Residue Analysis of Difenoconazole, Emamectin Benzoate and Fenazaquin on Tomatoes Using High Pressure Liquid Chromatography

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ABSTRACT

A supervised trial was conducted on tomato fruits to study the dissipation rates of three pesticides, difenoconazole (Score 25%EC), emamectin benzoate (Proclaim 5% SG) and fenazaquin (Pride 200, 20% SC) at Shanessa Village, Dhakahlia Governorate, Egypt. The tested pesticides were sprayed at recommended doses of 50 ml, 60 g and 300 ml in 100 liters water, for difenoconazole, emamectin benzoate and fenazaquin respectively on the tomato plants after three months of cultivation. The treated tomato fruits were randomly sampled in triplicates (100g per field replicate) after 1 hr, 1, 3, 6, 10, and 15 days period after pesticide application. Samples were extracted, cleaned up and then analyzed using HPLC method. The half-life values were calculated to be 3.16, 0.6 and 2.4 days for difenoconazole, emamectin benzoate and fenazaquin, respectively. The pre-harvest intervals (PHI) were determined to be 8, 3 and 1 days for tomatoes treated with difenoconazole, emamectin benzoate and fenazaquin under prevailed local field conditions, respectively.

INTRODUCTION

Egypt has a very suitable climate for growing a range of vegetable crops. Egypt is considered as one of the largest producers and consumers of vegetables. To maintain a higher agricultural productivity which appears inevitable as the increasing demand for food as a result of population increase in Egypt, pesticides seem to be a must to gain a high value of crop production (Abou Zeid *et al.*, 1993). Pesticides are necessarily applied to agricultural crops throughout the entire world to combat different pests, insects (Antonious, 2004), mites (Pereira, *et al*., 2005), fungi (Guan, *et al*., 2005), weeds (Owen and Zelaya, 2005) and nematodes (Giannakou and Karpouzas, 2003).

The tested pesticides, difenoconazole, emamectin benzoate and fenazaquin are registered and recommended in pest control program in Egypt to protect tomatoes *Lycopersicon esculentum var*. against different pests including *Alternaria Solani*, the Egyptian leaf worm, *Spodoptera littoralis* (Boisd) and red spider mite, *Latrodectus hasseltii*. On the other hand, the investigated pesticides possess limited biological persistence and are characterized by a very low mammalian toxicity (Codex, 2006).

Extensively use of pesticides in modern agriculture to combat plant pests has begun to receive much attention because pesticide residues in food commodities may be hazardous to human health (Mansour, 2004). It is well known that the pre-harvest intervals are critical periods before consuming food commodity (Lukassowitz, 2008). Thus, during the last two decades, considerable emphasis has been laid on increasing production vegetable to enhance export capabilities (Abou-Arab and Abou-Donia, 2001). However, the development of the export market is hindered by concerns about pesticide residues and inadequate monitoring programs (Karanth, 2002).

The present study, aims to determine the residue levels of the tested pesticides, difenoconazole, emamectin benzoate and fenazaquin on tomatoes at recommended dose. Also, the study aims to determine the pre-harvest intervals for the mentioned pesticides to avoid health hazards and to facilitate the national and international trade.

MATERIALS AND METHODS

Tested Pesticides:

Difenoconazole (Cis, Trans-3-chloro-4-[methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl-4-chlorophenyl ether) was provided as emulsifiable concentrate (Score 25%EC) obtained from Singenta Agro. Egypt.

Emamectin benzoate is a mixture containing 90% of (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R, 21R,24S)-6'-[(S)-sec-butyl]-21,24-dihydroxy- ,11,13,22-tetramethyl - 2 - oxo - 3,7,19 - trioxatetracyclo [15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'H-pyran)-12-yl 2,6-dideoxy-3-O-methyl-4-O-(2,4,6-trideoxy-3-O-methyl – 4 - methylamino-α-L-lyxo-hexopyranosyl)-α-L-arabino - hexopyranoside and 10% of (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S) - 21,24 - dihydroxy-6'-isopropyl-5',11,13,22-tetramethy l -2 - oxo-3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}] pentacosa -10,14,16,22-tetraene-6-spiro-2'- (5',6'-dihydro - 2'H - pyran)-12-yl 2,6-dideoxy-3-O-methyl-4-O- (2,4,6 - trideoxy-3-O-methyl-4-methylamino-α-L-lyxo-hexopyranosyl)-α-L-arabino-hexopyranoside. It was

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formulated as solid granules (Proclaim 5% SG) purchased from Singenta Agro. Egypt.

Fenazaquin (4-tert-butylphenethyl quinazolin-4-yl ether was used as suspension concentrate (Pride 200, 20% SC) produced by Sametrid Egypt.

Certified reference pesticides, difenoconazole and emamectin benzoate were purchased from Singenta Agro. (Swaziland), while fenazaquin was produced by Guan CIS (Portugal). The purity of these pesticides was 95%. These reference pesticides were used for HPLC standardization.

Field Experiments:

Tomato crops were planted at Shanessa Village, Dhakahlia Governorate, Egypt in April 19, 2008 in plots of 0.05 Fadden. Tomato plants were sprayed in July 25th,2008 under prevailed field temperature (approximately, 33°C). Untreated plots were left as control check. Difenoconazole (Score 25%EC), emamectin benzoate (Proclaim 5% SG) and fenazaquin (Pride 200, 20% SC) were sprayed at the rate of 50 ml, 60 g and 300 ml in 100 liters water respectively. A knapsack hand sprayer fitted with one nozzle boom was used.

Sampling:

Three replicate samples of treated and untreated tomato fruits were randomly picked up one hour and then 1, 3, 6, 10, and 15 days after pesticides spraying for residue determination. Each sample was chopped and divided into three sub samples (100 g) which were stored in individual polyethylene bags at -20 °C until residue analysis.

Methods of Analysis:

A-Extraction and Clean up Procedures for Difenoconazole and Emamectin benzoate

The frozen samples were left out to reach room temperature, then, macerated using Waring blender. One hundred grams of each macerated sample was placed in the blender and 200 ml of ethyl acetate was added to the blender and mixed thoroughly for 3 min., then filtered through a dry cotton pad into graduated cylinder. The filtrates were transferred into separatory funnel followed by an addition of 40 ml sodium chloride solution (20%) and then extracted three times with 50 ml redistilled methylene chloride. The organic phase was passed through cotton and anhydrous sodium sulfate. The filtrates were concentrated almost to dryness at 40?C using rotary evaporator.

Each of difenoconazole and emamectin benzoate residues was dissolved in 5 ml methanol and cleaned up according to Johnson, (1963) method with minor modification using coagulating solution (0.5g ammonium chloride and 1ml 85% orthophosphoric acid solution in 400 ml distilled water). The extract was thoroughly mixed with 10 ml of cooled freshly prepared coagulating solution and quantitatively transferred, and then filtered through a chromatographic columns of 2.5cm diameter packed with a 5cm layer of Hyflo-supercell. The column was eluted three times using a mixture of 5ml methanol and 10ml coagulating solution. The filtrates were then collected in 250 ml separatory funnel and partitioned with 3 x 50ml methylene chloride. The final extract was concentrated using rotary evaporator to dryness and dissolved in known volume of ethyl acetate prepared for HPLC analysis.

B- Extraction and Clean up Procedures for Fenazaquin

Tomatoes treated with fenazaquin were extracted according to Steinwandter (1985). Acetone (100 ml) was added to 100 g of fruit sample and the mixture was homogenized using Waring blender at high speed for 3 min. Thirty grams of sodium chloride and 150 ml dichloromethane were added to the contents and re-blended for 1-2min. The organic phase was filtered through anhydrous sodium sulfate on cotton pad and concentrated using rotary evaporator at 40°C to approximately 5ml.

To these contents, 5ml dichloromethane was added, mixed with 0.5g of activated charcoal, and then shaken for 2 min. The mixture was filtered through filter paper and the supernatant rinsed with 25ml dichloromethane (Al- Samariee *et al.,* 1988). The filtrates were collected and concentrated almost to dryness using a rotary evaporator at 40°C; the volume was adjusted to 2 ml for all samples and analyzed using HPLC.

C- Recovery Efficiency Studies:

The reliability of the analytical methods was tested by fortifying untreated samples with known quantities of the investigated pesticides, difenoconazole, emamectin benzoate and fenazaquin at 0.1 ppm levels, followed by the same procedures of extraction, clean-up and quantitation.

D- Chromatographic Determination of Tested Pesticides:

Samples were analyzed for determining the level of pesticide residues using HPLC, under the following conditions in Table (1).

RESULTS AND DISCUSSIONS

Dissipation of Tested Pesticides on Tomato Fruits:

The efficiency of analytical procedure was evaluated via determination of percent recovery of fortified samples at the level of 0.1 ppm for tested pesticides. The average recovery values were 86.9%, 78.95% and 89.2% for fortified tomato fruit samples

Table 1. High Pressure Liquid Chromatography Conditions For Different Tested Pesticides

Analytical Parameter	Value Corresponding to Each Parameter		
	Difenoconazole	**Emamectin benzoate**	**Fenazaquin**
1- Models	Agilent 1100 series	Agilent 1100 series	Agilent 1100 series
2- Column			
A) Type	ODS C18 Hypersil	ODS C18 Hypersil	ODS C18 Hypersil
B) Dimensions	4 mm (i.d) × 150 mm length	4 mm (i.d) × 150 mm length	4 mm (i.d) × 150 mm length
3- Detector			
A) Type	Diode Array Detector	Diode Array Detector	Diode Array Detector
B) wavelength	254 nm	245 nm	230 nm
4- Mobile phase			
A) Type	60% Acetonitrile + 40% Water	45% Acetonitrile + 40% Methanol+15%Water	50% Acetonitrile + 50%Water
B) Flow rate	0.8 ml/min.	0.5 ml/min.	1.0 ml/min.
5- Absolute retention time	2.78 min.	1.6 min.	2.8 min

treated with difenoconazole, emamectin benzoate and fenazaquin, respectively. These values are comparable with those obtained by Steinwandter, (1985); Al-Samariee *et al.,* (1988), Sannino *et al.,* (2004) and Frenich, *et al.,* (2008). It was reported by Hirahara *et al.,* (2005) that recoveries of 128 pesticides were > 70% at the level of 0.1 ppm using extracting method which is almost similar to that in the present study.

1. Residues of Difenoconazole:

Difenoconazole is a broad-spectrum fungicide being used for pathogen control in many fruits, vegetables, cereals and other field crops (NRS, 2006). Difenoconazole acts by inhibition of demethylation during ergosterol synthesis (FSANZ, 2007).

The data representing the residue levels of difenoconazole on tomato fruit was depicted out in Table (2). Such data indicate that the initial concentration was 1.77 ppm on fruit samples one hour after difenoconazole (25% EC) application at the rate of 50ml/100L water. The levels of difenoconazole residues on tomato fruits were 1.47, 0.93, 0.62, 0.02 and 0.01 ppm after 1, 3, 6, 10 and 15 days of treatment, respectively. Also, the data indicate that the persistence of difenoconazole decreased to 0.57% of the initial concentration after 15 days of application (Fig. 1). The half life time ($t_{0.5}$) of difenoconazole was calculated to be 3.16 days. The data show that tomato fruits could be safely consumed after 8 days of application according to the recommended maximum residue limit (MRL) for difenoconazole in tomato (0.5 ppm) (JMPR, 2007).

2. Residues of Emamectin benzoate:

Emamectin benzoate is semi-synthetic derivative from abamectin (Prabhu, *et al*., 1991). It is registered as an acaricide for different food commodities in different countries (Yoshii, *et al*., 2004).

The residue and degradation rate of emamectin benzoate (5% SG) in tomato fruits were presented in Table (3) and Fig. (1). The residue levels in fruit samples were 0.22, 0.05, 0.01 and 0.01 ppm after 1 hour, 1,3 and 6 days, respectively. No emamectin benzoate was detected in samples after 10 and15 days of application. The results show that emamectin benzoate

Table 2. Residue Levels of Difenoconazole on Tomato Fruits at Different Time Intervals

Time Intervals	Residue Concentration ppm (± S.D)	Dissipation (%)
1 hour	1.77 (± .032)	0.00
1 day	1.47 (± 0.021)	16.94
3 days	0.93 (± 0.026)	47.45
6 days	0.62 (± 0.01)	64.97
10 days	0.02 (± 0.01)	98.87
15 days	0.01 (± 0.006)	99.43
***$t_{0.5}$**	3.16 days	
MRL	0.5 ppm (JMPR, 2007),	
PHI	8 days	

*The half-life value ($t_{0.5}$) was calculated using following equation according to El-Henawy (1992): $t_{0.5}$ = ln2 / K = 0.693 / K (K is reaction rate constant)

Table 3. Residue Levels of Emamectin benzoate on Tomato Fruits at Different Time Intervals

Time Intervals	Residue Concentration ppm (± S.D)	Dissipation (%)
1 hour	0.22 (± .032)	0.00
1 day	0.05 (± 0.021)	77.27
3 days	0.01 (± 0.026)	95.45
6 days	0.01 (± 0.01)	95.45
10 days	*ND	100
15 days	*ND	100
$t_{0.5}$	0.6 day	
MRL	0.02 ppm (Codex, 2003)	
PHI	3 days	

*ND: Not detectable

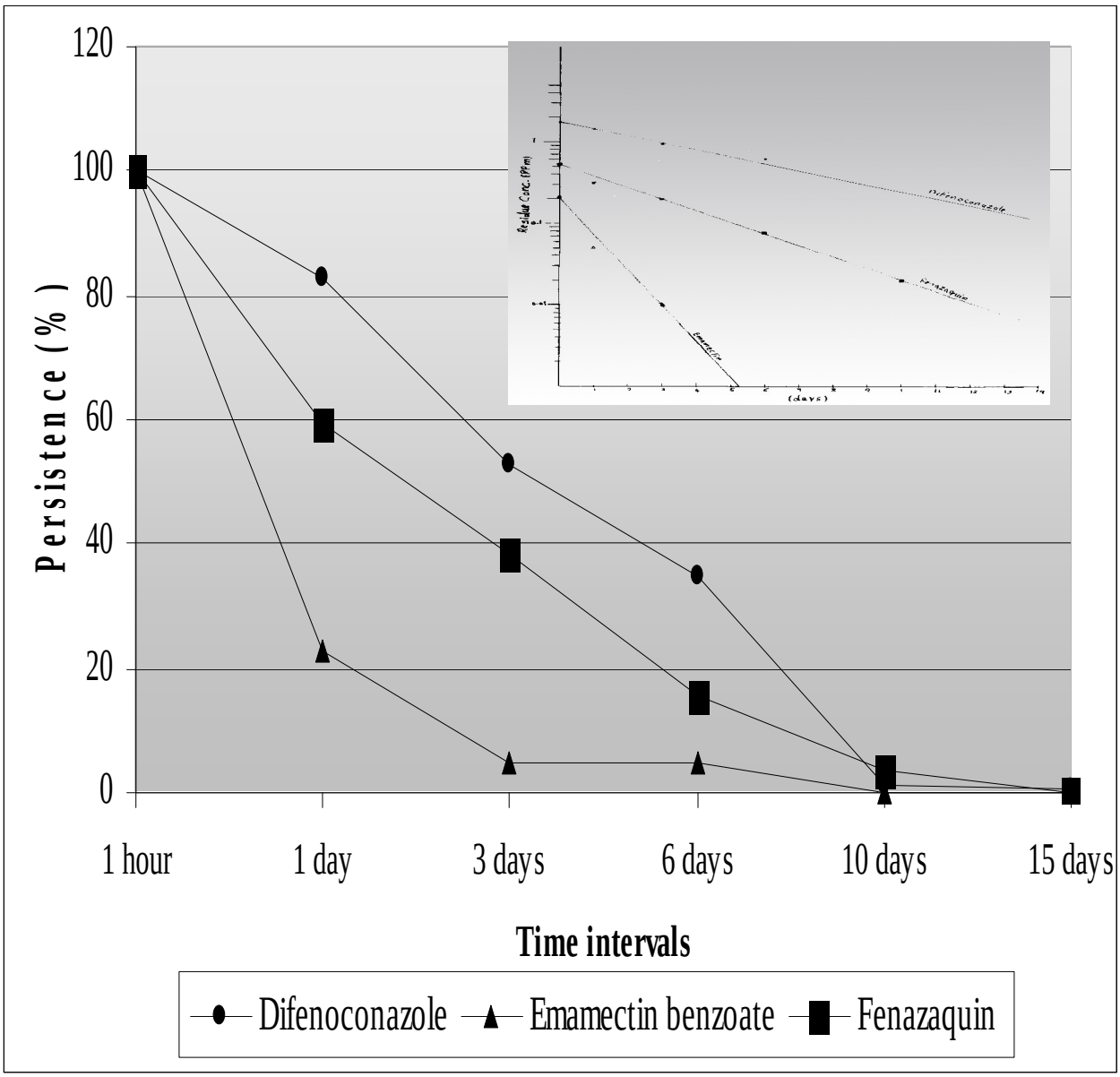

Figure 1. Disappearance Curves of Tested Pesticides on Treated Tomatoes

residues were decreased rapidly by time. The data indicate that the half life time ($t_{0.5}$) value of emamectin benzoate was 0.6 day. The maximum residue limit (MRL) for emamectin benzoate recommended by Codex, (2003) in tomato is 0.02 ppm. Data indicated that tomatoes could be consumed safely after three days.

3. Residues of Fenazaquin:

Fenazaquin is a non-systemic acaricide/insecticide and being used widely in control mites and other related pests in fruits, vegetables and tea (Kumar, *et al.,* 2006).

Tomato crop was treated with 300ml/100L water from fenazaquin (20% SC) as an acaricide against red spider mite, *Latrodectus hasseltii*. The results in Table (4) show that initial concentration of fenazaquin on tomato was 0.52 ppm after 1 hour of application. The residue levels of fenazaquin in the mature fruit of treated tomato crop decreased at 1, 3, 6 and 10 days after treatment to 0.31, 0.20, 0.08 and 0.02 ppm, respectively, while it was at 15 days undetectable limits. The persistence of fenazaquin in fruit sample decreased to become 3.85% of the initial concentration at 10 days of application (Fig. 1). The half life value ($t_{0.5}$) of fenazaquin was 2.4 days. The results indicate that tomatoes treated with fenazaquin could be consumed safely after one day of application, where MRL for fenazaquin in tomato crop is 0.5 ppm according to JMPR, (2007).

In recent investigations, a high performance liquid chromatographic (HPLC) method was applied for residue determinations of tested pesticides, difenoconazole, emamectin benzoate and fenazaquin in tomato crop. Kmell?r, *et al.,* (2008) carried out a validation study on tomato by using a rapid and sensitive liquid chromatographic method for the determination of 160 selected multi-class pesticides including the tested pesticides. Also, Liquid chromatography was reported for determining emamectin (Yoshii, *et al.,* 2001 & 2004), fenazaquin (Kumar, *et al.,* 2006) and certain pesticide residues in vegetables (Khan, *et al.,* 2009).

The present results indicate that difenoconazole was found to be more persistent on tomato compared with other two tested pesticides, data also reported that the lowest residue level (0.01 ppm) in tomato fruit was detected after 15 days of difenoconazole application, these results were supported by Sannino *et al*., (2004) who determined 24 new pesticides residues including difenoconazole on tomato by using liquid chromatography-electrospray ionization.

The present study also indicates that the residues of emamectin benzoate on tomatoes were rapidly decreased. These results are supported by Yoshii, *et al.,* (2001 & 2004) who determined the residues of emamectin and its metabolites, in tomato and Japanese radish by liquid chromatography, and found that emamectin rapidly degraded to its metabolites.

The present investigation reveals that, dissipation of fenazaquin on tomato fruits decreased to 0.02 ppm after 10 days of treatment and its half life time ($t_{0.5}$) was 2.4 days. This data is in agreement with those obtained by Kumar, *et al.,* (2006) who investigated the disappearance trend in tea crop treated with fenazaquin under field conditions. They cited that fenazaquin residues decreased sharply to minimum levels and the half-life values in green shoots were in the range of 1.43-1.70 and 2.10-2.21 days for wet and dry seasons, respectively.

Based on the dissipation pattern of tested pesticide residues in relation to their respective prescribed maximum residue limits, PHI values are 8, 3 and 1 days suggested for tomato treated with difenoconazole, emamectin benzoate and fenazaquin, respectively. A maximum residue level is only accepted if it is guaranteed that the concentration does not have any harmful effects on human health according to the latest scientific findings available. If these maximum residue levels are complied with, then the products are "safe" within the meaning of consumer health protection (Lukassowitz, 2008).

Table 4. Residue Levels of Fenazaquin on Tomato Fruits at Different Time Intervals

Time Intervals	Residue Concentration ppm (± S.D)	Dissipation (%)
1 hour	0.52 (± 0.020)	0.00
1 day	0.31 (± 0.006)	40.38
3 days	0.20 (± 0.015)	61.53
6 days	0.08 (± 0.010)	84.62
10 days	0.02 (± 0.001)	96.15
15 days	*ND	100
$t_{0.5}$	2.4 days	
MRL	0.5 ppm (JMPR, 2007)	

PHI	one day

*ND: Not detectable

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الملخص العربي

تحليل متبقيات الدايفنوكونازول والإيماميكتين بنزوات والفينازكوين على الطماطم باستخدام التحليل الكروماتوجرافي السائل ذو الضغط العالي

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اجريت تجربة على ثمار الطماطم في قرية شانيسا بمحافظة الدقهلية– مصر لدراسة معدل تحطم واختفاء ثلاثة مبيدات، الدايفنوكونازول (سكور٢٥% مركز قابل للإستحلاب) والإيماميكتين بنزوات (بروكليم ٥%حبيبات صلبة) والفينازكوين (برايد٢٠٠، ٢٠% مركز معلق). حيث تم رش المبيدات المختبرة بالجرعات الموصي بها لكل منها وهي بمعدل ٥٠ مل، ٦٠ جم و ٣٠٠مل في١٠٠لتر ماء للدايفنوكونازول و الإيماميكتين بنزوات و الفينازكوين على التوالي، وذلك على نباتات الطماطم بعد ثلاثة أشهر من الزراعة. وقد تم أخذ عينات عشوائية من ثمار الطماطم المعاملة بالمبيدات المختبرة في ثلاث مكررات(١٠٠جم لكل مكررة حقلية)وذلك على فترات زمنية بعد ساعة، ١، ٣، ٦، ١٠و ١٥ يوم من المعاملة بالمبيدات. تم استخلاص وتنقية العينات وتحليلها باستخدام الكروماتوجرافي السائل ذات الضغط العالي(HPLC). وقد كانت قيم نصف العمر للمبيدات المختبرة هي٣,١٦، ٠,٦ و٢,٤ يوم لكل من الدايفنوكونازول و الإيماميكتين بنزوات و الفينازكوين على التوالي. أما فترات ما قبل الحصاد (PHI) فقد قدرت فكانت ٨، ٣ و١ يوم للطماطم المعاملة بالدايفنوكونازول و الإيماميكتين بنزوات و الفينازكوين على التوالي.