In-Vitro Evaluation of selected Fungicides on the Growth and Sporulation of *Alternaria alternata* causing Blight Disease of Broad Bean (*Vicia faba* L.)

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Abstract— *Broad bean (Vicia faba L.) is an important leguminous cold season crop cultivated widely in different parts of the world and in India. This crop is grown especially in U.P., Bihar, Punjab, Haryana and in the foot hill ranges of Himalayan region including north eastern states. In Manipur, it is an important winter vegetable cum pulse crop. However, this crop suffers attack of various diseases of fungi, viruses and nematodes resulting in substantial reduction in yield. Hence, an in-vitro evaluation of selected fungicides on the Growth and Sporulation of Alternaria alternata causing blight disease of broad bean (Vicia faba L.) was under taken in the present investigation. A judicious application of Tricyclazole and Copper oxychloride at 1000ppm can effectively manages the blight disease of broad bean and prevent economic loss due to disease condition.*

Keywords— Alternaria alternata, broth media, solid media, sporulation, mycelium mat, radial growth, inhibition, fungicides, per cent disease incidence index.

I. INTRODUCTION

Broad bean (*Vicia faba* L.) is an important leguminous cold season crop cultivated widely in different parts of mild sub-tropical and temperate regions of the world. In India, this crop is grown especially in U.P., Bihar, Punjab, Haryana and in the foot hill ranges of Himalayan region including north eastern states. In Manipur, it is an important winter vegetable cum pulse crop. The protein rich tender green pods were consumed as vegetable and seeds as dal and snacks. However, this important pulse or vegetable crop suffers attack of various diseases of fungi, viruses and nematodes resulting in substantial reduction in yield. Important diseases of broad bean include leaf blight caused by *Alternaria alternata*, Rust caused by *Uromyces fabae*, Leaf spot caused by *Phoma exigua*, powdery mildew caused by *Erysiphe polygoni*, root knot diseases caused by *Meloidogyne javanica*, mosaic virus and little leaf virus diseases (Gupta, 1985). Among these diseases leaf blight caused by *Alternaria alternata* was found most serious in India and reported to cause losses upto 80% in sunflower (Agrawal, et al., 1979).

II. MATERIAL METHODS

The research work was carried out in the field and laboratory of Department of Plant Pathology, KVK-Senapati district, Manipur, ICAR, ATARI, Zone-VII, from 2017-2019. The details of experimental procedures adopted during the course of investigations are described as follows.

2.1 Collection of diseased specimen

During the month of October to December of *rabi* season 2017, Broad bean leaves showing typical blight symptoms was collected from the experimental plots of KVK-Senapati, Manipur and brought in the laboratory for isolation of fungus.

Isolation and purification of fungus associated with blight disease of Broad bean:

The blight disease infected leaves were cut into small bits of 2-3 mm and surface sterilized in 1% sodium hypochlorite for 1-2 minutes and wash with sterile water three times and then inoculated in sterilized petriplates containing fresh potato dextrose agar (PDA) and then incubated at 25 ± 10C for 72 hours. The fungus was purified by single hyphal tip culture method. The fungal culture thus obtained was stored at room temperature and periodically subcultured on fresh potato dextrose agar (PDA) slants from time to time.

2.2 Disease symptoms:

The disease first appeared as small circular usually at the leaf margin or tips or as scattered dark brown spot on surface of the broad bean leaves and progressed towards the mid rib. The spots coalesce together to form an elongated, irregular necrotic dark brown lesions. The affected leaf sometime showed chlorosis and later fell off or dries up along with the leaf stalk still

clinging on the plants. In severe condition young plant shows pale greenish leaves, start drooping, wilted and died. {Photo-I, II, III, IV].

PHOTO (I). Initial blight symptom

PHOTO (II). Leaf blight advance stage

PHOTO (III). Wilting of blight infected plant

PHOTO (IV). Death of blight infected plant

2.3 *In vitro* evaluation of selected fungicides on the Growth and Sporulation of *Alternaria alternata*

2.3.1 Broth media test:

The efficacy of selected 5 fungicides on the growth and sporulation of fungus was studied by using poisoned food technique (Sharvelle, 1960). The selected fungicides viz. Dithane M-45 (Mancozeb), Blitox 50 (Copper Oxychloride), Topsin M (Thiophenate methyle), Bavistin (Carbendazim), Beam (Tricyclazole) was prepared at uniform concentration of 1000ppm. 50ml of potato dextrose broth was dispelled in conical flasks and plugged with non absorbent cotton and autoclaved at 15 lb pressure per square inch for 20 minutes. And after cooling down at 45ºC each of these selected fungicides were incorporated and then with the help of sterilized inoculating needle three days old mycelial disc of 5mm diameter cut out by using sterilized cork borer was transferred aseptically. The medium without fungicides served as control. For each treatment four replication was maintained. The experimental flasks were incubated at 25±10C for 10 days shaken for 2 minutes at 24 hrs intervals. On completion of the incubation period mycelium mats was harvested by filtering through pre-weighed filter paper Qualigens No.651 A (11 cm diameter) and dried in hot air oven at 600C for 72 hours and then kept in a desiccator for 24 hours and weight was taken again. The differences of weight between pre-weight filter paper and filter paper weight containing dry fungal mycelium determine the test fungicides and its efficacy on growth inhibition of the test fungi. The per cent growth inhibition over control was calculated by following method described by Vincent (1927).

$$I = \frac{100(C - T)}{C}$$

Where I=per cent growth inhibition of test fungi, C=growth of test fungi in control, T=growth of test fungi in treatment fungicides.

2.3.2 Solid media test:

50ml of molten potato dextrose agar (PDA) was dispelled in 100ml capacity conical flasks and autoclaved at 15 lb per square inch for 20 minutes. After cooling down at 45ºC required concentration (1000ppm) of test fungicides was added to each of the experimented flasks and mixed thoroughly by gently shaking in circular motion. The poisoned molted about 15ml were then poured in 9cm diameter sterilized petriplates. The medium without fungicide served as control. Each poisoned media plates was inoculated with 5mm diameter mycelial discs of 3 days old test fungal culture cut with the help of sterilized cork borer. The mycelial discs were placed at the center of the plate facing the mycelial mat with the media. For each treatment four replication was maintained. The inoculated plates were incubated at 25 ± 10C and observations were made at 24 hours interval till the fungus in the control plate covered the whole plate. The per cent inhibitions of fungal radial growth were calculated by following method described by Vincent (1927). For estimation of fungal spores, method described by Devi (1991) was followed where 1sq.cm mycelial block was cut out from each of the experimented plates and the mycelial mats was detached and then transferred into a test tube containing 5ml distilled water and vigorously shaken for 5 minutes. The

spore density/ml of this solution was estimated by using Haemocytometer. Four replications were made while counting of the spores.

III. RESULTS AND DISCUSSION

TABLE 1
EFFICACY OF DIFFERENT FUNGICIDES ON THE GROWTH AND SPORULATION OF FUNGUS (BROTH MEDIA)

Treatment	Dose (%)	Growth (mycelium dry weight) mg*	% inhibition over control
Bavistin (Carbendazim)	1000ppm	257.5 (16.0)	55.2
Beam (Tricyclazole)	1000ppm	2.0 (1.5)	99.6
Blitox 50 (Copper oxychloride)	1000ppm	13.5 (3.7)	97.6
Dithane M-45 (Mancozeb)	1000ppm	250.0 (15.8)	56.5
Topsin M (Thiophenate methyl)	1000ppm	496.0 (22.2)	25.9
Control	-	575.0 (23.9)	-
C.D.$_{5\%}$	3.3		

Mean of four replication

Figure in the parenthesis are the square root transform value

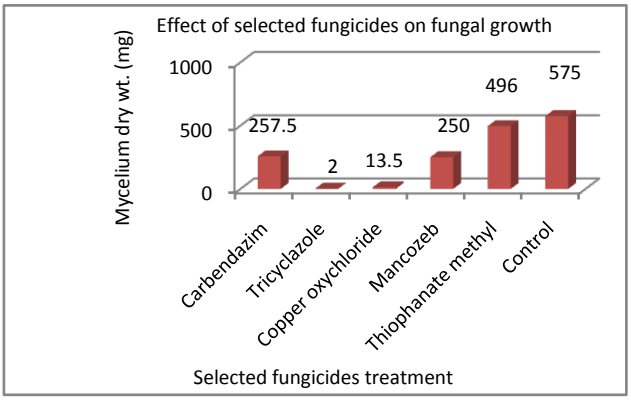

FIGURE 1: Selected fungicides and mycelium dry weight (mg)

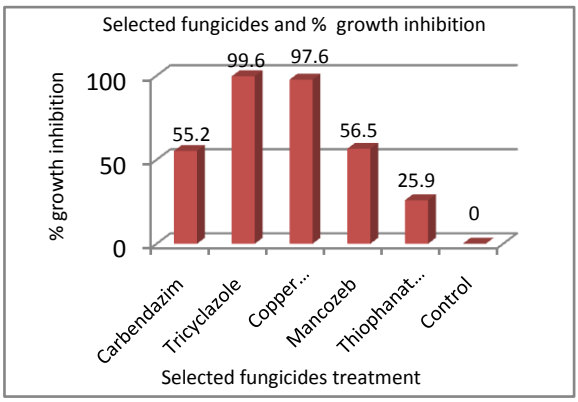

FIGURE 2: Per cent growth inhibition over control

The data presented in the above Table (1) and Fig.(1&2) is the result of efficacy of different fungicides on mycelium growth of *Alternaria alternata* in broth media. Among the selected fungicides, statistically highest significant inhibition on mycelial growth (minimum mycelium dry weight) was found in Tricyclazole (1.5mg) which was closely followed by copper oxychloride (3.7mg) with per cent growth inhibition of 99.6 and 97.6 respectively over untreated control whereas Mancozeb (15.8mg) and Carbendazim (16mg) with respective per cent growth inhibition of 56.5 and 55.2 and minimum significant mycelium growth inhibition was recorded in Thiophenate methyl (22.2mg) with per cent growth inhibition of 25.90 over untreated control. The present finding agreed with that of Misra and Singh (1965) who asserted fungicides like Blitox 50, Captan, Mancozeb and Zineb have got effective inhibitory properties on different isolates of *Alternaia tenui* during the *in vitro* experiment. Similar report was also reported by Paul and Mishra (1993).

TABLE 2

EFFICACY OF SELECTED FUNGICIDES ON GROWTH AND SPORULATION OF FUNGUS (SOLID MEDIA)

Treatment	Dose (%)	Colony diameter (mm)*	Spores/ml*	% inhibition over control	
				Colony diameter	Number of spores
Bavistin (Carbendazim)	1000ppm	55.2 (7.4)	1.1 x 10^3	38.2	82.5
Beam (Trycyclazole)	do	0.0 (0.7)	0.0	100.0	100.0
Blitox 50 (Copper oxychloride)	do	14.7 (3.8)	0.0	83.6	100.0
Dithane M-45 (Mancozeb)	do	41.7 (6.4)	0.6 x 10^3	53.6	90.0
Topsin M (Thiophanate methyl)	do	72.2 (8.5)	3.4 x 10^3	19.7	20.0
Control	-	90.0 (9.5)	6.3 x 10^3	-	-
C.D. 5%		0.4	-	-	-

Mean of four replication

Figure in the parenthesis is the square root transform value

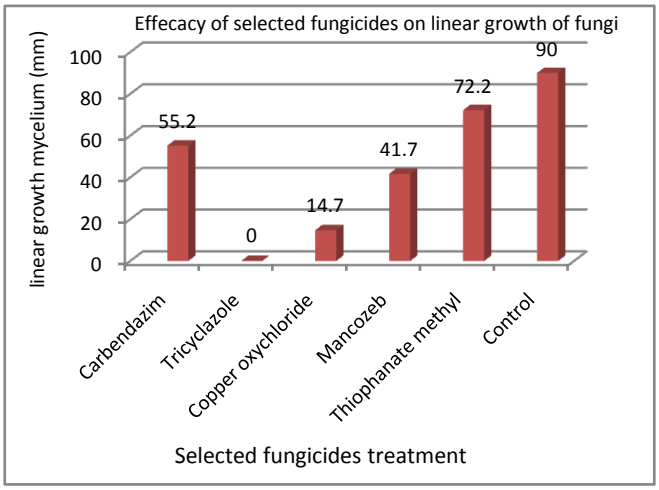

FIG.3.Selected fungicides on linear growth of fungi (*Alternaria ataernata*)

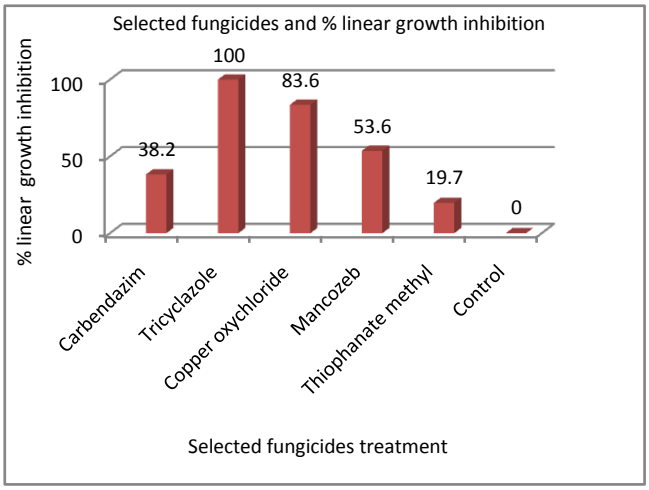

FIG.4. Selected fungicides and per cent linear growth inhibition

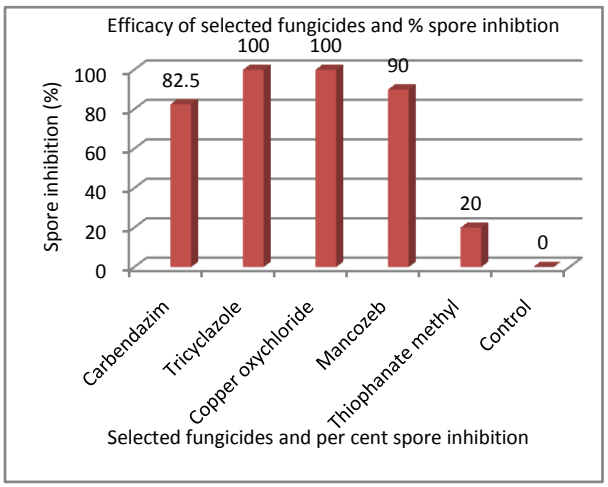

FIG.5. Selected fungicides and percent Spore inhibition of fungi (*A.alternata*)

Plate1.Mancozeb
Plate2.Tricyclazole
Plate3.Carbendazim
Plate4.Copper oxychloride
Plate5.Thiophanate methyl
Plate6.Control

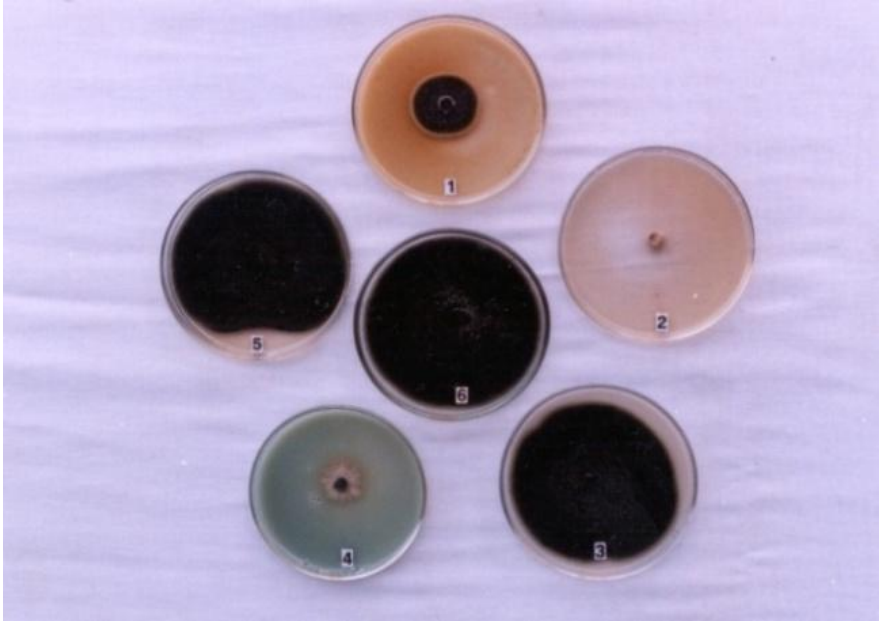

PHOTO (V). Selected fungicides treatment and linear growth inhibition of *Alternaria alternate*

The data presented in the above Table (2), Fig. (3,4 &5) and Photo (V) is the result of the effect of different fungicides on growth and sporulation of *Alternaria alternata* in solid media test. Among the selected fungicides statistically highest significant inhibition was found in Tricyclazole (0mm) where radial growth of mycelium and sporulation was completely suppressed followed by Copper oxychloride (14.7mm) with 83.6% radial growth inhibition with no formation of spore whereas Mancozeb (41.7mm) and Carbendazim (55.2mm) radial growth with respective radial growth inhibition of 53.6% and 38.2% and per cent spore inhibition of 90% and 82.5% over untreated control. A minimum significant effect on radial growth and sporulation of test fungus was recorded in Thiophanate methyl (72.2mm) with radial growth inhibition 19.7% and per cent spore inhibition of 20 over untreated control.

IV. CONCLUSION

Our present finding was in agreement with Misra and Singh (1965) who reported that fungicides like Blitox, Captan, Mancozeb and Zineb have got effective inhibitory properties on different isolates of *Alternaia alternata* during the *in vitro* experiment. Similar report also found by Paul and Mishra (1993). Rao and Rajagopalan (1982) reported Thiram was found most effective against the growth of *Alternaria helianthicola* with 95.9% inhibition of mycelium growth and 89.7% of spore germination during the *in vitro* test whereas Giri and Peshney (1993) reported Carbendazim, Mancozeb, Fosetyl-A and Iprodione could inhibit spore germination and mycelial growth of *Alternaria alternata* causing leaf spot of mungbean. Similary, Singh (1994) who reported Dithane M-45 @ 0.2% could completely inhibit mycelial growth and sporulation of *Alternaria Alternata* causing stalk rot of sunflower.

The present investigation therefore, revealed a judicious application of Tricyclazole and Copper oxychloride at 1000ppm can effectively manages the blight disease of broad bean and prevent economic loss due to disease condition and also will prevent indiscriminate used of non effective option of available fungicides against the target diseases.

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