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TECHNOLOGICAL AND MICROBIAL STUDIES ON SOME OXAMYL TREATED VEGETABLE CROPS

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ABSTRACT

This study was directed to investigate the impact of some technological processes on residue levels of Oxamyl and microbial counts in fresh and preserved kidney bean, squash and tomato fruits. Gas chromatography with electron capture detector (ECD) was used to determine Oxamyl residues on treated vegetable crops grown under plastic tunnels at zero time, 7 and 15 days of the application. The influences of technological processes on microbial counts of untreated and treated fresh and preserved vegetables were studied as well. Results revealed that washing of treated vegetables eliminated 23-42% at zero time, 41-54% after 7 days and reached to 63% of Oxamyl residues after 15 days of application to be within the established Maximum Residue Limits (MRL) of Oxamyl. Blanching, freezing and canning of treated washed vegetables occurred reductions ranged from 37% to 64% in residue levels to be lower than MRL of Oxamyl. Storage of preserved products minimized the remained residues to be mere traces in comparison with MRL of Oxamyl. The obtained data indicated that microbial counts of untreated and treated vegetable crops were significantly affected after washing, blanching, freezing, canning and after the experimental storage period of preserved products.

INTRODUCTION

Vegetable crops are the most important crops in the world because of their high nutritional value. In recent times, a lot of agrochemicals for plant protection and production are widely used throughout the world. Although pesticidal chemicals provide numerous benefits in terms of increased production and quality of the product, pesticides residues in the environment are of concern to everyone everywhere. Despite of all debates concerning pesticides to the contrary, the essentially of pest-control chemicals to adequate food production, manufacture, marketing and storage, especially in developing countries is out of questions. However, as a result of continuous surveillance, the Integrated Pest Management (IPM) Programs may help to answer the problems of plant foods contamination with different pesticidal chemicals for reducing residue levels on or in plant foods to be within the acceptable daily intake and lower than the safety tolerance level for protecting human health.

The technological processing and preservation of such contaminated plant foods play a great role in reducing the residue levels of many agrochemicals to be within or lower than the established MRL, and to be valid for human consumption without dangerous side effects as well (Bessar, 1984, Elkins, 1989, Bessar, 1992, Bessar and Tag El-Din, 1996).

The intent of the present study was to investigate the effect of some technological processes on residue levels of Oxamyl and microbial counts in fresh and preserved kidney bean, squash and tomato fruits grown under plastic tunnels.

MATERIALS AND METHODS

1. Raw material:

Green kidney bean, squash and tomato plants were grown at Rashid area, Behira Governorate under unheated plastic tunnels (50 x 7.5 x 2.5 m. dimension), these plastic tunnels erected in orientation North/South that produces better ventilation. Monthly average air temperature and relative humidity percentage under plastic tunnels during the experimental period (March and April, 2001) were as follow:

Month	Air temperature		Relative humidity percentage
	6 Am	12 Noon	
March	7.8	19.9	80.1
April	9.7	20.4	76.6

2. Pesticide:

Oxamyl [N, N-dimethyl-2-methyl carbamoyloxy-imino-2- (methylthio) acetamide] "Vydate" Insecticide/Nematicide, water-soluble liquid, 240 g a.i/L) was obtained commercially with high purity. Oxamyl is recommended to apply on green kidney bean, squash and tomato for its high effectiveness in controlling a broad range of insect and mite species and its short remaining time after application.

3. Treatment:

Vegetable plants of green kidney bean, squash and tomato were sprayed with the recommended field rate of Oxamyl 24% (5 ml/liter water) before 15 days of harvest time using knap sack sprayer.

4. Sampling:

Samples (2 kg) of treated vegetable fruits were randomly collected at the intervals one hour after application (zero time or initial deposit), 7 and 15 days post-treatment for residue determination and microbial counts as well.

Untreated fruits of the same vegetables were collected before spraying to serve as control samples. Contaminated fruits of each interval were divided into two parts with ratio 1: 4. The first part (one quarter) was kept without washing (as reference residue level) and the second part (three quarters) was washed with running tap water and left to dry for the technological procedures. Washed treated fruits divided into 3 portions for blanching, freezing and canning according to Saad *et al.* (1989). Preserved products of these vegetables were stored for three months. Oxamyl residues, microbial counts, molds and yeasts were measured in processed vegetables before and after the experimental storage period.

5. Residue analysis:

Oxamyl residue was chromatographically determined according to the method of Steinwander (1984). Extraction and clean up of Oxamyl residues were run according to Tag El-Din (1993).

6. Recovery values of Oxamyl residue:

The efficiency of the gas chromatographic analysis for Oxamyl residues on investigated vegetable fruits was run by adding 1 mg of pesticide/gm sample to a portion of untreated fruits and then these samples were put through the same procedures followed for residue determination. Average recovery value for Oxamyl was calculated as follow:

$$\text{Recovery value} = \frac{\mu g\ CS2/250\ ml\ sample\ solution}{\mu g\ CS2/250\ ml\ reagent\ solution} \times 100$$

Average recovery values of Oxamyl added to untreated portions of green kidney bean, squash, and tomato were 85.1%, 91.7% and 93.1%, respectively. All the obtained residue values were referred to calibration standard curve and corrected according to recovery value of oxamyl.

7. Microbial counts:

Total plate count was carried out using plate count agar (Difco) at the incubation temp. of 30°C for 48 hours according to Gilliland *et al.* (1976). Molds and yeasts were counted according to procedures of El-Demiry (1990). The changes in microbial counts, molds and yeasts of untreated and oxamyl treated vegetables were determined in preserved vegetable crops before and after storage period for three months.

RESULTS AND DISCUSSION

Influence of washing on Oxamyl residue levels on green kidney bean, squash and tomato:

Data of Table (1) show the role of washing with tap water in removing oxamyl residue from green kidney bean, squash and tomato at different waiting periods. Washing the contaminated vegetables grown under plastic tunnels remove major portions of Oxamyl residues that persisted on green kidney bean, squash and tomato fruits. Washing removed 23%, 39% and 42% of the initial residues found on unwashed green kidney bean, squash and tomato, respectively, after one hour of spraying (zero time), as well as, 54, 42 and 41%, respectively, of the initial residues found on unwashed treated vegetables were removed by washing the same mentioned vegetables after 7 days of oxamyl application. Washing of treated vegetables collected after the recommended waiting period (15 days) removed 54%, 63% and 55%, respectively, of the initial residues found on unwashed fruits of the same mentioned vegetable crops that grown under plastic tunnels. So, waiting period for 15 days after Oxamyl application was enough for safe consumption -after washing- all the experimental contaminated vegetables to minimize Oxamyl residues on green kidney bean, squash and tomato to be within the established MRL of Oxamyl (2 mg.kg) by Codex Alimentarius Commission (1993).

According to the obtained data (Table 1) and remarks of Elkins (1989), and Bessar and Tag El-din (1996) removal of pesticide residues by washing depends on several factors: character of the surface of the plant foods (Smooth of rough, waxy or non-waxy); Surface to volume ratio (washing is effective for bigger fruits); reference point of residue levels (higher levels easier to remove); chemical and physical properties of the applied pesticides; the length of time that the pesticide has been in contact with the plant foods: rate and number of applications and penetrability of pesticide into fruit tissues. Also, the obtained results are in agreement with findings of Bessar (1984), Elkins (1989), Tag El-Din (1990), Bessar (1992), Dogheim *et al.* (1993), Tag El-Din (1993), Bessar *et al.* (1994 a & b), Bessar and Tag El-Din (1996) and Tag El-Din and Salama (1996).

Table (1): Influence of washing on Oxamyl residue levels on green kidney bean, squash and tomato fruits.

Days after Application	Oxamyl residues (mg. kg)		
	Before washing Mean + SD*	After washing Mean + SD*	R% **
Green kidney bean			
Initial ***	6.92+0.68	5.34+0.91	23
7	4.29+0.55	1.98+0.25	54
15	1.25+0.19	0.58+0.11	54
Squash			
Initial ***	7.46+0.29	4.55+0.89	39
7	2.81+0.88	1.62+0.39	42
15	0.83+0.17	0.31+0.13	63
Tomato			
Initial ***	7.08+0.91	4.12+0.69	42
7	3.07+0.42	1.82+0.47	41
15	0.96+0.18	0.43+0.12	55

* : Data are mean values and standard deviations of 3 parallels.

** : Reduction percent = $\frac{\text{Residues before washing - Residues after washing}}{\text{Residues before washing residues}} \times 100$

*** : Initial residues determined after one hour of application.

Effect of some technological processes on Oxamyl residues in some products of the tested vegetables:

Technological processing of vegetable crops plays a vital role in minimization residue levels of many agrochemicals throughout thermal degradation during the processing of such plant foods. Blanching, canning and freezing as thermal technological processes share with major part in degradation and reduction of Oxamyl residue levels as shown in Table (2). Data indicated that Oxamyl residues were reduced by 57%, 66% and 42%, respectively, by blanching, canning and freezing of washed treated green kidney beans collected at zero time as well as, 61%, 69% and 33% respectively, of residues were reduced by the same mentioned processes after 7 days of treatment. The same trend of residue reduction was recorded after 15 days of Oxamyl application.

As for squash fruits, the main reduction percentage was occurred by the effect of canning where the reduction percents were 54%, 83% and 71% in canned squash at zero time, after 7 days and after 15 days of treatment, respectively and reduced by 37%, 59% and 48% in blanched squash and by 34%, 50% and 39% in frozen squash at the same mentioned intervals.

The same effect of canning, blanching and freezing was observed in tomato, where the reduction percentages ranged from 62% to 75% in canned tomato, from 42% to 49% in blanched tomato and from 23% to 42% in frozen tomato at the same mentioned intervals. The obtained data in Table (3) confirmed that, in spite of the reference points of Oxamyl residues were within its MRL by processing treated fruits after washing immediately post application and were below its MRL (2 mg/kg) by washing treated vegetables after one weeks of application, it could be concluded that thermal technological processes minimized Oxamyl residues to be mere traces in comparison with its MRL and to be very safe for human consumption without any hazardous effects on human beings. Results in Table (3) are in agreement with those findings of Ripley (1978), Newsome (1980), Bessar (1992), Shivankar and Kavadia (1992), and Mondy *et al.* (1993).

Table (2): Influence of some technological processes on Oxamyl residues in some vegetable crops.

Intervals (days)	Before processing	Oxamyl residues mg. Kg					
		After processing					
		Blanching		Freezing		Canning	
		Mean+SD	R%	Mean+SD	R%	Mean+SD	R%
Green kidney bean							
Initial	5.34+0.91	2.31+0.56	57	3.12+0.33	42	1.84+0.23	66
7	1.98+0.25	0.78+0.84	61	1.35+0.68	32	0.61+0.11	69
15	0.58+0.19	0.21+0.23	64	0.33+0.71	58	0.17+0.82	71
Squash							
Initial	4.55+0.89	2.87+0.25	37	3.01+0.89	34	2.08+0.42	54
7	1.62+0.39	0.66+0.19	59	1.81+0.21	50	0.27+0.33	83
15	0.31+0.31	0.16+0.42	48	0.19+0.23	39	0.09+0.03	71
Tomato							
Initial	4.12+0.69	2.09+0.55	49	2.42+0.98	41	1.55+0.48	62
7	1.82+0.47	1.05+0.32	42	1.06+0.31	42	0.46+0.56	75
15	0.43+0.23	0.24+0.21	44	0.33+0.72	23	0.11+0.32	74

Impact of storage of preserved products of tested vegetables on Oxamyl residues:

Role of storage for three months for canned, and frozen green kidney bean, squash and tomato was indicated in Table (3). Data show that great reduction in pesticide residues during the storage period was happened where the residues reduced by 45%, 69% and 71% in canned green kidney bean processed immediately after application and after 7 and 15 days of application, respectively and reduced by 30%, 27% and 36% in stored frozen products of green kidney bean which were frozen at the same mentioned intervals, respectively. As well as, in stored canned squash the residues were reduced by 43%, 59% and 67% and by 32%, 36% and 42% in frozen products at the same three previous intervals, respectively. As for stored tomato products the same trend of residue reduction was observed in canned and frozen tomato that processed at the same intervals post application. The results of Table (3) are in harmony with findings of many authors, i.e. Iwata *et al.* (1981), Bessar (1992), Bessar *et al.* (1994b) and Bessar and Tag El-Din (1996), where the storage for different periods (extended to three months) plays a key role in minimizing the residue levels of Oxamyl in canned and frozen vegetables to be lower than the established MRL of Oxamyl and to be very safe for human consumption without any dangerous side effects.

Table (3): Effect of storage for three months on degradation of Oxamyl residues in stored products of tested vegetables.

Intervals (days)	Oxamyl residues mg. Kg					
	Frozen products			Canned products		
	Before storage	After Storage		Before storage	After Storage	
	Mean+SD	Mean+SD	R%	Mean+SD	Mean+SD	R%
Green kidney bean						
Initial	3.12+0.33	2.17+0.22	30	1.84+0.23	1.02+0.85	45
7	1.35+0.68	0.98+0.37	27	0.61+0.11	0.19+0.41	69
15	0.33+0.71	0.21+0.62	36	0.17+0.82	0.05+0.21	71
Squash						
Initial	3.01+0.89	2.04+0.65	32	2.08+0.42	1.18+0.63	43
7	0.81+0.21	0.52+0.23	36	0.27+0.33	0.11+0.23	59
15	0.19+0.23	0.11+0.42	42	0.09+0.31	0.03+0.43	67
Tomato						
Initial	2.42+0.98	1.84+0.13	24	1.55+0.43	0.93+0.14	40
7	1.06+0.31	0.61+0.73	42	0.46+0.56	0.18+0.25	61
15	0.33+0.72	0.18+0.14	45	0.11+0.21	0.04+0.35	64

R % = 1- (Residues after storage/residues before storage) x 100

Effects of simple washing, processing and storage period of treated vegetables on total microbial counts, molds and yeasts:

The effect of washing with tap water, blanching, freezing and canning for untreated and treated green kidney bean, squash and tomato on microbial counts, molds and yeasts is shown in Table (4). The total microbial counts, molds and yeasts of fresh unwashed, washed, and processed fruits of previous tested vegetables were examined. The obtained results indicated that the total microbial counts; molds and yeasts were higher in untreated vegetables than in treated vegetables. Also total microbial counts, molds and yeasts were higher in unwashed fruits than washed fruits and blanched vegetables immediately after application and after 7 days of application and were very low after 15 days of treatment in frozen and canned products of tested vegetables. These recorded observations may be due to the toxic effect of bounded residues of Oxamyl with tissues of treated vegetables adding to the role of temperature degree used in preservation methods as was previously stated by El-Demiry (1990) and Bessar *et al.* (1994a).

As regards to the changes in microbial counts, molds and yeasts during the storage period for three months for vegetable products Table (5) indicates that with elapsing the time of storage for canned and frozen vegetables microbial counts, molds and yeasts increased in both frozen and canned products for treated and untreated vegetables. The obtained data confirm that the changes in microbial counts, molds and yeasts mainly due to the declination of bounded residues of Oxamyl with plant tissues and to the activity of some micro-organisms which attack plant tissues during the storage. The obtained results are in meeting with those findings of Foda *et al.* (1971), El-Kady *et al.* (1983), El-Demiry (1990), and Bessar *et al.* (1994a).

Table (4): Effect of simple washing and blanching of tested vegetables on total microbial counts, molds and yeasts (cells/gm).

Samples	Untreated samples					Treated samples				
Intervals	Unwashed	Washed	Blanched	Frozen	Canned	Unwashed	Washed	Blanched	Frozen	Canned
Total microbial count										
Green kidney bean										
I	27×10^3	18.3×10^3	8.2×10^3	3.7×10^3	2.80×10^3	13.1×10^3	7.7×10^3	3.0×10^3	2.2×10^3	1.4×10^3
7	22×10^3	15.4×10^3	3.7×10^3	2.82×10^3	1.02×10^3	11.0×10^3	5.2×10^3	1.4×10^3	1.1×10^3	0.48×10^3
165	12×10^3	9.2×10^3	2.1×10^3	0.96×10^3	0.73×10^3	7.5×10^3	4.6×10^3	0.36×10^3	0.22×10^3	0.08×10^3
Squash										
I	25.2×10^3	14.0×10^3	6.1×10^3	3.81×10^3	3.51×10^3	12.3×10^3	7.1×10^3	2.7×10^3	1.6×10^3	1.3×10^3
7	19.7×10^3	11.4×10^3	4.6×10^3	2.96×10^3	1.71×10^3	8.2×10^3	3.6×10^3	0.84×10^3	0.68×10^3	0.51×10^3
165	11.6×10^3	5.2×10^3	2.0×10^3	0.82×10^3	0.44×10^3	5.2×10^3	1.4×10^3	0.42×10^3	0.11×10^3	0.06×10^3
Tomato										
I	24.1×10^3	10.5×10^3	4.6×10^3	3.31×10^3	2.86×10^3	11.4×10^3	6.6×10^3	2.2×10^3	1.11×10^3	0.76×10^3
7	17.2×10^3	9.6×10^3	4.2×10^3	2.26×10^3	0.67×10^3	8.0×10^3	4.1×10^3	0.93×10^3	0.23×10^3	0.11×10^3
165	11.0×10^3	5.1×10^3	2.3×10^3	1.05×10^3	0.43×10^3	5.5×10^3	1.2×10^3	0.24×10^3	0.08×10^3	0.03×10^3
Molds and yeasts										
Green kidney bean										
I	7.2×10^3	3.6×10^3	1.2×10^3	0.43×10^3	0.31×10^3	0.83×10^3	0.5×10^3	0.21×10^3	0.12×10^3	0.08×10^3
7	4.0×10^3	2.3×10^3	0.51×10^3	0.31×10^3	0.11×10^3	0.41×10^3	0.32×10^3	0.05×10^3	0.06×10^3	-
165	1.8×10^3	1.3×10^3	0.17×10^3	0.14×10^3	-	0.23×10^3	0.12×10^3	-	0.04×10^3	-
Squash										
I	6.8×10^3	3.0×10^3	0.98×10^3	0.43×10^3	0.21×10^3	0.8×10^3	0.44×10^3	0.18×10^3	0.12×10^3	0.07×10^3
7	3.8×10^3	2.0×10^3	0.46×10^3	0.22×10^3	0.09×10^3	0.45×10^3	0.03×10^3	0.3×10^3	0.04×10^3	-
165	1.6×10^3	1.1×10^3	0.11×10^3	0.07×10^3	-	0.22×10^3	0.09×10^3	-	-	-
Tomato										
I	6.6×10^3	3.4×10^3	1.1×10^3	0.27×10^3	0.36×10^3	0.82×10^3	0.44×10^3	0.15×10^3	0.14×10^3	0.05×10^3
7	3.2×10^3	2.1×10^3	0.48×10^3	0.12×10^3	0.04×10^3	0.38×10^3	0.22×10^3	-	-	-
165	1.6×10^3	0.98×10^3	0.09×10^3	0.06×10^3	-	0.17×10^3	0.4×10^3	-	-	-

Table (5): Changes in microbial counts, molds and yeasts during storage of frozen and canned vegetables for three months.

Samples	Untreated samples		Treated samples	
Intervals	Frozen	Canned	Frozen	Canned
Total microbial count				
Green kidney bean				
I	6.2×10^3	5.3×10^3	3.1×10^3	2.1×10^3
7	3.4×10^3	2.2×10^3	1.5×10^3	0.87×10^3
15	1.78×10^3	1.1×10^3	0.43×10^3	0.21×10^3
Squash				
I	5.7×10^3	4.8×10^3	2.9×10^3	1.9×10^3
7	3.1×10^3	2.4×10^3	1.2×10^3	0.83×10^3
15	1.32×10^3	0.89×10^3	0.25×10^3	0.17×10^3
Tomato				
I	6.1×10^3	4.5×10^3	2.7×10^3	1.7×10^3
7	3.3×10^3	1.8×10^3	1.3×10^3	0.81×10^3
15	1.26×10^3	0.88×10^3	0.23×10^3	0.15×10^3
Molds and yeasts				
Green kidney bean				
I	0.73×10^3	0.52×10^3	0.23×10^3	0.14×10^3
7	0.38×10^3	0.23×10^3	0.11×10^3	0.09×10^3
15	0.19×10^3	0.11×10^3	0.09×10^3	-
Squash				
I	0.68×10^3	0.43×10^3	0.21×10^3	0.11×10^3
7	0.33×10^3	0.19×10^3	0.07×10^3	-
15	0.15×10^3	0.08×10^3	-	-
Tomato				
I	0.66×10^3	0.44×10^3	0.18×10^3	0.09×10^3
7	0.27×10^3	0.14×10^3	0.05×10^3	-
15	0.12×10^3	0.06×10^3	-	-

In general, it could be concluded that waiting period for 15 days after application and washing with tap water are enough to decline Oxamyl residues to be within the MRL. Also technological processes and storage period play an important role in reduction Oxamyl residues to be mere traces and became very save for human consumption without any hazardous side effects on human health. At the same time pesticide residues bounded with plant tissues caused various changes in microbial activity in comparison with untreated vegetables especially before the storage period.

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دراسة تكنولوجية وميكروبية على بعض محاصيل الخضر المعاملة بمبيد الأوكساميل

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تم إجراء هذا البحث لدراسة تأثير بعض التجهيزات الأولية والعمليات التكنولوجية على مستوى متبقيات مبيد الأوكساميل باستخدام التحليل الكروماتوجرافى الغازى وكذا على معدل النمو الميكروبى فى الثمار الطازجة والمحفوظة لمحاصيل الفاصوليا الخضراء ، والكوسة والطماطم المزروعة تحت الصوب البلاستيكية.

أوضحت النتائج المتحصل عليها ما يلى:

- أن غسيل ثمار الفاصوليا الخضراء والكوسة والطماطم المعاملة بمبيد الأوكساميل قد أزال من ٢٣-٤٢% من المتبقيات المترسبة على الثمار بعد ساعة واحدة من المعاملة وأزال من ٤١-٥٤% من المتبقيات الموجودة على الثمار بعد ٧ أيام من الرش ووصلت نسبة الإزالة إلى ٦٣% من المتبقيات الموجودة على الثمار بعد ١٥ يوم من تطبيق المبيد ليصبح مستوى المتبقيات على ثمار الخضراوات مقارب للحد الأقصى المسموح به لمبيد الأوكساميل وتكون صالحة للاستهلاك الآدمي.
- أحدثت عمليات سلق وتجميد وتعليب الخضراوات المعاملة بمبيد الأوكساميل بعد غسيلها انخفاضا فى مستوى المتبقيات ترواح من ٣٧% إلى ٦٤% ليصبح مستوى المتبقيات أقل من الحد الأقصى المسموح به لمبيد الأوكساميل دون أن يكون لها أي أثار جانبية ضارة على صحة الإنسان.
- وجد أن تخزين المنتجات المصنعة للخضراوات المعاملة قد خفض مستوى المتبقيات لتصبح مجرد آثار باقية إذا قورنت بالحد الأقصى المسموح به لمبيد الأوكساميل.
- وجد أن معدل النمو الميكروبى فى محاصيل الخضراوات الغير معاملة والمعاملة بمبيد الأوكساميل قد تأثر معنويا بعد عمليات الغسيل والسلق والتجميد والتعليب وكذا بعد فترة التخزين التجريبية للمنتجات المصنعة من هذه الخضراوات.