ISSN: 2348-1900
Plant Science Today
http://www.plantsciencetoday.online

OPEN ACCESS

Research Article

Synergistic interaction of *Glomus mosseae* T. and *Trichoderma harzianum* R. in the induction of systemic resistance of *Cucumis sativus* L. to *Alternaria alternata* (Fr.) K.

Abdulnabi A. A. Matrood[1], Mohammad Imad Khrieba[2] & Okon Godwin Okon[3*]

[1]Department of Plant Protection, College of Agriculture, University of Basrah, Iraq
[2]Department of Plant Pathology and Biological Control, National Commission of Biotechnology (NCBT), Damascus-Syria
[3]Department of Botany, Faculty of Biological Sciences, Akwa Ibom State University, Nigeria

Article history
Received: 28 August 2019
Accepted: 02 October 2019
Published: 03 February 2020

Publisher
Horizon e-Publishing Group

Correspondence
Okon Godwin Okon
okonokon@aksu.edu.ng

Abstract

Due to the various negative impacts of chemical fungicides, the reduction of its applications in agricultural production process is widely recommended. Thus, the need and application of bio-agents in disease control has increased tremendously. The current study aimed at investigating the role of both bio-agents *Glomus mosseae* (mycorrhizal fungi) and *Trichoderma harzianum* in protection of *Cucumis sativus* (cucumber plants) against the fungal pathogen *Alternaria alternata* which is an opportunistic pathogen and the causal agent of cucumber wilt disease. Results obtained from this work revealed the positive influence of using bio-agents treatments in the reduction of pathogenic effects of *A. alternata*. The results also showed that *G. mosseae* and *T. harzianum* combination had a positive synergistic influence in reducing the detrimental effects of *A. alternata* ny improving the biomass yield (e.g. fresh and dry weight of root); as well as, on disease severity on *C. sativus*. Bio-agents (*G. mosseae* and *T. harzianum*) increased resistance in *C. sativus* by raising the production of enzymes catalase and peroxidase. Conclusively, this research revealed that using a multifarious combination of bio-agents significantly (P =.05) increased the efficiency of biological control of *A. alternata* than using each of them exclusively. Thus, it is recommended that to get an effective result in the control of the pathogen *A. alternata* in crops as highlighted by the results of this work; a combination of two bio-agents should be used.

Keywords: Bio-control; Cucumber; Fungus; Mycorrhiza; Pathogen; Peroxidase

Citation: Matrood A A A, Khrieba M I, Okon O G. Synergistic Interaction of *Glomus mosseae* T. and *Trichoderma harzianum* R. in the Induction of Systemic Resistance of *Cucumis sativus* L. to *Alternaria alternata* (Fr.) K.. Plant Science Today 2020;7(1):101-108. https://doi.org/10.14719/pst.2020.7.1.629

Indexing: Plant Science Today is covered by Scopus, Web of Science, BIOSIS Previews, ESCI, CAS, AGRIS, UGC-CARE, CABI, Google Scholar, etc. Full list at http://www.plantsciencetoday.online

Introduction

The maintenance of high food quality and quantity yield of food crops is a necessity worldwide. Therefore, proper plant disease control measures are a crucial factor to food production and security. Several damages caused by pathogens hamper plant production and most of these events are reported mostly in field crops and vegetables

plants. However, disease control strategies have been applied to achieve such goal by using crop rotation; disease-free seeds; water and moisture management; pesticides and biological approaches. However, the issue of environmental pollution raises considerable challenges for pesticides applications; thereby, necessitating the search for an alternative approach to control plant diseases. Currently, there are strict regulations on chemical pesticides usage and there is a move by governments worldwide to remove the most hazardous chemical pesticides from the market around the world (1).

Several researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Amongst these alternative approaches are those referred to as biological control. A multiplicity of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people and the environment (2).

The management of plant diseases using bio-control agents offers a great promise (3, 4). These agents are vital component of sustainable agriculture (5), which colonize the rhizosphere (the site requiring protection) and leave no toxic residues as opposed to chemicals (4). Most species of the genus *Trichoderma* are well-known for their ability to act as bio-control agents against plant pathogens (6). *Trichoderma* spp have been used in reasonably large quantities in plant agriculture, both for disease control and yield increases. Recent trend of researches demonstrate that the effects of *Trichoderma* inoculation on plants includes induction of systemic or localized resistance to pathogens which are very important (7). Some species of *Trichoderma* have multiple interaction capabilities (mainly *T. harzianum*, *T. viride* and *T. hamatum*) with crop plants and soil borne fungal pathogens (8). Another important features of these fungal species is their ability to enhance plant growth and development, depending on several actions including elevated reproductive ability, capacity to modify the rhizosphere, ability to grow under adverse conditions, competence in the use of nutrients, strong aggressiveness against phytopathogenic fungi and efficacy in supporting plant growth and enhanced defense mechanisms (9, 10).

Several studies have properly documented that mycorrhizal fungi possess the ability to promote plant growth which is mainly attributed to its characteristic improvement of nutritional status of the plant (11), especially P nutrition. Additionally, several authors reported a higher tolerance of mycorrhizal plants to abiotic stresses, such as drought, salinity, heavy metals etc. (12). Evidence also has accumulated on the higher resistance of mycorrhizal plants to a wide range of belowground aggressors such as soil-borne fungal and bacterial pathogens, nematodes, or root-chewing insects (13). Arbuscular mycorrhizal fungi (AMF) have been reported by several researchers to form beneficial synergistic relationships with other mycorrhizal and fungal species as well as plant growth promoting rhizobacteria (PGPR). Symbiotic microorganisms such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can induce systemic resistance to both aerial and soil borne pathogens (14, 15). Moreover, the presence of both AMF and PGPR in the rhizosphere is known to be an important determinant of plant health in general (16), that is, of the ability of a plant to carry out its physiological functions to the best of its genetic potential (17).

Cucumis sativus L. (Cucumber) is a member of the *Cucurbitaceae* family and is grown in several countries worldwide. It is an important food crop grown in Iraq as well as Nigeria. Cucumber is a vegetable crop with a high nutritional value, its fruits are desirable for consumers worldwide and it is consumed fresh or used to make marinades thus increasing the demand for cucumber continuous throughout the year (18). Cucumber does well in well drained loamy soils having an optimum pH range of 6.5-7.5 with relevant soil nutrients, temperature range of 18-30°C, an optimum rainfall of about 800 mm; irrigation may be required to satisfy the consistent moisture requirements of the plant and about 6-8 hours of sunlight is also required (19).

The aim of this study is to evaluate the potentiality of bio-agents (*Trichoderma harzianum* and *Glomus mosseae*) synergy or singly in inducing resistance for *Cucumis sativus* (cucumber plants) against the fungal pathogen *Alternaria alternata*.

2. Materials and Methods

2.1 Study Location

The study was conducted at the College of Agriculture, University of Basrah,; located at Southern Iraq (Latitude 30° 56' 31.58'' Longitude 47° 74' 41.67'').

2.2 Isolation and Identification of *Alternaria alternata* Fungi and source of Mycorrhizal Fungi

The symptomatic leaves of cucumber plant and seedlings were collected form infected areas and transferred to the laboratory. Firstly infected plant materials were washed thoroughly with tap water and then sterilized with 10% of sodium hypochlorite (NaOCl) for 3-5 min and washed with sterilized distilled water, subsequently dried on filter paper and cut into small pieces (0.5-1 cm) and plated in a Petri dish containing a sterilized potato dextrose agar (PDA) and 250 mg/L. Inoculated plates were incubated at a temperature of 27 °C for five days. The fungal isolates were

removed by transferring parts of the fungus into sterile petrified dishes and then incubated in the incubator at 27 °C for three days. Identification of *Alternaria alternata* was based on the taxonomic characteristics mentioned in earlier works (20, 21).

The arbuscular mycorrhizal fungi were obtained from the Ministry of Science and Technology, Agricultural Research Department, Iraq.

2.3 Pathogenicity Test:

In this experiment, 5 kg plastic pots containing a mixture of soil and peat moss 1: 2 were used after sterilization of the mixture using commercial formalin by preparing a solution of 50: 1 formalin/ water. The solution was used with 3 liters of water/ m^3 soil.

The cucumber variety (Amal) was used and the seeds were planted and irrigated carefully for three weeks; then the seedlings were sprayed with a suspension of fungal conidia at 10^5 conidia/ml.

2.4 Antagonistic Ability of *T. harzianum* against *A. alternata:*

In order to identify the antagonistic ability of *T. harizanum* in the antifungal activity against the isolates of the fungus *A. alternata*, the dual cultures technique was used.

Both fungal isolates of *A. alternata* and *T. harzianum* were grown on the PDA medium at about 5 days. A plug of each fungal colony (0.5 cm) was plated at the edge of PDA plates (2 cm from the edge of plate) and incubated at 27 °C for 5 days. The antagonistic test repeated twice in triplicates, the degree of antagonistic character for each fungus was determined by the five-step standard of measurement. The degree of antagonism of each isolates was measured (22), as follow:

1. The bio agent fungi fills entire Petri dish
2. The bio agent fungi fills two-thirds of the Petri dish
3. The bio agent and the pathogenic fungus both fills half of the Petri dish
4. Pathogenic fungus fills two-thirds of the Petri dish
5. Pathogenic fungus fills entire the Petri dish.

2.5 Bio-effect of *Glomus mosseae* and *T. harizanum* on *A. alternata* in Plastic Pots

Plastic pots capacity of 5 kg were filled with a mixture of soil and peat moss as 1:2 and used in this experiment. *T. harzianum* was loaded into millet seeds; three days later, the soil was contaminated with fungus *G. mosseae* and added by 30 g of each seed from the fungus *G. mosseae* and other non-polluted sterile millet seeds and then planted with cucumber seeds and watered as needed.

Table 1. Pathological scales according to the number of spots on the plant

Class	Number of spots
0	0
1	1-3
2	4-6
3	7-9
4	Death of lower leaves

The germination percentage of cucumber seed was calculated after 10 days of planting and two weeks later the disease severity, fresh and dry weight of roots were calculated. Disease severity was measured according to methods described by Wheeler (23) (Table 1).

Table 2. Experiment parameters and number for each treatment

Treatments	Treatment number
Glomus mosseae + *Alternaria alternata*	1
Trichoderma harzianum + *Alternaria alternata*	2
Glomus mosseae. + *Trichoderma harzianum* + *Alternaria alternata*	3
Alternaria alternata only	4
Millet seeds only	5

The experiment was set up in a complete random design (CRD). The experiment included five treatments as explained in Table (2).

2.6 Enzymatic activity of cucumber inoculated with *G. mosseae*, *T. harzianum* and *A. alternata*:

2.6.1 Catalase activity:

Roots of cucumber plants were collected and placed in polyethylene bags and then in cooling box and brought to the laboratory. 300 mg fresh weight of roots was taken and washed with distilled water free of ions and 6 ml of 0.05 M potassium phosphate buffer solution (K_2PO_4 31g, K_2HPO_4 0.006, EDTA 0.1g, poly vinyl pyrolidon (pvp) 5g, ascorbic acid 0.2g, and adjust pH to 6) was added. Then centrifuged at 12000 rpm for 20 min for catalase activity the effectiveness of enzymatic activity in UV spectrophotometer was estimated at 240 nm (24).

2.6.2 Peroxidase activity:

For peroxidase activity, apply to the potassium phosphate buffer solution 250 µl from both 0.5% of Gauiacol pigment and hydrogen peroxide 0.3% v/v. Finally, the effectiveness of enzymatic activity in UV spectrophotometer was estimated at 470 nm (25). The enzymatic activity of both enzymes was estimated using the formula:

$$\text{Enzymatic activity} = \frac{\text{device reading}}{\text{Solution reading size x Extraction size}} \text{ x read size}$$

2.7 Statistical Analysis

All data obtained from this study results were subjected to Least Significant Difference (L.S.D) analysis. GenStat statistical software was used to analyze the data and Duncan's multiple range test was used to compare means (P = 0.05) between treatments obtained from enzyme activities data.

3. Results and Discussion

3.1 Identification and pathogenicity of *A. alternata*:

The results revealed the presence of three isolates of *A. alternata* in roots of cucumber seedlings. Based on pathogenicity most virulence isolate was no. 2. The results showed that the isolate of *A. alternata* was observed to be more virulent and reached 76 % of cucumber death while isolate no 1 reached 66.98 %. Based on the results, isolate 2 was selected.

3.2 Antagonistic capability of *T. harzianum* against *A. alternata*:

Antagonist activity (Dual culture assays) explained that *T. harzianum* significantly (P = 0.05) reduced the growth of the pathogens *A. alternata* (Fig. 1). *T. harzianum* was able to inhibit the growth of two isolates of the pathogen *A. alternata* (Table 3). The bio-agent *T. harzianum* has the capacity over the growth of plant pathogenic fungi resulting into complete degradation and growth inhibition (26). Based on pathogenicity most virulence isolate was no. 2 and was selected for the research.

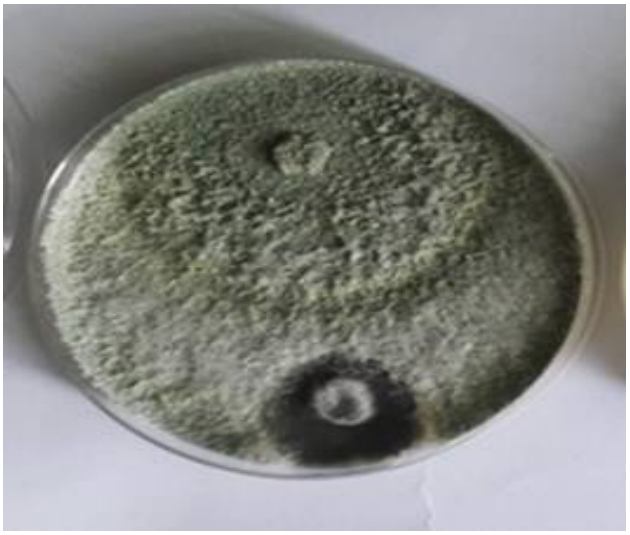

Fig 1. Antagonistic activity of *T. harizanum* against *A. alternata* grown on PDA after five days of inoculation at 28 °C.

Cucumber seeds could be penetrated by *T. harzianum* and induced systemic resistance (27). The bio-pesticide has a significant role in inhibiting pathogenic fungal growth. The results of this study are consistent with many researchers (28), stating that species of *Trichoderma* produce many antibiotics and these will be synergistic when they associate with cell wall analytic enzymes. Therefore, this would work as an "inhabitation" effect for many pathogens. Some strains of *Trichoderma* have been reported to elicit ISR and, moreover, colonized roots appear to be primed for an intense defense response to subsequent pathogen attack (29, 30).

Table 3. Antagonistic activity of bio-agent *T. harizanum* against isolates of *A. alternata*

Bio-agent fungi	*T. harizanum*
Alternaria alternata	Antagonism scale
Isolate-1	1
Isolate-2	1

3.3 ISR by *G. mosseae* and *T. harizanum* on Cucumber plants infected by *A. alternata* in plastic pots:

The results of this experiment showed that the fungus had a significant (P = 0.05) biological effect against *A. alternata* isolate 2. The results proved the efficiency of the synergy between fungus *G. mosseae* and *T. harzianum* in reducing the pathogenic effect of *A. alternata* on biomass yield (Table 4). The treatment *G. mosseae* + *A. alternata* were characterized by fresh weight of roots of Cucumber plant, which revealed that *A. alternata* + *G. mosseae, T. harizanum*+ *A. alternata* + *G. mosseae* and *T. harizanum* + *A. alternata* reached the highest average of 9.54, 11.05 and 11.78g respectively, compared to control treatments.

Results of the dry weight of roots showed a significant (P = .05) increase in the treatments of *A. alternata* + *G. mosseae*, *T. harzianum* + *A. alternata* which were reached 1.08 and 1.62 g respectively, but exhibited highest dry weight of the roots at *T. harizanum* + *G. mosseae* + *A. alternata* (1.99 g) when compared to the control treatments. *A. alternata* fungus and millet seeds reported the dry weight of seedling roots 1.87 and 1.21 g respectively.

Table 4. Biological effect of *Glomus mosseae* and *T. harizanum* on Cucumber plants infected by *A. alternata* in plastic pots

Treatments	**Fresh weight of roots (gm)**	**Dry weight of roots (gm)**	**% Infection Severity**
Glomus mosseae + *Alternaria alternata*	9.54	1.08	42.50
Trichoderma harzianum + *Alternaria alternata*	11.78	1.62	32.50
Glomus mosseae + *Trichoderma harzianum* + *Alternaria alternata*	11.05	1.99	59.93
Alternaria only	11.65	1.87	67.44
Millet seeds only	13.83	1.21	0
L.S.D 0.05	**4.80**	**0.61**	**11.97**

Disease severity was decreased (P = 0.05) significantly at the treatments *T. harizanum* + *G. mosseae* + *A. alternata*, *T. harizanum* + *A. alternata* and *G. mosseae* + *A. alternata* which reached 67.44, 32.50 and 42.50% respectively compared to the control treatment and *A. alternata* which reached 67.44 % (Table 4).

The inhibition activity between *T. harzianum* + *G. mosseae* against the pathogen was evident and could be attributed to their compatibility and synergy, in addition to the toxic and enzymatic capacity which enhance the ability of plant resistance against pathogen (31). Pre-transplant inoculation with mycorrhizal fungi can be a way to give efficient strains an immediate spatial advantage over the indigenous fungi which have to compete for root space (32). However, few researchers have reported that the simultaneous addition of AMF with pathogen could also reduce severity of some root diseases (4).

The combination between two bio-agents showed a significant (P = 0.05) reduction in the growth of the targeted pathogen through their ability to grow rapidly than the pathogenic fungi thus competing efficiently for space and nutrients. Starvation is the most common cause of death for microorganism, thereby making competition for limited nutrients the basis of biological control of fungal phyto-pathogens (33).

Bio-agents have a high inhibitory effect against a wide range of plant pathogens (28). The soil of sesame plant inoculated by *T. harzianum* and *Glomus* sp singly or combined led to increased host defenses in *Macrophomina phaseolina* by increasing the oxidation of enzymes and the total phenol (34). All demonstrated the possible role of chitinolytic and/or glucanases enzymes in biocontrol by *Trichoderma*, these enzymes function by breaking down the polysaccharides, chitin and glucans that are responsible for the rigidity of fungal cell walls thereby destroying cell wall integrity limiting the growth of the pathogen (35, 36).

In a study it was reported that *Trichoderma* + AMF treated pots showed significantly highest disease reduction under both prior (90.12%) and simultaneous inoculation (77.27%) among all treatments (4). This can be explained on the basis that *Trichoderma* fungi facilitated positive interactions (symbiosis with AM fungi and other beneficial microbes) in the rhizosphere which reduces biotic and abiotic damages (37) caused by soil borne pathogens and even prohibit entry of pathogens in the rhizospheric zone.

Enzymatic activity of catalase and peroxidase in plant roots showed the ability of *G. mosseae* and *T. harzianum* to induce resistance in Cucumber plants. The enzymatic activity of catalase was significant (P = 0.05) higher in treatments of *G. mosseae* + *A. alternata* (4.98 unit/g), *T. harzianum* + *G. mosseae* + *A. alternata* (1.78 unit/g) and *A. alternata* + *T. harzianum* (2.98 unit/g) respectively when compared to the control treatment (0.978 unit/g fresh weight). For peroxidase activity, the highest enzymatic activity reached 9.65 (*G. mosseae* + *A. alternata*), 3.876 (*T. harzianum* + *G. mosseae* + *A. alternata*) and 5.86 unit/g (*G. mosseae* + *A. alternata*) fresh weight. (Fig. 2).

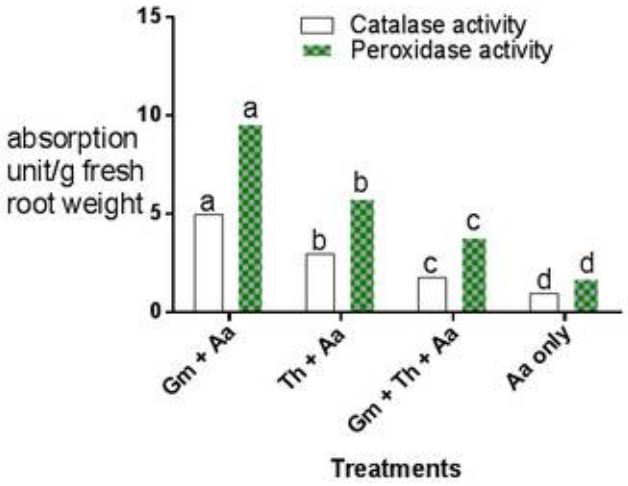

Fig. 2. Effect of *G. mosseae* and *T. harizanum* treatment on enzymatic activity (catalase and peroxidase) of Cucumber plants infected by *A. alternata* (absorption unit/g fresh root weight). Gm+Aa (*Glomus mosseae* + *Alternaria alternata*), Th+Aa (*Trichoderma harzianum* + *Alternaria alternata*), Gm+Th+Aa (*Glomus mosseae* + *Trichoderma harzianum* + *Alternaria alternata*), Aa only (*Alternaria alternata* only).

The interaction between bio-agents led to a significant (P = 0.05) production of catalase and peroxidase enzymes; this would indicate an increased effectiveness and inhibition against pathogenic fungi. Treating sunflower plants with *Glomus* sp. and *T. harzianum* fungi led to increase in the effectiveness of enzymes in defending against pathogens and found that inoculating plants with these bio-agents led to an increase in effectiveness of peroxidase which in turn increases resistance against *R. solani* (38).

Peroxidase is associated with the production of reactive oxygen species (ROS) which has a toxic effect towards the pathogens either directly or indirectly and it plays a pivotal role in reducing the spread of pathogen through increased lignin in the cell wall (39) and a mixture of several enzymes might be necessary for efficient cell wall lysis (33).

The catalase is considered as one of the defense enzymes which are produced by the plant because of exposure to the invasion of pathogens. This would also cause degradation to the cell walls of fungus and may enhance the antagonist activity of *T. harzianum* (40).

Trichoderma sp plays an important role in inducing mechanism of plant defenses. In the examination of the metabolism, toxic substances, volatile or non-volatile which is produced by

Trichoderma and that could have inhibited the settlement of micro-organisms leading to a synthesis of phyto-alexins, proteins and other compounds in the plant against plant pathogens (41). It was found that treating bean plants with *T. viride* will increase the phenolic substances level (42). The cotton seeds treated by isolates of *Trichoderma* had a clear effect in increasing peroxidase level (43).

Studies have reported that high efficacy of examined enzymes will conjugate with a high level of plant resistance. The peroxidase acts with hydrogen peroxide in breaking down of pathogenic enzymes, inducing phyto-alexins and building a structural defence to strengthen the cell walls. Such construction of lignin also interacts with cell wall proteins forming transverse bands and multiple compounds, which increase the hardness of cell wall (44). Other studies show mycorrhizal treatments stimulated antioxidative enzymes in leaves of various plants (45-49) in pepper plants such as CAT.

The inhibition of pathogenic fungi growth will increase by using a complex of compatible bio-agents, due to their capabilities in producing many antibiotics that works synergistically with different enzymes to degrade cell wall of a wide range of pathogenic fungi. Singh *et al*. (50) reported that phenolic compounds are major factors in disease resistance of many plant families. Peroxidase and polyphenol oxidase are associated in phenols oxidation to quinones which is more toxic to pathogens. These two enzymes are more effective in sunflower plants which used *T. harzianum* to control *R. solani*. This study indicates that high efficiency of these enzymes is associated with high level of resistance. In addition, peroxidase is associated with hydrogen peroxide in breaking down pathogenic enzymes such a pectinase that strengthens the cell wall. The induction of phyto-alexins gives strength and structural defense of walls as a result of lignin building and interaction with cell wall proteins (44).

The reasons for these increases may be that multiple inoculation with different microbes activated the host plant defense system greater than single or dual inoculation did before plants recognized the applied microbes as non-pathogenic ones, then host plants might take an advantage of the induced/primed defense system as an important mechanism to protect themselves against unfavorable conditions. Several workers reported that application of *Trichoderma* spp. can improve defense enzymes in plants (51, 52) and co-inoculation of AM fungi and *Trichoderma* spp. have synergistic impacts on controlling phytopathogens (53). Plants inoculated by *Glomus mosseae* or *Trichoderma harzianum* amended bioorganic fertilizer elevated significantly PPO and POD activity; however, the co-inoculation gained the highest PPO and POD level as compared to the non-inoculated control (54, 55).

4. Conclusion

Centered on results obtained from this study, it can be concluded that the use of a combination of bio-agents (*G. mosseae.* and *T. harzianum*) was more efficient in biological control of the opportunistic pathogen *A. alternata* than single treatment. This synergistic relationship developed a less advantageous environment for *A. alternata* and reduced the damaging effects of this pathogen. It can also be stated that the synergy between *G. mosseae.* and *T. harzianum* can activate a lot of machineries such as competition, reformed root exudations, anatomical and morphological variations in the root system and induced plant defense systems in the presence of the pathogen *A. alternata*. Thus, the inoculation of crops with a combination of *G. mosseae* and *T. harzianum* will go a long way to mitigate losses resulting from *A. alternata* attacks.

Funding source

This research work was sponsored by the authors.

Competing Interest

The authors declare that they have no competing interests.

Author's Contribution

AAM conceived the study and participated in its design, laboratory analysis and coordination, MIK drafted the initial manuscript. OGO ran the statistical analysis, edited and drafted the final version of the manuscript. All authors read and approved the final version of the manuscript.

References

1. Abass MH, Hameed MA, Ahmed AN. First report of *Nigrospora sphaerica* (Sacc.) Mason as a potential pathogen on date palm (*Phoenix dactylifera* L.). Can J of Plant Path. 2013;35(1):75-80. https://doi.org/10.1080/07060661.2012.732612
2. Chandrashekara KN, Chandrashekara C, Chakravathi M, Manivannan S. Biological Control of Plant Disease; book: Eco-friendly Innovative Approaches in Plant Disease Management, Chapter: 10, Publisher: International Book Distributors, 2012;147-166.
3. Prashar P, Kapoor N, Sachdeva S. Isolation and Characterization of *Bacillus* sp. with In-vitro Antagonistic Activity against *Fusarium oxysporum* from Rhizosphere of Tomato. J. Agr. Sci. Tech., 2013;15:1501-12.
4. Dehariya K, Shukla A, Sheikh IA, Vyas D. *Trichoderma* and Arbuscular Mycorrhizal Fungi Based Biocontrol of *Fusarium udum* Butler and Their Growth Promotion Effects on Pigeon Pea. J. Agr. Sci. Tech. 2015;17: 505-17.

5. Xu XM, Jeffries P, Pautasso M, Jeger MJ. Combined Use of Biocontrol Agents to Manage Plant Diseases in Theory and Practice: A Review. Phytopathol. 2011;101:1024–31. https://doi.org/10.1094/PHYTO-08-10-0216
6. Samuels GJ. *Trichoderma*: a review of biology and systematics of the genus. Mycol Res. 1996;100:923-35. https://doi.org/10.1016/S0953-7562(96)80043-8
7. Harman GE. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol. 2006;96:190-94. https://doi.org/10.1094/PHYTO-96-0190
8. Woo SL, Scala F, Ruocco M, Lorito M. The molecular biology of the interactions between *Trichoderma* spp., pathogenic fungi, and plants. Phytopathol. 2006;96:181-85. https://doi.org/10.1094/PHYTO-96-0181
9. Tripathi P, Singh PC, Mishra A, Puneet S, Chauhan S, Dwivedi S, et al. *Trichoderma*: a potential bioremediator for environmental cleanup. Clean Technol. Environ. Policy. 2013;15:541-50. https://doi.org/10.1007/s10098-012-0553-7
10. Keswani C, Mishra S, Sarma B, Singh S, Singh H. Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. Appl. Microbial. Biotechnol. 2014;98:533-44. https://doi.org/10.1007/s00253-013-5344-5
11. Linderman RG. Role of VAM fungi in biocontrol, pp. 1–26, in F. L. Pfleger and R. G. Linderman (eds.), Mycorrhizae and Plant Health. APS Press, 1994; St. Paul, MN.
12. Miransari M. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol. 2010;12:563–69. https://doi.org/10.1111/j.1438-8677.2009.00308.x
13. Whipps JM. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can. J. Bot. 2004;82:1198–1227. https://doi.org/10.1139/b04-082
14. Lioussanne L. The role of the arbuscular mycorrhiza-associated rhizobacteria in the biocontrol of soilborne phytopathogens. Span. J. Agric. Res. 2010;8(S1):51–61. https://doi.org/10.5424/sjar/201008S1-5301
15. D'Alessandro M. *et al*. Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant Cell Environ. 2013;37:813–26. https://doi.org/10.1111/pce.12220
16. Berta G. *et al.* Maize development and grain quality are differentially affected by mycorrhizal fungi and growth-promoting pseudomonas in the field. Mycorrhiza. 2014;24:161–70. https://doi.org/10.1007/s00572-013-0523-x
17. Alejandro P. *et al.* The interactive effects of arbuscular mycorrhiza and plant growth-promoting rhizobacteria synergistically enhance host plant defences against pathogens. Scientific Reports. 2017;7:1-10. https://doi.org/10.1038/s41598-017-16697-4
18. FAO. The state of the world's land and water resources for food and agriculture. Rome, ICARDA. 2005, cucumber production in protected agriculture. Aleppo, Syria. 2011.
19. Greenlife Crop Protection Africa. Cucumber Production. 2019. Available from: https://www.greenlife.co.ke/cucumber-production
20. Simmons EG. Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. Mycologia. 1967;59:67-92. https://doi.org/10.1080/00275514.1967.12018396
21. Simmons EG. Alternaria, an Identification Manual. CB S Biodiv. Ser. 2007;6:1-775.
22. Bell DK, Wells HD, Markham CR. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology. 1982;72(4):379-82. https://doi.org/10.1094/Phyto-72-379
23. Wheeler BEJ. An introduction to plant disease. John Wiley and Sons. Ltd. London: 1970; 374p.
24. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121- 26. https://doi.org/10.1016/S0076-6879(84)05016-3
25. Kim K, et al. The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol /Fe (III)/O_2 mixed-function oxidation system. J Biol Chem. 1988;263(10):4704-11.
26. Shafique HA, Sultana RN. Ara J. Effect of endophytic *Pseudomonas aeruginosa* and *Trichoderma harzianum* on soil-borne diseases, Mycorrhizae and induction of systemic resistance in Okra grown in soil amended with *Vernoniaan thelmintica* (L.) seed's powder Pak. J. Bot. 2015;47(6):2421-26.
27. Howell CR, Hanson LE, Stipanovic RD, Puckhober LS. Introduction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* seed treatment with *Trichoderma virens*. Phytopathol. 2000;35:49-60.
28. Vinale F, Marra R, Scala F, Ghisalberti EL, Loritoand M, Sivasithamparam K. Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett. Appl. Microbiol. 2006;43:143-48. https://doi.org/10.1111/j.1472-765X.2006.01939.x
29. Tucci M, Ruocco M, De Masi L, De Palma M, Lorito M. The beneficial effect of *Trichoderma* spp. on tomato is modulated by plant genotype. Molecular Plant Pathology 2011;12:341–54. https://doi.org/10.1111/j.1364-3703.2010.00674.x
30. Reglinski T, Rodenburg N, Taylor JT, Northcott GL, Chee AA, Spiers TM, et al. *Trichoderma atroviride* promotes growth and enhances systemic resistance to *Diplodia pinea* in Radiata pine (*Pinus radiata*) seedlings. For. Pathol. 2012;42:75–78. https://doi.org/10.1111/j.1439-0329.2010.00710.x
31. Eman FS, Abd El-Aziz Q, El-Deeb M. Bio-recycling of shrimp shellby *Trichoderma viride* for production of antifungal chitinase. Afr. J. Microbiol. Res, 2012;6(21):4538-45. https://doi.org/10.5897/AJMR12.148
32. Shukla A, Dehariya K, Vyas D, Jha A. Interactions between Arbuscular Mycorrhizae and *Fusarium oxysporum* sp. *ciceris*: Effects on Fungal Development, Seedling Growth and Wilt Disease Suppression in *Cicer arietinum* L. Arch. Phytopathol. Plant Prot. 2014. https://doi.org/10.1080/03235408.2014.884831
33. Siameto EN, Okoth S, Amugune NO, Chege NC. Antagonism of *Trichoderma harzianum* isolates on soil borne plant pathogenic fungi from Embu District, Kenya. J. Yeast Fungal Res. 2010;1(3):47-54.
34. El-Fiki, AII, Mohamed FG, El-Deeb AA, Khalifa MMA. Some applicable methods for controlling sesame charcoal rot disease (*Macrophomina phaseolina*) under greenhouse conditions, Egypt. J. Phytopathol, 2004;32:87-101.
35. Metcalf DD, Wilson CC. The process of antagonism of *Sclerotium cepivorum* in white rot affected onion roots by *Trichoderma koningii*. Plant Pathol. 2001;50:249-257. https://doi.org/10.1046/j.1365-3059.2001.00549.x
36. Sharon E, Bar-Eyai M, Chet I, Hewrra-Estrella A, Kleifeld O, Spiegal Y. Biological control of the rootknot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathol. 2001;91:687-93. https://doi.org/10.1094/PHYTO.2001.91.7.687

37. Shoresh M, Harman GE, Mastouri F. Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. Annu. Rev. Phytopathol., 2010;48:21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

38. Sirin U. Determining the effects of *Trichoderma harzianum* and some mycorrhizal fungi on plant growth and against *Rhizoctania solani* Kühn in *Lilium* under in vivo conditions. J. Biotechnol. 2011;10(67):15142-50. https://doi.org/10.5897/AJB11.2444

39. Hammond-Kosack KE, Jones JDG. Resistance gene-dependent plant defense responses. Plant Cell, 1996;8:1773-91. https://doi.org/10.1105/tpc.8.10.1773

40. Katatny MH, Somitsch W, Robra KH, El-Katatny MS, Gubitz GM. Production of chitinase and B-1,3- glucanase by *Tridoderma harzianum* for control of the phytopathogenic fungus *Sclerotium rolfsii*. Food Technol. Biotechnol. 2000;38:173-80.

41. Jayalakshmi R, Raju S, Usha R. Sreeramula, K. *Trichoderma harzianum* L., as a potential source for lytic enzymes and elicitor of defense responses in chickpea (*Cicer arietinum* L.) against wilt disease caused by *Fusarium oxysporum* f. sp. Cicero. Aust. J. Crop Sci. 2009;1:44-52.

42. Gailite A, Steinite I, Ievinsh G. Ethylene is involved in *Trichoderma* induced resistance of bean plants against *Pseudomonas syringae*. Biology, 2005;691:59-70.

43. Hamid FR. Study efficiency of isolates of *Trichoderma* spp. In inducing resistance against *Rhizoctonia solani* in four variety of cotton. M. Sc. Thesis. Coll. Educ., Univ. Baghdad, Iraq: 2002. 233pp.

44. Hibar K, Daami M, El-Mahjoud M. Introduction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *Radices lycopersici* by *Trichodermas* spp. Tunisian, J. Pl. Protect. 2007; 2:47-58.

45. Pedranzani H, Rodríguez-Rivera M, Gutiérrez M, mycorrhizal symbiosis regulates physiology and performance of *Digitaria eriantha* plants subjected to abiotic stresses by modulating antioxidant and jasmonate levels. – Mycorrhiza. 20Porcel R, Hause B, Ruiz-Lozano JM. Arbuscular.16;26(2):141–52. https://doi.org/10.1007/s00572-015-0653-4

46. Chu XT, Fu JJ, Sun YF, Xu YM, Miao YJ, Xu YF, et al. Effect of arbuscular mycorrhizal fungi inoculation on cold stress-induced oxidative damage in leaves of *Elymus nutans* Griseb. - South Afri. J. of Bot. 2016;104:21–29. https://doi.org/10.1016/j.sajb.2015.10.001

47. Jiang QY, Tan SY, Zhuo F, Yang DJ, Ye ZH, Jing YX. Effect of *Funneliformis mosseae* on the growth, cadmium accumulation and antioxidant activities of *Solanum nigrum*. – Appl. Soil Ecol. 2016;98:112–20. https://doi.org/10.1016/j.apsoil.2015.10.003

48. Hashem A, Abd_Allah EF, Alqarawi AA, Al-Huqail A, Egamberdieva D, Wirth S. Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. - Saudi J. of Biol. Sc. 2016;23(2):272–81. https://doi.org/10.1016/j.sjbs.2015.11.002

49. Sarkar J, Ray A, Chakraborty B, Chakraborty U. Antioxidative changes in *Citrus reticulata* L. induced by drought stress and its effect on root colonization by arbuscular mycorrhizal fungi. - European Journal of Biological Research. 2016;6(1):1–13.

50. Singh BN, Singh A, Singh, BR. *Trichoderma harzianum* elicits induced resistance in sunflower challenged by *Rhizoctonia solani.* J. Appl. Microbiol. 2013;116(3):654-666. https://doi.org/10.1111/jam.12387

51. Guler NS, Pehlivan N, Karaoglu SA, Guzel S, Bozdeveci A. *Trichoderma atroviride* ID20G inoculation ameliorates drought stress-induced damages by improving antioxidant defence in maize seedlings. - Acta Physiologiae Plantarum. 2016;38(6):132. https://doi.org/10.1007/s11738-016-2153-3

52. Gajera HP, Katakpara ZA, Patel SV, Golakiya BA. Antioxidant defense response induced by *Trichoderma viride* against *Aspergillus niger* Van Tieghem causing collar rot in groundnut (*Arachis hypogaea* L.). - Microbial Pathogenesis. 2016;91:26–34. https://doi.org/10.1016/j.micpath.2015.11.010

53. Srivastava R, Khalid A, Singh US, Shama AK. Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* F. sp. lycopersici for the management of tomato wilt. – Biol. Control. 2010;53:24–31. https://doi.org/10.1016/j.biocontrol.2009.11.012

54. Yuan S, Li M, Fang Z, Liu Y, Shi W, Pan B, et al. Biological control of tobacco bacterial wilt using *Trichoderma harzianum* amended bioorganic fertilizer and the arbuscular mycorrhizal fungi *Glomus mosseae*. Biological Control. 2016;92:164–71. https://doi.org/10.1016/j.biocontrol.2015.10.013

55. Duc NH, Mayer Z, Pék Z, Helyes L, Posta K. Combined Inoculation of Arbuscular Mycorrhizal Fungi, *Pseudomonas* Fluorescens And *Trichoderma* Spp. For Enhancing Defense Enzymes and Yield of Three Pepper Cultivars. Appl. Ecol. and Env. Res. 2017;15(3):1815-29. https://doi.org/10.15666/aeer/1503_18151829