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

SYNERGISM BETWEEN *TRICHODERMA HARZIANUM* RIFAI AND CHEMICAL FUNGICIDE FOR THE CONTROL OF *FUSARIUM OXYSPORUM* SCHLTDL. ON MUNG BEAN

[a]Umara Arain, [a]Aziz A. Ujjan*, [b]Abdul Q. Rajput, [b]Saleem Shahzad

[a] *Institute of Plant Sciences, Allama I. I. Kazi Campus, Jamshoro-76080, Pakistan.*
[b] *Department of Agriculture and Agribusiness, University of Karachi-75270, Pakistan.*

A B S T R A C T

The Present study was carried out to find out the compatible dose of the fungicide with *Trichoderma harzianum* (TH) and the study also found way to utilize the synergy of the fungicide (Copper Hydroxide) and the fungal biocontrol agent against soil borne plant pathogen causing *Fusarium* wilt in Mung bean (*Vigna Radiata* L.). TH is a well known fungicidal biocontrol agent. The efficiency TH strain was studied against a soil borne plant pathogen *Fusarium oxysporum* (FO). The biocontrol strain TH was mainly observed under *in-vitro* assays was found to be compatible with the Copper Hydroxide, an agro-fungicide formulation sold with a trade name Kocide (KD); TH tolerated to the fungicide KD as the mycelia reduced 50% growth (EC_{50}) at 176.1 ppm. KD increased the mycocidal potential of the TH at the highest tolerable dose (20 ppm < EC_{10} =58.2 ppm), from 24 to 26% against FO on food poisoned media, which was the evidence to the fungicide synergy (02%) with the biocontrol effects of the Under *in vivo* conditions, the synergy between KD and TH was found to be higher (26.1 %) in combating FO infection in mungbean plants in comparison to in vitro assay. Under *in vivo* conditions, the synergy between KD and TH was found to be higher (26.1 %) in combating FO infection in mungbean plants in comparison to in vitro assay. The combine use of KD and TH also positively impacted the plant growth where plants treated with both KD and TH had XX time dry weight (2.66 g) compared to single applications of TH (0.8 g) and KD (0.68 g). "The formulations, containing TH spore and the fungicide @ 20 ppm, would be integrated with crop managements".

Keywords: Fungicide tolerance, Copper Hydroxide, Fungi and fungicide synergism,

INTRODUCTION

Trichoderma is a famous mycoparasite and it has been utilized as commercial biocontrol agent. Among the species of *Trichoderma* the *T. harzianum* is the most effective and recognized biocontrol agent (Poveda, 2021). The most common types of *Trichoderma* as biocontrol agents are: *T. polysporum*, *T. harzianum*, *T. viride*, *T. atroviride*, *T. polysporum*. The numerous mechanisms of mycoparasitism were discovered that adopted by *Trichoderma* spp. as fungicide and plant protection ecology; these mechanisms could be, food struggle and location, the production of enzymes to degrade the fungal cell wall, the production of secondary metabolites, on the other side it triggers the immune responses through the stimulation of plant growth. *Trichoderma* has broad host ranges and is widely used to control many soil-borne diseases(Weindling, 1934; Błaszczyk *et al.,* 2014; Srivastava and Shahid, 2014; Lavanya *et al.,* 2017). The fungus also provides the plant with the resistance to biotic and abiotic pressure, plant growth and yield and improves the agro-ecosystem. In addition, the anticipated use of *Trichoderma*-based bio-compounds has not been only limited to agriculture, but italso been extended to other areas of the world and health sciences such as natural ecology (Lan *et al.,* 2012), medicine where toxins and antimicrobial activity may be required.

Fungicide resistance is a well known fact, it appeared during a disease control program and the broke new strata of fungicide inefficiency results at workshops in

Submitted: April 10, 2022
Revised: May 17, 2022
Accepted for Publication: June 15, 2022
* Corresponding Author:
Email: aziz.ujjan@usindh.edu.pk

Wageningen, 1980 and 1981(Fischer *et al.,* 2004). It was all about genetics and their expression in fungicide defense as some workers sorted specific multiple genes for this prospect which are developing fungal pathogens to resist the fungicides over the course of time(Molnar *et al.,* 1985). Using disease-resistant plant species are very effective measures to overcome the costly chemical control of *Fusarium oxysporum* f sp. *lycopersici* that was averted by developing resistant plants of tomato crop. (Neely and Beckman, 1990; Amini, 2009).

The pathotypes of *F. oxysporum* were identified as resistant to fungicides i.e. Benomyl and Thiabendazole. The fungicides were losing effects on the target organisms, which resulted in a heavy input doses in the field (Chung *et al.,* 2009). During a resistance assessment studies Su *et al.* (2019) reported the 30 moderately resistant strains of *F. graminiearum*; the Carbedazim fungicide was considered more effective to fungal pathogens but it was alarmingly hazardous to the environment and crop producers to use fungicides indiscriminately. The fungal resistance to highly efficient fungicides was developed in the European soils due to permanent inputs and residues. In the soils found with higher pesticide residue, other broad spectrum fungicides i.e. Boscalid, Expoxiconazole and Tebuconazole were found in agri-soils as the residues ranged from 0.1 to 1.0 ppm. The agri-soil situation in Europe could be compared with other areas of the world. As the fungicide residues were reportedly hindering the natural microbial competitions among friend and foe microbes for plant growth. Since biocontrol inundation of beneficial microbes, for example *Trichoderma* spp. would not efficiently survive under the conditions, where the fungicides present as residue. Meanwhile, the soil borne pathogenic fungi have had enough resistance to live with residual fungicides in soil. This situation could fearfully develop an economical loss to the farmers. Although the *Trichoderma* spp. has been induced for fungicide resistance and developing a chance to cope with traces of fungicides in agri. soils (Chaparro *et al.,* 2011; Jamil, 2021). The combined use of microbial antagonists with reduced doses of chemical fungicides was promising in earlier reports. The combination of biocontrol and commonly used fungicides were in positive association for reducing seed infection compared with fungicides and individual fungal antagonists. The mutual uses of chemical pesticides and biocontrol have combined additive effects in controlling pathogens in the soil (Kredics *et al.,* 2004). The Present study took up the tolerance assessment of *T. harzianum* to a of fungicide containing Copper Hydroxide; the study revealed the compatible doses of the fungicide and effects of the fungicide caused on biocontrol attributes of *T. harzianum* against the plant pathogenic *F. oxysporum* strain at dual culture experiments and pathogen-plant-*Trichoderma*+ low doses of fungicide (tritrophic *in-vitro*) interactions.

MATERIALS AND METHODS

A *T. harzianum* (TH) strain characterized for its compatibility with a fungicide Copper Hydroxide (Trade name Kocide). First, the amount (or concentration) of nonlethal doses were worked out using food poisoned technique as suggested by Schmitz. (1930). The Kocide fungicide was incorporated at predefined lower doses to the dual culture media for assessment of fungicide on biocontrol and plant pathogenic *F. oxysporum*(FO), which is well known tolerable for biocontrol agents. The additive effects were recorded that initiated by the fungicide "Kocide" commercial formulation (KD) and the biocontrol fungi TH to the plant pathogenic fungi FO on the protection of Mung bean plant. The experiments were carried out at the Mycology and Plant Pathology Laboratory, Institute of Plant Sciences, University of Sindh.

Fungal cultures: The fungal cultures were acquired from the culture collection facility at Department of Agriculture and Agribusiness Management, University of Karachi which is also exceedingly acknowledged. The fungal cultures were regularly maintained on Sabraud Dextrose Agar (SDA) medium poured slants, coded with accession number tags and incubated at 14 ºC for 30 days and again revived for fresh cultures at interval of 30 days. The Potato Dextrose Agar (PDA) and SDA were utilized during the study. The media was prepared according to suggested additions of ingredients in sterile water. Each formulation of the specific media was added with 200 ml hot water in a beaker to mix the ingredients homogenously and made up to 1000 ml by addition of distilled water. The homogenized media solution was poured into the Erlenmeyer flask, plugged with cotton swab and covered with Aluminum foil. The media filled flasks were placed in autoclave for sterilization at 121 ºC (15 lbs) for 15 minutes. The media was kept coolingat45 ºC. The antibiotic mixed in cooled molten medium @ 40 and 20 mg of Penicillin and Streptomycin per Liter media at the time of pouring, to stop bacterial contaminations. The fungicide Kocide™, a commercial formulation, was utilized during the study. The active ingredient of the fungicide was Copper Hydroxide 25%, in wet-able formulation, it was recommended by company to utilize the 500 g of the fungicide formulation per acre.

Compatibility test: The PDA media was amended with fungicide for the food poisoned media preparation with fungicide formulation. Each one Liter of PDA media was added with 300, 450, 900, 1800 and 2250 mg of the fungicide formulation before the transfer in the Petri plates, the outcome of the amended media was calculated as each amendment contained 15.6, 23.4, 46.8, 70.2, 93.6 and 117 ppm, respectively. The fungicide tolerance was observed using food poisoned media technique, the fungal strains were grown on the food poisoned as suggested by Schmitz. (1930).

The radial growths of the fungi on poisoned and empoisoned media (controls)were compared for the level of toxicity/compatibility (%) with the formula described as: A=C-T/C X 100, where the A stand for antagonism, C for radial growth of control fungi, T for treated fungi. Probit regression analysis was performed for the assessment of median lethal dose (EC_{50}) of the fungicides to the fungus on poised media. The concentration was selected that showed no inhibition to the fungi for assessment of the synergism between the fungicide and biocontrol agent on poisoned media dual culture assay. Empoison dual culture was kept as control treatment.

***In-vitro* synergism:** The synergism was a difference between poisoned and empoisoned media dual cultures. It was also calculated by a formula: S=APM-AEM, where the S stands for synergism, APM stands for antagonism on poisoned media and AEM stands for Antagonism on empoisoned media. The antagonistic activities of biocontrol agents against the test pathogens were evaluated by dual culture technique (Royse, 1978; Chand and Logan, 1984; Markey *et al.*, 2013).

***In-vivo* synergism:** A susceptible variety of Mung bean (*Vigna radiata* (L.) R. Wilczek) was selected; the healthy seeds surface sterilized for 05 minutes with 1% bleach ($NaCl_2$). The seeds were then rinsed three times with sterile water and air-dried. The seeds were coated with spore suspension supplemented with2% aqueous starch (*w/v*) as an adhesive. The 05mm disc of pathogen was amended with the critical number of fungal propagules to the sterilized 200 g soil in each pot with known amount of low concentration of fungicides. Five disinfected seeds were dressed with *Trichoderma* spp. spores ($1.2x10^7$ ml^{-1}) and sown in the 09 x 11 cm diameter plastic pots and watered (02ml) at alternate days. The treated and untreated pots were sown with test plant Mung bean seeds. The control pots were without inoculums either of pathogens or antagonism and fungicides. The pots were kept under screen house conditions (24±2°C) and irrigated regularly to avoid crop wilting. After 10 days the plants were uprooted gently under the slow shower of sterile water, the number of normal seedlings in each replication was counted and the germination was calculated and expressed in percentage. The roots of each treatment were cut into pieces of 02 cm and surface sterilized in 01% Sodium hypochlorite solution of water for 2 min., the roots were rinsed with sterile water five times and placed on PDA poured plates for observation of root colonization by *F. oxysporum*. The root colonization was calculated using formula as: RC= no. of roots recovered with the test fungi / total no of roots observed x 100. Root and shoot lengths/weights were measured from the central region to the tip of the primary root and shoot. The seedlings were used for growth measurement, those were air dried in a hot air oven maintained at 45±2°C for 16 hours; cooled for 30 min. The dry weight of seedlings was recorded using an electrically operated top pan balance. The mean dry weight of the seedlings was determined and recorded(Goodall, 1945; Haugland and Brandsaeter, 1996). The biomass/dry weight was also a determinative to plant growth which was also an indicator of synergism between the friend fungi and low doses of fungicides. The experiment laid out in a randomized block completed block design with five replicates and two repetitions. All the data were analyzed for significance levels $P<0.05$ to understand the quality of inference. The outcome of results was statistically Analyzed using analysis of variance (ANOVA).

RESULTS

Compatibility test: The fungicide stood less toxic at lower doses to the biocontrol fungi. *T. harzianum* (TH) tolerated the appropriate amount of Copper Hydroxide (EC_{50}176.1 ppm). The statistical analysis suggested that the data was highly significant and accepted TH as tolerable fungi with commercial fungicide Kocide (KD) (Figure 1, Table 1).

***In-vitro* synergism:** The fungicide KD (20 ppm) enhanced the potential of the food poisoned media dual culture to suppress the pathogen *F. oxysporum* (FO). The TH antagonized the FO growth 24% on empoisoned media, the synergism interaction enhanced the antagonistic potential of the TH strain up to 26% on poisoned media. The net synergism was observed between fungi and fungicide up to 02%. The synergism showed the prospect of combined applications (Figure 2 and 3). The microscopic examination revealed that TH invaded the FO colony and parasitized the pathogen hyphae. A huge number of chlamydospores were observed at the hyphal tips of TH. The Chlamydospores were prominent in those hyphae that were near the poisoned media floor. It could be suggested that the chlamydospores were responsible to sustain the TH growth on poisoned media with the KD fungicide. The FO hyphae was found with dense tubular cell aligned with the cell size, it was also reduced on poisoned media, the hyphal lyses was also seen at parasitized interactions. There was no sign of chlamydospores in FO at dual culture poisoned media studies (Figure 7).

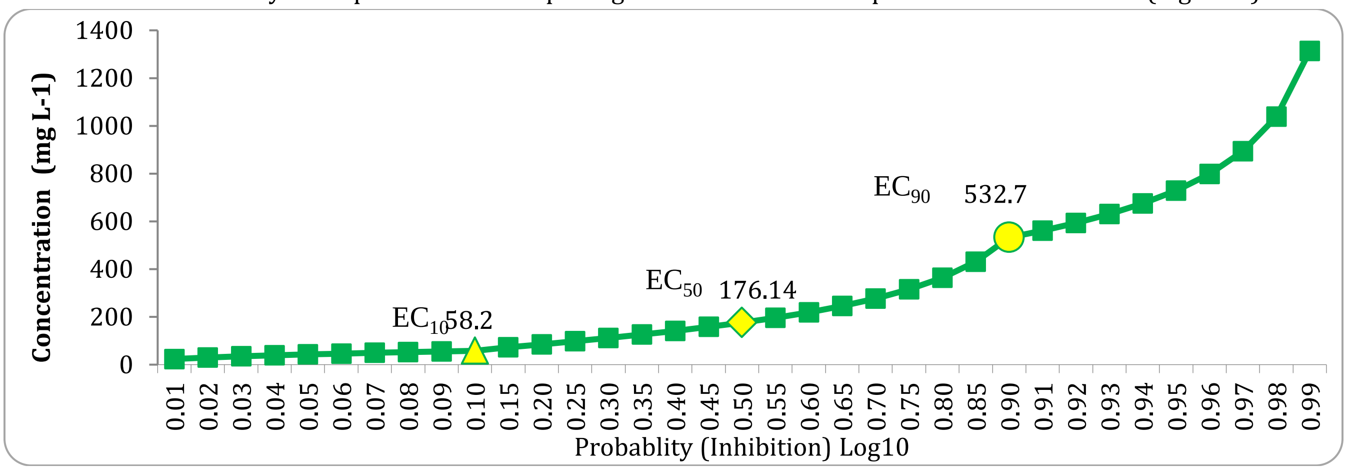

Figure 1. Probit regression output for different effective concentrations (EC_x) for the mycelia inhibition caused by fungicide KD to the fungus TH.

Table 1. Statistical representations of Probit Regression for dose response of the fungicides against *T. harzianum*

Parameter[b]		Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Concentration	2.666	0.115	23.218	0.000	2.441	2.891
	Kocide	-5.988	0.228	-26.301	0.000	-6.216	-5.760

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithm.) b. Corresponds to the grouping variable Fungicide.

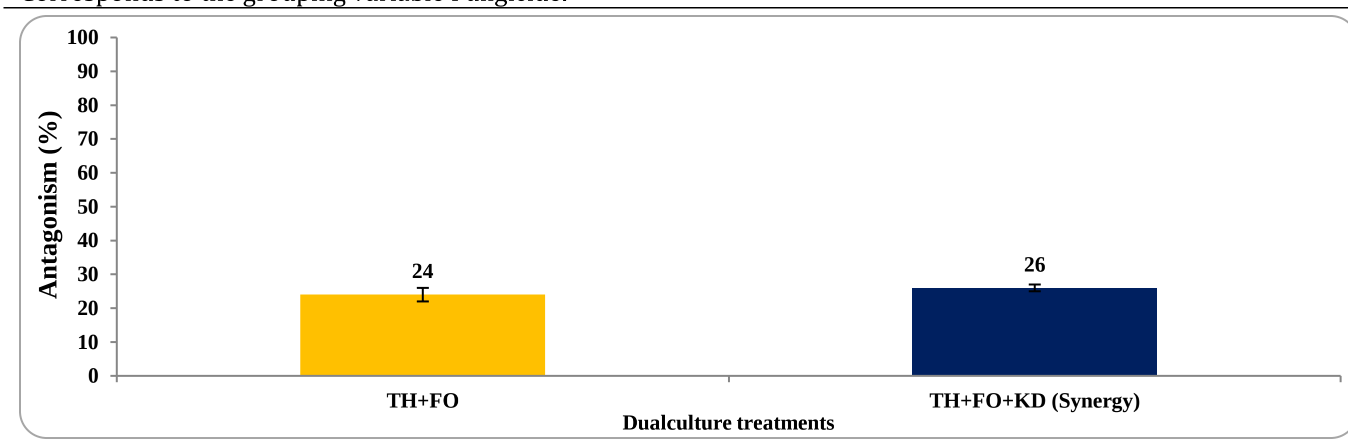

Figure 2. Antagonism (%) of *Trichoderma* spp. to the growth of the pathogenic fungi at *in-vitro* assessment

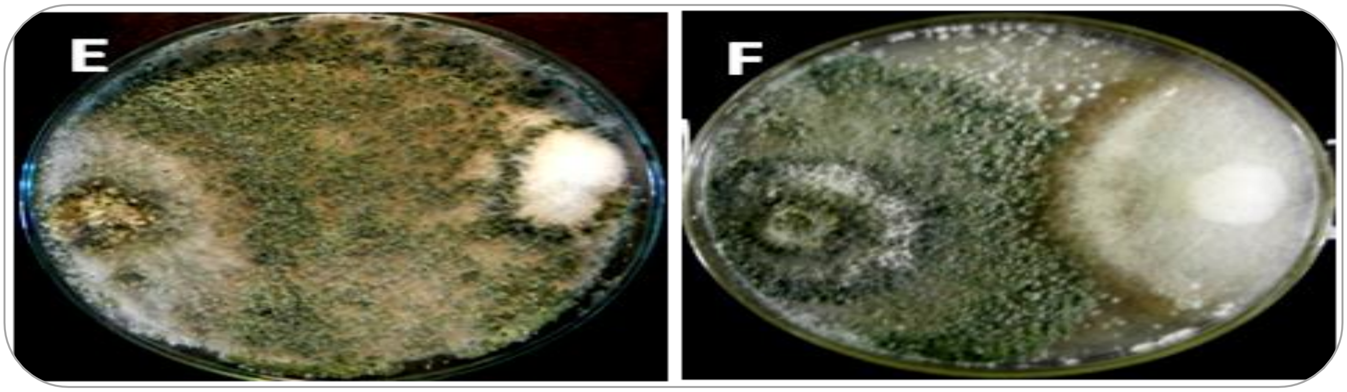

Figure 3. Inhibitions of FO caused by myco-fungicidal strain of TH on food poisoned media (E) and empoison media (F). The TH was placed on left side and FO on right side of the dual culture plates.

***In-vivo* synergism:** The Mung bean plant observed for growth in an amended soil with the fungicide KD, the biocontrol strain TH and the pathogenic fungi FO. The experiment calculated a negative impact of the pathogen and reduced the seed germination, plant growth and high infection rate at negative control treatment (95 in control to 19.7%). FO reduced the seed germination significantly. The addition of the fungicide KD to FO, suppressed the pathogenic effects and increased seed germination (40%), which was further enhanced to 86.7% when the fungicide KD and the biocontrol TH combined against FO. Thus the combination of KD and TH synergized protection from the pathogen FO to the seed germination (Figure 4).

Table 2. Plant dry weight of the Mung bean plant at *in-vivo* treatments

Treatment	N	Mean (g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
KD+TH+FO	3	2.66a	0.4642	0.2680	1.512	3.818	2.1	3.0
TH	3	1.43b	0.0900	0.0520	1.207	1.655	1.3	1.5
TH+FO	3	0.83c	0.0808	0.0467	0.636	1.037	0.8	0.9
Control	3	0.67cd	0.0500	0.0289	0.552	0.800	0.6	0.7
KD	3	0.61cd	0.0718	0.0415	0.439	0.796	0.5	0.7
KD+FO	3	0.68cd	0.0622	0.0359	0.533	0.841	0.6	0.8
FO	3	0.29d	0.1117	0.0645	0.013	0.567	0.2	0.4

The mean values followed by same letters are not significantly different DMRT p>0.05, DMRT uses harmonic mean sample size= 3.0.

The combined application increased the plant growth. The plant dry biomass increased (2.6 g), higher than negative control, where the soil was amended with the pathogen FO (0.29 g).The fungicide synergized the biocontrol against the pathogen, even the single doses of former (0.6 g) and later (1.4 g) exhibited reduced growth in the plant. The plant grew 0.67 g at untreated soil representing the positive control (Table 02).

The pathogen FO infested or infected plant roots. There was the higher FO root colonization in single FO amendment (65%). The fungicide KD reduced the root colonization of FO (58%); the seed treatment of plant with TH also effectively reduced FO root colonization (21%). The combined treatments of TH and KD efficiently reduced the root colonization of FO which was only 04% (Figure 05).

The fungicide KD was observed as stimulating agent to TH against plant pathogenic fungi FO. The fungicide KD (20 ppm) enhanced the antagonist characteristic of TH against FO on dual culture atmosphere; it also enhanced the TH efficiency to compete the pathogenic fungi FO in rhizospheric soil and resultantly reduced the root colonization of the pathogen. In other words, the fungicide and the biocontrol fungi were synergizing their effects for the protection of plant and hitting back the pathogenic effects of FO. Both synergized the efficiencies, about 02% at dual culture antagonism, about 26.3% at plant growth and 26.1% synergism of root colonization of FO. There was negative synergism between biocontrol and the fungicide for seed germination (-10%), the reasons could be higher germinations at single dose of TH than in combined applications. Finally, there were significant synergistic effects of lower doses of the fungicide KD and the biocontrol TH on FO disease suppression and vigor plant growth (Figure 4, 5 and 6).

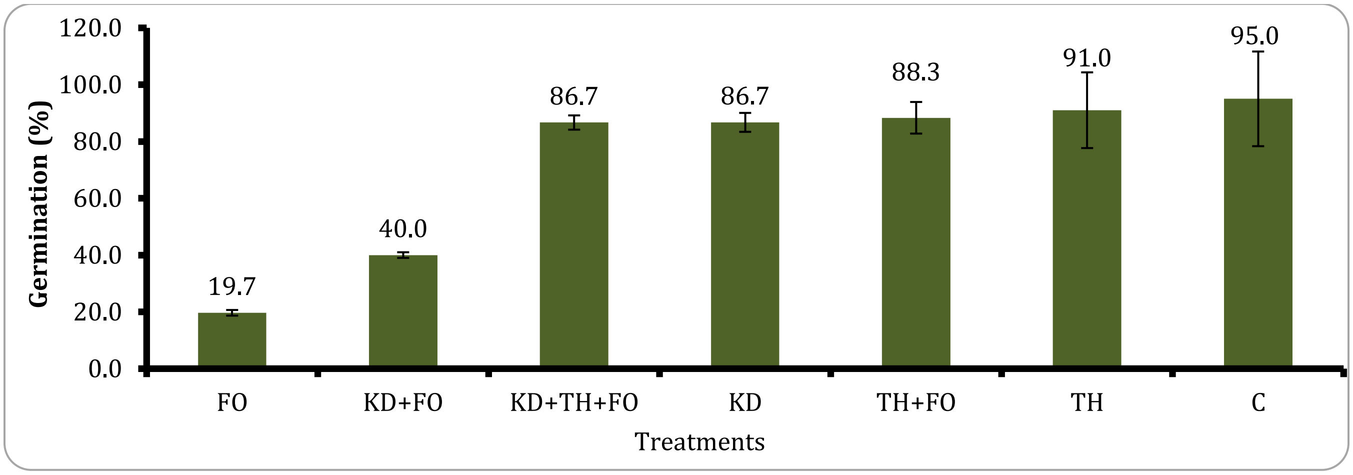

Figure 4. Effect of different treatments on seed germination of Mung bean

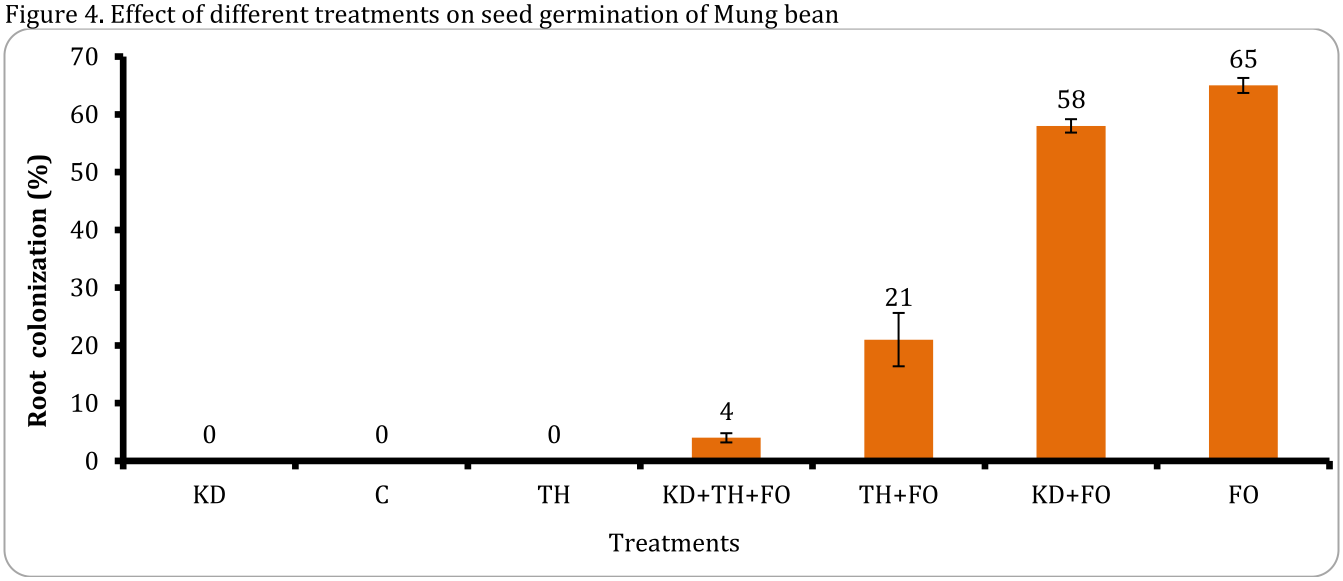

Figure 5. Root colonization of Mung bean plant by *F. oxysporum* at different treatments of the biocontrol and the fungicide

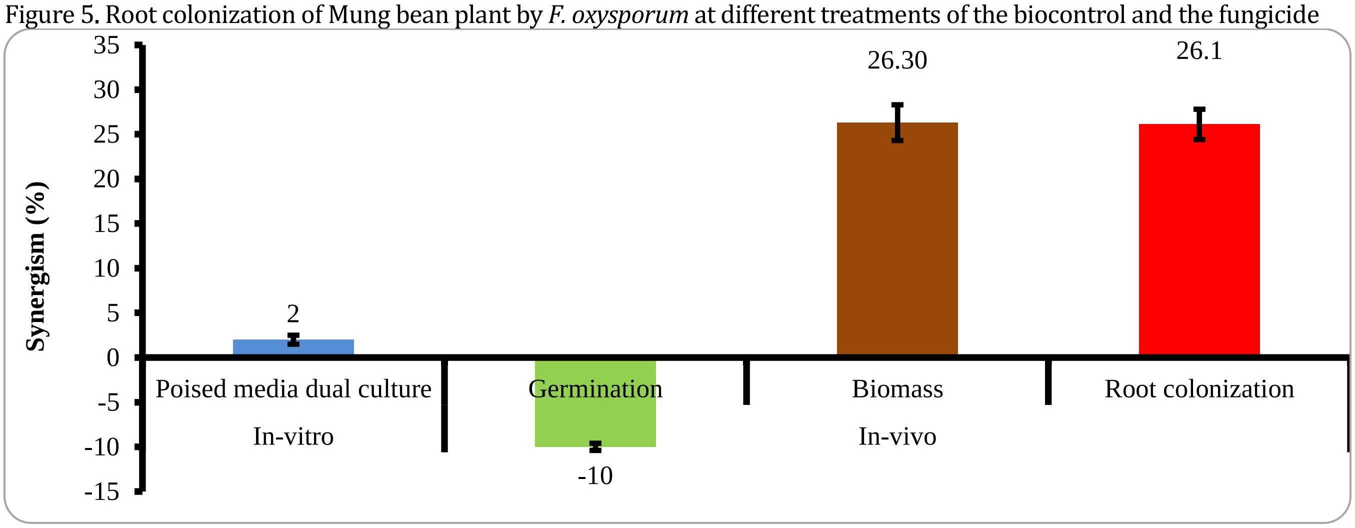

Figure 6. Synergism between the biocontrol fungi (TH) and fungicide (KD) to suppress the plant pathogenic fungi (FO).

Figure 7. The parasitism of TH on FO hyphae on poisoned food media; a chlamydospore is attached at the tip of TH hypha (A) that parasitized over FO hypha (B) and caused lyses.

DISCUSSION

There was a great deal of available literature on *Trichoderma* spp. antagonism. Previous findings are robust on control of fungal diseases with the *Trichoderma* spp. The fungicides have been utilized indiscriminately in agriculture worldwide. There were several reports suggesting fungicide residue in the soil. There was a consensus of researchers on negative impact of fungicide residue in soil on the potential of fungal biocontrol agents A group of researchers was suggesting Copper Hydroxide fungicide as slightly tolerable to *T. harzianum* at 500 ppm concentrations (Erayya *et al.,* 2020) and other species, like *T. viride*. The tolerant species of TH, were reported for the higher doses of the Cu-based fungicides (Valarmathi, 2013). Present findings were supporting the previous reports.

A trend of antagonism has been suggested in present study found TH antagonist up to 24% to FO at dual culture plate, while it was not merely lower for antagonist reactions; but the groups of researchers suggested a varied antagonism potential of the fungi, Carvalho *et al.* (2014) reported the inhibition (51%) of FO through TH, which is higher to present study; some other workers reported that the antagonism % of the TH isolates ranged from 24 to 70% to FO(Altinok and Erdoğan, 2015; VI, 2018). TH inhibition potential could be varied under laboratory conditions. Researchers were supporting the potential inhibition at *in-vivo* conditions using plant as a third biotic component. Therefore, the *in-vivo* experiments were conducted for the assessment of the TH strain for confirmation of this report.

There were fewer records available for the seed germination severity of FO, but some researchers provided a good information on soil borne FO effect on seed germination (Wall *et al.,* 1983), recent records are not available on the problem. The present study provided ample information on the effects of soil borne FO on seed germination of Mung bean. Hence the same treatments were simultaneously studied on plant growth parameters.

It is an accepted rule that has been further established during present study that "an appropriate level of crop protection could be ensured by the application of reduced amount of copper containing fungicides in combination with biocontrol Trichoderma strains with frame of integrated pest management" (Kredics *et al.,* 2004); this principal was expedited in the present study, where the fungicide Copper hydroxide enhanced the biocontrol efficacy of TH and controlled the FO infection in combined application strategy, where the low doses fungicide with the biocontrol agent effectively apprehended the infection on the plant. Thus the copper based fungicides are the best tolerable by TH biocontrol agent, it was supported by earlier and reports, it could reduce the infection rates when applied in combination with other species of the same genera (*T. asperellum*) against *Phytophthora infestans* a potent pathogen of late blight in potato (Ladi *et al.,* 2020). Present study was in concurrent results with earlier reports that the *Trichoderma* biocontrol agents were highly compatible with Copper hydroxide and effectively enhance their efficacy (synergized) for the control of pathogens and eventually promoted plant growth (Figure 5, 6 and 7). It was also reported that other fungal biocontrol species, of the same genera, were highly efficient to synergize with fungicides. *T. virens* effectively synergized with Caboxin

0.1% and additively reduced the FO disease incidences of corm rot in gladiolus plant (Mishra *et al.,* 2004). Hence, the present report stood confirmative in the terms of attributes exhibited by combined applications of KD and TH to suppress the FO infestation and disease development in Mung bean plant. Since there is no record of indigenous TH strains, that were found compatible with fungicides and it was not suggested for combined applications. It is therefore, suggested that the field application strategy of TH could be supportive for disease suppression and prevention. It is suggested that the crop seed would be treated with TH along with fungicide KD soil amendments for better plant growth and yields. The fungicide and biocontrol fungi are suggested for field disease prevention or suppression and vigor plant growth.

CONCLUSION

Hence, the present report stood novel in the term of attributes exhibited by combined applications of KD and TH to suppress the FO infestation and disease development in Mung bean plant. It is therefore, suggested that the field application strategy of TH could be supportive for disease suppression and preventions. It is suggested that the crop seed would be treated with TH along with fungicide containing Copper Hydroxide. The fungicide and biocontrol fungi are to be examined in field for plant vigor growth and disease prevention or suppression.

ACKNOWLEDGEMENTS

The executors of the research are highly thankful to chairman department of Agriculture and Agribusiness Management, University of Karachi for providing valuable cultures of the fungi. This support is highly acknowledged.

REFERENCES

Altinok, H. H. and O. Erdoğan. 2015. Determination of the in vitro effect of *Trichoderma harzianum* on phytopathogenic strains of *Fusarium oxysporum*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43: 494–500.

Amini, J. 2009. Physiological race of *Fusarium oxysporum* f. sp. *lycopersici* in Kurdistan province of Iran and reaction of some tomato cultivars to race 1 of pathogen. Plant Pathology Journal, 8: 68–73.

Błaszczyk, L., M. Siwulski, K. Sobieralski, J. Lisiecka, and M. Jedryczka. 2014. Trichoderma spp. application and prospects for use in organic farming and industry. Journal of Plant Protection Research, 54(4): 309–317.

Carvalho, D. D. C., M. Lobo Junior, I. Martins, P. W. Inglis, and S. C. M. Mello. 2014. Biological control of *fusarium oxysporum* f. sp. *phaseoli* by trichoderma harzianum and its use for common bean seed treatment. Tropical Plant Pathology, 39: 384–391.

Chand, T. and C. Logan. 1984. Antagonists and parasites of *Rhizoctonia solani* and their efficacy in reducing stem canker of potato under controlled conditions. Transactions of the British Mycological Society, 83: 107–112.

Chaparro, A. P., L. H. Carvajal, and S. Orduz. 2011. Fungicide tolerance of *Trichoderma asperelloides* </i> and <i>T. harzianum strains</i> Agricultural Science Journal, 2: 301–307.

Chung, W. H., W. C. Chung, P. F. Ting, C. C. Ru, H. C. Huang, and J. W. Huang. 2009. Nature of resistance to methyl benzimidazole carbamate fungicides in *Fusarium oxysporum* f.sp. *lilii* and *F. oxysporum* f.sp. *gladioli* in Taiwan. Journal of Phytopathology, 157: 742–747.

Erayya, N., S. A. A Shukla, K. Tewari and J. Kumar. 2020. Compatibility of Trichoderma spp. with copperchitosan graded combinations . Plant Disease Research, 35: 42.

Fischer, B. M., I. Salakhutdinov, M. Akkurt, R. Eibach, K. J. Edwards, R. Töpfer, and E. M. Zyprian. 2004. Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theoretical and Applied Genetics, 108: 501–515.

Goodall, D. W. 1945. The distribution of weight change in the young tomato plant. Annals of Botany, 9: 101–103.

Haugland, E. and L. O. Brandsaeter. 1996. Experiments on bioassay sensitivity in the study of allelopathy. Journal of Chemical Ecology, 22: 1845–1859.

Jamil, A. 2021. Antifungal and plant growth promoting activity of Trichoderma spp. Against *Fusarium oxysporum* f. sp. *lycopersici* colonizing tomato. Journal of Plant Protection Research, 61: 243–253.

Kredics, L., L. Hatvani, Z. Antal, A. Szekeres, L. Manczinger, and E. Nagy Lászlóné Antal. 2004. Protease over-production in the presence of copper by a *Trichoderma harzianum* strain with biocontrol potential. arthropod pests by BCAs IOBC/wprs Bull. Management of Plant Diseases, 27: 373–374.

Ladi, E., N. Shukla, Y. Bohra, A. K. Tiwari, and J. Kumar.

2020. Copper tolerant *Trichoderma asperellum* increases bio-efficacy of copper against *Phytophthora infestans* in dual combination. Phytoparasitica, 48: 357–370.

Lan, W. J., Y. Zhao, Z. L. Xie, and others. 2012. Novel sorbicillin analogues from the Marine fungus Trichoderma sp. associated with the seastar *Acanthaster planci*. Natural Product Communications, 7: 1337–1340.

Lavanya, S. N., S. Niranjan-Raj, S. C. Nayaka, and K. N. Amruthesh. 2017. Systemic protection against pearl millet downy mildew disease induced by cell wall glucan elicitors from *Trichoderma hamatum* UOM 13. Journal of Plant Protection and Research, 57: 296–308.

Liu, S., L. Fu, S. Wang, J. Chen, J. Jiang, Z. Che, Y. Tian, and G. Chen. 2021. Carbendazim Resistance of *Fusarium graminearum* from Henan Wheat. Plant Disease, 103: 2536–2540.

Markey, B., F. Leonard, M. Archambault, A. Cullinane, and D. Maguire. 2013. Clinical veterinary microbiology e-book, Elsevier Health Sciences. 222-229

Mishra, P. K., A. N. Mukhopadhyay and U. S. Singh. 2004. Suppression of *Fusarium oxysporum* f. sp. *gladioli* populations in soil by application of *Trichoderma virens* and in vitro approaches for understanding biological control mechanisms. Indian Phytopathology, 57: 44–47.

Molnar, A., M. Pesti, and L. Hornok. 1985. Isolation, regeneration, and fusion of *Fusarium oxysporum* protoplasts. Acta Phytopathology Academiae Scientiarum Hungaricae, 20: 175–182.

Neely, D. and C. H. Beckman. 1990. The Nature of Wilt Diseases in Plants. Mycologia, 82: 668.

Poveda, J. 2021. Trichoderma as biocontrol agent against pests: New uses for a mycoparasite. Biological Control, 159: 12–26.

Royse, D. J. 1978. The Influence of Fungi Isolated from Peach Twigs on the Pathogenicity of *Cytospora cincta*. Phytopathology, 68: 603.

Schmitz, H., E. E. Bateman, R. H. Colley, and others. 1930. A Suggested Toximetric Method for Wood Preservatives. Industrial and Engineering Chemistry Research, 2: 361–363.

Su, Z..., A. Bernardo, B. Tian, H. Chen, S. Ma. H. Wang, S. Cai, D. Liu, D. Zhang, T. Li and H. Trick. 2019. A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. *Nature Genetics*, *51*(7): 1099-1105.

Srivastava, M., and M. Shahid. 2014. Trichoderma Genome to Genomics: A Review. J. Data Mining Genomics Proteomics, 05: 12–26.

Valarmathi, P. 2013. Compatibility of Copper hydroxide (Kocide 3000) with Biocontrol Agents. IOSR Journal of Agriculture and Veternary Sciences, 3: 28–31.

VI, G. 2018. Evaluation of Antagonistic Effect of *Trichoderma Harzianum* against *Fusarium oxysporum* causal Agent of White Yam (*Dioscorearotundata poir*) Tuber Rot. A Trend in Technical and Scientific Research, 1: 12–26.

Wall, M. T., D. C. McGee, and J. S. Burris. 1983. Emergence and Yield of Fungicide-Treated Soybean Seed Differing in Quality 1. Agronomy Journal, 75: 969–973.

Weindling, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology, 24: 1153–1179.

Contribution of Authors:

Umara Arain	:	Collection samples and performed experiments
Aziz A. Ujjan	:	Wrote the manuscript
Abdul Q. Rajput	:	Analyzed and interpretation of data.
Saleem Shahzad	:	Morphological characterization of isolated microorganisms