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Field Application of Humic Acid and Thyme Essential Oil for Controlling Late Blight Disease of Tomato Plants under Field Conditions

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ABSTRACT

Effects of humic acid and thyme essential oil alone or in combination for controlling late blight diseased of tomato plants was tested under field conditions. The tested concentrations of humic acid had no inhibitory effect on *Phytophthora infestans*. While, thyme at concentrations 8.0 mL L⁻¹ caused complete inhibition in linear growth of *P. infestans*. The highest inhibition in linear growth was obtained with thyme at 6.0 mL L⁻¹ which reduced the linear growth by 92.2%. Moreover, under field conditions, results revealed that the most effective treatments are humic acid at 6.0 or 8.0 g L⁻¹ combined with thyme at 6.0 or 8.0 mL L⁻¹ which reduced the late blight incidence more than 81.3 and 78.4% during first and second growing seasons, respectively. Meanwhile, single treatments of thyme at 6.0 or 8.0 g L⁻¹ combined with humic acid at 6.0 or 8.0 g L⁻¹ combined with humic acid at 6.0 or 8.0 g L⁻¹ showed moderate effect. The highest increase in tomato yield was obtained with humic acid at 6.0 or 8.0 g L⁻¹ combined with thyme at 6.0 or 8.0 mL L⁻¹ which increased the tomato yield more than 53.3 and 46.9% during first and second growing seasons, respectively. Meanwhile, single treatments of humic acid at 6.0 or 8.0 mL L⁻¹ showed moderate increase. The highest increase in β ,1-3-glucanase activity was obtained with humic acid at 6.0 or 8.0 g L⁻¹ which increased the β ,1-3-glucanase activity of tomato plants more than 137.0%.

Key words: Humic acid, thyme, late blight diseased, tomato plants, field conditions

INTRODUCTION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is an economically important disease of tomato (*Lycopersicon esculentum* Mill.). Disease management strategies primarily depend on sanitary practices and well-timed fungicide applications (Winton *et al.*, 2007; Shtienberg *et al.*, 2010; Blandon-Diaz *et al.*, 2012). However, development of fungicide resistance within populations of *P. infestans* alternative approaches that can be incorporated into integrated pest management of this pathogen (Blandon-Diaz *et al.*, 2012).

Humic acid is a suspension, based on potassium-humates, 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 and pests (Scheuerell and Mahaffee, 2004, 2006; Abd-El-Kareem, 2007a; Abd-El-Kareem *et al.*, 2009). It also, stimulate plant growth through increased cell division, as well as optimized uptake of nutrients and water (Atiyeh *et al.*, 2002; Chen *et al.*, 2004). Moreover, humic acid stimulated the soil microorganisms (Atiyeh *et al.*, 2002;

Qualls, 2004; Chen *et al.*, 2004). Furthermore, Abd-El-Kareem *et al.* (2009) reported that humic acid treatment in potato plants induced resistance against early blight in addition to increased potato yield under field conditions.

The essential oil of thyme and its major component, thymol had antifungal activity against plant pathogenic fungi (Plaza *et al.*, 2004; Angelini *et al.*, 2006) as well as plant diseases of several fruits and vegetables (El Sherbieny *et al.*, 2002; Sergvic-Klaric *et al.*, 2007).

The purpose of the present study is evaluating the effects of humic acid and thyme alone or in combination for controlling late blight diseased of tomato plants under field conditions.

MATERIALS AND METHODS

Source of pathogenic fungus and tomato transplants: Pathogenic isolate of *Phytophthora infestans* the causal agent of late blight disease was procured by Department of Plant Pathology, (Project integrated management for controlling tomato fungal diseases under Egyptian and Tunisian conditions), Giza Egypt. Tomato transplants cv. Kastel rock were obtained from the Department of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

Laboratory experiments

Testing of different concentrations of humic acid and thyme essential oil on linear growth of *Phytophthora infestans:* Five concentrations of humic acid and thyme essential oil were tested to evaluate their inhibitory effect on radial growth of *P. infestans.* For this 0.0, 2.0, 4.0, 6.0 and 8.0 mL L⁻¹ of humic acid and thyme essential oil were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and poured in sterilized petri plates (9 cm diameter). Plates were inoculated at the center with disks of 10 days old culture of *P. infestans* and incubated at 25 ± 2 . Five replicates were used for each. The average radial growth of fungus was measured after 10 days.

Field experiments

Testing of combined treatments between humic acid and thyme on late blight severity and tuber yield of tomato plants under field conditions: Experiments were carried out, at the Experimental Farm of National Research Centre at El-Noubareia, Behera Governorate, Egypt.

The promising treatments were applied under field conditions to study their effect against late blight disease in addition to their effect on tomato yield during two growing seasons. Field experiments were conducted under natural infection in plots (4×10 m) each comprised of 8 rows (40 transplants/row) in a randomized complete block design with three replicates (plots) for each treatment.

Treatments: Humic acid and thyme essential oil at concentrations 6.0 and 8.0 mL L^{-1} were tested alone or in combination in addition to the Fungicides (Redomil-plus at 2 g L^{-1}) were applied as in Table 1.

Table 1: Testing of combined treatment between humic acid and signal and thyme at two concentrations

Single	Combined
Humic acid 6 mL L ⁻¹	Humic acid 6 mL L ⁻¹ +thyme 6 mL L ⁻¹
Humic acid 8 mL L^{-1}	Humic acid 6 mL L ⁻¹ +thyme 8 mL L ⁻¹
Thyme 6 mL L^{-1}	Humic acid 8 mL L ⁻¹ +thyme 6 mL L ⁻¹
Thyme 8 mL L^{-1}	Humic acid 8 mL L ⁻¹ +thyme 8 mL L ⁻¹
Fungicide (Redomil plus 2 g L ⁻¹)	
Un-treated plants (control)	

Application: Single or combined treatments were applied as foliar application on tomato plants which had 4-7 leaves and every 15 days up to 90 days of transplanting. Combined treatments between humic acid and thyme was carried out by spraying tomato plants with humic acid at 6 or 8 mL L^{-1} followed by thyme at 6 or 8 mL L^{-1} with 3 days interval.

Disease assessment: Late blight scale according to Yan *et al.* (2002) based on the leaf area infected was modified as follows:

0 = No leaf lesions 1 = 25% or less 2 = 26-50 3 = 51-75 4 = 76-100% infected leaf area

Disease was recorded up to 90 days of transplanting.

Determination of tomato yield: Accumulation of tomato yield (kg m^{-2}) for each treatment was determined.

Determination of \beta-1,3-glucanase activity activities: Two concentrations of humic acid or thyme essential oil, i.e., 6.0 and 8.0 mL L⁻¹ were applied alone or in combination to study their effect on β ,1-3-glucanase activity of tomato yield under filed conditions. Tomato plants after 70 days of transplanting were used as samples to determine β -1,3-glucanase activity.

Extraction of enzymes: Plant leaves (g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.1) (Goldschmidt *et al.*, 1966) at the rate of 1/3 w/v. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was used to determine enzyme activity.

 β -1,3-glucanase assay: The method of Abeles and Forrence (1970) was used to determine β -1,3-glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. The method was carried out as 0.5 mL of enzyme extract was added to 0.5 mL of 0.05 M of potassium acetate buffer (pH 5) containing 2% laminarin. The mixture was incubated at 40°C for 60 min. The reaction was stopped by adding 1 mL of dinitrosalicylic acid reagent and heating the tubes for 5 min at 100°C. The tubes were cooled and 3 mL of distilled water were added before assay. The optical density was read at 500 nm. The β -1,3-glucanase activity was expressed as millimolar glucose equivalent released/gram fresh weight tissues/60 min.

Statistical analysis: Tukey test for multiple comparisons among means was utilized (Neter *et al.*, 1985).

RESULTS

Effect of different concentrations of humic acid and thyme essential oil on radial growth of *Phytophthora infestans*: Five concentrations of humic acid and thyme essential oil i.e., 0.0, 2.0, 4.0, 6.0 and 8.0 mL L⁻¹ were tested to study their inhibitory effect against *Phytophthora infestans*. Results in Table 2 indicate that all tested concentrations of humic acid had

Treatments and concentrations	Linear growth	Reduction (%
Humic acid (mL L ⁻¹)		
2.0	$90.0^{a(1)}$	0.0
4.0	90.0^{a}	0.0
6.0	90.0^{a}	0.0
8.0	90.0^{a}	0.0
Thyme (mL L ⁻¹)		
2.0	65.0^{b}	27.8
4.0	34.0°	62.2
6.0	$7.0^{ m d}$	92.2
8.0	0.0^{d}	100.0
Control		
0.0	90.0^{a}	-

Table 2: Effect of humic acid and thyme essential oil solution on radial growth of Phytophthora infestans

⁽¹⁾Values with the same letter are not significantly different (p = 0.05)

Table 3: Late blight incidence of tomato plants as affected with integrated treatments between humic acid and thyme under field conditions

	First season		Second season	
Applications	Disease incidence	Reduction (%)	Disease incidence	Reduction (%)
Single treatment				
Humic acid 6 mL L^{-1}	$1.5b^{(1)}$	53.1	1.7^{b}	54.1
Humic acid 8 mL L^{-1}	1.4^{b}	56.3	1.6^{b}	56.8
Thyme 6.0 mL L^{-1}	1.1°	65.6	1.2°	67.6
Thyme 8.0 mL L^{-1}	1.0°	68.8	1.2°	67.6
Combined treatment				
Humic acid 6 mL L ⁻¹ +thyme 6.0 g L ⁻¹ mL L ⁻¹	0.6^{d}	81.3	0.8d	78.4
Humic acid 6 mL L ⁻¹ +thyme 8.0 mL L ⁻¹	0.4^{d}	87.5	0.6^{d}	83.8
Humic acid 8 mL L ⁻¹ +thyme 6.0 mL L ⁻¹	0.5^{d}	84.4	0.5^{d}	86.5
Humic acid 8 mL L^{-1} +Thyme 8.0 mL L^{-1}	0.4^{d}	87.5	0.5^{d}	86.5
Redomil-plus 2 g L^{-1}	0.6^{d}	81.3	0.8^{d}	78.4
Control	3.2	-	3.7^{a}	-

⁽¹⁾Values with the same letter are not significantly different (p = 0.05), 2: Late blight scale from 0-4 according to Yan *et al.* (2002)

no inhibitory effect on *Phytophthora infestans*. Meanwhile, thyme at concentrations 8.0 mL L⁻¹ caused complete reduction in linear growth of *Phytophthora infestans*. The highest growth inhibition was obtained with thyme at 6.0 mL L⁻¹ which reduced the radial growth by 92.2%.

Field experiments

Effect of combined treatment between humic acid and thyme essential oil on late blight incidence of tomato plants under field conditions: Two concentrations of humic acid or thyme essential oil, i.e., 6.0 and 8.0 mL L⁻¹ were applied alone or in combination to study their effect on late blight disease of tomato plants under filed conditions. Results in Table 3 reveal that both concentration of each treatment significantly reduced the disease incidence of tomato plants during two growing seasons. The most effective treatments are humic acid at 6.0 or 8.0 mL L⁻¹ combined with thyme at 6.0 or 8.0 mL L⁻¹ which reduced the late blight severity more than 81.3 and 78.4% during first and second growing seasons, respectively. Meanwhile, single treatments of thyme at 6.0 or 8.0 mL L⁻¹ showed moderate effect.

Effect of combined treatment between humic acid and thyme essential oil on tomato yield under field conditions: Two concentrations of humic acid or thyme essential oil, i.e., 6 and 8.0 mL L^{-1} were applied alone or in combination to study their effect on tomato yield under filed conditions. Results in Table 4 indicate that both concentration of each treatment significantly increase the tomato yield during two growing seasons. The highest increase was obtained with

Application	First season		Second season	
	Yield (kg m ⁻²)	Increase (%)	Yield (kg m ⁻²)	Increase (%)
Single treatment				
Humic acid 6 mL L^{-1}	$4.0^{b(1)}$	33.3	$4.1^{ m bc}$	28.1
Humic acid 8 mL L^{-1}	4.0^{b}	33.3	4.3^{b}	34.4
Thyme 6.0 mL L^{-1}	3.6°	20.0	3.8°	18.8
Thyme 8.0 mL L^{-1}	3.7°	23.3	$3.9.1^{\circ}$	21.9
Combined treatment				
Humic acid 6 mL L ⁻¹ +thyme 6.0 g L ⁻¹ mL L ⁻¹	4.6^{a}	53.3	4.7^{a}	46.9
Humic acid 6 mL L ⁻¹ +thyme 8.0 mL L ⁻¹	4.6^{a}	53.3	4.8^{a}	50.0
Humic acid 8 mL L ⁻¹ +thyme 6.0 mL L ⁻¹	4.7^{a}	56.7	4.7^{a}	46.9
Humic acid 8 mL L ⁻¹ +thyme 8.0 mL L ⁻¹	4.7^{a}	56.7	4.8^{a}	50.0
Redomil-plus 2 g L^{-1}	4.2^{b}	40.0	4.3^{b}	34.4
Control	3.0^{d}	-	3.2^{d}	-

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⁽¹⁾Values with the same letter are not significantly different (p = 0.05)

Table 5: Effect of integrated treatments between humic acid and thyme on β , 1-3-glucanase activity of tomato plants under field conditions

Treatments	β,1-3-glucanase activity	Increase (%)
Single treatment		
Humic acid 6 mL L^{-1}	$6.4^{a(1)}$	137.0
Humic acid 8 mL L^{-1}	6.6^{a}	144.4
Thyme 6.0 mL L^{-1}	4.2^{b}	55.6
Thyme 8.0 mL L^{-1}	4.1^{b}	51.9
Combined treatment		
Humic acid 6 mL L ⁻¹ +thyme 6.0 g L L ⁻¹ mL L ⁻¹	6.5^{a}	140.7
Humic acid 6 mL L ⁻¹ +thyme 8.0 mL L ⁻¹	6.7^{a}	148.1
Humic acid 8 mL L ⁻¹ +thyme 6.0 mL L ⁻¹	6.8^{a}	151.9
Humic acid 8 mL L ⁻¹ +thyme 8.0 mL L ⁻¹	6.8^{a}	151.9
Control	2.7	-

⁽¹⁾Values with the same letter are not significantly different (p = 0.05), 2: β ,1-3-glucanase activity expressed as millimolar glucose equivalent released/gram fresh weight/60 min

humic acid at 6.0 or 8.0 mL L^{-1} combined with thyme at 6.0 or 8.0 mL L^{-1} which increased the tomato yield more than 53.3 and 46.9% during first and second growing seasons, respectively. Meanwhile, single treatments of humic acid at 6.0 or 8.0 mL L^{-1} showed moderate increase.

Effect of combined treatment between humic acid and thyme on β ,1-3-glucanase activity of tomato plants under field conditions: Two concentrations of humic acid or thyme essential oil, i.e., 4.0 and 60.0 mL L⁻¹ were applied alone or in combination to study their effect on β ,1-3-glucanase activity of tomato yield under filed conditions. Results in Table 5 indicate that both concentration of each treatment significantly increase the β ,1-3-glucanase activity of tomato plants. The highest increase was obtained with humic acid at 6.0 or 8.0 mL L⁻¹ when applied as single or combined with thyme at 6.0 or 8.0 mL L⁻¹ which increased the β ,1-3-glucanase activity of tomato plants more than 137.0%. Meanwhile, single treatments of thyme at 6.0 or 8.0 mL L⁻¹ were less effective.

DISCUSSION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is an economically important disease of tomato (*Lycopersicon esculentum* Mill.) (Winton *et al.*, 2007; Shtienberg *et al.*, 2010; Blandon-Diaz *et al.*, 2012). However, development of fungicide resistance within populations of *P. infestans* has become a problem and alternative approaches that can be incorporated into integrated pest management of tomato late blight disease are needed (Blandon-Diaz *et al.*, 2012). In present study integrated treatments between humic acid as resistance inducer and thyme as protective safe chemical against late blight disease of tomato plants was evaluated.

Humic acid can be applied successfully in many areas of plant production as a plant growth stimulant, soil conditioner, i.e., enhanced natural resistance against plant diseases and pests (Scheuerell and Mahaffee, 2004, 2006; Abd-El-Kareem et al., 2009). In the present results indicated that all tested concentrations of humic acid had no inhibitory effect against *Phytophthora infestans* but it reduced late blight disease of tomato plants under field conditions. In this respect, Scheuerell and Mahaffee (2004, 2006) reported that the most effective treatments for suppression damping off in many plants and gray mould in Geranium was compost tea plus kelp extract and humic acid. The role of humic acid for reducing late blight diseases in addition to increase yield of tomato plants may be due to enhanced natural resistance against plant diseases and pests (Scheuerell and Mahaffee, 2004, 2006). In the present study results indicated the highest increase was obtained with humic acid at 6.0 or 8.0 mL L⁻¹, when applied as single or combined with thyme at 6.0 or 8.0 mL L⁻¹ which increased the β .1-3-glucanase activity of tomato plants more than 137.0%. In this respect, B-1,3-glucanases and chitinases are able to hydrolyze B-1,3-glucan and chitin, respectively, the major components of fungal cell walls (Kauffman et al., 1987; Legrand et al., 1987; Abd-El-Kareem et al., 2006; Abd-El-Kareem, 2007a). Abd-El-Kareem (2007b) reported that bean plants treated with humic acid induced resistance against root rot and Alternaria leaf spot in addition to increased bean yield under field conditions. Moreover, Abd-El-Kareem et al. (2009) reported that potato plants treated with humic acid induced resistance against early blight in addition to increased potato yield under field conditions.

On the other hand, humic acid stimulated plant growth through increased cell division, as well as optimized uptake of nutrients and water (Atiyeh *et al.*, 2002; Delgado *et al.*, 2002; Chen *et al.*, 2004) and stimulated the soil microorganisms (Atiyeh *et al.*, 2002; Chen *et al.*, 2004; Garcia-Gil *et al.*, 2004).

The essential oil of thyme and its major component, thymol, had antifungal activity against plant pathogenic fungi (Plaza et al., 2004; Angelini et al., 2006). In the present study results indicated that thyme at concentrations 8.0 mL L⁻¹ caused complete reduction in linear growth of *Phytophthora infestans.* The highest reduction in linear growth was obtained with thyme at 6.0 mL L^{-1} which reduced the linear growth by 92.2%. While, under field conditions results revealed that he most effective treatments are humic acid at 6.0 or 8.0 mL L^{-1} combined with thyme at 6.0 or 8.0 mL L⁻¹ which reduced the late blight severity more than 81.3 and 78.4% during first and second growing seasons, respectively. Meanwhile, single treatments of thyme at 6.0 or 8.0 mL L⁻¹ showed moderate effect. These treatments significantly increased the tomato yield. In this respect Soliman and Badeaa (2002) reported that thyme was tested for its inhibitory effect against Aspergillus flavus, A. parasiticus, A. ochraceus and F. moniliform. Results indicated that thyme at concentrations 500 ppm completely inhibited all tested fungi. Similar results was obtained by Daferera et al. (2003), they reported that thyme, clove and cinnamon essential oils completely inhibited P. digitatum and P. italicum growth either when added into the medium or by their volatiles. Moreover, Omidbaigi et al. (2007) reported that the fungistatic and fungicidal activities of the essential oils of thyme, myrtle, clove and lime against A. flavus and showed that the most effective essential oils were found from those of thyme and clove. Thyme and clove oils completely inhibited growth of A. flavus. Furthermore, thyme essential oil possesses a wide range spectrum of fungicidal activity against plant pathogenic fungi Aspergillus, Penicillium, Alternaria, Cladosporium pythium sp., F. oxysporum f. sp. lycopersici, Fusarium oxysporum f. sp. pisi, Verticilium albo-atrum, Rhizoctonia sp. and Clavibacter michiganensis sub sp. michiganesis (Tanovic et al., 2007). On the other hand, Liu et al. (2002) found that thymol was more effective for

controlling brown rot symptoms on apricots and fumigation of plumus with relatively low concentrations such as 2 or 4 mg L⁻¹ can greatly reduce postharvest decay without causing any phytotoxicity. Also, Daferera *et al.* (2003) reported that thyme and cinnamon essential oils applied at 5 mL L⁻¹ significantly reduced the incidence of green and blue moulds caused by *P. digitatum* and *P. italicum* respectively.

Sergvic-Klaric *et al.* (2007) reported that antifungal activities of the thyme essential oil, which contains p-cymene (36.5%), thymol (33.0%) and 1.8 cineole (11.3%) as a main components and pure thymol were determined by the dilution method and exposure to vaporous phase of the oil. Thymol exhibited approximately three times stronger inhibition than essential oil of thyme. Moreover, thyme essential oils was reported against plant diseases of several fruits and vegetables (El Sherbieny *et al.*, 2002; Sergvic-Klaric *et al.*, 2007).

It could be suggested that combined treatment between humic acid and thyme might be used commercially for controlling late blight disease of tomato plants under field conditions.

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