International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(4): 294-297

Received: 19-05-2021
Accepted: 21-06-2021

Karthikeyan R
M.Sc. Scholar, School of Agriculture (SOA), Uttaranchal University, Arcadia Grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

Sandeep Kumar
Assistant Professor, School of Agriculture (SOA), Uttaranchal University, Arcadia Grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

Rajendra Prasad
Assistant Professor, School of Agriculture (SOA), Uttaranchal University, Arcadia Grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

Mohsin Ikram
Assistant Professor, School of Agriculture (SOA), Uttaranchal University, Arcadia Grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

Jayanti Ballabh
Assistant Professor, School of Agriculture (SOA), Uttaranchal University, Arcadia Grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

Siddharth Shankar Bhatt
Assistant Professor, Research & Development (R & D), Uttaranchal University, Arcadia grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

Corresponding Author:
Karthikeyan R
M.Sc. Scholar, School of Agriculture (SOA), Uttaranchal University, Arcadia Grant, Chandanwari, Premnagar, Dehradun, Uttarakhand, India

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

Karthikeyan R, Sandeep Kumar, Rajendra Prasad, Mohsin Ikram, Jayanti Ballabh and Siddharth Shankar Bhatt

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 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 chlorothalanil was least effective in reducing fungal growth (53.94%). 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.

Keywords: *Alternaria,* fungicides, 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.

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 fungicides against the test pathogen were undertaken. The efficacy of five chemical fungicides such as Tebuconazole, Metalaxyl + Mancozeb, Tebuconazole, carbendazim and Mancozeb were taken at five different concentrations i.e. 50ppm, 100ppm, 250ppm, 500ppm and 1000ppm against *Alternaria brassicae* in order to show which chemical giving maximum growth inhibition at different concentrations. 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 respectively. Each of the treatment was replicated three times.

Isolation and maintenance of pure culture

Pathogen *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 fungicides

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) [7].

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

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 Opstat software (Sheoran *et al.*, 1998) [14]. 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 showing different days of incubation. 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. The following tables show the percent mycelial growth and percent mycelial growth inhibition at different concentrations at different days after incubation such as third, fifth and seventh days after incubation.

On 3rd day after inoculation T3 (Tebuconazole) proved to be the most effective fungicide showing complete inhibition (100%) followed by T5 (Mancozeb) (93.46%) at 1000ppm and T2 (Metalaxyl + Mancozeb) (90.91%) at 1000ppm. Minimum growth inhibition (84.63%) was obtained in the concentration at 1000 ppm of carbendazim followed by T1 (Chlorothalanil) shown in the table 1.

On 5th day after inoculation T3 (Tebuconazole) proved to be the most effective fungicide showing complete inhibition (100%) followed by T5 (Mancozeb) (95.38%) at 1000ppm and T2 (Metalaxyl + Mancozeb) (90.88%) at 1000ppm. Minimum growth inhibition (87.15%) was obtained in the concentration at 1000 ppm of carbendazim followed by T1 (Chlorothalanil) shown in the table 2.

On 7th day after inoculation T3 (Tebuconazole) proved to be the most effective fungicide showing complete inhibition (100%) followed by T5 (Mancozeb) (96.05%) at 1000ppm and T2 (Metalaxyl + Mancozeb) (92.10%) at 1000ppm. Minimum growth inhibition (82.22%) was obtained in the concentration at 1000 ppm of carbendazim followed by T1 (Chlorothalanil) shown in the table 3.

Similar result was recorded in findings of Panwar *et al.*, (2013) [10] 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) [3], Kantwa *et al.*, (2014) [7] obtained significant growth inhibition effect of *Alternaria* sp. in mancozeb. Similar inhibition was observed by Thawar*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) [5]. Higher inhibition effect of tebuconazole is due to inhibition of ergosterol biosynthesis, controlling the growth and reproduction of fungal pathogen (Muhamad *et al.*, 2010) [9].

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 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. Therefore, these fungicides could be a potentially used to control the harmful pathogen. However, these *in vitro* research finding should be verified in the field conditions before taking for field application.

Table 1: *In vitro* efficacy of different fungicides on growth of *Alternaria brassicae* on 3DAI

S. No	Treatments	Mean colony diameter(mm) at different concentrations (ppm)					Growth inhibition (%)					Mean growth inhibition (%)
		50	100	250	500	1000	50	100	250	500	1000	
1	Chlorothalanil	18.6	12.80	9.56	7.70	4.23	32.43	53.50	65.27	72.03	84.63	61.57
2	Metalaxyl + Mancozeb	9.30	7.13	5.40	2.90	2.50	66.21	74.10	80.38	89.46	90.91	80.21
3	Tebuconazole	0	0	0	0	0	100	100	100	100	100	100
4	Carbendazim	19.46	13.86	11.23	8.90	4.33	29.31	49.65	59.20	67.67	84.27	58.02
5	Mancozeb	9.20	7.13	6.66	3.53	1.80	66.58	74.10	75.80	86.08	93.46	79.20
6	Control	27.53	27.53	27.53	27.53	27.53	0.00	0.00	0.00	0.00	0.00	0.00
	C.D	3.90	3.81	3.72	3.58	3.65	-	-	-	-	-	-
	SEM(±)	1.25	1.24	1.16	1.15	1.17	-	-	-	-	-	-

Table 2: *In vitro* efficacy of different fungicides on growth of *Alternaria brassicae* on **5DAI**

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	28.30	16.03	12.50	10.16	7.13	49.00	71.11	77.47	81.67	87.15	73.28
2	Metalaxyl + Mancozeb	15.80	14.86	8.13	4.56	5.06	71.53	73.22	85.35	91.78	90.88	82.55
3	Tebuconazole	0	0	0	0	0	100	100	100	100	100	100
4	Carbendazim	32.35	15.93	13.26	10.6	6.83	41.71	71.29	76.10	80.90	87.69	71.53
5	Mancozeb	13.16	10.73	8.80	5.13	2.56	76.28	80.66	84.14	90.75	95.38	85.44
6	Control	55.50	55.50	55.50	55.50	55.50	0.00	0.00	0.00	0.00	0.00	0.00
	C.D	2.61	2.81	2.35	2.39	2.38	-	-	-	-	-	-
	SEM(±)	0.84	0.90	0.95	0.76	0.77	-	-	-	-	-	-

Table 3: *In vitro* efficacy of different fungicides on growth of *Alternaria brassicae* on 7 DAI

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	76.00	76.00	76.00	76.00	0.00	0.00	0.00	0.00	0.00	0.00
	C.D	2.12	1.82	2.15	2.46	1.59	-	-	-	-	-	-
	SEM(±)	0.68	0.58	0.69	0.79	0.51	-	-	-	-	-	-

Reference

1. Abhinandan D, Randhawa HS, Sharma RC. Incidence of 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. Biswas MK, Ghosh T. Evaluation of phyto-extracts, biological agents and chemicals against the devlopment of *Alternaria brassicae in vitro* and *vivo*. European Journal of Medicinal Plants 2018;22(9):1-9.
4. Gupta SK, Thind TS. Disease problems in vegetable production. Scientific publisher, Jodhpur 2006, 576.
5. 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.
6. Kamble SB, Pawar DR, Sankeshwar SB, Arekal JS, Sawant VK. *In-Vitro* efficacy of fungitoxicants against *Alternaria solani*. Internl. J Agri. Sci 2009;5(1):137-139.
7. 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.
8. Meena PD, Chattopadhyay C, Meena RL. Ecofriendly management of Alternaria blight in Brassica juncea. Indian Phytopath 2008;61(1):65-69.
9. Muhamad H, Zainol M, Sahid I. Determination of hexaconazole in field samples using gas chromatography –electron capture detection (GC-ECD) 2010.
10. 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.
11. 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.
12. Singh RS. Diseases of Vegetable Crops. Oxford IBH PUB. New Delhi 1987, 419.
13. 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.

14. Sheoran OP, Tonk DS, Kaushik LS, Hasija RC, Pannu RS. Statistical Software Package for Agricultural Research Workers. Recent Advances in informatio theory, Statistics & Computer Applications by D.S. Hooda & R.C. Hasija Department of Mathematics Statistics, CCS HAU, Hisar 1998, 139-143.