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ATTEMPTS TO ASSESS THE ROLE OF SOME BIOCONTROL AGENTS IN REDUCING CORN FUNGAL INFECTION AND IMPROVING ITS GROWTH PARAMETERS

Medhat S. Abd-Rabboh[1] and Yasser M. Abdel-Shafea[2*]

[1]Plant Pathology Research Institute, (ARC) Giza, Egypt.
[2]Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, EGYPT.
***Correspondence: E-mail:Yasser_773@hotmail.com**
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ABSTRACT

Fungal and bacterial plant diseases cause major losses in agricultural activities and food production through damaging plants and/or crops. This study was conducted to evaluate the antagonistic effect of some fungal, bacterial and yeast biocontrol agents (BA) (*Trichoderma harzianum*, *Pseudomonas fluorescens,* mycorrhizae *Glomus* sp. and *Saccharomyces serivisae*) and a mixture of them against damage of corn (*Zea mays* L.) caused by three isolates of *Fusarium moniliforme*. In the current experiment, significant reduction in the disease incidence such as number of infected plants and ears was observed in BA treatments in comparison with untreated control accompanied with enhancement of growth features such as plant height and weight, root length and weight and average weight of ears. The obtained results revealed that applying of Mycorrhizae *Glomus sp.* and *Sacchromyces serivisae* (Yeast) together showed significant superior effect to reduce diseases incidence and improving growth parameters followed by the BA mixture then *Trichoderma harzianum* and *Pseudomonas fluorescens,* respectively. Treatments increased peroxidase and polyphenol oxidase in corn leaves. Mycorrhizae was the most effective in this respect.

Key words: Biocontrol agents (BA), *Fusarium moniliforme,* Corn, Mycorrhizae, oxidase enzymes, *Pseudomonas fluorescens, Trichoderma harzianum* and Yeast.

INTRODUCTION

Maize (*Zea mays* L.), also called corn, it is believed to have originated in central Mexico 7000 years ago, it is grown throughout the world. It has been recognized worldwide as a major energy feed ingredient in the diets of poultry (***Dei, 2017***). About 65% of the corn grown worldwide is used for livestock feed (**FAO, 2005**). The stalks, leaves and immature ears are used as forage for ruminants (***Leonard and Martin, 1963***). Corn is recognized as giving the highest conversion of dry matter into meat, milk and eggs in relation to other cereal grains (***Kling, 1991***). Corn was considered as the third highest important staple food crop after wheat and rice by area and production in Egypt which grown in the summer or at late-summer. In Egypt, the total cultivated area of corn in 2018 was, about 1.05 million hectares (***Agricultural Statistics, 2019***). Its production is estimated to increase by 161 million ton to 1.2 billion ton by 2027 (***OECDFAO, 2018***). Corn is subjected to various types of diseases, mainly caused by fungi which minimize the quality of grains and may also produce mycotoxins that cause significant health hazards in humans and animals (***Li et al., 2019***). ***Mathur and Manandhar (2003***) listed several fungi on corn seeds as seed-borne in different countries, which include 82 species belonging to 43 genera; they added that in Egypt, *Fusarium moniliforme* and species of *Aspergillus*, *Penicillium* and *Rhizopus*. Fusarium ear rot and root rot and wilt are severe diseases caused by *F. oxysporum* and other *Fusarium* spp. which is the most destructive abundant disease associated with corn grains worldwide (***Lakshmanan and Slevaraj, 1986 and Leslie and Summerell, 2006***). In Egypt there are several plant diseases such as common smut, leaf blights, ear rot, stalk rot, bacterial stalk rot and late wilt. Fungal disease is the most important disease that affects the yield crop and cause considerable damage under the Egyptian conditions. Yield losses of susceptible plant may reach about 40% of the grain yield (***Samra et al., 1972***). It reduces output in corn by 10% typically and by 30-50% in severely affected areas, which characterized by discolored and a reduced number of grains, yield as well as the quality of the seeds (***Gai et al., 2018***). Pathogens can be survived in infected corn seed without causing apparent symptoms or killing seed tissues (by producing toxic molecules and lytic enzymes) and subsequently transmitted to growing seedlings causing blights and root, stem and ear rot diseases. Under field conditions, the pathogen is systemically transmitted easily through infected seeds to corn growing seedlings by transmitting through stalk up to the ear (***Thompson and Raizada, 2018***). Controlling corn diseases with chemical fungicides become a not good choice. Using of fungicides is costly, dangerous for human, animal health and environmentally toxic therefor strict regulations on using chemical pesticide and a pressure to remove the most hazardous chemicals from the market were developed (***Abd-Rabboh, 2006 and Al-Huqail et al., 2019***). The application of biocontrol agents (BA) as antagonistic microorganisms has proved to be

successful for controlling various plant diseases in many countries (***Sivan, 1987***). It can serve as the best control measure under greenhouse conditions. *Trichoderma* spp. were well documented as effective BA of plant diseases caused by soil-borne fungi (***Sivan and Chet, 1986, Whipps and Lumsden, 2001 and McLean et al., 2004***). BA was considered as a safe environmental application which increase food production and decrease the use of chemical fertilizers, pesticides, and other artificial supplements (***Esitken et al., 2005; Bhattacharyya and Jha, 2012***). Bacteria isolated from plant rhizosphere or rhizobacteria such as *Pseudomonas fluorescens* and its pathogenic antagonistic role has been studied for decades (***Boddey et al.,*** **1995**). These associative bacteria are considered as plant growth-promoting rhizobacteria because of their ability to stimulate plant growth through mechanisms such as biological N fixation, growth-regulating substance production, mineral and nutrient solubilization, increased root volume, and systemic induction of pathogen resistance (***Dobbelaere et al.,*** **2003**). The most important mechanism of *Trichoderma* spp. is the induction of plant defense response to specific pathogens (***Harman 2006; Inayati et al. 2020).*** Other than chemical and physical obstructions, plants have immune systems. The system is able to identify pattern that contain common structural features of all microbes but not present in their host plants. The defense response of plants is rapid, transitory and generalized. During biotic stress, host plant shows various cellular and physiological changes such as ion influx across the plasma membranes; activation of nitric oxide, defense-related genes; high production of ROS (reactive oxygen species), different phytohormones; biosynthesis of specific stress related proteins and production of antimicrobial chemicals such as phenolics (***Wu et al. 2014; Nishad et al. 2020***). Different biocontrol organisms may cause distinct molecular and cellular transformations in plants that enhance the resistance to biotic and abiotic stress (***Brotman et al. 2013; Kumar 2013).*** *The* activity of defense-related enzymes such as polyphenol oxidase (PPO) and peroxidase (PO) progressively enhanced in plants when inoculated with *T. viride* alone or in combination with *Pseudomonas fluorescens* against *M. phaseolina* (***Thilagavathi et al. 2007***). PO enzymes have been involved in the production of highly toxic compounds that are antifungal in nature, in the production of melanin and melanin like pigments (***Li, 1981***) The PO, and PPO enzymes are responsible for the production of phenolic compounds which contribute to the reinforcement of cell barriers ***(Souguir et al. 2011*****)**

Arbuscular Mycorrhizae (AM) is a nonpathogenic fungi which has a symbiotic relationship with plant root, formed by nearly all terrestrial plants roots and characterized by bidirectional transfer of nutrients between the plants and associated fungi, where plants provide sugar to the fungi and these help the plants on the acquisition of mineral nutrients from the soil (***Smith and Barker*** **2002**). AM increase uptake of organic and inorganic phosphorus from the soil and also, uptake of some trace elements like zinc and copper (***Oudeh et al., 2002***), potassium and magnesium (***Sharma et al., 2002)*** Several studies

indicated that AM can stimulate plant growth (***Gill et al.,* 2002**) and crops yield production (***Xavier and Germida, 2002***) by increasing the absorption of plant nutrition that is not normally available to non-mycorrhizael plants (***Clark,* 2002**). Hyphae of AM fungi are considered to contribute to soil structure by increasing aggregates and their stability (***Caravaca et al.,* 2002**), soil fertility (***Eriksson, 2001***) and soil reclamation (***Barea et al.,* 1996**). The objective of this study was to evaluate the ability of some antagonistic fungal and bacterial agents (*Trichoderma harzianum*, *Pseudomonas fluorescens, Glomus sp., Saccharomyces serivisae* and a mixture of them) to improve the growth and yield of corn and decrease the plant infection caused by three isolates of *Fusarium moniliforme* fungal corn pathogens under open greenhouse conditions.

MATERIALS AND METHODS

Plant materials:

Corn (*Zea mays* L.) seeds of local maize cultivar (Three ways cross 310) were used in this study from central administration for seeds agricultural research center (ARC), Giza, Egypt.

I. Isolation and identification of the pathogenic organisms:

The casual pathogenic organisms were isolated from naturally infected corn samples collected from three different districts (El Monofeya, El Sharkia and Pany souief) and labeled as *Fusarium moniliforme* (1, 2 and 3). Samples were surface sterilized with alcohol and flamed. The cortex was peeled off and small pith was cut aseptically and plated on potato Dextrose agar (PDA) medium supplemented with 0.1% yeast extract and incubated at 30 ºC for 5 days. Hyphal tip and single spore techniques were applied for fungal purification the isolates were sub cultured on PDA medium. The isolates were identified using cultural characteristics and morphology with reference to **De *Hoog et al.* (2000)** *and* ***Jay* (1998)**.

II. Biological control:

Isolation of the BA (Biocontrol Agents) microorganisms:

Healthy plants from epidemic fields were carefully uprooted and their roots were separated, transferred to sterile container and shaken to remove the excess soil from the root surfaces. One gram of soil dried in oven at 120 ºC for two hours, then used for isolation of biocontrol agents by adding in a test tube with 10.0ml sterile distilled water (SDW) to give 1×10^{-1} and diluted to 1×10^{-6}. For microbial isolation one ml from each of the following suspensions i.e., 1×10^{-1}, 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5} and 1×10^{-6} was poured in Petri dishes contained (PDA) medium. Suspensions were separated on the medium surface and the supernatant was eliminated and then incubated at 29^{o}C. Bacterial isolates were transferred after 24 to 48 hr., whereas fungal isolates were transferred after 3-5 days. The isolated biocontrol fungal isolates were identified according to the description given by **Rifai (1969)**. Bacterial isolates were

identified at Bacterial Diseases Department, Plant Pathology Institute. Agricultural Research Center Giza-Egypt.

III. Pathogenicity test:

For preparation of the fungal inoculate to the pathogenicity test, sorghum grains were washed, filled in 1.0 liter bottles at the rate of 250 g/bottle, then moistened with suitable amount of tap water and then autoclaved at 1.5 kg/Cm^2 for 30 min., sorghum grain medium was inoculated with pathogenic fungi isolates (*Fusarium moniliforme*). Each isolate was inoculated in ten bottles and incubated at 30 ºC for 20 days. The inoculum of each isolate was mixed with autoclaved soil [at the rate of 3: 100 (w/w), four pots were assigned for each treatment, and each pot was planted with five seeds of the obtained cultivar. Four un-inoculated pots were assigned for check treatment sown at the same rate.

IV. Laboratory experiment:

Relationship between the causal pathogenic fungi *F. moniliforme* isolates (1, 2 and 3) and BA (*Trichoderma harzianum*, *Pseudomonas fluorescens)* were studied. Petri dishes (9 cm in diameter) containing PDA medium were used in these trials. Five mm discs of six days old culture of 3 pathogenic fungi isolates were inoculated onto peripheral side of the PDA plate and same diameter disc of 5 days-old culture of the tested BA were inoculated onto the other side of the plate. Plates were incubated at 30 Cº for 6 days. The radial growth of pathogenic fungi was measured at two dimensions and then mean were estimated in mm.

V. Pathogenicity test of the causal fungi against corn in pots.

A. Under field conditions:

Effect of three BA (*T. harzianum, P. fluorescens* Mycorrhizae *Glomus* spp. and *Saccharomyces cerevisiae*) and a mixture of them on corn under field conditions were studied. These experiments were conducted at Zennara, Tala, Monofia at the 4^{th} week of May 2020. Ten m long 75 cm row spacing, 25 cm between hills and 2-3 seed/hill. 4 rows each one contain 100 plants on row for each treatment. Results were collected after 45 days and 90 days.

B. Under greenhouse condition:

In this experiment, 4 pots were used for each treatment and five seeds of Trihybrid 310 corn seeds were cultivated in each pot (30.0 cm in diameter) and sown in each pot. The tested fungi were grown on sterilized sorghum grains as mentioned before. The tested inoculum of both pathogens and the biocontrol fungi were prepared by mixing each of them at the rate 1:1 (w:w) then added to autoclaved soil at rate of 3%, 15 days before sowing. Results were obtained and investigated after 80 days. While bacterial strain inoculated in liquid pepton medium 200 ml/ 50 ml flask) and incubated at 27 ºC for 5 days. The inoculum was added to the potted soil at the rate of 1 flask per pot (2 x 107cell/ml), 15 days before sowing.

VI. Disease control using mycorrhizae:

Isolation and identification of vascular arbuscular mycorrhizae spores:

Soil samples were collected from rhizoplane of different crops. 100 gram soil sample was first stirred thoroughly in a bucket containing 5 L water and then allowed to settle for 15 sec. through 0.5 mm sieve into a second bucket, in which the suspension was swirled vigorously and again allowed to settle for another 15 sec., the supernatant was poured through a 0.036 mm sieve and the trapped material was washed into a beaker. After stirring, the material was transferred to 100 ml centrifuge tubes and centrifuged for 4 minutes at 3000 rpm. The supernatant was replaced with 5% sucrose solution. The tubes were stirred and then centrifuged for 15 seconds at 3000 rpm. The resulting supernatant was poured through a 0.036 mm sieve and finally the residue on the sieve was washed with water to remove the sucrose solution. The residue was washed into petri dishes and examined under a binuclear microscope. The isolated fungi were identified according to ***Gerdeman and Trappe (1979)***.

Preparation of mycorrhizael inoculum:

A mycorrhizal fungal inoculum belonging to *Glomus* genera was prepared by embedding in agar water medium in petri dishes. Agar medium which carries mycorrhizal spores of every petri dish was divided to small pieces 1 cm^2, each piece was inoculated to each pot. Five gm of yeast (*Saccharomyces cerevisiae*) containing $5x10^{12}$ yeast cells were applied together with AM to stimulate the growth of corn.

Effect of mycorrhizae against three of causal pathogenic fungi in greenhouse:

A pot experiment was conducted in corn disease department, agricultural research center, Giza, Egypt. Pots [30cm in diameter] were filled with Nile silt soil, previously individual infested with the tested pathogenic fungi were used. Two pieces of water agar medium (contained 60 Chlamydospores of mycorrhizal fungus) were added to each pot. Control treatment was carried out without adding the Chlamydospores. Six corn seeds were sown at each pot. Infection percentage was calculated after 80 days from sowing.

VII. Determination of some physiological characters

Peroxidase (PO) activity:

The procedure for determining the activity of peroxidase was adopted from ***Fehrmann and Dimond*** **(1967)**. Approximately 0.5 g fresh leaves of treated or non-treated (control) corn leaves ground in a pre-chilled mortar with 0.1 *M* ice cold phosphate buffer (20 mL) at pH 7.1. Later on, it was kept for centrifugation (3000 rpm) for 15 min. The supernatant (25 mL) was used for assay. Freshly prepared pyrogallol, reagent, enzyme extract and phosphate buffer were mixed in a cuvette tube and the blend was tuned to zero absorbance on a spectrophotometer. The activity of enzyme was measured as the alteration in absorbance per minute (ΔA/min) at 430 nm.

Polyphenol oxidase (PPO):

Enzyme extract (0.5 mL) and 0.1 *M* phosphate buffer (2.3 mL) were added to a cuvette, which was adjusted to zero absorbance on a spectrophotometer

(*Mahadevan and Sridhar 1982)*. A 0.2 mL aliquot of 0.1 *M* catechol was added and then reactants were rapidly mixed. The activity of enzyme was noted as variation in absorbance instantaneously after adding 0.1 *M* catechol (0.2 mL).

VIII. Physiochemical analysis of soil:

Soil samples were taken from 30 cm depth then air-dried and sieved (< 2 mm sieve) before analyses. Soil pH was measured in deionized water (1:1w/v) with a pH meter (Orion, 710A), while electrical conductivity (EC) was measured in a 1:5 soil:water solution with an EC-meter (Orion, 5 star) (***APHA,* 2005**). Inorganic anions (Phosphate, sulphate, chloride, nitrate and nitrite concentrations (mg/L) were determined according to (***USEPA 300, 1993 &USEPA 300.1, 1997)*** by using Thermo Scientific Dionex ICS-5000+ Ion Chromatography supplied with Dionex, Ion Pac AS23 analytical column protected by Dionex Ion Pac AS 23 guard column.

IX. Statistical analysis:

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between applied treatments and disease incidence. A general linear model option of the analysis system SAS (***SAS, 1996***) was used to perform the ANOVA. Duncan's multiple range tests at $P < 0.05$ level was used for means separation (***Winer*, 1971**).

RESULTS AND DISCUSSION

Corn is one of the most important crops all over the world for its essential uses in human consumption, animal feeding and industrial uses. Several fungal diseases infect corn crops from seedling to maturity stages. The majority of them are spread by seeds or transmitted from the soil, resulting in lower grain quality.

1- I*n vitro* laboratory experiment:

The antagonistic potential of BA was assessed against the fungal pathogens by dual culture assay. The data presented in Table (1) showed that significant reduction in Radial mycelial axis growth of pathogenic fungi (*F. moniliforme)* 3 isolates with a variable ratio after 6 days of incubation at 30 ºC against tested BA (*T. harzianum* followed by *P. fluorescens*) through creation of an inhibition zone and eventual over-growth. Such findings are consistent with those of ***Rasmy, 1991 and Xu et al.*, 1999.** As compared with control, also, data showed that *T .harzianum* had the greatest reduction zone than that of *P. fluorescens* with all pathogenic fungi. The growth reduction percentages were (89.63, 89.78 and 87) % for *F. monilifome* (1, 2 and 3) respectively. ***Gomathinayagam et al.* (2010)** reported that *Trichoderma* spp. was present in all soil and it was the most cultural fungi. *Trichoderma* species are strongly antagonistic to other phytopathogenic fungi. They produce hydrolytic enzymes which are believed to play an important role in the parasitism of phytopathogenic fungi. Some bioagents affect the pathogen by producing its metabolites in the medium, such as Tricodermin, Gliotoxin and some other antibiotics. These results are in harmony with those obtained by ***Pieta** and*

Pastucha (1994) *and* ***Abd-El-Khairet al. (2011)*** *and* ***Ragab et al. (2015*****).** They reported that, *T. album*, *T.hamatum*, *T. harizianum*and *T. viride* significantly reduced the mycelial growth of *R. solani*. ***El-Mougy et al.,*** **(2013)** reported that, *P. fluorescens* and *T. harzianum* reduced the growth area more than 90.6 and 87.4% for *F. solani* and *R solani*, respectively. The *P. fluorescens* has been reported by various researchers to restrict the growth of microorganisms causing disease *in vitro* (***Thomashow*** *and* ***Weller, 1988; Hebbar et al., 1992; Rosales et al., 1995****).* Several mechanisms could be suggested to interpret antagonistic potentiality of the tested antagonists. For example the ability to produce lytic enzymes (**Fridlender *et al., 1993***), antibiotics (***Bender et al.,*** **1999**), volatile compounds (***Claydon et al.,*** **1987; *Bakker*** and ***Schippers, 1987***) and phytotoxic substances (***Hoagland*** and ***Cutler, 2000***). In the case of bacterial antagonists, it has been suggested that associated with the production of antibiotics (***Bull et al., 1997*** and ***Janisiewicz et al., 1991***).

1- Field experiment

Data tabulated in Table (2) represent the effect of BA on corn growth parameters (Plant height, root length, root weight and stalk weight) under the field conditions after 45 days of sowing. Data revealed that the most superior effects were obtained by mycorrhizae and *S. serivisae* together on all growth parameters followed by the BA mixture, then *T. harzianum*, while the least effect was obtained by *P. fluorescens* comparing with control.

After 90 days from sowing, the growth parameters results tabulated in Table (3) were collected as an average of 10 plants revealed that there was a positive correlation between efficiency of BA on plant infection and growth vegetative (plant height and weight) mycorrhizae and *S. serivisae* and BA mixture still have the greatest effect on Plant height 209 cm and 195.8 cm, respectively and plant weight with 552.2 gm and 552.6 gm respectively followed by *T. harzianum* 522.4 gm for plant weight and 188.4 cm for plant height then *P. fluorescens* with 501.8 gm for plant weight and 181.2 cm for plant height comparing with control. Moreover, mycorrhizae and *S. serivisae* and BA mixture completely prevent plant and ear infections with 0% followed by *T. harzianum* then *P. fluorescens* comparing with control. Also, AM and BA mixture showed the best results in number and weight of ears per plant, whereas they gives 3 ears per plant with the greatest average weight with 915 gm/plant and 930gm/plant, respectively while the lowest effect was obtained by *P. fluorescens* with 525 gm/plant comparing with control. These results may be attributed to the role of AM fungi in increasing the activities of beneficial soil microorganisms, like N fixers and P mobilizers, and microorganisms in plant rhizosphere, and consequentially the uptake of N, P, and K promoted plant growth **(*Karthikeyan et al., 2007, 2008*).** Morphological root changes in mycorrhizae treated plants led to increasing the absorptive surface area of the whole host root system to water
and mineral nutrients supply,

particularly P, so that can improve biological N -fixation in soil ***Soliman et al., (*2011).** The increase in plant biomass by AM inoculation has been reported for perennial medicinal plants like palmarosa and kalmegh **(*Gupta and Janardhanan, 1991 and Arpana and Bagyaraj,* 2007**, respectively). Various mechanisms have been suggested for the nutrient uptake by AM plants, including the external hyphae of AM fungi which allow the root system to exploit a greater volume of soil nutrients (i) by extending away from roots and translocating nutrients from some distance to the rhizosphere and (ii) by exploiting smaller soil pores not reached by root hairs and adding surface area to adsorption system (**O'Keefe and Sylvia, 1990**). *Trichoderm* spp., a microorganism that can promote plant growth in rhizosphere is known to stimulate

producing growth regulators (***Jaleel et al., 2007; Karthikeyan et al., 2*010**).

2- Greenhouse experiment

Data in Table (4) clarify that the best results were achieved after 80 days of sowing in green house experiment in reducing the percent of infection caused by the *F. moniliforme* in corn were obtained when applying mycorrhizae and *S. serivisae* together and BA mixture with 0% against all pathogenic fungi, while antagonistic effects of *P. fluorescens* against *F. moniliforme 1, 2 and 3* gives (0, 20 and 20%) respectively. Thereafter, *T. harzianum* (15, 20 and 25%) with the same fungi respectively.

Enzymes activity:

The antagonistic effect of BA on the activity of PO and PPO enzymes in the infected plants by *F. moniliforme* are illustrated in **Table (5)**. The obtained results showed that PO and PPO activity in the infected plants seemed to be higher than that of the healthy plants. The treatment of BA especially mycorhhiza (1.005 and 0.648) significantly induced antioxidant defense enzymes in the plants leaves when compared with the untreated healthy plants, thus this may alleviating *F. moniliforme* oxidative damage and suppressing blast disease incidence. PO and PPO enzymes plays a significant role to initiate the plant defense response against various pathogens through production of highly toxic phenolic compounds and higher production of reactive oxygen species or establishment of structural barriers such as lignin accumulation (***Yusnawan et al. 2019; Inayati et al. 2020***).

Table (1): In vitro evaluation of biocontrol agents antifungal activity on radial mycelial growth of *F. moniliforme.*

Biocontrol / Fungi	Control (cm)	*P. fluorescens*		*T. harzianum*		LSD
		(cm)	% of reduction	(cm)	% of reduction	
F. moniliforme 1	8.97±0.09^{c}	3.03±0.03^{a}	66.22	0.93±0.03^{b}	89.63	0.20
F. moniliforme 2	9.10±0.06^{c}	2.80±0.06^{a}	69.23	0.93±0.02^{b}	89.78	0.18
F. moniliforme 3	9.00±0.06^{c}	2.13±0.03^{a}	76.33	1.17±0.03^{b}	87	0.15

Within the same row, various superscript letters indicate significant differences (Duncan, $P < 0.05$), Data are expressing as means ± SE.

Table (2): Effect of biocontrol agents on corn growth parameters in field after 45 days of sowing.

Parameters / Treatment	Plant height (cm)	Root length (cm)	Root weight (gm)	Stalk weight (cm)
mycorrhiza & *S. serivisae*	91.5±1.79^{a}	16.8±0.37^{a}	9.56±0.24^{a}	107.0±0.45^{a}
P. fluorescens	85±0.84^{c}	15.4±0.24^{b}	8.14±0.51^{d}	102±0.51^{c}
T. harzianum	82.5±0.84^{b}	15.6±0.24^{b}	8.28±0.20^{c}	104±0.87^{b}
BA mixture	91.2±1.67^{a}	17.2±0.20^{a}	9.44±0.24^{b}	107±0.95^{a}
Control	80.3±0.89^{d}	13.6±0.24^{c}	7.36±0.40^{e}	98±2.39^{d}
LSD	1.69	0.79	1.00	3.69

Within the same column, various superscript letters indicate significant differences (Duncan, $P < 0.05$), Data are expressing as means ± SE.

Table (3): Corn growth parameters in field after (90 days) from sowing

Parameters / Treatments	Total plants	Plant height (cm)	Plant weight (gm)	Infected plants (%)	Infected ears (%)	Ears				
						Ears No	Average weight of ears (gm)			
							1	2	3	Total weight
myco & *S. serivisae*	73	209±0.45[a]	552.2±1.98[a]	0.0	0.0	3	380	320	215	915
P. fluorescens	70	181.2±0.97[d]	501.8±1.02[c]	5	1	2	305	220	-	525
T. harzianum	73	188.4±0.51[c]	522.4±1.72[b]	3	1	2	325	210	-	535
BA mixture	73	195.8±0.58[b]	552.6±1.60[a]	0	0.0	3	370	330	230	930
control	70	171.8±0.92[e]	422.0±2.10[d]	6	5	2	280	210	-	490
LSD		2.12	5.09							

Within the same column, various superscript letters indicate significant differences (Duncan, *P <0.05*), Data are expressing as means ± SE.

Table (4): Infection of *F. moniliforme* isolates on corn plants after 80 days from sowing

Treatments	Pot 1		Pot 2		Pot 3		Pot 4		% of survival	% of infected	% of not germinated
	survival	infected	survival	infected	survival	infected	survival	infected			
mycorrhiza&yeast	5	0	4	0	4	0	5	0	90	0	10
T. harzianum	5	0	5	0	4	1	4	0	90	5	5
P. fluorescens	4	0	5	0	5	0	4	1	90	5	5
Mix	5	0	4	0	5	0	4	0	90	0	10
mycorrhiza&yeast& F.moniliforme1	4	0	4	0	4	0	5	0	85	0	15
T. harzianum&F. moniliforme1	4	1	4	1	4	0	4	1	80	15	5
P. fluorescens& F. moniliforme1	4	0	4	0	5	0	5	0	90	0	10
BA mixture & *F. moniliforme1*	4	0	4	0	5	0	4	0	85	0	15
mycorrhiza&yeast& F. moniliforme2	5	0	4	0	4	0	4	0	85	0	15
T. harzianum& F. moniliforme2	3	1	3	1	2	1	3	1	55	20	35
P. fluorescens& F. moniliforme2	3	1	3	1	3	1	3	1	60	20	20
BA mixture & *F. moniliforme2*	4	0	4	0	5	0	4	0	85	0	15
mycorrhiza&yeast& F. moniliforme3	5	0	4	0	5	0	4	0	90	0	10
T. harzianum& F. moniliforme3	4	0	4	1	3	2	3	2	70	25	5
P. fluorescens& F. moniliforme3	4	1	3	1	3	1	3	1	65	20	15
BA mixture & *F. moniliforme3*	4	0	4	0	4	0	4	0	80	0	20
F. moniliforme1	2	3	1	4	2	3	1	3	30	65	5
F. moniliforme2	2	2	2	2	2	3	2	3	40	50	10
F. moniliforme3	1	3	1	4	1	4	1	4	20	75	5
Control	2	3	2	3	2	3	2	2	40	55	5

Table (5): Effect of biocontrol agents on the activity of some oxidase enzymes in leaves of corn plants infected by *F. moniliforme* samples were taken after 80 days from sowing.

Enzymes / Treatment	Peroxidase D.D/gwt a. 3 min.	Polyphenol oxidase D.D/ gFwt a. 45min
Mycorrhiza & Yeast	1.005	0.648
BA mixture	0.885	0.386
Pseudomonas fluorscens	0.774	0.285
Trichoderma harzianum	0.999	0.513
Infected plants	0.835	0.260
Control (halthy plants)	0.294	0.150
L.S.D. at 5%	0.866	0.0790

Table (6): Chemical properties of soil before the biocontrol agents application (Initial) and at harvest (Final)

Soil parameters	Control		Biocontrol agents	
	Initial	Final	Initial	Final
PH	7.9 ± 0.0	8.2 ± 0.0	7.9 ± 0.0	8.0 ± 0.2
EC (µS cm^{-1})	826.0 ± 46.0^{bc}	466.0 ± 98.0^{a}	697.0 ± 57.0^{ab}	1078.0 ± 98.0^{c}
No_3^- (ppm)	306.0 ± 51.0^{b}	56.2 ± 7.3^{a}	319.0 ± 45.0^{bc}	514.0 ± 87.4^{c}
$No2^-$ (ppm)	2.8 ± 0.3^{b}	1.1 ± 0.2^{a}	4.1 ± 0.2^{c}	0.9 ± 0.1^{a}
Cl^- (ppm)	260.0 ± 29.0^{b}	40.1 ± 24^{a}	251.0 ± 49.0^{b}	378.0 ± 71.0^{c}
$Po4^{-3}$ (ppm)	28.3 ± 0.8^{a}	29.4 ± 5.6a	12.8 ± 2.0^{b}	8.6 ± 0.9^{c}
$So4^{-2}$ (ppm)	1212.0 ± 94.0^{c}	629.0 ± 380.0^{a}	981.0 ± 62.0^{b}	1216.0 ± 117.0^{c}

Values are the mean ± standard error (n=3). Means with different superscripts letters in the same row are significantly different ($P<0.05$).

Table (6) shows the soil properties before the BA application (initial) and after harvest (final). The EC values of
soils (876 µS cm-1 for control plots and 697µS cm-1 for treated plots); however, significant differences were observed in the final sampling between treatments in which the control and BA plot values decreased (466µS cm-1) and increased (1078µS cm-1), respectively. Given that the same volume and quality of irrigation water was applied to all plots, these results suggest that the BA probably promotes salt solubility, especially of chlorides. The pH values, which ranged from 7.9 to 8.0, were similar during the experiment and were not affected by the BA application. These results are similar to those reported by ***Mukhtar et al.* (2017)**, who found no differences in pH after applying phosphate-solubilizing biofertilizer to a wheat crop. According to ***Chen et al. (2004)*** *and* ***Hayat et al. (2010*)**, rhizobacteria promote phosphate solubilization by increasing the soil available P concentration. The primary mechanisms of P solubilization are H^+ excretion, organic acid production, and acid phosphatase biosynthesis **(*Behera et al.*, 2014).** Mycorrhiza fungi may infect plants in low Phosphorus soils easier than those in the high-P soils, and fungal species varied in the ability to adapt to different soil P contents **(*Miller et al., 1995)***.

Several researches showed the ability of *Trichoderma* to attack different fungi as shown by ***Durrell,* (1968)**. Which found that *T. harzianum* was an effective biocontrol agent for protecting a number of crop plants from damage induced by naturally infested soil with S. *rolfsii* and *R. solani* under both greenhouse and field conditions. It was capable of directly attacking and lysing both pathogens in culture. Moreover, ***Elad et al.* (1980)** found that application of *T. harzianum* in greenhouse experiments reduced disease incidence in bean seedlings by S. *rolfsii* up to 97% and *R. solani* 57%. Also under field conditions, *T. harzianum* significantly increased the yield and decreased disease incidence. Furthermore, the results obtained by ***(Elad,* 2000a, 2000b)** showed that the application of *B. subtilis* and *T. harzianum*, exhibited a significant antifungal activity (85–100%) against the fungal molds *in vitro* and *in vivo*, depended on isolate and host plant. Antagonistic effect of BA could be inhibit pathogens by antibiosis, some bioactive metabolites, and active degradation-related enzymes, which play crucial roles in inhibiting *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Also, *Trichoderma* spp. may inhibit fungal growth by three mechanisms: competition (for space and nutrients), parasitism (deriving nutrients from the host); and antibiosis (production of an inhibitory metabolite or antibiotic) ***Harman,* (2006)**. While one mechanism may predominate, this does not exclude the possibility that one or both of the other two mechanisms may also play a role in the antagonistic behavior. ***Angelica et al. (*2001)** tested several *Trichoderma* spp. and reported that all strains produced the enzyme amylase, which is partially responsible for the rapid growth of *Trichoderma* spp. on potato dextrose broth medium. *Trichoderma* spp. can also induce systemic and localized resistance as well as directly attacking or inhibiting the growth of plant pathogens **(*Harman et al., 2004 and Lo et al.*, 2000)**. In addition, certain *Trichoderma* strains have substantial influence on plant growth and development **(*Hedge and Hofreiter, 1962)***. In most cases, it is impossible to separate direct effects on plant growth from the control of

pathogenic or other deleterious microorganisms that reduce plant growth. However, there were reports indicating that *Trichoderma* spp. could also have the potential to stimulate plant growth independent of any plant disease **(*Ozbay and Newman, 2004)***. ***Windham et al. (*1986)** concluded that the *Trichoderma* spp. produced a growth-regulating factor which increased the rate of seed germination and dry weight of shoots and stems. ***Baker* (1988)** stated that plant growth responses induced by *Trichoderma* spp. appeared to be due to both the control of minor pathogens and production of a growth-regulating factor. ***Harman et al. (2004*)** indicated that *Trichoderma* spp. have evolved as opportunistic plant symbionts. So, application of *T. harzianum* in plant production may reduce the use of fungicides, growth regulators and labor which eventually will lower the production costs and environmental impact.

Mycoparasites may produce an array of cell wall lytic enzymes, such as gluconases, chitinases, protease, lipases **(*Chet, 1987*)** or antibiotic metabolites such as gliotoxin and glioviridin**(Di *Pietro et al.,* 1993)**. The chitinolytic and glucanolytic enzymes or the combination of lytic enzymes and toxins of the mycoparasites usually act synergistically rather than alone **(*Chiu and Tzeam, 1995)***. ***Singh et al.* (2018)** found that BA as *P. fluorescens* alleviated stalk rot of sorghum as well augmented plant growth. Use of BA protects environment by avoiding superfluous accumulation of chemicals, preventing residue accretion and pollution. This is supported by results found by ***Chen et al. (2004)*** *and* ***Hayat et al. (2010*),** who found that rhizobacteria like *P. fluorescens* promote plant development through mechanisms such as phytohormone production and pathogen biocontrol that help crop development and health.

Among the 3 biocontrol agents tested under both greenhouse and filed conditions, Application of AM fungi and yeast together had the greatest effect on decreasing percentage of plant infection and increase plant growth parameters (Plant height, root length, root weight and stalk weight) simultaneously. AM inoculation increased also, phosphate-solubilizing microorganisms that cause an inhibitory effect on pathogens development through releasing organic acids which are often accompanied with the release of other metabolites, mainly siderophores, phytohormones and lytic enzymes ***Mukerji and Ciancio (2007) & Akhtar and Siddiqui (2008*).** In addition, the increased phenolic compounds, phytoalexins, lignin, phenols, sugars and amino acids (phenylalanine and serine) in mycorrhiza treated plants have been suggested to play an important role in the plant defense mechanism ***Zhang et al. (*2008)**. AM have a direct effect on root morphology and plant growth through producing some mycorrhizal fungi (indole-acetic acid, gibberellin, Zeatin, Abscisic acid) ***Ludwing- Muller, 2000*** *and* ***Liuet al.,* 2002**). The hyphae associated with mycorrhizal plants can ramify a greater soil volume and provide a greater absorptive surface than non-mycorrhizal plants root hairs (**Al-*Karaki and Hammad, 2001***) Some mycorrhizal fungi have the capacity to breakdown phenolic compounds in soil that interfere with nutrient uptake (**Bending *and Read,* 1997**). Mycorrhizal fungi reduce plant disease severity through modifying root physiology which may arise through changes in lignin formation **(*Dugassa et al.,* 1996)**. ***Jeffries and*** *Rhodes* ***(1987)*** *and* ***Elshahat et al., (2010*)** reported that, the application of AM and yeast together stimulated sweet pepper growth and yield where yeast cells stimulated AM to grow well and increased its

efficiency to colonize in the root system of plant forming a good symbiotic relationship with plant. ***Manfred et al*. (2006)** concluded that dual inoculation of maize with yeast and AM resulted in increased shoot biomass depending on the combination of yeast species and AM isolate.

CONCLUSION

Our study clearly showed that there is a synergistic effects yielding from combination of both mycorrhiza and yeast together in maize displayed which triggers a stronger defense signals against *Fusarium moniliforme*, under both greenhouse and field conditions and could reduce the use of chemical fungicide, thereby reduce the hazards to consumers and environment, providing effective way to prevent and control maize fungal disease. Moreover, the use of fungi, bacteria and yeasts showed a great effect on various maize growth parameters and this appear clearly throughout our experiment.

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محاولات لتقييم دور بعض عوامل المكافحة الحيوية في الحد من الإصابة بفطريات الأذرة وتحسين معايير نموها

****مدحت سعيد عبد ربه * و ياسر محمد عبد الشفيع**

***معهد بحوث أمراض النبات, مركز البحوث الزراعية, الجيزة, مصر**
****المركز الأقليمي للأغذية و الأعلاف, مركز البحوث الزراعية, الجيزة, مصر**

تتسبب الأمراض الفطرية والبكتيرية والتي تصيب نبات الأذرة في خسائر كبيرة في الحقل علي النباتات النامية و علي محصولها وإنتاج الأغذية و ذلك عن طريق التسبب بتلف النباتات والمحاصيل علي حد سواء ، وقد أجريت هذه الدراسة لتقييم التأثير المضاد لبعض عوامل المقاومة الحيوية ومنها الفطرية والبكتيرية والميكوهيزا والخميرة وخليط منهم علي الأصابة بتلك الأمراض.
(Trichoderma harzianum, Pseudomonas fluorescens, mycorrhiza (Glomus sp.) and Saccharomyces serivise)
ضد تلف محاصيل الاذرة (Zea mays L.) الناجمة عن ثلاثة من الفطريات المسببة للأمراض المنقولة عن طريق التربة Fusarium moniliforme, Acrimonium stritum and Rizoctonia solani . وقد لوحظ في التجربة الحالية ، انخفاض كبير في العلامات المرضية للنبات مثل انخفاض عدد النباتات والكيزان المصابة في المعاملات الخاصة بعوامل المقاومة الحيوية بالمقارنة مع الكونترول و لوحظ ان ذلك كان ايضاً مصحوباً بتعزيز سمات النمو مثل ارتفاع النبات ووزنه ، وطول الجذور ووزنها ، ومتوسط وزن الكيزان. وكشفت النتائج المتحصل عليها أن استعمال mycorrhiza (Glomus sp.) و Sacchromyses serivisae معا أظهر تأثيرا متفوقا كبيرا للحد من الإصابة بالأمراض وتحسين سمات النمو في نفس الوقت يليه الخليط ثم Trichoderma harzianum و Pseudomonas florescens على التوالي. و قد أدت المعاملات ألي رفع الكفاءة الأنزيمية للنبات و كانت أكثر المعاملات تأثيراً المعاملة بأستخدام الميكوهيزا.