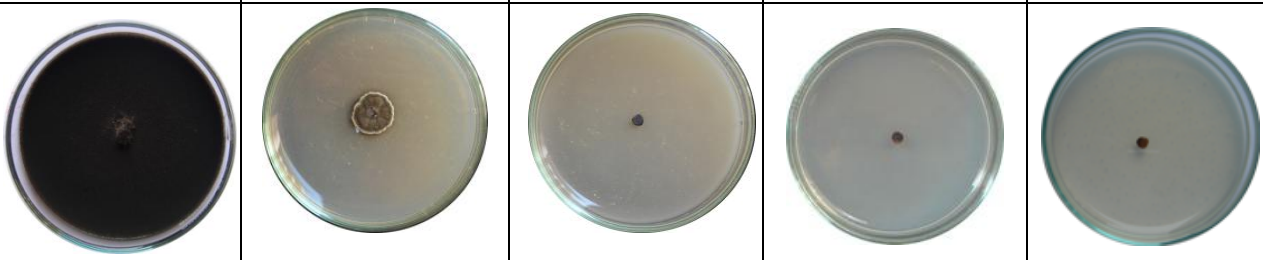
Isolate No.	Concentration (ppm)				
	Control	25	50	75	100
A1					
A2					
A3					
A4					
A5					

Fig. 3: Growth inhibition of *A.brassicicola* isolates by Hexaconozole at different concentrations.

Table 2. Growth inhibition of *A.brassicicola* isolates by Hexaconozole.

Isolate no.	Concentration (ppm)/ Inhibition (%)					Mean
	0	25	50	75	100	
A1	0.00	85.63	90.40	100.0	100.0	94.00
A2	0.00	88.90	100.0	100.0	100.0	97.22
A3	0.00	98.76	100.0	100.0	100.0	99.69
A4	0.00	100.0	100.0	100.0	100.0	100.0
A5	0.00	82.80	89.73	100.0	100.0	93.13
Mean	0.00	91.22	96.03	100.0	100.0	
	Isolate (I)			**Concentration (C)**		**Interaction (I*C)**
SEM	0.07			0.06		0.15
CD (5%)	0.2			0.2		0.4

This observation is equivalent to the earlier observation recorded by Singh and Singh (2006) who tested seven fungicides and among them Hexaconozole was very efficient as it caused 100% growth inhibition. Similar observations were reported by Mesta et al. (2009), Singh et al. (2013), Yadav et al. (2015) and also by Sadana and Didwania (2015) on different Alternaria species. Therefore it has been recommended that Hexaconozole is most effective for the management of Alternaria diseases in brassicas.

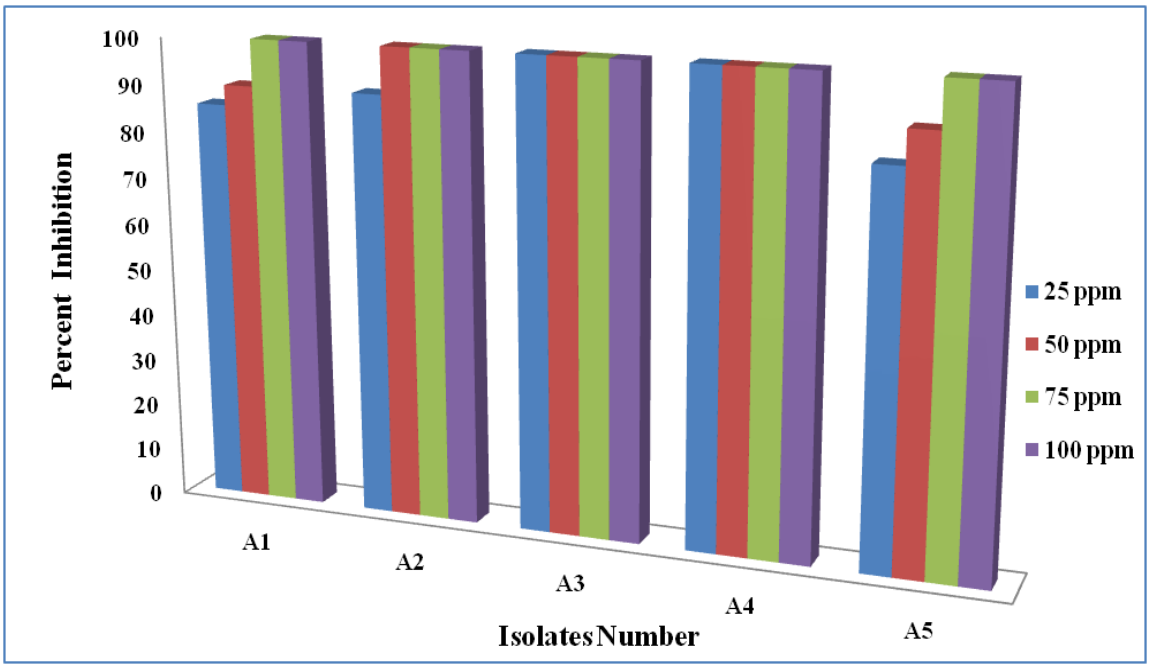


Fig. 4: Overall effect of different concentrations of hexocanozoles on isolates of *A. Brassicola.*

Conclusion

In vitro efficacy of a fungicide, namely, Hexaconozole (a systemic fungicide) was tested against the isolates of *Alternaria* sp. Based on the results, it can be concluded that Hexaconozole is effective in suppressing the growth of *Alternaria* sp. Both the biocontrol agents were unable to

control the growth of *A. brassicola* as they did not touch the magical mark of 50 percent. Only one isolate (A5) was suppressed more than 50 percent by *T. harzianum* and *T. viride*.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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