

## TOXICOLOGICAL STUDIES OF SOME PESTICIDES ON CERTAIN VEGETABLE CROPS PESTS II-AN ATTEMPT TO MINIMIZE ENVIRONMENTAL POLLUTION WITH PESTICIDES

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### ABSTRACT

Five formulated compounds Fenpyroximate, fenarimol , sulphur , mineral oil , Kz-oil and black pepper extract were studied for their acaricidal activity against different stages of the two-spotted spider mite *Tetranychus urticae* ,and their fungicidal activity against *Rhizoctonia solani* ,*Fusarium moniliforme* and *Alternaria solani* that associated with *T. urticae* under plastic tunnels conditions..The results exhibited in general that the sensitivity of the *T. urticae* eggs to the compounds was increased with increasing egg age , and that Fenpyroximate was the most potent against mite egg stages. The same compound and kz-oil exhibited high residual effect against moving stages of *T. urticae* on tomato , pepper and cucumber plants .When the seeds of these varieties of plants were treated with the tested compounds , fenarimol -treated pepper increased % seedling emergence in *R. solani* - infested soil more than the other compounds . All compounds – treated cucumber did not show a pronounced response against *R. solani* . % post emergence was 100%for tomato, while it was zero % in case of cucumber and pepper . On the other hand most of the tested compounds exhibited more protection for cucumber and pepper against *F. moniliforme* than that against *R. solani* . Fenarimol was the most protectant compound against *A.solani* when sprayed on tomato plants . The protection due to tested compounds in general , was more after inoculation with the pathogen than that of before inoculation.

### INTRODUCTION

*Tetranychus urticae* infested a wide range of economic plants in the field and under glass houses such as cucumber and tomatoes . Tomato, cucumber and pepper are considered as the most popular and important vegetables for fresh consumption in Egypt.( Veire *et al.*, 1992; Abbassy *et.al*, 1993 and Megali *et al.*, 1995 )The infestation by mites caused a great damage to these infested plants followed by a secondary infestation by various pathogens such as virus, bacteria and fungi. Fungi caused many diseases in the field crops and vegetables. *Alternaria solani* caused early blight disease of tomatoes and potatoes. Some important seed and soil born fungi particularly those causing damping-off disease, *Rhizoctonia solani* and *Fusarium moniliforme* are the most frequent fungi causing damping-off disease to many field crops and vegetables (Roman *et.al.*, 1982; De and Cattopadhyay, 1984; Ali *et al.*, 1992 and El-Shami *et al.*, 1993 ).

The present investigation was carried out to study the action of acaricides or the acaricidal action of fungicides as new method to control both fungi and mites in the same time under plastic tunnel conditions to minimize the use of pesticides.

## MATERIALS AND METHODS

### 1-Test organisms

#### 1-a. Spider mite ,*Tetranychus urticae*:

Spider mite *Tetranychus urticae* (Acarina: Tetranychidae) colonies were obtained from castor bean plants from Kafr El-Sheikh Governorate and reared under laboratory conditions on castor bean; *Ricinus communis* (L.) for about eight (8) months away from any contamination with pesticides before starting the experiments. 5-8 seeds of castor bean were planted in one pot for 10-15 days. The seedlings were infested by clean culture of red mites. Mites were transferred from old to young plants by cutting heavily infested leaves into small sections which were then placed on new plants. Contamination was prevented by placing these seedlings in special chambers, 50 x 50 x 60 cm. covered with muslin.

These cultures were maintained in a breeding room under a temperature of  $25 \pm 2$  °C , 60 – 70 % R.H. and 12 hours daily illumination by 2 fluorescent bulbs of 40 wts each.

Mites were collected by placing the infested castor bean leaves on white paper, then the full mature individuals were chosen and transferred by using a fine brush (No. 000).

#### 1-b .Isolation, purification and identification of Fungi:

Three fungi were used in this study namely *Alternaria solani* that causes early blight disease in potatoes and tomatoes and fruit rot of tomato and pepper, the fungus was isolated from tomato fruits and leaves, *Fusarium moniliforme* and *Rhizoctonia solani* that causes damping -off (Root-rot) in tomatoes, cucumber and pepper were isolated from tomatoes, cucumber and pepper roots. Infested pieces of leaves, fruits and roots were surface sterilized with 5% chlorox solution for 2 minutes, and washed several times with sterilized water. The surface sterilized pieces were then dried between two sterilized filter papers and allowed to grow in petri dishes which contain potato dextrose agar medium (PDA) amended with 25 mg/ml streptomycin sulfate to avoid bacterial contamination. The petri-dishes were incubated at  $27 \pm 3$  °C for 3-10 days and examined daily for the occurrence of fungus growth. The growing fungi were examined microscopically and purified using the hyphal tip technique, then transferred to PDA slants. Pathogenic isolates were identified according to their cultural and microscopical characters (Barnett and Hunter 1979). Slants were maintained in a refrigerator at 4 °C as stock cultures for further experiments.

### 2-Tested Compounds:

Five compounds in the formulated form were used and calculated on the basis of ppm. active ingredient.

**2-a-Fenpyroximate** (5% S.C)The IUPAC name is: tert-butyl (E)- $\alpha$ - (1, 3-dimethyl -5- phenoxy pyrazol-4-yl methylene-amino-oxy) = P-toluate.It was supplied by Nihon Nohyaku Company-Tokyo-Japan.

**2-b-Fenarimol** (12 % E-C) supplied by Eli Lilly Company Dow Elanco Germany

**2-c - .Flowable sulphur** 52% .S.C.) provided by stoller chemical Company –U.S.A

**2-d. - Mineral oil:** Kz-oil: formulated mineral oil supplied by kafr El-Zayat Co. for pesticides and chemicals as 95%E.C. This oil is recommended to control the scale insects in Egypt.

**2-e- Plant extract of black pepper (*Piper nigrum* fruits):**

The plant extract was prepared according to Abbassy *et al.* (1993).

**3-Toxicity of the tested compounds to different egg ages of the two-spotted spider mite, *Tetranychus urticae* (koch):**

Leaf disc dip method: according to Siegler (1947) was used. The treated eggs as well as the control were kept under laboratory conditions. After the untreated eggs (control) were hatched, (4 days after egg deposition) the % of unhatched eggs were calculated according to the following formula

$$\text{Percentage of unhatched eggs} = \frac{\text{No. of unhatched eggs}}{\text{No. of total eggs}} \times 100$$

Correction of control mortality was made using Abbott's Formula (1925).

**4-Toxicity of the tested compounds against the moving stages of two-spotted spider mite, *Tetranychus urticae* under plastic tunnel conditions**

These studies were carried out at the experimental farm of Faculty of Agric., Kafr El-Sheikh, Tanta Univ., during the growing season, summer 1999. Tested compounds were applied at half and field recommended doses using manual sprayer. A cultivated area grown with tomato, cucumber and pepper which was highly infested with the mite *Tetranychus urticae* was divided into plots of 40 m<sup>2</sup>. Each treatment was distributed in a complete randomized block design with four replicates. The infestation was determined by counting all the moving stages on 10 leaves which were taken for each replicate (4 from lower, 3 from the middle and 3 from the top of 4 random plants, the counts of mites were examined using hard lens. The mentioned inspection was carried out before spraying and continued 1, 3, 5, 7 and 14 days after spraying.

Percentages of reduction in the infestation of mites were estimated according to Henderson and Telton (1955).

**5-Plastic tunnel experiments**

This procedure was used to assay the tested compounds for the control of damping-off caused by *Rhizoctonia solani* and *Fusarium moniliforme* in a susceptible tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and pepper (*Capsicum annuum*) cultivars (kasel Rock, Beta-alpha and California wonder respectively).

The activities of these compounds were tested also against *Alternaria solani* the causative agent of early blight of tomato and pepper.

**6- Seed treatments :**

Seeds of tomato kasel Rock (*Lycopersicon esculentum*), cucumber (Beta-alpha) *Cucumis sativus* and pepper (California wonder) *Capsicum annuum*, were allowed to soak in water solutions of the tested compounds at rate of (1C<sub>75</sub>) for 6 hours and then dried at room temperature (Ramanathan and

Sivaprakasam, 1994). Seeds that were soaked in water for 6 hours before planting served as a check treatment.

#### **7- Soil infestation:**

Sterilized sandy and loam soils were mixed at ratio 1:2. Batches of soil were infested separately with inoculum of each isolate at the rate of 50 g/kg of soil. Infested soil was dispensed in 25 cm diameter plastic pots. For the control treatment, sterilized sand maize meal was mixed thoroughly with soil at the rate of 50 g/kg of soil. Ten seeds were sown in each pot of either infested soil with *Rhizoctonia solani*, *Fusarium moniliforme* or non-infested soil immediately after the seed drying, and the pots were regularly watered. Four pots were used for each treatment. Data of disease incidence were recorded 15 days after planting for pre-emergence damping-off and 30 days after planting for post-emergence damping-off according to: Shatla *et al.*, (1983)

#### **8-Activity against *Alternaria solani*:**

Seeds of tomato (kassel Rock) was sown in 15 cm diameter pots filled with autoclaved loamy soil, emerged seedlings were thinned to 3 per pot 15 days after sowing. A complete randomized design was adopted for this experiment. Three pots were served as an experimental unit. Plants at ten weeks stage were subjected to the artificial inoculation using the spraying technique, the method of Van Vliet and Meijssing (1974) was applied in which the plants were sprayed with spore suspension ( $2.5 \times 10^7$  spors/ml distilled sterile water) using a hand-sprayer. The plants were treated with the tested compounds at the concentration  $IC_{75}$  two days before or after inoculation. The symptoms were recorded 4-8 days after inoculations.

## **RESULTS AND DISCUSSION**

#### **1-Toxicity of the tested compounds to different egg stages of *Tetranychus urticae*:**

The data in table (1) indicated that fenpyroximate was potent toxic compound against 1-day, 2-days and 3-days old eggs followed by Kz-oil ,fenarimol and black pepper extract, while sulphur compound was the least effective compound on all egg stages. Also the sensitivity of *T. urticae* eggs to tested compounds increased by increasing of their age, it was found that 3-days old eggs were more sensitive than the other, except of fenarimol compound it was found that 1-day old eggs was more sensitive than the others. These results are in agreement with that of El-Monairy *et al.* (1994) who indicated that the 3-days old eggs were more susceptible for vertimec than one and two days old eggs. Ibrahim *et al.* (1994) reported also that 1-day old eggs of two-spotted spider mite *T. urticae* was more susceptible for Andalin than 2 and 3-days old eggs.

**Table (1) : Effect (LC<sub>50</sub>) of the tested compounds to different egg stages of *Tetranychus urticae* (koch.)**

Compounds	Concentrations p.p.m	LC <sub>50</sub>	unhatchability % of eggs		
			One day old eggs	Two days old eggs	Three days old eggs
Control	Water		3	2.5	3.2
Fenpyroximate	5.6		82.4	84.9	89.2
Fenarimol	2385		61.3	59	54.7
Sulphur	18298.7		19.8	31.7	35.9
Kz-oil	986		54.9	59.5	63.8
Black pepper extract	3735.3		36.7	45.4	52.5

These results are in agreement with that of Veire *et al.* (1992) who reported that fenpyroximate gave excellent control of *T. urticae* on tomatoes and cucumber in glasshouse. Gamieh and Saadon (1998) reported that Neron, Vertimec, Ortus and Sanmite were satisfactory in controlling the red spider mite *T. cucurbitacearum* infestation on soybean where they caused 90.99, 87.29, 86.23 and 85.12% reduction in the population density respectively. Osman (1997) reported that mineral oils, albulium 80% Ec, Kz-95% Ec, shokrona super 95% Ec and shokrona 95% Ec were more toxic to adult female spider mites, *Eutetranychus orientalis* and *T. urticae*. Abbassy *et al.* (1993) reported that the tested botanical extract could be effective in control the mite of *T. urticae* infested on cucumber plants.

### **3-Evaluation of the tested compounds against the tested Fungi under Plastic tunnel conditions**

#### **3-a. The effect of seed treatments with the tested compounds on the growth of tomato, cucumber and pepper plants grown in soil infested with *Rhizoctonia solani* under plastic tunnel conditions.**

Data in table (3) showed that seeds of pepper treated with fenarimol (IC<sub>75</sub>) increased the percentage of seedling emergence in soil infested with the tested fungus compared to the other tested compounds followed by fenpyroximate and black pepper extract. Also seed of tomato treated with fenarimol, fenpyroximate and black pepper extract (IC<sub>75</sub>) gave moderate increase in the percentage of seedling emergence in soil infested with the same fungus. On the other hand all tested compounds were ineffective when seed of cucumber treated with them and grown in soil infested with *R. solani*. Also the percentage of post-emergence were 100% in case of tomato plant but there was no effect on seedlings in both of cucumber and pepper plants. These results are in agreement with that Madkour *et al.*, (1988) who reported that fenarimol reduced the incidence of damping-off due to *Rhizoctonia solani* in cowpea seedlings when used as a soil drench.



**Table (3): Effect of tested compounds (IC<sub>75</sub> values) as seed treatments (tomato, cucumber and pepper) on seed germination in soil infested with *Rhizoctonia solani* and *Fusarium moniliforme* after 15 and 30 days from planting.**

Plants Treatments IC <sub>75</sub>	Fungus	Tomato, Casel Rock		Cucumber, Beta-alpha	Pepper, Ccalifornia wonder		
		Damping – off					
		*pre-emergence %	** post-emergence %	* pre-emergence %	** post-emergence %	*pre-emergence %	** post-emergence %
Fenarimol	<i>R.solani</i>	73.33 c	100	90 a	-	50 d	-
	<i>F.moniliforme</i>	76.67 b	100	46.67 c	85	53.33 c	-
Fenpyroximate	<i>R.solani</i>	80 c	100	96.67 a	-	70 c	-
	<i>F.moniliforme</i>	86.67 ab	100	56.67 bc	92.9	63.33 bc	-
Sulphur	<i>R.solani</i>	86.67 abc	100	100 a	-	80 bc	-
	<i>F.moniliforme</i>	90 ab	100	66.67 b	94.4	75 bc	-
Black pepper Extract	<i>R.solani</i>	83.33 bc	100	93.33 a	-	73.33 c	-
	<i>F.moniliforme</i>	86.67 ab	100	53.33 bc	92.3	66.67 bc	-
Kz-oil	<i>R.solani</i>	96.67 ab	100	96.67 a	-	93.33 ab	-
	<i>F.moniliforme</i>	96.67 a	100	73.7 b	96	76.67 b	-
Inoculated control	<i>R.solani</i>	100 a	100	100 a	-	96.67 a	-
	<i>F.moniliforme</i>	100 a	100	100 a	-	100 a	-
Control untreated	<i>R.solani</i>	-	-	-	-	-	-
	<i>F.moniliforme</i>	-	-	-	-	-	-

$$* \text{ pre – emergence \%} = \frac{\text{No. of non-emerged seed}}{\text{Total no-of germinated seed in the control}} \times 100$$

$$** \text{ post – emergence \%} = \frac{\text{No. of died-seedling}}{\text{Total no-of germinated seed}} \times 100$$

Generally, results of the plastic tunnel experiments revealed that most of the tested compounds gave good protection of cucumber and pepper against seedling disease in soil infested with *Fusarium moniliforme* compared with soil infested with *Rhizoctonia solani*. On the other hand the percentages of seedling emergence on tomato were decreased in all cases.

**3-c. Effect of tested compounds (IC<sub>75</sub> values) as spraying on tomato plants two days before or after inoculation with *Alternaria solani* under plastic tunnel conditions:**

The results in table (4) showed that fenarimol was the most potent compound to protect the plants from the disease (*Alternaria solani*) followed by fenpyroximate, black pepper extract and sulphur, also the effect of tested compounds that was sprayed two days after inoculation with pathogene was better than that sprayed two days before inoculation, where the percentages of reduction were 41.33%, 30.5%, 28.3%, 20.03% and 17.4% for fenarimol, fenpyroximate, black pepper extract, sulphur and kz-oil respectively. Guddewar *et. al.*, (1992) indicated that mancozeb (2 kg/ha), captan and copper oxychloride (2.5 kg/ha) were effective against *A. solani* when sprayed on potato plants. Muthulakshmi and Seetharaman (1994) found that leaf extract 10% of vilvam and prospis were effective on *Alternaria tenuis* when sprayed on the pepper plants in the pot culture.

**Table (4): Effect of tested compounds (Ic<sub>75</sub>) as sprays on the tomato plants two days before or after inoculation with *Alternaria solani*.**

Treatments	* before inoculation		After inoculation **	
	Incidence	Reduction %	Incidence	Reduction %
Control	76.7	-	76.7	-
Fenarimol	50	34.81 %	45	41.33%
Fenpyroximate	61.7	19.6 %	53.33	30.5%
Sulphur	63.3	17.4%	61.3	20.03%
Black pepper extract	60	21.4%	55	28.3%
Kz-oil	68.3	10.9%	63.3	17.4%

Where: \* = two days before inoculation

\*\* = two days after inoculation concentration of pathogen was  $2.5 \times 10^7$  spors

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## ٢- محاولة لتقليل تلوث البيئة بالمبيدات

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يهدف هذا البحث إلى دراسة تأثير بعض المبيدات الأكاروسية والفطرية وهى الفينبيروكسميت والفيناريمول والكبريت وبعض بدائل المبيدات ومنها الزيت المعدنى منتج شركة كفر الزيات للمبيدات والمستخلص النباتى لبذرة الفلفل الأسود ضد الحيوان الكامل للحلم العنكبوتى ذو البقعتين تحت ظروف الصوب البلاستيكية. وكذلك ضد فطريات الريزوكتونيا سولاتى والفيوزاريوم مونيليفورم التى تسبب عفن الجذور لمحاصيل الخضر (الطماطم – الخيار – الفلفل) بالإضافة إلى فطر الألترناريا سولانى الذى يصيب نباتات الخضر بعد إصابتها بالأكاروس تحت ظروف الصوب البلاستيكية وذلك بهدف الحصول على مركبات لها خواص ابادية على الأكاروس و الفطريات فى نفس الوقت وأوضحت النتائج أن مركب الفينبيروكسميت كان أكثر المركبات سمية على بيض عمر يوم، عمر يومين وعمر ثلاث أيام الزيت المعدنى (ك-زد) كان له سمية متوسطة على البيض عمر يوم واحد، عمر يومين، عمر ثلاثة أيام وكذلك أظهر مركب الفيناريمول تأثير متوسط على أعمار البيض المختلفة. المستخلص النباتى ومركب الكبريت كانت لهما سمية منخفضة على الأعمار المختلفة للبيض. وأوضحت النتائج أن كل المركبات المختبرة أدت إلى خفض تعداد الأطوار المتحركة للعنكبوت الأحمر خلال ٣ أيام الأولى من المعاملة على نباتات الطماطم والفلفل والخيار مع اختلاف نسب الخفض فى التعداد حسب نوع النبات المعامل وذلك فى حالة كل من نصف الجرعة والجرعة الحقلية الموصى بها لكل مركب. و دلت النتائج أيضاً أن مركب الفينبيروكسميت والزيت المعدنى (ك-زد) أعطت تأثير متبقى عالى ضد الأطوار المتحركة للعنكبوت المختبر بينما انخفضت فاعلية مركبات الفيناريمول والكبريت والمستخلص النباتى للفلفل الأسود ضد هذه الأطوار بمرور الوقت بعد التطبيق بالجرعة ونصف الجرعة الموصى بها حقلياً. ودلت النتائج أن مركب الفيناريمول كان أكفاً المركبات المختبرة فى زيادة نسبة إنبات بذور الفلفل فى تربة معدية بفطر الريزوكتونيا سولانى أما بالنسبة لتأثير المركبات المختبرة على زيادة نسبة الإنبات فى بذور الطماطم والخيار فكان ضعيفاً بعد ١٥ يوماً من الزراعة وكذلك فإن نسبة البادرات التى حدث لها موت بعد الإنبات فى الطماطم قد وصلت إلى ١٠٠% بينما لم يحدث موت لبادرات الخيار والفلفل بعد الإنبات حتى ٣٠ يوم من الزراعة. دلت النتائج أيضاً أن مبيد الفيناريمول كان أقوى المركبات المختبرة فى مكافحة فطر الفيوزاريوم وزيادة نسبة الإنبات فى بذور الخيار والفلفل بعد ١٥ يوم من الزراعة يليه كل من الفينبيروكسميت والمستخلص النباتى لبذور الفلفل الأسود بينما كانت مركبات الكبريت والزيت المعدنى (ك – زد) أقل المركبات تأثير على زيادة إنبات بذور الفلفل والخيار ولم يحدث موت لبادرات الفلفل بعد ٣٠ يوم من الزراعة. وأوضحت النتائج أيضاً أن هذه المركبات ليس لها تأثير معنوى فى زيادة نسبة الإنبات لبذور الطماطم بعد ١٥ يوم من الزراعة وبلغت نسبة موت البادرات بعد ٣٠ يوم من الزراعة ١٠٠% مع كل المركبات. وأوضحت النتائج المتحصل عليها أن مركب الفيناريمول كان أقوى المركبات المختبرة فى حماية نباتات الطماطم من الإصابة بالمرض يلية الفينبيروكسميت والمستخلص النباتى والكبريت وكذلك رش المركبات بعد العدوى بالمسبب الممرض بيومين كانت أفضل فى خفض نسبة الإصابة بالمسبب الممرض الالترناري عنه فى حالة رش المركبات المختبرة قبل يومين من العدوى بالمسبب الممرض.

Table (2): Effectiveness of the tested compounds (half and recommended doses) in controlling the moving stages of *Tetranychus urticae* infesting tomato, pepper and cucumber plants under plastic tunnel conditions (% Reduction):

Toxicants	Plant	% Reduction									
		Half dose					Recommended dose				
		After one day	After 3 days	After 5 days	After 7 days	After 14 days	After one day	After 3 days	After 5 days	After 7 days	After 14 days
Fenpyrox.	Tomato	85	93.6	87.5	78.98	71.05	100	100	98.4	94.4	80.14
	Pepper	83.5	90.7	86.5	76.7	67.2	96.6	100	95.2	88.6	76.2
	Cucumber	81.3	89.1	85.7	72.3	65.2	97.3	100	94.1	87.6	78.7
Kz-oil	Tomato	86.7	95.01	88.9	80.7	69.8	95.3	98.6	88.18	80.7	76.24
	Pepper	85.4	93.2	84.5	76.3	65.9	94.4	97.6	86.5	78.7	73.3
	Cucumber	80.8	87.5	75	68.6	58.7	93.9	97.4	85.4	75.14	65.9
Fenarimol	Tomato	74.5	81.91	65.35	56.23	48.4	79.9	90.95	78.9	67.07	56.93
	Pepper	69.6	77.9	63.5	52.9	46.5	77.5	84.6	70.1	63.8	54.3
	Cucumber	67.5	74.4	58.4	49.5	39.2	76.6	83.1	71.7	60.4	49.8
Sulphur	Tomato	72.84	63.79	52.39	46.56	37.39	84.49	78.78	68.52	59.45	49.18
	Pepper	71.8	59.12	47.02	41.5	33.3	82.8	76.1	65.42	55.8	40.7
	Cucumber	69.7	58.2	44.3	38.9	28.3	79.6	72.3	61.01	52.35	38.9
Black pepper extract	Tomato	80.94	69.19	59.98	47.5	39.53	90.98	83.07	74.15	64.85	52.34
	Pepper	75.9	59.7	49.2	44.7	34.7	89.8	78.5	69.5	57.9	46.9
	Cucumber	73.2	56.6	48.3	40.9	29.8	86.1	74.5	62.5	48.32	38.7