Comparative efficacy of three fungicides for *in vitro* control of *Curvularia lunata*

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Abstract

Curvularia lunata is a fungal pathogen that causes leaf spot, leaf blight and root rot diseases in plants, and sinusitis, mycotic keratitis, asthma and conjunctivitis in human. This *in vitro* study was conducted to assess the potential of three synthetic fungicides to control *C. linata.* The fungicides evaluated in this study were thiophenate methyl 70% WP, metalaxyl+mencozeb 72% WP and fosetyl-Al 80% WP. The concentrations of these fungicides used in food poisoning technique were 50, 100, 150, 200 and 250 ppm, which were compared with a control having 0 ppm concentration. Metalaxyl+mencozeb proved the best fungicide in inhibiting the fungal growth by 26–67% over control. Thiophenate methyl and fosetyl-Al showed poor activity against the targeted pathogen by suppressing its radial growth by just 15–26% and 8–12%, respectively.

Keywords: Chemical control, *Curvularia lunata,* Fungicides, Metalaxyl+mencozeb.

Introduction

Curvularia lunata is an important saprobic fungal pathogen that causes diseases in various plant species (Garcia-Aroca *et al.,* 2018). It is considered as the most common grain mold fungus. Being seed and soil-borne in nature it spreads throughout the world (Barupal *et al.,* 2020). It has the ability to cause leaf spot, seedling blight, leaf blight, seedling rot and sheath rot diseases in most of the plant species belonging to the family Cucurbitaceae, Leguminosae, Compositae, Malvaceae, Solanaceae and Gramineae (AbdElfatah *et al.,* 2021). It can cause damage from seedling to harvesting stages of the crop plants. The pathogen prevalence and amount of inoculum varies with climatic conditions and geographic locations (Almaguer *et al.,* 2021). It has long been recognized as a common plant pathogen, however, reports of human diseases caused by this organism are relatively uncommon (Dave *et al.,* 2020). *Curvularia* infections in humans include endocarditis, brain abscess, pneumonia, mycetoma, mycotic keratitis, keratomycosis, onychomycosis, sinusitis, asthma, conjunctivitis, cutaneous and allergic bronchopulmonary diseases (Alex *et al.,* 2013; Tóth *et al.,* 2020).

Different management strategies such as cultural and bio-control agents can be used widely for the control of pathogens (Wonglom *et al.,* 2019). As the pathogen overwinters on diseased crop residues left in the field, therefore, these practices have a very limited success rate in eliminating the population density of *C. lunata* (Barupal and Sharma, 2017). Hence, synthetic fungicides are applied frequently for the effective control of this pathogen (Adaangadi *et al.,* 2018). Many chemicals like carboxin, mancozeb, chlorothalonil, carbendazim, thiram, companion, metalxyl, propiconazole azoxystrobin, iprodion and copper oxychloride have been reported to retard the growth of *C. lunata* (Bisht *et al.,* 2018; Hao *et al.,* 2020). Among them, mancozeb and carbendazim are considered as the most effective control agents. Mostly fungicides involve the common mechanism of microtubule polymerization which results in control of fungal cell division for an effective crop protection program (Vela-Corcía *et al.,* 2018). The present study was carried out to determine the effect of thiophenate methyl 70% WP, metalaxyl+mencozeb 72% WP and fosetyl-Al 80% WP against *C. lunata* isolated from banana from Pakistan.

Materials and Methods

Three fungicides *viz.* metalaxyl+mancozeb 72% WP, thiophenate methyl 70% WP and fosetyl-Al 80% WP were purchased from local market of Lahore. Five concentrations of each fungicide *viz.* 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were tested against the target fungus using poisoned food technique. Malt extract agar (MEA) growth media (2%) was prepared in 250-mL flasks separately for every treatment and then autoclaved in saturated steam at 121 ºC and 103.4 kPa pressure for 30 min. Thereafter, the media was allowed to cool to about 60 ºC. To avoid bacterial growth, antibiotic streptomycin was added @ 100 mg L^{-1} into the media and then added fungicides in it. No fungicide) was added in control treatment (0 ppm). The media was gently swirled to mix properly. The media with different concentrations was then poured into sterilized 9-cm diameter Petri plates under sterilized environment.

Curvularia lunata culture was procured from Biofertilizers and Biopesticide Lab, Faculty of Agricultural Sciences, Punjab University Lahore. Originally the pathogen was isolated from infected banana fruits, identified on both morphological and molecular bases by sequencing its ITS and GAPDH regions, and deposited in the GenBank under accession No. MN752153 and MN787829, respectively (Khan and Javaid, 2020). The fungal culture was revived on MEA media to retain fungal pathogenicity. Revived fresh culture was inoculated at the center of MEA media plates using 5 mm borer disc. Four replicates were made for each treatment for better statistical results. All the Petri plates were arranged in a completely randomized design and incubated at 25 ºC for one week and then measured the fungal growth

Table 1: Analysis of variance (ANOVA) for the effect of different concentrations of three fungicides on the growth of *Curvularia lunata*.

Sources of variation	df	SS	MS	F values
Fungicides (F)	2	48.75	24.37	587*
Concentration (C)	5	33.87	6.77	163*
F × C	10	16.53	1.65	40*
Error	54	2.24	0.041	
Total	71	101.39		

*, Significant at $P \leq 0.001$.

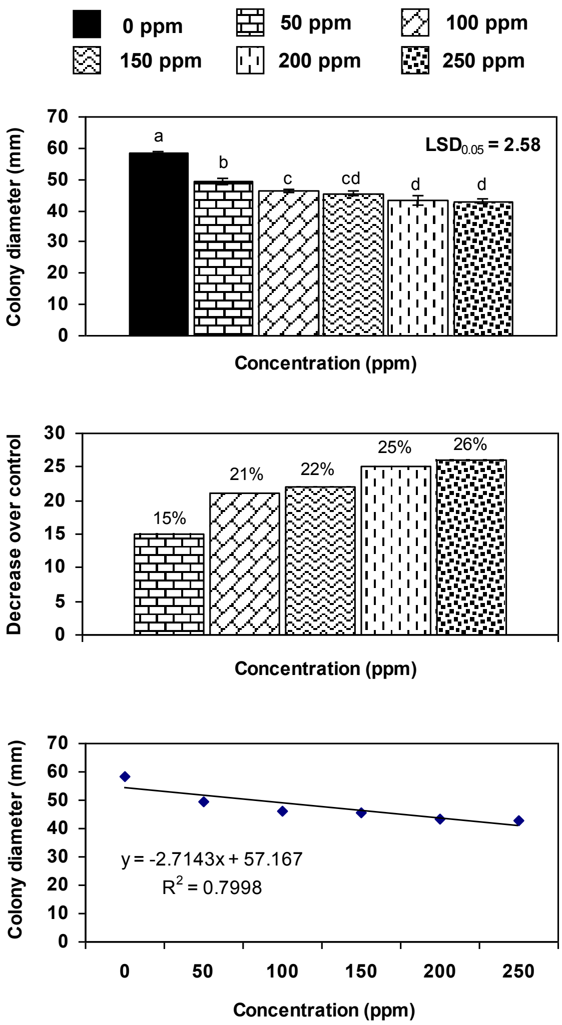

Fig. 1: Effect of thiophenate methyl on growth of *Curvularia lunata*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

Results and Discussion

Analysis of variance indicated that the effect of fungicides (F), their concentrations (C) as well as interaction (F × C) was significant ($P \leq 0.001$) for fungal growth as given in Table 1.

Fungicidal activity of thiophanate methyl

This fungicide was moderately effective against *C. lunata* (Fig. 4C). It controlled the fungal growth by 15–26% when applied in 50 to 250 ppm concentrations (Fig. 1A & B). A linear relationship was observed between concentrations and fungal growth with R^2 = 07998 (Fig. 1C). Thiophanate methyl belongs to class of thioureas. It is the dimethyl ester of (1,2-phenylenedicarbamothioyl) biscarbamic acid. It is a tubulin inhibitor fungicide falling into the FRAC (fungicide resistance action committee) Group 1 for benzimidazoles. It derives from 1,2-phenylenediamine (NCBI, 2021). Its mode of action is the inhibition of microtubule assembly. It is an effective fungicide against wide-ranging infections in turfs, vegetables, fruits, as well as in other crops comprising grey mould, eyespot, powdery mildew and scab. It is a far-ranging intrinsic fungicide that is immersed by hidden half and canopy of assessed plants. It exhibits mutually defensive and remedial modes of action. Subsequently, it aims a comprehensive array of infections and host plants. Since 1973, its widespread application has been extensively under practice. It is prescribed to substitute fungicides or spray mixture of this fungicide with other categories of fungicides having varying mechanisms of action. It develops a shielding layer on plants. It infiltrates as well as translocates through plant's vascular tissues i.e. xylem. It has defensive mode of action which comprises fresh growth and it has a marvelous health-giving action. It is applied on tubers, trees and vine crops. Householders employ it on turfs and landscape plants (Arena *et al.,* 2018).

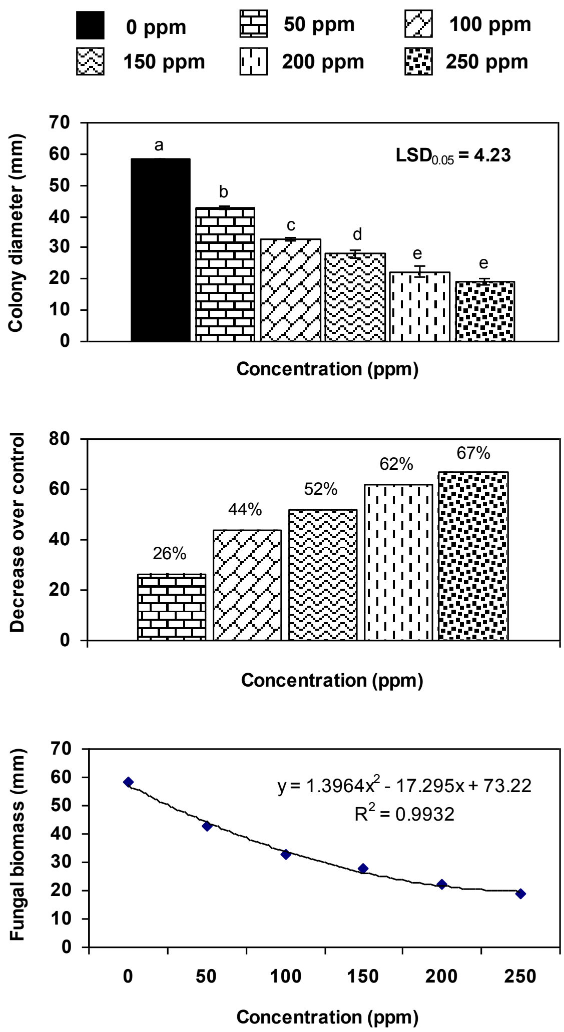

Fig. 2: Effect of metalaxyl+mencozeb on growth of *Curvularia lunata*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

Fungicidal activity of metalaxyl+mencozeb

This fungicide was highly effective against *C. lunata* (Fig. 4B). Its lowest concentration (50 ppm) significantly ($P \leq 0.05$) reduced growth of the fungus by 26% while the highest concentration (250 ppm) inhibited fungal growth by 67% (Fig. 2A & B). A polynomial relationship between concentration and fungal growth was recorded with R^2 = 099.32 (Fig. 2C). Metalaxyl is a generally used PA (phenylamides) fungicide. It suppresses uridine imbibition in RNA strand. It intrudes synthesis of nucleic acid by way of reticence of RNA polymerase I action. Hence, it hinders synthesis of rRNA at transcription interface of uridine. Extensive applications of PA fungicide may enhance pervasiveness of fungicidal resistance in phytopathogen population and numerous outbreaks of resistant isolates, as revealed by a contemporary research using markers for AFLP (amplified fragment length polymorphism) and SSR analysis. PA fungicides must be applied with risk avoidance, as the lateral influence of this fungicide on N cycling related microbes was testified (Yang *et al.,* 2011).

The forthright outcome of mancozeb upon principal biochemical pathways, contained by fungus, eventuates in reticence of spore germination. Mancozeb exhibits features of a characteristic multi-site defensive-unique fungicide. Because of subsequent use upon target plant, the chemical accumulates on foliar surface. Mancozeb has an outstanding profile of crop protection over wide-ranging crops and climatic situations. Its multi-site featured action evidences confirmed action against a wide-ranging fungi & fungus like organism comprising of ascomycetes, basidiomycetes, oomycetes, and fungi imperfecti. Its application and persistent use have increased over past 50 years. This fact has led to cataloguing and privileges of fungicidal efficacy in over 70 crops and 400 diverse maladies (Gullino *et al.,* 2010).

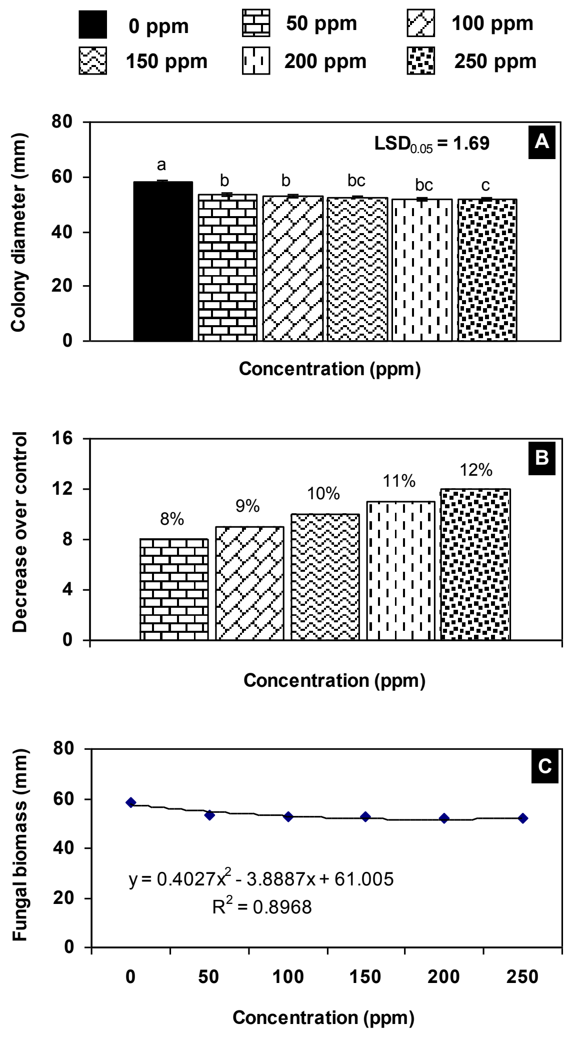

Fig. 3: Effect of fosetyl-Al on growth of *Curvularia lunata*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

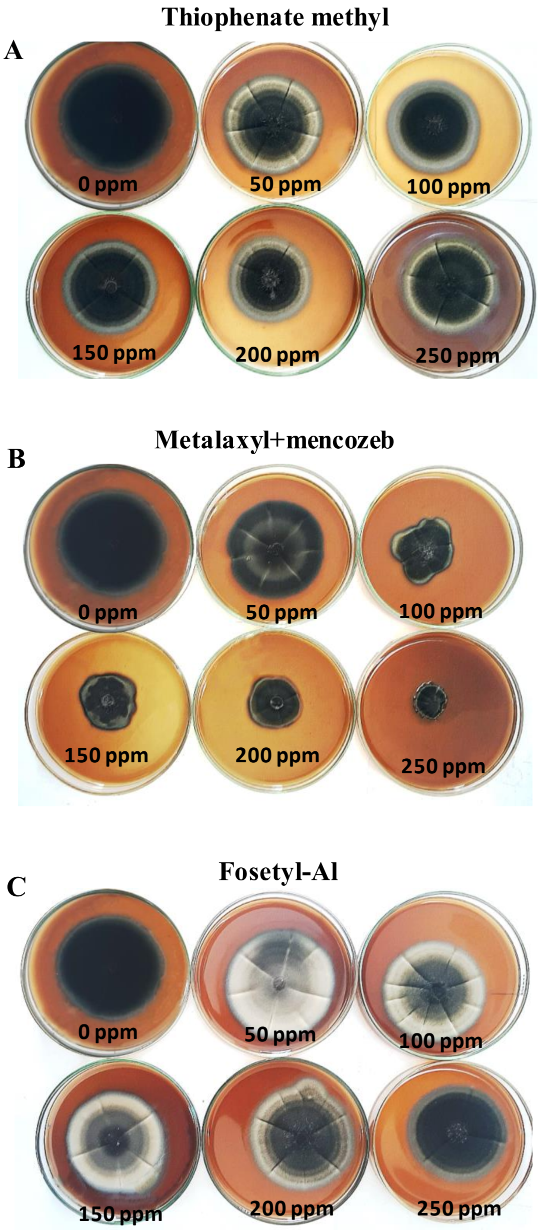

Fig. 4: Effect of thiophenate methyl, metalaxyl+mencozeb and fosetyl-Al on growth of *Curvularia lunata*.

Fungicidal activity of fosetyl-Al

Fosetyl-Al was the least effective against *C. lunata* among the three fungicides used in the present study (Fig. 4C). There was only 8–12% reduction due to different concentrations of the fungicide (Fig. 3A & B). This relationship between concentrations of the fungicide and fungal growth was polynomial with R^2 = 0.8968 (Fig. 3C). Fostyl-Al is a member of organophosphorus group and is a derivative of ethyl phosphate. Being a systemic fungicidal compound, it is frequently applied to manage plants infected by oomycetes prompting root decays since it is assimilated to below-ground part. Within plant, deionization of fosetyl-Al results into phosphonate as fosetyl-Al is a group member of phosphorous acid compounds (Müller *et al.,* 2010). Phosphorous acid is an anionic metabolite. It is produced as a result of ionization of fosetyl-Al. Acid is also translocated in plant tissues from epigeous to hypogeous part. Its chemical composition is comprised of ammonium salts, mono- and dibasic sodium, potassium. It affects oomycetes directly and indirectly (Arif *et al.,* 2021). Resurgence of resistant isolates in crop populations is reported neither for fosetyl-Al nor phosphorous acid (Türkölmez and Dervis 2017). Fosetyl-Al augments plant immune feedback against all encompassing ascomycetes e.g. *Alternaria* spp., *Podosphaera leucotricha*, *Stemphylium vesicarium*, *Venturia inaequalis*. Fosetyl-Al endeavors amazing persistence against the phytopathogenic fungus *S. vesicarium* in pears. It suppresses conidial inoculum of *V. inaequalis* on tender blossoms. This prospective marks fosetyl-Al a fascinating cohort in inclusive infection control tactic in pears and other crops. Through its PDE (Plant Defense Enhancement) outcome, fosetyl-Al deals sustenance to efficiency of appropriate fungicides. Hence, it may also be used explicitly against key pathogens in pome fruit (Petré *et al.,* 2014).

Conclusion

This study concludes that metalaxyl +mencozeb is highly effective in controlling the growth of *Curvularia lunata.*

References

AbdElfatah HAS, Sallam NM, Mohamed MS, Bagy HMK, 2021. *Curvularia lunata* as new causal pathogen of tomato early blight disease in Egypt. *Mol. Biol. Rep.,* **48**: 3001-3006.

Adaangadi KC, Harlapur SI, Savita C, Hadimani BR, 2018. Studies on evaluation of fungicides against *Curvularia lunata* in maize. *Int. J. Agric. Sci.,* **14**: 225-9228.

Alex D, Li D, Calderone R, Peters SM, 2013. Identification of *Curvularia lunata* by polymerase chain reaction in a case of fungal endophthalmitis. Med. *Mycol. Case Rep.,* **2**: 137-140.

Almaguer M, Diaz L, Fernández-González M, Salas S, 2021. Assessment of airborne *Curvularia* propagules in the atmosphere of Havana. *Cuba. Aerobiol.,* **37**: 53-69.

Arena M, Auteri D, Barmaz S, Belli S, Brancato A, Villamar-Bouza L, 2018. Peer review of the pesticide risk assessment of the active substance thiophanatemethyl. *EFSA J.,* **16**: Article 5133.

Arif M, Atta S, Bashir MA, Khan MI, Hussain A, Shahjahan M, Alwahibi MS, Elshikh MS, 2021. The impact of Fosetyl-Aluminium application timing on Karnal bunt suppression and economic returns of bread wheat (*Triticum aestivum* L.). *PloS One,* **16**: Article 0244931.

Barupal T, Sharma K, 2017. Effect of leaf extracts of *Lawsonia Inermis* Linn. on *Curvularia lunata*, caused leaf spot disease of maize. *Int. J. Innov. Res. Adv. Stud.,* **4**: 64-67.

Barupal T, Meena M, Sharma K, 2020. Comparative analysis of bioformulations against *Curvularia lunata* causing leaf spot disease of maize. *Arch. Phytopathol. Plant Prot.,* https://doi.org/10.1080/03235408.2020.1827657.

Bisht S, Balodi R, Ghatak A, Kumar P, 2018. Determination of susceptible growth stage and efficacy of fungicidal management of Curvularia leaf spot of maize caused by *Curvularia lunata. Maydica,* **61**: 1-5.

Dave VP, Joseph J, Pathengay A, Pappuru RR, Das T, 2020. Clinical presentations, diagnosis, and management outcomes of *Curvularia endophthalmitis* and a review of literature. *Retina,* **40**: 370-375.

Garcia-Aroca T, Doyle V, Singh R, Price T, Collins K, 2018. First report of Curvularia leaf spot of corn, caused by *Curvularia lunata*, in the United States. *Plant Health Prog.,* **19**: 140-142.

Gullino ML, Tinivella F, Garibaldi A, Kemmitt GM, Bacci L, Sheppard B, 2010. Mancozeb: past, present, and future. *Plant Dis.,* **94**: 1076-1087.

Hao JJ, Zhu WL, Li YQ, Liu JZ, Xie SN, Sun J, Dong ZD, 2020. Efficacy and profitability of fungicide use to manage Curvularia leaf spot of maize. *Crop Prot.,* **132**: Article 105126.

Khan IH, Javaid A, 2020. First report of *Curvularia lunata* causing postharvest fruit rot of banana in Pakistan. *Int. J. Agric. Biol.,* **24**: 1621-1624.

Müller F, Ackermann P, Margot P, 2010. Fungicides, Agricultural. Encyclopedia Industrial Chemistry. Wiley Online Library.

National Center for Biotechnology Information (NCBI) (2021). PubChem Compound Summary for CID 3032791, Thiophanate-methyl. Retrieved July 12, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Thiophanate-methyl.

Petré R, Labourdette G, Braun CA, Meredith R, Hauke K, Van Hemelrijck W, Schoofs H, Deckers T, Keulemans W, Schoevaerts C, Becker RC, 2014. Fosetyl-Al (Aliette®), a plant defense enhancer with good efficacy on bacteria and on ascomycetes in apples and pears. In: *XII Int. Pear Symp.* 1094: pp. 431-438.

Tóth EJ, Varga M, Takó M, Homa M, Jáger O, Hermesz E, Papp T, 2020. Response of human neutrophil granulocytes to the hyphae of the emerging fungal pathogen *Curvularia lunata*. *Pathogens,* **9**: Article 235.

Türkölmez S, Dervis S, 2017. Activity of metalaxyl-m+ mancozeb, fosetyl-al, and phosphorous acid against Phytophthora crown and root rot of apricot and cherry caused by *Phytophthora palmivora*. *Plant Prot. Sci.,* **53**: 216-225.

Vela-Corcía D, Romero D, de Vicente A, Pérez-García A, 2018. Analysis of β-tubulin-carbendazim interaction reveals that binding site for MBC fungicides does not include residues involved in fungicide resistance. *Sci. Rep.,* **8**: 1-12.

Wonglom P, Suwannarach N, Lumyong S, Ito SI, Matsui K, Sunpapao A, 2019. *Streptomyces angustmyceticus* NR8-2 as a potential microorganism for the biological control of leaf spots of *Brassica rapa* subsp. *pekinensis* caused by *Colletotrichum* sp. and *Curvularia lunata*. *Biol Control,* **138**: Article 104046.

Yang C, Hamel C, Vujanovic V, Gan Y, 2011. Fungicide: modes of action and possible impact on non-target microorganisms. Int. Schol. Res. Notices, **2011**: Article 130289.