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Role of hydrogen peroxide and Pharmaplant-turbo against cucumber powdery mildew fungus under organic and inorganic production

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Summary: Cucumber leaves have been sprayed with a solution of hydrogen peroxide (H_2O_2) or Pharmaplant-turbo combined with organic or inorganic fertilizers under plasic house. Under the influence of H_2O_2, leaves exhibited resistant against *Podoshaera fusca* fungus, the causal agent of cucumber powdery mildew. H_2O_2 (15 mM) was able to decrease the disease severity from 90.4% to 12% in two experiments conducted in two seasons. Pharmaplant-turbo (Turbo) is new chemical compound and used as an antifungal compound. Turbo in 1 ml/L was able to decrease the disease severity from 90.4% to 11.5% in the both experiments as well. Both of H_2O_2 and Turbo were combined with organic treatment (compost + compost tea + seaweed extracts) which showed significant effect against cucumber powdery mildew fungus and strongly suppressed it as compared to control leaves. Organic treatment produced higher vegetative growth characters and greater early and total yields as compared to inorganic treatment, also organic fruits produced the lower nitrate content and the higher ascorbic acid content as compared to inorganic fruits. Our study have indicated that, H_2O_2 and Turbo combined with organic fertilizers play a role in the resistance of cucumber against powdery mildew by decreasing the disease severity. We suggest to give more attention to the direct application of H_2O_2 in low concentration and Turbo against powdery mildew diseases and other plant diseases.

Key words: Hydrogen peroxide, Pharmaplant-turbo, cucumber powdery mildew, organic, inorganic production and cucumber

Introduction

Powdery mildew of cucumber caused by *Podoshaera fusca* (Fr.) U. Braun & Shishkoff is one of the most dangerous foliar diseases, attacking cucumber plants, in Egypt and in other countries (*Harfoush & Salama*, 1992; *Mosa*, 1997; *Reuveni* et al., 1997; *Verhaar* et al., 1997).

Cucumber (*Cucumis sativus* L.) is a favorite commodity exports markets and local consumption and represents one of the most important and economic vegetables in Egypt. It is grown in Egypt in the open field from March to November and under plastic houses from September to the end of May. The total cultivated area of cucumber in Egypt was about 26 656 hectares in 2005 according to the statistical data of FAO.

Fungicides and resistant or tolerant cultivars were used to control this disease; however, each of the used control methods has its limitations (*McGrath*, 1991). Therefore, powdery mildews of cucumber is remained the major problem for greenhouse producers worldwide.

The authors tried to find out alternative means to control the powdery mildew of cucumber using H2O2 comparing with a new microbicide compound namely: Pharmaplant-turbo in combination with organic and inorganic fertilizers to obtain healthy and clean fruits.

Gechev et al. (2002) found that it is possible to "immunize" tobacco plants by spraying leaves with low concentration of hydrogen peroxide (H_2O_2) against abiotic stresses. They have shown that a spray with 5 mM H_2O_2 induced augmented antioxidant activities in treated leaves which resulted in suppression of necrosis caused by a catalase inhibitor or high light intensity. *Geetha & Shetty* (2002) found that chemical induction of resistance in pearl millet against downy mildew disease (*Sclerospora graminicola*) is possible by treating seeds of highly susceptible cultivars with the resistance activator benzothiadiazole (BTH) (CGA 245704), calcium chloride ($CaCl_2$) and hydrogen peroxide (H_2O_2). BTH in 0.75%, 90 mM $CaCl_2$ and 1.0 mM H_2O_2 were effective in managing the disease by giving 78%, 66% and 59% protection, respectively.

It was possible to change the symptom expression of barley genotypes infected with powdery mildew (*Blumeria*

graminis f. sp. *hordei)* by spraying the leaves with 25 mM of H_2O_2. When the infected barley (cultivar Ingrid) expressing the genes *Mlo* (susceptible), *Mla12* (resistant with HR symptoms) and *mlo5* (resistant without HR) sprayed directly with H_2O_2 after establishment of infection (2–3 days after inoculation), leaves of the susceptible *Mlo* and *mlo5*-resistant plants exhibited HR-type symptoms with tissue necrosis. The *Mla12*-resistant genotype produced HR earlier and the number of necrotic lesions increased, as compared to untreated control leaves. Treatment with H_2O_2 before establishment of infection (one day after inoculation), resulted in all the three genotypes in inhibition of the pathogen and symptomless response (*Hafez & Király*, 2003, *Király & Hafez*, 2007).

Recently, *Oros* et al. (2003) developed a new preparation (microbicide) namely: Pharmaplant-turbo (40 fw) which used as an antifungal compound. In Navel orange fruits which pre-treated with Pharmaplant-turbo during the post harvest storage conditions, the disease severity of the artificially inoculated orange fruits with *Penicillium italicum* and *Botrytis cinerea* was significantly reduced under room temperature. The percentage of rotted fruits was significantly reduced from 88–42% during 2005 and 91–13% during 2006 seasons (*Belai* et al., 2006).

There is no doubt in long-term field experiments where mineral fertilizers have only been used, some problems could arise, especially increased soil erosion, soil compaction, environmental pollution and public health risk (*Top* et al., 2002). Therefore, it is essential to adopt a system of organic farming in vegetables due to increasing the objectives against the conventional farming as a main source of soil and water pollution as well as food products. As defined by the US Dept. Agric. in (1980), organic farming is a system that excludes the use of synthetic fertilizers, pesticides and growth regulators. Additions of organic amendments to agricultural soils can lead to improve soil quality and reduce severity of crop diseases (*Rotenberg* et al., 2005).

Several ir.vestigators indicated that addition of organic fertilizes increased vegetative growth characters, yield and fruit quality of vegetable crops (*Ozores-Hampton* et al., 1994; *Hsieh & Hsu*, 1995; *Yousef* et al., 2001; *Poudel* et al., 2002; *Aly*, 2002; *Bayoumi & Hafez*, 2006). On cucumber, potato, cabbage and beans, *Shiralipour* (1992) stated that soil incorporation of compost usually results in a positive effect on the growth and yield of these crops. On squash, *Ozores-Hampton* et al. (1994) showed that plants had increased yields when planted in municipal solid waste compost amended soil in spite of application of NPK fertilizers at recommended rates. On pepper, Hsieh and Hsu (1995) found that early and total yields of all organic sources were significantly higher than that of chemical fertilizers. In the same line on cucumber, *Aly* (2002) and *Bayoumi & Hafez* (2006) found that organic treatment produced significantly greater early and total yields than chemical treatment which produced the lower values. In the same line, *Jackson* et al. (2005) reported that yields of lettuce and broccoli in organic production system were usually typical of inorganic yields.

Organic fertilizers are claimed to produce higher nutritional quality of vegetables in forms of vitamin C, TSS, dry matter and acidity (*Vogtmann* et al., 1993; *Youssef* et al., 2001; *Bayoumi*, 2005). For nitrate content, *Clark* et al. (1999) and *Bayoumi & Hafez* (2006) found that nitrate content in tomato and cucumber fruits was the lowest in the organic system and the highest in the conventional system as the differences were highly significant.

Therefore, this investigation aimed to study the effect of H_2O_2 and Pharmaplant-turbo combined with organic treatment on the disease severity, growth, yield and fruit quality of cucumber crop comparing with the chemical treatment under plastic houses.

Materials and methods

This experiment was carried out in the experimental Farm of the Faculty of Agriculture, Kafrelsheikh University, Egypt. The experiment was conducted during the winter seasons of 2005 and 2006 on cucumber hybrid (Prince) cultivated under unheated plastic houses. Seedlings were transplanted on September 10th in the first season and September 5th in the second one on two sides of the ridge (6 meters in length and 1.5 meter in width) at spacing of 50 cm between plants within the row. Plant density was 3.5 plants per square meter. The harvesting time was after 40 days from transplanting and continuously for 6 weeks. Cucumber plants were left to infection with powdery mildew (*Podoshaera fusca*) naturally under plastic house conditions.

The treatments were arranged in 3 replications using split plot design as fertilizer sources were in the main plots and control methods in sub-plots and the treatments were:

A- Fertilizer sources:

1 – Organic treatment in which compost was used at the rate of 20 ton/fed. (*Mitchell* et al., 2000) accompanied with compost tea (it was sprayed directly on the plants and applied also to soil with irrigation 5 times, starting three weeks after transplanting) and also with seaweed extracts (which was used as foliar spray (2 ml/L) five times at two weeks intervals (starting three weeks after transplanting). Chemical analysis of compost was estimated immediately before its application (*Table 1*).

Table 1 Chemical properties of compost used in 2005 and 2006 seasons

Chemical analysis		Seasons	
		2005	2006
EC (dSm^{-1})		4.1	4.1
pH		7.5	7.6
O.M	(%)	33.9	32.7
Moisture	(%)	25.6	22.9
N	(%)	1.71	1.69
P	(%)	0.91	0.94
K	(%)	1.40	1.23
Fe	(ppm)	3380.4	3845.6
Zn	(ppm)	250.5	296.1
Mn	(ppm)	501.1	448.3

2 – Inorganic treatment, the recommended NPK fertilizers were used as known in cultivation area (90 kg N, 45 kg P_2O_5 and 150 kg K_2O/feddan).

B – Control treatments were carried out by direct application of H_2O_2 and Pharmaplant-turbo: cucumber leaves were sprayed with H_2O_2 solution (H_2O_2 in tap water) 10, 15, 20, 25 and 50 mM three times (after transplanting immediately, one and two weeks after transplanting).

Pharmaplant-turbo (microbicide) was obtained kindly from Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary and was sprayed at doses 1, 1.25 and 1.5 ml/L as recommended by *Oros* et al. (2003).

Measurements recorded:

1 – Disease severity % of the infection by cucumber powdery mildew after 90 days from transplanting according to Pandey *et al.* (1989).

2 – Microbial biomass of soil at 45 and 90 days after transplanting (mg CO_2/100 g soil/day).

3 – Vegetative growth parameters: stem length (cm), number of leaves/plant and leaf area/plant (dm^2) were determined at 45 and 60 days after transplanting.

Results and discussion

Effect of H_2O_2 and Pharmaplant-turbo on the disease severity (%) of the cucumber powdery mildew

When cucumber leaves were sprayed with H_2O_2 in different concentrations (10, 15, 20, 25 and 50 mM), It was found that most of the concentrations were effective against the powdery mildew. However, H_2O_2 in 15–20 mM was the best. H_2O_2 (15 mM) was able to decrease the disease severity from 90.4 % to 12 % in both seasons, i.e. 2005 & 2006 (Fig 2.). However, the other concentrations of H_2O_2 were either not effective (5 mM) or damaged leaf tissues a little (50 mM). Pharmaplant-turbo (1 ml/L) decreased the disease severity from 90.4 % to 11.5 % in the both seasons (*Figures 1 & 2*).

Interestingly enough, that the cucumber leaves which sprayed with Pharmaplant-turbo in different concentrations (1, 1.25 and 1.5 ml/L) showed strong resistance against the powdery mildew fungus. All the three concentrations were effective without significant differences between each other, therefore the Pharmaplant-turbo was used at 1 ml/L.

Figure 1 Effect of H_2O_2 and Pharmaplant-turbo combined with organic fertilizers on the disease severity percentage of cucumber powdery mildew. The left: control plants which infected only with *Podoshaera fusca.* The middle: cucumber plants infected with powdery mildew and treated with Pharmaplant-turbo 1 ml/L combined with organic fertilizer. The right: cucumber plants infected with powdery mildew and treated with 15 mM H_2O_2 combined with organic fertilizer

4 – Chlorophyll content in the leaves: relative green colour of one most recently matured leaf per plant was measured with SPAD meter (Minolta Corp, Ramsey, N.J.) after 45 and 60 days from transplanting.

5 – Fruit yield: early yield was considered as the number and weight of fruits per square meter of the first four pickings. Total yield was determined as number and weight of fruits /m^2 of all pickings.

6 – Fruit chemical quality: total soluble solids (TSS %) in juice of cucumber fruits was estimated by a hand refractometer, ascorbic acid content (mg/100 g f. wt) was estimated by titration with 2, 6-Dichlorophenol blue according to A.O.A.C. (1965) and nitrate content (ppm) was estimated by rapid colorimetric determination in fruits by nitration of salicylic acid (*Cataldo* et al., 1975).

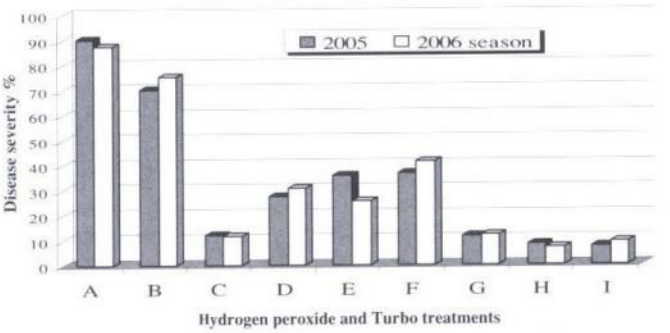

Figure 2 Effect of H_2O_2 and Pharmaplant-turbo on the disease severity (%) of the cucumber powdery mildew. A: control plants which infected only with the powdery mildew fungus. Cucumber plants infected with powdery mildew and treated with 5 mM H_2O_2 (**B**), 15 mM H_2O_2 (**C**), 20 mM H_2O_2 (**D**), 25 mM H_2O_2 (**E**) and 50 mM H_2O_2 (**F**) under organic fertilizer. Cucumber plants infected with powdery mildew and treated with Pharmaplant-turbo 1 ml/L (**G**), 1.25 ml/L (**H**) and 1.5 ml/L (**I**) under organic fertilizer.

Both of H_2O_2 and Pharmaplant-turbo were very effective against the powdery mildew fungus by killing or inhibiting the spore germination of the fungus. The obtained results are supported by similar results, where H_2O_2 was also effective against barley powdery mildew fungus (*Hafez & Király*, 2003, 2007; *Bayoumi & Hafez*, 2006) as well as the strong effect of Pharmaplant-turbo against rot diseases of orange fruits under storage conditions (*Belai* et al., 2006).

Effect of organic and inorganic fertilizers on microbial biomass

Data in *Figure 3*, showed that highly differences in microbial biomass of soil at 45 and 90 days after transplanting were obtained between both treatments in both seasons. Therefore, applying organic treatment produced higher value compared to chemical treatment which showed the lowest values in both seasons. Increasing microbial biomass by using organic treatment may be due to that the addition of compost to soil increased soil organic matter % which acts as a material for activity of various groups of microorganisms. In this respect, some reports showed that using organic manures as compost led to improve soil biological properties such as microbial communities (*Gaskell* et al., 2000; *Li* et al., 2000) as well as improve oil fertility.

Effect of fertilizer sources and control methods interaction on some vegetative growth characters of cucumber plants

Data in *Table 2* show that the interaction between fertilizer sources, i.e. organic and chemical and control treatments had no significant effect on cucumber growth characters (stem length, number of leaves/plant and leaf area/plant) and leaf chlorophyll content in most cases in both seasons. However, the highest values were obtained by applying organic treatment combined with Turbo, especially at the earlier stages. At the later stage, using chemical treatment with Turbo showed the highest value. Generally, the favorable effect of organic cultivation treatment on vegetative growth, especially at the early stages of cucumber growth may be due to that compost contains almost all of the macro-and micro-nutrients essential for plant growth, in addition to humic substances which increased soil fertility and cation exchange capacity, thus increased the availability of certain nutrients (*Seyedbagheri*, 1999). However, some of the major plant nutrients such as N, P and K may not present in adequate amounts for increasing plant growth.

Additionally, applying compost combined with compost tea and seaweed extracts had a beneficial effect on plant growth. This result might be related to the improvement of physical conditions of soil, providing energy necessary for microorganisms activity (*Figure 3*) and increasing the availability and uptake of nutrients, which positively reflected on vegetative growth (*Awad*, 1998; *Romero* et al., 2000). Chemical treatment showed mostly a favourable effect on plant growth characters at the last stages, as this may be due to more availability of macronutrients in chemical fertilizers than in organic treatment. In this concern, *Jokela* (1992) found that crop availability of N in organic manure is lower than availability of N from inorganic fertilizer, possibly due to slow release of organically bound N, immobilization of N and volatilization of NH_3 from surface applied manure.

Effect of fertilizer sources and control treatments interaction on early and total yields of cucumber

Figure 4 indicated that, the interaction had no significant effect on early and total yields in both seasons. However, the highest yields (number and weight of friuts/m^2) as early and total were obtained from organic treatment combined with any of control treatments especially with Pharma-plant turbo, while the lowest values were achieved with chemical treatment as the main factor combined with H_2O_2 application in both seasons.

Organic treatment (means of both control methods) had a significant increase in number and weight of fruits/m_2 of early and total yields compared to the chemical treatment in both seasons. The increase in cucumber yield may be attributed to applying organic treatment which increased biological activity in the soil (*Figure 3*) which leads to increasing phyto-hormones, organic acids and uptake of nutrients that promoted the vegetative growth and consequently, increased early and total yields (*Frankenberger & Arshad*, 1995). Also, the superiority of organic application treatment over the

Table 2 Effect of fertilizer sources and control treatments interaction on some vegetative growth characters of cucumber plants

Treatments: Fertilizer sources	Treatments: Control treatments	Stem length (cm)		No. of leaves/plant		Leaf area (dm²/plant)		SPAD green colour reading	
		Days after transplanting							
		45	60	45	60	45	60	45	60
2005 season									
Organic	H_2O_2	77.8	102.3	21.1b	28.2	23.3	26.0	44.6	42.4
	Turbo	80.1	106.6	24.0a	30.8	25.6	28.4	48.5	46.0
Chemical	H_2O_2	59.3	107.1	15.1c	26.1	15.3	23.5	41.8	43.9
	Turbo	63.2	110.3	19.8b	29.0	20.4	28.8	44.1	47.8
F. test		NS	NS	**	NS	NS	NS	NS	NS
2006 season									
Organic	H_2O_2	86.4	110.1	22.3	30.4	22.3	27.3b	46.4	45.0
	Turbo	88.6	114.9	24.4	34.4	26.9	29.8a	50.1	48.9
Chemical	H_2O_2	65.3	116.1	15.4	32.6	20.0	24.5c	42.8	48.4
	Turbo	70.1	117.8	19.1	35.2	24.6	29.4a	44.1	50.4
F. test		NS	NS	NS	NS	NS	**	NS	NS

Means designed by the same letter are not significantly different at the 5% level according to Duncan's test

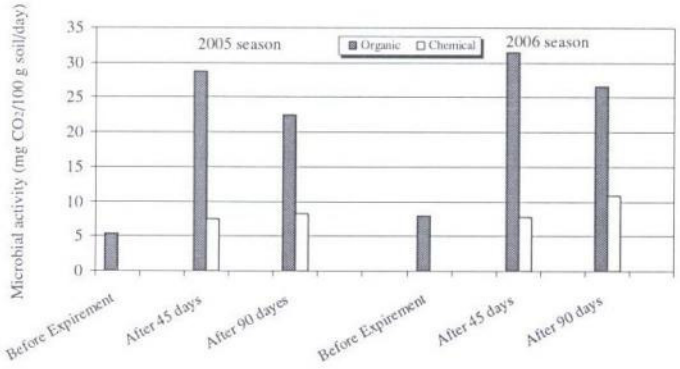

Figure 3 Main effects of organic and chemical fertilizers on microbial biomass activity of soil after organic and chemical fertilizers additions in both seasons

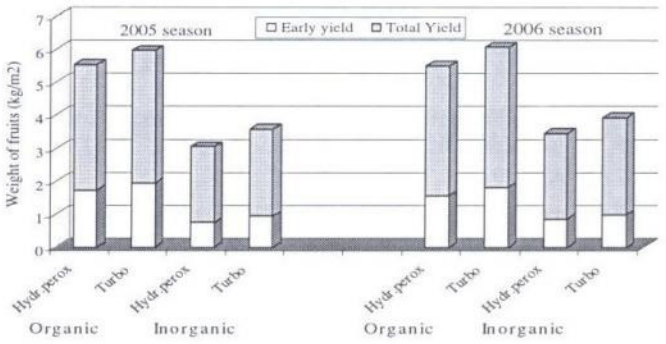

Figure 4 Early and total yields (weight of fruits/square meter) as affected by the interaction of fertilizer sources and control treatments in 2005 and 2006 seasons

chemical one attributed to the combined effect of compost, compost tea and seaweed extracts. So, compost contains higher micro-nutrients content (Fe, Zn and Mn), while seaweed extract had also higher values of K, Ca, Mg, S and Fe necessary for activity of meristimatic tissues (Bayoumi, 2005). Besides, using Turbo and H_2O_2 led to decrease disease severity (*Figure 2*). All these beneficial effects might lead to improve plant growth and consequently, fruit yield in organic treatment with Turbo and hydrogen peroxide. In this concern, *Cooke* (1972) stated that addition of organic materials improved the soil structure and encouraged the plants to have a good root development by improving the aeration in the soil which led to higher yield of plants. Also, *Cull* (1982) mentioned that micronutrients caused an increase in the enzymes activity and oxidation-reduction process in the plant tissues.

Table 3 Effect of fertilizer sources and control treatments interaction on some chemical characters of cucumber fruits

Treatments		Chemical characters		
Fertilizer sources	Control treatments	Ascorbic acid (mg/100g f.w)	TSS (%)	Nitrate content (ppm)
2005 season				
Organic	H_2O_2	14.0	4.5	105.8 b
	Turbo	15.1	4.6	100.7 b
Chemical	H_2O_2	11.8	4.8	210.5 a
	Turbo	13.4	4.9	215.6 a
F. test		NS	NS	**
2006 season				
Organic	H_2O_2	14.8	4.4	99.7 b
	Turbo	16.6	4.4	115.4 b
Chemical	H_2O_2	12.6	4.6	225.1 a
	Turbo	14.1	4.7	224.9 a
F. test		NS	NS	**

Means designed by the same letter are not significantly different at the 5% level according to Duncan's test

Effect of fertilizer sources and control treatments interaction on some chemical characters of cucumber fruits

Data in *Table 3* show that ascorbic acid content and TSS % were not affected by the interaction between fertilizer sources and control treatments in both seasons. However, organic treatment combined with Turbo showed the highest ascorbic acid content, while chemical treatment with H_2O_2 led to the least values.

Dealing with nitrate content in fruits, it was significantly affected by the interaction in both seasons, however using any of control methods with chemical treatments showed the highest nitrate content. Although, the values of nitrate fall below the recommended minimum level (270 mg/100 g dry wt.) in food substances as reviewed by *Lee* et al. (1971). Similar concolusion were done by *Vogtmann* et al. (1993), *Poudel* et al. (2002), *Bayoumi* (2005) and *Bayoumi & Hafez* (2006).

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