J. Plant Prot. and Path., Mansoura Univ., Vol. 4 (12): 1025 - 1033, 2013

RESIDUES AND PRE HARVEST INTERVAL OF ABAMECTIN, DINICONAZOLE, METHOMYL AND PHENTHOATE IN SWEET PEPPER UNDER GREENHOUSE CONDITIONS.

EL-Kabbany, Samia M.[1]; A. M. EL-Marsafy[2]; G. G. Mohamed[3] and M. M. Saadieh[4]

(1)Faculty of Agriculture, Cairo University.

(2)Director of Central laboratory of residue analysis of pesticides and Heavy metals in Food, Ministry of Agriculture, Dokki, Egypt.

(3)Inorganic and Analytical Chemistry, Dept. of Chemistry, Faculty of Science, Cairo University.

(4) Dept. of chemistry,faculty of sci. ,cairo univ..

ABSTRACT

The residual behavior of abamectin (Vertimec 1.8 %EC), diniconazole (Sumi-eight 5% EC) methomyl (Lannate 90 %SP) and phenthoate (**Cidial-L 50 % EC)** in sweet bell pepper fruits under the greenhouse condition of Egypt was studied. The tested pesticides were sprayed at recommended dose of 50 ml in 100 liter water for abamectin and diniconazole per 200 m^2, 100 ml and 50 ml in 100 liter water per 660 $m^2$for methomyl and phenthoate respectively on pepper fruits (foliar application). The treated sweet bell pepper fruits were randomly sampled in triplicates after one hour (initial), five hour, 1, 3, 5, 7, 10, 15 and 21 days period after pesticides application including control samples one hour before application. For abamectin 1 h, 3 hour, 5 hour, then 1, 3, 6, 9, 13 and 20 days. Samples were extracted using QuEChERS method and analyzed using LC/MS/MS. The four pesticides residues dissipated during 20 days post treatment, though at a slower rate and the residues amounts in fruits were much higher in case of methomyl and phenthoate. The pre-harvest interval (PHI) was determined to be 3, 10, 21 and 21 days for pepper fruits treated with abamectin, diniconazole, methomyl and phenthoate under greenhouse conditions, respectively.

Keywords: Pesticide residues; Maximum Residue Limit (MRL); (QuEChERS); (LC/MS/MS); Abamectin; Diniconazole; Methomyl; Phenthoate; PHI; Sweet bell pepper.

INTRODUCRION

Pesticides and their alternatives are an undeniable part of modern life, used to protect everything from flower gardens to agricultural crops from specific pests [1]. Pesticides comprise a large number of substances that belong to many different chemical classes, they are applied to crops at various stages of cultivation to provide protection against pests and during post-harvest storage to preserve quality, to ensure the safety of food for consumers and regulate international trade and legislation. Pesticides are considered to be essential for agricultural development; some of them can cause serious ambient contamination, principally in food [2] and [3]. This hazard, could be further increased in case of vegetable fruits which are usually consumed freshly e.g. consumed vegetable fruits freshly contaminated with pesticide residues, more than allowable tolerance [4]. One of the major disadvantages of pesticides use is their residues that may

remain on/ in food and feed with amounts above the maximum residue limits (MRLs) this could pose health hazards to consumers. The national monitoring programs for pesticide residues are the key means of ensuring compliance with regulations and also to create a database to assess the levels of the greatest number of pesticide residues and the level of residues intake. Thorough monitoring of pesticide residues is crucial for proper risk assessment of human exposure through food and if it was within MRLs [5]. Such information serves greatly to define human exposure to pesticide residues through dietary intake and also help in amending pesticide strategy in the country. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. The maximum residue levels (MRLs) limit and the types and amounts of residues that can be legally present on foods are set by regulatory bodies' worldwide [6]. Determination of abamectin, diniconazole, methomyl and phenthoate has become increasingly in the recent years because of the widespread use of these compounds, which is due to their wide ranging biological activity and relativity low persistence [7]. Abamectin, diniconazole, methomyl and phenthoate are recommended to apply against serious pests on several crops as sweet pepper [8]. The present work was carried out to assess the residues of the recommended pesticides under protected cultivation condition (greenhouse) following their foliar applications including investigate the suitable residue determination procedures for selected pesticides, using analytical techniques such as LC/MS/MS.

Material and method

Pesticides Used

- Abamectin, (Avermectin), (Vertimec 1.8% EC) obtained from Syngenta Agro. Egypt, Red Mite Spider.
- Diniconazole, (Azole), (Sumi-eight 5% EC) obtained from Sumitomo Corporation, Egypt, against Powdery Mildews.
- Methomyl, (Carbamate), (Lannate 90% SP) obtained from DuPont, Egypt, against Cotton leafworm.
- Phenthoate, (Organophosphate), **(**Cidial-L50%EC) obtained from Lotus Agricultural Development Egypt, against Cotton leafworm and Aphids.

Field Experiments

Sweet bell pepper was planted at the experimental station Ministry of Agriculture, Protected Cultivation, El Giza, Agricultural Research Center, Egypt. Plot (1) in September 2011 with an agricultural area was divided into 15 lines each line 40 m length about 600 m^2, plot (2) in Jun 2012 with agricultural area was divided into 33 lines each line its length 60 m about 1980 m^2 under greenhouse conditions. Sweet bell pepper was sprayed as foliar application under greenhouse condition using motor sprayer, 600 Lit capacity, temperature (approximately, between 20–25 ^oC). Control samples were taken one hour before application as control check. Abamectin and diniconazole were sprayed at the rate of 60 and 50 ml in 100 liters water per 200 m^2 in plot (1) while in plot (2) methomyl was 100 ml in 100 liters water per 660 m^2 and phenthoate was 100 ml in 100 liters water per 660 m^2, was

used The sweet pepper plants were sprayed after 45-60 days from sowing. The untreated control plots were sprayed with water only.

Sampling

Treated samples of Sweet bell pepper fruits were randomly picked up for all pesticides one hour (as initial), 5 hour, then 1, 3, 5, 7, 10, 15 and 21days after pesticides spraying including control sample one hour before treatment. For abamectin 1 h, 3 hour, 5 hour, then 1, 3, 6, 9, 13 and 20 days. Samples were transported to the laboratory immediately after collected (kept in an ice box). Three replicates sub-samples were taken from fruits, which weighed (50 g). Samples were kept in polyethylene pages in a deep freezer at -20ºC till residue analysis [9].

Pesticide Residues Analysis:

QuEChERS Method was followed [10]. Samples were weighed (10 g) of sweet bell pepper samples in a 50 ml PFTE tube and dissolved in 15 ml deionized water by shaking for one minute, 10ml of acetonitrile was added and shocked vigorously for one minute, buffer –salt –mixture needed and shake again vigorously for one minute, the samples was centrifuged at 4000 rcf for 5 min, 10 milliliter of the upper clear solution was transferred into 15 ml polyethylene tube containing 0.4 g primary secondary amine (PSA) sorbent and 0.6 g anhydrous magnesium sulphate. The tubes were caped and then mix the extract with the sorbent vigorously for one minute and centrifuged at 4000 rcf for 5 min. Filtrate of upper aqueous layer was taken using Acrodisc into vial. For spiking tests use samples that previously analyzed and proved to be abamectin / diniconazole / methomyl / phenthoate free, add appropriate volume of the spiking mixture, then 5µl of the sample was inject into LC-MS/MS system.

Quality Assurance:

The residue method developed complies with the performance characteristic requested for the analysis of this group of pesticides in vegetable samples. This means good selectivity, linearity in the response, recovery values (range between 70-120 % in most of the cases with good precision in the response R.S.D., < 20 %), good sensitivity (LODs range 0.01-0.004 mg/kg and confident identification criteria The average percentage recoveries for the tested pesticides from different commodities ranged from 70- 110% at spiking levels of 0.01, 0.05 and 0.1 mg/kg, with coefficients of variation (CV %) of 2-4%. The reproducibility expressed as relative standard deviation was less than 20%. The limit of quantification ranged between 0.01-0.05 mg/kg. The measurement uncertainty including random and systematic error at 95% confidence level was estimated to be less than 20%. Blank samples were fortified with the pesticides. The extraction method applied is simple, rapid and efficient and LC-MS-MS provides characteristic parent to production transition, enough for suitable pesticide conformation. The applicability of the method to routine analysis was tested in real samples with good results and quality control systems applied demonstrated a good performance and stability in the time.

Apparatus:

(a) Laboratory balance capable to weight (0.1 mg). **(b)** Polyethylene 15, 50 ml with screw cap tubes or similar quality. **(c)** Centrifuge (Heraues up to 4000 rcf). (d) Volumetric Graduated Pipettes 10 ml, Grade A. (e) Ministart RC 15 syringe filter (0.45 µm) Sartorius. (f) Liquid chromatography tandem mass spectrometry (LC-MS/MS) equipped with electrospray ion source (e.g. API 5500QTrape from Applied Biosystems, AB). Follow the instructions mentioned in the instrument log book for LC-MS/MS operation and conditions, with Electrospray ionization (ESI) in negative-ion mode, the mass spectrometer was operated in multiple reactions monitoring (MRM) mode. Two different MRMs are used for confirmation.

Reagents:

Solvents and chemicals: All reagents were of analytical (HPLC) grade; acetonitrile, acetone and hexane from (Lab-scan). Tetradecane, reagent grade anhydrous $MgSO_4$ (in powder form) and ACS-grade NaCl (Merck). $MgSO_4$ was baked for 5 hr at 500^{o}C in muffle furnace to remove phthalates. Primary secondary amine bulk sorbent (PSA), anhydrous sodium sulphate (Riedel-deHäen). De-ionized water was produced by Mille Q-unit (Mille Pore). Formic acid (Merck) was used as acid modifier of the LC mobile phase. Pesticide reference standards Purity was >95%.

Buffer–salt–mixture was prepared as follow: weight 4±0.2g anhydrous magnesium sulphate, 1±0.05g sodium chloride 1±0.05g trisodium citrate dehydrates and 0.51±0.03g of disodium hydrogen nitrate sesquihydrate into 25 ml glass tube.

RESULTS AND DISCUSSION

Residue of Abamectin:

Results in Table (1) and Figure (1,a) showed that the concentration of initial deposits of abamectin in pepper fruits were 0.04 mg/kg, then gradually decreased to 0.03 mg/kg five hours after application revealing 25.00 % loss. This value decline to 0.02 and 0.01 mg/kg recording the rate loss 50.00and 75.00 % at 1 and 3 days, respectively, after 20 days abamectin was not detected. The data show that pepper fruits could be safely consumed after 3.0 days of application according to the recommended maximum residue limit (MRL) for Abamectin in sweet bell pepper 0.02 mg/kg according to EU and Codex [9]. These results are in agreement with those of [11, 12].

Table (1): Mean residues (mg/kg) of the four tested pesticides on/in pepper fruits during the experiments.

Rate of application / times after application	Abamectin 50ml/100L/ 200 m^2	Diniconazole 50 ml/100 L/ 200 m^2	methomyl 100 ml/100L/650m^2	Phenthoate 50 ml/ 100L/650m^2
	Mean residue mg/kg	Mean residue mg/kg	Mean residue mg/kg	Mean residue mg/kg
Initial	0.04	0.11	3.7	2.99
5 h	0.03 3h	0.09	1.47	2.32
1 day	0.02 (5h)	0.09	1.04	1.79
3 days	0.01 (1day)	0.08	1.02	1.6
5 days	0.01 (3days)	0.07	0.86	1.3
7 days	0.01 (6days)	0.05	0.29	0.32
10 days	0.01 (9days)	0.04	0.29	0.18
15 days	0.01(13days)	0.04	0.12	0.14
21 days	ND (20days)	0.03	ND	ND

ND: not detected

Residues of Methomyl:

The data in Table (1), Fig (1,c) also showed the residues of methomyl in pepper fruits. The initial deposit of methomyl was 3.7 mg/kg one hour after application then decreased to 1.47, 1.04, 1.02, 0.86, 0.29 and 0.12 mg/kg indicating 60.27, 71.89, 72.43, 76.76, 92.16 and 96.76% loss after five hours, 1, 3, 5 ,7, 10 and 21 days respectively. The data indicated that pepper fruits could be consumed safely after 20 days after application, where (MRL) of methomyl residue in pepper fruits was 0.1 mg/kg according to EU. Such results are in agreement with those reported by several investigators [11, 21 - 22].

Residues of phenthoate:

The residues of phenthoate in pepper fruits as in Table (1) and Figure (1,d) revealed that the initial deposit of methomyl was 2.99 mg/kg one

hour after application then decreased to 2.32, 1.79, 1.60, 1.30, 0.32, 0.18, 0.14 and was not detected after 20 days recording 41.22, 40.13, 46.49, 56.52, 89.30, 93.98 and 95.32% loss after five hours, 1, 3, 5, 7, 10, 15 and 21 days respectively. The data indicated that pepper fruits could be consumed safely after 20 days after application, where (MRL) of phenthoate residue in pepper fruits was 0.1 mg/kg according to Codex[9]. Such results are in agreement with those reported by several investigators[11, 23 - 26].

Residue of Diniconazole:

The results given also in Table (1) and Figure (1, b) indicated the residues of diniconazole in pepper fruits. The initial deposits found after one hour was 0.11 mg/kg. The residue levels were decreased to 0.09, 0.8, 0.07, 0.05, 0.04, 0.03 and 0.01 mg/kg showing 18.18, 27.27, 36.36, 54.55, 63.64 and 72.73% loss after 5 hours, 3, 5, 7, 10, and 21 days, respectively. Maximum residue limits (MRL) for diniconazole on/in pepper fruits according to European Union was 0.05 mg/kg. Data indicated that pepper fruits could be consumed safely after 10 days. These results were generally in agreement with a number of researchers [5, 11 - 20].

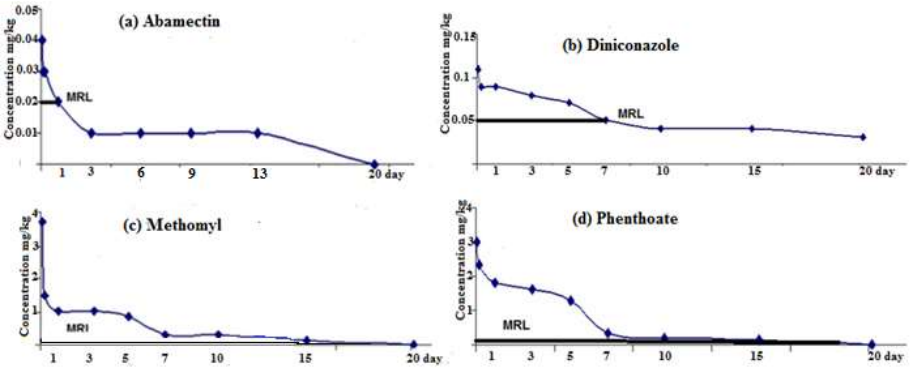

Fig (1) Behavior of abamectin, diniconazole, methomyl and phenthoate residues on/in sweet bell pepper fruits.

The present results indicated that: Methomyl and phenthoate were found to be more persistent on/in pepper fruits compared with the other two tested pesticides; data also reported that the lowest residue level 0.01mg/kg on/ in pepper fruits were detected after 21 days of application for methomyl, phenthoate and diniconazole, while the lowest residue of abamectin was 0.01 mg/kg within 3 days. Concerning the residues of abamectin, diniconazole, methomyl and phenthoate on/in sweet bell pepper fruits under greenhouse protected conditions, the residues amounts in fruits were much higher in case of methomyl and phenthoate than in case of abamectin and diniconazole. The rate of appearance (% loss) was faster in case of methomyl than the other tested pesticides [27]. Great interest to note that the loss of phenthoate in sweet bell pepper fruits more than 50% took place after 5 days following application. This phenomenon revealed that the levels of phenthoate residues

disappearance were higher in fruits. This statement agreed with that of [28] who stated that the dislodgeable phenthoate residues dissipated rapidly from fruits. All tested residues dissipated during 21 days post treatment on/in pepper fruits.

Conclusion:

The pre-harvest interval (PHI) was determined to be 3, 10, 21 and 21 days for pepper fruits treated with Abamectin, Diniconazole, Methomyl and Phenthoate under greenhouse conditions, respectively. It is important to respect the PHI so that the MRL for a given crop is not exceeded. Residues found in excess of the MRL on food would constitute a violation of the Regulations and could also pose a risk to consumers' health. In such situations, the harvested crop could be seized, destroyed or forbidden for export. Use pesticides only for the crops and pests listed on the product's label and make sure to follow the application rates, number of applications and PHI stated on the label.

REFERENCES

Saravi S. and Shokrzadeh M. (2011) Role of pesticides in Human life in modern age: A review. http://www.intechopen.com/books/pesticides-in-themodern-world-risks-and-benefits/role-of-pesticides-in-human-life-in-the-modern-age-a-review.

Saitta, M.; Di-Bella, G.; Salvo, F.; Lo-Curto, S. and Dugo, G. (2000) Organochlorine pesticide residues in Italian citrus essential oils. J. Agric. Food Chem., 48: 797-801 (2000).

Gonzalez,M.,Karina,S.B.M.,Julia,E.A.,Victor, J.M. (2003) J. Agric Food Chem., 51: 1353-1359.

Romeh, A.; Mekky, T. and Ayad, K. (2000) Effect of home preparative on diniconazole and pirimicarb residues. Annal of Agric. Sc., Moshtohor. 38: 2283-2292.

Hiemstra, M. and de Kok, A. (2007) Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography–tandem mass spectrometry. J. of Chrom.A, 1154: 3–25.

Rohan D.; Tangirala S. and Naishadham P (2012). Pesticide residue analysis of fruits and vegetables. J. of invironmental Chemistry and Ecotoxicology, 4: 19-28.

Pogačnik L, Franko M. (2003). Photochemical Bioanalytical Methods for Pesitcides Toxicity testing, Arh Hig Rada Toksikol 54:197-205.

http//www.capl.sci.eg.

Codex allimentarius committee for pesticide residues (CCPR) (1997). Guide to codex recommendations concerning pesticide residues part 3, maximum limits for pesticide residues.

Dasika, R.; Tangirala, S. and Naishadham, P. (2012). Pesticide residue analysis of fruits and vegetables. Journal of Environmental Chemistry and Ecotoxicology. 4: 19-28.

Commission Directive /60/ CE of 7 July (2006) amending Annexes to Council Directive 90/642/ EEC as regards the MRLs of trifloxystrobin, thiabendazole, abamectin, benomyl, carbendazim, thiophanate-methyl, myclobutanyl, glyphosate, trimethylsulfonium, fenpropimorph and chlormethequat, off. J. Eur. Union.

Reddy, E.; Sharma, D. and Kumar, K. (2007). Residues of insecticides on sweet pepper and tomato grown under greenhouse and open field cultivation. Pesticides research journal 19: 239-243.

Mahmoud, H. and Eissa, F. (2007). Diniconazole residues in field-sprayed and household processed cucumber and pepper fruits. Annals Agric. Sci., 52: 253-260.

Walgenbach, J.; Leidy, R. and Sheets, T. (1991). Persistence of insecticides on tomato foliage and implications for control of tomato fruitworm (Lepidoptera: Noctuidae). J. of Eco. Entomology. 84: 978-986.

Hegazy, M.; Abdel-Razik, M.;El-Hadidi, M.; Abu-Zahw, M.; Shokr, S. and Ibrahim, Y. (1999). Residual behavior of certain pesticides on/in grape leaves. Egypt. J. Agric. Res. 77: 159-168.

Talebi, K. (2002). A laboratory investigation of the side effects of fourpesticides on predatory bug, Orius albidipennis (Het. Anthocoridae). Iranian Journal of Agricultural Science 33: 651-659.

Hegazy, M. and N. Nasr. (2003). Pesticide residues on and in moloukhia leaves grown under field conditions and the effect of processing on the residues Bull.Fac.Agric. Cairo univ., 54:283-292.

Hu, J.; Zhang, W. and Li, J. (2005). Residue analysis and dissipation of a new fungicide 2-allylphenol in tomato. J. Environ. Sci. china, 17: 491-493.

Li، J.; Wu, X. and Hu, J. (2006). Determination of fungicide kresoxim-methyl residues in cucumber and soil by capillary GC with nitrogen-phosphorus detection. J. Environ. Sci. Health B. 41: 427-436.

El-Sawi, A. and N. Zidan. (2007). Residual behavior of fenarimol fungicide on cucumber and pepper fruits grown under greenhouse conditions.J. Egypt. Soc. Toxicol. 36: 7-11.

Zwieg, G. (1963). Analytical methods for pesticides, Plant Growth Regulation, and Food Additives, 1: 42-43, Principles, Methods, and General Applications, Academic Press, New York, USA.

Awad, T.; Vision, S. and Brazzel, J. (1967). Effect of environmental and biological factors on persistence of Malathion applied as ultra-low vol. or emulsifiable concentrate to cotton plants. J. Agric. Food and Agriculture, 84: 1253-1259.

Iwata, Y.; Carman, G. ; O'Neal, J. ; Barkley, J. ; Düsch, M. and Gunther, F. (1981). Phenthoate applied to California citrus trees: residue levels on foliage and soil, in air, and on and in fruit, J. Agric Food Chem., 29: 135-45.

Iwata, Y.; Westlake, W.; Barkley, J.; Carman, G. and Gunther, F. (1977). Behavior of phenthoate (Cidial) deposits and residues on and in grapefruits, lemons and lemon leaves, oranges and orange leaves, and in the soil beneath orange trees. J. Agric. Food Chem., 25: 362-368.

www.mhlw.go.jp/english/topics/mrls/second/dl/mrls33.PDF.
Attalla, I. (2006). Residues of phenthoate and abamectin on orange fruits. Egypt. J. of Appl. Sci. 21: 754-757.
Al-Khalaf, Y.; Al-Kadi, S.; Khan, P. and Abd El-Razik, M. (1995). Residues of some insecticides and fungicides in tomato-fruits grown in controlled environment. Int. Conf. of pest Control, Mansoura, Egypt.
Allshalaby, A. (2010). Studies on the fate of chlorfenapyr and phenthoate pesticides in peach and under some environmental conditions. M.Sc. thesis, Fac. Of Agric. Cairo Univ.

متبقيات وفترة ماقبل الحصاد لمبيدات الابامكتين، الديكونازول، الميثوميل و الفينثوات لثمار الفلفل الحلو تحت ظروف المحميات البلاستيكية

سامية محمد القباني[1] – أشرف محمود المرصفى[2] – جهاد جنيدي محمد[3] و محمود محمد سعديه[4]

1- قسم المبيدات - كلية الزراعة – جامعة القاهرة.
2- قسم الكيمياء – مدير المعمل المركزي لتحليل متبقيات المبيدات و العانصر الثقيلة في الاغذية - وزارة الزراعة – الدقي- مصر.
3- قسم الكيمياء الغير عضوية و التحليلية - قسم الكيمياء- كلية العلوم- جامعة القاهرة.
4- قسم الكيمياء – كلية العلوم – جامعة القاهره

تم تقدير سلوك متبقيات مبيدات الابامكتين ، الديكونازول، الميثوميل و الفينثوات عقب معاملة ثمار الفلفل الحلو بالجرعة الموصى بها تحت ظروف المحميات البلاستيكية. تم رش المبيدات وفق الجرعات الموصى بها، في حالة الابامكتين والديكونازول 50 مل/ 100 ليتر ماء/200 متر مربع و 100 مل/ 100 لتر ماء/ 660 متر مربع في حالة الميثوميل، اما في حالة الفينثوات كانت الجرعة 50 مل / 100 لتر ماء / 660 متر مربع (معاملة ورقية). و جمعت الثمار المعاملة بعد رشها بساعة و 5 ساعات ثم 1، 3، 5، 7، 10، 15، 21 يوم متضمنة اخذ عينات قبل الرش بساعة (عينة المقارنة). انفردت عينات الاباماكتين حيث أخذت وفق الترتيب: ساعة، 3، 5 ساعات ثم بعد 1، 3، 6، 9، 13 و 20 يوم من الرش. الاستخلاص تم وفقا لطريقة (QuEChERS) (من احدث الطرق المستخدمة في الاستخلاص : سريعة، سهلة، غير مكلفة، فعالة، مرنة وآمنة) مع أستخدام جهاز LC/MS/MS لتحليل و تقدير المتبقيات. و قد أوضحت النتائج المتحصل عليها تناقص متبقيات المبيدات الاربعة في ثمار الفلفل الحلو بإطراد مع مرور الوقت خلال 20 يوم من المعاملة مع ملاحظة ان معدل التناقص كان الابطأ في حالة الميثوميل و الفينثوات. قدرت فترة ما قبل الحصاد (PHI) للمبيدات ب 3، 10، 21 و 21 لكل من مبيدات الابامكتين، الديكونازول، الميثوميل و الفينثوات تحت ظروف المحميات البلاستيكية، على التوالي. اعتمادا على قيم الحد الاقصى لمتبقيات المبيدات (MRLs) وفقا لقوانين الاتحاد الاوربي (EU) ، منظمة الصحة العالمية/منظمة الاغذية و الزراعة (FAO/WHO) .

قام بتحكيم البحث

أ.د / عادل عبد المنعم صالح	**كلية الزراعة – جامعة المنصورة**
أ.د / عطا على مرسى شلبى	**كلية الزراعة – جامعة الزقازيق**