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Original Research Article

In vitro Evaluation of different Fungicides, Combi Products and Botanicals Efficacy against *Alternaria alternata* on Asalio

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Keywords

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A B S T R A C T

Alternaria, the fungal pathogen has wide host range generally attacks the aerial parts of plants causing leaf spots. Among the different diseases affecting Asalio, leaf blight caused by *Alternaria alternata* is an important one. Very little information is available on the management aspects of this disease. Therefore, we evaluate two combi products, two individual fungicides and two botanicals was evaluated on potato-dextrose-agar media for sensitivity aganist *A. alternata*. Among these treatments combi products *i.e.*, Metiram 70% + Pyraclostrobin 20% WG (0.35 % concentration) was found most effective in inhibiting mycelial growth of *A. alternata In vitro.*

Introduction

Asalio (*Lepidium sativum* Linn; Family: Brassicaceae) is a medicinal plant. *Lepidium* name derives from Greek word 'lepidion' means small scale probably it refers to the form of fruits and *sativum* is derived from 'serere' meaning to cultivate, to plant or to sow. It is known as “Common cress”, “Land cress”, “Haliv”, “Garden cress” or “Chandrasur” in some regions of India (Gokavi *et al.,* 2004).

The crop is mainly affected by diseases like Alternaria leaf spot & white rust. The Rabi season Asalio crop in India is commonly affected by Alternaria leaf spot. The disease causes extensive damage to the quality and quantity of the foliage and grains. If persistent cloudy and cool weather prevails the blight appears after flowering stage of the crop. Alternaria genus is the largest genus. It is distributed worldwide and has been reported on 115 plant genera from 43 plant families that cause blight and leaf spot disease (Neergaard, 1945). Its species have wide host range fungi and it is causing crop loss in economically important crop plants by causing leaf spot diseases. Alternaria pathogen have a large host range, in terms of total number of host plants it has 10th rank in all the pathogens (Farr *et al.,* 1989).

Alternaria genus causes different diseases, in which leaf spot disease is most severe to the crop and losses to the tune of 32-57 per cent in yield on a wide range of crops (Conn and Tewari, 1990). In some parts of the world It is reported to have has 4 per cent fungal diversity and have been reported to cause losses up to 80 per cent (Rotem, 1994).

There is a need to identify efficient management strategies of this disease. In vitro evaluation of fungicides helps to identify effective fungicide for application in fields. In the present investigation an attempt was made to identify effective fungicides to manage *A. alternata.*

Materials and Methods

At the Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, the study on Management of Alternaria leaf spot of Asalio Caused by *Alternaria alternata* was undertaken during Rabi 2018-2019. The laboratory experiments were carried out in Department of Plant Pathology.

Isolation, purification and pathogenicity tests of the pathogen

The infected samples of Asalio showing symptoms of *Alternaria* leaf spot disease were collected from the farmer's fields around Udaipur. Fungal pathogen was isolated from diseased samples of Asalio using standard methodology on potato dextrose agar (PDA) medium. Small bits of infected portions were surface sterilized for 1 minute in mercuric chloride solution (0.1%) and washed thrice in sterilized distilled water under totally aseptic conditions in a laminar air flow. These were then dried by keeping in two folds of sterilized filter papers then aseptically transferred to PDA in Petri plates. The plates were incubated at 27±1ºC for 7-8 days. For Sub-culturing 5 mm bit of the culture were cut from the periphery of the mycelial growth of 6-7 days old colonies and transferred on to the (PDA) slants.

The culture of *A. alternata* thus isolated and identified was further tested on pot grown plants for its pathogenicity and fulfilling the Koch's postulates. An inoculum with load of 1×10^3 conidia ml^{-1} concentration of the spores was inoculted in pot grown Asalio. The typical concentric ring symptoms appeared within 7-10 days after inoculation. Re-isolation was done from infected plant parts collected 10 days after inoculation. The resultant cultures were compared with the original ones to confirm the pathogenicity and then these cultures were sent for final identification to "Indian Type Culture Collection Identification/Culture supply Services, Division Of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012". Dr. T. Prameela Devi and Dr. Deeba Kamil identified these cultures as *Alternaria alternata*. The identification number is 10,856.18 is allotted by ITCC.

In vitro Evaluation of Different Fungicides and Botanicals in Amended Plain Agar Media against *Alternaria alternata*

In vitro evaluation of different fungicides and botanicals was carried out using poisoned food technique. Test Fungicides and botanicals as per the treatments were evaluated on potato dextrose agar (PDA) medium. The flasks containing melted and sterilized PDA was amended with the desired concentration of each test fungicide and botanical. 20 ml of the amended PDA was poured in 90 mm sterilized Petri-plates and allowed to solidify. From the periphery of 7-days-old actively growing and sporulating culture of *A. alternata*, 5 mm disc was cut and placed in the centre of each experimental

Petri-plate under totally aseptic conditions. The experiment was conducted in completely randomized design (CRD) with five replications in each treatment.

These were then incubated at 27±1ºC in BOD. After 7 days when the control plates were full of fungal growth, the radial diameter was measured diagonally in each of the control and treatment plates.

Per cent inhibition of mycelial growth was calculated by using formula given by Bliss (1934) as:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

Results and Discussion

In vitro Bioassay of different Fungicides and Botanicals in Amended Potato Dextrose Agar Media against *Alternaria alternata*

Efficacy of different fungicides and botanicals viz., Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC (0.07 % concentration), Azoxystrobin 23 SC – (0.1 % concentration), Difenoconazole 25 EC - (0.06 % concentration), Metiram 70% + Pyraclostrobin20% WG – (0.35 % concentration), Azadirachtin - (0.5 % concentration), and Neem oil – (0.5 % concentration), (as discussed in Materials and Methods) were tested in vitro against mycelial growth of A. alternata by Poisoned Food Technique. The Results envisage that the combi products Metiram 70% + Pyraclostrobin 20% WG –(0.35 % concentration) with the colony diameter 18.64 mm followed by Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC – (0.07 % concentration), with colony diameter 28.04 mm at given concentrations were significantly superior in inhibiting the mycelial growth of the test fungus over the individual chemicals and control. Among the individual chemicals Difenoconazole 25 EC (0.06 %concentration) with colony diameter 36.48 mm was significantly superior in inhibiting the growth of test fungus followed by Azoxystrobin 23 SC (0.1% concentration) with colony diameter 38.51 mm. All the test chemicals as combi products or individually were found to inhibit the growth of A. alternata significantly over botanicals and control. As for botanicals Neem oil –(0.5% concentration) was significantly more effective within test botanicals with colony diameter 45.90 mm then Azadirachtin – (0.5 % concentration) with colony diameter 65.84 mm in inhibiting mycelial growth of *A. alternata*.

The Results reveal that the combi products Metiram 70% + Pyraclostrobin 20% WG – (0.35 % concentration) with the per cent growth inhibition 79.28 followed by Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC – (0.07 % concentration), with the per cent growth inhibition 68.84 at given concentrations were significantly superior in inhibiting the growth of the test fungus over the individual chemicals and control. Among the individual chemicals Difenoconazole 25 EC (0.06 % concentration) with the per cent growth inhibition 59.24 was significantly superior in inhibiting the growth of test fungus followed by Azoxystrobin 23 SC (0.1% concentration) with the per cent growth inhibition 57.21 mm.

Table.1 Treatment details

Number	Name of treatments	Concentrations
T1	Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC	0.07 %
T2	Azoxystrobin 23 SC	0.1 %
T3	Difenoconazole 25 EC	0.06 %
T4	Metiram 70% + Pyraclostrobin 20% WG	0.35 %
T5	Azadirachtin	0.5 %
T6	Neem oil	0.5 %
T7	Control	

Design-CRD, Replication-5, Treatment: 7

Fig.1 *In vitro* efficacy of different fungicides and botanicals against *Alternaria alternata* after 7 days inoculation at 27±1°C

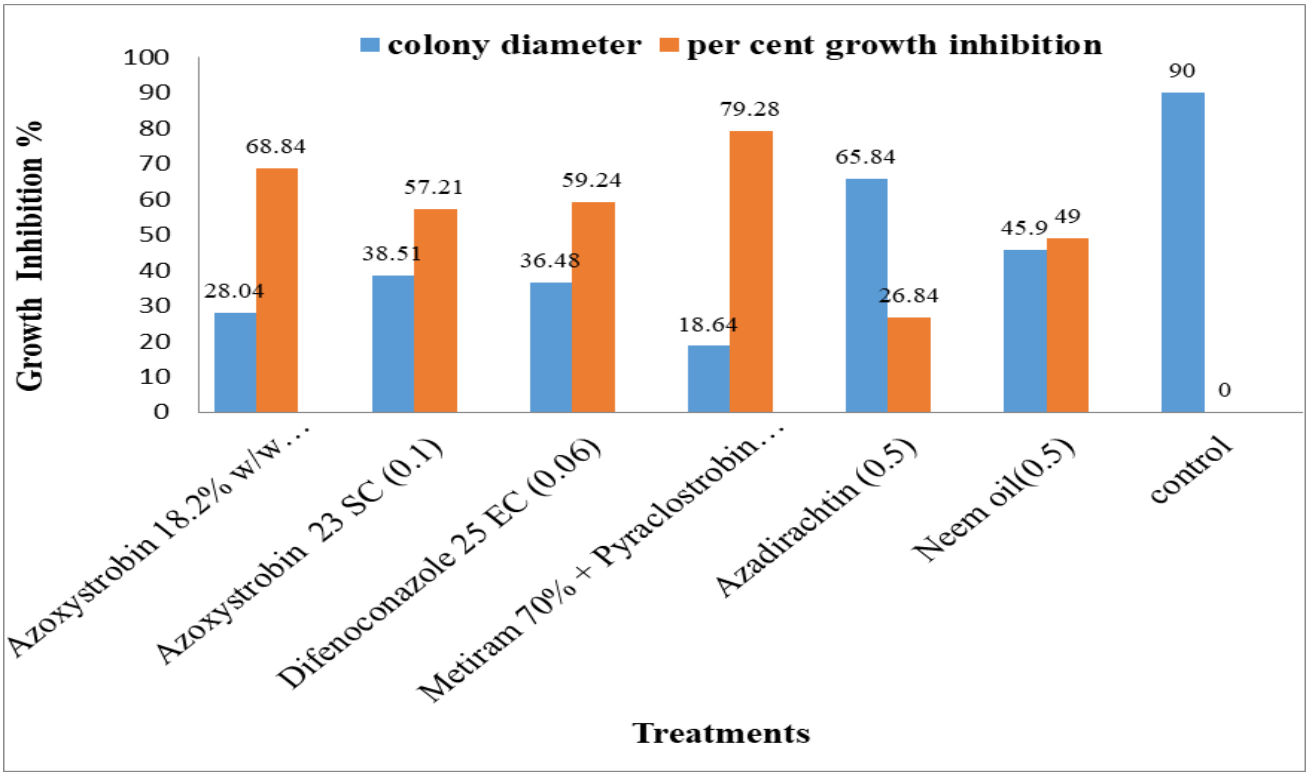
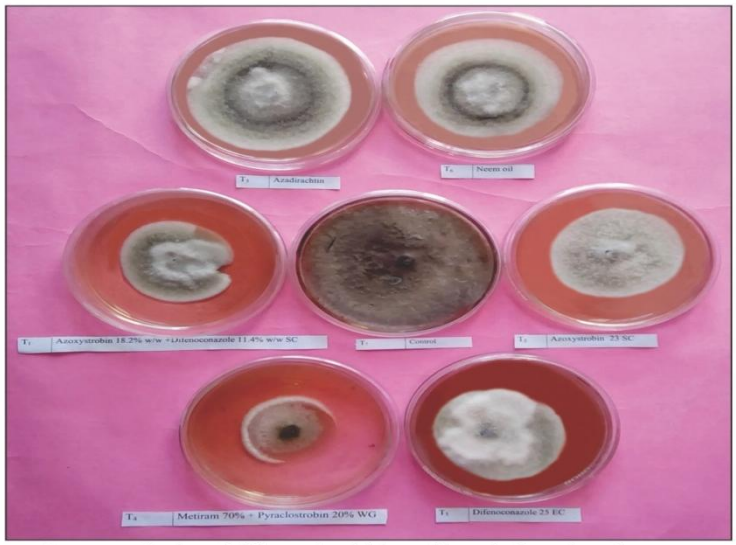


Fig.2 *In vitro* evaluation of different fungicides and botanicals against *A. alternate*



In vitro evaluation of different fungicides and botanicals against *Alternaria alternata* after 7 days at 27 ± 1°C
T_1 Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC (0.07)
T_2 Azoxystrobin 23 SC (0.1) T_3 Difenoconazole 25 EC (0.06)
T_4 Metiram 70% + Pyraclostrobin 20% WG (0.35)
T_5 Azadirachtin (0.5) T_6 Neem oil (0.5)
T_7 Control

Among botanicals tested Neem oil – (0.5% concentration) was significantly more effective within test botanicals with the per cent growth inhibition 49.00 then Azadirachtin – (0.5 % concentration) with the per cent growth inhibition 26.84 in inhibiting the growth of *A. alternata* (Table-4, Plate-5 and Fig-4).

As narrated earlier there have been no reports in the review of literature for management of *A. alternata* causing Alternaria leaf spot of Asalio. Although, management of *A. alternata* causing leaf spots in other crops is listed here. Surviliene and Dambrauskiene (2006) reported efficacy of Azoxystrobin 250 SC against various Alternaria species viz., *Alternaria alternata*, *Alternaria brassicae*, Alternaria dauci on potato dextrose agar under *In vitro* conditions and reported 87% to 96% inhibition of colonies of different Alternaria species by Azoxystrobin at 7 days after inoculation. Mane (2008) reported that Mancozeb (0.2%) and Propiconazole (0.05%) completely inhibited the growth of *A. alternata* causing leaf blight of chilli. Kamanna *et al.*, (2010) observed that Chlorothalonil (0.2%) was effective for the control of chrysanthemum leaf blight under field conditions caused by *A. alternata*. The present study are liken with the farther said reported reviews.

In view of the increasing disease incidence, in Asalio growing areas, attempts were made to evaluate two combi products and two fungicides and two botanicals formulations against *A. alternata*, which were first tested In vitro, combi products Metiram 70% + Pyraclostrobin 20% WG -0.35 % and Azoxystrobin 18.2% w/w +Difenoconazole 11.4% w/w SC - 0.07 %, were most effective in inhibiting growth of *A. alternata* In vitro with per cent inhibition of growth as 79.28% and 68.84%, respectively. Azadirachtin has been reported to be safe for the environment (Khalid and Shad, 2002; Morgan, 2009) but no information is available on efficacy of botanicals in suppressing *A. alternata* causing Alternaria leaf spot disease of Asalio. Among botanicals Neem oil effectively inhibited the

growth of *A. alternata* in present study with per cent growth inhibition as 49.00%. When treated with fungicides, botanicals and combi products showed variations in suppressing *A. alternata*. The observations suggest that sensitivity to combi products is greatly prevalent in natural population of *A. alternata*.

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