Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

IN VITRO EVALUATION OF FUNGICIDES AND BIOCONTROL AGENTS AGAINST *CURVULARIA LUNATA* CAUSING LEAF BLIGHT AND FRUIT ROT DISEASES IN TOMATO

[a]Rabia Safdar, [a]Salman Ahmad, [b]Ejaz Ashraf, [c]Malik. A. Rehman, [d]Yasir Ali, [e]Umbreen Shahzad, [f]Muhammad E. U. Haq, [g]Muhammad A. Zardari

[a] *Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, Pakistan.*
[b] *Department of Agri. Extension and Rural Studies, College of Agriculture, University of Sargodha, Sargodha, Pakistan.*
[c] *Citrus Research Institute, Sargodha, Punjab, Pakistan.*
[d] *Department of Plant Pathology, College of Agriculture, University of Layyah (31200), Layyah, Pakistan.*
[e] *Department of Horticulture, College of Agriculture, University of Layyah (31200), Layyah, Pakistan.*
[f] *Plant Pathology Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*
[g] *Agriculture Extension Department, Government of Sindh, Sindh, Pakistan.*

A B S T R A C T

Fungal pathogens cause economically important diseases in tomato. Fungus *Curvularia lunata* has been reported the cause of tomato leaf blight and fruit rot. The objective of this study was *in vitro* evaluation of fungicides and biocontrol agents against leaf blight and fruit rot pathogen. For this purpose, surveys of different tomato fields were conducted for the collection of samples from infected tomato fields. For the isolation of *C. lunata*, potato dextrose agar (PDA) media was used. Pathogen was identified on genus level on the basis of macroscopic and microscopic characteristics. *In vitro* pathogenicity assays were conducted to check the virulence of isolates. Poison food technique was used for the evaluation of five different fungicides against *C. lunata*. All fungicides significantly inhibited mycelial growth of *C. lunata* at 3rd, 5th and 7th day at different concentrations. Nativo showed best results against *C. lunata* and inhibited mycelial growth to 50.64%, 55.65% and 58.9% followed by Metalaxyl+Mancozeb 37.33%, 43.57% and 45.98% at 3rd, 5th and 7th day, at 50, 100 and 150 ppm concentrations, respectively. Minimum percent inhibition was given by Topsin-M 33.43%, 36.15% and 43.9% at same concentrations. Dual culture technique was used for the evaluation of biocontrol agents (BCAs) against *C. lunata*. *Trichoderma harzianum* showed maximum antagonistic potential to suppress the growth of pathogen *C. lunata* (70.72%). All tested BCAs inhibited the growth of pathogen more than 60% in dual culture assays. The present study revealed that Nativo fungicide and BCA *T. harzianum* have the potential to control *C. lunata*.

Keywords: Biocontrol agents, virulence, percent inhibition, *Trichoderma harzianum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop which is widely grown around the globe, and the 2nd most common vegetable eaten globally after potatoes (Adhikari *et al*., 2017). It is a member of the *Solanaceae* family. It is native to the Andes, where it occurs naturally in Central America and Bolivia (Mehta, 2017). Tomatoes are farmed because of their eatable fruit, that is eaten freshly and also used to make different products like ketchup, soup, paste, sauce, powder along with entire preserved tomatoes. Tomatoes lower the chance of developing heart and other harmful diseases in humans, because they contain vitamin A, C and essential minerals (Shidfar *et al*., 2011).

Tomato fruit is known for its flavor, taste and nutritional benefits (Olaniyi *et al*., 2010; Naseer *et al.,* 2021; Ahmad *et al.,* 2021a). Tomato crop is easy to cultivate and produce large returns on annual basis mostly in hotter climates (Naika *et al*., 2005). Because of Pakistan's varied climatic, good standard tomatoes are being

Submitted: July 08, 2023
Revised: November17, 2023
Accepted for Publication: December 01, 2023
* Corresponding Author:
Email: yasirklasra.uca@gmail.com

produced annually. Pakistan yearly produces 599,700 tonnes of tomatoes (Malik *et al*., 2022).

Diseases caused by biotic and abiotic factors seriously harm crops every year and have a serious impact on the global Agrarian system (Nazarov *et al*., 2020; Ahmad *et al.,* 2021b). Several fungi-related disorders including leaf blight, powdery mildew, damping off and fruit rot, Fusarium wilt, anthracnose, Pythium damping off and fruit rot, Buckeye fruit and root rot have been observed on tomato crop Early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Septoria leaf spot (*Septoria lycopersici*), wilt (*Fusarium oxysporum* f. sp. *lycopersici*) are some of the fungi-related disorders that affect tomatoes and pose a danger to their yield (Sokhi *et al*., 1990).

Leaf blight and fruit rot are the damaging diseases of tomato brought on by pathogens belonging to genus *Alternaria* such as *A. solani*, *A. alternata* and *A. tomatophila* (Adhikari *et al*., 2017) along with *Curvularia lunata* var *aeria* (Iftikhar *et al*., 2016; AbdElfatah *et al*., 2021). These diseases are significantly reducing tomato crop production (Nashwa and Abo-Elyousr, 2013). The main symptoms these pathogens produce include wilting and death of leaves, wilting of twigs, early leaf and fruit drop and tiny black destructive abrasions on leaves, fruit, stems and branches that frequently have dense circles (Yadav *et al*., 2014; Upadhyay *et al*., 2009). Brown to black decaying spots are the first early blight disease symptoms to occur on basal older leaves. As the crop ages, the infection progresses on the upper portion of crop. Symptoms on fruit include deep, black, crusty spots on fruit surface where they enlarge quickly and penetrate the fruit's flesh extensively. The majority of diseased tomatoes fall before they are fully developed.

The majority of threadlike fungi in the world's tropical and subtropical regions belong to genus *Curvularia* (Bisht *et al*., 2013). They exhibit dark brown spongy culture on PDA media (Gadeeyya and Kumar, 2014), disseminate naturally around the plants since they are soil and seed-borne (Barupal and Sharma, 2017). Within majority of crops from the families *Solanaceae, Cucurbitaceae, Gramineae, Compositae, Malvaceae* and *Leguminosae, Curvularia* possesses the capacity to produce leaf blight, leaf spots, sheath rot, seedling blight and seedling rot (AbdElfatah *et al*., 2021).

There are several ways to manage plant diseases like use of resistant varieties, biological control agents, use of synthetic chemicals and plant extracts. Majorly agrochemicals are being employed to manage fungal problems. Mixture of Metalaxyl and Mancozeb gives good results against *C. lunata* (Hassan *et al*., 2021). For the management of plant diseases biocontrol serves as a harmless alternative. Rhizospheric microbes, i.e., bacteria, fungi and viruses are potentially efficient biocontrol agents that are economically affordable and ecologically non-hazardous (Nicolopoulou-Stamati *et al*., 2016), and able to withstand the harsh environmental conditions even lasts for longer periods with no residual effects. Rhizobacteria, i.e., *Bacillus subtilis* gives good results against pathogenic *C. lunata* (Xie *et al*., 2020). *T. harzianum* is also effective to inhibit the mycelial growth of *C. lunata* (Iftikhar *et al*., 2017).

The objective of this research was to evaluate suitable fungicides and biocontrol agents that prove to work efficiently against pathogenic *C. lunata* to reduce the losses caused by *Curvularia* leaf blight and fruit rot diseases in tomato.

MATERIAL AND METHODS

Present research was carried out in the Fungal Bank and Plant Disease Diagnostic Lab located in College of Agriculture, University of Sargodha, Sargodha.

Field survey and collection of diseased samples: Tomato fields were visited in the vicinity of Sargodha District and neighboring areas of Sargodha for the collection of diseased samples. Leaves and fruits showing disease symptoms were collected in sterilized polythene zipper bags.

Isolation and identification of pathogen from diseased samples: PDA (Potato dextrose agar) media was used for the isolation of pathogen. Leaf blight and fruit rot pathogen (*C. lunata*) was isolated by using tissue planting technique.

Tissue planting technique: The diseased leaves and fruit samples were washed by using the tap water to remove debris and soil particles. Samples (brownish and rotted part) were cut into small pieces of size ranging 2-3 mm. The cut pieces were disinfected by soaking in 2 percent sodium hypochlorite solution for 1-2 minutes. Two consecutive washings with distilled water were performed for the removal of sodium hypochlorite from diseased samples. Three to four surface sterilized samples were transferred into petri plates containing 15 ml PDA media. All the task was done in completely sterilized environment under laminar flow chamber. Plates were wrapped and kept at 25 - 27ºC in incubator and growth was observed after every 24 hours (Ghosh and Shamsi, 2014).

Sub culturing: When growth was initiated on PDA media,

mycelial bits were again transferred to petri plates having PDA for purification and multiplication.

Identification: Fungus was identified on the basis of cultural and morphological characters like colony color, shape, size, hyphal morphology etc.

***In vitro* pathogenicity test on detached leaves:** For pathogenicity assays on leaves, moist chamber detach leaf method was employed. Healthy tomato leaves were surface sterilized and inoculated with media plugs of fungal culture of *C. lunata* to be tested, in parallel leaves were inoculated with plane PDA plugs to serve as a check. The data regarding the development of lesions on leaves was recorded (Mostafa *et al*., 2022).

***In vitro* pathogenicity test on detached fruits:** Healthy tomato fruits were inoculated with media plugs of fungal culture *C. lunata* to be tested, in parallel fruits were inoculated with plain PDA plugs to serve as a check. The data on the progression of lesions was recorded.

***In vitro* evaluation of fungicides**

Poison food technique: Poison food technique was used to check the efficacy of 5 different fungicides (Metalaxyl+Mancozeb, Topsin-M, Nativo, Copper Oxychloride and Success). *In vitro* experiment was conducted by preparing three different concentrations, i.e., 50 ppm, 100 ppm and 150 ppm. For each treatment, three replicates were used. Potato dextrose agar was prepared and amended with fungicides before pouring into the petri plates. Three plates were poured for each concentration of fungicide. After solidification, 5mm mycelial plugs of fungi were placed in the center of plates (Gautam *et al*., 2017). Plates were incubated at 25±2ºC and data was taken after 3 days, 5 days and 7 days. Percentage inhibition was measured by using the formula given below:

$$\text{Percentage inhibition} = \frac{C - T}{C} \times 100$$

C= Colony diameter in control

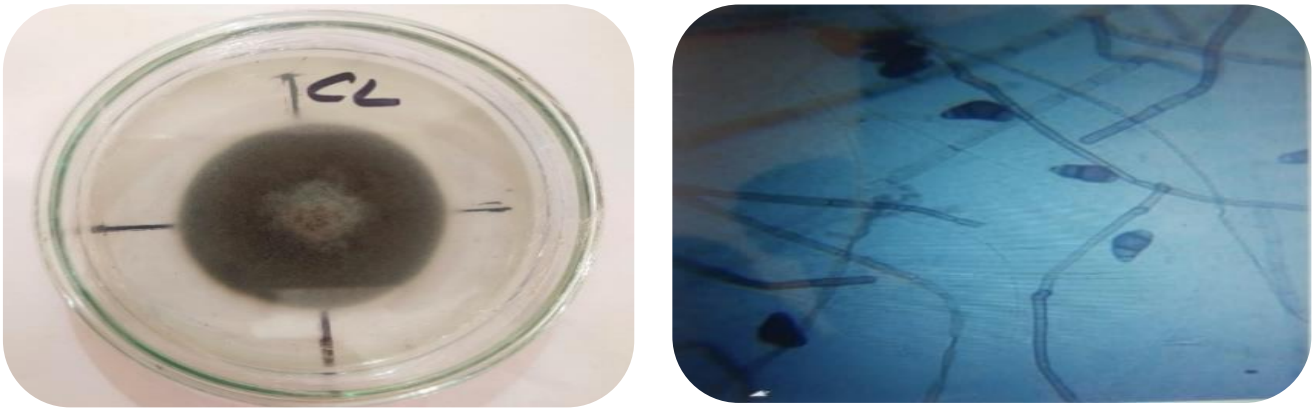
T= Colony diameter in treatment

***In vitro* evaluation of biocontrol agents: Isolation of biocontrol agents:** Biocontrol agents were isolated from the rhizosphere soil of healthy tomato plants by soil serial dilution method (Vehapi *et al*., 2023). For the preparation of dilutions, 9ml of distilled water was added in four autoclaved test tubes. One-gram soil sample was added to first test tube, shaken vigorously to allow the homogeneous mixing of soil properly. Serial dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were prepared by repeating the procedure taking 1 ml from tube- 1 to 9ml of tube- 2 and so on. For the isolation of antagonistic fungi, 3rd and 4th dilutions were used to pour 100µL from each dilution using micro pipet and smeared on the media with sterilized glass rod. To check the antagonistic activity of biocontrol agents, dual culture experiment was performed.

Dual culture assays: For this purpose, 5mm media plugs of biocontrol agents and target pathogen were inoculated three centimeters away from the outer wall parallel to each other on 9 mm freshly poured media plates, with one plate only inoculated with target pathogen to serve as a control treatment for comparison. At 3rd, 5th and 7th days interval after inoculation, growth appearance data of fungal inhibition by biocontrol agents was collected to calculate the percentage inhibition (Abdelmoteleb *et al*., 2023).

RESULTS

Characterization of isolated fungal pathogen: Fungus that cause tomato leaf blight and fruit rot diseases was characterized by colony morphology and microscopic observation. Pathogenic fungus was isolated from diseased samples and identified as *C. lunata*. Pure culture of *C. lunata* and its spores are shown in Figure 1.



A B

Figure 1. Pure culture of *C. lunata* on PDA media (A) and microscopic view of spores (B).

Virulence of isolated pathogens: Results of pathogenicity test showed that pathogen *C. lunata* infected tomato leaves and fruit in moist chamber. Lesion diameter (cm) on detached leaves and fruit is shown in Table 1.

Table 1. Lesion diameter (cm) of *C. lunata* on detached tomato leaves and fruit

Pathogenicity test	Lesion diameter in cm	Control
Leaves	1.23±0.08 A	0±0 B
Fruits	1.83±0.09 A	0±0 B

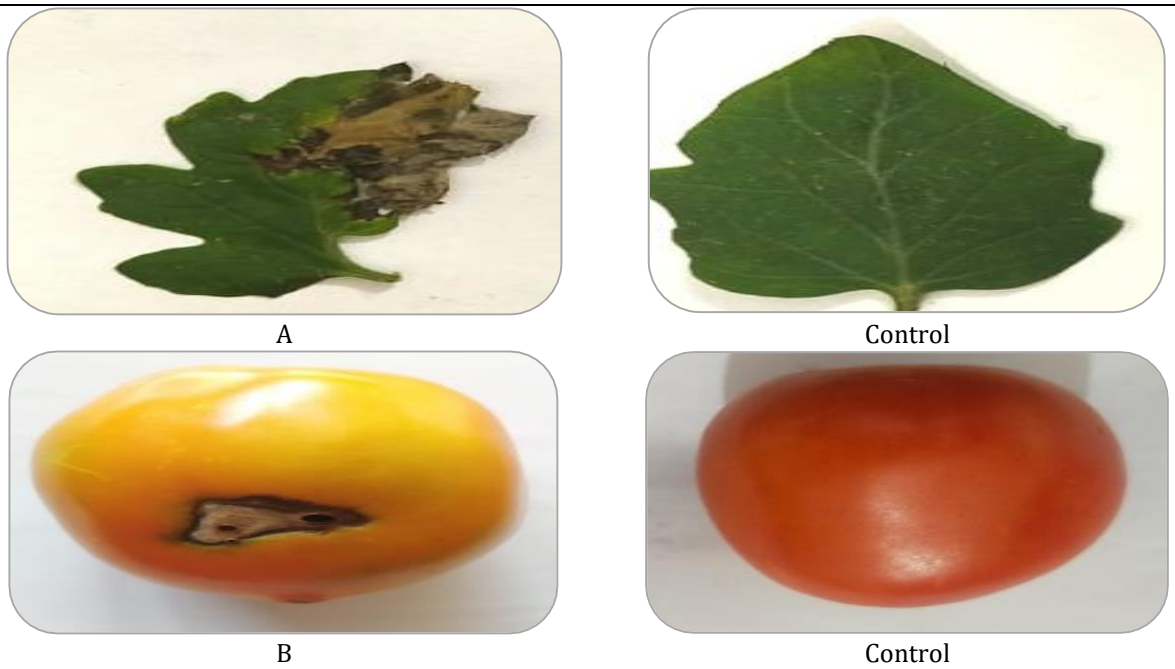


Figure 2. *In vitro* pathogenicity test of *C. lunata* on detached tomato leaves (A) and fruit (B) with their control treatments.

Evaluation of fungicides against *C. lunata*: Results showed that all fungicides significantly (P<0.05) inhibited mycelial growth of *C. lunata* at 50, 100 and 150 ppm concentrations; however, their efficacy varies at 3rd, 5th and 7th day. Among all fungicides evaluated, Nativo was found most effective as compared to others and it showed maximum percent inhibition (82.69%) at 7th day after the treatment at 150 ppm concentration followed by the same fungicide when used at 100 ppm (78.84%) and 50 ppm (72.43%). Topsin-M was the least effective fungicide (Figure 3).

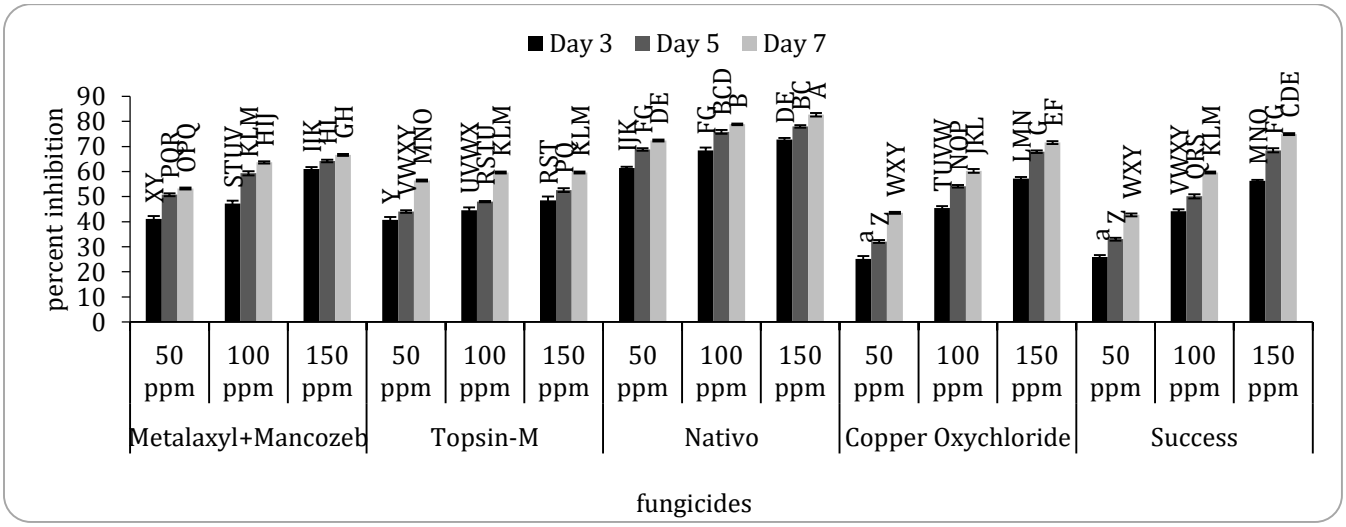


Figure 3. Percent inhibition (mean±SE) of mycelial growth of *C. lunata* on PDA amended with different fungicides at different concentrations and at different time intervals after treatment. Means with similar letters are not significantly different at P<0.05.

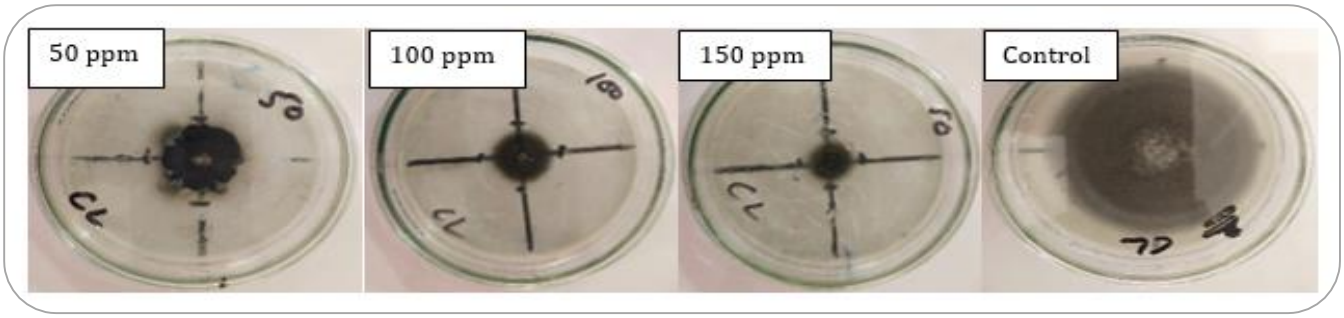


Figure 4. Pictorial views of *C. lunata* colonies on PDA amended with Metalaxyl+Mancozeb at 7th day after treatment.

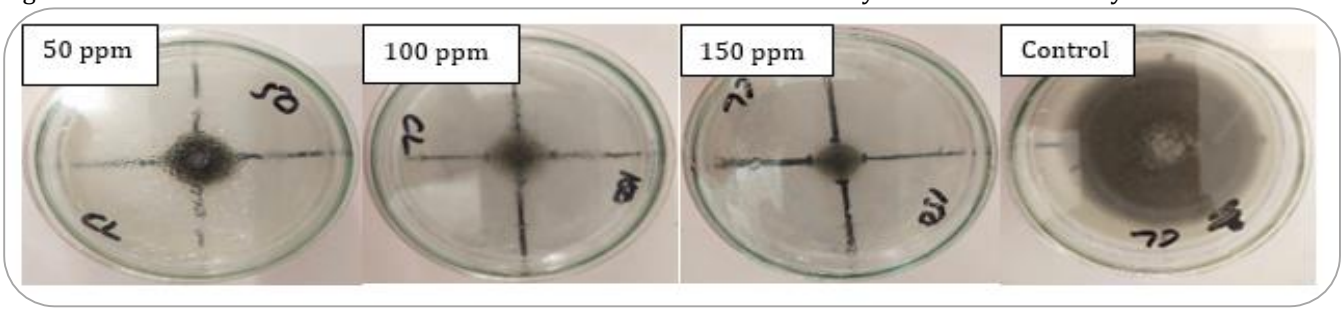


Figure 5. Pictorial views of *C. lunata* colonies on PDA amended with Topsin-M at 7th day after treatment.

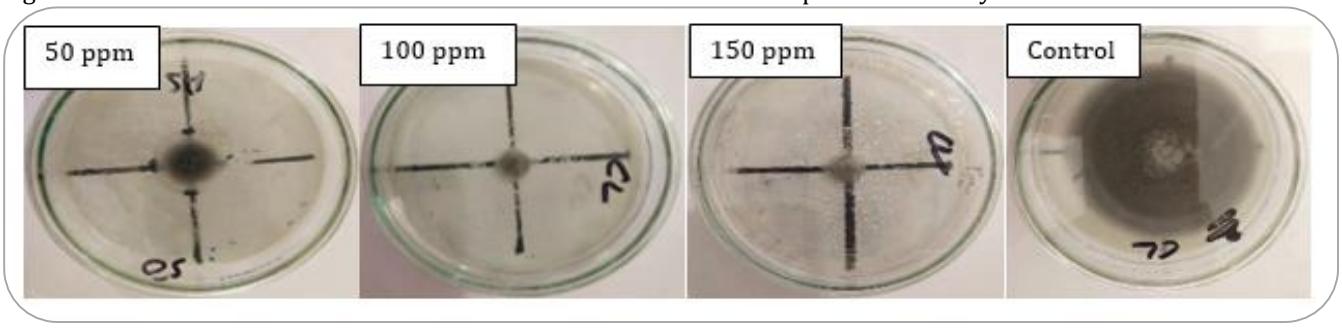


Figure 6. Pictorial views of *C. lunata* colonies on PDA amended with Nativo at 7th day after treatment

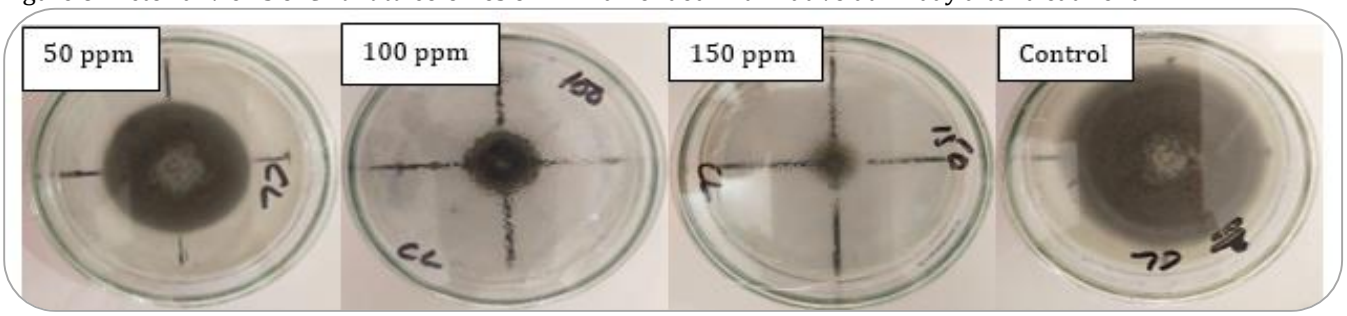


Figure 7. Pictorial views of *C. lunata* colonies on PDA amended with Copper Oxychloride at 7th day after treatment

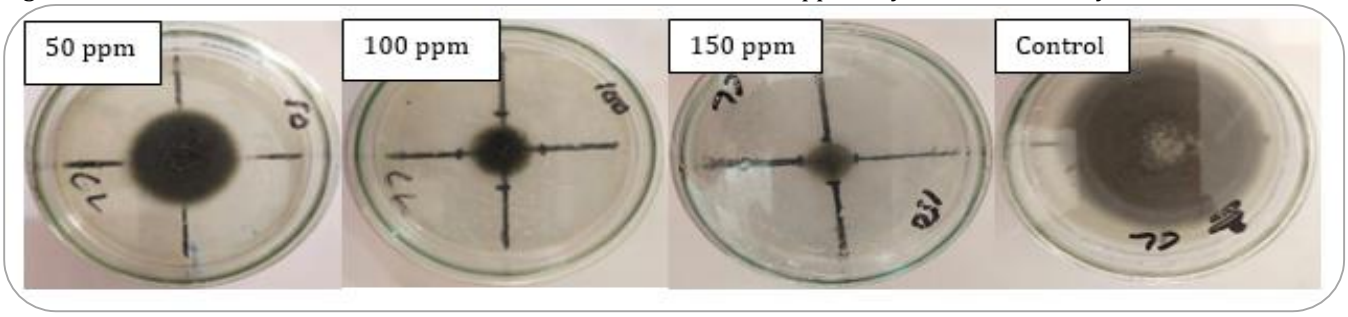


Figure 8. Pictorial views of *C. lunata* colonies on PDA amended with Success at 7th day after treatment.

Potential of biocontrol agents against *C. lunata*: Different species of *Trichoderma* and *Paecilomyces lilacinus* were used in dual culture assay *in vitro* as biocontrol agent to suppress the growth of *C. lunata*. The biocontrol agents were morphologically identified on the basis of their growth pattern. Moreover, microscopic observations were conducted to confirm biocontrol strains at genus level. Identified strains of biocontrol agents are shown in Table 2.

Table 2. Strains used as biocontrol agents.

Sr. No	Specie name	Growth pattern	Colony color
1	*T. harzianum*	Concentric rings	Dark green
2	*T. atroviridae*	Thin in center, thicker at edges	Green
3	*T. viridae*	Dark green	Circular
4	*T. asperellum*	Cottony	Dull green
5	*P. lilacinus*	Purplish pink	Scattered spots

All isolated biocontrol agents significantly ($P<0.05$) inhibited mycelial growth of *C. lunata* at 3rd, 5th and 7th day. Percent inhibition was higher at 7th day of treatment and lower at 3rd and 5th day. Among all biocontrol agents, T1 (*T. harzianum*) showed maximum percent inhibition (70.72%) against *C. lunata*. T3 (*T. viridae*) was the second best biocontrol agent against *C. lunata* and it showed (67.3%) inhibition. T4 (*T. asperellum*) gave least inhibition (62.6%) of pathogen (Figure 9).

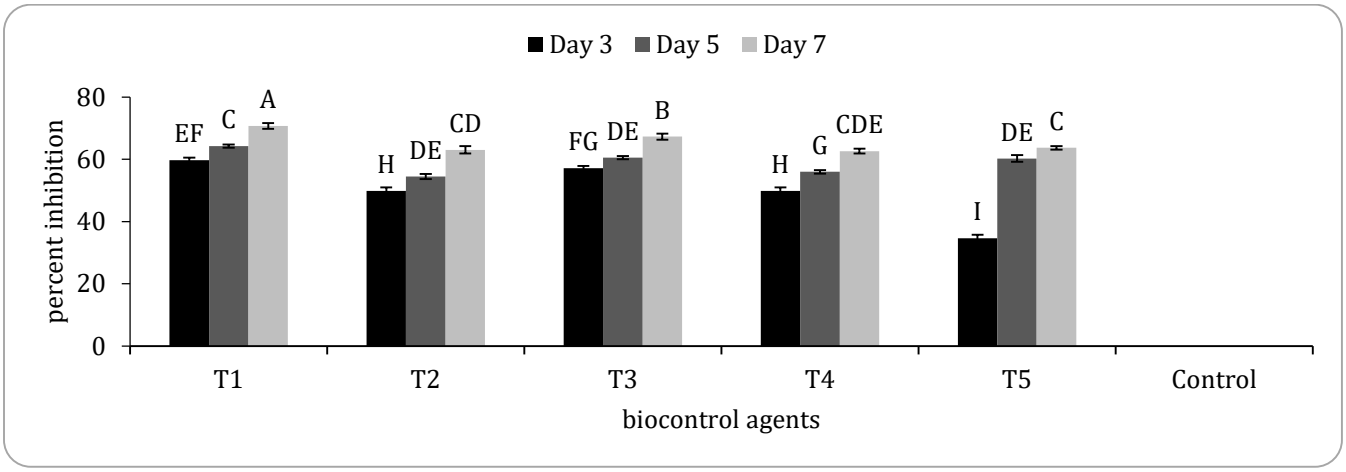


Figure 9. Percent inhibition (mean±SE) of mycelial growth of *C. lunata* with *Trichoderma* species and *P. lilacinus* at different time interval after treatment.

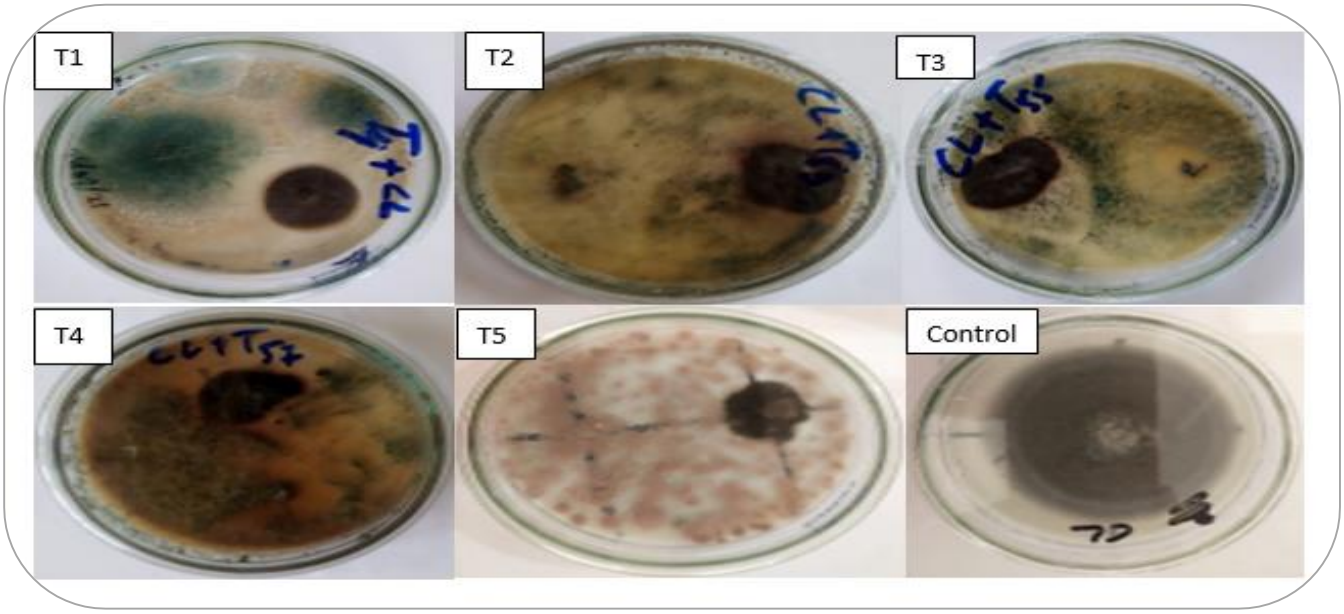


Figure 10. Dual cultures of pathogenic *C. lunata* with biocontrol agents (*Trichoderma* species and *P. lilacinus*) and their control.

DISCUSSION

Diseases caused by biotic factors are serious threat to tomato crops. *C. lunata* is the most important causal organism of tomato diseases. Tomato leaf blight and fruit rot are diseases caused by this pathogen. These diseases are present in all tomato producing regions of the world and producing 35-78% losses (Jones *et al*., 1993). There are several characteristics of *Curvularia* which make it the worst plant enemy. These characters include rapid production of inoculum, production of chlamydospores for their survival outside plant tissues, wide host range, availability of host plant all around the year, ability of single species to cause diseases in multiple hosts and ability to cause more than one disease in single host. Without managing leaf blight and fruit rot diseases, it is impossible to get maximum yield of tomato. Nativo gave higher percent inhibition against *C. lunata* in present study. It is a broad-spectrum systemic fungicide and found very effective against many fungi. This fungicide significantly inhibited mycelial growth of *C. lunata* at different concentrations and mycelial growth inhibition increased with increasing concentration. These results are similar to the studies conducted previously (Rao *et al*., 2018; Hao *et al*., 2020; Nahar and Shamsi 2020). Nativo (Tebuconazole + Trifloxystrobin) has been found effective against *Curvularia* leaf spot of maize (Hao *et al*., 2020). They concluded that Nativo significantly suppress the growth of *C. lunata* and its efficacy increases with increasing concentration. Nahar and Shamsi (2020) used poison food technique to check the efficacy of fungicides and botanicals against different pathogens of cotton. Results of their study showed that Nativo significantly suppressed the spore germination and mycelial growth, and at higher concentrations this fungicide was most effective against the fungus.

Rao *et al*. (2018) checked the activity of Nativo and other fungicides against *C. lunata* that causes rice discoloration. They also reported that this fungicide significantly suppresses the growth of *C. lunata*. *T. harzianum* inhibited *C. lunata* the maximum in present findings. These results were similar to the studies conducted previously (Tekade *et al*., 2017; Yassin *et al*., 2021;). Tekade *et al*. (2017) conducted *in vitro* experiments to check the efficiency of microbial antagonists and fungicides against *C. lunata* causing blight disease of coleus. They reported that *T. harzianum* gave maximum inhibition of pathogen. *T. harzianum* and *T. viride* were found effective against different fungal pathogens including *C. lunata* in sorghum (Yassin *et al*., 2021).

CONCLUSION

Results of present study showed that all evaluated fungicides and biocontrol agents are effective against *C. lunata*; however, their efficacy varies with day's interval. Among fungicides, Nativo was found most effective against pathogenic fungus *C. lunata*. Among biocontrol agents, *T. harzianum* was the most effective.

REFERENCES

AbdElfatah, H. A. S., N. Sallam, M. S. Mohamed and H. M. Bagy. 2021. *Curvularia lunata* as new causal pathogen of tomato early blight disease in Egypt. Molecular Biology Reports, 48: 3001-3006.

Abdelmoteleb, A., L. Moreno-Ramírez, B. Valdez-Salas, M. F. Seleiman, S. El-Hendawy, K. J. Aldhuwaib, M. Alotaibi and D. González-Mendoza. 2023. New Bacillus subtilis Strains Isolated from *Prosopis glandulosa* Rhizosphere for Suppressing *Fusarium* Spp. and Enhancing Growth of *Gossypium hirsutum* L. Biology, 12(1).

Adhikari, P., Y. Oh and D. R. Panthee. 2017. Current status of early blight resistance in tomato: an update. International Journal of Molecular Sciences, 18(10).

Ahmad, S., M. Yousaf, R. Anjum, W. Raza, M. A. Rehman and Y. Ali. 2021a. Prevalence of Fusarium Wilt of Tomato in Major Tomato Growing Areas of Punjab, Pakistan. International Journal of Phytopathology, 10(3): 225-230.

Ahmad, S., M. Yousaf, R. Anjum, W. Raza, Y. Ali and M. A. Rehman. 2021b. Evaluation of fungicides against *Fusarium oxysporum* f. sp. *lycopersici* the cause of Fusarium wilt of tomato. Journal of Plant and Environment, 3(2): 125-135.

Barupal, T. and K. Sharma. 2017. Effect of leaf extracts of *Lawsonia Inermis* Linn. on *Curvularia lunata*, caused leaf spot disease of maize. International Journal of Innovative Research and Advanced Studies, 4: 64-67.

Bisht, S., P. Kumar, A. Srinivasanraghvan and J. Purohit. 2013. *In vitro* management of *Curvularia* leaf spot of maize using botanicals, essential oils and bio-control agents. Bioscan, 8: 731-733.

Gadeeyya, G. and P. K. R. Kumar. 2014. Isolation and characterization of mycoflora from infected weeds. The Journal of Plant Protection Sciences, 6: 1-19.

Gautam, C., H. V. Prabhu and V. B. Nargund. 2017. *In vitro* evaluation of fungicides, botanicals and bioagents

against *Peziotrichum corticolum* causing black banded disease of mango. International Journal of Current Microbiology and Applied Science, 6: 652-661.

Ghosh, A. and S. Shamsi. 2014. Fungal diseases of rose plant in Bangladesh. Journal of Bangladesh Academy of Sciences, 38: 225-233.

Hao, J. J., W. L. Zhu, Y. Q Li, J. Z. Liu, S. N. Xie, J. Sun and Z. D. Dong. 2020. Efficacy and profitability of fungicide use to manage *Curvularia* leaf spot of maize. Crop protection, 132: 105126.

Hassan, M. A. U., I. Butt, I. H. Khan, A. Javaid and N. Shad 2021. Control of *Curvularia lunata.* Mycopath, 18: 47-52.

Iftikhar, S., A. A. Shahid, K. Nawaz and S. W. Ali. 2016. First report of *Curvularia lunata* causing fruit rot of tomato (*Lycopersicum esculentum*) in Pakistan. Plant Disease, 100: 1013-1014.

Iftikhar, S., A. A. Shahid, K. Nawaz and W. Anwar. 2017. Potential of *Trichoderma* species as biocontrol agent against *Curvularia lunata* causing fruit rot of tomato (*Lycopersicum esculentum* Mill.). In 5thInternational Conference on Food, Agricultural, Biological and Medical Science, pp. 77-83.

Jones, J. B., J. P. Jones, R. E. Stall and T. A. Zitter. 1993. Compendium of tomato diseases, St. Paul. American Phytopathological Society.

Malik, M. S., S. Haider, A. Rehman, S. U. Rehman, M. Jamil, I. Naz. and M. Anees. 2022. Biological control of fungal pathogens of tomato (*Lycopersicon esculentum*) by chitinolytic bacterial strains. Journal of Basic Microbiology, 62: 48-62.

Mehta, R. 2017. History of Tomato (Poor Man's Apple). IOSR Journal of Humanities and Social Science, 22: 31-34.

Mostafa, Y. S., S. A. Alamri, S. A. Alrumman, M. Hashem, M. A. Taher and Z. A. Baka. 2022. *In vitro* and *In vivo* biocontrol of tomato Fusarium wilt by extracts from brown, red, and green macroalgae. Agriculture, 12.

Nahar, M. N. and S. Shamsi. 2020. *In vitro* screening of fungicides and plant extracts against six pathogenic fungi isolated from cotton (*Gossypium arboreum* L.) seed. Bangladesh Journal of Botany, 49: 197-204.

Naika, S., J. V. L de Jeude, M. de Goffau, M. Hilmi, and B. Van Dam. 2005. Cultivation of tomato. Production, processing and marketing, Agromisa/CTA. Revised edition.

Naseer, S., I. Nadeem, A. Mustafa, J. A. Shah, S. Ahmed, M. Saqib, T. Abbad and Y. Ali. 2021. Management of tomato leaf curl virus and its *Bemisia tabaci* vector in relation to prevailing environmental conditions of Faisalabad. Pakistan Journal of Phytopathology, 33(2): 313-324.

Nashwa, S. M. and K. A. Abo-ElyouSr. 2013. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Protection Science, 48: 74-79.

Nazarov, P. A., D. N. Baleev, M. I. Ivanova, L. M. Sokolova and M. V. Karakozova. 2020. Infectious plant diseases: etiology, current status, problems and prospects in plant protection. Acta Naturae, 12: 46-59.

Nicolopoulou-Stamati P, S. Maipas, C. Kotampasi, P. Stamatis and L. Hens. 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture.

Olaniyi, J. O., W. B. Akanbi, T. A. Adejumo and O. G. Akande. 2010. Growth, fruit yield and nutritional quality of tomato varieties. African Journal of Food Science, 4: 398-402.

Rao, S. S., K. M. Reddi, P. Madhusudhan and R. B. Reddy. 2018. Evaluation of bio-efficiency of rice based fungicides against rice discoloration causing pathogen *Curvularia lunata* (Wakker) Boedijn. International Journal of Current Microbiology and Applied Sciences, 7: 1373-1379.

Shidfar, F., N. Froghifar, M. Vafa, A. Rajab, S. Hosseini, S. Shidfar and M. Gohari. 2011. The effects of tomato consumption on serum glucose, apolipoprotein B, apolipoprotein AI, homocysteine and blood pressure in type 2 diabetic patients. International Journal of Food Sciences and Nutrition, 62: 289-294.

Sokhi, S. S., G. D. Munshi, R. K. Grewal, T. S. Thind and K. S. Sandhu. 1990. Current fungal disease problems of important vegetables in India. Current Fungal Disease Problems of Important Vegetables in India, 382-404.

Tekade, A., M. Koche, R. Kothikar and A. Surpam. 2017. Efficacy of fungicides and bioagents against *Curvularia lunata* causing blight of coleus under laboratory conditions. Journal of Medicinal Plants, 5: 189-191.

Upadhyay, P., P. C. Singh, B. Sinha, M. Singh, R. Kumar, K. Pandey and M. Rai. 2009. Sources of resistance

against early blight (*Alternaria solani*) in tomato (*Solanum lycopersicum*). Indian Journal of Agricultural Sciences, 79: 752-753.

Vehapi, M., B. İnan, S. Kayacan-Cakmakoglu, O. Sagdic and D. Özçimen. 2023. Production of *Bacillus subtilis* soil isolate as biocontrol agent under bioreactor conditions. Archives of Microbiology, 205.

Xie, S., J. Liu, S. Gu, X. Chen, H. Jiang and T. Ding. 2020. Antifungal activity of volatile compounds produced by endophytic *Bacillus subtilis* DZSY21 against *Curvularia lunata*. Annals of Microbiology, 70: 1-10.

Yadav, O. P., M. R. Dabbas and L. B. Gaur 2014. Screening of tomato advanced lines, genotypes against *Alternaria solani*. Plant Archives, 14: 553-555.

Yassin, M. T., A. A. F. Mostafa and A. A. Al-Askar. 2021. *In vitro* antagonistic activity of *Trichoderma harzianum* and *T. viride* strains compared to carbendazim fungicide against the fungal phytopathogens of Sorghum bicolor (L.) Moench. Egyptian Journal of Biological Pest Control, 31: 1-9.

Contribution of Authors:

Rabia Safdar	:	Wrote the manuscript
Salman Ahmad	:	Designed the experiment
Ejaz Ashraf	:	Analyzed the data
Malik. A. Rehman	:	Help in data analysis
Yasir Ali	:	Edit the manuscript
Umbreen Shahzad	:	Review manuscript
Muhammad E. U. Haq	:	Prepared figures and help in performing experiment
Muhammad A. Zardari	:	Proofread the manuscript
Muhammad S. Fareed	:	Designed the experiment