

## Fungicidal Effect of Some Promising Agents in Controlling Maize Late Wilt Disease and their Potentials in Developing Yield Productivity

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**A**NTIFUNGAL activities of cyanobacterial filtrate, compost tea, H<sub>2</sub>O<sub>2</sub>, garlic oil, barnyardgrass root exudates and Premis fungicide were tested against *Cephalosporium maydis*, the pathogenic fungus of late wilt disease of maize plants. *In vitro* and two field trials were carried out during 2015 and 2016 growing seasons under disease nursery conditions. *In vitro*, cyanobacterial filtrate and 30% premis fungicide were the superior treatments, by them linear growth of *C. maydis* were prevented totally and fulfillment inhibitions (100 %) were resulted with lowest IC<sub>50</sub> values. Cyanobacterial filtrate and 3% H<sub>2</sub>O<sub>2</sub> were more effective in developing grain germination. Disease incidence showed better efficacy due to use 30 % Premis fungicide followed by 3 % H<sub>2</sub>O<sub>2</sub> with massive disease reductions reached 83.21 and 75.37 %, respectively during 2015 season. Effectiveness of the 3 % H<sub>2</sub>O<sub>2</sub> dose was extended to the 2016 season with 5.11 % disease incidence and 73.39 % disease reduction. For grain productivity, remarkable enhancements in the weights of both 10 ears and 1000 grains due to all treatments compared to control were recorded in both seasons. Due to their antifungal activities, qualitative analysis of Cyanobacterial filtrate and barnyardgrass root exudates was assayed on Gas chromatography mass-spectrum (GC-MS). Malonic acid, 2,3-Butandiol, Hexestrol, 12-Crown-4-ether and cis-Vaccenic acid were the major compounds extracted from the cultured blue-green algae. Whereas, Nadolol, Quinine,  $\alpha$ -Methylionol, Phyllocladene, alcohols, acids, phenols and 2,6-dihydroxy benzoic acid were the most abundant antimicrobial agents in the barnyardgrass root exudates.

**Keywords:** Maize late wilt, Cyanobacteria, Compost tea, H<sub>2</sub>O<sub>2</sub>, Premis, Garlic oil, *Cephalosporium maydis*, Disease reduction, Productivity, Biochemical assay.

### Introduction

*Cephalosporium maydis* is a soil-borne vascular wilt pathogen that penetrates root tissue and colonizes the xylem causes late wilt disease of maize plants. It can be transmitted through the seeds as seed-borne and may occasionally cause seed rot or pre-emergence damping-off under heavy inoculum potential (El-Shafey and Claflin, 1999). Nowadays, a great attention has been focused on the possibility for using natural and safely agents to overcome the harmful effects of the plant pathogens and in the same time to promote growth of these plants. Utilization of these agents became positive alternative to chemical pesticides and safely used for human, animal and environment (Whipps, 2001). In the last few decades, several possible plant-microbe interactions were developed to benefit

plants and colonize their roots and enhance their growth through wide variety of mechanisms, such as the production of plant growth regulators (phytohormones), antifungal activities, siderophores, phosphate solubilization, nutrient uptake and availability (Bowen and Rovira, 1999).

Compost or its extracts are the most promising bio-products recently responsible for developing different management programs as plant pest, disease and fertility (Sheurell and Mahafee, 2002). Decomposition processes of compost are actually enhanced via inoculation with beneficial microorganisms acted, themselves or their metabolites, as plant growth-promoting rhizobacteria (PGPR) (Brinton, 1995). Compost tea was also has a great potential for controlling different diseases in addition to improvement growth of the plants (Ghobrialet *al.* 2009).

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DOI : 10.21608/JENVBS.2017.1849.1013

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Weeds are one the main suppressive factor in maize (*Zea mays* L.) production and cause yield losses in the cultivation system (Zoschke, 1990). Barnyardgrass (*Echinochloa crus-galli*) belongs to the same family of maize (Gramineae), which may be one of the reasons for its superior ability to compete with maize or rice plants. Furthermore this noxious weed may release growth inhibitors that inhibit the emergence of maize, rice and other growth parameters (Li *et al.*, 1992; Xuan *et al.*, 2006). Some weeds contain and release phytotoxic compounds (allelochemicals) into the environment that inhibit the growth of crops (Tang *et al.*, 1981). Allelopathy was defined as any process involving secondary metabolites produced by plant, algae, bacteria, and fungi that influence the growth and development of agriculture and biological system (IAS, 1993) or sometimes the substances which originated from plant could play as a crop protection role.

Cyanobacteria are photosynthetic cosmopolitan prokaryotic organisms that have been isolated from aquatic (freshwater, brackish and marine), terrestrial (soil, lichen-associated and the surface of leaves), and different aquatic and terrestrial extreme environments (hot springs, high salinity, deserts) (Whitton and Potts, 2000; Kaasalann *et al.*, 2012). In these environments, cyanobacteria face competitors and predators, including parasitic fungi, such as chytrids. The production of oligopeptides by *Planktothrix* spp. is believed to contribute to the defense against chytrid fungi (SØnstebØ and Rohrlack, 2011; Rohrlack *et al.*, 2013). In cyanobacteria, the antifungal hassallidin is synthesized by NRPSs and tailoring enzymes (Vestola *et al.*, 2014). Interestingly, a single hassallidin gene cluster encoded in the biosynthetic pathway for more than 40 chemical variants of hassallidin in *Anabaena* sp. SYKE748A (Vestola *et al.*, 2014).

The objective of this study is to investigate the fungicidal and biochemical activities of certain agents for induce resistance and controlling late wilt disease on maize plants. Enhancement of plant growth and nutritive values of maize productivity were also aimed in the presented study.

## Materials and Methods

### Pathogen

A virulent isolate of late wilt disease pathogen was isolated from naturally infected maize (*Zea mays*, the susceptible “Baladi” variety) in Kafr El-Env. *Biodiv. Soil Security* **Vol.1** (2017)

Sheikh governorate and used through this study. The pathogen was isolated and cultured on potato dextrose agar (PDA) medium supplemented with yeast extract according to the methods described by Shalaby *et al.* (2009). Cultures were incubated at 28 °C ± 2 °C for 3-7 days and then purified by the hyphal tip technique according to Booth (1977). Pure cultures were examined microscopically and maintained on PDA slants supplemented with 0.1 % yeast extract at 4 °C for further experiments. The obtained fungal isolates were identified as *Cephalosporium maydis* by morphological characteristics and microscopic examination according to (Samraet *et al.*, 1962) and confirmed by comparing these isolates with the culture collection of maize, sugar and foliage crops Res. Dis. Dept., Plant Path. Res. Inst., Agric. Res. Center, Giza, Egypt.

### Sterilization of treatments

Except Premis fungicide and Hydrogen peroxide, the tested treatments were sterilized by exposure to the vapor of chloroform by putting every 3 small bottles (20ml in size) on conical flask (250ml) containing 25 ml of chloroform, then closed with parafilm for 5 days to kill all cells of any contaminated microorganisms according to the technique of Vidaver *et al.* (1972) and was adapted by El Bakery (2010).

### Treatments

#### Compost tea

For compost tea, rice straw was watered and firstly inoculated with *Trichoderma viridi* and *T. harzianum*. After that, the liquid cultures of the plant growth promoting rhizobacteria (PGRP) of *Azotobacter chroococcum*, *Zosperillum brasilense* and *Paenibacillus polymyxa* was added according to Badawi (2003). After maturation, enriched compost was filtered to obtain its extract. As foliar spraying, compost tea was diluted, five times using distilled water, and regular applied every 15 days during the vegetative stage. At the flowering stage, foliar spraying was stopped to avoid falling of the flowers. After that, further spraying was done. Chemical and biological properties of the supplemented compost tea are presented in Table (1). One kilogram of the maturated compost was immersed in 10 L water to obtain tea compost. Crude concentration of compost tea was diluted into 10 g L<sup>-1</sup> before application based on Ghobrialet *et al.* (2009).

**TABLE 1. Chemical and biological characters of the supplemented compost tea.**

Character	Value
pH	8.20
EC (ds m <sup>-1</sup> at 25 °C)	3.51
C/N ratio	14.05
Total Nitrogen (ppm)	148.5
Total Phosphorus (%)	0.11
Total soluble Nitrogen (ppm)	103.7
Available Phosphorus (ppm)	19.80
Cross seed germination test (%)*	91.20
Total count of bacteria (cfu/ml)	8.7 x 10 <sup>7</sup>
Total count of fungi (cfu/ml)	1.3 x 10 <sup>6</sup>
Total count of Actinomycetes (cfu/ml)	1.2 x 10 <sup>6</sup>

\*Cross germination test was carried out using *Erucasativum* seeds after 72 hr.

#### *Cyanobacteria*

Mixed strains of Cyanobacteria known as *Anabaena oryzae*, *Nostocmuscorum* and *N. calcicola* were kindly obtained from the stock culture collection of Biological Nitrogen Fixation Unit, Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt. Nitrogen-fixing Cyanobacteria have been cultured routinely in a modified Allen's BG-11 free-nitrogen medium (Allen and Stainer, 1968). For the growth in the dark, 1% glucose was added to the medium. Flasks containing Allen's medium (pH 7) were inoculated with 20 ml of homogenized combined culture of the three tested Cyanobacteria strains to get 500 ml total volume. Cultures were incubated at 35°C for 20 days and illuminated on a 16 / 8 h light / dark cycle using fluorescent tubes with a light intensity of 3500 to 4500 Lux at the surface of the vessels (Abdel-Raouf and El-Shafey, 2009). Cultures were manually stirred twice for a few minutes daily. After that, number of cells was counted and adjusted at 10<sup>8</sup> cell ml<sup>-1</sup> using Haemocytometer. Cultures were used to impregnate sterilized soil (121°C for 30 min.) at the rate of 52 ml liquid culture per 100 g soil. Inoculated soil was well mixed and maintained at room temperature for 48 h. To store Cyanobacteria, the mixed culture was inoculated into nutrient agar medium and incubated in dark for 3 days to activate its growth before maintaining at 4°C in the refrigerator. Cultures were filtered using cloth sheets directly before application without dilution either individual treatment or in combination with the 10 % compost tea treatment.

#### *Hydrogen peroxide*

Antifungal effects of three concentrations (1, 2, and 3 %) of the well-known induce resistance agent H<sub>2</sub>O<sub>2</sub> (30%) were also investigated. The required concentrations were obtained by adding

appropriate amount of the row dose (1, 2 and 3 ml) to complete 100 ml portions of autoclaved PDA medium cooled to about 45°C. A non-amended PDA medium was acted as control.

#### *Garlic oil*

Garlic oil (produced by CAP-Pharm, Reg. No. 2849/2002) was purchased from local market. The required concentrations (1.5, 3 and 6 %) were obtained by adding appropriate amount of the row plant oils (1.5, 3 and 6 ml) to complete 100 ml portions of autoclaved PDA medium cooled to about 45°C. A non-amended PDA medium was acted as control.

#### *Barnyardgrass root exudates*

Barnyardgrass (*Echinochloa crus-galli*) is a well-known weed plant commonly naturally grows during summer cultivations of field crops. The grains of Barnyardgrass were grown in jars containing Cooper nutritious solution (Cooper, 1973) and glass granules under room conditions. After their growth, Barnyardgrass was filtered using filter paper Whatman No.1 then sterilized it.

#### *Chemical fungicide*

Chemical fungicide known as Premis was tested against the investigated pathogenic isolate. Trade and common name, chemical and molecular formula and recommended dose of the tested chemical fungicide premis in Table (2) were done. Successive concentrations represented as 10, 20 and 30 % were tested. To set these concentrations, a stock solution of 10 g L<sup>-1</sup> was first prepared using sterilized distilled water. Then, 10, 20 and 30 ml of the stock solution were added to flasks containing still melted sterilized PDA medium to complete their volumes up to 100 ml, respectively using membrane filter syringe of 0.2 µm.

After thorough handling shaking, medium was poured in 9 cm in diameter Petri-dishes. As replicates, three dishes of each treatment were inoculated centrally with 5 mm agar discs bearing mycelium of 7-days old cultures of the pathogen, then incubated at 28±2°C. Petri-dishes free from fungicides were acted as check (control) treatment. Growth was daily observed and the maximum linear growth was measured at the time of full growth in the control treatment. Net growth data of each concentration were calculated and percentages of inhibition (I %) was calculated by the same equation mentioned above by Ferreira *et al.* (1991).

To describe the relationship between

**TABLE 2. Trade and common name, chemical and molecular formula and recommended dose of the tested chemical fungicide premis.**

Trade name	Common name	Molecular formula & weight	Chemical formula
Premis	Triticonazole	$C_{17}H_{20}ClN_3O$ (317.81)	(RS)-(E)-5-(4-Chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) Cyclopentanol

inhibition percentage (I %) and concentration of the  $H_2O_2$ , garlic oil and the fungicide premis as mathematical basis, derived equation from the well-known Michaelis-Menten kinetics was applied as follows:

$$\mu = \mu_{\max} * C_S / (K_S + C_S)$$

where:  $\mu$  = Specific growth rate

$\mu_{\max}$  = Maximum specific growth rate.

$C_S$  = Concentration of the substrate.

$K_S$  = Constant affinity.

It was well applied by Shalaby *et al.* (2015) after modification into:

$$I \% = I \%_{\max} * C_S / (K_S + C_S)$$

where: I % = inhibition percentage

$I \%_{\max}$  = maximum inhibition percentage.

$C_S$  = Concentration of the tested control agents.

$K_S$  = Constant affinity of the control agents.

In which,  $\mu$  and  $\mu_{\max}$  were replaced by I % and  $I \%_{\max}$ , respectively.

For this purpose, the linear and nonlinear least squares fitting routine of MicroCal's ORIGIN® software package was used and regression degree ( $R^2$ ) of each fungicide was achieved. Based on the regression data and their slope values, concentration of each antagonist caused inhibition of 50 % of the fungal growth ( $IC_{50}$ ) could be plotted.

#### *Antagonistic effects of the tested treatments under laboratory conditions*

To test their antagonistic effects against *C. maydis*, certain concentration of each treatment was added to flask containing certain part of autoclaved PDA medium (supplemented with 0.1 % yeast extract) cooled to about 45°C by using membrane filter syringe 0.2  $\mu$ m. After thorough handling shaking, medium was poured

in 9 cm in diameter Petri-dishes (15ml/dish). After solidification, Petri-dishes were inoculated centrally with 5 mm discs of 3 days-old cultures of *C. maydis*, and then incubated at 28-30°C. These trials were represented by three replicates. The non-amended Petri-dishes of compost tea were acted as check control. Linear growth was daily observed and measured till a time of full growth in the control treatment. However, percentage of inhibition (I %) was calculated for each concentration of all tested treatments in this study according to identical formula of Ferreira *et al.* (1991) as follows:

$$I \% = [(A - B)/A] \times 100$$

where:-

I % = Percentage of inhibition.

A = Mean diameter growth in the control.

B = Mean diameter growth in a given treatment.

#### *Seed germination test*

Maize grains were surface sterilized by soaking into liquid solution of sodium hypochlorite (0.5 %) for 3 min., rinsed 3 times with distilled sterilized water before planting. Maize grains were immersed in each tested treatment for 12 hours. Grains were allowed to dry in a laminar flow cabinet for 1-2 h before sowing. Grains soaked in water were served as controls. Four grains were sown at a suitable depth into polyethylene boxes filled with clay-sand soils watered when needed. Each treatment was represented by three replicates. The boxes were incubated for 10 days at room temperature. Then, germination indexes and percentage of germination were calculated. Germination index was calculated according to the formula of Walker-Simmons and Sesing, (1990). Number of seeds that germinated was counted daily for ten days. Germination was defined as pericarp rupture over the embryo. A weighted germination index was calculated first and less weight to these those germinated subsequently:

$$\text{Germination index} = \frac{(10 \times n_1 + 9 \times n_2 + \dots + 1 \times n_{10})}{(\text{Total days} \times \text{Total seeds})}$$

where,  $n_1 \dots n_{10}$  are the number of seeds that germinated on the first, second and subsequently days until the 10<sup>th</sup> days, respectively. 10, 9, 8 are that wt. given to the No. of seeds germinated on the first, second and respectively. The maximum germination index is 1 and the minimum is 0.

#### *Disease assessment under disease nursery field conditions*

Field trials were carried out during 2015 and 2016 seasons. Maize grains immersed for overnight in certain concentrations of the tested treatments were sowed in plots of 9.6 m<sup>2</sup> in area containing two rows each with 6 m. long and 80 cm. apart and watered under disease nursery field conditions (El-Shafey *et al.*, 1988). Grains soaked in sterilized distilled water were served as control. Fifty grains were planted for each row in the disease nursery. Three replicates were used in these experiments. Degrees of disease assessment were recorded as percentages of disease incidence; percentages of survival plants and disease reduction were also recorded. Plants were thinned to one plant per hill 45 days from sowing and 25 cm between hills. Degrees of disease incidences of the survival plants were recorded, as percentage of infected plants, 35 days after silking (95 days after planting) (El-Shafey, *et al.*, 1988) as follow:

$$\text{Disease incidence (DI \%)} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

Disease incidence data were reused to calculate percentages of disease reduction (Efficiency) of each treatment based on this formula:

$$\text{Disease reduction \%} = \frac{\text{DI \% of control} - \text{DI \% of treatment}}{\text{DI \% of control}} \times 100$$

On the other hand, weights of 10 ears and the 1000 grains yielded from plants of each treatment were determined.

#### *Biochemical tests*

Analysis of blue-green algae and root exudates of *Echinochloa crus-galli* (Barnyard grass) on GC/MS (Agilent 7000 Triple Qvad) located at

Regional center for food and feed, ARC, Giza, Egypt was done. Prior to GC-MS analysis, each sample was dissolved in toluene. GC-MS was performed in a fused-silica capillary column (BP5; 0.25  $\mu\text{m}$ , 30 m x 0.25 mm; SGE Ltd, Agilent 7000 Triple Qvad) using a temperature gradient (120  $^{\circ}\text{C}$  for 5 min, 100 to 160  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}$   $\text{min}^{-1}$ ). The GC injector was kept at 280  $^{\circ}\text{C}$ , and the GC-MS interface was kept at 250  $^{\circ}\text{C}$ . Samples (one  $\mu\text{l}$  each) were injected in split mode (1: 50) and helium (1 ml  $\text{min}^{-1}$ ) was used as the carrier gas (Abhayet *et al.*, 2007).

#### *Statistical analysis*

Data were subjected to statistical analysis of variance (ANOVA) test. A complete randomize design was applied and Duncan's multiple range tests were used for comparing means (Gomez and Gomez, 1984).

### **Results and Discussion**

#### *Laboratory antagonism*

Inhibitory effects of the tested control agents on the linear growth of the pathogenic fungus of maize late wilt disease under laboratory conditions were done and data were tabulated in Table (3). It illustrates that the algal filtrate and 30% of premis fungicide were the superior treatments, by them linear growth of *C. maydis* were prevented totally and fulfillment inhibitions (100 %) were resulted. As well as, great inhibitory effects against the pathogenic fungus due to use 6 % of garlic oil and barnyard grass extract of 98.35 and 97.25 %, respectively, followed by 3 %  $\text{H}_2\text{O}_2$  (89.56 %) were obtained compared with control. The reminder agents were varied in their effects and considered less magnitudes compared to the previous. Superiority data of the cyanobacterial extracts were in full agreement with the findings of Abed *et al.* (2009), who indicated that the antifungal compounds detected in cyanobacterial extracts, such as fischerellin A, hapalindole, hassallidin/balticidins, carazostatin, phytoalexin, tolytoxin, scytophycin, toyocamycin, tjipanazole, nostocyclamide, nostodione and nostofungicide have great efficiencies against most pathogenic fungi. On the other hand, premis has a great potential, as all chemical fungicides, against hyphal cells. Potential impacts of such fungicides against the fungal cells via their ultrastructure were investigated by Abd El-Ghany and Tayel (2009). Disorder and striking changes in the cell wall of hyphae, phialides and conidiophores were

observed on *Fusariumsolani*, *Rhizoctoniasolani* and *Penicilliumcitrinum*. These alterations in the wall were not detected with the untreated hyphae. Disorganization of the cytoplasm was also recorded. Additionally, vacuoles were completely disappeared under influence of the fungicides. For compost tea, presented results were less magnitudes in reducing growth mycelium of the pathogen for 23.63 % instead of 31.58 % by Shalaby *et al.* (2011).

To investigate if the inhibitory effects of *C. maydis* due to the tested agents will be extended by increasing their doses, experimental data were fitted. The fitted functions of each tested agent with the experimental data of mycelium inhibition were plotted in Fig. (1) H<sub>2</sub>O<sub>2</sub>, Fig. (2) Premis and Fig. (3) for garlic oil. As well as, concentration of each agent caused 50 % growth inhibition (IC<sub>50</sub>) of the pathogen was therefore determined. For this purpose, nonlinear least squares fitting routine of MicroCal's ORIGIN® software package was used. Due to their kinetic functions, Michaelis-Menten enzyme relations (Monod, 1949) were applied and found to be the least deviation curve giving the most fitting acceptable model for H<sub>2</sub>O<sub>2</sub> and Premis.

It is worthy to note that the resulted IC<sub>50</sub> values were reached to 0.7 % of H<sub>2</sub>O<sub>2</sub> and 7.0 % for Premis with 0.97 and 0.90 regression (R<sup>2</sup>),

respectively values obtained due to the nonlinear fitting of 1 % data.

Destroying of cell wall and/or cytoplasm was probably the common mode of action due to H<sub>2</sub>O<sub>2</sub> and Premis. This was in agreement with the findings of Abd El-Ghany and Tayel (2009). They suggested that Disorder and striking changes in the cell wall of hyphae, phialides and conidiophores were observed on *Fusariumsolani*, *Rhizoctoniasolani* and *Penicilliumcitrinum*. These alterations in the wall were not detected with the untreated hyphae. Disorganization of the cytoplasm was also recorded and vacuoles were completely disappeared in presence of the fungicide. Antifungal activity of H<sub>2</sub>O<sub>2</sub> was also in full agreement with the data obtained by Leinaet *al.* (1998) who stated that spore germination in *Pseudocercosporaabelmoschi* and *Pseudocercosporacruenta*, pathogens of *Hibiscus esculentus* L. and *Vignasinensis ssp. sesquipetalis* (L.) van Eseltine, was significantly inhibited when incubated with different levels of hydrogen peroxide. They also found significant reduction in mycelial growth in both fungi at different levels of H<sub>2</sub>O<sub>2</sub>. Spore germination and mycelial growth were inhibited significantly at higher levels of peroxidase.

For garlic oil (Fig. 3), fitting linear was found to be the most acceptable (0.98 regression (R<sup>2</sup>)), indicating that the tested dose was not limited yet

**TABLE 3. Inhibitory effects of the tested control agents on the linear growth of the pathogenic fungus of maize late wilt disease under laboratory conditions.**

Treatment	Concentration %	Linear growth (Cm)				Inhibition (%)
		R1	R2	R3	Mean	
Algal filtrate	100	0.0	0.0	0.0	0.00	100.00
compost tea	10	4.3	4.8	4.8	4.63	23.63
	3	0.7	0.6	0.6	0.63	89.56
H <sub>2</sub> O <sub>2</sub>	2	2.1	2.0	1.9	2.00	67.03
	1	2.4	2.6	2.4	2.47	59.34
	30	0.0	0.0	0.0	0.00	100.00
Premis	20	2.1	2.4	2.1	2.20	63.74
	10	2.9	2.8	2.6	2.77	54.40
	6	0.1	0.0	0.2	0.10	98.35
Garlic oil	3	2.4	3.1	2.6	2.70	55.49
	1.5	5.1	5.1	4.8	5.00	17.58
Barnyardgrass root exudates	100	0.1	0.2	0.2	0.17	97.25
Control	0.0	6.2	5.9	6.1	6.07	0.00

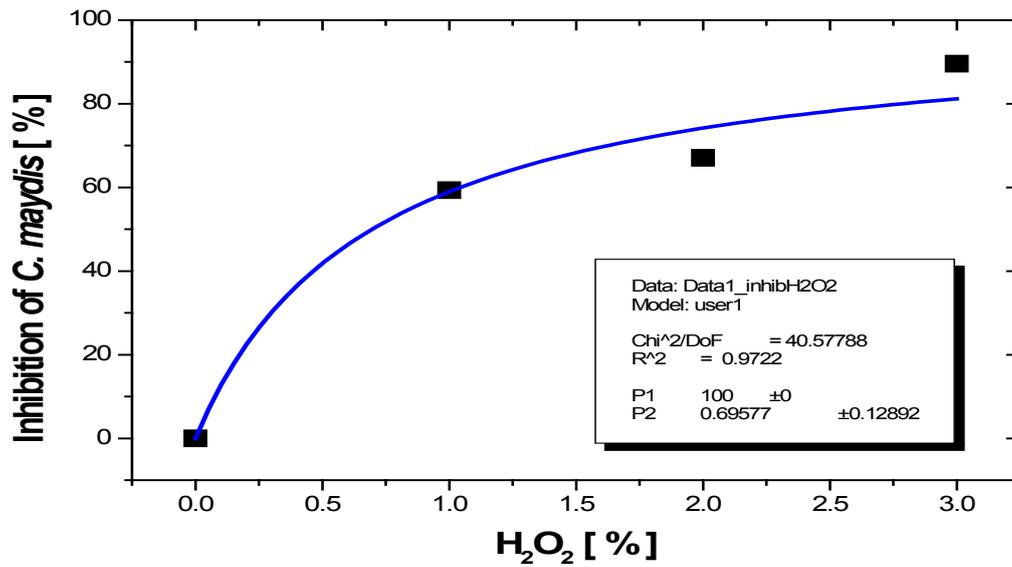


Fig. 1. Inhibitory effects of H<sub>2</sub>O<sub>2</sub> against *C. maydis* in relation to its concentrations. Symbol refers to the experimental data and line refers to fitted data with Monod's equation.

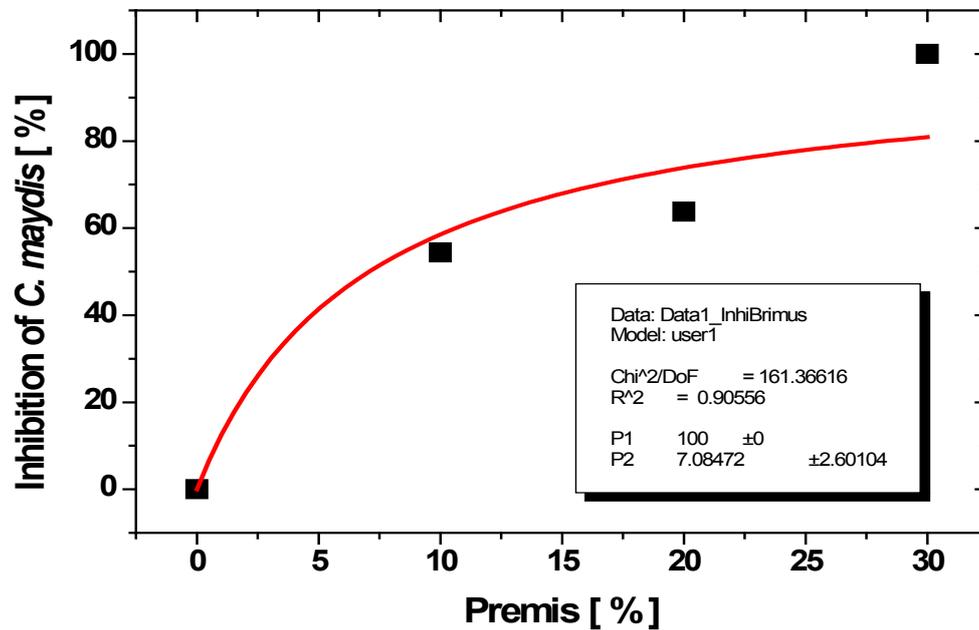


Fig. 2. Inhibitory effects of Premis against *C. maydis* in relation to its concentrations. Symbol refers to the experimental data and line refers to fitted data with Monod's equation.

to the growth of the pathogenic fungus and more concentrations were needed to reach maximum inhibition. Here,  $IC_{50}$  value due to garlic oil reached 3.2 % (Data not shown). Singh *et al.* (1997) found that growth of *Fusariumoxysporium* f. sp. *ciceri* and *Sclerotiniasclerotiorum* in liquid media incorporated with 5000 to 7000 ppm of garlic cloves juice was greatly reduced. Fungicidal activities of the ethanol extracts of *Allium sativum*, were *in vitro* achieved against *Fusariumsolani*, *Rhizoctoniasolani* and *Macrophominaphaseolina* (Dawaret *al.*, 2008). They found also that ethanol extract of spices was more effective in the control of root rot pathogens as compared to aqueous extract.

Fitting data of  $H_2O_2$ , premis and garlic oil with the very good regression ( $R^2$ -values) were expressed by the simple kinetic parameters, which based on a limitation of growth rate by substrate concentrations. Similar explanation was confirmed by Barr and Aust (1994), who found that the metabolism of chemicals by bacteria and fungi involves mostly enzymatic conversions; pollutant degradation often follows Michaelis-Menten-type kinetics, which represented the enzymatic form of Monod kinetics (Monod, 1949). So, higher enzyme sensitivity of the pathogens toward  $H_2O_2$ , premis and garlic oil were compatible with greatest antagonistic effects. Nesci *et al.* (2003) explained that they may inhibit the functions of

several enzymes by the oxidized compounds or/ and by more nonspecific interactions with the proteins. Based on regulation of the enzyme activity explained by Schlegel (1992), the cells may be containing sensitive system to adjust activity of these enzymes, in addition to regulation of its levels.

#### Seed germination test

Effect of the tested control agents on germination indexes and percentages of maize grains were tested during two successive seasons and data were presented in Table (4). Data of germination index and percentages showed varied readings with superiority of 3%  $H_2O_2$  treatment reached 1.52 and 54.73 %, respectively at 2015 season compared with control (1.43 and 49.87%, respectively). Superiority of 3%  $H_2O_2$  was delayed to the second level with 1.75 germination indexes and 55.40 % germination percentages compared to 1.58 and 52.99 %, respectively for control at 2016 season. The highest corresponding values (1.88 and 63.07 %) were recorded by the blue-green algal filtrates. Compost tea in the single form and in its combination with the algal filtrate were the following effective treatment with 1.67 and 1.66 indexes and 54.27 and 54.93 % percentages, respectively at the same season.

Although the tested agents have great antagonistic effects, data of the germination

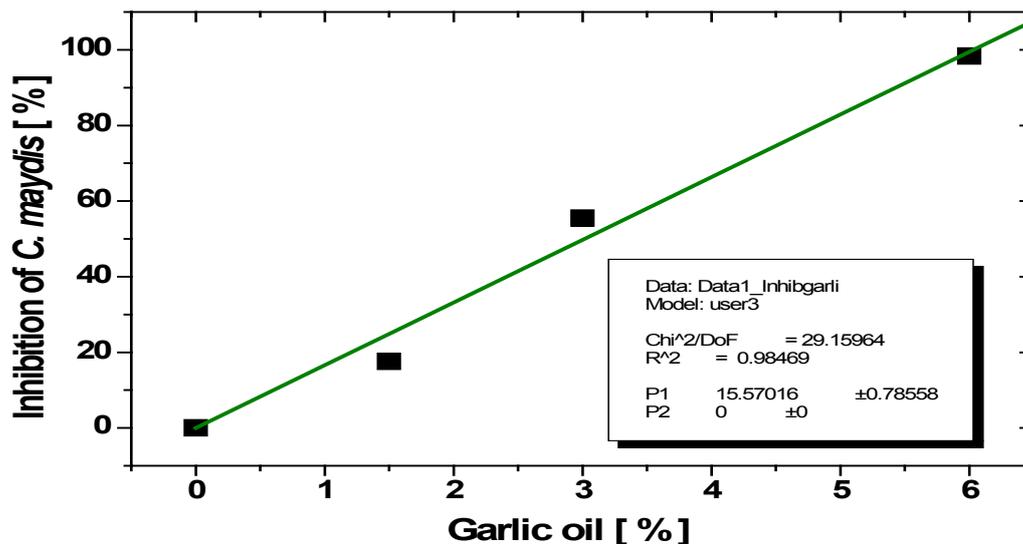


Fig. 3. Inhibitory effects of garlic oil against *C. maydis* in relation to its concentrations. Symbol refers to the experimental data and line refers to fitted data with Monod's equation.

test proved that their role played for promoting growth parameters doesn't appear clearly yet. Low germination of maize grains might be due to high available ammonium in polyethylene boxes (Ells *et al.*, 1991).

#### *Disease assessment*

Effectiveness of the selected control agents was also evaluated against *C. maydis* under disease nursery conditions. During two successive growing seasons of 2015 and 2016, field experiments were carried out. Their efficacy towards late wilt disease expressions were represented by percentages of disease incidence, survival and disease reduction (Table 5). It is worthy to note that disease incidences of late wilt in control plants were nearly identical in both studied seasons (19.29 and 19.20 %), indicating equal severity of *C. maydis* inoculated the disease nursery in both seasons.

Data of disease incidence showed better efficacy of 30 % Premis fungicide followed by 3 % H<sub>2</sub>O<sub>2</sub> and dual treatment between compost tea and algal filtrate with 3.24, 4.75 and 5.10 % respectively during 2015 season, by them massive disease reductions reached 83.21, 75.37 and 73.54 %, respectively were done. It indicates that the defensive capacity of maize grains was increased due to suppressive effects of such treatments against the late wilt pathogenic fungus. Effectiveness of the 3 % H<sub>2</sub>O<sub>2</sub> dose was extended to the 2016 season with 5.11 % disease incidence and 73.39 % disease reduction. Whereas, the 2% H<sub>2</sub>O<sub>2</sub> dose was the superior causing reduce of disease incidence to 4.42 % or about 76.99 % disease reduction giving the highest value of 95.58 % survival plants at 2016 season.

Moreover, significant efficacy for reducing disease incidence reached 7.06 causing 63.23%

**TABLE 4. Effect of the tested control agents on germination indexes and percentages of maize grains during 2015 and 2016 seasons in polyethylene box trials.**

Treatment	Concentration %	Germination 2015		Germination 2016	
		Index	%	Index	%
Algal filtrate	100	1.26	46.67 abc	1.88	63.07 a
compost tea	10	1.28	46.60 abc	1.67	54.27 ab
Compost tea + Algal filtrate	1 : 1	1.41	51.33 ab	1.66	54.93 ab
	3	1.52	54.73 a	1.75	55.40 ab
H <sub>2</sub> O <sub>2</sub>	2	1.21	43.00 abcd	1.50	51.80 abc
	1	1.31	46.33 abc	1.40	46.53 bcde
	30	1.18	41.60 abcd	1.27	40.80 de
Premis	20	1.39	48.87 abc	1.49	48.53 bcd
	10	1.38	49.40 abc	1.58	53.20 abc
	6	1.01	36.80 bcd	1.07	36.27 ef
Garlic oil	3	0.93	33.00 cd	0.87	28.73 fg
	1.5	0.85	29.80 d	0.81	26.53 g
Barnyardgrass root exudates	1	1.42	49.60 abc	1.26	41.33 cde
Barnyard cultivation	-	1.39	48.40 abc	1.46	53.20 abc
Control	0	1.43	49.87 abc	1.58	52.99 abc

Averages in a column followed by a different letter are significantly different at 0.05 level after a Duncan's multiple range test.

**TABLE 5.** Effects of the tested control agents on rating of late wilt disease index parameters of maize plants in the disease nursery during 2015 and 2016 growing seasons

Treatment	Concentration %	Disease expressions ( % )					
		Disease incidence	2015 Survival plants	Disease reduction	Disease incidence	2016 Survival plants	Disease reduction
Algal filtrate	100	8.75 bc	91.25	54.65	10.63 ab	89.37	44.63
compost tea	10	12.26 b	87.74	36.41	7.06 b	92.94	63.23
Compost tea + Algal filtrate	1 : 1	5.10 c	94.90	73.54	11.65 ab	88.35	39.28
H <sub>2</sub> O <sub>2</sub>	3	4.75 c	95.25	75.37	5.11 b	94.89	73.39
	2	6.27 c	93.73	67.51	4.42 b	95.58	76.99
	1	7.41 bc	92.59	61.58	12.63 ab	87.37	34.21
	30	3.24 c	96.76	83.21	7.96 b	92.04	58.53
Premis	20	6.20 c	93.80	67.86	11.10 ab	88.90	42.18
	10	8.11 bc	91.89	57.93	9.41 b	90.59	50.96
	6	6.92 c	93.08	64.12	7.56 b	92.44	60.61
Garlic oil	3	8.55 bc	91.45	55.64	10.69 ab	89.31	44.29
	1.5	8.36 bc	91.64	56.67	12.17 ab	87.83	36.57
Barnyardgrass root exudates	1	6.43 c	93.57	66.67	7.12 b	92.88	62.91
Barnyard cultivation	-	9.02 bc	90.98	53.23	9.13 b	90.87	52.44
Control	0	19.29 a	80.71	0.00	19.20 a	80.81	0.00

Averages in a column followed by a different letter are significantly different at 0.05 level after a Duncan's multiple range test.

disease reductions and 92.94 % survival percentage due to use compost tea during 2016 season. This was in agreement with Kone' *et al.* (2010), who attributed effect of compost teas to the physical and chemical properties of its nutrients. This may improve the nutritional status of plants, be directly toxic to the pathogen, and/or induce systemic resistance to the pathogen. A hypothesis stated that compost tea seems to act as a bio-control of pathogens by favoring the growth of beneficial bacteria (Diánzet *et al.*, 2007). Therefore, compost tea considered one of the most promise bio-products recently responsible for developing different management programs as plant pest, disease and fertility, in addition to promote growth of the plants. Accordingly, antagonistic effect of compost tea was confirmed against *Pythiummultimum*, *Rhizoctoniasolani*, *Phytophthoraspp*, *Fusariumoxysporum* and *Verticilliumdahliae* (Noble and Coventry, 2005). Similarly of compost tea, Barnyard grass extract, 6% garlic oil and 30 % premis were considered of the most effective control agents of late wilt disease at 2016 season, respectively. By yeast strain, role played for controlling late wilt disease,

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but with non-significant magnitudes.

#### *Grain Productivity*

Due to their relative convergence, weight of both 10 ears and 1000 grains of both experimental seasons of maize plants were investigated. Concerning weights of 10 ears, Fig. (4) Indicated great enhancements due to use all treatments compared to control. As well as, data presented in Table (6) showed majority weights of the 1000 grain yield reached 0.461 Kg due to use 1 % H<sub>2</sub>O<sub>2</sub> application during 2015 season compared to 0.357 Kg for control. Whereas, the maximum weight of 1000 grains reached 0.471 Kg was recorded by using 1.5 % garlic oil compared to 0.408 Kg for control during 2016 season. It was in the same trend indicated general improvements of the 1000 grains weights due to most tested agents during both seasons. Here, it is worthy to notice that the great suppressive efficiencies of both 2 and 3 % H<sub>2</sub>O<sub>2</sub> doses did not reflex to enhance grain productivity, but reduction was resulted. It indicated toxicity of the plant tissues due to use higher doses of H<sub>2</sub>O<sub>2</sub> leading to reduce their ability to form new grain primordia cells in

maize ears. On contrary with findings of Shalaby *et al.* (2011), effect of compost tea for increasing weight of 1000 grains was less magnitude in the presented study.

#### Biochemical analysis

Due to their remarkable antagonistic efficiencies against *C. maydis* and yield enhancing role, chemical compositions of both blue-green algae and root exudates of barnyard grass was assessed. Blue-green algae and root exudates of *Echinochloa crus-galli* (Barnyard grass) were assayed on Gas chromatography mass-spectrum (GC-MS) for making qualitative analysis. Results presented in Table (7) and Fig. (5) indicated that the major compounds extracted from the cultured blue-green algae were summarized from 19 to seven compounds. Malonic acid, 2,3-Butandiol, Hexestrol, 12-Crown-4-ether and cis-Vaccenic acid were the most abundant compounds. Their peaks appeared at 3.69, 4.60, 5.08, 6.72 and 16.86 minutes of retention time, respectively. For absolute quantification, the amount of endogenous compound can be calculated from peak areas. Malonic acid and 12-Crown-4-ether have maximum peak areas (18.53 and 18.56 % of peak area, respectively) and then highest concentrations between the compounds which extracted from the blue-green algae. The cis-Vaccenic acid ranks in the second order as concerns its concentration with 14.65 % of peak area. The reminder 4 compounds recorded lower peak areas ranged between 7.87 to 5.18 % ranked at the third order. There are 12 other compounds (Data not shown) recorded lowest concentrations in comparison with the other compounds, indicating weak antimicrobial activities.

The screening for antifungal compounds

produced by cyanobacteria led us to discover new strains producing scytophycins and hassallidins. Antifungal compounds were detected from strains belonging to the Nostocales and Stigonematales orders, such as Anabaena, Fischerella, Nostoc and Scytonema (Guggeret *et al.*, 2002). The presented results were in agreement with Abed *et al.* (2009), who indicated that the antifungal compounds have been previously detected in cyanobacterial extracts, such as fischerellin A, hapalindole, hassallidin/balticidins, carazostatin, phytoalexin, tolytoxin, scytophycin, toyocamycin, tjipanazole, nostocyclamide, nostodione and nostofungicide. Root exudates of *Echinochloa crus-galli* (Barnyard grass) were assayed and data were recorded in Table (8) and Fig. (6). It demonstrated that the major compounds were summarized from 39 to 14 compounds. Thymol and Vanillin were the first and second compounds appeared their peaks after 5.28 and 7.10 minutes of retention time from beginning, respectively. On the other hand, 3,5-di-t-Butyl-Catechol was the last one appeared its peak at 23.85 min. of retention time. For absolute quantification, the amount of endogenous compound can be calculated from peak areas. Nadolol has a maximum peak area (1000376571.2 of peak area) and then highest concentration between the compounds which extracted from the barnyard root exudates. Quinine ranks in the second order as concerns its concentration with 108216727.0 of peak area.  $\alpha$ -Methylionol and Phyllocladene ranked at the third order with 71330793.5 and 68315202.2 of peak areas. The other compounds included alcohols, acids, phenols, 2,6-dihydroxy benzoic acid or some metabolites represent antimicrobial agents, although they have lower concentrations.

The results indicated that the extracts of

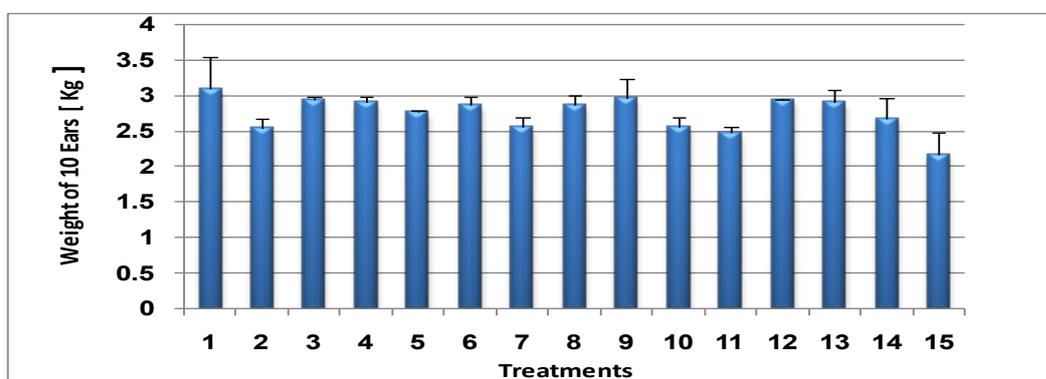


Fig. 4. Effect of the tested agents on the average weight of a 10 ears of maize plants in late wilt disease nursery and their significant power during the investigated seasons. Where: 1= Algal filtrate, 2= compost tea, 3= Compost tea + Algal filtrate, 4, 5, 6= 3, 2, 1% H<sub>2</sub>O<sub>2</sub>, 7, 8, 9= 30, 20, 10% premis, 10, 11, 12= 6, 3, 1.5 % garlic oil, 13= Barnyard grass extract, 14= Barnyard cultivation and 15= control.

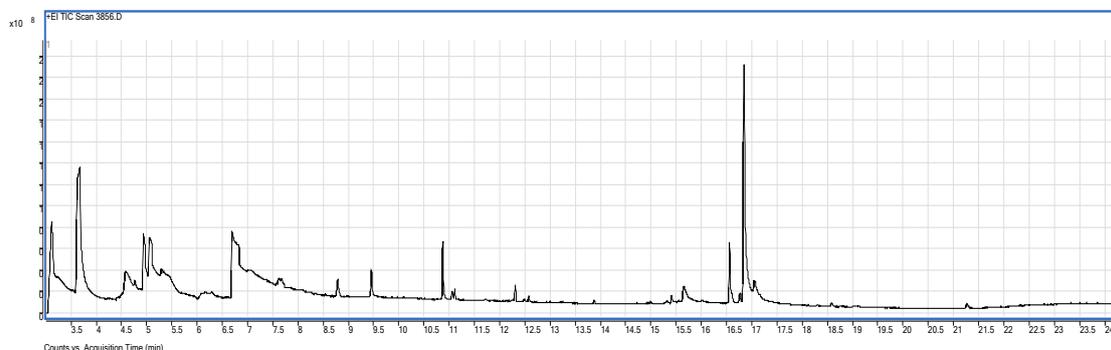
**TABLE 6.** Effect of the tested control agents on weight of 1000 maize grains in the disease nursery during 2015 and 2016 growing seasons.

Treatment	Concentration %	Weight of 1000 grains ( Kg )	
		2015	2016
Algal filtrate	100	0.389 bcd	0.416 abc
compost tea	10	0.395 bc	0.449 ab
Compost tea + Algal filtrate	1 : 1	0.386 bcd	0.451ab
	3	0.399 bc	0.382 bc
H <sub>2</sub> O <sub>2</sub>	2	0.376 cd	0.365 c
	1	0.461a	0.457 ab
	30	0.385 bcd	0.419 abc
Premis	20	0.397 bc	0.425 abc
	10	0.339 e	0.454 ab
	6	0.364cde	0.428 abc
Garlic oil	3	0.368 cde	0.421 abc
	1.5	0.395 bc	0.470 a
Barnyardgrass root exudates	1	0.417 b	0.434 ab
Barnyardgrass cultivation	-	0.402 bc	0.454 ab
control	0	0.357 de	0.408 abc

Averages in a column followed by a different letter are significantly different at 0.05 level after a Duncan's multiple range test.

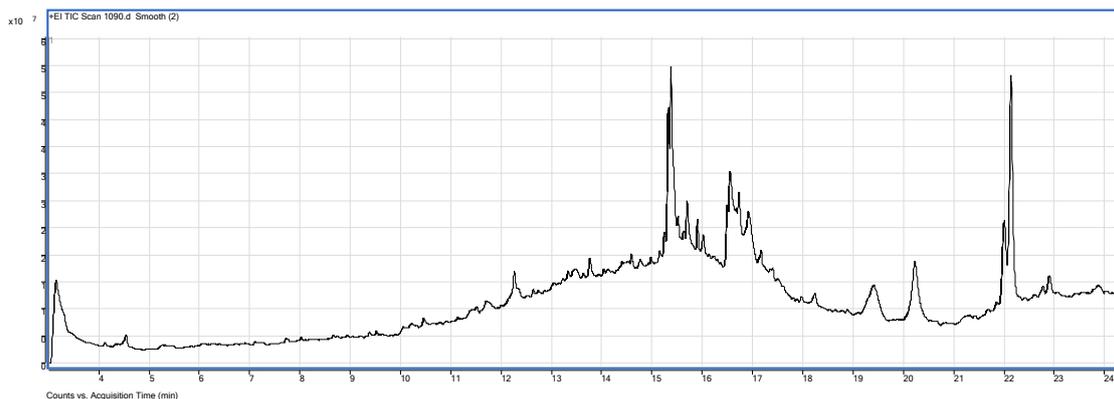
**TABLE 7.** Chemical compositions of the blue-green algae extract analyzed by the laboratory of organic pollutants, ARC, Cairo, Egypt (Sample No. 3856). (7 from 19 compounds).

No.	RT (min)	Name	Area sum %
1	3.69	Malonic acid	18.53
2	4.60	2,3-Butandiol	7.87
3	5.08	Hexestrol	7.70
4	6.09	Polyneuridinedioldiacetate	5.33
5	6.72	12-Crown-4-ether	18.85
6	16.86	cis-Vaccenic acid	14.56
7	17.06	cis-11-Eicosenoic acid	5.18

**Fig. 5.** Determination of the extracted compounds from the blue-green algaeon Gas chromatography mass-spectrum (GC-MS).

**TABLE 8. Chemical compositions of the *Echonochoa crus-gali* (Barnyardgrass) root exudates analyzed by the laboratory of organic pollutants, ARC, Cairo, Egypt (Sample No. 1090). (14 from 39 compounds)**

No.	RT (min)	Name	Area
1	5.28	Thymol	10516841.2
2	7.10	Vanillin	11233775.4
3	10.22	3,3-Dinitro-4,4-dihydroxy-diphenylsulphone	13658067.9
4	12.25	p-Cresol-2,2-methylenebis[6-tert-butyl]	38158890.8
5	15.33	Cyanidin cation	26467829.6
6	16.01	Argyrophilic acid methyl ester	11605552.3
7	16.55	3-Hydroxypyridine	34817531.1
8	16.93	Phyllocladene	68315202.2
9	19.40	Nadolol	1000376571.2
10	20.24	Quinine	108216727.0
11	21.23	2,6-dihydroxy benzoic acid	28999106.2
12	21.87	Phenol, 4-tert-butyl	25909859.8
13	22.02	$\alpha$ - Methylionol	71330793.5
14	23.85	3,5-di-t-Butyl-Catechol	20890823.3

**Fig. 6. Determination of the extracted compounds from the *Echonochoa crus-gali* (Barnyardgrass) root on Gas chromatography mass- spectrum (GC-MS).**

barnyardgrass had the phytotoxic influence on maize plants. Varied antifungal degrees due to different areas of such barnyard grass root extracts were done. The degree of inhibition was largely dependent on barnyardgrass tissue types and the treated maize plants. These results specified that barnyardgrass release allelopathic substances such as 2,6-dihydroxy benzoic acid. These results were in full agreement with the finding of Li *et al.*, (1992) and Yamamoto *et al.*, (1999) who suggested that allelopathy of barnyardgrass against crops was correlated with the amounts of phenolic acids released specially phydroxybenzoicacid.

Weeds especially barnyardgrass can produce many kinds of secondary metabolites through its tissues. These metabolites in barnyardgrass

root extracts possess multiple functions on the chemical interactions among organisms in the environment. Our results were in agreement with results of Xuan *et al.*, (2006) who suggested that p-Hydroxybenzaldehyde, phydroxybenzen and p-hydroxybenzoicacid as major allelochemicals in barnyardgrass. They recommended that these compounds may be, at least, a key factor in barnyardgrassallelopathy on rice and other crops. The amount of nitrogen content is often equivalent by protein content in crop production. By using of allelopathy definition, it is obvious that allelochemicals could affect all phases of nitrogen biological fixation, mineralization and nitrification (Reigosaet *al.*, 2006). So, applicability of such effective and environmentally safe tested agents for overcome, or at least reduces, the

harmful effects of the chemical fungicides applied for controlling late wilt disease and enhancement maize productivity was preferred to recommend as promising agents.

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(Received 16/10/2017;  
accepted 20/11/2017)