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Study of Bifenazate, Indoxacarb and Emamectin Benzoate Residues on Tomato

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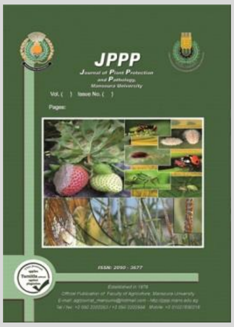

ABSTRACT

Experiments were conducted on the tomato to study the residues and dissipation rates of bifenazate, indoxacarb, and emamectin benzoate during the summer plantation of 2021 at different intervals (2h), 1, 3, 6, 9, 12, and 15 days. QuEChERS method was used for extraction and clean-up and analyzed using HPLC. Results revealed that the initial amounts of bifenazate, indoxacarb, and emamectin benzoate in leaves and fruits were3.641, 1.463; 2.592, 0.943, and 1.721, 0.215, respectively. Loss percentages in residues were higher in tomato fruits than leaves. The half-life (t½) values of bifenazate, indoxacarb, and emamectin benzoate were 1.86, 2.16; 1.83, 3.01, and 0.973, 1.16 days in tomato fruits and leaves, respectively. No residues were detected in processed tomato paste from contaminated tomato fruits with the tested pesticides indicating 100% removal. Also, washing tomato fruits resulted in a 22.92–42.45, 5.57–21.31, and 15.79–33.02% removal percentage from the residues of bifenazate, indoxacarb, and emamectin benzoate, respectively. Contaminated tomatoes could be consumed safely after 3 days for unwashed and washed fruits contaminated with the three tested pesticides according to the maximum residues limit (MRL) of the EU pesticides database - European Commission.

***Keywords**: Bifenazate, indoxacarbe, emamectin, tomato, paste.*

INTRODUCTION

In Egypt, tomato is one of the most important and the largest grown vegetables, which is considered one of the largest producers and consumers of vegetables. Tomato is well known as an important food for its component such as low fat and calories, free of cholesterol, and rich in vitamins A and C, β-carotene, lycopene, and potassium (Pawar *et al.*, 2012). Tomato plants are liable to investigate different pests such as white fly, aphids, *Tuta absoluta*, and powdery mildew which are considered serious pests causing drastic decreases in yield. Therefore farmers used pesticides to protect crops from harmful pests; however the use of pesticides can also be associated with risks to environment and human health mainly by the exposure of farmers or contamination of foods (Radriguezcabo *et al.*, 2018 and Villaverde *et al.*, 2018)

Bifenazate is used for controlled phytophagous mites (both eggs and motile stages) on crops including citrus, tree fruits, vines, hops, nuts, vegetables, ornamentals, cotton, and maize. Bifenazate is a non-systemic acaricide with predominantly contact action and long residual action. Indoxacarbe is used for broad-spectrum control of Lepidoptera in cotton, vegetables, and fruit, the mode of action is to block sodium channels in nerve cells. Emamectin benzoate is used for control of Lepidoptera on vegetables, brassicas, fruit, maize, tea, grapes, and cotton considered a non-systemic insecticide that penetrates leaf tissues by translaminar movement, Paralyses the Lepidoptera, which stop feeding within hours of ingestion, and die. (MacBean, 2012)

Therefore, the present study was undertaken to determine the levels of bifenazate, indoxacarb, and emamectin benzoate residues in tomato fruits and leaves as well as estimation of their dissipation rate, half-life values t½, and pre-harvest intervals PHI. The effect of washing and paste preparation as home processing on the residues of the investigated pesticides was also studied. This will help implement measures that can protect the consumers from the toxic effects of pesticide residues in tomatoes.

MATERIALS AND METHODS

I- Pesticide selected:

Bifenazate (Solo 24% SC); indoxacarbe7.5% and emamectin benzoate 1.5% were used as mixture in commercial formulation Penny9%SC. The pesticides were acquired from Central Agricultural Pesticides Laboratory, agricultural Research Center, Dokki – Giza, Egypt.

2- Field experiment and sampling:

Experiments were carried out at a private tomato field (*Solanum lycopersicum* var Hagen) located at El-Tahra village, Zagazig district, Sharkia governorate, Egypt. The experimental area was divided into plots of 1/100 of fed. each and arranged in randomized blocks design with three replicates for each treatment and the untreated control. The pesticides used and their rates from the commercial formulations were:

a. Bifenazate (Solo 24% SC) 80 cm / 100 l water.

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b. Indoxacarbe and emamectin benzoate was used as a mixture called Penny 9% SC at the rate of 150 g/fed.

Rates of pesticides application were chosen based on recommended rates according to the recommendation of the ministry of agriculture and land reclamation

Tomato plants were sprayed at the fruiting stage during summer plantation (June 2021) one time using a knapsack hand sprayer fitted with one nozzle, and the rate of water as pesticide diluent was 200 liters per fed. Representative samples of tomato fruits and leaves (500 g fruits and 25 g leaves/replicate) were taken randomly from the experimental plots at intervals of 2 hr, 1, 3, 6, 9, 12, and 15 days after application to determine the residues of the tested pesticides.

3- Extraction, clean up procedures and residues determination:

Tomato samples were extracted and cleaned up using the QuEChERS method (Anastassiades*et al*., 2003). A homogenized tomato fruits and leaves samples of 10 g of fruits and leaves were taken into a centrifuge tube (50-mL). Fifteen milliliters of acetonitrile containing 1.0% acetic acid were transferred to the centrifuge tube and vigorously shaken for 1 min. Then, 4 g magnesium sulfate anhydrous and 1 g sodium acetate were added, and then the mixture was shaken vigorously for 5 min. The mixture was centrifuged at 3000 rpm for 5 min. Five milliliters of the supernatant were transferred to a centrifuge tube (15 ml) and shaken with 50 mg primary secondary amine (PSA), 10 mg graphitized carbon black, and 150 mg magnesium sulfate. Thereafter, the tube was centrifuged for 10 min at 6000 rpm. The supernatant of bifenazate, indoxacarbe, and emamectin benzoate was taken for analysis by HPLC with an Agilent 1260 HPLC system (USA), with a quaternary pump, autosampler injector, thermostat compartment for the column, and photodiode array detector. The chromatographic column was Zorbax C18 XDB (250 × 4.6 mm, 5 mm). The column was kept at room temperature. Mobile phase (acetone/water) and wavelength were 65:35, 230nm; 75:25, 254 nm, and 95:5, 190nm, forbifenazate, indoxacarbe, and emamectin benzoate, respectively. The flow rate of the mobile phase was 1 ml min-1, and the injection volume was 20 µl. Under these conditions good separations and high sensitivity were obtained with retention times of 5.084, 3.987, and (3.531 and 4.890) min for bifenazate, indoxacarbe, and emamectin benzoate (1 and 2), respectively (fig. 1).

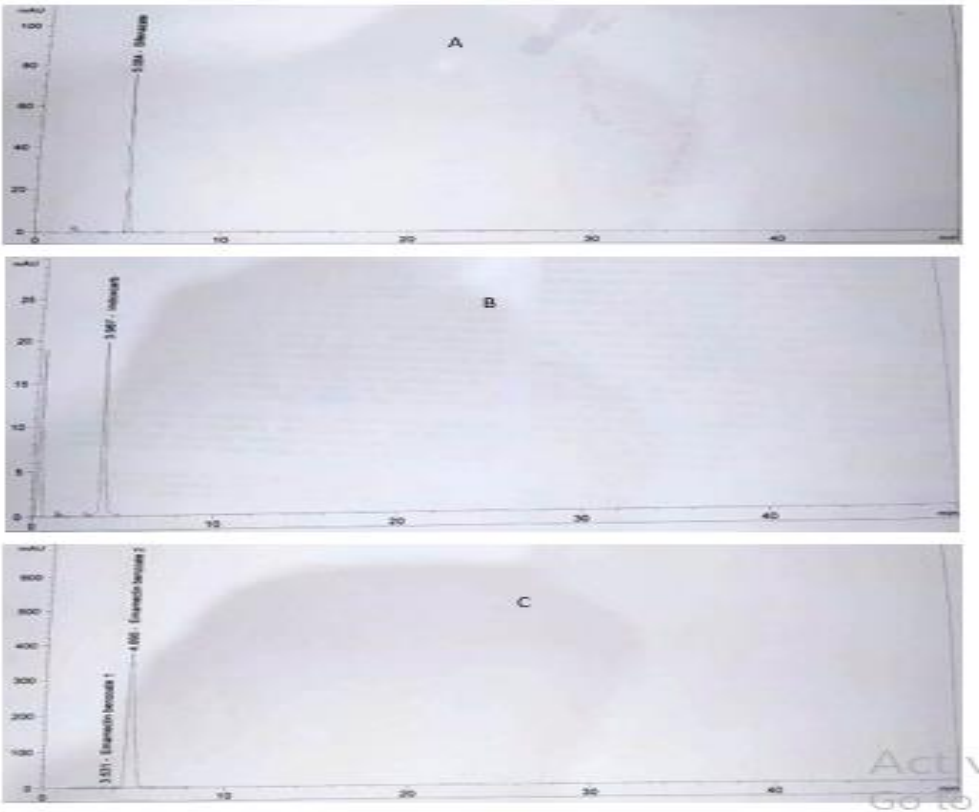

Fig. 1. HPLC, Chromatogram of bifenazate (A), indoxacarbe (B) and emamectin benzoate (C)

To study the removal effects of washing and preparing tomato paste on the residues of the tested pesticides: 1) two kilogram tomato fruits were taken two hrs after spraying from each treatment and crushed into small pieces in a warring blender then the tomato juice was concentrated at 100 ˚C until form paste with the addition of 2.5% NaCl (Ismail *et al*., 1993), 2) subsamples for each treatment during the first three sampling dates (2 hr, 1 and 3 days) after spraying were washed with tap water (2 min) because the washing effect was decreased with time elapse . The washed fruits and paste were analyzed as described before.

4- Recovery rates and statistical analysis

To determine the effectiveness of the used extraction, clean up and final determination procedures, three untreated samples from each fruit and leaves were spiked with recognized concentrations (0.5, 1, and 5 mg/kg) of the active ingredient from the standard solutions of the three investigated pesticides. The extraction, clean up and detection steps were performed as described before, and the average recovery rates for leaves and fruits were 94.26, 90.33; 91.62, 89.84 and 93.81, 88.73% for bifenazate, indoxacarbe and emamectin benzoate, respectively. Results obtained were corrected according to their mean of recovery. The rates of degradation (k) and half-life (t½) periods of each pesticide were calculated according to (Gomaa and Belal, 1975).

RESULTS AND DISCUSSION

Residues of the investigated pesticides on tomato:

The residues and the correlated criteria of bifenazate24% SC; indoxacarbe7.5% and emamectin benzoate 1.5% were used as mixture in commercial formulation Penny 9% SC in tomato are presented in Tables (1, 2 and 3) and (fig. 2, 3 and 4). It is obvious that there was a positive correlation between the uptake of the tested pesticides on the recipient surface of the leaves or fruits and their used rates. Data also show that the initial amounts of each pesticide on and in kg fruits were much lower than those in leaves such differences may be due to the differences in the area, morphology and chemistry of the recipient two surfaces. These phenomena clearly noticed in the same Tables; the initial amounts determined two hours after spraying in leaves and fruits were 3.641, 1.463; 2.592, 0.943 and 1.721, 0.215 for bifenazate, indoxacarbe and emamectin benzoate, respectively. Many investigators recorded that lower content of pesticide residues were determined on fruits compared with leaves on several vegetable and field crops shalaby 1998, shalaby, *et al*., 1998, Nasr *et al*., 2009, Abd El-zaher*et al*., 2011).

Table 1. Residues of bifenazate detected in tomatofruits andleaves.

Days after treatment	leaves mg/kg	% loss	fruits unwashed mg/kg	% loss	washed mg/kg	% loss by washing	Tomato paste
Initial (2 hrs)	3.641		1.463		0.842	42.45	UND (100% loss)
1	2.281	37.35	0.816	44.22	0.564	30.88	
3	1.537	57.78	0.384	73.75	0.296	22.92	
6	0.606	83.36	0.135	90.77	-	-	
9	0.213	94.15	0.054	96.31	-	-	
12	0.074	97.96	0.013	99.11	-	-	
15	0.028	99.23	UND	-	-	-	
K	0.3201		0.3730				
t½	2.16		1.86				
MRL(mg/kg)			0.5				
PHI (Days)			3				

UND = Undetectable Amounts, K = Degradation Rate, t½= Half-life, MRL= Maximum Residue Limit, PHI= Preharvest Interval.

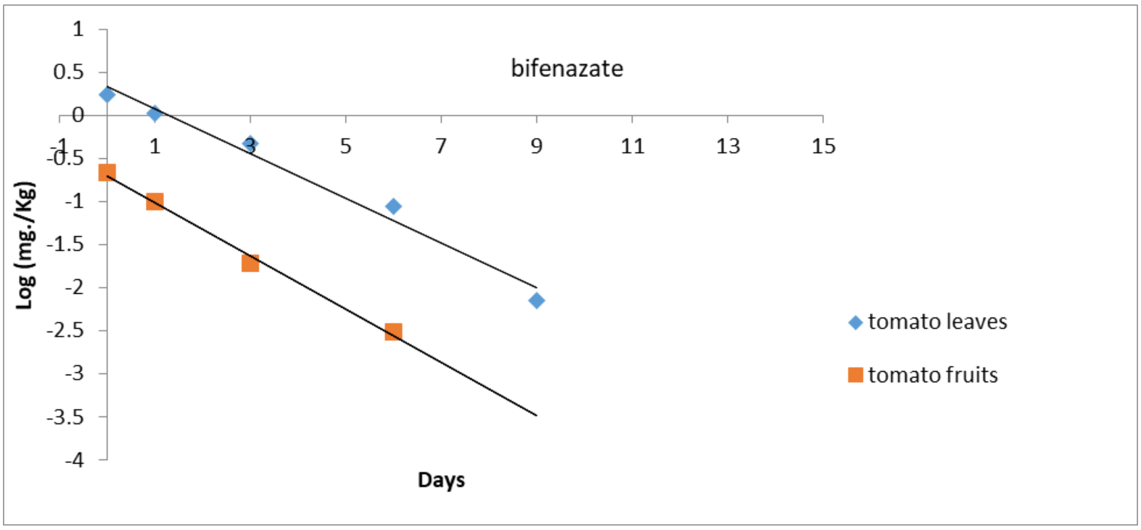

Figure 2. Log., residue - day regression line of bifenazatein tomato leaves and fruits.

Table 2. Residues of indoxacarbdetected in tomatofruits and leaves.

Days after treatment	leaves mg/kg	% loss	fruits unwashed mg/kg	% loss	washed mg/kg	% loss by washing	Tomato paste
Initial (2 hrs)	2.592		0.943		0.742	21.31	UND (100% loss)
1	1947	24.88	0.614	34.89	0.556	9.45	
3	1.583	38.92	0.341	63.83	0.322	5.57	
6	0.893	65.55	0.114	87.91	-	-	
9	0.405	84.38	0.037	96.08	-	-	
12	0.196	92.44	0.009	99.05	-	-	
15	0.073	97.18	UND	-	-	-	
K	0.2303		0.3776				
t½	3.01		1.83				
MRL(mg/kg)			0.5				
PHI (Days)			3				

UND = Undetectable Amounts, K = Degradation Rate, t½= Half-life, MRL= Maximum Residue Limit, PHI= Preharvest Interval.

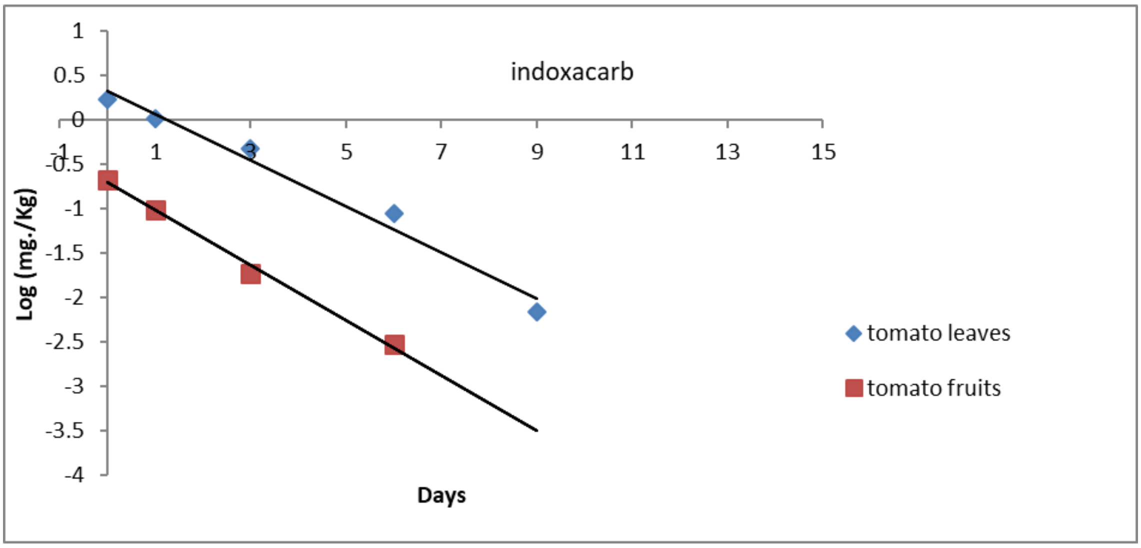

Figure 3. Log., residue - day regression line of indoxacarbin tomato leaves and fruits.

Table 3. Residues of emamectin benzoate detected in tomatofruits and leaves

Days after treatment	leaves mg/kg	% loss	fruits unwashed mg/kg	% loss	washed mg/kg	% loss by washing	Tomato paste
Initial (2 hrs)	1.721		0.215		0.144	33.02	UND (100% loss)
1	1.036	39.80	0.098	54.42	0.078	20.41	
3	0.473	72.51	0.019	91.16	0.016	15.79	
6	0.088	94.88	0.003	98.60	-	-	
9	0.007	99.59	UND	-	-	-	
12	UND		UND	-	-	-	
15	UND		UND	-	-	-	
K	0.5964		0.7116				
t½	1.16		0.973				
MRL(mg/kg)			0.2				
PHI (Days)			3				

UND = Undetectable Amounts, K = Degradation Rate, t½= Half-life, MRL= Maximum Residue Limit, PHI= Preharvest Interval.

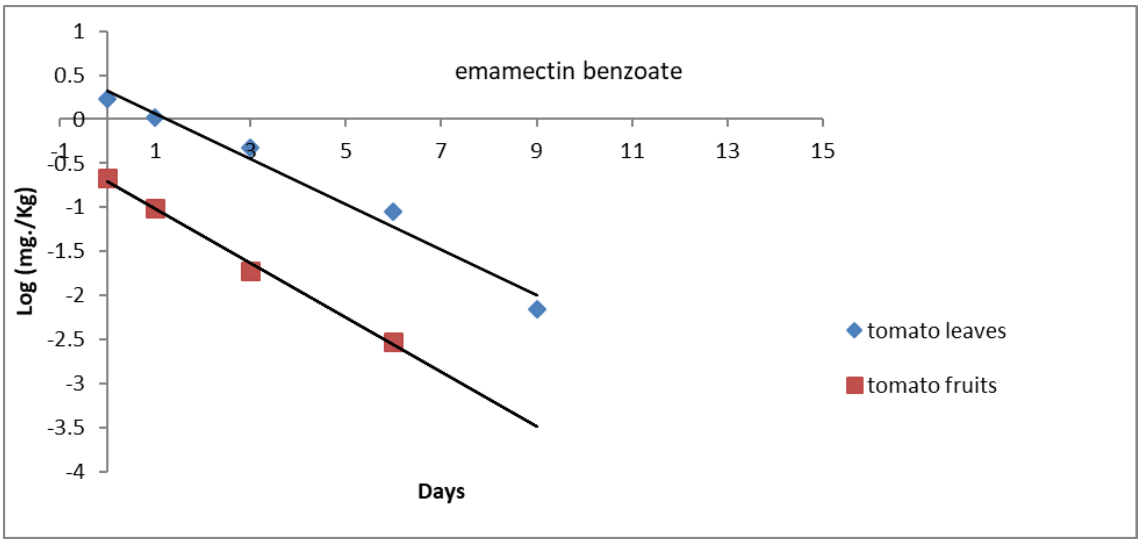

Figure 4. Log., residue – day regression line of emamectin benzoate in tomato leaves and fruits.

Levels of the tested pesticide residues decreased gradually during the experimental period. Loss percentages in residues were higher in tomato fruits than leaves; this may be due to differences in metabolism since the role of natural degradation in both targets was similar. The figures of the rate of pesticide degradation as well as the half-lives support this phenomenon; the figures of the rate of tested pesticide degradation in the case of fruits are higher than those of leaves, whereas the inverse case could be observed with figures of half-lives. As could be noticed in Tables 1, 2, and 3 the rates of degradation (k) in fruits and leaves were 0.3730, 0.3201; 0.3776, 0.2303, and 0.7116, 0.5964 forbifenazate, indoxacarb, and emamectin benzoate, respectively. The corresponding half-lives (T½) was 1.86, 2.16; 1.83, 3.01, and 0.973, 1.16 days, respectively. These results are in harmony with these obtained by many investigators working with the same tested pesticides or others on several vegetables and field crops (Amer *et al.*, 2007, Wang *et al*., 2007, Zhang *et al.*, 2008, Chi *et al.*, 2009, Abd-Alrahman and Ahmed 2012, Wang *et al*., 2012, Shao *et al.*, 2013, Satheshkumar *et al.*, 2014, Sdeek and Hanan

2018, Badawy *et al*., 2019, Dalia *et al*., 2019, Elhefny *et al*., 2021, Wang *et al*., 2021)

Concerning the effect of washing and process tomato paste, data show that washing with tap water resulted in removing reasonable amounts of residues, especially with the first three samples. The effect of washing was more pronounced with the acaricides bifenazate (22.92 – 42.45%) followed by emamectin benzoate (15.79 – 33.02%) and indoxacarb (5.57 – 21.31%). These findings may be due to the difference in physic-chemical properties of the tested pesticides. As expected, the effect of washing was less noticeable as time elapsed from the onset of spraying; this could be elucidating based on the increased rate of permeability with time elapsion. No residues were detected in processed tomato paste from treated tomato fruits with the tested pesticides recording 100% removed. Several investigators pointed out that washing or paste processes resulted in removing magnitude amounts of residues present on the surface of many vegetables, fruits, and field crops (Abou-Arab and Abou-Donia 2001, Antonious 2004, Guardia-Rubio *et al*., 2007, Kaushik *et al.,* 2009, Aguilera *et al*., 2012, Kong *et al*., 2012, Zhao *et al.*, 2014, Huan *et al.*, 2015, Jankowska *et al.*, 2019, Liu *et al.*, 2019).

Based on the figures of maximum residues levels of the used pesticides (0.5, 0.5, and 0.02 mg/kg) forbifenazate, indoxacarb, and emamectin benzoate, respectively presented in the EU Pesticides database - European Commission the contaminated tomato could be harvest safely after 3 days for unwashed and washed fruits contaminated with the three tested pesticides.

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دراسه لمتبقيات مبيدات البيفينازات والإندوكساكارب والإيمامكتين بنزوات على الطماطم

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[3] قسم بحوث متبقيات المبيدات وتلوث البيئة ـ المعمل المركزي للمبيدات ـ مركز البحوث الزراعيه ـ الدقي الجيزه ـ مصر.

الملخص

أجريت التجارب على الطماطم لدراسة متبقيات ومعدلات الاختفاء للبيفينازات ، الإندوكساكارب والإيمامكتين بنزوات خلال العروة الصيفية لصيف 2021 على فترات مختلفة (ساعتان) ، 1 ، 3 ، 6 ، 9 ، 12 ، 15 يوم. وقد تم استخدام طريقة QuechERS للاستخلاص والتنظيف وتم التقدير النهائي بإستخدام HPLC. وقد أظهرت النتائج أن الكميات الأولية من البيفينازات والإندوكساكارب وإيمامكتين بنزوات في الأوراق والثمار كانت 3.641 ، 1.463 ؛ 2.592 ، 0.943 ؛ 1.721 ، 0.215 على التوالي. وكانت نسب الفقد في المتبقيات أعلى في ثمار الطماطم عنها في الأوراق. وأدى تصنيع ثمار الطماطم المعاملة إلى صلصة إزالة متبقى المبيدات الثلاث بنسبة 100% ازالة ، كما أدى غسيل ثمار الطماطم المعاملة بالمبيدات المستخدمة إلى إزالة كميات من متبقي المبيد بمقدار 22.92-42.45 و 5.57-21.31 و 15.79-33.02% فى حالة كل من البيفينازات ، الإندوكساكارب والإيمامكتين بنزوات على التوالى. وكانت قيم نصف العمر (t½) للبيفينازات ، والإندوكساكارب ، وبنزوات الإيمامكتين 1.86 ، 2.16 و 1.83 ، 3.01 و 0.973 ، 1.16 يوم في ثمار الطماطم وأوراقها على التوالي. ويمكن استهلاك الطماطم الملوثة بأمان بعد 3 أيام للثمار غير المغسولة والمغسولة الملوثة بالثلاث مبيدات المختبرة وفقًا للحد الأقصى للمتبيقيات (MRL) لقاعدة بيانات الاتحاد الأوروبي - المفوضية الأوروبية لمبيدات الآفات.