Egypt. J. Appl. Agric. Res. (*NRC*),**Vol. 1**, No. 1,pp. 57-67 (2008)

Effect of Combinations among Two Fungicides, the Biocides Rhizo-N and the Biofertilizer Humix on Suppressing Damping-off and Root-Rot Diseases of Tomato

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BOTH FUNGICIDES Rizolex-T and Topsin-M70 caused similar significant reduction to the linear growth of *Fusarium solani* (Mart.) Sacc. and *Rhizoctonia solani* Kuhn compared with unpoisoned medium Potato Dextrose Agar (PDA). The tested fungicides, the biocide Rhizo-N and the biofertilizer Humix, each alone or in different combinations resulted in significant reduction to both damping-off and root-rot diseases of tomato (cv. Castle Rock)caused by the two tested fungi compared with control treatment. In addition, the different combinations were more efficient in this regard, especially when more than two treatments were combined, compared with applying one treatment alone. This reduction in both damping-off and root-rot diseases was reflected on the estimated crop parameters, *i.e.* plant height, foliage fresh weight and root length, where considerable increase was found due to using the corresponding treatments.

Keywords: Plant diseases, *Fusarium solani*, *Rhizoctonia solani*, Pathogen control.

Tomato (*Lycopersicon esculentum* Mill.) is one of the major Solanaceous crops grown allover the world including Egypt. It is considered most suitable crop that can be grown at all times of the year in Egypt. The cultivated area with tomato during 2007 in Egypt amounted to about 495381 feddan which produced about 8391223 ton fruits with an average of 16.94 ton/feddan (Ministry of Agriculture, Reports of Econ. Agriculture and Statis. Department, Egypt, 2007).

Tomato fruits can be used as fresh or canned. In addition, it is considered one of the highest nutritional Solanaceaus crops that has high vitamin C content, many chemical compounds and elements which are not found in the other members.

Under Egyptian conditions, tomato plants are vulnerable to many fungal, bacterial, viral and nematode diseases as well as physiological disorders (Fletcher & Harris, 1979 and Assal *et al.*, 1995).

However, fungal diseases, especially damping-off and root-rot diseases, caused by soil-borne fungi are the most important ones that can cause great reduction in plant stand as well as fruit yield (El-Helaly *et al.*, 1970). During the last few years, several complains from tomato growers have constantly been received, especially under conditions of no crop rotation. They are mainly due to low seed germination, high death percentage of the seedlings as a result of infection by damping-off and root-rot diseases.

This work tested the possibility of reducing damping-off and root-rot diseases of tomato by using the combinations among two fungicides, the biocide Rhizo-N and the biofertilizer Humix and estimating their effect on some crop parameters.

Material and Methods

Effect of two fungicides on the radial growth of both Fusarium solani(Mart.) Sacc and Rhizocotnia solani Kuhn

Both isolates of *Fusarium solani* and *Rhizoctonia solani* were kindly provided by Department of Vegetable Disease Research, Plant Pathology Research Institute, ARC. Egypt.

Different concentrations, (i.e. 0.0, 25, 50, 100, 200 and 300 ppm) of the fungicides Rizolex-T (Tolcolofos-methyl) and Topsin M-70 (Thiophanate-methy) according to their active ingredients were added to accurate amounts of Potato Dextrose Agar (PDA) medium after sterilization (at 45°C) then poured in sterilized Petri-dishes (9 cm in diameter), plates were inoculated after solidification at the center with 5 mm discs taken from the edge of 7-days old cultures of any of the two tested fungi. In addition, medium free from any fungicide was also inoculated with the tested fungi and left as control. Four Petri-dishes were prepared for each treatment and incubated at 30±1°C for 5 days, then, the linear growth in mm was recorded.

Effect of the combinations among two fungicides, the biocide Rhizo-N and the biofertilizer Humix on the incidence of damping-off and root-rot diseases (greenhouse)

Both *F. solani* and *R. solani* were grown on sterilized barley medium in glass bottles (500 ml) for two weeks. Disinfested soil by 5% formalin was infested by the inoculum of each of the two tested fungi, separately, and distributed in sterilized plastic pots (25 cm in diameter). Tomato (cv. Castle Rock) seeds were moistened by 10% super film as sticker and dressed by Rizolex-T or Topsin-M70, at the rate of 1.5 and 1.0 g/kg seeds, respectively then sown in the plastic pots at the rate of 5 seeds/pot. Untreated seeds with the tested fungicides were also sown at the same rate as a control. In addition, a mixture was prepared from both the commercial products of the biocide Rhizo-N (*Bacillus subtilis* $30x10^6$/g) and the biofertilizer Humix (12% humic acids, 1% manganese, 1% zinc, 1% iron, 0.5% magnesium, copper 200 ppm, calcium 200 ppm, boron 100 ppm and traces of sulphur, molybdenum and cobalt) at the rate of 2 g and 10 ml/L water for both products respectively. Hundred ml from the mixture were added to each sown pot

in different combinations with the two tested fungicides. Three pots were prepared for each treatment. All agricultural practices such as irrigation and fertilization were applied as recommended by the Ministry of Agriculture.

Disease assessment

The number of emerged seedlings was counted two weeks after sowing and the percentages of pre-emergence damping-off were calculated referring to the number of sown seeds. Four weeks after sowing, the number of dead seedlings was also counted and the percentages of post-emergence damping-off were assessed referring to the number of sown seeds. Also, ten weeks after sowing, the number of dead plants was counted and the percentages of dead plants were taken as criteria for assessing root-rot referring to the number of the sown seeds.

At the end of the experiment, the plants were irrigated with excess water and the pots converted gently to get out the plants with their roots. The roots of the collected plants were carefully examined for root-rot and root-rot severity was calculated based on the 0-4 scale devised by Castanho and Butler (1978) using the following formula:

$$\text{Disease severity (\%)} = \frac{\Sigma (n \times v)}{4\,N} \times 100$$

where:

n = Number of plants in each category.
v = Numerical values of symptoms of each category.
N = Total number of plants.
4 = Maximum of numerical values of symptoms categories.

Also, foliage fresh weight (g), plant height (cm) and root length (cm) were assessed at the end of the experiment

Statistical analysis

Data were statistically analyzed according to the standard procedures for split design and complete randomized blocks (Snedecor and Cochran, 1967). The averages were compared using least significant differences (LSD) at the 5% level (Fisher, 1948).

Reults and Discussion

1. Effect of two fungicides on the radial growth of both F. solani and R. solani

Data presented in Table 1 show that both Rizolex-T and Topsin-M70 caused significant reduction to the linear growth of the two tested fungi. This reduction gradually increased by increasing the concentration of the two tested fungicides. In addition, both Rizolex-T and Topsin-M70 caused almost complete inhibition to *F. solani* and *R. solani* at 300 (Table 1).

TABLE 1. Effect of the fungicides Rizolex-T and Topsin-T70 on both *Fusarium solani* and *Rhizoctonia solani*, 5 days after incubation at 30 ± 1°C.

Fungi	Fungicides	Linear growth (mm)							
		Concentration (ppm)							
		0.0	25	50	100	200	300	Mean	General mean
F.solani	Rizolex-T	90.0	65.6	51.0	30.4	18.2	0.0	42.5	37.1
	Topsin-M70	90.0	57.0	28.6	14.0	0.0	0.0	31.6	
R. solani	Rizolex-T	90.0	54.8	28.0	15.0	0.0	0.0	31.3	36.6
	Topsin-M70	90.0	64.0	50.0	29.6	17.0	0.0	41.8	
Mean	Rizolex-T	90.0	60.2	39.5	22.7	9.1	0.0	36.9	--
	Topsin-M70	90.0	60.5	39.3	21.8	8.5	0.0	36.7	
General mean		90.0	60.4	39.4	22.3	8.8	0.0	--	--

L.S.D. at 5% for: Fungi (I) = n.s, Concentration (C) = 3.2, Fungicides (F) = n.s., I x C = 2.8, I x F = 3.1, C x F = 4.0 and I x C x F = 4.8.

No significant difference was found concerning the effect of Rizolex-T and Topsin M-70 on the two pathogenic fungi. Also, no significant variation was recorded between the average of the linear growth of *F. solani* and *R. solani* due to the effect of the two tested fungicides, being 37.1 and 36.6 mm, respectively.

The inhibitory effect of both Rizolex-T and Topsin-M70 on the two fungi as pathogens of peanut plants was reported early by El-Deeb *et al.* (2002).In addition, the fungicide Rizolex-T has high inhibitory effect on fungi forming sclerotia such as *R. solani*. Therefore, this fungus was completely inhibited at 200 ppm of Rizolex-T, whereas at 300 ppm for *F. solani*.

2. Effect of the combination among two fungicides, Rhizo-N and the biofertilizer Humix on the incidence of damping-off and root-rot diseases

Data presented in Table 2 show that the two tested fungicides (Rizolex-T and Topsin-M70), Rhizo-N and the biofertilizer Humix, each alone or in different combinations, resulted in significant reduction to both pre- and post-emergence damping-off as well as dead plants (root-rot) of tomato (cv. Castle Rock) caused by *F. solani* and *R. solani* compared with control treatment. In addition, using any of the aforementioned treatments alone was of low efficiency in reducing the infection by the two fungi compared with the different combinations. In this regard, the combinations among [Rhizo-N + Rizolex-T + Topsin-M70 + Humix], [Rhizo-N + Rizolex-T + Humix] and [Rhizo-N + Topsin-M70 + Humix] were the most efficient treatments, which no pre-emergence damping-off was recorded, while 2.2, 3.3 and 3.3% post-emergence damping-off and 1.1, 1.1 and 2.2% dead plants, on the average, were recorded, respectively. Meanwhile, Rhizo-N, Rizolex-T, Topsin-M70 and Humix recorded 20.0, 11.1, 12.2 and 16.6%, on the averages pre-emergence damping-off; 13.3, 7.8, 7.8 and 11.1%, on the average, post-emergence damping-off and 13.3, 12.2, 11.1 and 13.3%, on the average, dead plants, respectively. Control treatment recorded 31.1, 17.8 and 14.4%, on the average, respectively.

TABLE 2. Effects of the different combinations among two fungicides, the bioagent Rhizo-N and the biofertilizer Humix compounds on incidence of damping-off and dead plants caused by *F. solani* and *R. solani* in a greenhouse experiment.

Treatments	* % pre-emergence damping-off			** % post-emergence damping-off			*** % dead plants (root-rot)			% root-rot severity		
	F. solani	*R. solani*	Mean	*F. solani*	*R. solani*	Mean	*F. solani*	*R. solani*	Mean	*F. solani*	*R. solani*	Mean
Rhizo-N (1)	22.2	17.8	20.0	15.5	11.1	13.3	13.3	13.3	13.3	15.3	15.3	15.3
Rizolex-T (2)	15.5	6.7	11.1	8.8	6.7	7.8	13.3	11.1	12.2	11.0	11.0	11.0
Topsin-M70 (3)	13.3	11.1	12.2	8.8	6.7	7.8	8.8	13.3	11.1	12.0	10.0	11.0
Humix (4)	17.8	15.5	16.6	13.3	8.8	11.1	13.3	13.3	13.3	20.0	18.6	19.3
(1) + (2)	11.1	4.4	7.7	6.7	6.7	6.7	11.1	8.8	10.0	8.3	8.3	8.3
(1) + (3)	11.1	11.1	11.1	6.7	6.7	6.7	6.7	8.8	7.8	9.0	6.6	7.8
(1) + Humix	13.3	11.1	12.2	4.4	6.7	5.6	6.7	11.1	8.9	9.6	9.6	9.6
(2) + (3)	4.4	2.2	3.3	2.2	4.4	3.3	2.2	4.4	3.3	4.0	3.5	3.8
(2) + Humix	11.1	0.0	5.5	4.4	6.7	5.6	2.2	4.4	3.3	6.6	4.6	5.6
(3) + Humix	13.3	0.0	6.5	6.7	4.4	5.6	4.4	4.4	4.4	7.3	4.6	6.0
(1) + (3) + (4)	0.0	0.0	0.0	2.2	4.4	3.3	2.2	2.2	2.2	5.0	2.2	3.6
(1) + (2) + (4)	0.0	0.0	0.0	4.4	2.2	3.3	2.2	0.0	1.1	3.0	2.0	2.5
(1) + (2) + (3) + (4)	0.0	0.0	0.0	2.2	2.2	2.2	0.0	2.2	1.1	1.0	1.0	1.0
Control (infected)	33.3	28.9	31.1	17.7	17.8	17.8	15.5	13.3	14.4	39.3	33.3	36.3
Mean	11.1	7.7	--	7.0	6.8	--	7.2	7.9	--	10.8	9.3	9.4

* Assessed 2 weeks after sowing, ** assessed 4 weeks after sowing and *** assessed 10 weeks after sowing

L.S.D. at 5% for:

Treatments (T)	=	1.8	3.2	3.1	1.8
Disease (D)	=	2.3	n.s	n.s	1.1
T x D	=	3.5	3.1	2.7	2.4

The obtained data revealed that using any of the tested fungicides, the bioagent Rhizo-N and the biofertilizer Humix alone caused different degrees of reduction of both damping-off and root-rot (dead plants) diseases. Meanwhile, the combination between two items increased their efficiency and using the three different tested compounds resulted in low values of disease hazard. Therefore, it could be recommended to use such material in controlling damping-off and root-rot diseases of tomato, at least in the nurseries or greenhouses that produce tomato transplants for tomato growers, where using healthy transplants is the first successful step in obtaining excellent foliage growth and fruit production, especially when the other recommended criteria are applied. The fungicides were frequently used for managing damping-off and root-rot diseases of tomato (Souza-Filho, 1978, Satija & Indra, 1987 a&b, Davis, 1989 and Kapoor & Kumar, 1991). In addition, different bioagents or biocides were used for the same purpose (Roberti & Selmi, 1999, Montealegre *et al.*, 2002, El-Abbasi *et al.*, 2003, Attia *et al.*, 2004, Szczech & Shoda, 2004, Domenech *et al.*, 2006, Omar *et al.*, 2006 and Tao-Jing *et al.*, 2006). But in most cases, each of the aforementioned materials was used alone or as a combination between fungicides and bioagents (Sabet *et al.*, 2000, Raut & Patil, 2005 and Slusarski, 2005). Herein, a combination among two fungicides, the biocide Rhizo-N and the biofertilizer Humix was made in order to increase or to sustain the efficiency of these compounds, where both fungicides and the biocide Rhizo-N caused drastical effect on the tested pathogens and the biofertilizer provided the grown plants by enough essential elements. (Castanho &Butler, 1978, Pacuta *et al.*, 2001 and Feckova *et al.*, 2005). In previous research work, they used the biofertilizers Biogin, Microbin, Phosphorin and Rhizocetrin in decreasing the infection by strawberry root-rot caused by soil-soil pathogens. El-Kolaly (2003) and some biofertilizers in controlling many soil-borne pathogens (Abo Elwafa and Abd El-Latief, 2006).

Table 2 shows also that values of root-rot severity were, in most cases, in the same trend of the percentages of dead plants. In this regard, the combination among the two fungicides + Rhizo-N and Humix resulted in the lowest values of root-rot severity (1%) followed by the combination among the two fungicides + Humix (2.5%). Meanwhile, Humix alone recorded the highest values of root-rot severity (19.3%) followed by Rhizo-N (15.3%).

3. Effect of the combination among two fungicides, the biocide (Rhizo-N) and biofertilizer Humix compounds on fresh weight, plant height and root length

Table 3 shows that the incidence of damping-off and root-rot diseases of tomato caused by *F. solani* and *R. solani*, respectively, was reflected on the assessed crop parameters, i.e. foliage fresh weight, plant height and root length. In this respect, using any of tested fungicides, the biocide Rhizo-N and the biofertilizer Humis, each alone was of low efficiency in increasing the assessed crop parameters compared with using, at least, two of the tested materials and the highest values of crop parameters were recorded for using the combination among the four compounds.

TABLE 3. Effects of the different combinations among two fungicides, the bioagent Rhizo-N and the biofertilizer Humix compounds on fresh weight, plant height and root length of tomato plants grown in soil infested with *F. solani* and *R. solani* in a greenhouse experiment.

Treatments	Plant height (cm)			Foliage fresh weight (g)			Root length (cm)		
	F. solani	*R. solani*	Mean	*F. solani*	*R. solani*	Mean	*F. solani*	*R. solani*	Mean
Rhizo-N (1)	13.3	14.8	14.1	10.4	13.3	11.9	10.8	8.3	9.6
Rizolex-T (2)	20.8	15.3	18.1	23.6	20.9	22.3	14.7	10.2	12.5
Topsin-M70 (3)	19.8	16.9	18.4	22.0	18.5	20.3	12.5	10.1	11.3
Humix (4)	17.7	10.3	14.0	11.4	15.1	13.3	11.4	9.3	10.4
(1) + (2)	22.0	23.5	22.8	24.3	22.5	23.4	15.4	11.0	13.2
(1) + (3)	24.8	24.6	24.7	25.1	23.1	24.1	16.5	16.6	16.6
(1) + Humix	20.8	21.7	21.3	24.5	20.0	22.3	17.0	15.0	16.0
(2) + (3)	24.8	22.5	23.7	26.0	33.5	29.8	16.2	16.7	16.5
(2) + Humix	25.0	23.5	24.3	19.5	25.5	22.5	17.2	17.5	17.4
(3) + Humix	27.0	21.7	24.4	25.0	26.7	25.9	20.0	17.7	18.9
(1) + (3) + (4)	30.0	23.5	26.8	26.5	28.4	27.5	22.4	20.7	21.5
(1) + (2) + (4)	31.0	28.0	29.5	35.0	31.3	33.2	24.4	29.0	26.7
(1) + (2) + (3) + (4)	38.0	30.0	34.0	38.4	36.5	37.5	25.0	32.0	28.5
Control (infected)	11.0	9.0	10.0	7.1	8.3	7.7	8.0	5.5	6.8
Mean	23.3	20.4	--	23.0	23.1	--	16.5	15.7	--
L.S.D. at 5% for:									
Treatments (T) =			2.1			2.3			2.7
Disease (D) =			2.0			1.1			n.s.
T x D =			1.8			2.7			2.2

The obtained data were documented our expectations, where seed dressing with either of the two tested fungicides is not enough to protect the roots of the emerged seedlings from invasion by the tested pathogenic fungi, at least, for a long period. The same applies for using the biocide Rhizo-N and the biofertilizer Humix. Yet, using more than one of these factors, especially from the two fungicides and the biocide Rhizo-N could cause more effect on the causal pathogens and gave capability to the grown plants to resist the infection by the pathogens, especially if the biofertilizer Humix is added.

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(*Received* 17/4/2008;
accepted 9/7/2008)

تأثير الجمع بين مبيدين فطريين والمبيد الحيوى ريزو-إن والسماد الحيوى هيومكس على تثبيط مرضى سقوط البادرات وعفن الجذور فى الطماطم

مرفت رفعت هلال و سامى حسين مصطفى
معهد بحوث أمراض النباتات - مركز البحوث الزراعية – القاهرة – مصر .

أحدث المبيدين الفطريين ريزولكس-تى ، توبسين إم-٧٠ إنخفاضاً معنوياً للنمو الطولى للفطرين *Rhizoctonia solani, Fusarium solani* مقارنة لنموهما على البيئة غير المسممة بالمبيدين ، ولم يكن هناك فرق معنوى نتيجة للتأثير المثبط للمبيدين على الفطرين المختبرين.

أوضحت نتائج الصوبة أن استخدام كل من المبيدين المختبرين ، والمبيد الحيوى ريزو-إن والسماد الحيوى هيومكس وكل منهم منفرداً أو باتحاداتهم المختلفة أحدث إنخفاضاً معنوياً لكل من أمراض سقوط البادرات وعفن الجذور المتسببة عن الفطرين المختبرين مقارنة بالنباتات غير المعاملة. بالإضافة لذلك فإن استخدام أكثر من معاملتين كانت أكثر فاعلية فى خفض الإصابة مقارنة باستخدام معاملة واحدة. وقد انعكس هذا الإنخفاض على القياسات المحصولية (طول النبات والوزن الطازج للمجموع الخضرى وطول الجذر) حيث أدى إلى زيادة ملحوظة فى هذه القيم نتيجة لاستخدام هذه المعاملات.

كلمات مفتاحية: أمراض النبات ، *Rhizoctonia solani, Fusarium solani*، مكافحة الأمراض.