Minufiya J. Agric. Res. Vol. 33 No.6: 1295-1304 (2008) "http://www.mujar.net"

DETERMINATION OF PESTICIDE RESIDUES IN OLIVE FRUITS AND OILS

Azza A. A. Ahmed and Nahed M.M. Atta
Fat and Oil Res. Dept. Food Tech. Ins., Agric Res. Center, Giza Egypt
(Received: Oct. 12, 2008)

ABSTRACT: ***Residue of diazinon insecticide in olive fruits and oils (Koronaki and Picual vars) under normal field conditions were studied. The samples were taken at different intervals of 1, 15, 30 and 45 days after treatment.***

The data indicated continuous loss of residues with elapse time. The level of diazinon residues in koronaki var were higher than in the picual var. (fruits and oil). Safety period for consumption was 45 days after pesticide treatment. The results ascertained that, the diazinon insecticide had no clear effect on the chemical composition of olive fruits as well as on the physical and chemical properties of virgin olive oils under study.

Keywords: ***Diazinon, Olive fruits oil and pesticide residues.***

INTRODUCTION

Olive oil is the most difficult vegetable oil to be analysed compared to that of sunflower, corn and soybean oils. This is not only due to the relatively high amount of lipids eluted from the clean-up, but also because of the potential lipid interference at the Gc determination stage. (Lentza-Rizos and Avramides 1995).

Organophosphorus insecticides are widely used on vegetables and fruits for the control of insect pests because of their fast action and prolonged protection. These chemicals are generally short persistent and do not accumulate in the animal tissue and in the environment (Hegazy et al., 2004). Organophosphorus insecticides namely chloryrifos-methyl, diazinon and phenthoate are widely used in Egypt to control the economic pests as aphids (Aphids gossypii), white fly, cotton leaf worm (Spodopetra littorals and Zeuzera pyrina Ministry of Agriculture Recommendation 2001).

The possibility of detecting pesticide in oil as a consequence of pre-harvest treatment for pests or diseases is becoming increasingly more important in the concept of quality. The presence of pesticide residue in oil, as in any other foodstuff, is a constant consumer concern. (Lopez-villalta 1999).

According to the codex Alimentarius, pesticide residue means "any specified substance in edible olive oil resulting from the use of a pesticide".

When a pesticide is applied to a crop, some of the formulation remains on the plant organs. How much depending on the following, amongst other things, i.e. the dose applied, chemical properties of the pesticide and on what

conditions the treatment is applied particularly drop size, nature and size of plant organ and climatic conditions. (Lopez-villalta 1999).

Olive trees are usually attacked by several pests, mainly the olive fruit fly Bactocera (Dacus) oleae, and receive treatment with seveera pesticide. Those more extensively used belong to the class of organ ophosphorus insecticides and are mainly fenthion, diazinon methidathion and azinphos-ethyl (Rastrelli *et al.,* 2002).

Many studies have been conducted for the estimation of the level of each pesticide at the harvest time of olives and in the oil, Montemurro et al. (2002).

Maximum residual level (MRL) from pesticide for oils is not defined, therefore it is necessary to consider the (MRL) for olives opportunely corrected on the base of concentration or dilution factors caused by the extraction process. (Parlati et al., 2000).

Olive oil quality is of great concern and many studies have been conducted on the organoleptic characteristics and healthy value (Lentza-Rizos 1999).

The consumer requires detailed quality organoleptic characteristics, safety and absence of pesticides (Tsimidou 1998).

The aim of this paper is to determine residues of diazinon pesticide on and in olive fruits and oil and to study, its effect on the chemical composition of olive fruits as well as the physical and chemical properties of the oils.

MATERIALS AND METHODS

Materials:

1. Diazinol insecticide (Basudin E.C. 60% trade name).

A) Chemical composition of diazinon :

O,O-diethyl-O-(2-isopropyl-4-methyl-1-6-pyrimidinyl) phosphorothioate.

2. Olive fruits (Koranaki and Picual vars.).

Methods:

Insecticidal application and sampling:

Olive fruits of picual and koranaki varieties were obtained from Kom-Oshim, Egypt under normal field conditions. An atomizer was used in applying the diazinon insecticide after diluting with water. The recommend rates 1.5 cm./1L water [Ministry of Agriculture Recommendation 2001]. About were 1.5 kg of olive fruits of each treatment (including the control), were collected randomly at internals of 1, 15, 30 and 45 days after insecticidal application then transferred to laboratory until oil extraction from the fruits.

Oil extraction:

The olive fruits of koranakii and picual cultivars were crushed and packed in cheese cloth then pressed using a hydraulic laboratory press [carver]. The extracted oil was dried over anhydrous sodium sulfate, filtered through a

whatman filter paper No.1 then kept frozen in brown glass bottles till the analysis.

Analysis:

1- General chemical analysis of olive fruits:

The olive fruits of koranaki and picual were finally ground and the following analyses were conducted.

A) The moisture content, total lipids, crude proteins, crude fibers and ash were determined according to the methods described in the A.O.A.C. (1990).

B) Determination of total hydrolysable carbohydrates: Total carbohydrates were determined (as glucose) after acid hydrolysis spectrophotometrically using phenol sulfuric acid reagent according to the method reported by Dubois *et al.* (1960).

2- Residual determination of tested insecticides on olives and olive oil:

A) Olives:

Sample preparation: 1.5 kg of harvested fruits were first roughly ground using a homogenizer, so as to separate the woody endocarps. After manual separation of the end carps, the pulp was put again in the same equipment to obtain a finer paste. The paste was then preserved in a freezer (- 18°C) for further use.

Extraction: olives were extracted according to Nasr and Hegazy (2002) who used methanol instead of acetone as a solvent for the extraction of insecticide under study. Samples (100 gm) for each were added to distilled methanol (200 ml) for extraction.

The sample was blended for three minutes at high speed then filtered through a dry pad of cotton into a graduated cylinder. A known volume of the filtrate (100 ml) was taken and partitioned successively with 100, 50 and 50 ml of dichloromethane for water separating from methanol extract in a 500 ml separatory funnel after adding 30 ml of saturated sodium chloride solution. The combined dichloromethane phase was dried after filtration through a pad of cotton and anhydrous sodium sulfate, until evaporation to dryness on a rotary evaporator at 40°C.

B) Olive oils

Determination of residues in virgin olive oils accoding to the method of Molinari *et al.* (1998).

Clean-up procedure:

The florisil column cleans up procedure of Nasr and Hegazy (2002) was used for cleaning the sample extract from the insecticide in use. A 18 mm

(i.d.) x 40 cm glass column chromatography was filled with 6 gm of activated florisil (60-100 mesh) and topped with anhydrous sodium sulfate and compacted thoroughly. The column was pre washed using 50 ml n-hexane. The sample extract was dissolved in 10 ml of the same solvent and transferred into the column and then eluted with 200 ml eluate [50% dichloromethane: 48.5% n-hexane: 1.5% acetonitrill] at the rate of 5 ml/min. The eluate was evaporated to dryness by rotary evaporator at 40°C and the residues were ready for chromatographic determination.

3- Physical and chemical properties:

3-1 Refractive index at 25° C, free fatty acids (as oleic acid %), peroxide value (as millie equivalent/kg oil), unsaponifiable matters (%) were determined as described in the A.O.A.C (1990).

3-2 Color: A Lovibond Tintometer was used to measure the colour using 6-25 inch cell according to the method described in the A.O.A.C (1990).

3-3 Absorbency in ultraviolet: The degree of oxidation of olive oil is reflected by its specific extinction $E^{1\%}_{1cm}$ at 323nm and 270 nm [FAo/ WHO (1970), and by using Shimadzu spectrophotometer [UV-vis-120-02].

3-4 The fatty acid composition of the oil: The fatty acids of the analyzed oil samples were determined by Gc-capillary column according to the method reported by IOOC (2001).

3-5 The oxidative stability of olive oil was estimated using a 679 Rancimat (Metrohm Herisou, Co. Switzerland , at 100°C with an air flow rate of 20 L/hr according to the method described by Mendez *et al.* (1997).

3-6 Determination of total tocopherols: The total tocopherols content in olive oil was determined according to the method of Wong *et al.* (1988).

RESULTS AND DISCUSSION

Residues of diazinon in olive fruits and oils:

Data in table (1) show the residues of diazinon insecticide in olive fruits and oils (Koranaki and picual vars) at different intervals after using the insecticide (1, 15, 30 and 45 days). The results show that the concentration of the residue of diazinon insecticide were 2.2 and 1.8 ppm in olive fruits of koranaki and picual varieties after one day from the application respectively. These amounts decreased to 1.2, 0.53 and 0.02 ppm in koranaki var. and to 0.65, 0.12 and 0.01 ppm in picual var after 15, 30 and 45 days from application, respectively. On the other hand, no detectable amounts were found from diazinon residue at one day after application in olive oils of the aforementioned varieties, but it was detectable after 15, 30 and 45 days being (0.95, 0.44 and 0.02 ppm respectively in oil of koranaki var. and after 15 and 30 days (0.75 and 0.24 ppm respectively) in picual var. while after 45 days it was not detectable in the oil of the same var. Also the data indicate continuous loss of the residues with elapsed time. This may be due to the fast degradation of pesiticide with elapsed time.

Table (1): Residues of diazinon in olive fruits and olive oil of koranaki and picual varieties.

Time after application (days)	Koranaki				Picual			
	Fruits		Oil		Fruits		Oil	
	ppm	Loss %	ppm	Loss %	ppm	Loss %	ppm	Loss %
1	2.2	00	ND	0.0	1.8	0.0	ND	0.0
15	1.20	45.45	0.95	0.0	0.65	63.88	0.75	0.0
30	0.53	75.9	0.44	53.68	0.12	93.33	0.24	68.00
45	0.02	99.09	0.02	97.87	0.01	99.44	ND	100

ND: Not detectable

Also from the results the level of diazinon residue in koronaki var. (fruit and oil) was higher compared to those of picual var. These results would probably be due to the higher degradation and volatilization of diazinon in fruits of picual var. more than in koranaki fruits, and that may be due to the large surface and size of picual fruits compared to those of koronaki (Lopez-villalta 1999).

According to the European communities (EC) (2006) the maximum level of residues (MRLs) is 0.02 ppm for diazinon on olive fruit. The olive fruits of both karanaki and picual varieties should not be pricked for consumption not before 45 days of pesticide treatment, in order to realize safety.

Effect of diazinon insecticide on the chemical composition of olive fruits (Koranaki and picual vars.):

Results in tables (2 and 3) show the effect of diazinon on the chemical composition of olive fruits (koranaki and picual vars.). From the data in tables (2 and 3), it could be noticed that, no clear effect of the diazinon insecticide was noticed on the moisture content, crude oil, crude protein, crude fiber, total soluble carbohydrates and ash of all samples compared to control samples. These results in agreement with those obtained by (Hegazy *et al.,* 2004).

Table (2): Effect of diazinon insecticide on chemical composition (on dry basis) of olive fruits of koranaki variety.

Treatment (days) / Chemical composition %	Control				Diazinon			
	1	15	30	45	1	15	30	45
Moisture content	59.64	57.33	55.21	52.64	59.66	58.43	56.30	53.58
Crude oil	35.34	36.71	37.25	38.26	35.34	36.93	36.84	36.99
Crude protein	11.85	11.65	11.48	11.36	11.84	11.40	11.29	11.25
Crude fibers	20.87	19.96	19.76	19.26	20.89	20.05	19.72	19.34
Total hydrolysable carbohydrates	18.48	18.24	18.15	18.03	18.47	17.94	17.85	17.70
Ash content	13.46	13.46	13.36	13.09	13.47	13.99	14.30	14.72

Table (3): Effect of diazinon insecticide on chemical composition (on dry basis) of olive fruits of picual variety.

Treatment (days) / Chemical composition %	Control				Diazinon			
	1	15	30	45	1	15	30	45
Moisture content	58.39	54.5	51.08	49.37	58.36	56.42	53.60	50.24
Crude oil	34.80	35.51	37.51	39.88	34.74	35.23	36.83	38.65
Crude protein	11.83	11.74	10.94	10.75	11.84	11.60	10.76	10.7
Crude fibers	20.82	20.74	20.43	19.32	20.8	20.6	20.39	19.12
Total hydrolysable carbohydrates	18.80	18.56	17.58	16.94	18.85	18.35	17.49	16.89
Ash content	13.75	13.45	13.49	13.11	13.77	14.22	14.53	14.64

Effect of diazinon insecticide on the physical and chemical properties of virgin olive oils (Koranaki and picual vars.):

Data in tables (4 and 5) indicate the effect of diazinon at different intervals after application (1, 15, 30 and 45 days) on the physical and chemical properties of virgin olive oils (kotanaki and picual vars.). From these results, no change was observed in referactive index, colors (red and blue), UV absorbance at 232 and 270 nm., free fatty acids, peroxide values, unsaponifiable matters, total tocopherols and stability as a result of diazinon insecticide effect in all samples compared to the control. This may be only due to the diazinon pesticide effect on the chemical metabolism of insects, which caused infestation Montemurro *et al.* (2002) and Molinari *et al.* (1998).

Table (4): Effect of diazinon insecticide on physical and chemical properties of virgin olive oil of koronaki variety.

Treatment (days) / Properties	Control				Diazinon			
	1	15	30	45	1	15	30	45
Refractive index 25°C	1.4707	1.4702	1.4692	1.4685	1.406	1.4700	1.4690	14683
Color Yellow	35	35	35	35	35	35	35	3.5
Red	3.0	2.8	2.5	2.4	3.0	2.9	2.7	2.6
Blue	2.1	2.0	1.8	1.5	2.2	2.1	1.9	1.7
Uvabsotbance 232 nm	0.85	0.90	0.93	0.95	0.85	0.93	0.96	0.97
Uvabsotbance 270 nm	0.050	0.054	0.058	0.061	0.052	0.062	0.068	0.073
Free fatty acid (as oleic acid)	0.25	0.23	0.20	0.18	0.25	0.26	0.30	0.35
Peroxide value (meq/kg)	3.37	3.96	4.42	5.75	3.38	4.21	4.63	6.43
Unsaponifiable matter	1.39	1.35	1.33	1.30	1.38	1.34	1.30	1.26
Total tocopherol content	172.52	166.43	153.61	140.33	172.30	162.05	150.2	137.40
Stability (hours)	28.2	26.90	25.4	23.82	28.1	26.52	25.0	22.41

Table (5): Effect of diazinon insecticide on physical and chemical properties of virgin olive oil of picual variety.

Treatment (days) / Properties	Control				Diazinon			
	1	15	30	45	1	15	30	45
Refractive index 25°C	1.4705	1.4701	1.4695	1.4689	1.4705	1.4698	1.4692	1.46855
Color Yellow Red Blue	35 3.0 2.2	35 2.9 2.1	35 2.7 1.8	35 2.3 1.6	35 3.5 2.3	35 3.0 2.2	35 2.8 2.0	35 2.5 1.8
Uvabsotbance 232nm Uvabsotbance 270nm	0.80 0.043	0.84 0.052	0.90 0.050	0.92 0.068	0.81 0.043	0.86 0.060	0.93 0.064	0.94 0.072
Free fatty acid (as oleic acid)	0.27	0.25	0.21	0.19	0.26	0.28	0.34	0.37
Peroxide value (meq/kg)	3.01	3.8	4.23	5.42	3.05	4.15	5.42	6.66
Unsaponifiable matter	1.36	1.34	1.30	1.28	1.37	1.32	1.2	1.22
Total tocopherol content	169.5	155.21	138.80	125.36	168.80	152.45	134.9	122.62
Stability (hours)	24.85	23.51	22.05	21.25	24.82	22.95	21.85	20.65

Effect of diazinon insecticide on the fatty acids composition of oils:

Results in tables (6 and 7) show that the effect of diazinon on the fatty acids composition of virgin olive oils (Koranaki and Picual vars.) at different intervals after insecticide application (1 and 45 days), compared with fatty acid composition of virgin olive oils (control samples). These results ascertained no effect on fatty acid composition of samples after one day of insecticide application compared with the control samples (zero). On the other hand, after 45 days of insecticide diazinon application a slight increase occurred in saturated fatty acid ($C_{16:0}$ and $C_{18:0}$) and caused a slight decrease in unsaturated fatty acid $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$) compared with the control samples (45 days).

Table (6): Effect of diazinon insecticide on fatty acid composition of virgin olive oil of koranaki variety.

Fatty acid %	Control		Diazinon	
	Zero	45 days	(After one day) Zero	45 days
$C_{14:0}$	0.62	0.54	0.63	0.66
$C_{16:0}$	13.26	11.25	13.22	14.32
$C_{18:0}$	1.96	1.33	1.96	2.43
$C_{18:1}$	70.56	72.61	70.62	68.74
$C_{18:2}$	13.21	14.06	13.17	13.42
$C_{18:3}$	0.39	0.21	0.4	0.23
Total saturated F.A	15.84	13.12	15.81	17.41
Total unsaturated F.A.	84.16	86.88	84.36	82.59

Table (7): Effect of diazinon insecticide on fatty acid composition of virgin olive oil of picual variety.

Fatty acid %	Control		Diazinon	
	Zero	45 days	(After one day) Zero	45 days
$C_{14:0}$	0.58	0.53	0.60	0.65
$C_{16:0}$	13.68	13.25	13.70	14.50
$C_{18:0}$	1.93	1.55	1.97	2.44
$C_{18:1}$	77.63	78.69	77.60	76.75
$C_{18:2}$	6.18	5.75	6.13	5.45
$C_{18:3}$	-	0.23	-	0.21
Total saturated F.A	16.19	15.33	16.27	17.59
Total unsaturated F.A.	83.81	84.67	83.73	82.41

CONCLUSION

The olive fruit oil of picual variety after 45 days from application contained no detectable residues of the diazinon pesticide, but koranaki variety contained 0.02 ppm residues of this pesticide.

REFERENCES

A.O.A.C (1990). Official methods of analysis of the Association of Official Analytical Chemists, Fifteenth ed., Published by the Association of Official Analytical Chemists, Arlington Virginia, USA.

Dubois, M.A., W.F. Gedde and F. Smith (1960). The carbohydrates of the gromincae. A Quantitative study of carbohydrates of wheat germ, Cereal Chem., 37, 557.

EC (European communities) regulation (2006). http:/mrldatabase.con/query.cfm.(8/2/2006)

FAO/WHO (1970). Recommended international standard for olive oil, virgin and refined and refined olive residue oil. Codex Almentarius commission CAC/RS 33-1970. Food and Agriculture Organization. World heath organization, Rome, Italy.

Hegazy, M.E.A., A. M. R. Afify, A.A. Hamama and T.F. A. El-Refahey (2004). Effect of food processes pesticides in cucumber fruits. Egypt. J. Agric. Res., 82 (2), 525-542.

IOOC (2001). Method of Analysis of the International Olive oil Council. Preparation of the fatty acid methyl esters from olive oil and olive-pomace oil. Col/T. 20/Doc. No. 24.

Lentza-Rizos, C.H. (1999). Pesticide residues in olives and olive oil from a European perspective. In pesticides in food in Mediterranean countries P. 125-145.

Lentza-Rizos, CH and E.J. Avramides (1995). Pesticides residues, in olive oil. Reviews of Enviromental Contamination and Toxicology, 144, 111-134.

Lopez-villalta, M. C. (1999). Influence of pests and Diseases on olive oil quality (179-196) P. in: olive pest and disease management, published by looc, Madrid, spain.

Mendez, E., J. Sanhveza, Il. Speisky and A. Valenzvela (1997). Comparison of oxidative stability of fish oil, J. Am. Oil chem.. Soc., 74 (3) 331-332.

Ministry of Agriculture, Recommendation, A. R.E. (2001). Pest control program (in Arabic).

Molinari, G.P., S. Cavanna and L. fornara (1998). Determination of fenthion and its oxidative metabolites in olives and olive oil: errors caused by matrix effects. Food Additives and contaminant vol. 15, (6), 661-670.

Montemurro, N., P. Benedetto and G. Lacertosa (2002). Quality of olive oils production in Basilicata Region Chemical Characteristics Investigations. Acta Hort. 586: SHS 533-536.

Nasr, I.N. and M.E. Hegazy (2002). Residues and half-lives of certain insecticides on and in some vegetables under field condition. Egypt, J. Agric. Res. (In press.).

Parlati, M.V. Pandolfi, S. Leandri, A., Pompi, V. And L., Forchelli (2000). Provedi controllo di Bactrocera olea (Gme L.) in Italia centrale: efficacia residui sulle olive nell'olio. Atti Giornate Fitopatologiche 1: 181-188.

Rastrelli, L., K. Totaro and F. Desimone (2002). Determination of organophosphorus pesticide residues in cilento (campania it aly) virgin olive oil by capillary gas chromatography. Food chemistry 79 (1) 303-305.

Tsimidou, M. (1998). Polyphenols and quality of virgin olive oil in retrospect. Italian Journal of food science. X2: 199-116.

Wong, L.M., J.E. Timms, E. Rand and ME. Goh (1988). Colorimetric determination of total tocopherols in palm oil, J. Am. Oil Chem. Soc. 65 (2), 318-321.

تقدير متبقيات المبيدات فى ثمار وزيت الزيتون

عزة عبد الله أحمد – ناهد محمد محروس عطا

قسم بحوث الزيوت والدهون – معهد بحوث تكنولوجيا الأغذية

مركز البحوث الزراعية – جيزة – مصر

الملخص العربى:

تناول هذا البحث دراسة بقاء المبيد الحشرى ديازينون على ثمار وزيت الزيتون صنف كروناكى وبيكوال وذلك تحت الظروف الحقلية وقد تم أخذ العينات بعد ١، ١٥، ٣٠، ٤٥ يوم من المعاملة بالرش وقد وجد الآتى:

- **أشارت النتائج إلى حدوث فقد مستمر للمتبقيات بمرور الوقت.**
- **متبقيات المبيد ديازينون كانت أعلى فى الصنف كروناكى عن الصنف بيكوال سواء كان فى الثمار أو الزيت.**
- **تحققت حالة الأمان للمستهلك بعد ٤٥ يوم من المعاملة بالرش للمبيد ديازينون.**
- **كما أوضحت النتائج أن المبيد ديازينون ليس له تأثير واضح على التركيب الكيميائى للثمار وعلى الخواص الطبيعية والكيميائية لزيت الثمار موضع الدراسة.**