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CHEMICAL MANAGEMENT OF ALTERNARIA LEAF BLIGHT OF SUNFLOWER

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ABSTRACT

Sunflower is an important oilseed crop of Pakistan, comprising 20% proteins and 38-45% oil contents. Alternaria leaf blight (ALB) caused by *Alternaria alternata*, is one of the devastating diseases of sunflower. Six different fungicides viz., difenoconazole, hexaconazole, azoxystrobin, dimethomorph + mancozeb, myclobutanil and Sulphur were tested at different concentrations (10, 20, 30 ppm) in the laboratory and the greenhouse. Fungicides performed best in the laboratory were also investigated in greenhouse against *Alternaria* leaf blight of sunflower. In *in vitro* study, Hexaconazole showed 100 % growth inhibition of *A. alternata* at 30 ppm followed by 70% at 20 ppm and 62% at 10 ppm. Difenoconazole proved as the 2nd best fungicide with 77% fungal inhibition at 30 ppm, 75.8% at 20 ppm and 71% at 10 ppm. Azoxystrobin was the least effective fungicide with 24%, 28%, 34% fungal inhibition at 10, 20 and 30 ppm, respectively. Twelve cultivars of sunflower were screened against blight disease in pot experiment to check the fungicides at a different level of susceptibility in the greenhouse. Screening result showed that FH 702 was the highest susceptible variety with mean value 7.6. Greenhouse study of disease inhibition effect of fungicides also showed that hexaconazole fungicide produced the best results against *A. alternata* with 42% disease reduction in FH 702 cultivar and 25 % in FH 696 cultivar as compared to control (83%). The results showed that no fungicide provided full disease inhibition, so, further investigation is needed to find the new chemistry against blight disease of sunflower crop.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a member of the *Asteraceae* family and is originated about 3000 B.C ago from Eastern North America. In the late 1800s, it was introduced as a food crop by Russian farmers and made substantial advances in its cultivation (Fernández-Luqueño *et al.*, 2014). Sunflower seed is enriched with 20% protein and 38-45% oil contents. The oleic acid contents in the seed are 25.1 %, linoleic acid contents are 66.2 % (Javed *et al.*, 2003). The world annual production of sunflower is nearly 45 million metric tons and the area under its cultivation is 26 million hectares (Konyalı, 2017). In Pakistan, the total area under sunflower cultivation is almost 257,000 acres, with 133,000 tonnes seed production and 51,000 tonnes oil production annually (GOP, 2019).

Alternaria leaf blight (ALB) is considered as a major disease and has been reported to cause yield losses up to 80% (Calvet *et al.*, 2005; Kgatle *et al.*, 2020; Waghe *et al.*, 2015). The disease symptoms are characterized by irregular, necrotic greyish brown lesions surrounded by a chlorotic halo on leaves, stem and even on florets resulting in premature defoliation and stem breakage. Use of resistant cultivars is normally the most economical management option against most of the plant diseases. Unfortunately, such resistance against

ALB is not usually expressed in commercially available sunflower hybrids till now (Iacomi-Vasilescu *et al.*, 2004). Therefore, farmers rely on other methods for management of ALB disease like a combination of cultural practices, disease-free seeds along with synthetic chemical fungicides (Avenot *et al.*, 2019). Many fungicides are being used as both seed and spray treatments including Iprodione, Hexaconazole, Mancozeb, Carbendazim, Ridomil MZ, Chlorothalonil, Captan and Strobilurin etc. to curtail ALB disease of sunflower (Kgatle *et al.*, 2020).

Therefore, the present study aimed to determine the fungicidal potential of twelve fungicides in laboratory and greenhouse trials. Moreover, locally available germplasm was also evaluated for resistance against ALB of sunflower.

MATERIALS AND METHODS

Isolation and identification of associated fungi

Sunflower leaf samples showing symptoms of blight were collected from the botanical garden of Bahauddin Zakariya University, Multan. Pakistan. Infected leaf portion (2 cm) was excised with some healthy portion and surface sterilized by rinsing in 1% mercuric chloride ($HgCl_2$) solution for about 40 seconds. Then, it was thoroughly washed and blot dried with sterile blotter paper. Afterwards, diseased samples were transferred aseptically on potato dextrose agar (PDA) medium plates and incubated for one week at 27±1 °C.

Olive green circular growth of the fungus was observed after 72 hr of incubation. The fungal growth was examined and identified based on morphological characters (Barnett and Hunter, 1972; Ellis, 1971). Purified fungal colonies were transferred to PDA slants and preserved at 4 °C till further use. Pathogenicity of isolated fungi was confirmed following Koch's postulates.

In vitro activity of fungicides against *A. alternate*

Poisoned food technique was used for evaluation of six fungicides viz., Difenoconazole, Hexaconazole, Azoxystrobin, Dimethomorph + Mancozeb, Myclobutanil and Sulphur against *A. alternata*. Different concentrations were prepared for each fungicide i.e.10 ppm, 20 ppm and 30 ppm were prepared. 1 ml of each concentration of each fungicide was dispensed in sterilized PDA medium and after solidification of the medium, culture disc of *A. alternata* was taken from a week-old culture and placed in the center of poisoned PDA plates. Plates were replicated three times. Plates without fungicide served as a control. All Petri plats were incubated at 27±1°C. Radial growth was measured on a daily base till the control plate was full of mycelial growth of fungi by the given formula of percentage inhibition (Vincent, 1947).

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

C: Control;
T: Treatment

Screening of sunflower cultivars

Twelve sunflower cultivars including FH-669, FH-676, FH-677, FH-678, FH-680, FH-685, FH-696, FH-697, FH-700, FH-702, FH-703, were obtained from AARI, Faisalabad (Ayub Agricultural Research Institute) and a local variety (Desi) from Multan. Sunflower seeds were sown in earthen pots filled with soil: sand: FYM @ 2:1:1. After germination, 4 weeks old healthy seedlings were selected with three replications per variety for inoculation purpose. The spore suspension was prepared from ten days old culture of *A. alternata* after passing through the thin pore muslin cloth to remove the residues. The suspension was diluted by adding distilled water to make 1×10^6 spores per ml. Inoculation was done by spray method and control plants were sprayed with water. The temperature and humidity of greenhouse were maintained at 27±1°C and 80-90%, respectively (Waghe *et al.*, 2015). The disease score was calculated 5 days after inoculation and expressed as percent Disease Index (Gopalakrishnan *et al.*, 2010).

$$\text{Percentage Disease Intensity (\%)} = \frac{\text{Summation of numerical rating}}{\text{Total No. of observation} * \text{ max. rating scale}} \times 100$$

Disease severity percentage was recorded using 0 to 9 disease rating scale developed by Mayee and Datar (1986). For this purpose, two leaves located at the bottom, two at middle and two at the top of the plant were chosen and scored as per scale given: 0 = no symptoms; 1 = leaf area infected with necrotic spot covering 1% or less of the leaf; 3 = spots enlarging, covering 1–5% of the leaf area; 5 = spots enlarging, dark brown, target like appearance covering 6–25% of leaf area; 7 = spots dark brown, coalescing and cover 26–50% of leaf area; 9= half of the leaf become infected covering 51% or more of leaf area.

Greenhouse experiment

For greenhouse experiment, susceptible varieties FH676, FH696, FH702, Desi were selected based on screening. Best *in vitro* performed fungicide Hexaconazole at a maximum concentration (30 ppm) was prepared as 15 ml of fungicide and 485 ml water were mixed and sprayed on plants as a curative. Afterwards, spore suspension of *A. alternata* @ 10^6 spores/ml and applied on the potted susceptible varieties. All the treated seedlings were covered with polythene bags for 24 hrs to conserve the humidity. After every seven days' severity of the disease was observed using a disease rating scale mentioned above.

Statistical analysis

The data was statistically analyzed by Analysis of Variance (ANOVA) followed by LSD test using the Statistix 8.1 program. Results were expressed as average ± Standard Deviation (Steel *et al.*, 1997).

RESULTS

***In vitro* efficacy of fungicides**

The poisoned food technique results of six fungicides against *A. alternata* showed that hexaconazole fungicide showed the best results with 100 % inhibition with results at 30 ppm, 70% inhibition at 20 ppm and 62.3% at 10 ppm. The next most effective was difenoconazole was found the 2nd best fungicide with 77% inhibition at 30 ppm, and 75.8% and 71% at 20 and 10 ppm, respectively. However, Azoxystrobin was observed least effective among all of them with, 23.6, 28 and 33.8% inhibition at 10, 20, 30 ppm, respectively (Table 1).

Table 1. Effect of fungicides on mycelial growth inhibition of *Alternaria alternata* at different concentrations.

Percent Inhibition Growth (±SD)						
Dose (ppm)	Dimethomorph+ Mancozeb	Myclobutanil	Difenoconazole	Azoxystrobin	Hexaconazole	Sulphur
10	60.5±0.13b	59±1.7b	71±1.10b	23.6±1.12c	62.3±0.33c	52.2±1.1c
20	62.7±1b	65±2a	75.8±0.7a	28±1.3b	70±0.5b	65.5±2b
30	65.9±0.8a	70±0.4a	77±0.8a	33.7±1a	100±0a	71.3±0.7a
Control	9 a ± 0.1					

Varietal screening

The susceptibility of twelve sunflower varieties was tested against *A. alternata* and observed that variety FH702 showed the highest mean susceptibility according to the disease rating scale. Furthermore, FH776, desi variety, and FH696 with the disease scoring mean of 7.6, 7.2, 7 and 6.8, respectively (Table 2, Figure 1).

Table 2. Disease severity index and varietal response of different sunflower varieties against Alternaria leaf blight disease.

Variety	Disease severity index (Mean)	Disease reaction
FH-696	6.8	Susceptible
FH-703	5	Susceptible
FH-685	5	Susceptible
FH-680	4.3	Susceptible
FH-678	6.7	Susceptible
FH-702	7.6	Highly susceptible
FH-697	5.7	Susceptible
FH-669	4.8	Moderately resistant
FH-700	6.1	Susceptible
FH-676	7.2	Highly susceptible
FH-677	5.2	Susceptible
DESI	7	Susceptible

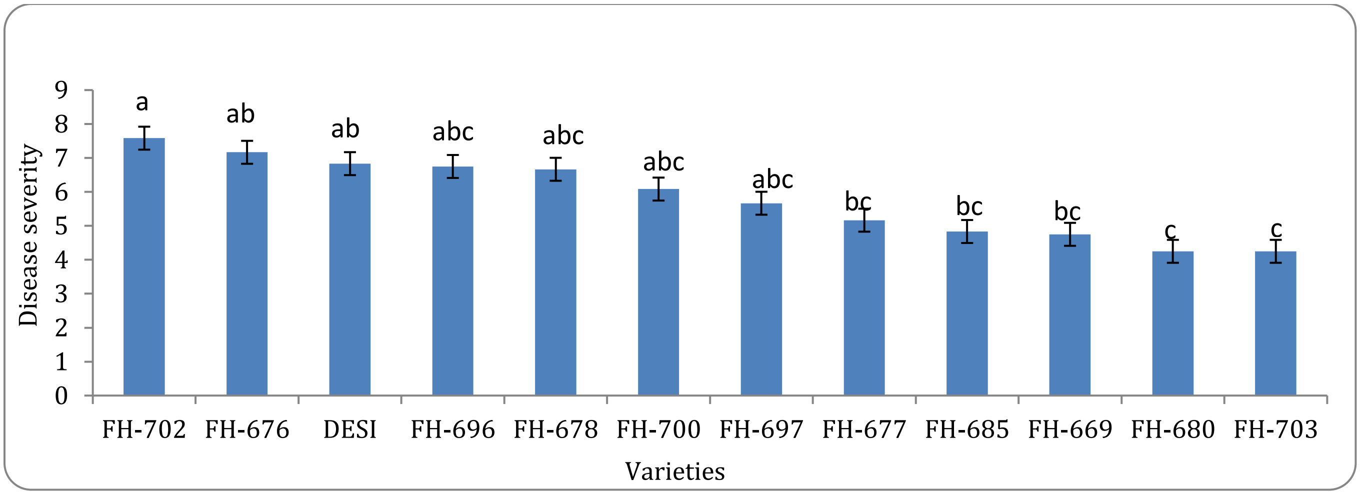

Figure 1. Graph showing the variation in disease severity among different varieties. The bar shows standard error. The means with same letters are not significantly different according to LSD test at $P > 0.05$

Evaluation of fungicides in greenhouse

On basis of screening results 4 susceptible varieties viz., FH676, FH696, FH702 and Desi, were selected for greenhouse experiment. Hexaconazole @ 30ppm significantly reduces disease severity over control, at all the tested cultivars. Maximum PDI (61.6%) was observed in case of FH702, followed by FH676 showing disease intensity of 61% over control (Table 3).

Table 3. Disease severity and disease reduction percentage of sunflower cultivars against Alternaria leaf blight.

Varieties	Week 1	Week 2	Week 3	Week 4	% disease index	% Disease reduction
	*D. S	D. S	D. S	D. S		
FH676	0.33	1.7	3	3	61.10	39
FH696	0.7	0.33	1	3	57	43.1
FH702	1.33	1.7	2.33	3	61.6	38.44
Desi	0.33	1.7	1	1.7	55.1	45

*D. S: Disease severity.

DISCUSSION

In view of the economic losses caused by *A. alternata*, the present study was conducted under *in-vitro* conditions and in the greenhouse, the efficacy of six fungicides viz., dimethomorph + mancozeb, myclobutanil, diphenoconazole, azoxystrobin, hexaconazole and Sulphur, was evaluated against ALB of sunflower. In the laboratory, Hexaconazole performed the best results followed by difenoconazole. Present results are analogous to an early investigation conducted by Mesta *et al.* (2009), who reported that systemic fungicides such as hexaconazole and propiconazole out of other fungicides showed 72.87% and 76.53% growth inhibition respectively against *A. helianthi*. They also reported that among non-systemic fungicides; iprodione + carbendazim (58.39%) recorded notably highest inhibition followed by Mancozeb (45.34%). Similarly, findings of Choudhary *et al.* (2020); Kumar *et al.* (2011) and Zaine (2011) also support our results. They all proposed hexaconazole as best fungicide against various fungal diseases. Our results signify that this fungicide is still efficient against the *Alternaria alternata* in Pakistan and can be an effective tool against ALB of sunflower.

The efficiency of the above-mentioned fungicides against ALB of sunflower, some researchers observed the effectiveness of test fungicides against other fungal pathogens of various crops. For example, Hoffman and Wilcox (2003) and Molitor *et al.* (2011) reported that Myclobutanil gave the best effect against grapevine powdery mildew (*Uncinula necator*) and grapevine black rot (*Guignardia bidwellii*). It also gave the best control of diseases of stone fruit, cereal seeds, cucurbits sugar beet, rose and cucurbits. So, it can be further tested in against other *Alternaria* species causing different plant diseases in Pakistan.

Contrary in our results, myclobutanil showed somehow better result with 69.9 % inhibition against *A. alternata* as reported by Molitor *et al.* (2011). Kongcharoen *et al.* (2020) reported that azoxystrobin has least fungicidal activity against rice blast disease, reducing its severity by 32–33%. These findings are in agreement with the present study in which results revealed that azoxystrobin gave minimum 34.7% inhibition even at a higher dose. Furthermore, Latinović *et al.* (2013) stated that Sulphur application at the pre-flowering, post fruit-set and drupe enlargement (September) stages, performed the best, followed by Mancozeb, and Copper oxychloride. We also found Sulphur good with 71% inhibition as compared to Dimethomorph + Mancozeb which gave 65.9% inhibition at 30 ppm. Some work has already been documented with regard to the *in vitro* effect of Hexaconazole, Difenoconole + Mancozeb, Propiconazole, Myclobutanil and Sulphur fungicides on mycelial growth inhibition of *A. helianthi* and other pathogenic fungi (Chattopadhyay, 1999; Feng and Zheng, 2007; Mirza and Hoes, 1996; Morris *et al.*, 1983; Savitha *et al.*, 2012; Sujatha *et al.*, 2008).

Out of the twelve genotypes tested under greenhouse, only one showed moderate resistance, nine were found susceptible and the rest of the two were found to be highly susceptible. However, all the genotypes exhibited a varied level of resistance to ALB disease on sunflower (Mesta *et al.*, 2011; Van der Waals *et al.*, 2001). Foliar application of Hexaconazole @ 30 ppm in the greenhouse on all the four tested varieties greatly reduced ALB disease severity. It has been previously reported that foliar spray of Carbendazim (Chattopadhyay, 1999), Hexaconazole @ 0.1% (Amaresh and Nargund, 2002), difenoconazole @ 0.05% (Karuna *et al.*, 2012), SAAF (Mancozeb + Carbendazim) (Waghe *et al.*, 2015) and Propiconazole@ 0.1% (Pathare *et al.*, 2019) significantly reduce the severity of Alternaria blight resulting in the lowest disease severity of ALB on the sunflower.

This study shows that Hexaconazole-treated (non-systemic fungicide) sunflower plants displayed prolonged greening that yields higher as more photosynthetic efficient leaves are retained longer. ALB reduce the amount of green leaf area may be due to the determinate number of leaves in sunflower. Consequently, a greater ALB disease severity results in the decrease in sunflower yield. Alternaria leaf blight was observed to reduce the amount of green leaf area resulting in the decreased level of photosynthesis (Kgatle *et al.*, 2020; Leite *et al.*, 2006). Hexaconazole fungicide might have a mode of action that inhibits mitochondrial respiration, preventing spore germination, reducing mycelial growth and are active against many plant pathogenic fungi (Mahoney *et al.*, 2015).

Thus, Hexaconazole and Difenoconazole fungicides showed a good fungal Alternaria leaf blight disease inhibition against the devastating disease of sunflower. All the tested plant varieties were susceptible to the pathogen. Azoxystrobin showed the least effectiveness due to high fungal resistance. Still, no fungicide provided full disease reduction. Therefore, further investigation is needed to find some new chemistry against this nasty disease of sunflower.

REFERENCES

Amaresh, V. S. and V. B. Nargund. 2002. Field evaluation of fungicides in the management of *Alternaria* leaf blight of sunflower. Annals of Plant Protection Sciences, 10: 331-336.

Avenot, H. F., M. Luna and T. J. Michailides. 2019. Phenotypic and molecular characterization of resistance to the SDHI fungicide fluopyram in populations of Alternaria alternata from pistachio orchards in California. Crop Protection, 124: 104838.

Barnett, H. L. and B. B. Hunter. 1972. Illustrated Genera of Imperfect Fungi 3rd ed., Burgess Publishing Company, Minneapolis.

Calvet, N., M. Ungaro and R. Oliveira. 2005. Virtual lesion of *Alternaria* blight on sunflower. Helia, 28: 89-100.

Chattopadhyay, P. 1999. Beyond direct and symmetrical effects: The influence of demographic dissimilarity on organizational citizenship behavior. Academy of Management Journal, 42: 273-287.

Choudhary, M., R. P. Ghasolia, T. Bajaya and M. Shivran. 2020. Efficacy of natural products and fungicides against powdery mildew of ber. International Journal of Chemical Studies, 8: 913-915.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycology Institute Kew, England.

Feng, W. and X. Zheng. 2007. Essential oils to control Alternaria alternata in vitro and in vivo. Food control, 18: 1126-1130.

Fernández-Luqueño, F., F. López-Valdez, M. Miranda-Arámbula, M. Rosas-Morales, N. Pariona and R. Espinoza-Zapata. 2014. An introduction to the sunflower crop. Sunflowers Edited by: JI Arribas. Nova Science Publishers, Inc: 1-18.

GOP. 2019. Pakistan oilseed development board. Agricultural Statistics of Pakistan. Statistics Division, Islamabad, Pakistan.

Gopalakrishnan, C., N. Manivannan, P. Vindhiyavarman and K. Thiyagarajan. 2010. Evaluation and Identification of Alternaria leaf spot resistant sunflower genotypes. Electronic Journal of Plant Breeding, 1: 177-181.

Hoffman, L. E. and W. F. Wilcox. 2003. Factors influencing the efficacy of myclobutanil and azoxystrobin for control of grape black rot. Plant Disease, 87: 273-281.

Iacomi-Vasilescu, B., H. Avenot, N. Bataillé-Simoneau, E. Laurent, M. Guénard and P. Simoneau. 2004. In vitro fungicide sensitivity of *Alternaria* species pathogenic to crucifers and identification of *Alternaria brassicicola* field isolates highly resistant to both dicarboximides and phenylpyrroles. Crop Protection, 23: 481-488.

Javed, M. S., A. Ali and H. Badar. 2003. Factors affecting the yield of sunflower in Punjab. Pakistan Journal of Social Sciences, 1: 42-44.

Karuna, K., K. S. Jagadish, K. N. Geetha and Y. G. Shadakshari. 2012. Evaluation of efficacy of chemical fungicides and a plant product for the management of *Alternaria* blight of sunflower. Indian Phytopathology, 65: 305-306.

Kgatle, M., B. Flett, M. Truter and T. Aveling. 2020. Control of Alternaria leaf blight caused by Alternaria alternata on sunflower using fungicides and Bacillus amyloliquefaciens. Crop Protection: 105146.

Kongcharoen, N., N. Kaewsalong and T. Dethoup. 2020. Efficacy of fungicides in controlling rice blast and dirty panicle diseases in Thailand. Scientific Reports, 10: 16233.

Konyalı, S. 2017. Sunflower Production and Agricultural Policies in Turkey. Sosyal Bilimler Araştırma Dergisi, 6: 11-19.

Kumar, G. S. A., B. C. Kamanna and V. I. Benagi. 2011. Management of chrysanthemum leaf blight caused by *Alternaria alternata* (fr.) Keissler under field condition. Plant Archives, 11: 553-555.

Latinović, J., A. Mazzaglia, N. Latinović, M. Ivanović and M. L. Gleason. 2013. Resistance of olive cultivars to *Botryosphaeria dothidea*, causal agent of olive fruit rot in Montenegro. Crop Protection, 48: 35-40.

Leite, R. M. V. B. C., L. Amorim and A. Bergamin Filho. 2006. Relationships of disease and leaf area variables with yield in the *Alternaria helianthi* sunflower pathosystem. Plant Pathology, 55: 73-81.

Mahoney, K. J., R. J. Vyn and C. L. Gillard. 2015. The effect of pyraclostrobin on soybean plant health, yield, and profitability in Ontario. Canadian Journal of Plant Science, 95: 285-292.

Mesta, R. K., V. I. Benagi, K. Srikant and M. P. Basavarajappa. 2011. Management of *Alternaria* blight of sunflower through fungicides. Karnataka Journal of Agricultural Sciences, 24: 149-152.

Mesta, R. K., V. I. Benagi, K. Srikant and I. Shankergoud. 2009. In vitro evaluation of fungicides and plant extracts against *Alternaria helianthi* causing blight of sunflower. Karnataka Journal of Agricultural Sciences, 22: 111-114.

Mirza, M. S. and J. A. Hoes. 1996. AGAINST Altenraria trclionthri. Helia, 19: 87-92.

Molitor, D., O. Baus and B. Berkelmann-Löhnertz. 2011. Protective and curative grape black rot control potential of pyraclostrobin and myclobutanil. Journal of Plant Diseases and Protection, 118: 161-167.

Morris, H., K. Gatter, G. Sykes, V. CASEMORE and D. Mason. 1983. Langerhans' cells in human cervical epithelium: effects of wart virus infection and intraepithelial neoplasia. BJOG: An International Journal of Obstetrics & Gynaecology, 90: 412-420.

Pathare, I., S. Akash, T. Ingle and R. J. Choudhary. 2019. Efficacy of fungicides and *Trichoderma viride* mutants against *Alternaria helianthi* causing leaf blight of sunflower. Journal of Pharmacognosy and Phytochemistry, 8: 1041-1044.

Savitha, A., M. Naik and K. Ajithkumar. 2012. Alternaria sesami, causing blight of sesame produces toxin and induces the host for systemic resistance. Asian Journal of Research in Chemistry, 5: 1176-1181.

Steel, R. G. D., J. H. Torrie and D. A. Dicky. 1997. Principles and Procedures of Statistics: A Biometrical Approach 3rd ed., McGraw Hill Book Co. Inc., New York.

Sujatha, M., A. V. Reddy and A. Sivasankar. 2008.

Identification of sources of resistance to *Alternaria* blight in sunflower. Current Biotica, 2: 249-260.

Van der Waals, J. E., L. Korsten and T. A. S. Aveling. 2001. A review of early blight of potato. African Plant Protection, 7: 91-102.

Vincent, J. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159: 850-850.

Waghe, K., S. Wagh, D. Kuldhar and D. Pawar. 2015. Evaluation of different fungicides, bioagents and botanicals against Alternaria blight caused by Alternaria helianthi (Hansf) of sunflower. African Journal of Agricultural Research, 10: 351-358.

Zaine, D. 2011. The effect of some fungicides on *Trichoderma* spp. that use in biocontrol. (Unpublished) thesis, Tishreen University, Syria.

CONFLICT OF INTEREST

There is no conflict of interest among authors.

AUTHORS CONTRIBUTIONS

Hafiz M.Saqib and Muhammad Abid conducted research experiments and wrote the paper. Both authors equally share the first authorship. Sobia Chohan supervised the present research work.