

EFFECT OF SOME APPLICATOR TYPES ON METALAXYL RESIDUE IN CUCUMBER WITH SPECIAL REFERENCE TO ITS TOXICOLOGICAL EFFECTS

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ABSTRACT: *Application of Ridomil plus on cucumber plants using different amounts of its suspension, at the rate of 150g/100 liters water, by the three applicator types i.e. ultra low volume (U.L.V), low volume (L.V) and high volume (H.V) to control cucumber Downy mildew disease resulted in different level efficacy depending upon the applicator type. However the applicator ULV which applied the least amount of the spray suspension (100 liters/feddan) showed the highest disease control efficacy followed by the LV applicator while the HV applicator showed the least disease control efficacy, however it applied the highest suspension amount (400 liters/feddan)used.*

Metalaxyl residues in the fruits differed from one applicator to another. The least amount of metalaxyl residues were found in fruits resulted from plants sprayed using the ULV applicator followed by fruits resulted from plants sprayed using the LV applicator while the highest amount was found in fruits picked from plants sprayed using HV applicator. The fungicide residues, in all the fruits picked from all the different plots, at any time were in the range of 0.081 to 0.585 mg/kg. These amounts are less than the official safety maximum residues level (0.5mg/kg).

Studying the effect of feeding rats on cucumber contaminated with different concentrations of metalaxyl on enzymes activity and biochemical substances in blood serum revealed that, Cholinesterase activity was affected even at very lower metalaxyl concentration (0.025 mg/kg). The effect on transaminase enzymes (ALT and AST) was not clear, since no significant effect on ALT was noticed while the effect on AST reversed after 21 days. No effect on albumin was found; while cholesterol content and alkaline phosphatase increased gradually as metalaxyl residues in the fruits increased. Concentration of 0.100 mg/kg showed significant effect on the amount of total protein either after 2 or 21 days.

It was concluded that using ULV applicator reduces the amount of fungicides needed to perform good control of the disease to 25% of the recommended dose and reduced the fungicide residues in the fruits. However metalaxyl residues in the fruits was found to be extremely less than the official safety maximum residues level, the data in this study represent new parameters of the hazards of this fungicides on human health, which have to be taken in to consideration.

Key words: Metalaxyl, Residues, Toxicological effects, Downy mildew, Applicator types.

INTRODUCTION

In the recent years, both legislators and consumers all over the world showed great interest in clean environment and safety food products free from different contaminants. As a matter of fact pesticides have become universal contaminants that found in all segments of the environment and food chain. In previous studies Moustafa and Ismail (2003) and Moustafa *et al.* (2003) studied the effect of applicator types on the efficacy of fungicides and their role in environmental pollution. They found that, using the ULV applicator resulted in reduction of the fungicide amount needed to perform appropriate control of the late blight of potato from 400 liters/feddan (the recommended amount) to 50 liters/feddan and lowered the amount of the fungicide which run-off, this in turn save the environment and decrease the crop protection costs.

High concentration of some pesticides residues have been reported in different food items in different part of the world.(Panel 1977, Hadjidemetriou 1988, Hasegawa *et al*; 1992, Dejonckheere *et al*; 1996a and b, Bayoumi *et al*; 1997 and 2003). Many pesticides can accumulate in the human body via the food chain resulting in chronic toxicity, and estimation of such residue became of great interest, because of their potential (Wayland and Hayes, 1975). In this respect cucumber constitutes a special class of vegetables, not only because it is rich in different vitamins, but also because it is consumed fresh without any cooking; thus, contamination of cucumber with pesticides create a serious health means for man.

The objective of this study was directed to determine the role of the different pesticides applicator types in contamination of cucumber with metalaxyl fungicide residues. In addition to investigate some toxicological effect of such residues on certain enzyme activities (cholinesterase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase). Also to determine its effect on total protein, albumin and cholesterol.

MATERIALS AND METHODS

Cucumber var. Belta Alfa was planted on march 2002 under the normal field conditions in El – Ghrabeia governorate . The field was divided into three plots to serve as replicates, each of them was divided into four subplots; three of them were sprayed with Ridomil plus 50 % wp. which contains 35 % copper oxychloride and 15% Metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) -DL-alanine methyl ester (CAS)] at the concentration of 150 g in 100 liters/ feddan, using one of the three applicator

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types namely hand operated knapsack (high volume), motorized knapsack (low volume) and spinning disk (ultra low volume) in ten days intervals. The fourth subplot was treated with water only. Disease severity was estimated periodically using the same scale and procedure used by Mustafa *et al.* (2003) and mean severity of disease in each subplot was calculated at the end of the experiment.

1- sampling

Three fruits of cucumber (about 300 g) were collected randomly from each subplot at intervals of 2, 4, 6, 8 and 10 days after the fungicide application. The collected samples were weighted, labeled and preserved in clean new polyethylene bags, which were kept in a deep – freezer until time of the analysis.

2-Rearing of animals

White albino healthy male rats (a pure strain) of 3-4 months old with the mean average weight of 180-200 gm were purchased from Medicine Faculty, Tanta Univ. The rats were reared under the laboratory conditions ($25 \pm 5^{\circ}\text{C}$ and $65 \pm 5\%$ RH.) in metallic cages 45 x 35 x 20 cm, fed daily with balanced ratio consisted of bread, lettuce, cucumber and water soaked wheat.

3- Extraction of the fungicide residue:

The extraction procedure was performed according to Ramsteiner (1976), in which certain weight of each sample was blend with 5 folds of methyl alcohol in high-speed homogenizer. The methanol extract was diluted with water and the mixtures were filtered through filter papers. The extract filtrates were cleaned up by water-methanol/dichloromethane partitioning. The solvent was evaporated in rotary evaporator and the residues were dissolved in certain amount of methanol and used for the further studies.

4- Treatments of the rats:

The male rats were divided into groups (3 rats / group) equal in age and weight. Different of concentrations of metalaxyl (technical material) 0.0, to 1.00 ppm were prepared in methanol; these concentrations were used to create the standard curve.

The rats were fasted for 4 days; then they were fed on cucumber contaminated with the different metalaxyl concentration either the technical material or these which were reisolated from the samples (each two rats group received one treatment). After 2 and 21 days the rats were sacrificed. The blood from each group was collected in tubes containing heparin, centrifuged at 4500 rpm for 15mins. to separate the serum from the plasma. The serum samples were kept at -4°C .

5-Determination of metalaxyl residues in the samples:

In order to determine the metalaxyl residues in cucumber fruits a biotest based on estimation of the reaction of cholinesterase (ChE) activity in the

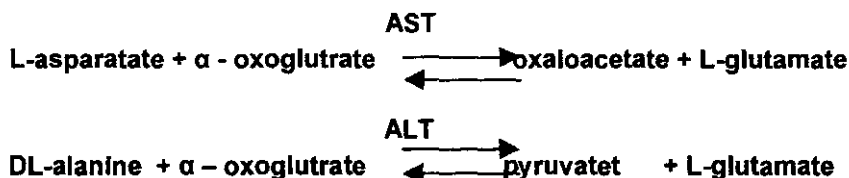
Blood of rats fed on the samples containing the residues using the method described by Ellman *et al.* (1961), which is based on the reaction between 5.5-dithiobis(nitrobenzoic acid) with acetylthiocholine iodide and the enzyme to form 2-nitro-5-mercaptobenzoate which can spectrophotometrically be determined at 405 nm. Comparing the data obtained from the samples with the data obtained from the standard curve gives the concentration of the metalaxyl residues in the different samples which can be used in calculation of the residues in the fruits as mg/kg fruits.

6- Effect of different concentrations of metalaxyl on the activity of some enzymes:

Effect of different concentrations of metalaxyl on the activity of Transaminases (AST and ALT), alkaline phosphatase enzymes; in addition to concentration of albumin, cholesterol, and total protein in the blood.

a- Transaminases determination:

Transaminases (AST and ALT) activity was determined by Diamond-diagnostics kits. based on the method of Reitman and Frankel. (1957) in which :



The pyruvate or the oxaloacetate formed is measured spectrophotometrically as its derivative, 2,4-dinitrophenylhydrazone at 550 nm.

b- Alkaline phosphatase determination:

Alkaline phosphatase activity was determined using the Kind and King (1954) method in which the reagent p-nitrophenyl phosphate is split by the enzyme into phosphate and the yellow colored indicator p-nitrophenol measured at 500 nm.

c-Albumin determination:

Albumin was determined according to Doumas and Watstan. (1971), which is based on the production of a green colored complex as a result of the reaction of the albumin with bromocresol green. The color density is proportional to the amount of albumin in the samples and can be estimated at 623-630 nm.

d- Determination of total protein:

The total protein amount in the blood of test rats was determined using Diamond diagnostic kit according to the method of Gornall *et al.* (1949). In this method alkaline cupric sulphate reacts with the protein to produce a violet

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color, which its intensity can be measured at 550 nm.

e- Determination of cholesterol :

Cholesterol was determined using Diamond diagnostic kit according to Watson (1960), which is based on the measurement of the color, formed as a result of the reaction of the cholesterol with acetic acid and sulfuric acid, at 578 nm.

Results and Discussion

Application of Ridomil plus on cucumber plants using different amounts of its suspension, at the rate of 150g/100 liters water, by the three applicator types to control cucumber downy mildew disease resulted in different control efficacy depending upon the applicator type. However the ULV applicator applied the least amount of the spray suspension (100 liters/feddan) showed the highest disease control efficacy (82.17%) followed by the LV applicator (200 liter/ feddan), resulted in (78.02 %) and the HV applicator (400 liter / feddan) which showed the least disease control efficacy (75.8%), however it applied (400 liter/feddan). No significant difference was found between the disease severity in plots sprayed using ULV and LV applicators; but significant difference was found between the disease severities in plots sprayed using ULV and HV applicators (Table 1).

Table 1: Cucumber downy mildew disease severity and fungicide efficacy as a result of using different applicator types and different amount of spray suspension.

Applicator type	ULV	LV	HV
Amount of spray suspension	100 liter/feddan	200 liter/feddan	400 liter/feddan
Disease severity	9.33	11.5	12.66
Fungicide efficacy	82.17	78.02	75.80

LSD at 0.05 for disease severity = 2.19

Determination of metalaxyl residues in cucumber fruits picked from treated plants after different periods of fungicide application

It was found that the metalaxyl residues in the fruits differed from one applicator to another (table2). it was obvious that the least mean amount of metalaxyl in fruits resulted from plants sprayed using the ULV applicator was (0.081 mg/kg), while the mean amount of metalaxyl in fruits resulted from plants sprayed using the LV applicator was 0.152 mg/kg and 0.271 mg/kg in fruits picked from plants sprayed using HV applicator. Such difference can be noticed after each picking interval.

It was found also, that the fungicide residues, in all the fruits picked from all the different plots, decreased gradually as the period between the fungicide application and the fruits picking prolonged.

Table 2: Amount of metalaxyl residues in cucumber fruits picked periodically from treated plants using three applicator types.

Period between the application of the fungicide and fruits picking.	Metalaxyl residues amount in mg/kg fruits		
	ULV	LV	HV
2 days	0.183	0.315	0.585
4 days	0.087	0.166	0.308
6 days	0.063	0.126	0.227
8 days	0.042	0.085	0.145
10 days	0.034	0.072	0.094
Mean	0.081	0.152	0.271

L. S. D. at 5% Time = 0.017 Applicator = 0.012 Time X applicator = 0.027

Effect of metalaxyl residues of some enzymes activities and biochemical substances in blood serum:

The effect of metalaxyl residues on some enzymes activities and biochemical substances in blood serum of fed and non-fed rats on contaminated cucumber fruits with metalaxyl fungicides after 2 and 21 days of feeding are shown in (table 3).

a- Total protein:

Feeding the rats on 0.025 or 0.050 mg/kg metalaxyl in cucumber fruits showed no significant effect on amount of total protein neither after 2 nor 21 days of feeding; however feeding the rates on 0.1 mg/kg resulted in significant increase in total protein amount either after 2 or 21 days of feeding.

b- Albumin:

According to albumin content in blood serum it was found that 0.025 mg/kg had no effect on it neither after 2 nor 21 days after feeding, in contrast to 0.050 and 0.100 mg/kg, resulted in significant increasing in albumin contents after 2 days of feeding. This increase was correlated positively with the increasing in the metalaxyl residues. After 21 days of feeding all the metalaxyl concentration showed no significant effect on albumin content in blood serum.

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c- Cholesterol:

Drastically increasing in cholesterol amounts was recorded in blood serum of the rats fed on contaminated cucumber fruits either after 2 or 21 days of feeding was noticed. The cholesterol contents increased from 160.6 and 189.5 mg, in blood serum of the rats received clean cucumber fruits when the determination was applied after 2 and 21 days of feeding respectively, to 306.3, 326.98 then 344.4 mg after 2 days and 255.0, 270.0 then 1032.8 mg after 21 days respectively depending upon the concentration of metalaxyl in the fruits.

d- Alanine transaminase (ALT) activity:

Concerning the effect of different concentrations of metalaxyl on the activity of alanine transaminase enzyme the tested concentrations of metalaxyl showed no significant difference neither after 2 nor 21 days of feeding.

e- Asparagines trans minase (AST) activity:

Regarding to AST after two days of feeding the low concentration of metalaxyl (0.025 and 0.050 mg/kg) showed no effect on its activity. Higher concentration (0.100 mg/kg) resulted in significant increase of its activity. This effect was reversed after 21 days of feeding since the enzyme activity decreased gradually as the concentration of metalaxyl increased.

Table 3 : Effect of metalaxyl residues on some enzymes activities in blood serum of non-fed and fed rats on cucumber fruits contaminated with metalaxyl fungicides after 2 and 21 days of feeding.

Conc. Mg/kg	After 2 days					After 21 days				
	0.000	0.025	0.050	0.100	LSD at 5%	0.000	0.025	0.050	0.100	LSD at 5%
Total protein	7.88	7.98	7.80	10.30	1.79	7.797	9.00	8.90	11.0	1.44
Albumin	3.85	3.86	6.53	7.63	0.13	3.35	3.50	3.38	3.32	NS
Cholesterol	160.6	306.30	326.98	344.40	2.13	189.5	255.0	270.0	1032.8	2.72
ALT	0.064	0.097	0.064	0.081	NS	0.078	0.063	0.264	0.229	NS
AST	0.134	3.130	0.135	0.217	0.073	0.105	0.079	0.076	0.032	0.049
Alkaline phosphatase.	24.72	28.40	30.70	32.30	0.19	25.35	25.30	26.80	26.70	1.20
Choline esterase	1.328	1.467	1.643	1.967	0.046	0.576	1.663	1.632	1.694	0.027

f- Alkaline phosphatase activity:

Increasing of metalaxyl concentration in the contaminated fruits resulted in increased activity of the enzyme. After 2 days of feeding the enzyme activity increased from 24.72 mg in blood of rats fed on clean cucumber to 28.40, 30.70 and 32.30 in blood of rats fed on 0.025, 0.050 and 0.100 mg/kg respectively. Similar data were obtained after 21 days of feeding; however the low metalaxyl concentration (0.025 mg/kg) showed no significant changing in enzyme activity.

g- Cholinesterase activity:

Gradually increasing in concentration of metalaxyl in the contaminated fruits resulted in gradual increase in choline esterase activity either after 2 or 21 days of feeding.

Application of Ridomil plus (Which contain 15% metalaxyl) on cucumber plants using different amounts of its suspension, at the rate of 150g/100 liters water, by the three applicator types to control cucumber Downy mildew disease resulted in different control efficacy depending upon the applicator type. However the applicator ULV which applied the least amount of the spray suspension it showed the highest disease control efficacy followed by the LV applicator while the HV applicator showed the least disease control efficacy although applying the highest suspension amount. This data is in harmony with that reported by Moustafa and Ismail (2003) and Moustafa et al. (2003). Metalaxyl residues in the fruits differed from one applicator to another. The least amount of metalaxyl residues were found in fruits resulted from plants sprayed using the ULV applicator followed by fruits resulted from plants sprayed using the LV applicator and the highest amount was found in fruits picked from plants sprayed using HV applicator. This difference in fungicide residues may be due to that the amount of fungicide needed to perform good control of the disease using ULV applicator is half of that needed using LV applicator and equal to 25% of the amount needed for using HV applicator.

In this study, methanol and water were used for the extraction of the fungicide residues; since copper oxychloride is insoluble in water, or in organic solvent (Worthing, 1979), therefore all the residues were found to be metalaxyl or its metabolites. The fungicide residues, in all the fruits picked from all the different plots, at any time were in the range of 0.081 to 0.585 mg/kg. Those results are in harmony with that found by Ramsteiner (1976). Those residues were less than the official safety maximum residues level (5mg/kg.) as reported by Anonymous (1992). Cholinesterase activity was affected even at very lower metalaxyl concentration (0.025 mg/kg). This sensitivity of choline esterase to very low metalaxyl concentration was of a great value to be used in the biological test and proved that the data obtained using this technique are very accurate.

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Concentration of 0.100 mg/kg showed significant effect on the amount of total protein either after 2 or 21 days. Ganong (1995) stated that some of the protein function in the transport of thyroid, adrenocortical, gonadal, and other hormones. Binding keeps these hormones from being rapidly filtered through the means on which the tissues can draw. In addition, albumin serves as carrier for metal, ions, fatty acids, amino acids, bilirubin, enzymes, and drugs; thus any effect on the plasma protein will be correlated with disturbances in these functions. However, no effect of the tested concentration of metalaxyl on albumin was found.

Cholesterol content and alkaline phosphatase increased gradually as metalaxyl residues in the fruits increased. Cholesterol and alkaline phosphatase are excreted in the bile. In patients with jaundice due to intra- or extrahepatic obstruction of the bile duct, the blood levels of these two substances usually rise (Ganong, 1995). The effect on transaminase enzymes (ALT and AST) was not clear, since no significant effect on ALT was noticed while the effect on AST reversed after 21 days.

It is to be concluded that using ULV applicator reduces the amount of fungicides needed to perform good control of the disease to 25% of the recommended dose and reduced the fungicide residues in the fruits. However metalaxyl residues in the fruits was found to be extremely less than the official safety maximum residues level, our data represent new parameters of the hazards of this fungicide on human health, which have to be taken into consideration.

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تأثير نوع آلة رش المبيدات على الأثر الباقي للميتالاكسيل في ثمار الخيار مع التركيز على التأثيرات السامة لها

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المخلص العربي

برش مبيد الريدوميل بلاس على نباتات الخيار بواسطة ثلاث آلات رش مختلفة باستخدام ثلاث كميات من محلول الرش بتركيز ١٥٠ جم/١٠٠ لتر ماء لمكافحة مرض البياض الزغبي على الخيار، وجدت اختلافات في كفاءة محلول الرش في مقاومة المرض تبعا لنوع آلة الرش المستخدمة.

بالرغم من أن آلة الرش ذات القرص الدوار (ULV) استهلكت أقل كمية من محلول المبيد (١٠٠ لتر/ فدان) فقد أظهرت أعلى كفاءة في مكافحة المرض، تبعها موتور الظهر (LV) ثم رشاشة الظهر اليدوية (HV) والتي أظهرت أقل كفاءة رغم استهلاكها لأكثر كمية مبيد (٤٠٠ لتر/ فدان).

اختلفت متبقيات مبيد الميتالاكسيل في ثمار الخيار تبعا لاختلاف آلة الرش المستخدمة. ووجدت أقل كميات من متبقيات مبيد الميتالاكسيل في ثمار الخيار التي جمعت من نباتات تم رشها باستخدام آلة الرش ذات القرص الدوار (ULV) تلاها ثمار الخيار التي جمعت من نباتات تم رشها باستخدام موتور الظهر (LV) وكانت أكبر كمية من المتبقيات في ثمار الخيار التي جمعت من نباتات تم رشها باستخدام رشاشة الظهر اليدوية (HV). إلا أنه في جميع الحالات كانت كمية المتبقيات في الثمار أقل بكثير من حد الأمان المسموح به.

بدراسة تأثير تغذية فئران التجارب على ثمار خيار ملوثة بتركيزات مختلفة من ميتالاكسيل على نشاط بعض أنزيمات الدم وكذلك بعض المركبات الحيوية بة بعد يومان و ٢١ يوم من التغذية وجد ازدياد ملحوظ في نشاط أنزيم الكولين أستريز متناسب طرديا مع زيادة جرعة المبيد. بالنسبة لإنزيمات الترانس أمينيز لم يكن للتأثير واضحا تماما حيث لم يكن هناك

الاختلاف معنوي في نشاط الإنزيم (ALT) بينما زاد نشاط الإنزيم (AST) بعد يومين بزيادة تركيز الجرعة ولكن بعد ٢١ يوما انعكس هذا التأثير حيث بدأ انخفاض نشاط الإنزيم بزيادة الجرعة.

لم يلاحظ أي تغيير على كمية الألبومين بينما ازدادت كميات الكولستيرول والبروتين الكلي في الدم . كما أن زيادة جرعة المبيد أدت كذلك الى زيادة نشاط إنزيم الألكالين فوسفاتيز. من النتائج السابقة يمكن استخلاص أن استخدام آلة الرش ذات القرص الدوار (ULV) تقلل كمية المبيد اللازمة لرش الفدان الى ٢٥% من الكمية الموصى بها وتعطي أعلى كفاءة في مكافحة المرض ، كما تؤدي الى تقليل الأثر الباقي للمبيدات في الثمار. رغم أن كميات الأثر الباقي للمبيدات في الثمار كان أقل بكثير من الحد الآمن المسموح به إلا أن تأثير المبيدات على أنزيمات ومكونات الدم يجب أن يوضع في الاعتبار عند دراسة سمية أي مركب على الإنسان.