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# Influence of Some Bioagents and Chitosan Nanoparticles on Controlling Maize Late Wilt and Improving Plants Characteristics

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**The** high environmental risks of fungicides were a reason for encouraging biological control and its biofactors and recommending its use in the field of plant diseases. Chitosan NPs, *Trichoderma viride* and mycorrhizae (*Glomus mosseae*) are one of those factors that have been used in this study separately or in combination to control maize late wilt caused by *Cephalosporium maydis*. The experiments were conducted during seasons 2017 and 2018 in a greenhouse and infested fields at the Agric. Res. Stat. of Gemmieza. Tested treatments showed a significant decrease in the incidence of late wilt compared to untreated control in either greenhouse or field trials. In this regard, the treatment with chitosan NPs was the best when combined with the mycorrhizae (VAM) which gave the highest effect in controlling late wilt in the greenhouse and field trials followed by treatment with chitosan NPs+ *T. viride* in addition to the positive effect on plant growth parameters compared to the other treatments. Experiments have also showed that using of *T. viride* with mycorrhizae together in one treatment led to a law effect on disease control, although their use each alone has a great effect on disease control and improving plant growth. The data also showed that the association of mycorrhizae with maize plants was significantly affected by the infection by *C. maydis*, in addition to an increase in the activities of peroxidase and catalase enzymes compared to healthy plants. The results obtained from the affected plants revealed that the activities of both enzymes were still less in the plants treated with mycorrhizae than untreated plants. Finally, these results suggest that the use of chitosan NPs in combination with mycorrhizae is one of the methods that can be adopted to achieve the goal of sustainable agriculture in controlling maize late wilt and improve its growth.

**Keywords:** *Cephalosporium maydis*, *Magnaporthiopsis maydis*, Arbuscular mycorrhizal fungi, chitosan nanoparticles, maize, *Trichoderma viride*, biological control.

Maize (*Zea mays* L.) is one of the world's top crops. Maize provides not only the fast-foods of western society - breakfast cereals, sweet corn and popcorn, but also

the staple foods for much of the world's population in developing countries, where it is used to make porridge, bread and tortillas. All around the world, maize grain is a basic livestock feed, and the crop can be cut while still green to make silage as a winter feed (Roth and Heinrich, 2001).

Late wilt is a vascular wilt disease of maize caused by the phytoparasitic fungus *Cephalosporium maydis* Samra, Sabet, & Hingorani, synonyms are *Magnaporthiopsis maydis* (Klaubauf *et al.*, 2014 and El-Shafey and Clafin 1999), *Harpophora maydis* (Gams, 2000). Late wilt was reported in Egypt (Sabet *et al.*, 1961). The pathogen can cause severe economic losses, with 80-100% infection and total yield loss reported when heavily infested fields were planted with sensitive maize cultivars (El-Hosary *et al.*, 2015). Late wilt is characterized by relatively rapid wilting of maize plants, typically at the age of 70 to 80 days, before teaseling and until shortly before maturity. First symptoms appear approximately 60 days after sowing (Sabet *et al.*, 1970).

In general chemical controls are the most procedures used to reduce the pathogen's impact on commercial production (Degani and Cemica, 2014 and Sharma *et al.*, 2015). Fungicidal applications cause hazards to human health and increase environmental pollution. Therefore, alternatives, eco-friendly approaches for control of plant diseases are needed such as biological control and induced resistance (Mahmoud *et al.*, 2014&2015).

Chitosan is a natural linear biopolymer obtained by alkaline deacetylation of chitin and it is a homopolymer consists of β-(1,4)-linked N-acetyl-glucosamine units (Islam *et al.*, 2017). Chitosan is considered as the most abundant natural polymer with a dual effect: It controls pathogenic microorganisms by preventing growth, sporulation, spore viability, germination and disrupting cell and inducement of different defense responses in host plant inducing and/ or inhibiting different biochemical activities during the plant-pathogen interaction (Hassan and Chang, 2017). Numerous researchers have assayed chitosan for control pre and post-harvest diseases of many crops (Bhaskara *et al.*, 1999; Bhuvaneshwari and Sivasubramanian, 2013 and Li *et al.*, 2017).

Arbuscular mycorrhizal fungi can be act as bioprotectors of plants. These Zygomycetous fungi that form specialized structures such as arbuscules and/ or vesicles are obligate biotrophs and utilize host photosynthates for their growth. They are ubiquitous and co-exist with over 80% of terrestrial plants including agricultural or horticultural crops. Numerous reports mentioned that they improved resistance to a variety of stresses including drought, salinity, temperature, metals, and diseases due to fungal symbiosis (Maya and Matsubara, 2013; Salam *et al.*, 2017 and Liu *et al.*, 2018). Moreover formation of hyphal network by the mycorrhizae with plant roots significantly enhances the access of roots to a large soil surface area, causing improvement in plant growth (Bowles *et al.*, 2016).

Fungi belonging to the genus *Trichoderma* have been known since 1920s for their capabilities to function as bio-control agents against plant pathogens (Samuels, 1996). Some species of genus *Trichoderma* have the multiple interactions with crop plants and soil borne fungal pathogens (Woo *et al.,* 2006). The different species of this genus have long been known not only for the control of plant diseases, but also for their capability to enhance plant growth and development, elevated reproductive ability, capacity to modify the rhizosphere, capability 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 (Schuster and Schmoll, 2010; Pandya *et al.,* 2011; Daguerre *et al.,* 2014 and Keswani *et al.,* 2014). The species of genus *Trichoderma* have been evaluated against many pathogens and have exhibited greater potential in managing diseases (Kaur and Mukhopadhayay, 1992; Harman and Björkman, 1998; Woo *et al.,* 2006 and Keswani *et al.,* 2014).

The presented work aimed to understand the role of chitosan NPs, mycorrhizae (*Glomus mosseae*) and *Trichoderma viride* in a biological control of late wilt caused by *C. maydis*.

## Materials and Methods

*1. Isolation, purification and identification the pathogen:*

Samples of maize plants which have symptoms of late wilt were collected from Gharbia, Kalubia and Giza Egyptian governorates during the previous growing seasons. These samples were transferred to the laboratory in clean plastic bags and were stored at 4°C till fungal analysis. Pathogen was isolated from the lower internodes of symptomatic plants by splitting a surface-sterilized stalk (wiped with a cloth soaked in 5% Na-hypochlorite, then dipped in 70% ethanol and flamed) with a sterile knife and transferring a small portion of the discolored vascular bundle to PDA+ yeast extract. Plates were incubated at 28°C for 4 days in the dark. (Zeller *et al*., 2002). Symptom recognition is based on the dull green, desiccated leaves, streaked and "collapsed" stalk, and discolored pith tissues. Symptoms are not definitive, morphological and microscopic characteristics are still used to identify *C. maydis* according to the keys of Samra *et al.* (1962).

*2. Synthesis and characterization of chitosan nanoparticles:*

Chitosan nanoparticles were prepared using the ionic gelation method; approximately 2% of the polymer was dissolved in a 1.0% (v/v) acetic acid solution. A sodium TPP solution was also prepared in distilled water at a concentration of 5 mg/mL. The sodium TPP solution was added dropwise using a burette to the chitosan solution while stirring, followed by sonication for 20 min. The resulting suspension was subsequently centrifuged at 15,000 rpm for 10 min and then dried at room temperature approximately 28°C (Ghadi *et al.,* 2014). Maize grains were coated and soaked in chitosan nanoparticles (0.1g/ml).

Transmission electron microscope (TEM) The morphological and particles size of chitosan and chitosan nanoparticles were demonstrated by using TEM model JEM-1230, Japan, operated at 120 kV, with maximum magnification of 600×103 and a resolution until 0.2 nm. A drop of an aqueous dispersion of the nanomaterial was placed on a carbon-coated copper grid and allowed to dry in air before characterization. Particle size and zeta potential were measured using a Zetasizer Nano-ZS-90 (Malvern Instruments, UK).

*3. Preparation of Trichoderma viride:*

An isolate of *T. viride* was obtained from Vegetables Plant Diseases Department, Plant Pathol. Res. Inst. Spore suspension of the tested *T. viride* was prepared by culturing mycelial disces (5mm) of the tested fungus (4 days old culture) in conical flasks 250 ml potato dextrose medium and incubated at 25±2°C for 10 days. The growth of tested fungus was blended using an electric blender and then filtered through two folded piece of cheese cloth to obtain spore suspension only. Spore suspension was adjusted to $10^6$ spore/ mL with the aid of Haemocytometer (Ibrahim, 1993). Then, applied by soaking seeds before planting.

*4. Source of mycorrhizae.*

The previously identified local strain of mycorrhiza was obtained from Bot. Dept. Fac. of Science, Mansura University, Egypt. The inoculum consisted of AM colonized root fragments from the stock culture with guania grass (*Megathyrsus maximus*), rhizosphere soil having extrametrical mycelium and spores at rate 10 spores/ mg of soil (Gerdeman and Nicolson, 1963). Open-pot culture method was used for the mass production of AMF as described by Gerdeman and Nicolson (1963). After two months, the inocula were taken from soil rhizosphere containing extrametrical mycelium and spores by making small trench (just under the seed planting depth). 25g of two month old mycorrhiza were used as inoculum for coating 100g of maize grains (Scott *et al.*, 1991).

*5. Properties of soil and grains.*

Sandy loamy soil was collected from reclaimed soil in Sharkia governorate and autoclaved at 121°C (15-lbs/$in^2$) for one hour. The soil was analysed as follows: physical analysis was done as described by Jackson (1973) and was sand 80%, silt 15% and clay 5%. chemical analysis was done according to Jackson (1973) and (1985) and was nitrogen 0.85 g/ Kg, potassium 0.186 g/ kg, phosphorus 0.32g/ kg.

Grains of the tested maize Baladi cv. were obtained from Agron. Dept. Agric. Res. Centre, Giza, Egypt. They were surface sterilized with 0.01% NaClO, washed 2-3 times with distilled water and sown in black plastic pots 30cm diameter, containing 3000g of soil (Boby *et al.*, 2008).

*6. Preparation the inoculum of C. maydis:*

Each isolate of *C. maydis* was grown on potato dextrose agar (PDA) + 2g yeast extract/ liter for 6 days after the isolate had initially covered the surface of the plate

at 28˚C in the dark. One $cm^2$ diameter agar disk from this culture was used to inoculate 500-ml bottle containing 70 g of autoclaved sorghum grain (autoclaved for 30 min). Inoculated bottles were incubated at 26±2˚C until the sorghum grain was completely colonized with the pathogen, two weeks and saved until soil inoculation (Zeller *et al*., 2002).

*7. Pots experiments*

This experiment was performed in the greenhouse of Maize and Sugar Crop Dis. Res. Dept. Plant Pathol. Res. Inst., ARC, Giza, during 2017 growing season. Clay pots (30 cm diameter) were filled with a mixture of approximately 3 kg of sterilized El-Sharkia soil as shown before, 2.5g superphosphate and 2g $K_2SO_4$ were add. Approximately 50 g (wet weight) of sorghum grains colonized by the causal fungus inoculum per pot were added to the soil. The grains and soil were mixed thoroughly by stirring. Inocula of the pathogen were obtained from 3 governorates of Egypt as mentioned before and were mixed with each other by equal ratio. Pots were sown with susceptible Baladi cv.

Seed coating technique adopted by Fravel *et al*. (1985) was used to identify the most effective and practical formulation for application of control agents. Soil was infested with *C. maydis* as described before.

Antagonistic formulations, *i.e*. chitosan NPs, *Trichoderma viride* and *Glomus mosseae* each alone or combined with each other by ratio (1:1) were entrapped on the surface of maize grains treated with Arabic gum at the rate of 8 mg/20g grains and left them overnight before sowing. Five grains were planted per pot.

Four replicates were used. Untreated seeds were used as control. Plants were fertilized 21 days after sowing at the rate of 3g urea (46%N)/ pot and regularly watered using tap water. Percentages of infected plants were recorded 90 days after sowing. Samples were taken 45 and 90 days after sowing.

*8. Field experiment:*

This experiment was performed in the experimental farm of Gemmieza Agric. Res. Stat., A.R.C., during 2017 and 2018 growing seasons. Complete randomized Plot design with three replicates was used in this experiment, Plots comprised of 4 rows, 5 m long rows spaced 60 cm apart and 20 cm between plants. Baladi cv. was sown after treatment by the tested bio-agents *T. viride*, mycorrhiza and chitosan nanoparticles. Seed coating technique adopted by Fravel *et al*. (1985) was used in all tested agents, each alone or combined with each other (1:1 ratio). The normal methods of sowing and agricultural practices were applied as recommendations.

*9. Plant mycorrhizal colonization, mycorrhizal dependency, drybiomass.*

The whole plant was used for biomass measurement 45 and 90 days after sowing. The plant samples were washed with distilled water to remove the adhering soil particles and separated from the above-ground parts and the root parts. Firstly,

samples were air-dried in cool and well ventilating places, then they were oven dried (68°C± 2°C for 72 h). The dry weight of each part was weighed to calculate the biomass. Before drying, the weighed subsamples of fresh roots and leaves were used for root mycorrhizal colonization by morphological and molecular methods and acid and alkaline phosphatiseactivity assessment (Gianinazzi-pearson and Gianinazzi, 1976). Root samples were collected at an interval of 45 days, AMF colonization of root was examined after staining with 0.5% trepan blue in lactophenol (Phillips and Hayman, 1970) method. Root segments, each approximately 1-$cm^2$ long, were selected at random from a stained sample and mounted on microscopic slides. Slides with stained root segments were carefully examined using the microscope under suitable magnification. Mycorrhizal percentage root colonization (frequency of mycorrhizal infection F%, Mycorrhizal intensity M% and Arbuscular activity A%) was determined by gridline intersect slide method (Giovannetti and Mosse, 1980). Spores produced by AMF in the root zone soil were estimated by wet sieving and decanting method (Gerdeman and Nicolson, 1963). The mycorrhizal dependency (MD) of plants were calculated according to Gerdemann (1975).

*10. Enzyme assays*

Leaves and roots of control and treated seedlings were removed at 45 and 90 days and were extracted with Tris-HCl buffer by grinding in a pre-cooled mortar and pestle as described previously (Sathiyabama and Manikandan, 2016). The extract was centrifuged at 10,000 rpm for 10 min at 4°C. The soluble portion was removed and used for enzyme assays. Defense enzymes such as catalase and peroxidase, were assayed as described earlier (Sathiyabama and Manikandan, 2016). Fresh leaves of treated plants were harvested from the selected plants treated with various amendments for estimation of enzymes including, acid phosphatase 45 and $90^{th}$ days of experimentation (Weimberg, 1975).

*11. Statistical analysis.*

Data of growth, physiological parameters and mycorrhiza levels in maize inoculated with AMF and Chitosan NPs in the presence of *C. maydis* were subjected to analyze by One- way ANOVA and LSD (Least Significant Deference) according to Kautsoyiannis (1981).

## Results

*1. Isolation, purification and Identification the pathogen:*

After isolation from the three governorates, Gharbia, Kalubia and Giza during previous growing seasons, then purification, and identification by Maize and Sugar Crops Dis. Res. Dept. Plant Pathol. Res. Inst., ARC, Giza, three isolates were obtained from the pathogen which was identified as *Cephalosporium maydis* .Thus, they were all used during the present study.

*2. Synthesis and characterization of chitosan nanoparticles:*

A representative Transmission Electron Microscope (TEM) micrograph of chitosan nanoparticles obtained after adding TPP to chitosan under magnetic stirring is presented in (Fig.1a). The micrograph shows nanoparticles with variable shapes, most of them present in spherical and some others having occasionally oval in nature with homogenous population of nanoparticles.

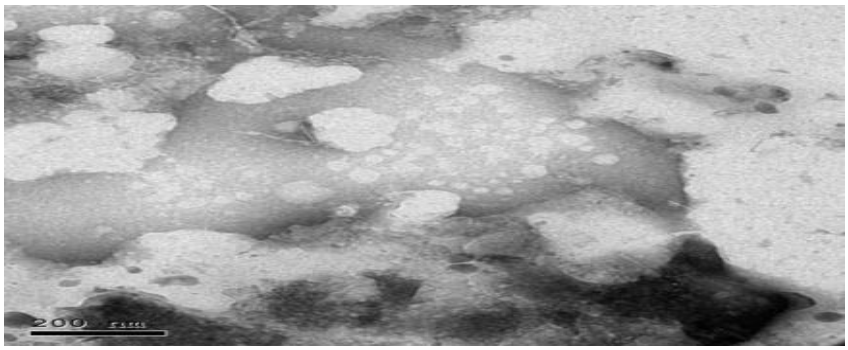


**Fig. (1a): TEM image of chitosan nanoparticles synthesized.**

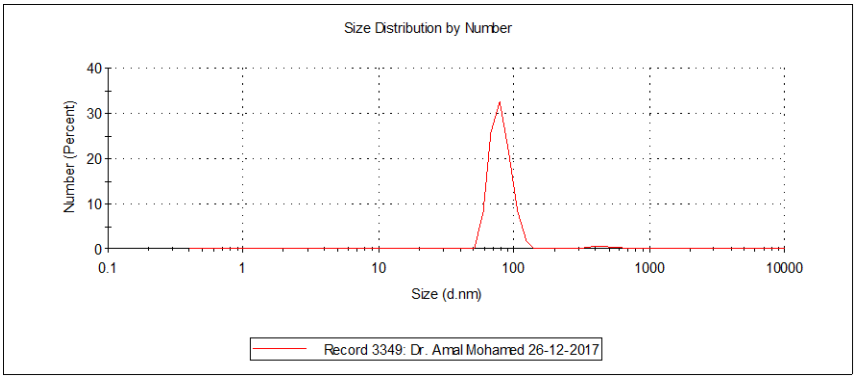


**Fig. (1b): Zetasizer average diameter of chitosan NPs.**

**Table (1): Zetasizer average diameter of chitosan NPs.**

| Size (nm) | Percentage % |
|---|---|
| 50.748 | 0.0 |
| 58.771 | 8.056 |
| 68.061 | 25.747 |
| 78.82 | 32.527 |
| 91.28 | 21.528 |
| 105.709 | 8.299 |
| 122.42 | 1.725 |
| 141.772 | 0.117 |
| 164.183 | 0.0 |

Zeta average diameter was used for measurement of average hydrodynamic diameters and particle size distribution (poly disparity indexes). Fig.(1b) and Table (1) show the size distributions of nanoparticles in colloids, which ranged from 58.77 to 141.772 nm. The most of them had a diameter of approximately 78.82 nm by 32.527% of all colloidal solution. However, 25.747% of all colloidal solution had a diameter of approximately 68.061 nm and the size of 91.28 nm represents 21.528% of colloidal solution. Whereas, the particle size greater than 100 nm represents only 10% of all solution. On the other hand, poly disparity index (Pdi) recorded 0.7.

*3. Effect of different treatments on incidence of maize late wilt:*

This study was carried out in the greenhouse of Maize and Sugar Crop Dis. Res. Dept. Plant Pathol. Res. Inst., ARC, Giza during 2017 growing season and in the research experiment farm in Gemmieza Agric. Res. Stat. during 2017 and 2018 growing seasons using the susceptible maize cv. Baladi. Seven treatments in addition to control were applied.

Data in Table (2) show the efficiency of the tested treatments against late wilt under greenhouse and field conditions. Disease incidence of late wilt was significantly reduced in all tested treatments compared to untreated control in both trials. In this respect, chitosan NPs when combined with the mycorrhizae (VAM) achieved the highest effect in controlling late wilt in the greenhouse and in the field followed by chitosan+ *T. viride*.

Unexpectedly, the treatments with VAM + *T. viride* gave unpredictable results, being 65% in the greenhouse experiment and 65 and 70% in the field experiments during 2017 and 2018 growing seasons. Also, the treatment with chitosan + VAM + *T. viride* resulted in high disease incidence, being 60 % in the greenhouse and 65% in both seasons of field experiments.

**Table (2): Effect of chitosan nanoparticles, VAM and *T. viride*, each alone or in combination, in greenhouse and field during 2017 and 2018 growing seasons at Gemmieza on the incidence of late wilt of maize plants, 90 days after sowing.**

| Treatments | Greenhouse experiment | | Field experiment 2017 | | Field experiment 2018 | |
|---|---|---|---|---|---|---|
| | DI% | Efficiency % | DI% | Efficiency % | DI% | Efficiency % |
| Chitosan NPs | 25 | 72.22 | 30 | 64.7 | 35 | 58.8 |
| VAM | 30 | 66.67 | 40 | 52.9 | 35 | 58.8 |
| *T. viride* | 40 | 55.56 | 40 | 52.9 | 40 | 52.9 |
| Chitosan NPs + *T. viride* | 15 | 83.33 | 20 | 76.5 | 20 | 76.5 |
| Chitosan NPs + VAM | 10 | 88.89 | 20 | 76.5 | 15 | 82.35 |
| VAM + *T. viride* | 65 | 27.78 | 65 | 23.53 | 70 | 17.65 |
| Chitosan NPs + VAM + *T. viride* | 60 | 33.33 | 65 | 23.53 | 65 | 23.53 |
| Control | 90 | - | 85 | - | 85 | - |
| LSD at 5% | 4.0 | | 3.0 | | 3.0 | |

*4. Effect of different treatments on growth of healthy maize plants:*

Data of this work are represented in Fig (2). This image was taken 47 days after sowing, showing that all plants in the pots are healthy (not infected). Seven treatments were used in addition to control without treatment. When chitosan NPs and VAM were used each alone (pots B and C, respectively), or combined (pot E) many small male flowers appear constituting the male inflorescence(tassel) approximating in 47 days after sowing , where, control pot (A) formed tassel after 60 days. Furthermore, there was a general relationship between the increase in elongation of stalk maize plants treated with chitosan NPs and VAM each alone or in combination.

On contrast, there was no pronounced effect on plants in case of pot (G) that was previously treated with both *T. viride* + VAM. While there was a little effect in elongation and flowering in pot (F), which was treated with chitosan NPs+ *T. viride*. The work gift was in pot (H), treated with chitosan NPs+ VAM+ *T. viride*

represented in unexpected appearance of the ears compared to other pots including untreated pot (control).


**Fig. (2): Effect of chitosan NPs , VAM and *T. viride* on the growth of maize plants.**

*5. Effect of different treatments on activity of arbuscular mycorrhizal fungi (AMF):*

Data presented in (Table, 3) show that frequency of mycorrhizal root segments (F%), the intensity of mycorrhizal colonization in root tissues (M %) and arbuscule frequency in root systems (A%) were increased in the response of the treatment with chitosan NPs irrespective to presence and absence of *C. maydis* and rate of increase at 90 days were higher than that at 45 days. In contrast, in the presence of *T. viride* the values of F, M and A% at 90 days were lower than that at 45 days in case of healthy and infected plants.

Data also show that the mycorrhizal association with maize plants was significantly influenced by *C. maydis*, where the values of F, M and A% were reduced in plants infected by *C. maydis* compared to healthy plants regardless of the treatments.

Results presented in Table (3) indicate that mycorrhizal dependencies for plant dry mass were increased in case of *C. maydis*. In healthy maize plants the increase was varied from 238 to 269% in absence and presence of chitosan NPs, respectively. While the mycorrhizal dependence was increased in infected plants with *C. maydis* from 247 to 278% in absence and presence of chitosan NPs, respectively. By highlighting on the negative role of *T. viride*, the mycorrhizal dependence was increased from 358 to 365% in healthy and infected plants, respectively. MD

reached the highest level due to using chitosan NPs+ *T. viride*, which recorded 457 and 878% in healthy and infected plants, respectively.

**Table (3): Effect of *C. maydis* on AMF development and mycorrhizal dependence (MD) of mycorrhizal plants supplemented with and without chitosan NPs separately or combined with *T. viride*.**

| Periods (days) / Treatments | | F%[1] | | M%[2] | | A%[3] | | MD |
|---|---|---|---|---|---|---|---|---|
| | | 45 | 90 | 45 | 90 | 45 | 90 | 90 |
| Maize plants (con.) | I[4] | 68 | 70.5 | 28.4 | 29.6 | 7.9 | 8.3 | 247 |
| | H[5] | 80 | 83 | 31.8 | 33.32 | 9.4 | 9.8 | 238 |
| Chitosan-NPs | I | 70 | 85.7 | 28 | 36.3 | 8.9 | 17.4 | 278 |
| | H | 81.3 | 93.2 | 30 | 41.4 | 10.6 | 20.5 | 269 |
| *T. viride* | I | 68 | 45 | 12.2 | 9.8 | 3.9 | 3.45 | 365 |
| | H | 75 | 55 | 12.2 | 10.8 | 5.5 | 5.65 | 358 |
| Chitosan-NPs+ *T. viride* | I | 78 | 81 | 13.8 | 0.52 | 5.9 | 9.8 | 878 |
| | H | 81 | 88 | 18.3 | 27.4 | 8.9 | 14.6 | 457 |

(1) F% Frequency of mycorrhizal root segments; (2) M% intensity of mycorrhizal colonization; (3) A% arbuscule frequency in root systems; (4) I: Infected maize plants; (5) H: Healthy maize plants not infected.

*6. Effect of different treatments on activity of peroxidase, catalase and acid phosphatase enzymes:*

The results (Fig.,3) show that the activity of peroxidase and catalase enzymes was increased in the infected maize plants with *C. maydis* compared with the healthy plants. The results obtained from infected plants also revealed that the activities of both enzymes were still lower in VAM treated plants than non-VAM treated plants. The maximum activity was recorded for peroxidase and catalase enzymes, being 0.8 and 0.3 unit/ mg protein, respectively in infected plants with *C. maydis*

On the other hand, the activity of phosphatase enzyme was significantly reduced by the presence of *C. maydis* in non-VAM treated plants either in the absence or the presence of chitosan NPs or *T. viride* (Fig.3). It is also quite obvious that inoculation of maize plants with the VAM led to stimulation of phosphatases enzymes, although the activity was slightly decreased in infected plants by *C. maydis*. Moreover, the results also revealed that co-inoculation of VAM + *T. viride* + chitosan NPs to maize plants caused an increase in acid phosphatases compared with other treatments of infected plants.

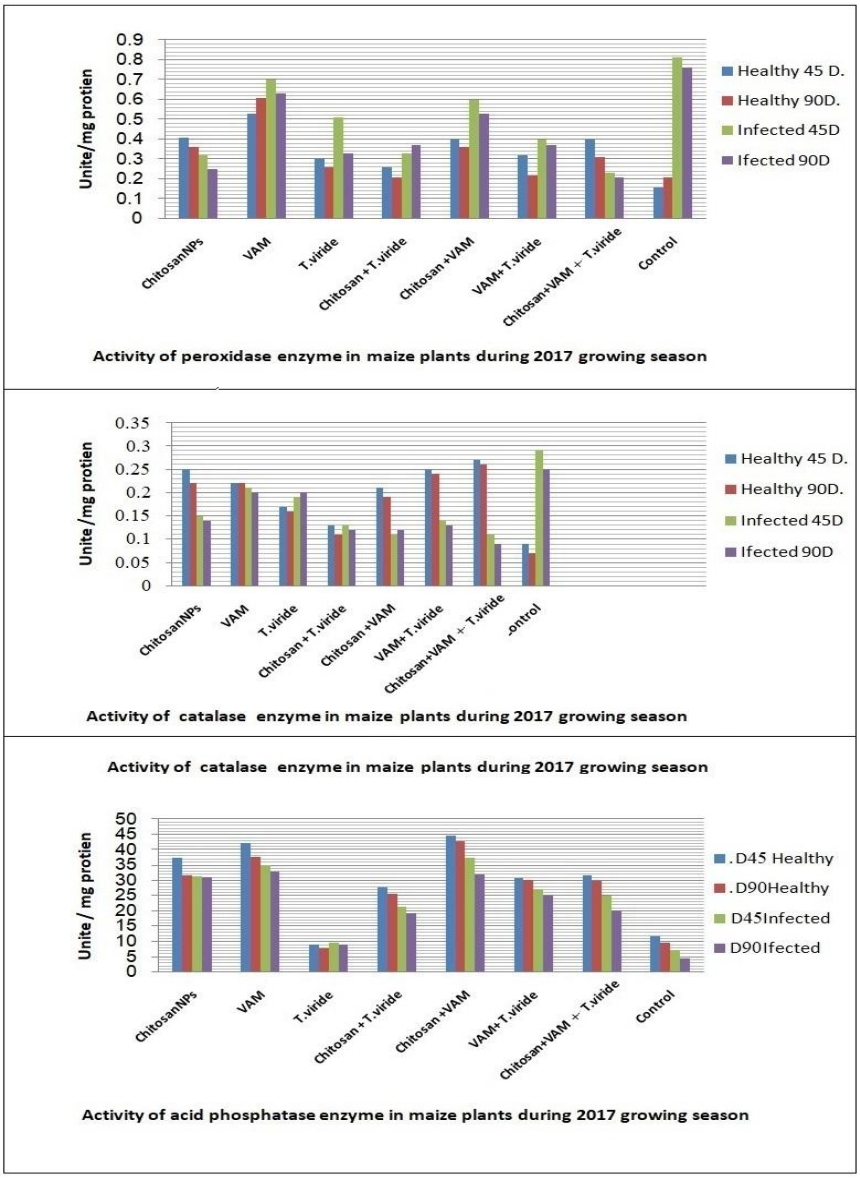


**Fig. (3): Activity of peroxidase, catalase and acid phosphatase enzymes in healthy and infected maize plants in pots trial.**

## Discussion

Late wilt has been considered one of the most economically important fungal diseases of maize in Egypt, where yield losses approached 40% and the infection arrived 80-100% when cultivated under heavily infested fields and planted with sensitive maize cultivars (El-Hosary *et al.*, 2015). The control of this pathogen is still major problem for farmers and attracts a strong attention of Egyptian researchers and for environmental safety the non-chemical control measures are being used in this work (El-Hosary *et al.*, 2015).This study examines the potential of controlling of late wilt in Baladi cultivar of maize plants biologically by using chitosan nanoparticles, mycorrhiza and *Trichoderma viride* as a bio-factor each alone or combined with each other, during 2017 and 2018 growing seasons under field condition and in the greenhouse .

Nanometer particles, such as chitosan, have drawn the attention of scientists because of their extensive application to new technologies in agriculture at general and specifically in plant protection. Using transmission electron microscope (TEM) can provide further morphology, characterize and size details of the chitosan nanoparticles (Balaji *et al*., 2009). Chitosan nanoparticles were examined using TEM and the results showed that the majority of the nanoparticles were variable in shape, most of them present in spherical and some others having occasionally oval in nature with homogenous population of NPs. Moreover, the size distributions of nanoparticles in colloids ranged from 58.77 to 141.772 nm. In addition, poly disparity index (Pdi) was recorded as 0.7. The results are in agreement with the obtained data by Deng *et al*. (2006); Ing *et al.* (2012) and Ghadi *et al.* (2014) who admitted that size distribution ranged around 90 and 164nm.

Presented results revealed the reality of using chitosan nanoparticles in controlling late wilt in susceptible maize cultivars, where all the treatments that contain chitosan alone or combined with other treatments have highest, percentage of efficiency. This confirms what many researchers have got from the ability of chitosan to control diseases (Al-Hetar *et al.*, 2011; Sunpapao and Pornsuriya, 2014; Silva *et al.*, 2014). This may be due to the multiple activities of chitosan that lead to the antimicrobial effects such as: agglutination, disruption of the cell membrane, inhibition of H+-ATP as activity, inhibition of toxin production and microbial growth, inhibition of the synthesis of messenger RNA and proteins, and blockage of nutrient flow (Malerba and Cerana, 2016).

Data also clarify the important role of VAM when used alone or combined with chitosan NPs for controlling *C. maydis.* This is due to the role of VAM in disease control by eliminating pathogens or reducing their effectiveness by interacts directly with soil borne pathogens, or indirectly by stimulating other natural antagonistic (Mohan, 2000). In addition to, VAM improves host resistance by stimulation of plants to produce phytoalexin (Sundaresan *et al.*, 1993), or plant growth hormones,

improving rhizospheric and soil conditions (Lindermann, 1994), altering the physiological and biochemical properties of the host like water absorption capacity of plants by increasing root hydraulic conductivity and favorably adjusting the osmotic balance and composition of carbohydrates (Ruiz-Lozano, 2003) and defending roots against soil-borne pathogens (Dehne, 1982). Moreover it enhances the uptake of nutrients of low mobility in the soil solution such as P, Ca, Zn, and Cu (Bethlenfalvay, 1992).

Data also indicated that *T. viride* can effect on late wilt, the results have already been supported and interpreted by Harman *et al.* (2004); Ruiz *et al.* (2007); Abd El-Motty *et al.* (2010); Li *et al.* (2017) and Elshahawy *et al.* (2017). That because *Trichoderma* fungi play an important role in controlling plant diseases by producing more than 43 secondary metabolites, which exhibit antibiotic activity such as chitinase and chitinolytic enzymes, which could be used as a bioagent against plant pathogenic fungi (Kubicek e*t al.*, 2001). *Trichoderma* spp. also compete with plant pathogens on nutrients and reduce of pathogen by producing antibiotics, parasitizing on pathogens, or by inducing resistance in the host plants (Berg *et al.*, 2007). But this study illustrated that *T. viride* when combined with VAM in the same treatment led to reduce their effect on controlling *C. maydis* .This may be due to the ability of *T. viride* to parasitize on the mycelium of mycorrhiza fungus and compete for nutrients (Jaeger *et al.*, 2011).

It has been found that chitosan NPs have a good effect when used separately or in combination with VAM or *T. viride* on healthy maize plants by elongation of stem and early flowering compared to untreated control. This is in agreement with the obtained data by Amborabe *et al*. (2004); Algam *et al*. (2010); Mondal *et al*. (2012); Mondal *et al*. (2013) and Ramkissoon *et al*. (2016), who stated that chitosan acts on depolarization of plasma membrane that is manifested by the influx of $Ca_2$+ and $H^+$, and efflux of $Cl^-$ and $K^+$. Activation of ion channels causes changes in intra and extracellular media. A decrease of calcium in pectocellulosic walls increases their extensibility; which leads to cell growth or elongation.

This study revealed the fact that the mycorrhiza enhanced acid phosphatase activities in all treatments, which leads to higher uptake of phosphate from soil. This enhanced uptake of nutrients led to increase growth of plants inoculated with AM fungi, similar results were obtained by Saito *et al.* (2004). In this connection, Ezawa *et al.* (2001) reported that absorption of P by external hypha from soil is the first step, followed by translocation along hypha and the final exchange for sugar in arbuscules.

In this study, inoculation of plants with *C. maydis* alone caused oxidative-stress at 45and 90 days after inoculation. On the other hand, inoculation with Chitosan NPs or *T. viride* alone causes slightly oxidative-stress at last period of inoculation only. These results are in agreement with data obtained by Jacob *et al*. (2001) who

stated that the increase of POD and CAT led to an elaborate antioxidant defense system (enzymatic antioxidant system) which induced to maintain the cellular redox balance. In this respect, the process of detoxification of the reactive oxygen protects cell against harmful concentration of hydroperoxides (Castillo, 1992). Based on these data, it is conceivable to conclude that the AM fungi play a prime role for induction and activation of the antioxidative system in the plants grown under stress conditions.

Data of the present study revealed that the mycorrhizal infection (F%) of AM plants exhibited significantly higher inverse correlation with acidic phosphatase activity. This result is in agreements with obtained data by Saito *et al*. (2004). In addition, the intensity of mycorrhizal infection (M%) of AM plants revealed significantly higher inverse correlation with peroxidase enzyme, this is similar with that reported by Hause *et al*. (2007). Activity of mycorrhizal infection (A%) of AM plants was significantly higher inversely correlated with catalase activity, this connected with that reported by Sreenivasulu *et al.* (1999).

## Conclusion

It can be said that this study gives indications that both of chitosan NPs and mycrrohiza can be used to control late wilt of maize as well as improve maize growth as one of the bio factors, which are environmentally safe.

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# تأثير بعض العوامل الحيوية و النانوشيتوزان في مكافحة الذبول المتأخر في الذرة الشامية وتحسين خصائص النباتات

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المخاطر البيئية العالية لمبيدات الفطريات كانت سببا في تشجيع استخدام المكافحة البيولوجية وما تحويه من عوامل حيوية والتوصية باستخدامها في مجال مكافحة أمراض النباتات. النانوشيتوزان وفطر *Trichoderma viride* والميكوريزا (*Glomus mosseae*) هي واحدة من تلك العوامل التي تم إستخدامها في هذه الدراسة بشكل منفصل ومجتمعة لمكافحة مرض الذبول المتأخر في الذرة المتسبب عن *Cephalosporium maydis*. أجريت التجارب في الصوبة والحقول المصابة خلال موسمي ٢٠١٧ و ٢٠١٨ في محطة البحوث الزراعية بالجميزة. أظهرت المعاملات المختبرة إنخفاضاً ملحوظاً في نسبة الإصابة بمرض الذبول المتأخر مقارنة بالكنترول الغير معامل سواء في تجارب الصوبة أو الحقل بموسميه. في هذا الصدد أعطت المعاملة بالنانوشيتوزان مقترنة مع الميكوريزا أعلى تأثير في مكافحة مرض الذبول المتأخر في تجارب الصوبة والحقل تلاها المعاملة بالنانوشيتوزان مقترنة مع فطر *T. viride* بالإضافة إلى ما حدث من تأثير إيجابي على نموالنباتات مقارنة بباقي المعاملات. كما أظهرت التجارب أيضاً أن استخدام فطر *T. viride* مع الميكوريزا مقترنين في معاملة واحدة يؤدي إلي ضعف تأثيرهما علي مكافحة المرض على الرغم من أن استخدام أي منهم بشكل منفصل له تأثير كبير على مكافحة المرض وتحسين نمو النباتات ولكن يبدو أن هناك منافسة بينهما في حالة إقترانهما معاً أدت إلى التأثيرالمثبط على أنشطتهم وقللت من فاعليتهما في المكافحة. وأظهرت البيانات أيضا أن ارتباط الميكروهيزا مع نباتات الذرة قد تأثرت بشكل كبير بـالإصابة بالفطر الممرض بالإضافة إلى حدوث زيادة في أنشطة إنزيمات البيروكسيديز والكتاليز مقارنة بالنباتات السليمة. وكشفت النتائج المتحصل عليها من النباتات المصابة أن أنشطة كلا الإنزيمين كانت لاتزال أقل في النباتات المعاملة بالميكوريزا عن تلك النباتات الغير معاملة. وأخيراً تشير هذه النتائج إلى أن استخدام النانوشيتوزان مقترناً مع الميكوريزا هي أحد الوسائل التي يمكن إعتمادها لتحقيق هدف الزراعة المستدامة في السيطرة على مرض الذبول المتأخر في الذرة الشامية وتحسين نموه.