International Journal of Plant Pathology and Microbiology

E-ISSN: 2789-3073
P-ISSN: 2789-3065
IJPPM 2021; 1(1): 16-19
Received: 13-11-2020
Accepted: 10-01-2021

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In vitro evaluation of fungicides and botanicals *against Alternaria brassicae* causing leaf blight of mustard

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Abstract
Alternaria leaf blight caused by *Alternaria brassicae* is one of the destructive diseases of mustard and causes considerable loss in the yield and quality of the produce. An experiment was conducted in *in vitro* to evaluate the efficacy of five chemical fungicides at five different concentrations i.e. 50ppm, 100ppm, 250ppm, 500ppm and 1000ppm and five botanical extracts at three concentrations i.e. 5%, 10% and 15%, against *Alternaria brassicae*. The study was carried out using poisoned food technique for botanical extracts and chemical fungicides Completely Randomized Design (CRD). Among the fungicides Tebuconazole proved to be the most effective chemical fungicide recording 100% growth inhibition at all the tested concentrations which was followed by Mancozeb at 1000 ppm (96.05) and followed by Metalaxyl + Mancozeb at 1000ppm (92.10) whereas, 50 ppm Chlorothalonil was least effective in reducing fungal growth (53.94). Among the botanical extracts, maximum inhibition (74.26%) of mycelial growth was observed in Turmeric at 15% concentration followed by 15% Veld grape (69.20%) and minimum inhibition percent (39.46%) was observed in *Aloe vera* at 5% concentration. The study indicated better performance of some chemical fungicides even at lower concentrations i.e. 100ppm and 250ppm. So, such effective fungicides could be used to minimize hazardous effect. Significant effect of some botanical extracts against pathogen growth suggests their application as potential control agent alternative to chemicals.

Keywords: *Alternaria,* fungicides, botanicals, poisoned food technique

Introduction
Mustard (*Brassica juncea*) is one of the major *Rabi* oilseed crops of India which occupies a prominent place, being next in importance to groundnut, both in area and production. It is extensively grown traditionally as a pure crop as well as intercrop (mixed crop) in marginal and sub marginal soils in the eastern, northern and north-eastern states of India. Cool and moist climate of winter months is the major factor for luxuriant growth and productivity of mustard in these states (Wadhwani and Dudheja, 1982). Mustard seeds are known by different names in different places e.g. sarson, rai or raya, torai or lahi.

It fulfils the oil requirement of about 50 per cent population in the states of Uttar Pradesh, Punjab, Rajasthan, Madhya Pradesh, Bihar, Orissa, West Bengal and Assam. Assuming normal weather conditions through harvest, India is expected to produce 7.2 million metric tons (MMT) of mustard in marketing year on planted area of 6.9 million hectares. Mustard's nutritional profile boosts a plentiful supply of essential minerals including calcium, iron, manganese, phosphorus and zinc. In addition to being a very good source of omega-3 fatty acids it also supplies tryptophan, phosphorus, iron and protein. Mustard seeds are also a very good source of selenium. The Mustard crop is attacked by different bacterial, fungal, and viral disease. Of these, diseases *Alternaria* blight incited by *Alternaria brassicae* is an economically important and widely distributed disease throughout the world.

Alternaria blight caused by *Alternaria brassicae* has been reported to inflict heavy yield losses to the tune of 35-60% (Kadian and Saharan, 1983; Kolte *et al.*, 1987; Tripathi, *et al.*, 1987; Ram and Chauhan, 1998 and Kumar, 1999) in mustard crop. Typical symptoms of *Alternaria* blight disease are the formation of spots on leaves, stems and siliquae. The concentric black spots produced were usually gray coloured and their characters varied with host and environmental factors. Initially symptoms appeared on the lower leaves as black points, later which enlarged to develop into prominent, round, concentric spots of various sizes. As disease progressed, the lower leaves defoliated and disease appeared on middle and upper leaves. At the later stage of the plant growth, spots appear on siliquae and stem. The spots were round black and quite conspicuous and the siliquae turn completely black.

On the stem, black, elongated spots became visible in the form of black streaks with or without necrotic gray centres.
Considering economic importance of the mustard and destructive nature of *Alternaria* blight incited by *Alternaria brassicae* in mustard, present studies on *in vitro* evaluation of botanicals and fungicides against the test pathogen were undertaken. Therefore, the present study conducts in a view of the above evidence of alternaria blight caused by *A. brassicae*, the objectives/purpose of this investigation are as follow.

Materials and Methods

The experiment was carried out in Plant Pathology Laboratory of Uttaranchal University Dehradun in Completely Randomized Design using poisoned food technique. Five chemical fungicides i.e. Chlorothalanil, Metalaxyl + Mancozeb, Tebuconazole, Carbendazim and Mancozeb were evaluated at five different concentrations such as 50, 100, 250, 500 and 1000 ppm and five botanical extracts (i.e. Neem, Aloe, Tulsi, Veld Grape and Turmeric) were evaluated at three different concentrations *viz*. 5%, 10% and 15% respectively. Each of the treatment was replicated three times.

Isolation and maintenance of pure culture

Pathogenic *Alternaria brassicae* was isolated from infected leaf of mustard collected from field Uttaranchal University Dehradun. Spores were teased from infected portion for microscopic examination to check the presence of pathogenic fungus. After confirming the presence of *Alternaria brassicae*, leaves were cut into small pieces (1-1.5cm) with sterile blade. These pieces were disinfected with 0.5% sodium hypochlorite (NaOCl) solution for two minutes followed by three washings with distilled water and excessive moisture was removed using sterile blotting paper. The sterilized leaf pieces were placed on PDA medium using sterilized forceps and incubated at 27 ± 1 °C for 7 days. On the basis of morphological characters of conidia as described by Yu (2015); Corlett and MacLatchy, (1996a, 1996b) pathogen was identified as *Alternaria brassicae*. Then the culture was purified by transferring small piece of agar containing spore to another petriplate containing media and incubated at 27 ± 1 °C for 7 days. The pathogen was sub cultured three times to obtain pure culture and pure culture thus obtained was preserved in PDA slant at 4 °C.

In vitro evaluation of botanical extracts and chemical fungicides

Botanical extract was prepared as per methods used by Ul-Haq *et al.*, (2014) and Thaware *et al.*, (2010). Fresh and healthy leaves, bulbs and rhizomes were collected, thoroughly washed in tap water followed by sterilized distilled water, then air dried and grounded with mortar and pestle with the addition of distilled water at the ratio of 1:1 w/v. Then the extract obtained was filtered through double layered muslin cloth. Extract was centrifuged at 4000 rpm for 5 minutes. The supernatant was then filtered through Whatman's filter paper No. 1 and then. Boiled at 80 °C for 10 minutes in a hot water bath. Thus obtained filtrate was taken as 100% basic stock solution. After autoclaving PDA media and cooling it to 50 °C required amount of this standard solution was mixed into PDA to get final concentration of 5%, 10% and 15% for poisoned food technique. Similarly for the evaluation of chemical fungicides, calculated amount of stock solution was mixed in sterilized PDA to make final concentration of 50ppm, 100ppm, 250ppm, 500ppm and 1000ppm. Twenty ml of amended PDA was poured in each 90mm sterilized petri plate and allowed to solidify. Control treatment was maintained without adding plant extracts or chemical fungicides on PDA. A circular disc of 7mm diameter from 9 days old culture of *Alternaria brassicae* was cut with sterilized corn borer and inoculated in the centre of solidified amended as well as control media. Each treatment was replicated in three petri plates. Then the petriplates were incubated at 27 ± 1 °C for seven days.

Growth inhibition test

The observation on mycelial growth was recorded after 7 days of incubation in each treatment using vernier caliper scale. The percent growth inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947, as cited in Kantwa *et al.*, (2014); Roopa *et al*., 2014) [14].

$$I = \left[\frac{C - T}{C}\right] * 100$$

Where
I = Percent growth inhibition,
C = Growth of hyphae in control (mm) and
T = Growth of hyphae in treatment (mm)

Statistical analysis

All the data were entered in MS Excel (2013) and analysis of variance was done using Op stat software. Mean comparison was done using Fisher-LSD test at 0.05 level of significance.

Results and Discussion

In vitro evaluation of fungicides

The efficacy of different fungicides was shown in the table below.

Table 1: *In vitro* efficacy of different fungicides on growth of *Alternaria brassicae*

S. No	Treatments	Mean colony diameter(mm) at different concentrations (ppm)					Growth inhibition (%) at different concentrations (ppm)					Mean growth inhibition (%)
		50	100	250	500	1000	50	100	250	500	1000	
1	Chlorothalanil	35.00	22.5	16.5	13.00	10.20	53.94	70.39	78.28	86.30	86.57	75.09
2	Metalaxyl+Mancozeb	19.50	17.00	12.00	8.00	6.00	74.34	77.63	84.21	89.47	92.10	83.55
3	Tebuconazole	0	0	0	0	0	100	100	100	100	100	100
4	Carbendazim	30.50	29.50	22.50	20.00	13.50	59.86	61.18	70.39	73.68	82.22	69.46
5	Mancozeb	15.00	12.50	10.80	6.00	3.00	80.26	83.55	85.78	92.10	96.05	87.54
6	Control	76.00					0.00					0.00
	CD, CV(%), SEM(±)	5.972, 1.975, 5.598										

The efficacy of different chemical fungicides against test fungus was evaluated *in vitro* using poisoned food technique. The data on inhibition percent is presented in table. An insight into data reveals that all the tested chemical fungicides showed significant effect (p<0.001) against pathogen growth over control (76.00 mm). The extent of mycelial growth inhibition increased with increase in their concentration. Among the chemicals tested Tebuconazole proved to be the most effective fungicide showing complete inhibition (100%) followed by Mancozeb (96.05%) at 1000ppm and Metalaxyl + Mancozeb (92.10%) at 1000 ppm. Minimum growth inhibition (82.22%) was obtained in the concentration at 1000 ppm of carbendazim followed by Chlorothalanil.

Similar result was recorded in findings of Panwar *et al.*, (2013) [13] who reported complete growth inhibition of *Alternaria* in tebuconazole followed by mancozeb and least inhibition in carbendazim. Similarly, Tu (2015) recorded complete inhibition of *A. brassicae* by tebuconazole, mancozeb at 250, 500 and 1000 ppm and by metalaxyl + mancozeb at 500 and 1000 ppm and least inhibition at carbendazim. Biswas and Ghosh (2018) [4], Kantwa *et al.*, (2014) [20] obtained significant growth inhibition effect of *Alternaria* sp. in mancozeb. Similar inhibition was observed by Thaware *et al.*, (2010) against *A. alternata.* Synthetic fungicides bring about the inhibition of pathogens either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogen and hence are effective (Kakraliya *et al.*, 2018) [8]. Higher inhibition effect of tebuconazole is due to inhibition of ergosterol biosynthesis, controlling the growth and reproduction of fungal pathogen (Muhamad *et al.*, 2010) [12].

In vitro evaluation of botanical extracts

Five different botanical extracts were evaluated at three concentrations for their efficacy against *Alternaria brassicae in vitro*. The result revealed that, all the tested botanicals inhibited the growth of pathogen over untreated control. Extracts from different plant species used in the experiment showed to possess different level of fungicidal effect against test fungus. Growth inhibition ranged from 39.46% to 74.26% irrespective of concentrations. Significant difference (p<0.001) was obtained among different botanical extracts in their inhibition effect. Increase in effectiveness was observed with increase in concentration

Among three concentrations used, maximum reduction of mycelial growth (74.26%) was observed at 15% concentration of turmeric which was significantly superior over rest of concentrations. Among the botanical extracts, maximum mean growth inhibition (64.98%) of tested pathogen was recorded in Turmeric followed by Veld Grape (57.95%) and Tulsi (56.83%) while, minimum growth inhibition (44.76%) was recorded in *Aloe vera*. In case of interaction effect all concentrations i.e. 5%, 10% and 15% of Turmeric proved to be superior with highest inhibition.

Table 2: *In vitro* efficacy of different botanicals on growth of *Alternaria brassicae*

S. No	Treatments	Concentrations						Mean Growth inhibition (%)
		5%		10%		15%		
		Mean colony diameter (mm)	Growth inhibition (%)	Mean colony diameter (mm)	Growth inhibition (%)	Mean colony diameter (mm)	Growth inhibition (%)	
1	Neem	46.75	40.82	32.05	59.43	26.32	66.68	55.64
2	Aloe vera	47.82	39.46	43.82	44.53	39.25	50.31	44.76
3	Tulsi	42.50	46.20	31.12	60.60	28.67	63.70	56.83
4	Veld grape	41.67	47.29	33.67	57.37	24.44	69.20	57.95
5	Turmeric	33.63	54.01	26.33	66.67	20.33	74.26	64.98
6	Control	79.00	0	79.00	0	79.00	0	0
	SEM (±), CV (%)	5.88, 18.191						

The result was in confirmatory to finding of Pitipong, (2009) who reported maximum inhibition of *Alternaria* sp. in *Turmeric* Similarly, Kavita and Dalbeer (2015), Waghe *et al.*, (2015), Kakraliya *et al.*, (2018) [8] revealed significant inhibition effect of neem. Chethana *et al.*, (2012), Thaware *et al.*, (2010) and inhibition effect of 93.64%, 96.68% and 99.91% respectively. This was followed by all concentrations (5%, 10% and 15%) of neem (75.87%, 76.00% and 80.15%) and 15% of garlic extract (77.34%) which were statistically at par with each other.

Conclusion

Alternaria leaf blight is a worldwide disease of economic importance in mustard crop. Different chemical fungicides are commercially available in market to control this disease. This study revealed significant inhibition effect of all the tested chemical fungicides and botanical extracts over control. Indiscriminate application of chemical fungicides have resulted several health hazards, negative impacts in environment so, the use of effective chemical at possible lower concentrations could be safer way to minimize health hazards and environmental pollutions Botanical extracts such as Turmeric, Tulsi and Veld Grape exhibited inhibition of *Alternaria brassicae*at higher percentage. Therefore, this biological agent and plant extracts could be a potential to be used as novel fungicides alternative to harmful chemical fungicides. However, these *in vitro* research finding should be verified in the field conditions before taking for field application.

References

1. Abhinandan D, Randhawa HS, Sharma RC. Incidence of Early Blight on Mustard and Efficacy of commercial fungicides for its control. Ann. Biol. 2004;20(2):211-218.
2. Agrios GN. Plant Pathology, 3rd edition. Acad. Press. California, USA 1988.
3. Ahila Devi P, Mohan S, Thiribhuvanamala G. Antifungal activity of plant extracts against Alternaria helianthi. J Biopest 2013;6(2):231-236.
4. Biswas MK, Ghosh T. Evaluation of phyto-extracts, biological agents and chemicals against the development of *Alternaria brassicae in vitro* and *in vivo*. European Journal of Medicinal Plants 2018;22(9):1-9

5. Bushra S, Farooq A, Muhammad A. Effect of Extraction solvent/Technique on the Antioxidant activity of selected medicinal plant extracts 2009.
6. Ganie SA, Ghani MY, Nissar Q, Rehman S. Bioefficacy of plant extracts and bio control agents against Alternaria. African Journal of Microbiology Research, 2013;7(34):4397-4402.
7. Gupta SK, Thind TS. Disease problems in vegetable production. Scientific publisher, Jodhpur 2006, 576.
8. Kakraliya SS, Choskit D, Pandit D, Abrol S. Effect of bio-agents, neem leaf extract and fungicides against Alternaria leaf blight of wheat (*Triticum aestivum* L.) 2018.
9. Kamble SB, Pawar DR, Sankeshwar SB, Arekal JS, Sawant VK. In-Vitro efficacy of fungitoxicants against Alternaria solani. Internal. J Agri. Sci. 2009;5(1):137-139.
10. Sukhadia V. Photo catalytic degradation of copper surfactant. Int. J Adv. Chem. Res. 2020;2(2):53-55. DOI: 10.33545/26646781.2020.v2.i2a.63
11. Meena PD, Chattopadhyay C, Meena RL. Ecofriendly management of Alternaria blight in Brassica juncea. Indian Phytopath. 2008;61(1):65-69.
12. Muhamad H, Zainol M, Sahid I. Determination of hexaconazole in field samples using gas chromatography-electron capture detection (GC-ECD) 2010.
13. Panwar V, Yadav RS, Singh H, Shukla AC. *In vitro* evaluation of fungicides and bio agents against Alternaria alternata – an incident of leaf spot of Aloe vera 2013.
14. Patni CS, Kolte SJ, Awasthi RP. Efficacy of botanicals against Alternaria blight (*Alternaria brassicae*) of mustard. Indian Phytopath 2005;58(4):426-430.
15. Phalisteen S, Ishaq S, Amardeep K, Arif J, Sami S. Evaluation studies of some medicinal plant extracts and fungicides against A. solani. African Journal of Clinical and Experimental Microbiology 2008;9(1):19-32.
16. Prasad R, Khandelwal GL, Jain JP. Morphology, physiology and control of *Alternaria brassicae*on taramira. Indian Phytopath 1970;23:105-110.
17. Prasad Y. Studies on variability, pre and post-harvest management of early blight of mustard. M.Sc.(Agri) Thesis, Univ. Agric. Sci, Dharwad (India); c2002. p. 153.
18. Singh RS. Diseases of Vegetable Crops. Oxford IBH PUB. New Delhi; c1987. p. 419.
19. Sohbat B, Seifolahpour B, Reza A. Antifungal effects of some medicinal and aromatic plant essential oils against Alternaria solani. Journal of Crop Protection 2016;5(4):603-616.
20. Kantwa SL, Tetarwal JP, Shekhawat KS. *In vitro* effect of fungicides and phyto-extracts against Alternaria alternata causing leaf blight of groundnut. IOSR Journal of Agriculture and Veterinary Science 2014;7(6):28-31.