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Detection of insect infestation and insecticides residues in pulses seeds from different governorates in Egypt

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ABSTRACT

Insect infestation was detected in samples of broad beans, cowpeas and chickpeas, obtained from local markets of six governorates in Egypt (Giza, Cairo, Behaira, Qalubia, Ismailia and Menya). Two insect species have been detected, they were the cowpea weevil, *Callosobruchus maculatus* and Chinese bruchid, *C. chinensis.* The highest insect infestation was occurred in cowpea seeds obtained from Qalubia governorate followed by chickpea from Cairo governorate, while, the lowest infestation was recorded in the broad bean from Cairo and Chickpeas from Menya. No infestation was recorded in Giza samples. Also, insecticide residues were analyzed in all the pulses samples by using the quechers method for pesticides analysis. Results showed that faba beans, cowpea and chickpea samples were found either clear of all organochlorine and carbamates insecticides or they were below the level of quantification (LOQ). Organophosphorus pesticides tested have been detected and represented in chloropyrifos, while neonicotinoids were involved and represented in acetamiprid. However, the levels of the two insecticides were below the maximum residue limits (MRLs) established by the Egyptian Organization for standardization and Quality (EOS).

Key words: Insects infestation-Insecticides -Residues-Pulses seeds

INTRODUCTION

Coleopteran insects of the family Bruchidae are the major bruchids associated with the seeds of pulses crops (Sales *et al.*, 2000). Callosobruchus *maculatus* and *Callosobruchus chinensis* infest pulses in the field, even before the crop is harvested (Kawuki *et al.* 2005), and this infestation is carried into warehouses, resulting in further infestation and deterioration of the stored pulses. These insects multiply at a rapid rate in suitable environmental conditions such as high humidity and optimum temperature conditions **(**Appleby and Credland, 2004)**.**The increasing demand for enhanced food productivity to meet the needs of the global population has led farmers to use sophisticated agricultural technology in which pesticides play a crucial role. Generally, pesticide use has a positive and dramatic impact on agricultural production through protection of crops against insect pests, but every effort must be made to ensure that insecticide application is safe for human and environmental health. Undesirable level of insecticides residue used for insect control on pulses and grain may accumulate and persist during storage. The level of insecticides residues depends largely on the type and quantity of the chemical used, formulation in which it was applied, temperature and moisture content of the grain during storage and the length of time elapsing between application of pesticide and consumption of the food (Sinhaand Muir, 1973; Quinlan *et al.*, 1979; Abdel-Kader and Webster, 1980; Dikshit, 1985).

The present study aimed to evaluate the levels of insect infestation and also to determine the levels of insecticides residues in broad bean, cowpea and chickpea seeds collected from 6 different governorates in Egypt.

Materials and methods

1. **Detection of insect infestation in the pulses samples:**

Samples of broad beans, cowpeas and chickpeas seeds were collected from local markets of six governorates in Egypt (Giza, Cairo, Behaira, Qalubia, Ismailia and Menya) during July-2012 to Aug-2014. Samples of 100 gm. of the collected seeds were placed in a plastic cup (5.5 cm diameter X 8 cm height) that was covered with a piece of muslin. Four replicates were used for each sample and the cups were kept under controlled conditions of temperature (28±2°C) and relative humidity (70±5% R.H.). The seeds in the cups were sieved after two months incubation period to determine the total number of adult insects and identify them.

2. Analysis of pesticide residues in pulses samples

Residues of pesticides in the collected pulses samples were determined according to the quick, easy, cheap, effective, rugged, and safe (quenchers) method for pesticides analysis (Lehotay *et al*., 2005) in the Central Lab. of Residue Analysis of Pesticides and Heavy Metals in Food at Giza, Egypt. Samples of seeds of each pulse were grinded in waring blender. Powdered samples (10 g) were taken in 50 ml centrifuge test tube and mixed with 10 ml of double distilled water and allowed to macerate for 10 minutes, then 10 ml of ethyl acetate, 4g of activated anhydrous $MgSO_4$ and 1.0g activated NaCl were added and shaken for 10 min. at 50 rpm. The extract was centrifuged for 10 min at 8,000 rpm. and 1 ml aliquot of extract was cleaned with the mixture of 100 mg primary secondary amine (PSA), 150 mg anhydrous $MgSO_4$ and 10 mg activated charcoal. The extract was again shaken for 10 min. at 50 rpm and centrifuged for 10 min at 8,000 rpm. The supernatant was collected in 2 ml GC vial and mixed with 5µl acidified ethyl acetate. 1µl , clean extract was injected in gas chromatography equipped with Electron capture detector (ECD) for the analysis of pesticide residues and further confirmed by GC-MSGC.

Analytical condition:

Residues were analyzed on Shimadzu GC-2010 equipped with fused silica capillary column, DB-1 (30 mt. × 0.25mm. id) coated with 100% dimethyl-polysiloxane (0.25 µm film thickness) using electron capture detector (ECD). General operating conditions were as follows; Injector port temperature: 280 ºC; detector temperature 300 ºC; using carrier gas Nitrogen (N2); Total flow 7.7 ml/min, Column 0.79 ml/min, purge flow 3.0 ml/min, make up flow 30 ml/min column temperature program: initially 165 ºC for 1.50 min, increase at 2.70ºC/min to 210 ºC hold for 2.70 min, then increase to 265 ºC at 2.70 ºC /min and hold for 1.90 min; injection volume: 1µl split ratio 1:5. The total run time was 46.83 min and Shimadzu, GC Solution software was used for instrument control and data analysis. Quantification of the pesticides was done by peak area using the external standard method and further confirmed by GC-MS.

Results and discussion

1. Detection of insect infestation in the pulses samples

In the present study insect pests were detected, identified and recorded in samples of broad beans, cowpeas and chickpeas, obtained from local markets of six governorates in Egypt (Giza, Cairo, Behaira, Qalubia, Ismailia and Menya) during the investigated period. Results revealed that two insect species have been detected, they were the cowpea weevil, *Callosobruchus maculatus* and Chinese bruchid, *C. chinensis* (O.: Coleoptera, F.: Chrysomelidae). In agreement with these results several authors detected the infestation of pulses seeds with the two insect species (Singh *el al.*, 1989; Ishimoto *et al.*, 1996 and Adugna, 2006). Results revealed that infestation of pulses with *C. maculatus* was more abundant than infestation with *C. chinensis* (Fig. 1). These results are in agreement with Tuda *et al.* (2005) who recorded that *C.*

maculatus was more able to develop and adapt on different types of pulses seeds than *C. chinensis.* This adaptability is attributed to the ability of *C. maculates* to live and reproduce in dry beans and seeds without the requirement of food and water. Data in Table (1) showed that the highest insect infestation was occurred in chickpea seeds obtained from Cairo governorate with a mean number 47.25 adults followed by cowpea obtained from Qalubia governorate with a mean number 26.25 adults. While, the lowest infestation was recorded in the broad bean samples obtained from Cairo, cowpeas from Ismailia and chickpea from Menya governorates with a mean number 0.25. Results also revealed that no infestation was recorded in Giza samples. The variation of insect's infestation from one governorate to another may be due to the difference of the environmental condition as temperature and relative humidity and also the storage methods. Kawuki *et al.* (2005) recorded that high percentage of infestation occurs due to unhygienic stores. Also, the variation of insect's infestation with the type of the pulse seeds may be due to difference in physical and chemical characteristics of the tested pulses seeds. Several authors found that the growth and development of the bruchid weevils influenced by the physical and also the chemical characteristics of the pulses seeds (Lambrides and Imrie, 2000; Chakraborty *et al.*, 2004; Abdel Fattah and Ahmed, 2007).

Table (1). Mean number of insect species (adults only) recorded in pulses seeds obtained from six governorates.

Governorate	Broad bean	Cowpea	Chickpea	Total no. of adults
Giza	0.00	0.00	0.00	0.00
Cairo	0.25	0.00	47.25	47.50
Behaira	0.00	0.00	1.50	1.50
Qalubia	0.00	26.75	0.00	26.75
Ismailia	0.25	0.25	0.00	0.50
Menya	0.00	0.00	0.25	0.25

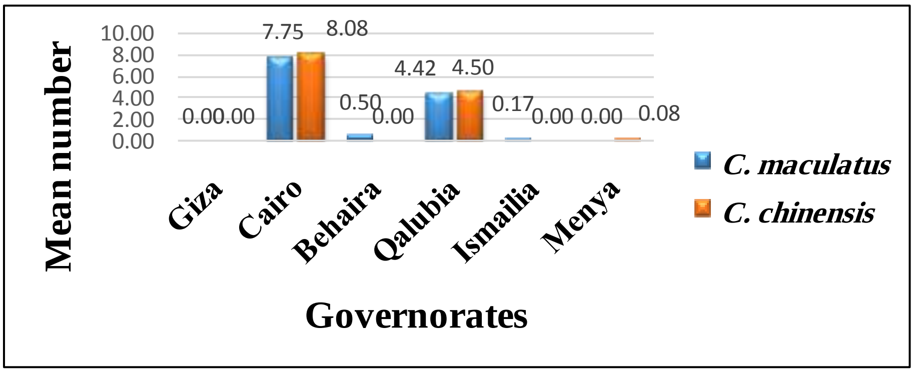

Fig. (1): Mean number of insect species (adults only) recorded in six governorates for all pulses types.

2. Detection of insecticide residues in investigated samples

Data in Tables (2-4) showed the amount of pesticide residues in samples of faba beans, cowpea and chickpea obtained from six governorates in Egypt (Giza, Cairo, Behaira, Qalubia, Ismailia and Menya). Results showed that faba beans samples (Table 2) were found either clear of all Organochlorine pesticides (OCs) and

Carbamates or they were below Level of Quantification (LOQ). Organophosphorus pesticides have been detected and represented in chloropyrifos that was found in Giza sample at 0.01 mg/kg which was below the maximum residue limits (MRLs) established by the Egyptian Organization for standardization and Quality (EOS) (2007). Also, Srivastava (2014) detected chlorpyriphos in pulse seeds, they were frequently encountered in 39 % of total analyze (120) samples.

Table (2). Mean levels of insecticide residues detected in mg/kg in broad bean samples collected from six governorates.

Insecticides	Governorates					
	Giza	**Cairo**	**Behaira**	**Qalubia**	**Ismailia**	**Menya**
Organchlorines						
Aldrin	-	-	-	-	-	-
Butachlor	-	-	-	-	-	-
P,P DDD	-	-	-	-	-	-
P,P DDE	-	-	-	-	-	-
P,P DDT	-	-	-	-	-	-
Endosulfan-alpa	-	-	-	-	-	-
Endosulfan-beta	-	-	-	-	-	-
Endrin	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-
Mirex	-	-	-	-	-	-
Organophosphorus						
Diazinon	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-
Chloropyrifos	0.01	-	<LOQ	-	-	<LOQ
Chloropyrifos-Methyl	-	-	-	-	-	-
Fenitrothion	-	-	-	-	-	-
Malathion	-	-	-	-	-	-
Profenofos	-	-	-	-	-	-
Dimethoate	-	-	-	-	-	-
Carbamates						
Aldicarb	-	-	-	-	-	-
Carbaryl	-	-	-	-	-	-
Neonicotinoids						
Acetamiprid	-	-	-	-	0.02	-

Results also revealed that neonicotinoids were represented in acetamiprid that was detected in Ismailia sample, the mean value of residues was 0.02 mg/kg which is also below the MRLs (Table 3). Detection of insecticides residue in cowpea seeds samples revealed that three samples of governorates, Behaira, Qalubia and Menya were found contaminated with detectable pesticides belonging to different groups (Table 3). Organophosphorus pesticides have been detected and represented in chloropyrifos that was found in Behaira and Menya samples at 0.01 mg/kg and also in Qalubia sample at 0.02 mg/kg which are all below the MRLs. All Organochlorine pesticides (OCs), Carbamates and Neonicotinoids were either not detected or they were below Level of Quantification (LOQ). On the other hand, results in Table (4) showed that chickpea samples were either clear of all other pesticides and that include (OCs), (OPs), Carbamates and Neonicotinoids or polluted with them were below Level of Quantification (LOQ). In agreement with these results, Kaphalia *et al.* (1990) recorded that the residue levels of hexachloro-cyclohexane (HCH) and 2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane (DDT) were either very small (less than 0.06 ppm) or not detected at all in tested pulses. They mentioned that this was due to the fact that pulses contained low moisture content, thus not enabling pesticide residues to persist. Also**,** Aldana-Madrid *et al.* (2008) found that most pesticide levels were within regulation limits. Kamil *et al*. (1996) recorded the degradation of malathion and pirimiphos methyl and concluded that stored broad beans safely used for human consumption after 90 days when the insecticide residues reached safe levels. In contrary to the present study, Sonchieu *et al*. (2010) detected organochlorine pesticides more frequently and in higher concentrations on stored maize, cowpea and millet, and more than 75% of samples contained pesticide residues above the maximum residue limit (MRL).

Table (3). Mean levels of insecticide residues detected in (mg/kg)in cowpea samples collected from six governorates.

Pesticides	**Governorate**					
	Giza	**Cairo**	**Behaira**	**Qalubia**	**Ismailia**	**Menya**
Organochlorines						
Aldrin	-	-	-	-	-	-
Butachlor	-	-	-	-	-	-
P,P DDD	-	-	-	-	-	-
P,P DDE	-	-	-	-	-	-
P,P DDT	-	-	-	-	-	-
Endosulfan-alpa	-	-	-	-	-	-
Endosulfan-beta	-	-	-	-	-	-
Endrin	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-
Mirex	-	-	-	-	-	-
Organophosphorus						
Diazinon	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-
Chloropyrifos	-	-	0.01	0.02	-	0.01
Chloropyrifos-Methyl	-	-	-	-	-	-
Fenitrothion	-	-	-	-	-	-
Malathion	-	-	-	-	-	-
Profenofos	-	-	-	-	-	-
Dimethoate	-	-	-	-	-	-
Carbamates						
Aldicarb	-	-	-	-	-	-
Carbaryl	-	-	-	-	-	-
Neonicotinoids						
Acetamiprid	-	-	-	-	-	-

Table (4). Mean levels of insecticide residues detected in (mg/kg) in chickpea samples collected from six governorates.

Insecticides	Governorates					
	Giza	Cairo	Behaira	Qalubia	Ismailia	Menya
Organchlorines						
Aldrin	-	-	-	-	-	-
Butachlor	-	-	-	-	-	-
P,P DDD	-	-	-	-	-	-
P,P DDE	-	-	-	-	-	-
P,P DDT	-	-	-	-	-	-
Endosulfan-alpa	-	-	-	-	-	-
Endosulfan-beta	-	-	-	-	-	-
Endrin	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-
Mirex	-	-	-	-	-	-
Organophosphorus						
Diazinon	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-
Chloropyrifos	-	-	-	-	-	<LOQ
Chloropyrifos-Methyl	-	-	-	-	-	-
Fenitrothion	-	-	-	-	-	-
Malathion	-	-	-	-	-	-
Profenofos	-	-	-	-	-	-
Dimethoate	-	-	-	-	-	-
Carbamates						
Aldicarb	-	-	-	-	-	-
Carbaryl	-	-	-	-	-	-
Neonicotinoids						
Acetamiprid	-	-	-	-	-	-

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الكشف عن الإصابة الحشرية ومتبقيات المبيدات فى عينات البقول التي تم جمعها من محافظات مختلفة في مصر

محمد عادل حسين ، هدى محمد عبد الفتاح ، أمانى سليمان خالد ، شمس محمد فوقى ، سامح نجاح الغنيمى
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المستخلص

تم الكشف عن الإصابة الحشرية في عينات من الفول واللوبيا والحمص والتي تم الحصول عليها من الأسواق المحلية من ست محافظات في مصر (الجيزة، القاهرة، البحيرة والقليوبية والإسماعيلية والمنيا). وقد اظهرت النتائج وجود اثنين من أنواع الحشرات، وهما: *C. chinensis* , *Callosobruchus maculates* وقد سجلت اعلى إصابة بالحشرات في بذوراللوبيا التى تم الحصول عليها من محافظة القليوبية يليها الحمص من محافظة القاهرة، بينما سجلت أدنى إصابة في الفول من محافظات القاهرة والإسماعيلية. ولم تسجل أية إصابة في عينات الجيزة والمنيا. أيضا، تم تحليل متبقيات المبيدات الحشرية في جميع عينات البقول. وأظهرت النتائج أن عينات حبوب الفول، اللوبيا والحمص إما انها خالية من جميع المبيدات الكلورية العضوية والكارباماتية .اوكانت أقل من المستوى الكمي (LOQ). وقد تم الكشف عن المبيدات الفسفورية العضوية ووجد انها تمثلت في الكلوربيروفوس، وايضا مبيدات النيونيكوتينويدات والممثلة في أسيتامبريد. وقد وجد ان مستويات هذه المبيدات في عينات البقول دون الحدود القصوى لمتبقيات المبيدات (الحدودالقصوى) التي وضعتها المنظمة المصرية للمواصفات والجودة.