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## A Modern Tactic for Reducing the Biotic Stress on Cucumber Plants Caused by *Fusarium oxysporum*

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**Abstract:** Both biological control and the use of antioxidants represent a modern tactic to induce disease resistance in plants against several pathogens especially when applied together. In the present study, cucumber plants infected by *Fusarium oxysporum* were inoculated with Arbuscular Mycorrhizal (AM) fungi and/or seed presoaked in antioxidant GAWDA<sup>®</sup> formulation at concentration of (3 g L<sup>-1</sup>) for 12 h before sowing in infested soil with the fungus. Results revealed significant increases in growth parameters, levels of mycorrhizal colonization, content of the photosynthetic pigments. The results suggested that the combined treatment of antioxidant GAWDA<sup>®</sup>+AM may be used as a tactic to enhance plant resistances against *Fusarium oxysporum*, improve plant growth and increase the phenolic compounds in the vegetative growth of cucumber.

**Key words:** Biological control, antioxidants, *Fusarium oxysporum*, cucumber, arbuscular mycorrhizal fungi, Biotic stress

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### INTRODUCTION

Cucumber (*Cucumis sativus* L.) is subjected to the attack of a number of soil born plant pathogens including *Fusarium oxysporum* f. sp., *radicis-cucumerinum* (Pavlou and Vakalounakis, 2005).

Since, the fungal attacks significantly affect plant health viz., reduction in growth parameters and physiological activities of the plant, several attempts were made to reduce or retard the biotic stress on the plants using non-conventional method. Yedia et al. (1999), demonstrated that inoculating roots of 7-day-old cucumber seedlings with spores of *T. harzianum* (T-203) at a concentration of 105 per ml enhanced plant defense in both roots and leaves of treated plants. They demonstrated that the hyphae of this candidate were able to penetrate the epidermis and upper cortex of the cucumber root and increases peroxidase and chitinase activity as well as the deposition of callose-enriched wall appositions on the inner surface of cell walls.

In the present era, it was found that enhancing the protective mechanisms in plants to eliminate or reduce stresses on the physiological activities is a successful and promising trend for the control of plant diseases (Heil and Bostock, 2002).

A number of researchers have published different methods for reducing the biotic stresses occur on the plants. One of these is the use of antioxidants as a seed

treatment or as foliage spray to control some plant diseases including root and pod rot in peanut (Elwakil, 2003), *Fusarium* wilt in chickpea (Sarwar et al., 2005), Faba bean chocolate spot (Hassan et al., 2006), peanut root rot (Mahmoud et al., 2006), *Fusarium* wilt in tomato (El-Khallal, 2007; Mohamed et al., 2007), root rot and leaf blight in lupine (Abdel-Monaim, 2008), damping-off in pepper (Rajkumar et al., 2008). In this regard, Mostafa (2006) found that the application of antioxidants, e.g., ascorbic, salicylic, coumaric, benzoic acids and propylgalate either in the form of seed soaking or soil drenching gave acceptable protection in cumin plants against *Fusarium oxysporum*, f. sp., *Cumini* and *Acremonium egyptiacum*. This information may addresses the use of antioxidants as an effective method for the control plant disease as it is cost efficient, effective in promoting the plant growth, environmentally safe and disappear before the end of the life span of the annual crop.

The effective role of mycorrhizal colonization in reducing the damage produced by some soil born plant pathogen was presented in a number of studies. Under greenhouse conditions *Glomus fasciculatum* and *Gigaspora margarita* decrease root rot incidence caused by *Fusarium oxysporum* f. sp., *asparagi* and *Helicobasidium mompa* in asparagus (*Asparagus officinalis* L.) (Matsubara et al., 2000, 2001). Chandanie et al. (2006) found that inoculation of

cucumber (*Cucumis sativus* L.) seedlings with arbuscular mycorrhizal fungus *G. mosseae* 10 days before transplanting into soil infested with *R. solani* overcomes the damping-off syndrome.

These data bases directed our attention here to study the role of antioxidant formulation GAWDA<sup>®</sup> designed and patented in Egypt, as well as the use of mycorrhizal colonization solitary and/ or in combination with GAWDA<sup>®</sup> formulation on reducing the biotic stresses on cucumber plants caused by *Fusarium oxysporum* f. sp., *radicis-cucumerinum*. In this context, the increases in the photosynthetic pigments and total phenol contents in the vegetative growth of the plants will be taken as indicator for the enhancement of plant defense mechanism against the attack of the pathogen as indicated by (Schutzenduble and Polle, 2002).

## MATERIALS AND METHODS

**Fungal isolation:** A pure culture of *Fusarium oxysporum* f. sp., *radicis-cucumerinum* isolated from the rhizosphere of wilted cucumber beta alpha hybrid was used in these investigations.

**Source of arbuscular mycorrhizal (AM) and its inoculation:** Pure cultures of AM (Multi-VAM) *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, *G. intraradices* Schenck and Smith and *Gigaspora margarita* (Becker and Hall) were obtained from Plant Pathology Institute, Agricultural Research Center, Egypt. An inoculum of each culture consisted of 30 spores 2 g-1 moist soil was placed at the bottom of the seed-bed of each pot at the time of transplanting.

**Antioxidant formulation:** Water solution of the antioxidant formulation GAWDA<sup>®</sup> which consists (Tri-Sodium Orthophosphate 1 mM+Tartaric acid 2 mM+Hydroxyquinoline 1 mM+Calcium Chloride 6 mM+Magnesium Chloride 5 mM+Calcium Borate 5 mM) was selected antioxidant formulation used in this research.

**Pot experiments:** Pots of 30 cm diameter were filled with 6 kg sterilized sandy clay soil each, then inoculated with the isolated fungus at the upper 5 cm layer of the soil at a rate of 4% (w/w) potential inoculums, then maintained freely for one week for fungal adaptation. Five healthy seeds of cultivar Beta Alph hybrid were sown in each pot. The first treatment was seeds soaked in the antioxidant formulation (GAWDA<sup>®</sup>) for 12 h while the second treatment presented seeds received AM inoculum as a seed bed. All treatments kept in open field while day temperature was approximately 25 and 20°C at night with

13 h photoperiod. Plants watered when necessary. Ten pots presented one replicate in each treatment. The 8 treatments were designed as follows; (control), (GAWDA<sup>®</sup>), (M), (GAWDA<sup>®</sup>+M), (*F. oxysporum*), (GAWDA<sup>®</sup>+*F.oxysporum*), (M+*F.oxysporium*) and (GAWDA<sup>®</sup>+M+*F.oxysporium*).

**Analysis of data:** Fifteens plants of each treatment of 4 and 8 weeks old were harvested, washed in a running tap water to remove soil particles and kept to air dry. The growth parameters: presenting shoot and root lengths (cm), shoot fresh and dry weights (g), root fresh and dry weights (g) and leaf area (cm<sup>2</sup>) were recorded.

**Disease assessment:** Disease Severity (DS) and Disease Incidence (DI) of *Fusarium* root and stem rot disease were assessed using one and 4 weeks old plants of each treatment. Disease Incidence (DI) (%) was estimated as a percentage of diseased cucumber plants while disease severity was assessed as a number of diseased plants depending on the designed visual disease scale which started from 0-2, where 0: no symptoms, 1: vascular disease symptoms in the stem, root and stem rot, wilt or without wilt, 2: dead or almost dead (Pavlou and Vakalounakis, 2005).

**Estimation of mycorrhizal colonization:** The rate of mycorrhizal root infection was microscopically estimated according to the method described by Trouvelot *et al.* (1986). The method calculates three parameters of infection as follows:

- F:** Frequency of root infection (percentage of root segments infection)
- M:** Intensity of cortical infection (proportion of cortical infection in all of the mycorrhizal root system)
- A:** Arbuscul frequency in roots (percentage of arbuscular infection of the root system)

**Estimation of photosynthetic pigments:** Photosynthetic pigments were extracted from cucumber leaf samples presenting the different treatments and determined by the method of Harborne (1984).

**Estimation of total phenol:** Total phenols were estimated in the roots of all treatments using Folin Ciocalteau reagent following the method described by Maliak and Singh (1980).

**Statistical analysis:** Data was statistically analyzed using the statistical analysis system (COSTAT, 2005). All multiple comparisons were first subjected to analysis of

variance (ANOVA), comparisons among means were made using Duncan's multiple range test ( $p = 0.05$ ) (Duncan, 1955).

**RESULTS**

**Changes in the growth parameters:** The treatments of mycorrhizal colonization and/or GAWDA® formulation significantly reduced the depletion in the growth parameters in the infected cucumber plants with *F. oxysporum*. Moreover, the tested parameters significantly increased in the second harvest than the first one (Table 1). Four weeks after inoculation with *F. oxysporum* the increases in shoot length and shoot fresh weight achieved when the addition of either mycorrhiza or GAWDA® or both were applied. Shoot length, shoot dry weight and leaf area were the measured growth parameters. Also root length, root fresh and dry weights were significantly increased. The check treatments in which healthy plants were treated with GAWDA® showed significant increases in shoot and root length, shoot fresh weight, leaf area and root fresh and dry weights.

Eight weeks after inoculation with the pathogen, significant increases in shoot and root lengths, shoot fresh and dry weight and leaf area were shown in either mycorrhiza or GAWDA® treatments or both. The magnitude in the growth was more pronounced in all treatments in which mycorrhiza and GAWDA® formulation were combined together.

**Disease assessment:** Disease incidence and disease severity in non colonized mycorrhizal plants were significantly higher than those of colonized mycorrhizal plants at the two harvests (Table 2). In spite of the fact that the treatment of the infected plants with GAWDA® formulation significantly decreased disease incidence and disease severity, treatment with either mycorrhiza alone or with GAWDA® significantly reduced both disease incidence and disease severity in one to four weeks old plants. In this connection, the massive reduction in disease incidence and disease severity was significantly found in the treatments in which mycorrhiza was associated with GAWDA® formulation.

Table 1: Effect of mycorrhizal colonization and GAWDA® formulation on growth parameters of cucumber plant infected with *Fusarium oxysporum*

Weeks after planting	Treatment	Shoot length (cm plant <sup>-1</sup> )	Shoot Fresh weight (g plant <sup>-1</sup> )	Shoot dry weight (g plant <sup>-1</sup> )	Root length (cm plant <sup>-1</sup> )	Root fresh weight (g plant <sup>-1</sup> )	Root dry weight (g plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )
4	Control	36.6 <sup>def</sup>	13.8 <sup>bcd</sup>	3.4 <sup>bcd</sup>	10.8 <sup>de</sup>	2.68 <sup>bcd</sup>	0.526 <sup>c</sup>	535 <sup>cd</sup>
	GAWDA®	44.8 <sup>bc</sup>	15.6 <sup>bc</sup>	5.2 <sup>ab</sup>	16.4 <sup>b</sup>	3.28 <sup>ab</sup>	0.642 <sup>a</sup>	562 <sup>b</sup>
	M	50.6 <sup>b</sup>	17.2 <sup>b</sup>	5.6 <sup>a</sup>	17.8 <sup>ab</sup>	3.58 <sup>a</sup>	0.716 <sup>a</sup>	586 <sup>a</sup>
	M+GAWDA®	57.0 <sup>a</sup>	21.6 <sup>a</sup>	5.2 <sup>a</sup>	19.6 <sup>a</sup>	3.78 <sup>a</sup>	0.744 <sup>a</sup>	603 <sup>a</sup>
	<i>F. oxysporum</i>	25.2 <sup>e</sup>	7.20 <sup>e</sup>	2.0 <sup>d</sup>	6.4 <sup>f</sup>	1.08 <sup>e</sup>	0.220 <sup>af</sup>	311 <sup>ah</sup>
	<i>F.ox</i> +GAWA®	32.0 <sup>efg</sup>	12.4 <sup>bcd</sup>	3.0 <sup>cd</sup>	7.8 <sup>e</sup>	2.12 <sup>d</sup>	0.342 <sup>e</sup>	476 <sup>ef</sup>
	<i>F.ox</i> +M	33.4 <sup>ef</sup>	13.2 <sup>bcd</sup>	3.4 <sup>bcd</sup>	8.4 <sup>ef</sup>	2.32 <sup>cd</sup>	0.440 <sup>d</sup>	495 <sup>e</sup>
	<i>F.ox</i> +M+GAWA®	35.8 <sup>def</sup>	14.2 <sup>bc</sup>	3.6 <sup>bcd</sup>	8.8 <sup>def</sup>	2.54 <sup>bcd</sup>	0.522 <sup>c</sup>	519 <sup>d</sup>
	Control	68.2 <sup>cd</sup>	26.6 <sup>ab</sup>	5.0 <sup>abcde</sup>	14.8	3.98 <sup>abc</sup>	0.828 <sup>cd</sup>	658 <sup>e</sup>
	GAWDA®	72.8 <sup>bc</sup>	32.0 <sup>bc</sup>	6.0 <sup>ab</sup>	17.2 <sup>bc</sup>	4.02 <sup>abc</sup>	0.896 <sup>ab</sup>	698 <sup>e</sup>
8	M	79.8 <sup>b</sup>	34.0 <sup>b</sup>	5.6 <sup>abc</sup>	20.2 <sup>ab</sup>	4.36 <sup>ab</sup>	0.914 <sup>e</sup>	742 <sup>e</sup>
	M+GAWDA®	84.2 <sup>a</sup>	44.2 <sup>a</sup>	6.2 <sup>a</sup>	23.2 <sup>a</sup>	4.88 <sup>a</sup>	0.940 <sup>e</sup>	763 <sup>e</sup>
	<i>F. oxysporum</i>	37.2 <sup>e</sup>	14.6 <sup>c</sup>	2.8 <sup>e</sup>	9.2 <sup>e</sup>	2.14 <sup>d</sup>	0.426 <sup>h</sup>	433 <sup>e</sup>
	<i>F.ox</i> +GAWA®	64.8 <sup>de</sup>	21.4 <sup>f</sup>	3.8 <sup>cde</sup>	12.8 <sup>defg</sup>	2.92 <sup>bcd</sup>	0.674 <sup>f</sup>	552 <sup>e</sup>
	<i>F.ox</i> +M	61.0 <sup>def</sup>	23.4 <sup>ef</sup>	4.4 <sup>bcde</sup>	11.0 <sup>efg</sup>	3.28 <sup>abcd</sup>	0.732 <sup>e</sup>	570 <sup>de</sup>
	<i>F.ox</i> +M+GAWA®	64.8 <sup>de</sup>	28.4 <sup>cd</sup>	4.8 <sup>bcde</sup>	13.6 <sup>def</sup>	3.48 <sup>abcd</sup>	0.792 <sup>d</sup>	592 <sup>d</sup>

*F.ox*: *Fusarium oxysporum*, M: mycorrhizal fungi, Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ( $p < 0.05$ ), each value represents the mean of 10 replicates

Table 2: Effect of mycorrhizal colonization and GAWDA® formulation on disease incidence and disease severity of cucumber plant infected with *Fusarium oxysporum*

Treatments	Disease incidence (%)	*Disease severity (%)	Disease incidence (%)	Disease severity (%)
Control	0.0 <sup>e</sup>	0.0 <sup>d</sup>	0.0 <sup>e</sup>	0.0 <sup>e</sup>
GAWDA®	0.0 <sup>e</sup>	0.0 <sup>d</sup>	0.0 <sup>e</sup>	0.0 <sup>e</sup>
M	0.0 <sup>e</sup>	0.0 <sup>d</sup>	0.0 <sup>e</sup>	0.0 <sup>e</sup>
M+GAWDA®	0.0 <sup>e</sup>	0.0 <sup>d</sup>	0.0 <sup>e</sup>	0.0 <sup>e</sup>
<i>F. oxysporum</i>	68.9 <sup>a</sup>	59.4 <sup>a</sup>	71.1 <sup>a</sup>	63.3 <sup>a</sup>
<i>F.ox</i> +GAWDA®	60.8 <sup>b</sup>	53.3 <sup>b</sup>	64.1 <sup>b</sup>	55.8 <sup>b</sup>
<i>F.ox</i> +M	40.3 <sup>c</sup>	31.9 <sup>c</sup>	55.9 <sup>c</sup>	48.4 <sup>c</sup>
<i>F.ox</i> +M+GAWDA®	31.8 <sup>d</sup>	28.8 <sup>c</sup>	35.1 <sup>d</sup>	33.1 <sup>d</sup>

*F.ox*: *Fusarium oxysporum*, M: mycorrhiza, Values were taken after 1 and 4 weeks of planting, \*Disease severity was estimated according to Carling *et al.* (1999), Values of the same dependent variable followed by the same letter(s) are not significantly different according to Duncan's multiple range test ( $p = 0.01$ ), each value represents the mean of 5 replicates

**Level of mycorrhizal colonization:** The effect of different treatments on the levels of mycorrhizal colonization in cucumber roots are presented in Table 3. Data was expressed as frequency of root colonization (F), intensity of cortical colonization (M) and arbuscules frequency in roots (A).

The results showed that, the level of mycorrhizal root colonization increases with the increase in plant age. It was also noticed that the establishment of mycorrhiza significantly reduces in the treatments in which (*F. oxysporum*+M), (M+GAWDA®) and (*F. oxysporum*+M+GAWDA®) were applied in compare with M treatment. In this connection, it is reported here that application of GAWDA® formulation accelerated the potential of mycorrhizal colonization in the infected cucumber plants (*F. oxysporum*+M+GAWDA®) when compared to (*F. oxysporum*+M) in the 4 week old plants. The efficiency of mycorrhizal colonization was shown in the treatment in which (*F. oxysporum*+M+GAWDA®) were integrated.

Table 3: Effect of different treatments on the levels of mycorrhizal colonization of cucumber plant infected with *Fusarium oxysporum*

Treatments	F (%)	M (%)	A (%)	F (%)	M (%)	A (%)
Control	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>f</sup>	0 e	0 <sup>d</sup>	0 <sup>d</sup>
GAWDA®	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>f</sup>	0 e	0 <sup>d</sup>	0 <sup>d</sup>
M	88.2 <sup>a</sup>	39.5 <sup>a</sup>	28.7 <sup>a</sup>	99 <sup>a</sup>	72.0 <sup>a</sup>	31.8 <sup>b</sup>
M+GAWDA®	76.7 <sup>b</sup>	30.6 <sup>c</sup>	20.4 <sup>b</sup>	93.3 <sup>b</sup>	53.5 <sup>c</sup>	22.8 <sup>c</sup>
<i>F. ox</i>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>f</sup>	0 e	0 <sup>d</sup>	0 <sup>d</sup>
<i>F.ox</i> +GAWDA®	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>f</sup>	0 e	0 <sup>d</sup>	0 <sup>d</sup>
<i>F.ox</i> +M	61.3 <sup>c</sup>	33.9 <sup>bc</sup>	18.6 <sup>b</sup>	87.7 <sup>c</sup>	51.5 <sup>c</sup>	30.03 <sup>b</sup>
<i>F.ox</i> +M+GAWDA®	76.3 <sup>b</sup>	36.6 <sup>bc</sup>	22 <sup>b</sup>	82.3 <sup>d</sup>	61.4 <sup>b</sup>	38.3 <sup>a</sup>

*F.ox:* *Fusarium oxysporum*, M: mycorrhiza, Values were taken after 8 week of planting. Values of each column followed by the same letter (s) are not significantly different according to Duncan's multiple rang test (p = 0.05), Each value represents the mean of 10 replicates

**Photosynthetic pigments:** Cucumber plant infected with *F. oxysporium* showed depletion in pigment contents (chl. a, chl. b and carotenoid) in the 4 week old plants (Table 4). The application of M or GAWDA® or there combination (M+GAWDA®) in the infected cucumber plants showed a significant increase in pigments contents compared to their level in the control (infected plants but not treated with any of (M or GAWDA®). However, there are no significant difference in chl. a and Carotenoid in treatments (*F.ox*+GAWDA®), (*F.ox*+M) and (*F.ox*+M+ GAWDA®) in the 4 weeks old plants. The application of (M) or (GAWDA®) also accelerated the level of pigments content with no significant difference in chl. a and chl. b in treatment (*F.ox*+GAWDA®), (*F.ox*+M). Generally, the magnitude of increase in pigment contents was more pronounced in (*F.ox*+M+GAWDA®) treatment.

**Total phenols:** The effect of mycorrhizal colonization and/or GAWDA® formulation on the formation of total phenol in 7-day-old infected cucumber plants is illustrated in Table 5. The total phenols content of infected plants pretreated with GAWDA® were significantly higher than that of the uninfected plants. In the meanwhile, the total phenols content in the infected plants treated with mycorrhiza and/or GAWDA® formulation show a marked increase than of the untreated ones. As compared to the uninfected plants, the highest total phenols content was markedly shown in (M+GAWDA®) treatment while the highest total phenols content in infected plants was recorded when (*F. oxysporium*+M+GAWDA®) were integrated together in the treatment.

Table 4: Effect of mycorrhizal colonization and GAWDA® formulation on pigments content (mg g<sup>-1</sup> d.wt.) of leaves of cucumber plant infected with *Fusarium oxysporum*.

Weeks after planting	Treatments	Chl a	Chl b	Carotenoid
4	Control	0.656 <sup>ab</sup>	0.270 <sup>abcd</sup>	0.17 <sup>ab</sup>
	GAWDA®	0.701 <sup>ab</sup>	0.341 <sup>abc</sup>	0.192 <sup>a</sup>
	M	0.764 <sup>ab</sup>	0.380 <sup>ab</sup>	0.203 <sup>a</sup>
	M+GAWDA®	0.793 <sup>a</sup>	0.391 <sup>a</sup>	0.221 <sup>a</sup>
	<i>F. oxysporum</i>	0.41 <sup>b</sup>	0.156 <sup>d</sup>	0.065 <sup>b</sup>
	<i>F.ox</i> +GAWDA®	0.521 <sup>ab</sup>	0.243 <sup>bcd</sup>	0.120 <sup>ab</sup>
	<i>F.ox</i> +M	0.626 <sup>ab</sup>	0.257 <sup>abcd</sup>	0.147 <sup>ab</sup>
	<i>F.ox</i> +M+GAWDA®	0.643 <sup>ab</sup>	0.277 <sup>abcd</sup>	0.150 <sup>ab</sup>
8	Control	0.940 <sup>ab</sup>	0.377 <sup>bc</sup>	0.197 <sup>abc</sup>
	M	1.147 <sup>a</sup>	0.486 <sup>ab</sup>	0.207 <sup>abc</sup>
	GAWDA®	1.164 <sup>a</sup>	0.541 <sup>ab</sup>	0.232 <sup>ab</sup>
	M+GAWDA®	1.184 <sup>a</sup>	0.563 <sup>a</sup>	0.264 <sup>a</sup>
	<i>F. oxysporum</i>	0.620 <sup>c</sup>	0.216 <sup>c</sup>	0.083 <sup>c</sup>
	<i>F.ox</i> +GAWDA®	0.802 <sup>bc</sup>	0.330 <sup>bc</sup>	0.149 <sup>abc</sup>
	<i>F.ox</i> +M	0.830 <sup>bc</sup>	0.362 <sup>bc</sup>	0.176 <sup>abcd</sup>
	<i>F.ox</i> +M+GAWDA®	0.901 <sup>abc</sup>	0.381 <sup>abc</sup>	0.189 <sup>abcd</sup>

*F.ox:* *Fusarium oxysporum*, M: mycorrhizal fungi, Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (p = 0.05), each value represents the mean of 10 replicates

Table 5: Effect of mycorrhizal colonization and GAWDA® formulation on the content of total phenols ( $\mu\text{g g}^{-1}$  fresh wt) in the root of cucumber plants infected with *Fusarium oxysporum*

Treatment	Total phenols ( $\mu\text{g g}^{-1}$ fresh wt.)
Control	17.70 <sup>d</sup>
GAWDA®	23.00 <sup>abcd</sup>
M	25.20 <sup>abcd</sup>
M+GAWDA®	28.90 <sup>ab</sup>
<i>F. oxysporum</i>	18.40 <sup>d</sup>
<i>F.ox</i> +GAWDA®	25.03 <sup>abcd</sup>
<i>F.ox</i> +M	27.50 <sup>abc</sup>
<i>F.ox</i> +M+GAWDA®	31.00 <sup>a</sup>

*F.ox: Fusarium oxysporum* M: Mycorrhiza, Values of each column followed by the same letter(s) are not significantly different according to Duncan's multiple rang test (p-value = 0.05). Each value represents the mean of 10 replicate, data was reported in 7 day old seedling

## DISCUSSION

The present study highlights a modern tactic for amplifying the plant defense responses induced by mycorrhiza colonization or antioxidants against *Fusarium* root and stem rot of cucumber plants. The results presented here revealed that application of arbuscular mycorrhiza AM and/or GAWDA® formulation greatly reduced percentage of disease incidence of infected plants as compared with the untreated plants. It was also shown that the treatment improved plant health and reduced the disease symptoms, vascular invasion and sporulation of the pathogen. The results are in harmony with the finding of Buzi *et al.* (2004), Jayaraj *et al.* (2004), Segarra *et al.* (2006) and Chandanie *et al.* (2006) who indicated that, pre inoculation with AM fungi led to a significant reduction in disease severity caused by *R. solani* in mung bean, potato and cucumber, respectively.

When applying arbuscular mycorrhiza AM and GAWDA® formulation together, a great enhancement on the growth vigor's of both root and shoot of the cucumber plants, even under the stress of the fungal invasion, was significantly shown.

The results suggest that AM fungi may increase host survival via compensating the loss of root biomass depleted by pathogen. This result represents an indirect contribution to the bio-control defense through the conservation of root system function, both by mycorrhizal hypha growing out into the soil and/or increasing the absorbing surface of the roots. Also, maintaining the root cell activities through arbuscular formation as described by Cordier *et al.* (1998), Whipps (2004), Morgan *et al.* (2005). On the other hand, plants treated with antioxidants cause induction in defense pathways as indicated by Baldwin (1998), Redman *et al.* (2001). In this research GAWDA® formulating antioxidants contribute to a better growth of the plant and this could be mediated by effects on the level of cytokinins, the growth factor, for cell

division as shown by Haberer and Kieber (2002). The synergistic action found between AM fungi and antioxidant on the growth promotion and protection of cucumber against *Fusarium*, might also attributed to antibiotic production, induced systemic resistance or site competition between mycorrhiza and the pathogen (Morgan *et al.*, 2005).

## CONCLUSION

In light of the presented data, the potential of arbuscular mycorrhizal colonization being elicited when antioxidants seed treatment was integrated in our trial to reduce the biotic stress on cucumber plants caused by *F. oxysporum*. Subsequently, this tactic proved to be a modern alliance for suppressing *Fusarium* root and stem disease of cucumber plants. However, we recommend this method as a new approach in managing *Fusarium* root and stem disease of cucumber plants with a beneficial affect upon the ecosystem of the soil (s).

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