Non-traditional Methods for Controlling Maize Late Wilt Disease Caused by *Cephalosporium maydis*

Amany M. Hamza[*]; G. A. El-Kot[**] and S. El-Moghazy[***]

[*]Pesticides Chemistry Department, Faculty of Agriculture, Kafr El-Shiekh University, 33516 Egypt
[**]Agricultural Botany Department, Faculty of Agriculture, Kafr El-Shiekh University, 33516 Egypt
[***]Plant Pathology Research Institute, Agriculture Research Centre, Giza, Egypt
(Received: February 26, 2013 and Accepted: March 14, 2013)

ABSTRACT

In an attempt to search for alternative control methods to pesticides, certain bio-control formulations (*Bacillus subtilis 1, B. ubtilis 3, B. pumilus, Pseudomonas fluorescens* and *Epicoccum nigrum*), bentocide, zinc oxide nanoparticles and nanosilica were tested against *Cephalosporium maydis*, the causative fungus of late wilt disease of maize, under field conditions of Kafr El-Sheikh Governorate, Egypt in 2011 and 2012 growing seasons. Results showed that the bio-control formulations were the most effective treatments against the disease, followed by nanosilica, bentocide and nanozinc oxide, descendingly in both growing seasons, with respect of pre-emergence damping off, disease incidence and crop yield. As well, the bio-control formulations showed the highest level of defense enzymes activity in maize post treatment, followed by nanosilica, nanoznic oxide and bentocide, descendingly in both seasons. Assayed materials represented potential effective alternatives to fungicides for controlling late wilt of maize.

Key words: Late wilt disease, *Cephalosporium maydis*, Maize, Alternative, Control, Egypt.

INTRODUCTION

Maize (*Zea Mays*) is one of the most important cereals for both human and animal consumption and grown for grain and as forage. Late wilt of maize, caused by the fungus *Cephalosporium maydis* (Samra *et al.*, 1963) is one of the most important fungal diseases in Egypt. This disease also was reported in India (Payak *et al.*, 1970) and in Hungary (Pecsi and Nemeth 1998). *C. maydis* reproduces asexually, and no perfect state has been identified. Saleh *et al.* (2003) stated that the pathogen is clonal in Egypt and the Egyptian population contained four lineages, three of which were widely distributed throughout the country.

Generally, corn diseases are controlled or reduced by management decisions and practices made prior to planting; however, there are several exceptions. Foliar fungicides are frequently used in seed production fields to control economically important foliage diseases. Chemical compounds have been used to control plant diseases (chemical control), but abuse in their employment has favored the development of pathogens resistant to fungicides (Tjamos *et al.*, 1992). By contrast, the use of microorganisms that antagonize plant pathogens (biological control) is risk-free as it results in enhancement of resident antagonists (Monte, 2001). Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as bio-control agents.

Nanotechnology has offered an important role for improving the existing crop management techniques (Nair *et al.*, 2010). It is a promising technique in plant pathology either by providing controlled delivery of functional molecules or as diagnostic tool for disease detection, an important step in plant disease treatment (Sharon *et al.*, 2010).

The present study was carried out to test the efficacy of certain bio-control agents, bentoicide, zinc oxide nanoparticles and nanosilica against *C. maydis* emergency percentage, disease severity reduction and corn yield increase, as well on the simulation of defense enzymes in corn plants after treatment.

MATERIALS AND METHODS

Tested materials

The nanosilica and zinc oxide nanoparticles were obtained from Egypt Nanotech Company Limited, Cairo, Egypt with a purity of 99.99%. The bentocide formulation [a diatomite, siliceous sedimentary rock] is produced by the El Ahram Mining Company, Cairo, Egypt. *Bacillus subtilis* 1, *B. subtilis* 3, *B. pumilus*, *Ps. fluorescens* and *E. nigrum* as bio-control agents were obtained from the Plant Pathology Research Institute, Agriculture Research Centre, Giza, Egypt.

Preparation of bio-control formulations

The talc-based formulation of each bio-control agent was prepared by some modifications of the method developed by Commare *et al.*, (2002). The selected bio-control formulations were inoculated into the KB broth and incubated in a rotary shaker at 150 rpm for 48 hr at room temperature (28± 2°C). One kg of talc powder was

taken in a sterilized metal tray and its pH was adjusted to neutral by adding $CaCO_3$ at the rate of 15 g kg^{-1}. Ten grams of carboxy methyl cellulose (CMC) were added to 1 kg of talc and mixed well and the mixture was autoclaved for 30 min on each of two consecutive days. The 400 ml of 48 hr grown suspension containing 9 $x10^8$ cfu ml^{-1} for bacterial isolates and 12 x 10^8 spore/ml were mixed with carrier cellulose mixture under aseptic conditions. After drying (approximately to 35% moisture content) for overnight, it was packed in polypropylene bag, sealed and stored at room temperature (28 ± 2°C).

Isolation and identification of the causal pathogen

Samples of the third to fifth internodes of diseased plants were washed by running water, divided into small pieces, surface sterilized using 0.5 sodium hypochlorite solution for 3 minutes. Surface sterilized internal plant tissues were transferred into Petri dishes, containing potato dextrose agar (PDA) medium mixed with 2 g yeast extract/ liter. Plates were incubated at 28 ± 2°C for 3-7 days and fungal growth was daily observed. Using hyphal tip technique (Booth,1977), mycelium was purified , transferred to PDA slant supplemented with 1% yeast extract and kept in the refrigerator at 4±2°C as stock cultures for maintaining the culture growth. According to Singh (1982), the isolated fungus was identified as *C. **maydis*** at Plant Pathology Branch, Agricultural Botany Department, Faculty of Agriculture, Kafr El-Sheikh University and at the Department of Maize, Sugar and Foliages Corp Diseases, Plant Pathology Institute, Agriculture Research Centre, Giza, Egypt.

Evaluation of the tested treatments against late wilt of maize under field conditions

Field experiments were carried out at the disease nursery of the Experimental Farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt in 2011 and 2012 growing seasons. Soil infection with *C. maydis* was carried out according the technique of El-Shafey *et al.*, (1988), one week before planting. Infection was carried out by mixing thoroughly with surface soil layer of the disease nursery in plots (9.6 m^2) and watered before planting. Each plot comprised of two rows, with 6 cm long and 80 cm apart. Three replicates were used for each treatment. Germination test of maize grains, used in this study, was carried out and the germination percentage was 80% (unpublished data). One week post soil infection, grains of the highly susceptible maize cultivar (Balady) were soaked in all tested agents (*B. subtilis 1, B. subtilis 3, B. pumilus, Ps. fluorescens* and *E. nigrum*, bentoicide, zinc oxide nanoparticles and nanosilica) for 8 hours before sowing. Soaking in bacterial bio-formulations was carried out in suspension (1 $x10^8$ cfu/ ml), made by adding 5 g of the prepared bioformulation/L tap water of all the tested bacterial agents. While, soaking in the fungal bio-agent *(E. nigrum)* was made by adding 5 g of the prepared bioformulation /L tap water ($3x10^5$ spore/ ml). Soaking of maize grains in bentoicide, zinc oxide nanoparticles and nanosilica were carried out by preparing liquid solutions of these treatments at a concentration level of 2 g/L for bentoicide, and 1 g/l for zinc oxide nanoparticles and nanosilica. No reference fungicide was used in this study to compare the efficacy of tested materials because there was no specific recommended fungicide for this plant pathogen. Grains soaked in sterilized distilled water acted as control. One week post soil infection, soaked grains were planted and sixty grains were used for each row in the disease nursery. Two weeks post planting germination rates were recorded and pre-emergence damping off percentage was calculated according to the eq. 1

% Pre-emergence damping off = [No. of non emerged seeds/ no of sown seeds] x 100 **(1)**

Afterwards, the emerged plants were thinned to one plant per hill and 25 cm between hills. Disease incidence in the survived plants was recorded as percentage of infected plants, (35 days post planting) (El-Shafey *et al.* 1988) as shown in eq. 2

Disease Incidence (D.I. %) = [No of infected plants/no of total plants] x100 **(2)**

Disease Incidence data were re-used to calculate the percentage of disease reduction for each treatment as shown in eq. 3

% Reduction = [(% D.I. of control - % D.I. of treatment)/ % D.I. of control] x 100 **(3)**

Furthermore, weight of ten cobs were determined and the efficacy of each treatment with respect to cob yield productivity relative to control was calculated as described by El-Assuity *et al.*, (1986) in eq. 4

Efficacy = [(yield in treatment- yield in control)/ yield in control] x 100 **(4)**

Biochemical tests

1. Peroxidase (POX) activity

Three grams of fresh leaves from the second growing season were ground in a pre-cooled mortar and pestle containing 9ml of 0.1M phosphate buffer (pH 7.0). The extract was centrifuged at 12,000 rev min^{-1} at 5 °C for 15 min. The supernatant was

collected and used as enzyme extract for the assay Thimmaiah (1999). POX activity was determined by measuring the oxidation of pyrogallol to pyrogalline in the presence of hydrogen peroxide. Peroxidase activity was measured, following the changes in absorbance at 425 nm every min. up to 3 minutes. All measurements were assayed using Beckman Spectrophotometer Du®7400. Activity was calculated according to equation 5

$$\text{Enzyme activity} = A_{425nm}\ min^{-1} / 6.58 \qquad (5)$$

2. Polyphenoloxidase (PPO) activity

Three grams of fresh leaves from the second growing season were ground in a pre-cooled mortar and pestle containing 6ml of 0.1M sodium phosphate buffer (pH 7.1). The extract was strained through four layers of cheesecloth and filtered. The filtrate was centrifuged at 12,500 rev min^{-1} at 6 °C for 20 min and the supernatant was used as enzyme extract for the assay (Sadasivam and Manickam (1991). PPO activity was determined according to the method of Walter and Purcell (1980). Activity was calculated as Δ A450 min^{-1} g (leaf fresh weight)$^{-1}$.

Statistical analysis

Data were analyzed statistically by the analysis of variance test (ANOVA) using Irristat version 93 and the different means were compared by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Efficacy of tested materials against the late wilt disease of maize under field conditions

a- Emergency percentages

As shown in table (1) bio-control formulations were the most effective treatments against late wilt pathogen, followed by nanosilica, bentocide and nanozinc oxide, descendingly in 2011 and 2012 growing seasons. Among the tested bio-control agent, *B. subtilis 3* was the most effective one, followed by *E. nigrum, B. subtilis 1, B. pumilus* and *Ps. fluorescens*, descendingly in both seasons.

b- Disease severity

Data in table (2) showed that the bio-control formulations were the most effective treatments against late wilt pathogen, followed by nanosilica, nanozinc oxide and bentocide, descendingly in both growing seasons. Among the tested bio-control agent, *B. pumilus* was the most effective one, followed by *Ps. fluorescens, E. nigrum, B. subtilis 3* and *B. subtilis 1*, descendingly in both seasons. The efficacy of the tested materials against late wilt disease of maize was generally higher in the first season than in the second one.

c- Maize yield

As shown in table (3), bio-control formulations were the most effective treatments against late wilt disease of maize, followed by nanosilica, nanozinc oxide and bentocide, respectively in both growing seasons relative to control. The increase of maize yield in all treatments relative to the control was generally higher in the first season than in the second one.

Effect of tested treatments on stimulation of defense enzymes (peroxidase and polyphenoloxidase) of maize plants

Data in tables (4 and 5) showed effect of the tested treatments on defense enzymes activity in maize plants relative to control treatment. The bio-control formulations showed highest level of defense enzymes activity, followed by nanosilica, nanozinc oxide and bentocide, respectively in both tested seasons.

The results showed that, nanosilica had a significant reduction against late wilt of maize relative to control, with no adverse effect on treated maize plants. This may be due to that silicon (Si) is known to be absorbed into plants to increase disease resistance and stress resistance (Brecht *et al.*, 2003). Aqueous silicate solution, used to treat plants, was reported to exhibit excellent preventive effects on pathogenic microorganisms. Moreover, it promoted the physiological activity and growth of plants and induced diseases and stress resistance in plants (Kanto *et al.*, 2004). Use of such nanomaterial is more acceptable since they are safe for plants and cause less environmental pollution in comparison to conventional chemical pesticides.

Zinc oxide nanoparticles showed high efficacy against late wilt of maize under field conditions as indicated by reduction of disease severity. Zinc oxide nanoparticles have been reported to be effective to control plant pathogens (Wani and Shah 2012). The ability of ZnO to inhibit bacterial growth by generation of radical oxygen species was well documented [Seven *et al.*, 2004]. ZnO is a semiconductor with a wide band gap. As with other semiconductors, radiation of ZnO with higher photon energy than its band gap caused movement of electrons from the valence band (vb) to the conduction band (cb) of the particle (Derbalah, 2009). Formation of a positive area that can be described as a hole (h^+) in the valence band and a free electron in the conduction band might be a result of this process. On the surface of the ZnO particles, these holes react with hydroxyl groups and absorb water to create a hydroxyl radical. In the presence of oxygen, the lone electron in the conduction band created a superoxide ion, which

Table (1): Effect of the tested treatments on emergency percentages of maize seeds relative to control at Kafr El-Sheikh Governorate, Egypt in 2011 and 2012 maize growing seasons

Treatments	First season		Second season	
	Mean percentage survival plants	Pre-emergence damping off %	Mean percentage survival plants	Pre-emergence damping off %
Bacillus subtilis 1	63.0± 0.1 b	37.0± 0.12 cd	67.0± 0.11 b	33.0± 0.14 e
Bacillus subtilis 3	72.0± 0.2 a	28.0±0.13 f	62.0± 0.12 bcd	38.0± 0.17f
Bacillus pumilus	66.0± 0.3 b	34.0±0.14 e	72.0± 0.13 a	28.0±0.18 d
Pseudomonas fluorescens	52.0± 0.1 bc	48.0±0.15 a	64.0 ± 0.21 bcd	36.0± 0.19d
Epicoccum nigrum	72.0± 0.2 a	28.0± 0.17 f	67. 0±0.23	33.0±0.3 e
Bentocide	62.0± 0.3 b	38.0± 0.2 bc	56.0 ±0.24 cd	44.0±0.31 b
Nanozinc oxide	61.0± 0.4b	39.0± 0.3 b	61.0 ± 0.31 bcd	39.0±0.21c
Nanosilica	64.0± 0.2b	36.0± 0.41 d	58.0± 0.34 cd	42.0±0.23 b
Control	47.0± 0.1 a	53.0± 0.23 g	53.0±0.33 d	47.0± 0.24a

Table (2): Effect of the tested treatments on disease incidence relative to control at Kafr El-Sheikh Governorate, Egypt in 2011 and 2012 maize growing seasons

Treatments	First season		Second season	
	Mean Percentage of D. I.	% Disease reduction	Mean Percentage of D. I.	% Disease reduction
Bacillus subtilis 1	8.20±0.1 cd	51.80 ± 0.2g	15.0± 0.02bc	54.0 ± 0.21d
Bacillus subtilis 3	5.10±0.1 d	70.0±0.22 c	19.50± 0.3e	40.20±0.23 h
Bacillus pumilus	4.10±0.02 d	75.90±0.23 a	9.2 0±0.4b	71.80±0.24a
Pseudomonas fluorescens	4.40±0.3 d	74.10±0.24 b	11.70± 0.5cd	64.10±0.25 c
Epicoccum nigrum	5.50±0.04d	67.60± 0.32d	10.70±0.5 cd	67.20± 0.35b
Bentocide	15.10±0.2 bc	11.20± 0.33h	16.90± 0.1b	48.20±0.33 f
Nanozinc oxide	6.20±0.05 d	63.50±0.34 f	15.40±0.11 bc	52.30± 0.34e
Nanosilica	6.0± 0.1d	64.60±0.44 e	18.0± 0.12b	44.80±0.45 g
Control	17.0± 0.11d	0.00± i	32.0± 0.22a	0.00± i

* D. I. = disease incidence

Table (3): Effect of the tested treatments on maize yield relative to control at Kafr El-Sheikh Governorate, Egypt in 2011 and 2012 maize growing seasons

Treatments	First season		Second season	
	Mean corn yield	Percentage of yield increase	Mean Corn yield	Percentage of yield increase
Bacillus subtilis 1	2.50 ±0.25bc	19±0.1 b	2.0 ± 0.01bcd	14.3± 0.01b
Bacillus subtilis 3	2.3 ± 0.01bcd	9.50± 0.11d	2.0±0.02bcd	14.3 ±0.01b
Bacillus pumilus	2.7±0.02 a	28.60±0.13 a	2.20±0.03 a	23.80±0.11 a
Pseudomonas fluorescens	2.4± 0.03bcd	14.30± 0.14c	2.0± 0.04bc	14.30±0.12 b
Epicoccum nigrum	2.5± 0.04bc	19.00± 0.15b	1.9±0.05 bcd	9.5± 0.021c
Bentocide	2.2± 0.04e	4.8±0.12 e	1.80± 0.06e	4.8± 0.12e
Nanozinc oxide	2.30±0.02 cd	9.50± 0.18d	1.9±0.03 bcd	9.5± 0.014cd
Nanosilica	2.2± 0.03cd	4.80±0.10 e	1.80±0.02 bcd	4.8±0.05 e
Control	2.10± 0.04cd	0.00± g	1.70± 0.1bcd	0.00± f

Table (4): Determination of peroxidase activity in the leaves of maize plants grown under different treatments

Treatments	Activity of Pox as mg enzyme /ml		
	1 min	2 min	3 min
Bacillus subtilis 1	0.0601±0.002a	0.080±0.002a	0.104± 0.001a
Bacillus subtilis 3	0.0512 ± 0.001b	0.075 ±0.002b	0.105 ±0.001a
Bacillus pumilus	0.044 ± 0.001 c	0.058 ±0.001c	0.069 ±0.003b
Pseudomonas fluorescens	0.032 ± 0.002d	0.050 ±0.003e	0.060 ±0.003c
Epicoccum nigrum	0.029 ± 0.002e	0.052 ±0.002d	0.055±0.002 d
Bentocide	0.009 ±0.001h	0.018 ±0.001h	0.033 ±0.002g
Nanozinc oxide	0.012 ±0.001g	0.024 ±0.001g	0.035±0.002 f
Nanosilica	0.016 ± 0.002f	0.044 ±0.001f	0.052 ±0.003e
Control	0.007 ± 0.001i	0.013 ±0.001i	0.022±0.001 h

Table (5): Determination of polyphenoloxidase activity in the leaves of maize plants grown under different treatments

Treatments	Activity of PPO as Darkening after		
	1 min	2 min	3 min
Bacillus subtilis 1	0.088± 0.003b	0.089± 0.002 b	0.048± 0.001d
Bacillus subtilis 3	0.092± 0.002a	0.099 ±0.002a	0.090± 0.001a
Bacillus pumilus	0.044±0.001 c	0.048 ± 0.001c	0.055± 0.003c
Pseudomonas florescense	0.020± 0.001e	0.030± 0.001e	0.033±0.001 f
Epicoccum nigrum	0.038 ±0.002c	0.047± 0.001 c	0.057 ± 0.002b
Bentocide	0.019± 0.001e	.025± 0.002f	0.020±0.001 h
Nanozinc oxide	0.038± 0.002c	0.041±0.003 d	0.030 ±0.001g
Nanosilica	0.035 ±0.003d	0.047 ±0.001c	0.037 ±0.003e
Control	0.017±0.001 f	0.017±0.002 g	0.010±0.002 i

could also become a hydroxyl radical, and so forth. Derivatives of this active oxygen damage the bacterial cell Seven *et al.*, (2004) and Gordon *et al.*, (2011. Results indicated also that zinc nanoparticles may distort and damage the bacterial cell membrane,resulted in a leakage of intracellular contents and eventually the death of bacterial cells (Jin *et al.* 2009).

Chemical content analysis of the bentocide formulation revealed the presence of silica (46.5%), with a high percentage relative to other detected oxides (Hamza, 2012). The antifungal activity of the biocide against late wilt disease may be due to the presence of silica, which has been known as effective antimicrobial agent (Derbalah *et al.* 2012 a and b).

Moreover, using the bio-formulations of the bio-control agents showed high efficacy against the tested pathogen relative to control. The efficacy of the tested bio-control agents against late wilt pathogen may be due to the successful antagonism of the plant pathogen by the saprophytic microorganisms operate by nutrient competition, hyperparasitism, and/ or antibiosis (Falk *et al.*, 1995). One of the strategies used to control pathogens is mycoparasitism (Harman, 2000), whereby a species or strain of fungus directly attacked and fed on other fungi (Kendrick, 1992). Another mechanism involved the production of antibiotics or enzymes that can inhibit the growth or reduce the competitive ability of other organisms (Harman, 2000). Control may also be achieved through competition for space and resources with highly competitive BCAs quickly colonizing plant surfaces, creating an effective 'living barrier' to subsequent pathogen invasion (Cook, 1988). Another mechanism was the mobilization of nutrients in the soil, a process that made compounds in the soil more available for plant uptake and resulted in increasing of general health and disease resistance. BCAs may also induce changes in the plant that increase disease resistance similar to the phenomena of induced and systemic acquired resistance (Harman, 2000).

Some isolates of *B. subtilis* were reported as a growth-promoter or antagonist of several pathogens and BCAs of numerous diseases (Romero *et al.*, 2008). *B. subtilis* QST 713 strain was active against fungi and bacteria that cause scab, powdery mildew, sour rot, downy mildew, early leaf spot, early and leaf blight, bacterial spot and walnut blight diseases (Environmental Protection Agency, 2005). Antagonism by *B. subtilis* QST 713 maybe achieved

in several ways, including nutrient competition, site exclusion, colonization and attachment of the bacteria to the fungal pathogen and induction of the plant's natural systemic resistance.

Moreover, disease severity varied in the two seasons and this was attributed to the variables of incidence and severity of pests and pathogens in maize within and among the seasons (Cardwell *et al.,* 1997). Significant increase in maize yield under different treatments, relative to control in both seasons, was found that maybe due to significant disease reduction and/or significant increase in defense enzymes (POX and PPO) in maize plants under different treatments.

An increase in defense-related enzymes was observed in treated maize plants relative to control. The role of defense related enzymes in disease resistance was reported earlier (Chen *et al.,* 2000). Biochemical analysis of rice plants raised from seeds treated with *P. fluorescens* showed an early induction of POX activity (Nandakumar *et al.,* 2001). Sivakumar and Sharma (2003) reported that POX and PPO activities were higher in plants raised from *P. fluorescens* treated seeds than in pathogen alone inoculated ones.

Foliar application of Zn and silica showed substantial influence on both yield and particularly grain quality (Khoshgoftarmanesh *et al.,* 2010 and Xiao-Fang *et al.,* 2011).

In conclusion, the results indicated that tested bio-control agents were most effective against the late wilt disease of maize with respect to disease severity and crop yield. Moreover, nanosilica and zinc oxide nanoparticles can be considered promising non-traditional control agents as an increase in defense-related enzymes was observed in treated maize plants relative to control. The assayed materials can represent potential safe control methods for late wilt disease of maize.

REFERENCES

Booth, C. 1977. *Fusrarium*; Laboratory guide to the identification of the major species, Commonwealth Mycological Institute, Kew, Surry England.

Brecht, M., L. Datnoff, R. Nagata and T. Kucharek 2003. The role of silicon in suppressing tray leaf spot development in St.Augustine grass. Publication in Univ. of Florida, 1-4.

Cardwell, K. F., Schultless, F., Ndemah, R. and Z. Nogok 1997. A system approach to assess crop health and maize yield losses due to pests and disease in Cameron. Agric. Ecosyst. and Environ. 65: 33-47.

Chen, C., R. R. Belanger, N. Benhamou and T. Paulitz 2000. Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol. and Mol. Plant Pathol., 56: 13–23.

Commare, R. R., R. Nandakumara, A. Kandana, S. Sureshb, M. Bharathib, T. Raguchandera and R. Samiyappan 2002. *Pseudomonas fluorescense* based bio-formulation for the management of sheath blight disease and leaf-folder insect in rice. Crop Protect., 21: 671–677

Cook, R. J. 1988. Biological control and holistic plant-health care in agriculture. Am. J. Alter. Agric. 3: 51–62.

Derbalah, A. S. 2009. Chemical remediation of carbofuran insecticide in aquatic system by advanced oxidation processes, Journal Agric. Res. Kafr El Sheikh Univ., 35: 308-327.

Derbalah; A. S, S. M. Kamel, S. M. Morsy, S. Z. and M. M. El-Sawy 2012a. Alternatives to control powdery mildew and early blight diseases of tomato under greenhouse conditions. Egypt J. Biol. Pest Cont., 22: 185-190.

Derbalah; A. S. Morsy, S. Z. Kamel, S. M. and M. M.El-Sawy 2012b. Some recent approaches to control powdery mildew in pepper under greenhouse conditions. Egypt. J. Biol., Pest Cont., 22: 205-210.

Duncan, D. B. 1955. Multiple ranges and multiple F. test. Biometrics, П: 1-42.

El-Assiuty, E. M., T. H. Abd El-Moity and H. A. El-Shaafey 1986. Mycoparasitic effect of *Trichoderma harzianum* Rifai against *Cephalosporium acremonium Corda*. Egytian J. of Microbial., 21: 111-116.

El-Shafey, H. A., F. A. El-Shorbagy, I. I. Khalil and E. M. El-Assuity 1988. Additional sources of resistance to the late wilt disease of maize caused by *Cepalosporuim maydis*. Agric. Res. Rev., 66: 221-230.

Environmental Protection Agency (EPA) 2005. *Bacillus subtilis* strain QST 713. Biopesticide Registration Action Document 006479.

Falk, S. P., D. M. Gadoury, P. Cortesi, R. C. Pearson and R. C. Seem 1995. Parasitism of *Uncinula necator ascomata* by the mycoparasite *Ampelomyces quisqualis*. Phytopathol., 85: 794–800.

Gordon, T., B. Perlstein, O. Houbara, I. Felnerc, E. Banin and S. Margel 2011. Synthesis and characterization of zinc/iron oxide composite nanoparticles and their antibacterial properties. Colloids and Surfaces A: Physicochem. Eng. Aspects 374: 1–8.

Hamza, Amany. M. 2012. Efficacy and safety of non-traditional methods as alternatives for control of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in rice grains. Egypt J. Biol. Pest Control 22: (2) 103-109.

Harman, G. E. 2000. Myths and dogmas of bio-control: changes imperceptions derived from research on *Trichoderma harzianum*T-22. Plant Dis., 84: 377–393.

Jin, T., D. Sun J. Y. Su, H. Zhang and H. Sue 2009. Antimicrobial efficacy of zinc oxide quantum dots against *L. monocytogenes, S. enteritidis* and *E. coli* O157:H7. J. Food Sci., 1: M46–52.

Kanto, T., A. Miyoshi, T. Ogawa, K. Maekawa and M. Aino 2004. Suppressive effect of potassium silicate on powdery mildew of strawberry in hydroponics. J. Gen. Plant Pathol., 70: 207-211.

Kendrick, B. 1992. The Fifth Kingdom, Mycologue Publications, Waterloo, Ontario, Canada, Mycologue Publications.

Khoshgoftarmanesh, A. H., Schulin, R. Chaney, R. L. Daneshbakhsh, B. and M. Afyuni 2010. Micronutrient efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A Review Agronomy for Sustainable Develop., 30: 83–107.

Monte, E. 2001. Understanding *Trichoderma*: between biotechnology and microbial ecology. Inter. J. Microbiol., 4:1-4.

Nair, R., S. H. Varghese, B. G. Nair, T. Maekawa, Y. Yoshida and D. S. Kumar 2010. Nanoparticulate material delivery to plants. Plant Sci., 179: 154–163.

Nandakumar, R., S. Babu, R. Viswanathan, T. Raghuchander and R. Samiyappan 2001. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol. and Biochem., 33: 603–612.

Payak, M. M., S. Lal, J. Lilaramani and B. L. Renfro 1970. *Cephalosporium maydis,* a new threat to maize in India. Ind. Phytopathol., 23:562–569.

Pecsi, S. and L. Nemeth 1998. Appearance of *Cephalosporium maydis* Samra, Sabet and Hingorani in Hungary. Facult Landbouw en Toegepaste Biolog Wetenschappen, Univer Gent., 63:873–877.

Romero, D., A. De Vicente, H. Zeriouh, F. Cazorla, M. Fernández-Ortuño, D. J. A. Torés and A. Pérez-García 2008. Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. Plant Pathol., 56 (6): 976-986.

Sadasivam, S. and A. Manickam 1991. Biochemical Methods. New Age International Publishers, Chennai, India, 256 pp.

Saleh, A. A., K. A. Zeller, A. S. M. Ismael, Z. M. Fahmy, E. M. El-Assiuty and J. F. Leslie 2003. Amplified fragment length polymorphism (AFLP) diversity in *Cephalosporium maydis* from Egypt. Phytopathol., 93:853–859.

Samra, A. S., K. A. Sabet and M. K. Hingorani 1963. Late wilt disease of maize caused by *Cephalosporium maydis*. Phytopathol., 53: 402–406.

Seven O., B. Dindar, S. Aydemir, D. Metin, M.A. Ozinel, S. Icli 2004. Solar photocatalytic disinfection of a group of bacteria and fungi aqueous suspensions with TiO_2, ZnO and Sahara desert dust. J. of Photochem. and Photobiol. A Chem., 165: 103–107.

Sharon M., Kr. Choudhary A. and K. Rohit 2010. Review article nanotechnology in agricultural diseases and food safety. J. Phytol., 2(4): 83–92.

Singh, R. S. 1982. Plant Pathogens "The Fungi" Oxford and IBH publishing Co. New Delhi Bombay Calcutta pp 443.

Sivakumar, G. and R. C. Sharma 2003. Induced biochemical changes due to seed bacterization by *Pseudomonas fluorescens* in maize plants. Indian Phytopathol., 56: 134–137.

Thimmaiah, S. K. 1999. Standard Methods of Biochemical Analysis. Kalyani Publishers, New Delhi, India, 545 pp.

Tjamos, E. C., G. C. Papavizas and R. J. Cook, (eds) 1992. Biological Control of Plant Diseases. Progress and Challenges for the Future. Plenum Press, New York.

Walter, W. M. and A. E. Purcell 1980. Effect of substrate levels and polyphenoloxidase activity on darkening in sweet potato cultivars. J. Agric. Food Chem., 28: 941-944.

Wani, A. H. and M. A. Shah 2012. A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. Journal of Applied Pharmaceutical Science 02:40-44.

Xiao-Fang, G., Ze-Bin, W. Qi-Tang, W. Jin-Rong, Q. and Z. Jian-Li 2011. Cadmium and zinc accumulation in maize grain as affected by cultivars and chemical fixation amendments. Pedosphere 21: 650–656.