In vitro screening of some fungicides against *Alternaria brassicae* causing Leaf blight in mustard

Embadi Maruthi, Dr.Jai Prakash Mishra & Dr. Rajendra Prasad

Master of Science Agriculture (Plant Pathology)

Department of Agriculture,Uttaranchal College of Agicultural Science,

Uttaranchal University, Dehradun (Uttarakhand) India.

Abstract :

The efficacy of five fungicides evaluated at range of 1000, 2000, 3000, 4000, 5000 ppm concentration by poisoned food technique against *Alternaria brassicae* under *In-vitro* conditions. The observations on colony diameter and percent inhibition of linear growth of *Alternaria brassicae*. Mancozeb, combination of Mancozeb Metalaxyl, Bavistin and Chlhorothalonil in which combination two fungicide viz Mancozeb, Mancozeb and Metalaxyl were effective against *Alternaria brassicae.* Mancozeb was significantly inhibited mycelial growth of *Alternaria brassicae* at 2000 ppm. The combination of both Mancozeb and Metalaxyl was also completely inhibited mycelial growth at 2000 ppm. Bavistin and chlorothalonil were showed least effective against *Alternaria brassicae* at all concentrations compared to all fungicides. The Mancozeb and combination of Mancozeb and Metalaxyl were found most effective in inhibition of the mycelial growth and Bavistin and Chlorothalonil fungicides were found least effective.

Key words : Mustard, leaf blight, *Alternaria brassicae*, fungicides, Management.

Introduction

Indian mustard (*Brassica junceae* (L.) is also called as rai or laya belongs to the Brassicaceae family. Rapeseed and mustard are the most important rabi oilseed crop of India. Among oil seed crops Indian mustard is a major group of oilseed crops of Indo -Gangetic plains grown in various agro-climatic conditions. Among oilseed crops grown in India, Brassicae rank second in acerage with 6.5 million ha, superseded by groundnut only with 7.5 million ha. The crop requires relatively cooler temperature within our agro-climatic conditions is therefore, grow extensively in the north-eastern and central parts of India. Generally, the crop is grown in the rabi season from September-October to February-March. The mustard crop grows well in area having 25 to 40 cm of rainfall and thrives best in light to heavy-loam soil.

The mustard seeds contain 37-49% oil. The seeds and oil are used in the preparation of pickles and curries. The oil utilized for human consumption throughout the North India. It is also used in the preparation of hair oil, medicines and soap making. After extraction of oil from seeds it is used as animal feed and manure for crops.

As per reports of (**Directorate of Economics and Statistics, DAC&FW**) Rapeseed and mustard occupy 5.76 million hectares all over the India and its production about 6.82 million tones Rapeseed and mustard is Important oilseed crop in uttarakhand state and it is occupy 3000 hectares and Productivity of mustard is 83.22 lakh tones in rabi season.Yield about 1000 kg/ha **(Directorate of Economics and Statistics, DAC & FW2017-2018**) According to **USDA** 100 grams of mustard oil contain 100 g of fat, carbohydrates 0.0 g, Energy 884 k cal, Fatty acid- 11.582g.

Forty two fungal pathogens are associated with rapeseed and mustard out of these blight caused by *Alternaria brassicae* (Berk.) Sacc. is major disease and it causes heavy loss in all brassica species. Some other species of Alternaria viz.,*A.brassicae* (Berk) Sacc, *A.brassicicola* (Schw) Wiltsh. *A. raphanin* (Groves) and *A.alternata* (Fr) Keissler have been reported for the cause of Alternaria blight.).

Alternaria leaf blight of mustard is an important disease in India (Chahal, 1986; Kolte, 1985). Not only the quantitative losses in yield (Kolte et al., 1987), but also affects the quality of seed and its germination (Ansari et al.,1988). Leaf spot of mustered caused by *Alternaria brassicae* causes severe damage up to 35% (Kolte et al., 1987).

Production of rapeseed and mustard in Uttarakhand is affected by several factors, among them Alternaria leaf blight caused by *Alternaria brassicae* (Berk.) Sacc. is an important disease which causes 10-70% yield loss. It is assumed that Alternaria blight have been divulged from Nepal (Shrestha et al 2005). All most all cruciferous crops, viz., broccoli, cauliflower, field mustard, leaf or chinese mustard, Chinese or celery cabbage, cabbage, rapeseed, radish etc.,are mainly affected by *Alternaria brassicae.*

The fungal pathogen infects mainly on upper leaves, stem, pods. It develops dark brown colour spots with concentric ring. Symptoms vary with the host and environmental conditions. Symptoms& signs appear s mainly on older lower leaves as tiny black points, that develops into pre-eminent, circular and homocentric co- axial spots in many different sizes. On basis of environmental conditions, diseases progress to lower,

middle, upper leaves and than lower leaves starts being deprived of leaves. To make head way these round black visible spots that develops on stem and siliquae.

Keeping all the above mentioned aspects in focus the present investigation has been carried out by undertaking the following objects.

1) To screen different concentration of available fungicides against *Alternaria brassicae* using poisoned food technique in laboratory.

Materials and methods

3.1.1 Materials

All glassware's used for experiments were Borosilicate. The glassware was sterilized before and after use as per standard procedure . All Chemicals and culture media used in present study were purchased from Himedia (India). Sprit lamp, inoculation wire loop, colony counter, micro pipette, funnels, forceps, needle and Cork borer, Electric balance, BOD incubator, autoclave, hot air oven, refrigerator, compound microscope, stereo bi nocular microscope, laminar air flow, pH meter were used for experimental work. The laminar air flow first surface sterilized by 70 % alcohol then exposed to U.V radiation for 20 mins.

3.2 Methods

3.2.1 Isolation of pathogen

The infected leaves were collected from the mustard field,(Practical crop) Uttaranchal College of Agricultural science,Uttaranchal university. The collected leaf sample were washed in sterile zed distilled water. The leaf samples were cut into small pieces of 3-5 mm in size from the healthy with diseased portion. These leaf pieces were passed through 70 % alcohol solution for one minute for surface sterilization and washed three times in three changes of sterilized distilled water. These leaf cuttings were aseptically placed on Potato Dextrose Agar (PDA). In each plate a single piece was placed and incubated at 25± 2 °C for 7 days. After appearance of mycelial growth it was transferred on to fresh PDA slant.

3.2.2 Pathogenicity test

The seedlings of mustard were grown in separate pots containing sterilized soil under control environmental condition. Pure cultured of Alternaria were inoculate at 3-4 leaf stage (15 – 20 DAS) in order to prove the pathogenicity of fungal pathogen. The seedlings were observed every day for 20 days for the development of disease symptoms. After development of symptoms in seedlings the pathogen was isolated from the infected leaves. The morphology of pathogen were observed under compound microscope in laboratory and similarity of isolated pathogen with original inoculated pathogen in step -2 , confirms its pathogenicity.

3.2.3 *In- vitro* screening of fungicides against *A. brassicae*

Five fungicides namely mancozeb 75 %WP (Dit,hane M-45) Mancozeb 8 % + Meatalaxyl 64 % WP (Partner), Chlorothanoil 75% WP (Kavach), Bavistin 50% WP (Carbendazim) were evaluated *in- vitro* condition against *Alternaria brassicae* by poisoned food technique (Nene and Thapliyal, 1979). The different concentration of fungicide were prepared in PDA and allowed to solidification. A 7 mm disk of active culture *Alternarria brassicae* was placed on the petri dishes. To maintain the growth of pure culture the plates were kept in upside down position.Control plate was observed without any fungicide. The radial growth of fungus and percentage of inhibition was calculated with the help of formula : $I = C - T / C \times 100$

Where I = Percentage of inhibition

C = Colony diameter in control plate

T = Colony diameter in treatment

EXPERIMENTAL RESULTS

The different fungicides namely Mancozeb and Mancozeb + Metalaxyl, Bavistin, chlorotalonil were tested against *Alternaria brassicae* in present study showed variable inhibitory effect at various concentrations. The data is shows that at 7thday after inoculation Mancozeb, Mancozeb + Metalaxyl were exhibited 100 % inhibition of growth of *Alternaria brassicae* at 2000ppm followed by bavistin and

chlorotalonil. Therefore Mancozeb and Mancozeb + Metalaxyl were significantly superior over all other fungicides against *Alternaria brassicae*.

Table -1: Colony diameter (cm) of *Alternaria brassicae* in PDA Plates

S.No	Concentration (ppm)	Mancozeb	Mancozeb+Metalaxyl	Bavistin	Chlorotalonil
1	1000	1.1	1.4	3.1	3.4
2	2000	0	0	2.6	2.9
3	3000	0	0	2.2	2.6
4	4000	0	0	2.0	2.2
5	5000	0	0	1.8	2.0
6	Control	6.84	6.84	6.84	6.84
SE m±		0.02	0.01	0.30	0.26
CD		0.07	0.06	0.09	0.08

Table -2 : Growth inhibition of *Alternaria brassicae*

Growth inhibition % of fungal colony					
S.No	Concentration	Mancozeb	M+M	Bavistin	Chlorothanoil
1	1000	83.3	80.9	50.2	54.2
2	2000	100	100	59.5	64.1
3	3000	100	100	63.8	67.6
4	4000	100	100	67.7	70.7
5	5000	100	100	74.0	70.4
SE m±		0.19	0.56	1.29	1.58
CD		0.56	1.76	4.1	5.04

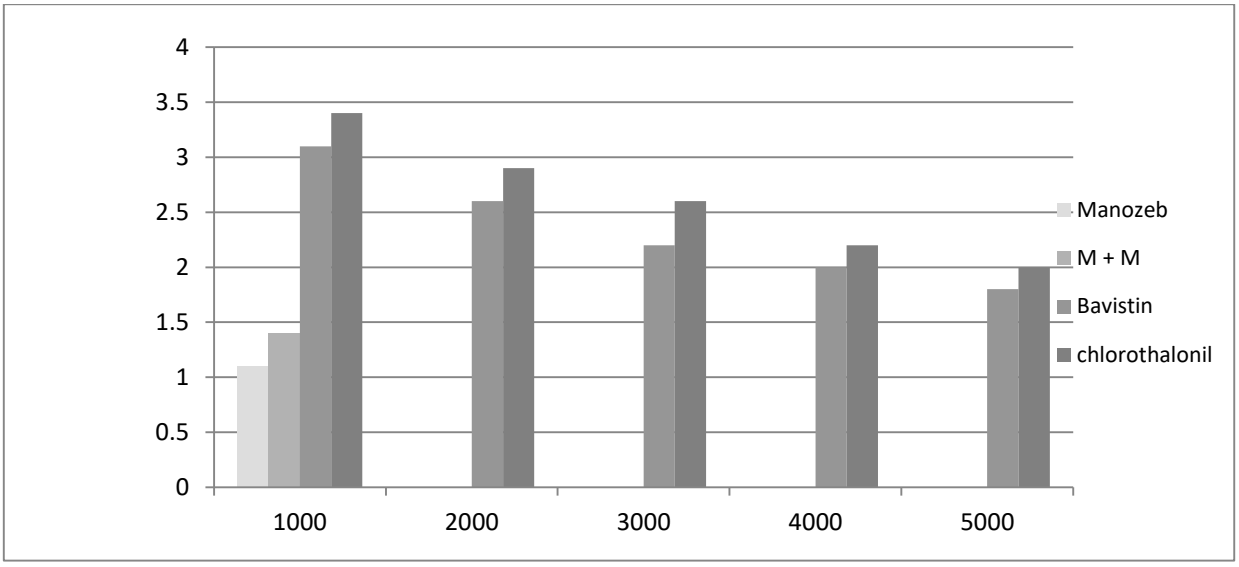

Fig 1 : Graph shows of colony diameter (cm) of *Alternaria brassicae* in PDA contains different ppm of different fungcides

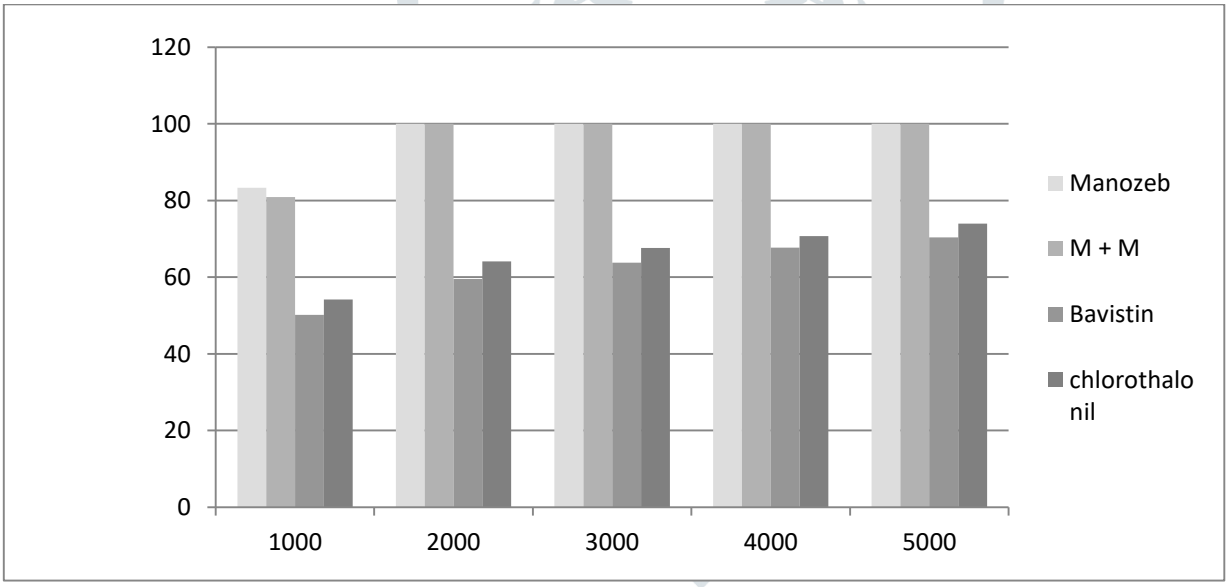

Fig 2 : Graph shows Percent of inhibition of *Alternaria brassicae* in PDA contains different ppm of different fungicides

Discussion

The relative efficacy of five fungicides evaluated at 1000, 2000, 3000, 4000, 5000 ppm concentration by poisoned food technique against the *Alternaria brassicae in-vitro* condition. The observation regarding on colony diameter and percent inhibition are presented in table 1 and table

Among all tested fungicides Mancozeb and combination of Mancoozeb and Metalaxyl were significantly reduced the mycelial growth compared to of Bavistin, Chlorothalonil but all the fungicides and there concentration significantly differ with each other. Mancozeb & combination of Mancoozeb and Metalaxyl completely inhibited mycelial growth *Alternaria brassicae* at 2000 ppm. The percent of inhibition of Mancozeb and Mancozeb + Metalaxyl at 2000 ppm was 100 percent followed by Bavistin and chlorothalonil.

Conclusion

In-vitro tested different fungicides viz, Mancozeb and Mancozeb + Metalaxyl absolutely checked the mycelial growth at 2000 ppm followed by bavistin, chlorothalonil. It means Mancozeb and the combination of Mancozeb and Metalaxyl was found most effective against *Alternaria brassicae* in *in -vitro* conditions.

References

(1) Ansari N A, Khan M W and Muheet A (1990) Evaluation of some fungicides for seed treatment and foliar application in management of damping-off of seedlings and blight of rapeseed caused by Mycopathol110(3): 163-167.

(2) Ayub A, Dey T K, Jahan M, Ahmed H U and Alam K B (1997) Foliar spray of fungicides to control Alternaria blight of mustard. Ann Bangladesh Agric 6(1): 47-50.

(3) Ayub A, Dey T K, Jahan M, Ahmed H U and Alam K B (1997) Foliar spray of fungicides to control Alternaria blight of mustard. Ann Bangladesh Agric 6(1): 47-50.

(4) Brazauskiene I and Petraitiene E (2004) Effects of fungicide application timing on the incidence and severity of Alternaria blight (*Alternaria brassicae*) and on the productivity of spring oilseed rape (Brassica napus L) Agronomy Res 2(2): 121-133.

(5) Chahal A S (1986) Losses and chemical control of Alternaria in rapeseed-mustard in Punjab. Pl Dis Res 1:46-50.

(6) Chattopadhyay A K and Bhunia C K (2003) Management of Alternaria leaf blight of rapeseed- mustard by chemicals. J Mycopathol Res 41(2): 181-183.

(7) Girish G, Gowda M B, Saifulla M, Mahesh M and Satheesh (2007) Management of white rust and alternaria leaf blight of mustard using fungicides. Environ Ecol25S: 830-833.

(8) Godika S and Pathak A K (2002) Efficacy of some fungicides against Alternaria blight disease of mustard. Pl Dis Res 17(1): 138-140.