EFFICACY OF SOME ORGANIC COMPOUNDS IN CONTROLLING FUSARIUM WILT DISEASE, GROWTH AND YIELD PARAMETERS IN CHICKPEA PLANTS

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* causes considerable yield loss of chickpea. The possibility to reduce wilt severity in chickpea cv. Giza 3 using some organic compounds *viz.*, Inicium, Bio-Health, Alga Grow-4, Humic acid and tea of date palm leaves compost was investigated. The obtained data indicated that all tested organic compounds were able to reduce area under wilt progress curve (AUWPC) caused by *F. oxysporum* f. sp. *ciceris* under greenhouse and field conditions. Humic acid recorded the highest reduction of AUWPC followed by Inicium. While, Alga Grow-4 gave the lowest reduction in this respect. On the other hand, all organic compounds significantly increased the tested growth and yield parameters except protein content in seeds during growing seasons 2014-2015 and 2015-216. Humic acid recorded the highest increase of all tested growth and yield parameters in both growing seasons. While Alga Grow-4 gave the lowest ones in this respect. In biochemical studies, activity of defense-related enzymes, including peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), pathogenesis related (PR) protein as well as phenolic compounds (total, free, conjugated phenols) were increased in plants treated with organic compounds and grown in pots infested with *F. oxysporum* f. sp. *ciceris* compared with untreated plants (control). In general, Humic acid recorded the highest activity of PO, PPO, PAL, β-1,3 glucanase, phenolic contents followed by Inicium, while Inicium recorded the highest chitinase activity followed by Humic acid. On contrary, Alga Grow -4 gave the lowest activity of PO, PPO, and PAL. The lowest activity of chitinase and β-1,3 glucanase and phenolic contents was recorded in case of compost –T treatment.

Keywords: Chickpea, Organic compounds, Oxidative enzymes, Pathogenesis related (PR) protein, plant growth and yield parameters, Wilt disease

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important crops growing in the world, but the yield and quality of chickpea are influenced by Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Sato & Matuo (Salem *et al.*, 1991). Fusarium wilt is one of the most important and destructive vascular disease of chickpea (Omar *et al.*, 1999). This disease causes considerable yield losses which may reach up to 100 % under conditions favorable for high disease severity**.** There are eight races of *F. oxysporum* f. sp. *ciceris* (0, 1A, 1B/C, 2, 3, 4, 5 and 6) which are identified by reaction on a set of differential chickpea cultivars**.** The most efficient method for the management of disease is using resistant cultivars**,** although new races of the pathogen appear to overcome resistant genes. Fungicides however used for effective control of Fusarium wilt of chickpea (Maitlo *et al*., 2014), heal ever, there are concerns of the harmful effects of these chemicals to environment, human health, and microbial ecology, including pathogen resistance to fungicides (Ha and Huang, 2007). Other control methods such as the use of tolerant cultivar and cultural practices were often unsuccessful. Therefore, it is important to seek alternative methods that are effective and environmentally friendly for management of this disease.

Such strategy includes the use of effective microorganisms to control fungal plant diseases as well as other fungicides alternatives such as plant resistance inducers organic compounds (Abdel-Monaim *et al*., 2014).

Organic supplements, however, are one of the biological means for the control of soil borne diseases. Bio-agents produce biologically active compounds (antibiotics & toxic substances) that have antifungal activity, besides bioactive compounds such as plant growth regulators like gibberellin, auxin, cytokinin, ethylene, abscisic acid, jasminic acid, protein, vitamins and minerals (Noble and Coventry, 2005). Alga Grow-4, Bio-Health, Humic acid, Inicium and compost –T are organic compounds which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases, stimulation of plant growth through increased cell division, as well as optimizing uptake of nutrients and water. Moreover, such treatments stimulated growth of the useful soil microorganisms as mentioned by Abdel-Monaim *et al*. (2014).

The present study aimed to evaluate some organic compounds as resistance inducers in chickpea plants against wilt disease under greenhouse and field conditions. Also, the effect on growth and yield parameters were also studied in the field.

MATERIALS AND METHODS

Source of chickpea seeds and growth of plants.

Chickpea (*Cicer arietinum* L.) susceptible cultivar Giza 3 used in this study was obtained from Legume Crop Res. Dep., Field Crop Res. Inst., Agric. Res. Center, Ministry of Agric., Egypt. Seeds were planted in plastic pots 30 cm in diameter (2.4 kg soil), filled with a sterilized mixture of loamy sand soil (84.27% sand, 8.25% silt, 7.48% clay). Five seeds were sown in each pot and these pots were irrigated every three days.

Source of fungal pathogen

The highly pathogenic *F. oxysporum* f. sp. *ciceris* isolate isolated from diseased chickpea plants collected from New Valley Governorate was used in this study.

Preparation of fungal inoculum

The inoculum of *F. oxysporum* f. sp. *ciceris* isolate was prepared from one week old culture grown on 50 mL potato dextrose broth (PDB) medium in conical flask (250 mL) and incubated at 25 ± 1°C. The content of flask were homogenized in a blender for one min. Plastic (30 Cm) pots were filled with sterilized soil and mixed with fungal inocula (100 mL homogenized culture per pot), seven days before planting.

Source of Organic Compounds

In this experiment, four organic compounds were evaluated under greenhouse and field conditions to control wilt disease on chickpea cv. Giza 3 are listed in Table (1).

Preparation of date palm leaves compost tea:

Date palm leaves compost was obtained from Soil and Water Research Department, New Valley Res. Station. Date palm compost tea was prepared by mixing date palm leaves compost with tap water at a ratio of 1:5(w/v) followed by fermentation for one week. Then, it was stirred once every day and allowed to ferment at the New Valley Agric. Res. Station at 25°C. After 7 days, the solution was filtered through cheese cloth. Then the prepared compost -T was ready for application use.

Effect of organic compounds on wilt disease under greenhouse conditions:

The experiments were carried out in the greenhouse at the New Valley Agric. Res. Station. Pot experiments were conducted to investigate the influence of chickpea seeds treatment with each of organic compounds listed in Table (1) on the wilt disease caused by *F. oxysporum* f. sp. *ciceris*. Chickpea seeds cv. Giza 3 were sterilized with sodium hypochlorite (5%) for 2 minutes, washed several times with sterile distilled water. Chickpea seeds were soaked in the following treatments, Inicium, Bio-Health, Alga Grow-4, Humic acid and Compost -T at the recommended concentrations, for 6 hours. Five seeds were grown in each pot and 4 replicates were used for each treatment. In addition, untreated seeds were grown in pots containing infested soil (infected control). Plants were irrigated when needed and fertilized as usual.

Disease assessments:

Disease reactions were assessed based on the severity of symptoms at 20 days intervals, for 100 days using a 0 to 4 rating scale based on the percentage of foliage yellowing (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, and 4 = dead plant). Disease severity index (DSI) described by Liu *et al*. (1995) was adopted and calculated as follows:

$$DSI= \Sigma d/(d\ max \times n) \times 100$$

Where: (d) is the disease rating of each plant, (d) max the maximum disease rating and (n) the total number of plants examined in each replicate.

The mean of area under disease progress curve (AUWPC) for each replicate was calculated as suggested by Pandy *et al.* (1989).

$$AUWPC= D\ [1/2\ (Y1+Yk) + (Y2+Y3+\dots\dots+Yk-1)]$$

Where D= Time interval; Y1= First disease severity; Yk= Last disease severity;

Y2, Y3,……Yk-1= Intermediate disease severity.

Table 1. Trade name, common name, active ingredient % and recommended doses of the used organic compounds.

Trade name	Common name	% Active ingredient	Recommended doses	Company
Inicium	Amino acids and phosphorus pentoxide.	10%FS	5 mL/L	Bioiberica Co., Spain
Bio- Health	*T. harzianum*, *B. subtilis*, Amino acids, Trace elements, Humic acid, Fulvic acid, Vitamins, Auxin and Cytokinin.	100% WSG	2.5 g/L	Humin Tech Co. Germany
Alga Grow-4	Algae extract, nitrogen, phosphorus pentoxide and potassium oxide.	16% FS	1 mL/L	Beijing Multigrass Formulation Co., LTD, China
Humic acid	Potassium humate soluble granule	85% WSG	4 g/L	Broadtech Chemical International Co. Ltd., Inner Mongolia, China

FS= Flowable concentrates for Seed treatment **WSG**= Water soluble granule

Field experiments:

Field experiments were carried out at New Valley Res. Station Farm during 2014-2015 and 2015-2016 growing seasons, to evaluate the efficiency of organic compounds listed in Table (1) for controlling wilt disease and its effect on growth and yield parameters under field conditions. The experimental design was a complete randomized block design with three replicates. The experimental unit area was 10.5 m^2 (3.5 × 3m). Each unit included 5 rows; each row was 3.5 m in length and 60 cm width. Chickpea seeds (cv. Giza 3) were soaked in treatments described above for 6 hrs. The treated seeds were sown in hills 25 cm apart on one side of the row in both seasons, two seed per hill. In control treatment, chickpea seeds were soaked in water for 6 hrs. and sown at the same rate. The normal cultural practices of growing chickpea were followed. The mean of area under wilt progress curve (AUWPC) for each replicate was calculated as above. At harvest, plant height (cm), number of branches/plant, number of pods/plant, number of seeds/plant, 100-seed weight (seed index) and total yield (kg/plot) were recorded.

Total crude protein (%) in chickpea seeds was determined. The determined nitrogen of dry seeds was used for calculating total crude protein by multiplying N-values by 6.25 (A.O.A.C., 2000).

Biochemical changes associated with organic compounds treatments:

After 15 days from planting, fresh samples were taken from plants grown from previously treated and untreated chickpea seeds and extracted according to Maxwell and Bateman (1967). Then the extracts were used for assaying biochemical change associated with the tested treatments with organic compounds, the activities of peroxidase enzyme (Hammerschmidt *et al*. (1982), polyphenoloxidase enzyme (Gauillard *et al*., 1993), phenylalanine ammonia lyase enzyme (Cavalcanti et al., 2007), chitanase enzyme (Wirth and Wolf, 1992) and β-1,3-glucanase (Pan *et al*., 1991) were determined.

Protein concentration

Total protein content of the samples was quantified according to the method described by Bradford (1976).

Phenolic contents

Phenolic compounds were colorimetrically determined using phosphotungestic phosphomolybdic acid (Folin Ciocalteau) reagents according to Snell and Snell (1953). Standard curve of catechol was used to calculate the amount of phenolic compounds in different tested samples. Obtained results were expressed as catechol equivalents in milligrams per gram fresh weight.

Statistical analysis:

Analyses of variance were carried out using MSTATC, 1991 program ver. 2.10. Least significant difference was employed to test for significant difference between treatments at $p \leq 0.05$ (Gomez and Gomez. 1984).

RESULTS

Effect of organic compounds on wilt disease under greenhouse and field conditions:

The effect of some organic compounds applied as seed soaking on infection of chickpea plants grown in artificially infested soil with a highly pathogenic isolate of *F. oxysporum* f. sp. *ciceris* was investigated. Results in Table (2) showed that all treatments led to significant reductions in the area under wilt progress curve (AUDPC) compared to the check treatment (control) under greenhouse conditions. Humic acid and Inicium resulted the lowest AUWPC, recorded 152.60 and 199.70 AUWPC compared with 955.40 in control, respectively. While, Alga Grow-4 gave the lowest reduction of infection (485.80 AUWPC). On the other hand, the same trend was obtained under field conditions during growing seasons 2014-2015 and 2015-2016 (Tables 3 and 4).

Table 2. Effect of soaking chickpea seeds (cv. Giza 3) in organic compounds on wilt severity and area under wilt progress curve (AUWPC) caused by *F. oxysporum* f. sp. *ciceris* under greenhouse conditions.

Treatments	% Wilt severity after ;[a)]					AUWPC[b)]
	20 days	40 days	60 days	80 days	100 days	
Inicium	0.00c	1.67d	2.65cd	3.33d	4.67d	199.70d
Bio- Health	0.00c	2.33c	3.00cd	4.43c	6.00cd	255.20c
Alga Grow-4	2.33b	3.33b	5.69b	6.77b	14.67b	485.80b
Humic acid	0.00c	0.00e	2.13d	3.00d	5.00d	152.60e
Compost-T	0.00c	2.67c	3.44c	4.50c	7.42c	286.40c
Control	3.67a	6.67a	10.76a	15.67a	25.67a	955.40a

Different letters indicate significant differences among treatments within the same color column according to least significant difference test ($P \leq 0.05$).

[a)]DSI= $\Sigma d/(d\ max \times n) \times 100$

[b)] AUWPC= $D\ [1/2\ (Y1+Yk) + (Y2+Y3+........+Yk\text{-}1)]$

Table 3. Effect of soaking seeds in organic compounds on wilt severity and area under wilt progress curve (AUWPC) during seasons 2014-2015 under field conditions.

Treatments	% Wilt severity after;					AUWPC
	20 days	40 days	60 days	80 days	100 days	
Inicium	0.00c	0.00d	2.19c	2.66d	3.88cd	135.80de
Bio- Health	0.00c	2.01c	2.36c	3.48c	4.27cd	199.70c
Alga Grow-4	2.36b	2.95b	3.95b	5.25b	8.59b	352.50b
Humic acid	0.00c	0.00d	1.39d	2.89cd	3.48d	120.40e
Compost-T	0.00c	0.00d	2.36c	3.59c	4.94c	168.40cd
Control	4.69a	6.27a	8.26a	12.18a	19.93a	780.40a

Different letters indicate significant differences among treatments within the same color column according to least significant difference test ($P \leq 0.05$).

Table 4. Effect of soaking seeds in organic compounds on wilt severity and area under wilt progress curve (AUWPC) during seasons 2015-2016, under field conditions.

Treatments	% Wilt severity after;					AUWPC
	20 days	40 days	60 days	80 days	100 days	
Inicium	0.00c	0.00d	1.67d	2.67d	3.89de	125.70e
Bio- Health	0.00c	1.92c	2.63c	3.79c	4.86c	215.40c
Alga Grow-4	2.15b	2.88b	3.64b	5.42b	6.21b	322.40b
Humic acid	0.00c	0.00c	1.36d	2.56d	3.07e	109.10e
Compost-T	0.00c	0.00c	2.05cd	3.18cd	4.53cd	149.90d
Control	4.25a	6.28a	7.56a	10.36a	17.37a	700.20a

Different letters indicate significant differences among treatments within the same color column according to least significant difference test ($P \leq 0.05$).

Effect of organic compounds on growth and yield parameters under field conditions.

Soaking chickpea seeds cv. Giza 3 in the tested organic compounds gave significant increases in plant growth characters (plant height, number of branches per plant) and yield components (number of pods, seeds per plant, seeds weight per plant, weight of 100 seeds and seed yield per plot) compared with control during both growing seasons 2014-2015 and 2015-2016 (Tables 5 and 6). The obtained results indicated that Humic acid recorded the highest growth and yield parameters during both growing seasons (2014-2015 and 2015-2016) followed by Inicium, Bio-Health and date palm leaves compost –tea.

Table 5. Effect of soaking seeds in organic compounds on growth, yield parameters and protein contents during season 2014-2015 under field conditions.

Treatments	Plant height (cm)	Number of branches/plant	Number of pods/plant	Number of seeds/plant	Seed weight/plant	Weight of 100 seeds	Seed yield /plot (Kg)	Protein contents (%)
Inicium	80.24b	5.82b	28.14b	57.45a	13.04ab	23.26ab	2.25c	20.79a
Bio- Health	78.36b	5.96b	27.45bc	50.18b	11.86b	22.69ab	2.10d	20.14a
Alga Grow-4	75.36c	5.63b	25.36c	45.36c	10.25c	22.69ab	1.97e	20.25a
Humic acid	89.23a	7.22a	32.85a	60.12a	14.21a	24.63a	2.63a	20.15a
Compost-T	80.24b	5.96b	29.02b	51.25b	12.17b	23.85ab	2.37b	20.69a
Control	66.96d	4.90c	20.36d	39.63d	9.02c	21.59b	1.76f	20.36a

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

While, Alga Grow -4 gave the lowest increase in plant growth and yield components in both growing seasons 2014-2015 and 2015-2016 compared with the other organic compounds. On the other hand, no significant differences between organic compounds and control in case of protein contents in seeds of both growing seasons. Also, the results recorded in season 2015-16 were nearly similar to those of 2014-15 growing season.

Table 6. Effect of soaking chickpea seeds in organic compounds on growth, yield parameters and protein contents during season 2015-2016, under field conditions.

Treatments	Plant height (cm)	Number of branches/plant	Number of pods/plant	Number of seeds/plant	Seed weight/plant	Weight of 100 seeds	Seed yield /plot	Protein contents (%)
Inicium	79.32b	6.36b	27.54b	52.14b	12.42ab	22.56a	2.16c	20.42a
Bio- Health	73.25c	5.75c	26.05b	47.52c	11.52b	23.85a	2.02d	20.02a
Alga Grow-4	70.05c	5.29d	26.03b	42.63d	9.73c	22.41a	1.90e	20.55a
Humic acid	88.42a	7.01a	33.14a	58.26a	13.56a	24.06a	2.62a	20.00a
Compost-T	77.96b	6.29b	27.52b	50.36bc	12.25b	23.41a	2.31b	20.54a
Control	65.24d	4.36e	19.63c	34.52e	7.29d	20.63b	1.64f	19.92a

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Effect of chickpea seed treatment with organic compounds on activity of oxidative enzymes, pathogenesis related (PR) protein and phenol content:

A) Oxidative enzymes*:*

Results in Table (7) clearly showed that all tested organic compounds increased activity of peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) enzymes in artificial inoculated plants with *F. oxysporum* f. sp. *ciceris* compared to the control. Plant treated with Humic acid and Inicium recorded the highest increase in activity of all tested oxidative enzymes. Humic acid increased PO, PPO, and PAL by 83.29, 149.65, and 92.19% over the untreated check, respectively, followed by Inicium treatment which increased these enzymes by 66.47, 119.30, 80.04% over the untreated check, respectively. Chickpea plants treated with Alga Grow-4 showed the lowest increase in activity of PO, PPO, PAL enzymes compared with the other organic compounds, at 19.28, 67.55, 10.99 % over the untreated check, respectively. On the other hand, all organic compounds showed a considerable increase in PPO activity more than the increase of PO and PAL.

B) Pathogenesis related (PR) protein

The obtained results in Table (8) clearly indicated that all organic compounds increased pathogenesis related (PR) protein (chitinase and β-1,3 glucanase) compared with untreated plants under artificial inoculation with *F. oxysporum* f. sp. *ciceris*. Humic acid and Inicium recorded the highest increase in activity of chitinase and β-1,3 glucanase, while increase of both enzymes with 70.76 and 94.70% and 48.41 and 49.02% over the untreated chick, respectively. On the other hand, chickpea plants treated with Compost-T recorded the lowest increase in both chitinase and β-1,3 glucanase activity compared with the other treatments (20.76 and 23.73%) over the untreated check,. In general, all organic compounds except Compost-T increased the activity of chitinase more than β- 1,3 glucanase.

Table 7. Effect of soaking chickpea seeds in organic compounds on the activity of oxidative enzymes in chickpea plants grown in soil infested with *F. oxysporum* f. sp. *ciceris* under greenhouse condition (artificially infection).

Treatments	PO activity (Enzyme unit min^{-1} mg $protein^{-1}$)		PPO activity (Enzyme unit min^{-1} mg $protein^{-1}$)		PAL activity (Enzyme unit min^{-1} mg $protein^{-1}$)	
	Activity	% Increase	Activity	% Increase	Activity	% Increase
Inicium	1.425	66.47	1.568	119.30	6.523	80.04
Bio- Health	1.196	39.72	1.325	85.31	4.569	26.11
Alga Grow-4	1.021	19.28	1.198	67.55	4.021	10.99
Humic acid	1.569	83.29	1.785	149.65	6.963	92.19
Compost-T	1.186	38.55	1.259	76.08	5.428	49.82
Control	0.856	-	0.715	-	3.623	-

Table 8. Effect of soaking chickpea seeds in organic compounds on pathogenesis related (PR) protein (chitinase and β-1,3 glucanase) in chickpea plants grown in soil infested with *F. oxysporum* f. sp. *ciceris* under greenhouse condition (artificially infection).

Treatments	Chitinase activity (Enzyme unit min^{-1} mg $protein^{-1}$)		β- 1,3 glucanase activity (Enzyme unit min^{-1} mg $protein^{-1}$)	
	Activity	% Increase	Activity	% Increase
Inicium	6.865	94.70	5.862	48.41
Bio- Health	5.012	42.14	4.693	35.56
Alga Grow-4	5.210	47.76	4.852	37.68
Humic acid	6.021	70.76	5.932	49.02
Compost-T	4.258	20.76	3.965	23.73
Control	3.526	-	3.024	-

C) Phenol contents

The content of phenolic compounds (free, conjugated and total phenols) was greatly increased in plants treated with different organic compounds, compared with untreated plants (Table 9). Maximum increase in free phenols, and total phenols (112.63, 125.44 % over the untreated check, respectively) followed by Inicium treatment (82.69 and 105.98%, respectively). While, Inicium treatment recorded the highest increase of conjugated phenols (193.30% over the untreated check) followed by Humic acid (173.44). Bio-Health and Alga Grow-4 gave moderate increase of total phenols compounds being 71.50 and 72.28% over the untreated check, respectively). On the other hand, chickpea seeds treated with compost -T gave the lowest increase of total phenolic compounds while recording 33.95% over the untreated check.

Table 9. Effect of soaking chickpea seeds in organic compounds on phenolic compounds in chickpea plants grown in soil infested with *F. oxysporum* f. sp. *ciceris* under greenhouse condition (artificially infection).

Treatments	Phenolic contents (mg gm^{-1} fresh weight)					
	Free phenols	% Increasing	Conjugated phenols	% Increasing	Total phenols	% Increasing
Inicium	2.965	82.69	1.270	193.30	4.235	105.98
Bio- Health	2.568	58.23	0.958	121.25	3.526	71.50
Alga Grow-4	2.419	49.04	1.123	159.35	3.542	72.28
Humic acid	3.451	112.63	1.184	173.44	4.635	125.44
Compost-T	1.795	10.60	0.959	121.48	2.754	33.95
Control	1.623	-	0.433	-	2.056	-

DISCUSSION

Chickpea wilt disease caused by *F. oxysporum* f. sp. *ciceris* is found everywhere in the world wherever chickpea is grown, causing losses up to 12-15% annually (Landa *et al.,* 2004). Because high disease pressure and high crop value require frequent applications of chemical pesticides, significant environmental pollution and development of resistant pathogen strains are among the main problems encountered in the fields. This situation has prompted the search for biological alternatives that could be efficient either for conventional disease management programs or for integration with other methods (Hibar *et al*., 2006). Many researchers have used organic compounds as means of protection against soil- borne diseases as an alternative control method to fungicides (Abdel-Monaim *et al*., 2014).

Data obtained in this study demonstrated that all organic compounds *viz.* Inicium, Bio-Health, Alga Grow-4, Humic acid and Compost-T significantly reduced area under wilt progress curve (AUWPC) caused by *F. oxysporum* f. sp. *ciceris* under artificial infection in pots and natural infection in the field during growing seasons 2014-15 and 2015-16. Humic acid followed by Inicium recorded the highest protection against wilt disease in chickpea plant either under artificial infection with *F. oxysporum* f. sp. *ciceris* or natural infection under field conditions., Alga Grow- 4 gave the lowest protection in this respect.

On the other hand, all organic compounds significantly improved chickpea plant growth *viz.* plant height, number of branches per plant and increased yield components *viz.* numbers of pods, seeds, seeds weight per plant, weight of 100 seeds (seed index) and seed yield per plot compared with control. Protein content in seeds was not affected. Humic acid recorded the highest plant growth and yield parameters during the two growing seasons, while Alga Grow -4 gave the lowest increase of plant growth and yield components.

Similar studies have been conducted for other pathogens using the same organic compounds (Abdel-Monaim *et al.*, 2014). The role of the tested organic compounds in reducing root rot/wilt diseases severity and stimulating plant growth may be due to enhanced natural resistance against plant diseases and pests, stimulated plant growth through increased cell division, as well as optimized uptake of nutrients, water and stimulated soil microorganisms (Okorski *et al.*, 2010; Abdel-Monaim *et al.*, 2014). Application of Humic acid enhanced the activity of antioxidants such as α - tocopherol, α - carotene, superoxide dismutases and ascorbic acid concentrations in turf grass species (Piccolo *et al.*, 1992). These antioxidants may play a role in the regulation of plant development, flowering and disease resistance. This positive action of promoting organic compounds tested may be primarily attributed to their stimulating bioagants such as *Trichoderma harzianum* and *Bacillus subtilis*, besides their role as effective fertilizers and plant growth promoters.

On the other hand, the obtained data in this study clearly indicated that all tested organic compounds stimulated the activity of oxidative enzymes *viz*. PO, PPO, PAL and pathogenesis related (PR) protein (chitinase and β-1,3 glucanase) and increased the accumulation of phenolic compounds (total, free and conjugated phenols) compared with untreated plants under artificial inoculated plants with *F. oxysporum*. The increase in oxidative enzymes and pathogenesis related (PR) protein activity has an important function in secondary cell wall biosynthesis by polymerizing hydroxyl and methyl hydroylcinnamic alcohols into lignin and forming rigid cross –links between cellulose, pectin hydroxyproline in glycoproteins (HPLGP) and lignin (Grisebch, 1981). The role of oxidative enzymes could be explained as an oxidation process of phenolic compounds to oxidized products (quinones) which may be limiting the fungal growth. β-1,3-glucanase and chitinase are well-studied PR proteins which are produced by plants in response to pathogen attack. These enzymes are important determinants of the resistance of plants to fungal diseases. It has been demonstrated that genetically engineered - over expression of PR proteins can increase resistance in plants. The activation of defense mechanisms in plants is considered to be consequent upon an initial recognition event in which the host plant detects molecular components of the pathogen, known as elicitors (Van't Slot and Knogge, 2002).

In conclusion, this study has shown the potential of using organic compounds in reducing the most important disease in chickpea plants. The results obtained from pot and field experiments indicated that all tested organic compounds were able to reduce the area under wilt progress curve caused with *F. oxysporum* f. sp. *ciceris* and improved growth and yield of chickpea plants.

REFERENCES

1. Abdel-Monaim, M.F., M.A. Abdel-Gaid, and S.A. Zayan. 2014. Effectiveness of organic compounds in controlling root rot/wilt diseases, growth and yield parameters of pepper. International J. of Agric. Sci., 4 (4): 143-150.
2. A.O.A.C. 2000. Official Methods of Analysis Association of Official Analysis Chemists. 25th Edn., AOAC, Washington DC, USA.
3. Bradford, M. 1976. A rapid and sensitive method for the quantitation of Microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72:248-250.
4. Cavalcanti, F.R., M.L. Resende, C.P. Carvalho, J.A. Silveira, J.T. Oliveira. 2007. An aqueous suspension of *Crinipellis perniciosa* mycelium activates tomato defense responses against *Xanthomonas vesicatoria.* Crop Prot., 6:729-38.
5. Gauillard, F., F. Richard-Forget, J. Nicolas. 1993. New spectrophotometric assay for polyphenol oxidase activity. Anal Biochem, 215:59-65.
6. Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for Agricultural Research. Interscience Publication. New York.
7. Grisebach, H. 1981. The Biochemistry of Plants. Academic Press, NY, USA
8. Ha, M.T. and J.W. Huang, 2007. Control of Fusarium wilt of asparagus bean by organic soil amendment and microorganisms. Plant Pathol. Bull., 16: 169-180
9. Hammerschmidt, R.E.M. Nuckles, J. Ku. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol., 20:73-82.
10. Hibar, K., M. Daami-Remadi, W. Hamada, and M. El-Mahjoub. 2006. Bio-fungicides as an alternative for tomato Fusarium crown and root rot control. Tunisian J. Plant Protection, 1: 19-29.
11. Landa, B.B., J.A. Navas-Cortés and R.M. Jiménez- Díaz. 2004. Integrated management of Fusarium wilt of chickpea with sowing date, host resistance, and biological control. Phytopathology., 94(9): 946–960.
12. Liu, L., J.W. Kloepper, and S. Tuzun. 1995. Introduction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology, 85: 695-698.
13. Maitlo, S.A., R.N. Syed, M.A. Rustamani, R.D. Khuhro and A.M. Lodhi. 2014. Comparative efficacy of different fungicides against Fusarium wilt of chickpea (*Cicer arietinum* L.). Pak. J. Bot., 46(6): 2305-2312.

14. Maxwell, D.P. and D.F. Bateman. 1967. Change in the activities of some oxidases in extracts of Rhizoctonia infected bean hypocotyls in relation to lesion maturation. Phytopathology, 57: 132-136.
15. Noble, R. and E. Coventry. 2005. Suppression of soil-borne plant diseases with composts. Biocontrol Science and Technology, 15: 3 – 20.
16. Okorski, A., J. Olszewski, K. Głowacka, S. Okorska, and A. Pszczółkowska. 2010. The effect of the application of the biological control agent EM1 on gas exchange parameters and productivity of *Pisum sativum L.* infected with *Fusarium oxysporum* Schlecht. Acta Agrobotanica, 63 (2): 105–115.
17. Omar, S.A., D.D. Salem, and A.A. Aly. 1999. Quantitave measurement of host-pathogen interaction in Fusarium wilt of chickpea. Minofiya J. Agric. Res., 24 (1): 1-11.
18. Pan, S.Q., X.S. Ye, and J. Kuc. 1991. Association of ´ β-1,3-glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. Physiol. Mol. Plant Pathol., 39: 25–39.
19. Pandy, H.N., T.C.M. Menon, and M.V. Rao. 1989. Simple formula for calculating area under disease progress curve. Rachis, 8 (2): 38-39.
20. Piccolo, A., S. Nardi, and G. Concheri. 1992. Structural characteristics of humic substances as regulated to nitrate uptake and growth regulation in plant systems. Soil Biochem., 24:373–380.
21. Salem, D.E., S.A. Omar, and A.M. Khattab. 1991. Screening for resistance to root rot/wilt disease complex in chickpea (*Cicer arietinum* L.). Egypt J. Agric. Res., 69: 581-593.
22. Snell, F.D. and C.T. Snell, 1953. Colorimetric methods of analysis including some uribidmetric and nephelmetric methods. Vol. 111. D. Van Nostrand Company. Inc., New York, London, Organic. 606 pp.
23. Van't Slot, K.A.E., and W. Knogge. 2002. A dual role for microbial pathogen-derived effector proteins in plant disease and resistance. Crit Rev Plant Sci., 21:229–271.
24. Wirth, S.J. and G.A. Wolf. 1992. Micro-plate calorimetric assay for endo-acting cellulase, xylanase, chitinase, β-1, 3-glucanase and amylase extracted from forest soil horizons. Soil. Biol. Biochem*.,* 24:511-519.

فاعلية بعض المركبات العضوية فى مكافحة مرض الذبول الفيوزاريومى وتأثيرها على صفات النمو والمحصول فى الحمص

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يعتبر مرض الذبول الفيوزاريومى والذى يسببه فطر *Fusarium oxysporum* f. sp. *ciceri* من أخطر الأمراض التى تؤثر على إنتاجية الحمص. فى هذة الدراسة تم إستخدام بعض المركبات العضوية مثل الانسيوم وبيوهلث والجاجرو–٤ وحمض الهيوميك وشاى الكمبوست لأوراق النخيل فى خفض حدوث مرض الذبول فى الحمص تحت ظروف الصوبة والحقل.

تبين من النتائج المتحصل عليها إن جميع المركبات العضوية المستخدمة أدت الى خفض المساحة الموجودة تحت المنحنى المرضى المتسببة عن فطر *Fusarium oxysporum* f. sp. *ciceris* سواء تحت ظروف الصوبة أو الحقل. سجل المعاملة بحامض الهيوميك أعلى إنخفاض فى المساحة الموجودة تحت المنحنى المرضى يليها المعاملة بالانسيوم فى حين أعطى معاملة الجاجرو–٤ أقل نسبة انخفاض فى المساحة الموجودة تحت المنحنى المرضى سواء فى الصوبة او الحقل.

من ناحية اخرى ادى استخدام كل الاحماض العضوية الى زيادة معنوية فى صفات النمو والمحصول فيما عدا نسبة البروتين فى البذور خلال موسمى الزراعة (٢٠١٤–٢٠١٥ و٢٠١٥–٢٠١٦). وكانت معاملة بذور الحمص بحامض الهيوميك اكثر المعاملات زيادة فى طول النبات وعدد الافرع والقرون والبذور لكل نبات ووزن المائة بذرة والمحصول الكلى لكل وحدة تجريبية فى كلا" موسمى الزراعة. فى حين أدت المعاملة بالجا جرو–٤ أقل زيادة فى تلك الصفات.

تبين من الدراسات الفسيولوجية للنباتات الناتجة من بذور معاملة بالأحماض العضوية والمنزرعة فى تربة معدية بالفطر الممرض إلى زيادة نشاط إنزيمات البيروكسيديز والبولى فينول اوكسيديز والفينيل الانين أمونيا ليز والبروتينات المرتبطة بالمرضية (شيتينيز والجلاكونيز) مقارنة بالنباتات الغير معاملة. عموما أعطى حامض الهيوميك أعلى زيادة فى نشاط إنزيمات البيروكسيديز والبولى فينول أوكسيديز والفينيل الانين أمونيا ليز والشيتينيز متبوعا بالمعاملة بالأنسيوم فى حين أعطت المعاملة بالأنسيوم أعلى زيادة فى نشاط إنزيم الجلاكنيز يليه المعاملة بحامض الهيوميك . على العكس من ذلك سجلت المعاملة بالجا جرو–٤ أقل زيادة فى نشاط إنزيمات البيروكسيديز والبولى فينول أوكسيديز والفينيل الانين أمونيا ليز بينما سجلت المعاملة بشاى الكمبوست أقل زيادة فى نشاط الشتينيز والجلاكونيز. من ناحية أخرى أدت المعاملة بالمركبات العضوية إلى زيادت محتوى النباتات من المركبات الفينولية سواء الكلية أو الحرة أو المرتبطة مقارنة بالنباتات الغير معاملة. وأعطت المعاملة بحامض الهيوميك أعلى زيادة فى المركبات الفينولية بينما سجلت المعاملة بشاى الكمبوست أقل زيادة فى محتوى المركبات الفينولية.

فى النهاية نخلص من هذة الدراسة إلى إن إستخدام المركبات العضوية يلعب دور هام فى مكافحة مرض الذبول الفيوزاريومى عن طريق تحفيز المقاومة المستحثة فى نباتات الحمص.