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DETERMINATION OF PROFENOFOS AND METALAXYL RESIDUES IN GREEN ONION

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ABSTRACT

Profenofos (Selecron 72 % E.C.), and metalaxyl (Ridomil Gold Plus (42.5 % W.P.) were sprayed on field – grown onion plants at the recommended rates of 0.54 and 0.17 Kg. a.i./ Fed., respectively. Commercial products from (Ridomil Gold Plus) contained mixtures of metalaxyl as the active ingredient with low rates of residual protective fungicides, such as copper. Samples were collected at hour to 18 days after application and analyzed to determine the content and dissipation rate of aforementioned pesticides. Profenofos and metalaxyl residues were quantified by using gas chromatography, while copper by atomic absorption. The results showed that the amount of residues recorded during the experimental period, varied for each insecticide to another. The initial deposits of profenofos; metalaxyl, and copper on and in green onion were 3.50; 4.04, and 95.80 mg/kg, respectively. These amounts decreased gradually till reached 0.08; 0.53 and 1.18 mg/kg. after 14 days of spraying. The dissipation of profenofos was faster than metalaxyl, and copper. The corresponding half-life values were 0.91; 3.92, and 7.70 days, respectively.

According to the maximum residues limits (MRLs) of profenofos (0.5 mg/kg.), metalaxyl (2.0 mg/kg.) and copper (30 mg/kg), were reached after 12, 12 and 5 days, respectively.

Keywords: Residues; Profenofos; Metalaxyl; copper; Onion

INTRODUCTION

Onion *(Allium cepa L.)* is one of the most important field crop, which grow on a large scale in Egypt, particularly in Upper Egypt, and plays an important role in the Egyptian diet either as green or dry, also as an export crop. Several insects and numerous diseases attack onions, and control of these currently relies on the frequent use of pesticides. Thrips, *Thrips tabaci* (lind) is one of the main economic sucking pest which attacks onion. Onion Thrips are tiny, thin insects up to 1/10 long which feed by taking sap from the leaves. Thrips are black when fully grown but yellow during their nymphal stages. Thrips feeding causes a silvery-white discoloration of the leaves (Broocks and Halstead, 1983; and Ananthakrishnan, 1993). On other hand, the major disease of onion in Egypt is downy mildew *(Peronospora destructor)*.It is an especially devastating disease because it spreads rapidly and is not readily controlled. Downy mildew first infects older leaves, occurring as pale, elongated patches that may have a grayish-violet fuzzy growth appear early in the morning during moist periods. Infection can occur systemically, with stored bulbs becoming soft, wrinkled, watery and amber in color. (Howard *et al*., 1994; and Schwartz and Mohan, 1995).

Profenofos is an organophosphorous insecticide widely used to control various insect pests, particularly Lepidoptera, and mites (Anonymous, 2000 – 2001) on field and vegetable crops. While metalaxyl is a fungicide used to control of many fungal diseases in field crops, and had high activity

against fungal pathogens of the order Peronosporales, which cause early and late blights, downy mildews, damping-off and root, stem and fruit rots of many plants. The compound is taken up by the roots, leaves, green stems and shoots and transported acropetally within the plant (Urech *et al*., 1977).Commercial products first introduced as foliar sprays contained only metalaxyl as the active ingredient or were mixtures of metalaxyl with low rates of residual protective fungicides, such as copper or folpet.

In Egypt, both profenofos and metalaxyl are used in controlling Onion Thrips, and fungal diseases caused by, downy mildew *(Peronospora destructor),* respectively.(Anonymous, 2001). Using pesticides could contaminate onion plants and caused hazard to consumers. Analysis of pesticide residues after application should be followed to determine the safety period between application and harvesting to be sure that residues are below tolerance levels and the edible parts are safe for human consumption.

There are numerous studies in the literature that have examined profenofos behaviour in fresh and processed edible crops such as potatoes (El-Tantawy *et al.,* 1992; and Soliman, 2001), tomatoes (Ramadan, 1991; El-Nabarawy *et al.,* 1992 and Ismail *et al.,* 1993; Soliman *et al.* 2005), moloukhia (Sallam and El–Nabarawy, 2001.; and Shokr, 2006).; and pepper and eggplant (Radwan *et al.,* 2005).

The present investigation aimed to throw light on the persistence of the profenofos insecticide and metalaxyl fungicide residues on and in green onion plants; to give an idea about the pre-harvest interval (PHI) that should pass following application and before marketing in order to minimize health hazards.

MATERIALS AND METHODS

1- Pesticides used:

a- Insecticide, Profenofos: O – (4 – bromo – 2 – chlorophenyl) – O – ethyl – S – propyl phosphorothioate. Selecron formulation (72 % E.C.), was used at the rate of 375 ml / 100 liter water.

b-Fungicide, metalaxyl: methyl N-(2,6-dimethylphenyl)-N- (methoxyacetyl)-DL-alaninate. Ridomil Gold Plus + Copper oxychloride formulation (42.5 % W.P.) was used at the rate of 200 g / 100 liter water.

2- Field experiment and sampling

A field experiment was carried out in the Agricultural Experimental Farm of the Faculty of Agriculture, Sohag University. Onion plants (*Allium cepa* L.) Var. Giza 6 seedling was cultivated during the winter cultivation, season of 2007, under normal field and agriculture practices. The experimental area was divided into plots of 42 m^2 (1 / 100 Fed.). The experimental area design was a complete randomized blocks with three replicates for each pesticide. Three plots were left untreated to serve as control. The pesticide applications were carried out on February, 25, 2007 (plant age of 55 days) at the rates mentioned above (recommended dose). Spraying was carried out using a Knapsack- sprayer (Cp-3) provided with one nozzle delivering 200 liters of water per feddan, which has proved to be sufficient to give good coverage on the treated plants.

Representative samples (500 g were sampled per replicate) of onion plants were collected from the treated plots after one hour of application (initial deposits) and 1, 2, 5, 8, 12, 14, and 18 days after spraying. Clean polyethylene bags were used for preserving the collected samples. The samples were stored at – 20 °C in a deep freezer until analysis.

3- Residue analysis:

A- Extraction and clean – up

A.1. Profenofos: The extraction procedure used is the general method suitable for organophosphorus compounds (Anonymous, 1988).The frozen samples were left to reach room temperature. Fifty grams of samples were placed in the blender cup with 50 g. anhydrous sodium sulphate and 150 ml. ethyl acetate, and then blended for five minutes. The liquids were decanted through a funnel with a plug of cotton into a graduated cylinder, and then evaporated to near dryness using a rotary evaporator at 40 °C. The residues were dissolved in 5 ml. of n- hexane and clean- up was done according to (Mills, *et al*. 1972). Through chromatography column 10 g. of activated florisil 60 – 100 mesh 3.5 % moisture covered with 2 g. of anhydrous sodium sulphate. The elution solvent system was dichloromethane: n – hexane: acetonitrile at the ratio of 50: 48.5: 1.5 (V /V /V). The eluant was evaporated just to dryness as previously described and the residues were ready for chromatographic determination after redissolved in an appropriate volume of ethyl acetate.

A Pye Unicam 4500 gas chromatograph equipped with a flame photometric detector (FPD) operated in the phosphorus mode (526 nm filter) was used for determination of profenofos. The column (1.5 m × 4 mm i.d. pyrex) was packed with 4 % SE – 30 + 6 % OV – 120 on gas chromosorb Q (80 – 100 mesh). The following conditions: Injector temperature 230 °C, Column temperature 240 °C, Detector temperature 240 °C and gas flow was 30, 30, and 30 ml /min. for nitrogen, hydrogen, and air, respectively. The limit of detection of standard profenofos under these conditions was 0.018 mg/kg. Identification of insecticide residue was accomplished by retention time (Rt_{50}) and compared with known standard at the same conditions. The quantities were calculated on peak height basis. Using these conditions, the retention time of profenofos was 7.89 minutes.

A.2. Fungicide (metalaxyl))

Commercial products from (Ridomil Gold Plus) contained mixtures of metalaxyl as the active ingredient with low rates of residual protective fungicides, such as copper. Metalaxyl residues can be determined in onion by applying the method of Ambrus *et al* (1981). The samples (50 g) are extracted with 100 ml acetone in a high speed homogenizer. The acetone extract is diluted with water containing 2% Na_2SO_4 and partitioned into dichloromethane. The extracts have to be cleaned up on neutral alumina column (V. activity grade).The elution solvent by water-methanol /dichloromethane partitioning. The appropriate fraction is then examined for metalaxyl by gas chromatography equipped with FPD. The limit of determination is 0.01-0.02 mg/kg.

Results were corrected according to the rate of recovery which was determined in fortified untreated samples at levels ranged from 0.1 to 1 ppm. Following the techniques previously mentioned, the rate recovery for profenofos, and metalaxyl was 85.3; and 81.9 %, respectively.

A.2.1. Copper determination: Samples were analyzed according to method of (Anonymous, 1990) for determination of copper in onion plants. About (2.0 g samples were digested with concentrated nitric acid: concentrated per chloric acid at a ratio of 2: 1 and heated till colorless then diluted to known volume deionized water. Then determination carried out using Thermo Jarrell Ash Atomic Absorption (model: AA- Scan 1) using a specific hollow cathode for copper.

RESULTS AND DISCUSSION

1- Recovery percentage of Profenofos and metalaxyl:

Recoveries of profenofos and metalaxyl from fortified samples (50 g) are shows in Table (1). Recoveries ranged from 86.3 to 84.5 % for profenofos, whereas metalaxyl was 83.1 to 80.4%. These results agree with those obtained by Soliman, *et al*. (2005); Sallam and El- Nabarawy (2001); and Shokr, (2006) used the same method in determination of profenofos in tomato, moloukhia, and moloukhia. They found that recovery percentage of profenofos were 90.6; 83.4; and 83.5 %, respectively

Table (1):Recovery percentages of profenofos and metalaxyl from green onion plants

Added (mg/kg)	Profenofos	metalaxyl
1.0	85.2	83.1
0.5	86.3	80.4
0.1	84.5	82.2
Average	85.3	81.9

2-Residues of profenofos, metalaxyl and copper on and in green onion plants:

Results in Tables (2 & 3) showed that the concentration of the initial deposits of profenofos, metalaxyl and copper were 3.50, 4.03, and 95.80 mg /kg., respectively. The amount of residues decreased to 2.02, 3.60, and 79.27 mg /kg., respectively within the first 24 hours after spraying. These figures decreased gradually until reached 0.04, 0.53 and 1.18 mg /kg after two weeks of application. The amount of residues recorded during the experimental period varied for each pesticide to another. These levels depended on the initial deposits, the rate exposure of the fruits to the environmental factors and the reaction between the treated surface and the chemical applied. Stevens, *et al*. (1988) demonstrated that uptake of pesticides on plant surface is affected by the chemical structure, formulation as well as the rate of used insecticide, the nature of recipient surface, the used spraying equipment and the climatic conditions; especially the ambient temperature during pesticide application.

The results also indicated that loss of residues increased as time lapsed from the onset of spraying until the end of the experimental period. After one

day from the onset of spraying loss percentages were 42.29, 10.67, and 17.25 %, with profenofos, metalaxyl, and copper, respectively. After the eighth day more than 70 % of profenofos, and copper, and 35 % of metalaxyl of the initial deposits were disappeared. At the end of experiment loss percentages reached more than 95 % of the initial deposits.

Table (2): Residues (mg /kg) of profenofos on and in green onion plants

Days after spraying	Residues	Reduction %
O (1 h)*	3.50	00.00
1	2.02	42.29
2	1.84	47.43
5	1.33	62.00
8	0.91	74.00
12	0.13	96.29
14	0.08	97.71
18	0.04	98.86

* **Initial deposits of the insecticide.**

Table (3): Residues (mg /kg) of metalaxyl and copper on and in green onion plants

Days after spraying	Metalaxyl		Copper	
	Residues	%Dissipation	Residues	%Dissipation
O (1 h)*	4.03	00.00	95.80	00.00
1	3.60	10.67	79.27	17.25
2	3.20	20.60	32.47	66.11
5	2.61	35.24	17.29	81.95
8	2.50	37.97	14.89	84.46
12	1.12	72.21	6.37	93.35
14	0.53	86.85	1.18	98.77
18	0.08	98.01	0.40	99.58

***Samples were taken one hour after application = Initial deposits of the insecticide.**

The same phenomenon was observed by Shiboob (1995), he found that loss percentages of profenofos residues in tomato and cucumber fruits ranged from 99.1 to 99.3 % after 12 days from spraying. El-Disouky (1995) mentioned that, the compound profenofos at the recommended rate was completely dissipated after 10 days of application. Radwan, *et al*. (2005) found that initial deposits of profenofos in / on hot pepper, sweet pepper; and eggplant were 11.62, 10.67, and 4.50 ppm, respectively. A rapid degradation of profenofos residues was noticed, after one day of application was values of 46.04, 46.29, and 26.67 5 reductions, respectively. The progression of time after application resulted in more dissipation of residues. At the end of experiment (two weeks), hot pepper fruits contained negligible residues (0.021 ppm), whereas no residues were detected in sweet pepper and eggplant fruits. Soliman, *et al.* (2005) and Shokr (2006) found that, the first three days, however, the most critical period at which most amount of residues from profenofos were dissipated.

The values of half-life were obtained from calculations of Moye*, et al.* (1987). The initial disappearance of profenofos, metalaxyl and copper appeared to follow first order kinetics with different rates of reaction of 0.759,

0.177 and 0.090 day -1, respectively. The corresponding half – lives (t ½) were 0.91, 3.92 and 7.70 days (Table 4).The dissipation of profenofos was faster than metalaxyl may be due to vapor pressure (1.24 ´ 10^{-1} mPa (25 °C) for profenofos and (0.75 mPa (25 °C) for metalaxyl. These results are in agreement with those obtained by Sallam and El –Nabarawy (2001) who found that the half – life values of profenofos on moloukhia leaves was 52.08 hours, while with Shokr (2006) was 38 hours. Radwan, *et al.,* (2005) found that the half – life values of profenofos residues was 1.84, 1.74, and 1.96 days on hot pepper, sweet pepper and eggplant, respectively.

According to the maximum residue limits (MRLs) of profenofos (0.5 ppm) , metalaxyl (2 ppm) and copper (30 ppm) in onion and similar vegetables, presented in Anomyomus,(2003).Data tabulated in Table (4) show that the periods (days) after which onion plants sprayed with profenofos and metalaxyl can picked up for human consumption after 12days, while copper after five days. The results of this study are quite comparable with those reported by El-Sayed, *et al*. (1977); Soliman *et al.* (2005) and Radwan *et al*.(2005). El-Sayed, *et al*. (1977) reported that waiting periods between application of organophosphorus insecticides and harvesting for marketing were defined for the consumer safety and avoiding health hazards, ranged between one and twelve days according to kinds of pesticides and vegetables**.** Soliman *et al.* (2005) stated that the safety period after which sprayed tomato fruits with chlorpyrifos-methyl and profenofos were 5 and 3 days, respectively. Radwan *et al*.(2005) detected that only 10 and 14 days were enough to reduce the residues of profenofos on sweet pepper and hot pepper below the permissible limits(0.5 ppm) on peppers.

Table (4): legal limits of tested pesticides and calculated half-life period on and in onion plants,

Pesticides	Rate of decomposition (K)*	Half-life time (t ½) ** (days)	(MRLs)***	(PHI)****
Profenofos	0.759	0.91	0.5	12
Metalaxyl	0.177	3.92	2.0	12
Copper	0.090	7.70	30.0	5

***k = 1/tx . Lin a/bx where k = rate of decomposition, a = initial residue, tx = time in days, and bx = residue in tim (x)**

**** t1/2 = lin 2 / k = 0.693 / k**

***** MRLs = Maximum Residue Limits According to Codex Alimentarius Commission, 2003**

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تقدير متبقيات البروفينوفوس والميتالكسيل في البصل الأخضر

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رشت نباتات البصل في الحقل بالمبيد الحشري البروفينوفوس (السيليكرون ٧٢% مركز قابل للاستحلاب والمبيد الفطري ميتالكسيل (رودوميل جولد بلاس ٤٢,٥ % مسحوق قابل للبلل) بالمعدلات الموصي بهما وهي (٠,٥٤ ، ٠,١٧ كجم مادة فعالة /فدان) علي التوالي. المنتج التجاري من المبيد الفطري رودوميل جولد بلاس يتكون من مخلوط من (الميتالكسيل والنحاس). قدرت متبقيات كل من البروفينوفوس والميتالكسيل عقب الرش مباشرة وعلي فترات مختلفة بعد الرش بواسطة جهاز التحليل الكروماتوجرافي الغاز بينما تم تقدير النحاس بواسطة جهاز الامتصاص الذري، ودرس معدل الاختفاء وتحديد فترات الأمان للمبيدات السابقة الذكر. وأوضحت النتائج مايلي:

تختلف كميات متبقيات المبيدات المتحصل عليها أثناء التجربة باختلاف المبيد المرشوش، حيث كانت كميات المتبقيات الأولية لمبيدي البروفينوفوس ،الميتالكسيل والنحاس هي ٣,٥٠ ، ٤,٠٤، ٩٥,٨٠ ملجم / كجم علي التوالي، وانخفضت هذه المتبقيات تدريجياً حتى وصلت إلي ٠,٠٨، ٠,٥٣، ١,١٨ ملجم/كجم بعد ١٤ يوم من الرش،وكان معدل اختفاء البروفينوفوس أسرع من الميتالكسيل والنحاس حيث كانت فترة نصف العمر لهما هي ٠,٩١، ٣,٩٢، ٧,٧٠ يوم علي التوالي.

من خلال استعراض نتائج متبقيات المبيدات خلال فترة التجربة بالكامل ومقارنة مستوي هذه المتبقيات بالحدود المسموح بها دولياً من قبل لجنة دستور الأغذية والزراعة (الكودكس) (البروفينوفوس ٠,٥، الميتالكسيل ٢,٠، النحاس ٣٠,٠ ملليجرام / كيلو جرام) وجد أن فترات الأمان للبروفينوفوس والميتالكسيل هي ١٤ يوم بعد الرش، بينما كانت للنحاس خمسة أيام. ومن وجهة النظر العملية فإنه يجب أن يسمح بشيء من التحفظ قبل استهلاك البصل الأخضر بفترة لا تقل عن الفترات المذكورة.