

Original Research Article

Evaluation of different Fungicides, botanicals and Combi Products Efficacy for the Management of *Alternaria* Leaf Spot Disease on Asalio

Poonam Kumari^{1*}, Amit Trivedi¹, Vimal Kumar¹ and Anupriya²

¹Department of Plant Pathology, Rajasthan College of Agriculture, MPUAT, Udaipur-313001, India

²Department of Plant Pathology, Sri Karan Narendra Agriculture University, Jobner, Jaipur, Rajasthan-303328, India

*Corresponding author

ABSTRACT

Diseases especially caused by fungal pathogens are the main biological constraints in Asalio production and *Alternaria* leaf spot disease caused by *Alternaria alternata* is one of the most economically important diseases of Asalio. Fungicides, botanicals and combi products of fungicides application are an effective way to control this disease. The current study tests the efficacy of various fungicides for controlling the *Alternaria* leaf spot disease of Asalio. Two combi products, two individual fungicides and two botanicals was evaluated on pot grown Asalio for sensitivity against *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 pot grown Asalio. In pot experiment treatment consisting combination of fungicide (found most effective In vitro) and botanical (found most effective In vitro) was observed to be most effective for managing *Alternaria* leaf spot disease of Asalio.

Keywords

Alternaria alternata, PDI, *Lepidium sativum*, pot, Combi products, fungicides and botanicals

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).

It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant

derived components were discovered after following up on ethano medicinal use of the plants (Ncube *et al.*, 2008). Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Lis-Balchin and Deans, 1996; Maoz and Neeman, 1998; Hammer *et al.*, 1999).

In the year 1967 the *Alternaria* leaf spot of Asalio was first reported from Kaffa province, Ethiopia and the pathogen was identified as *Alternaria brassicae* (Berk.)

Sacc.(Stewart and Dagnalechew, 1967). Melkania (1980) reported that *Alternaria alternata* caused Alternaria leaf spot on leaves of cress at Almora (H.P.) for the first time in India and in the same year 1980, Singh reported that Alternaria leaf spot caused by *Alternaria alternata* on cress at College Farm, Banaras Hindu University, Varanasi (U.P.)

Alternaria is included in order Hyphomycetes, family Dematiaceae, genus dictyosporic and it is a Fungi Imperfecti. *Alternaria* genus was first reported by Nees (1816). *A. alternata* belongs to Longicatenatae according to Neergaard (1945). Melkania (1980) reported that Alternaria leaf spot on leaves of Asalio was caused by *A. alternata* and Utikar and Padule (1980) described its morphology. He reported that conidiophores of *A. alternata* were simple, light brown, variable in length ranging from 17.10 to 61.56 μm and mostly 2-3 septate rarely 4-5 septate. Conidia were found light to dark brown in colour, uniform with 0-2 longitudinal septa and 1-6 transverse septa, and variable in shape and size, mostly oval shape with rudimentary beak and in size measuring about 10.26-77.52 x 4.56-14.82 μm . Simmons and Roberts (1993) observed three-dimensional sporulation patterns of *A. alternata* in electron microscope at 50 magnification.

Melkania (1980) reported that Alternaria leaf spot on leaves of Asalio was caused by *A. alternata*. The first initiation of symptoms were small, discoloured oval lesions, irregular in shape which later increase in size and brown in colour. The initial infection starts when lower leaves of plant touch the irrigation channels. Later on these symptoms are also seen on the seed coat and stem. The disease is initially seed borne but the inoculum is also contributed by infected plant debris for reoccurrence of disease. The

severity of the disease increases up to mid January. At this stage, the stem and floral parts of plant also become diseased. Finally, whole plant shows typical blight symptoms. Fungal colonizes in xylem of the host plant, and as a result, blockage and breakdown of the xylem lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant.

More number of plant species has been reported to possess natural substances that are toxic to many fungi causing plant diseases (Amadioha, 2000; Kagale *et al.*, 2004).

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 pot experiments were carried out at cage house in Department of Plant Pathology.

Against Alternaria Leaf Spot of Asalio under Pot Culture on Inoculated Plants

For management of Alternaria leaf spot of Asalio, different test fungicides and botanicals were evaluated as treatment solutions using spray applications. Susceptible local Asalio landrace was sown in 30 cm earthen pots having a Soil and FYM (3:1) mixture and kept in the cage house at Department of Plant Pathology, RCA Udaipur during Rabi season 2018. The experiment was laid out in completely randomized design (CRD) with three replications. A population of ten plants per pot was maintained throughout the growing season. 30 day-old Asalio plants were inoculated with an inoculum concentration 1×10^3 conidia ml^{-1} of *A. alternata* spore

suspension using spray inoculation technique with the help of hand held atomizer. The symptoms of *Alternaria* leaf spot started appearing on foliar parts of plant after 36 hours of inoculation of the pathogen. Foliar applications of solutions with desired concentration of fungicides and botanicals individually and in combination was done as per following treatments. A suitable untreated control was also maintained for comparison.

Design – CRD, Treatments details – 8 treatments with each have 3 Replication.

Disease inoculation 30 days after sowing

Disease intensity was recorded on a standard 0-5 disease rating scale [0 to 5 scales of Gawande and Patil (2003)].

The per cent disease index (PDI) and per cent efficacy of disease control (PEDC) were calculated by using following formula given by McKinney 1923.

Per cent disease index (PDI)

$$\text{PDI} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants assessed} \times \text{maximum rating}} \times 100$$

Results and Discussion

Evaluation of Different Fungicides and Botanicals against *Alternaria* Leaf Spot of Asalio under Pot Culture on Inoculated Plants

Different treatment solutions of fungicides (as combi products and individuals), botanicals and their combinations were assessed against *A. alternata* as foliar applications for management of *Alternaria* leaf spot of Asalio. A pot experiment was laid out with three replications of each treatment following completely randomized design

(CRD). Susceptible local Asalio landrace was sown in 30 cm earthen pots having a Soil and FYM (3:1) mixture and kept in the cage house at Department of Plant Pathology, RCA, Udaipur during Rabi season 2018. A population of ten plants per pot was maintained throughout the growing season. 30 day-old Asalio plants were inoculated with an inoculum concentration of 1×10^3 conidia ml^{-1} of *A. alternata* spore suspension using spray inoculation technique with the help of hand held atomizer. The symptoms of *Alternaria* leaf spot started appearing on foliar parts of plant after 36 hours of inoculation of the pathogen. Foliar applications of solutions with desired concentration of fungicides and botanicals individually and in combination was done. Foliar applications of solutions of fungicides and botanicals such as (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% + Pyraclostrobin 20% WG) – (0.35 % concentration), Azadirachtin – (0.5 % concentration), Neem oil – (0.5 % concentration), Fungicide and botanicals most effective *In vitro*, a suitable untreated control was also maintained (the concentrations were kept in same proportion as discussed in *In-vitro* studies).

A standard 0-5 disease rating scale was used for recording disease severity, after 10-days of inoculation the observation were recorded and per cent disease index (PDI) and Per cent Efficacy of Disease Control (PEDC) were calculated. All the treatments were found to significantly suppress *Alternaria* leaf spot of Asalio over un-treated inoculated control. In un-treated inoculated control exhibited maximum PDI 72.66 as compared to the other treatments. The Results revealed that the treatment having combination of fungicide and botanical which were most

effective *In vitro* with PDI 15.33 was most effective against *A. alternata*. This treatment exhibited significant superiority over individual applications of test combi products, individual fungicides and untreated control. Among combi products tested Metiram 70% + Pyraclostrobin 20% WG – (0.35 % concentration) with the PDI 16.67 followed by Azoxystrobin 18.2% w/w + Difenconazole 11.4% w/w SC – (0.07 % concentration), with PDI 20.00 at given concentrations were significantly superior in managing the disease over the individual chemicals and botanicals. (Table-3, Plate-4). Among the individual chemicals Difenconazole 25 EC (0.06 % concentration) with PDI 25.33 was significantly superior in managing the disease followed by Azoxystrobin 23 SC (0.1% concentration) with PDI 29.33.

All the test chemicals as combi products or individually were found to manage the disease significantly better over botanicals and control. As for botanicals Neem oil – (0.5% concentration) was significantly more effective within test botanicals with PDI 38.00 then Azadirachtin – (0.5 % concentration) with PDI 48.00 in managing *Alternaria* leaf spot of *Asalio*.

The Results revealed that the treatment having combination of Fungicide and botanical which were most effective *In vitro* with PEDC 78.89 was most effective against *A. alternata*. This treatment exhibited significant superiority over individual applications of test combi products, individual fungicides and untreated control.

Among combi products tested Metiram 70% + Pyraclostrobin 20% WG – (0.35 % concentration) with the PEDC 77.06 followed by Azoxystrobin 18.2% w/w + Difenconazole 11.4% w/w SC – (0.07 %

concentration), with PEDC 72.47 at given concentrations were significantly superior in managing the disease over the individual chemicals and botanicals. Among the individual chemicals Difenconazole 25 EC (0.06 % concentration) with PEDC 65.13 was significantly superior in managing the disease followed by Azoxystrobin 23 SC (0.1% concentration) with PEDC 59.62. All the test chemicals as combi products or individually were found to manage the disease significantly better over botanicals and control. As for botanicals Neem oil – (0.5% concentration) was significantly more effective within test botanicals with PEDC 47.69 then Azadirachtin – (0.5 % concentration) with PEDC 33.93 in managing *Alternaria* leaf spot of *Asalio* (Table-5, Plate-6 and Fig-5).

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. Neem (*Azadiracta indica* L.) is widely used and well known tree and seed extracts and oils are commonly used to control the insects and pathogens. A high content of Azadiractin, its active ingredient can be found both in the oil and in the extract (Mordue and Nisbet, 2002). Govindachari *et al.*, (1998) also studied the antifungal activity of neem oil towards Drechsleraoryzae, *Fusarium oxysporum* and *Alternaria alternata*. Neem oil yields various acids, sulphur, etc. Meliantiol and azadiractin are obtained from seeds and decatylinbin also contains quecetin and sitosterol. The fungicidal spectrum of *Azadiracta indica* has been attributed to azadiractin which belongs to C25 terpenoides (Subramanian and SrinivasaPai, 1953). The present study are liken with the farther said reported reviews.

Table.1 Treatments details

Number	Name of treatments	Concentrations
T1	Azoxystrobin 18.2% w/w + Difenconazole 11.4% w/w SC	0.07 %
T2	Azoxystrobin 23 SC	0.1 %
T3	Difenconazole 25 EC	0.06 %
T4	Metiram 70% + Pyraclostrobin 20% WG	0.35 %
T5	Azadirachtin	0.5 %
T6	Neem oil	0.5 %
T7	Fungicide and botanicals most effective In vitro	
T8	Control	

Fig.1 Evaluation of different fungicides and botanicals against *Alternaria* leaf spot of Asalio on pot culture on artificially inoculated plants

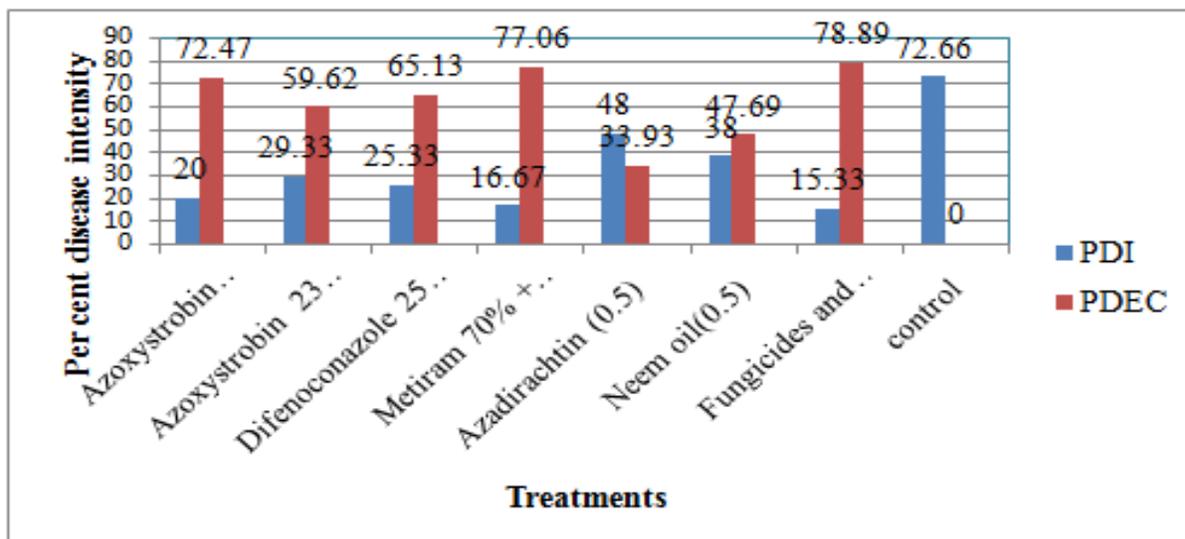


Fig.2 Evaluation of different fungicides and botanicals against *A. alternata* under artificially inoculated Asalio plants in pot culture



Evaluation of different fungicides and botanicals against *Alternaria* leaf spot of Asalio on pot culture on artificially inoculated plants
 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 Fungicide and botanicals most effective *in vitro*
 T₈ Control.

In pot experiment, conducted on local landrace of Asalio under inoculated conditions, maximum reduction in disease severity (78.89 % efficacy of disease control), was obtained with spray combination of (Metiram 70% + Pyraclostrobin 20% WG) -0.35 % and Neem oil 0.5%. Among fungicides combi products Metiram 70% + Pyraclostrobin 20% WG - 0.35 % and in botanicals Neem oil- 0.5% were found effective for managing *Alternaria* leaf spot of Asalio. It was found that suppression of *Alternaria* leaf spot through application of combination of fungicides and botanicals fared better than their individual application for suppression of *Alternaria* leaf spot disease of Asalio. The results in present investigation are modulate with the onward said reported reviews. Elsewhere, the evaluation of various fungicides revealed that azoxystrobin, chlorothalonil, hexaconazole, difenoconazole, dodine were highly effective

against *Alternaria* spp. (Tiwari *et al.*, 2004). Integrated treatments were evaluated by Patni *et al.*, (2005) in mustard which is another cruciferous crop. Two divided spray-doses of 3% neem oil at the on-set and fortnight later, retarded the development, spread and disease index of leaf blight of onion (*Aternaria alternata*); strongly improving the crop performance and yield (Ramjegathesh *et al.*, 2011). Similar results on the efficacy of plant extracts against *Alternaria* spp. have been reported by Baraka *et al.*, (2011) causing root rot of date palm, Nashwa *et al.*, (2012) in purple blotch of onion, and Ravi kumar and Garampalli (2013) in early blight of tomato. The present investigation of various fungicides, combi products and botanicals inhibiting the growth of *A. alternata* is in line with the earlier findings (Amaresh, 2000; Singh and Majumdar, 2001; Rao, 2006; Pramod Kumar, 2007).

References

- Amadioha, A. C., 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Protect., 19: 287-290.
- Amaresh, Y. S., 2000. Epidemiology and management of Alternaria leaf blight and rust of sunflower (*Helianthus annuus* L.). Ph.D. Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India.
- Baraka, M. A., Fatma, R. M., Shaban, W. I. and Arafat, K. H. 2011. Efficacy of some plant extracts, natural oils, biofungicides and fungicides against root rot disease of date palm. Biol. Chem. Environ. Sci. 6(2): 405-429.
- Eddouks, M., Maghrani, M., Zeggwagh, N. A. and Michel, J. B. 2005. Study of the hypoglycaemic activity of *Lepidium sativum* L. aqueous extract in normal and diabetic rats. Journal Ethnopharmacology. 97: 391-395.
- Gokavi, S. S, Malleshi N. G. and Guo, M. 2004. Chemical composition of garden cress (*Lepidium sativum*) seeds and its fractions and use of bran as a functional ingredient. Journal of Plant Food and Human Nutrition. 59: 105-111.
- Govindachari, T. R., G. Suresh, G. Gopalakrishnan, B. Banumathy and S. Masilamani, 1998. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. Phytoparasitica, 26: 109-116.
- Hammer, K. A., C. F. Carson and T. V. Riley, 1999. Antimicrobial activity of essential oils and other plant extracts. J. Applied Microbiol., 86: 985-990.
- Kagale, S., T. Marimuthu, B. Thayumanavan, R. Nandakumar and R. Samiyappan, 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Daturametel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiol. Mol. Plant Pathol., 65: 91-100.
- Kumar, A. D. 2006. Ayurvedic Drug Plants, Daya Books Publisher, New Delhi, India, 97.
- Lis-Balchin, M. and S. G. Deans, 1996. Antimicrobial effects of hydrophilic extracts of Pelargonium species (Geraniaceae). Lett. Applied Microbiol., 23: 205-207.
- Mane, V. A. 2008. Studies on Alternaria blight and *Fusarium wilt* diseases of chilli (*Capsicum annun* L.) in Konkan region. M. Sc. (Ag.) Thesis, Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri (M.S.).
- Maoz, M. and I. Neeman, 1998. Antimicrobial effects of aqueous plant extracts on the fungi *Microsporium canis* and *Trichophyton rubrum* and on three bacterial species. Lett. Applied Microbiol., 26: 61-63.
- Melkania, N. P. 1980. *Lepidium sativum* Linn - A new host record for *Alternaria alternata*. (Fr.) Keissler. Curr.Sci..49 : 27-28.
- Mordue (Luntz), A. J. and A. J. Nisbet, 2000. Azadiractin from the neem tree *Azadirachta indica*: Its action against insects. Ann. Braz. Soc. Entomol., 29: 615-632.
- Nashwa, S. M. A. and Abo-Elyousr, K. A. M. 2012. Evaluation of Various Plant Extracts against the Early Blight Disease of Tomato Plants under Greenhouse and Field Conditions. Plant Protection Science. 48: 74-79.
- Ncube, N. S., A. J. Afolayan and A. I. Okoh, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr. J. Biotechnol., 7: 1797-1806.
- Nees, V. E. 1816. Das System der Pilze und Schwamme. Stahelsche,

- Wurzberg.pp. 329.
- Patni, C. S., Kolte, S. J. and Awasthi, R. P. 2005.Efficacy of botanicals against *Alternaria* blight (*Alternaria brassicae*) of mustard. *Indian Phytopath.* 58: 426-430.
- Prajapati, P. and Sharma, K. 2003. In A hand book of medicinal plants. Published by Agrobios (Indian).pp. 81.
- Pramod Kumar, T., 2007. Biological management of *Alternaria* blight of onion. M.Sc. Thesis, University of Agricultural Science, Dharwad, India.
- Ramjegathesh, R., Ebenezer, E. G. and Muthusamy, M. 2011. Management of onion leaf blight by *Alternaria alternata* (FR.) Keissler by botanical and bio-control agents. *Plant Pathology Journal*, 10: 192-196.
- Rao, M. S. L., 2006. Management of *Alternaria* leaf spot in sunflower. Annual Report 2005-2006, Directorate of Oilseeds Research, India, pp: 54.
- Ravikumar M. C. and Garampalli, R. H. 2013. Antifungal activity of plants extracts against *Alternaria solani*, the causal agent of early blight of tomato. *Archives of Phytopathology and Plant Protection*. 46: 1897-1903.
- Rotem, J. 1994. The genus *Alternaria*: Biology, Epidemiology and Pathogenicity. The American Phytopathological Society. USA. 325.
- Simmons, E. G. and Roberts, R. G. 1993.*Alternaria* themes and variations. *Mycotaxon*. 48: 109–140.
- Singh, J. and V. L. Majumdar, 2001. Efficacy of plant extracts against *Alternaria alternata*, the incitant of fruit rot of pomegranate (*Punica granatum* L.). *Indian J. Mycol. Plant Pathol.*, 31: 346-349.
- Stewart, R. B. and Dagnalechew Y. 1967. Index of plant diseases in Ethiopia. Exp, Station Bull, No, 30, College of Agriculture, Alenaya, Ethiopia (Spices, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance, Ed. Jansen, P.C.M., Centre for Agricultural Publishing and Documentation, Wageningen, 1981, PP. 216-224).
- Subramanian, C. V. and K. V. Srinivasa Pai, 1953.Relation of nitrogen to growth and sporulation of *Fusarium vasinfectum* ATK. *Proc. Plant Sci.*, 37: 149-157.
- Utikar, P. G. and Padule, D. N. 1980. A virulent species of *Alternaria* causing leaf blight of onion. *Indian Phytopathol*. 33: 335-336.