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Detection of fungal pathogen, *Alternaria alternata* associated with mungbean and its management

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The damaging potential of *Alternaria alternata* on different mungbean genotypes was accessed and its management was done under *in vitro* and *in vivo* conditions through suitable fungicides. Seeds of fifty different mungbean genotypes were sown in lines. There were fifteen replications for each line. The data on disease incidence and percent disease intensity were accessed on all the genotypes. Infection was observed on all genotypes, none of them were found to be immune. Fourteen lines were found to be the most susceptible (14114, 14117, 14128, 14198, 14203, 14205, 14250, 14265, 14266, 14295, 14305, 14306, 14368, 14438). Infection was observed on all genotypes, none of them were found to be immune. The maximum PDI (Percent Disease Intensity) was recorded 35.30% while minimum was 2.43%. For detached leaf assay, the conidial suspension of 10^5 conidia/mLwas prepared and sprayed on detached leaves. The results revealed that maximum infection percentage was observed in 14306, 14198 genotypes while minimum was observed in 14128, 14203. The lesion area was found to be maximum in 14306, 14198 (0.23, 0.22) while minimum in 14128 (0.12) respectively. For management experiments, different fungicides; Propiconazole (Tilt), Propineb (Antracol), Difenconazole (Score), Thiophanate methyl (Topsin M), Mancozeb (Dithane M-45) were tested under *in vitro* and *in vivo* conditions. Significant decrease in mycelial growth with the increase in concentration of fungicides was observed. Percent inhibition of mycelial growth was maximum in Propiconazole at its highest concentration (1000 ppm) under *in vitro* conditions. A significant decline in disease incidence was observed as compared to control treatment. The minimum percent disease incidence was recorded in Propiconazole (10.34) with PDC (67.31) while the maximum percent disease incidence was observed in Thiophanate methyl (25.46). So, Propiconazole could be used successfully for the management of *A. alternata* under field conditions.

Keywords: Association, mungbean, incidence, lesion, management.

INTRODUCTION

Pakistan is an agrarian nation producing a diversity of crops. Mungbean (*Vigna radiata* L.) is an important pulse crop in many Asian countries including Pakistan, where the diet is mostly cereal based. Mungbean (*Vigna radiata* L.) similar to other pulses, is a good source of protein, vitamins, minerals and calories (Khan *et al*., 2020). A wide variety of fungus isolates has been identified that infect the mungbean crop causing dry root rot, charcoal rot and cercospora leaf spot (Nair *et al.,* 2019). The most prominent fungal disease of mungbean is the leaf spot disease. The symptoms include the formation of spots of different kind and shape on leaves (sometimes on stems and fruits; depending upon the type of the fungus). The genus *Alternaria* has its various species that are known to cause a significant damage of crops in the field as well as at late harvesting stage. It is well known pathogen affecting pulses, cereals fruits and vegetables that result in severe agricultural losses (Barkai-Golan *et al.,* 2008).

A wide variety of research based on epidemiological studies have revealed that *A. alternata* spores are the most commonly recognized fungal spore in the environment (Woudenberg *et al.,* 2015). *Alternaria* black rot, *Alternaria* leaf spot and *Alternaria* brown spot are the most significant phenotypes of plant diseases caused by *A. alternata* spores (Logrieco *et al.,* 2009). *Alternaria* leaf spot damage is more prominent on aerial parts and approximately upto 80% losses were recorded (Singh, 1987).

Different strategies were used for the management of *Alternaria*, but the chemical application seems the most efficient as it stops the further proliferation of disease. The effect of various fungicides was checked against *Alternaria*

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under *in vitro* conditions (Chohan *et al.,* 2015; Gazanafar *et al.,* 2016). Among the fungicides Propiconazole was found to be the most effective against *A. alternata* (Singh and Majumdar, 2002).

Similarly various researchers focused on the use of fungicides for the management of *Alternaria* and found this method suitable for securing their crops from the damage (Gorawar *et al.,* 2006b; Phapale *et al.,* 2010; Sanjeev *et al.,* 2017; Yasmeen *et al*., 2021). Hence the current research was focused on accessing the damaging effects of *A. alternata* on different genotypes of mungbean and also the management of *A. alternata* under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

***Evaluation of Mungbean germplasm against Alternaria alternata infection*:** Seeds of fifty mungbean genotypes were sown in lines by using 1 ft row to row distance. There were fifteen replications for each line. Inoculation of *A. alternata* was done on one month old mungbean plants with a conidial suspension of 10^5 conidia/mL in sterile distilled. Five mL of solution was sprayed on each plant. The disease incidence was recorded by counting the total number of leaves and the number of infected leaves. The percent disease incidence was calculated as per formula by Kalloo (1997):

Percent disease incidence = Number of infected leaves × 100 /Total number of leaves examined

The percent disease intensity was recorded by visual observation using 0-5 scale adopted by Singh *et al.* (1996), wherein 0 = no disease, 1, 2, 3, 4and 5 represented 1-10, 11-25, 26-50, 51-75and > 75 leaf area affected by disease, respectively. The percent disease intensity (PDI) was calculated as per the formula:

Percent disease intensity (PDI) =

Σ (nxv)× 100/ N×S

Where, Σ = Summation; n = Number of leaves in each category; v = Numerical value of each category; N = Number of leaves examined, and S = Maximum numerical value.

***Detached leaf assay*:** For detached leaf assay, the conidial suspension of 10^5 conidia/mL was prepared and sprayed on detached leaves from 3-week-old mungbean plants until surface run-off, and the leaves were placed in Petri plates processed with moistened blotting papers and incubated at temperature 26± 1 ºC. Observations were recorded after 7 days of spray.

***In vitro evaluation of fungicides*:** The efficacy of different fungicides [Propiconazole (Tilt), Propineb (Antracol), Difenconazole (Score), Thiophanate methyl (Topsin M), Mancozeb (Dithane M-45)] was tested on *A. alternata* by poisoned food technique (Nene and Thapliyal, 1993). Three concentrations of fungicides were prepared (250, 500,1000 ppm). Approximately twenty ml of poisoned media was poured in the sterilized Petri plates (9 cm dia.). Then inoculation of fungus in each plate was done by adding 5 mm mycelia discs from actively growing culture of the fungus under aseptic conditions and incubated at 26±1ºC. Plates containing non poisoned medium served as control. Three replications were kept for each treatment. The efficacy of fungicides was recorded by calculating percent inhibition over control through following formula by Vincent (1927):

$$I = \frac{C - T}{C} X\ 100$$

Where I= Percent inhibition, C= Radial growth in control (mm), T= Radial growth in treatment.

***In vivo evaluation of fungicides*:** The fungicides tested under *in vitro* experiment at different concentrations, were also tested under *in vivo* conditions at their concentration which showed significant control of fungus in laboratory experiment. The treatments comprised of five fungicides; Propiconazole (Tilt), Propineb (Antracol), Difenconazole (Score), Thiophanate methyl (Topsin M), Mancozeb (Dithane M-45) and control (untreated). There were ten replications for each treatment. Data on disease intensity were recorded according to the previously mentioned formula.

Percent Disease Control (PDC) was also recorded on the basis of following formula:

PDI in control – PDI in treatment = x 100/PDI in control

All the experiments were repeated and statistically analyzed data were presented in the Tables through Minitab version 18.1.

RESULTS

***Evaluation of Mungbean germplasm against Alternaria alternata*:** The effect of *A. alternata* was determined on fifty genotypes of mungbean. The results revealed that the susceptibility behavior of different genotypes was significantly varied (Table 1). The data on disease incidence and percent disease intensity were accessed on all the genotypes. Fourteen lines were found to be most susceptible (14114, 14117, 14128, 14198, 14203, 14205, 14250, 14265, 14266, 14295, 14305, 14306, 14368, 14438). Infection was observed on all the genotypes, none of them were found to be immune. The maximum Percent Disease Intensity was recorded 35.30 while minimum was 2.43 (Table 1). The percent disease incidence was maximum in 14306 (77.46).

Statistical analysis showed a differential relationship between mungbean genotypes and percent disease incidence /percent disease intensity (Fig.1, Fig. 1.1). The highest value of percent disease incidence was observed in 14306 genotype.

***Detached leaf assay*:** Observations were recorded after seven days of inoculation, significant differences in lesion area were observed among the lines. The infection percentage was also calculated that revealed the differential behavior of lines (Table 2). The size of lesion correlated with the infection percentage. The results revealed that maximum infection percentage was observed in 14306, 14198 lines (14.70,

Table 1. Evaluation of Mungbean germplasm against *Alternaria alternata*

Sr. No	Germplasm	Percent disease incidence	Percent disease intensity	Sr. No.	Germplasm	Percent disease incidence	Percent disease intensity
1	14104	45.00lm	20.83gh	26	14266	53.70ij	22.43fg
2	14108	35.63o	16.67k	27	14279	6.30yz	3.50tu
3	14113	12.96vwx	6.40qr	28	14289	14.30vwx	6.20qr
4	14114	63.06f	26.60d	29	14291	26.36r	11.33no
5	14115	23.70s	11.53no	30	14292	14.50v	6.23qr
6	14117	57.53g	23.60ef	31	14294	45.33lm	27.53cd
7	14123	32.36q	13.43lm	32	14295	71.30c	31.73b
8	14128	54.03ij	22.46fg	33	14299	13.26vwx	3.36tu
9	14129	12.50wx	7.43pq	34	14300	21.43tu	8.16p
10	14133	23.36s	12.53mn	35	14304	34.33op	12.43mn
11	14134	4.73z	2.76tu	36	14305	56.70gh	24.46e
12	14141	32.56pq	6.83pqr	37	14306	77.46a	35.30a
13	14150	12.40x	5.33rs	38	14310	31.26q	14.30l
14	14165	45.00lm	6.40qr	39	14311	22.96st	10.46o
15	14180	21.16tu	6.23qr	40	14314	6.73y	2.60tu
16	14198	73.50b	27.00cd	41	14349	40.33n	16.43k
17	14203	52.40j	33.10b	42	14368	64.36ef	26.60d
18	14205	55.50hi	23.30ef	43	14369	32.40q	19.33hi
19	14208	19.56u	4.26st	44	14378	14.36vw	6.43qr
20	14215	6.46yz	2.43u	45	14438	67.36d	28.36c
21	14243	46.53kl	19.33hi	46	14443	45.90klm	18.56ij
22	14250	66.26de	28.40c	47	14473	26.56r	6.23qr
23	14256	31.80q	12.30mn	48	14478	13.36vwx	3.30tu
24	14261	44.43m	17.63jk	49	14480	47.46k	20.46h
25	14265	64.40ef	28.40c	50	14540	23.60s	11.33no

[1]Means within a column sharing the same letter are not significantly different from each other at $P = 0.05$

Table 2. Evaluation of Mungbean germlasm against *Alternaria* by detached leaf assay

Sr. No.	Germplasm	Lesion Area (cm^2)	Leaf Area (cm^2)	Infection percentage
1	14114	0.19b	1.51cd	12.91bc
2	14117	0.18bc	1.57ab	11.30e
3	14128	0.12f	1.60a	7.53i
4	14198	0.22a	1.55abc	13.17b
5	14203	0.14ef	1.54bcd	8.86h
6	14205	0.15de	1.44ef	10.91ef
7	14250	0.20b	1.54bcd	12.91bc
8	14265	0.16cde	1.49de	9.92g
9	14266	0.15de	1.52cd	10.79ef
10	14295	0.12f	1.49de	9.00h
11	14305	0.16cd	1.56abc	10.64f
12	14306	0.23a	1.53bcd	14.70a
13	14368	0.18bc	1.43f	12.43cd
14	14438	0.18bc	1.52cd	12.05d

[1]Means within a column sharing the same letter are not significantly different from each other at $P = 0.05$

13.17), while minimum was observed in 14128, 14203 (7.53, 8.86) respectively. The lesion area was found to be maximum in 14306, 14198 (0.23, 0.22) while minimum in 14128, 14203 (0.12, 0.14) respectively (Table 2).

Variation in lesion area was observed statistically significant in mungbean genotypes (Fig.2). Leaf area variations were also seen in graphical representation of data. Infection percentage was varied significantly in different mungbean genotypes (Fig 3).

Evaluation of fungicides under in vitro conditions against A. alternata: Different fungicides were tested against *A. alternata* under *in vitro* conditions by following poisoned

food technique. Significant differences were observed in growth inhibition of *A. alternata*. The growth inhibition varied significantly at different concentrations of fungicides (Fig. 3). Significant decrease in mycelial growth with the increase in concentration of fungicides was observed. Percent inhibition of mycelial growth was maximum in Propiconazole (97.45%) at its 1000 ppm concentration. At the concentrations of 500ppm and 250ppm the mycelia growth inhibition was recorded 78.18 % and 62.36% respectively. Significant reductions in growth of fungus were recorded in Difenconazole at its all concentrations with the highest of 96.23% at 1000 ppm concentration. Propineb and Mancozeb also inhibited the growth of *A. alternata*, significantly maximum inhibition was recorded in 1000 ppm concentration (87.45, 92.67%) respectively. Minimum inhibition in mycelial growth was observed in Thiophanate methyl treatment at its all concentrations, percent inhibition was recorded 42.46 % at its 1000 ppm concentration while 18.42% at 250 ppm concentration (Table 3). Statistical analysis showed a significant increase in inhibition zone with increasing concentrations of fungicides (Fig. 3.1).

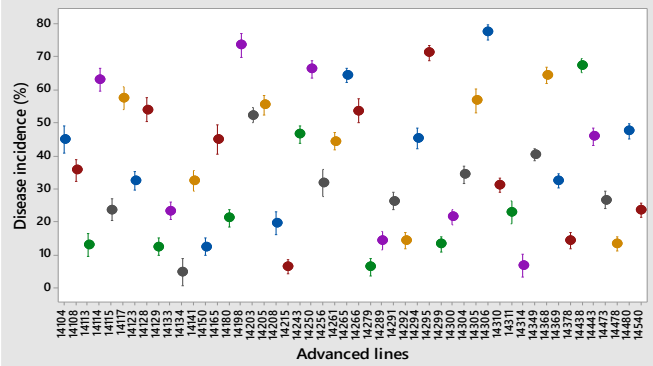

Figure 1. Evaluation of advanced lines of mungbean against *Alternaria* leaf spot under field conditions

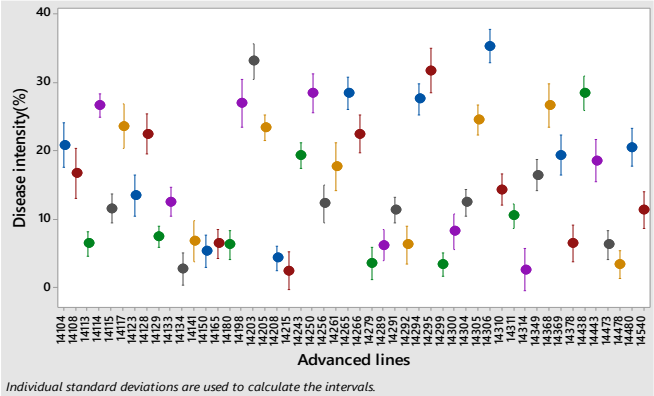

Figure 1. Evaluation of Percent Disease Intensity and mungbean genotypes infected by *A. alternata*.

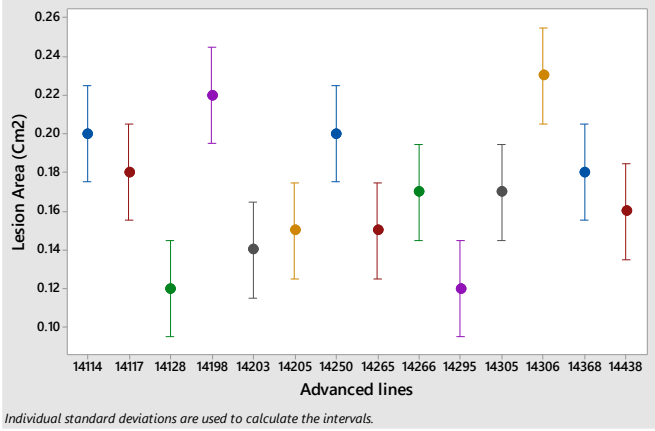

Figure 2. Lesion area in mungbean genotypes induced by *A. alternata* infection

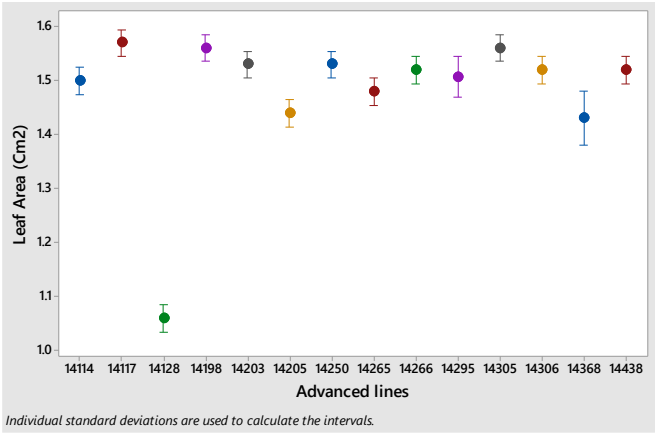

Figure 2. Leaf area of mungbean germplasm infected by *A. alternata*.

Evaluation of fungicides under in vivo conditions against A. alternata: The fungicides were also evaluated under *in vivo* conditions for the management of *A. alternata.* The results showed that the behavior of all the fungicides tested was significantly varied from each other. A significant decline in disease incidence was observed as compared to control treatment. The data were analyzed on the basis of percent disease incidence and percent disease control. All the fungicides significantly reduced the PDI (Percent Disease Incidence) (Table 4). The minimum percent disease incidence was recorded in Propiconazole (10.34) with PDC (67.31). The maximum percent disease incidence was observed in Thiophanate methyl (25.46). The other fungicides response was varied. In Propineb the percent disease incidence was (20.26) recorded while in Difenconazole and Mancozeb (14.34, 17.33) respectively (Table 4). Variation in percent disease incidence was clearly observed in graphical representation of PDI with mungbean genotypes (Fig.4). Maximum percent disease over control was observed in Propiconazole (Fig. 4.1).

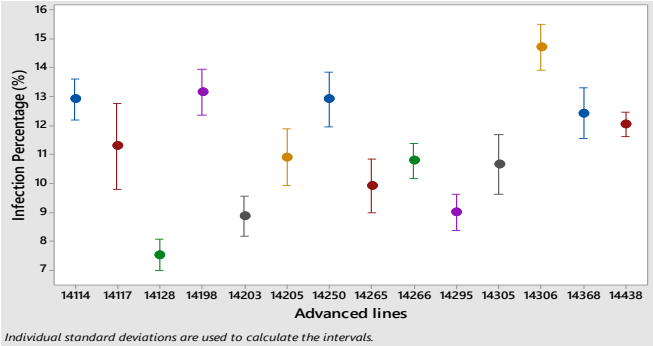

Figure 3. Infection Percentage in mungbean genotypes induced by *A. alternata*

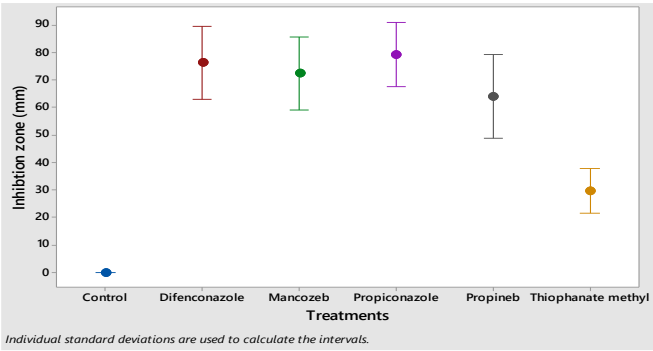

Figure 3. Evaluation of different fungicides against *A. alternata* through inhibition zone technique.

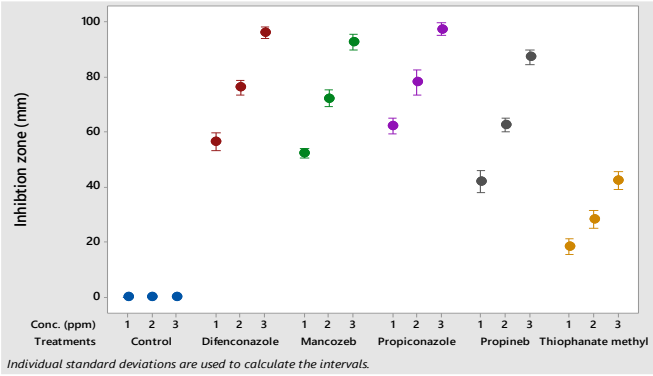

Figure 3. Percent inhibition of *A. alternata* mycelium over control at different concentrations of fungicides.

Table 4. Evaluation of fungicides against *A. alternata* under field conditions

Sr.	Treatments	PDI	PDC
1	Propiconazole (Tilt)	10.34f	67.31a
2	Propineb (Antracol)	20.26c	43.59d
3	Difenconazole (Score)	14.34e	63.41b
4	Thiophanate methyl (Topsin M)	25.46b	28.84e
5	Mancozeb (Dithane M-45)	17.33d	55.45c
6	Control	35.48a	-

[1]Means within a column sharing the same letter are not significantly different from each other at $P = 0.05$

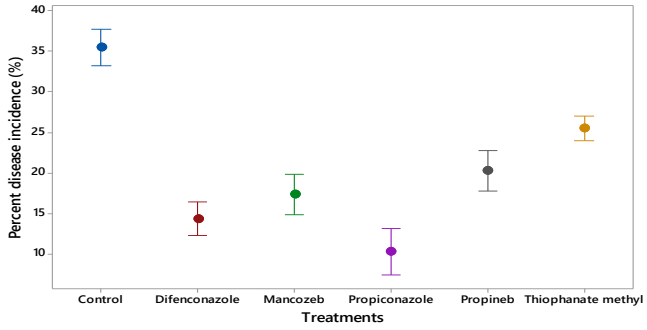

Figure 4. Effect of different fungicides on percent disease incidence in mungbean genotypes under field conditions.

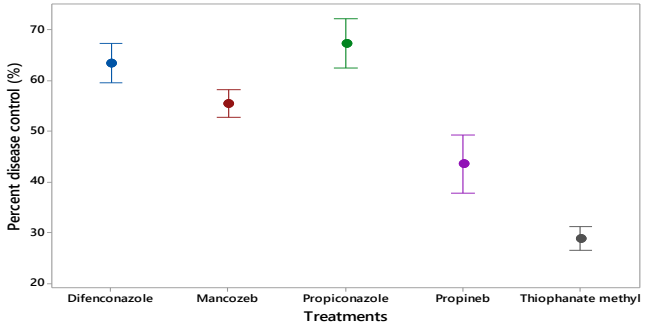

Figure 4. Effect of different fungicides on percent disease control in mungbean genotypes under field conditions.

Table 3. Evaluation of fungicides under i*n vitro* conditionsagainst *A. alternata*

Sr. #	Treatments	Percent inhibition of mycelium over control Concentrations (PPM)			Mean
		250	500	1000	
1	Propiconazole (Tilt)	62.36g	78.18d	97.45a	79.34a
2	Propineb (Antracol)	42.14j	62.59g	87.45c	64.06d
3	Difenconazole (Score)	56.53h	76.34e	96.23a	76.37b
4	Thiophanate methyl (Topsin M)	18.42l	28.35k	42.46j	29.76e
5	Mancozeb (Dithane M-45)	52.38i	72.33f	92.67b	72.46c
6	Control	0.00	0.00	0.00	0.00

[1]Means within a column sharing the same letter are not significantly different from each other at $P = 0.05$

DISCUSSION

Fungal pathogens cause several diseases that are now becoming epidemic in various plants due to the change in climatic conditions. The proper use of fungicides can combat the disease and lessen the losses caused by fungal pathogens. The focus should also be on the use of resistant or tolerant varieties to minimize the disease losses. The effect of *A. alternata* was determined on various genotypes of mungbean. The results revealed that the susceptibility behavior of different genotypes was significantly varied. Infection was observed on all the genotypes, none of them were found to be immune while fourteen lines were found to be the most susceptible. Infection of *A. alternata* was also observed on mungbean causing leaf spot disease (Jung *et al.,* 2019).

Significant differences in lesion area were observed among the lines under the detached leaf assay technique, which is also frequently used for the assessment of disease on leaf area (Prasad *et al.,* 2008). Different *Alternaria* species also attack on other plants (Pryor and Gilberson, 2000; Quayyum *et al.,* 2005; Kumar *et al.,* 2008) and also cause leaf blight disease, causing yield losses that varies from 32-57 % (Conn and Tewari, 1990).

Different fungicides were tested against *A. alternata* under *in vitro* conditions. Significant differences were observed in growth inhibition of *A. alternata*. Percent inhibition of mycelial growth was found to be maximum at the higher doses of fungicides. Decrease in mycelial growth with the increase in concentration of fungicides was observed that found to be similar with others findings (Chohan *et al.,* 2015; Gazanfar *et al.,* 2016; Sanjeev *et al.,* 2017; Bokal *et al*., 2020). Percent inhibition of mycelial growth was maximum in Propiconazole (100%) at its highest concentration. These results are in collaboration with Khan *et al.,* 1995 who reported maximum inhibition of the growth of *A. alternata* with the use of Propiconazole.

Response of Propiconazole was also evaluated at different concentration (250,500,1000ppm) and showed the complete inhibition of the growth of *A. alternata* (Phapale *et al.,* 2010). Present study also supported the findings of previous researches (Gorawar *et al.,* 2006b; Pairashi, 2007; Thaware *et al.,* 2010). Growth inhibition of *Alternaria* was also reported by Murthy and Shenoi (2001) through Propiconazole, Difenconazole and Mancozeb.

The results of fungicides tested under *in vivo* conditions showed that a significant decline in disease incidence was observed as compared to control treatment. The minimum percent disease incidence was recorded in Propiconazole following Propineb. Propineb was also tested against *A. alternata* causing leaf blight and found effective for the management of the disease under field conditions (Arun-Kumar *et al.,* 2011). Various fungicides (Propineb, Copper oxychloride and Mancozeb) were also tested against *Alternaria* leaf spot disease and effectively reduce the disease incidence (Bhattiprolu and Rao, 2014). Propiconazole has been tested by various researchers and found effective for the management of *A. alternata* in different crops (Singh *et al.,* 1998; Murthy and Shenoi, 2001; Singh and Majumdar, 2002). Different fungicides were also tested against *Alternaria* in combination with other management strategies at their different doses. Pun *et al.,* 2020 tested the efficacy of Mancozeb with Carbendazim and Metalaxyl and found them effective for the management of *Alternaria.* Mancozeb was also found to be effective by other researchers (Waghe *et al.,* 2015; Biswas and Ghosh, 2018; Gautam *et al.,* 2018). Mancozeb is classified as dithiocarbamate fungicide that acts as a multi-site action fungicide it generates ethylene bisisiothiocynate sulfide and ethylene bisisiothiocynate after it reacts to water. That interferes with the sulphydryl groups of enzymes that are involved in biochemical processes (Gullino *et al.,* 2010). Significant reductions in growth of fungus were recorded in Difenconazole at its all concentrations. It acts as a demethylation inhibitor that effects sterol 14 a demethylase which is dogmatic enzyme in the biosynthetic pathway of ergosterol (Munkvold, 2009).

***Conclusion*:** So, it is concluded from the findings of present research that tolerant varieties should be used to reduce the crop losses. The proper use of effective fungicides can cause significant inhibition of mycelial growth of *A. alternata*.

Authors Contributions statement: HA, NN and MSNR planed, designed and executed experimental work, TS draft preparation and SL editing and data analyses.

Conflict of interest: The Authors declare that there is no conflict of interest.

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