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Depreciation, Risk Assessment Study of Pymetrozine, Pyriproxyfen, and Acetamiprid Residues using QuEChERS and HPLC (DAD) and their Biochemical Effects on Eggplant under Greenhouse Conditions

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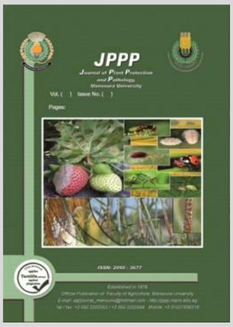

ABSTRACT

Eggplant is economically highly rated in both Asia and Africa because of its richness in vitamins and antioxidants. The work purpose is to conduct a gap period, dietary risk assessment, and effect on some quality parameters of pymetrozine, pyriproxyfen, and acetamiprid on eggplant under greenhouse conditions. The QuEChERS method was appropriate for residue extraction for used insecticides. Recoveries ranged from 86 -105 % with relative standard deviation(RSD) rated from 1.52 to 10.26%. High-performance liquid chromatography (HPLC) was used for analysis. Pymetrozine, acetamiprid, and pyriproxyfen reached half-life by 1.15, 1.62, and 4.64 days consecutively. Pre-harvest interval guided to 3, 5, and 7 days for the three insecticides respectively. All tested insecticides decreased significantly the levels of chlorophyll a, chlorophyll b, N, P, and K content in leaves of eggplant compared with the control. Pyriproxyfen recorded the highest decrease. Pymetrozine and acetamiprid significantly decreased the total soluble solids(T.S.S.), ascorbic acid(vitamin C), carbohydrates, and total soluble sugars content in eggplant fruits compared with control, and did not significantly decrease total nitrogen, crude protein, and Fe content. Pyriproxyfen significantly decreased all parameters except % acidity and recorded the highest decrease compared with the control. Eggplant can be harvested safely after 3 to 7 days after application with tested insecticides. Risk quotient(RQ) was higher than 1 till the 5th and 7th day after application then became less than 1 for the rest of the tested interval days which indicates low long-term risk. The effect on plant quality needs to be studied in depth in future studies.

Keywords: Insecticide residual behavior, eggplant, risk assessment, quality.

INTRODUCTION

Vegetables are commonly used as a source of vitamins and fiber to make the balanced diet requirement (Bemph, and Augustine, 2011). Eggplant (*Solanum melongena* L.) is an essential vegetable crop that is extensively grown worldwide.

The top eggplant producers in 2007 were China, India, and Egypt with 18, 8.5, and 1 million tons (t), respectively (Prado-Lu, and Leilanie, 2015). It is rich in vitamins B1, B3, folic acid, and C as well as K, Fe, Ca, Cu, Mn, and Zn (Kowalski *et al.*, 2003). Moreover, eggplant is very rich in antioxidants and has a big role in avoiding colon cancer, managing type 2 diabetes, and reducing the level of cholesterol, and is also used in dieting systems to lose weight (Dome, 2013).

Like several crops, eggplant (aubergine or brinjal) is attacked by various pests and diseases, which negatively affect production and reduce about 20-40% of crop yields globally. Furthermore, the world needs to increase food production by approximately 60 % to counter population growth by 2050 (FAO, 2012).

Neonicotinoid insecticides (acetamiprid) are considered the systemic class of insecticides that are spreading the fastest on the market since the use of pyrethroids (Muccio *et al.*, 2006). The new class of insecticides is highly effective in controlling various insects (Lepidoptera, Hemiptera, Coleoptera, and Thysanoptera) on several fruit and vegetable crops (Kim *et al.*, 2003).

Pymetrozine is a new selective insecticide used against sucking insects in vegetables, fruits, and field crops (Fuog *et al.*, 1998). It affects feeding behavior's neurological regulation, causing death after some days (Horowitz *et al.*, 2011). At present, pymetrozine has been used as a broad-spectrum and indispensable insecticide in the control of sucking pests by the European Food Safety Authority (EFSA 2018).

Pyriproxyfen is considered a broad-spectrum regulator of insect growth which, mimics the actions of the juvenile hormone which interferes with the insects' hormonal system, preventing the insects from developing and becoming adults (Devillers, 2009).

Since residue of pesticides may persist in edible plant components, monitoring of residue dissipation is necessary to evaluate the potential risks to human health and provide safe food.

Pesticide residues are extracted and cleaned up in a wide range of matrices by the quick, easy, cheap, effective, rugged, and safe (QuECHERS) approach (Prodhan *et al.*, 2018). Comparing this strategy to the other ones that are now in use, its popularity is growing daily.

In general, the work presented here aims to investigate the pre-harvest interval (PHI), half-life (RL50), the decline and dietary risk assessment of pymetrozine, pyriproxyfen,

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and acetamiprid on and in eggplant fruits under greenhouse conditions as well as the side effects of tested insecticide residues on certain eggplant quality parameters.

MATERIALS AND METHODS

Insecticides used:

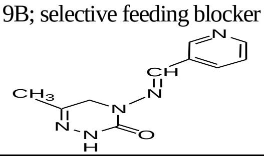
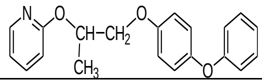
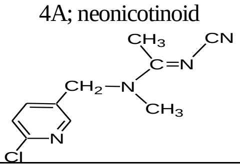
The common and trade name, Empirical Formula, group of insecticide, and rate of application are displayed in Table (1).

Reagents and Standards

Standards of pymetrozine, pyriproxyfen, and acetamiprid (≥99.9% purity) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC grade of organic solvents utilized and obtained from Scharlau (Barcelona, Spain). The solvent's reasonability was approved by comparing the reagent blank parallel to the actual analysis. Sodium chloride, anhydrous magnesium sulfate, disodium hydrogen citrate sesquihydrate, sodium citrate dehydrates and C18 were obtained from Merck (Germany). Primary secondary amine (PSA, 40 µm Bondesil) was obtained from Supelco (Bellefonte, PA).

The stock solution of pymetrozine, pyriproxyfen, and acetamiprid was prepared using acetonitrile at a concentration rate of 100 µg/ml and maintained at 4°C in a refrigerator. A standard stock solution was diluted several times in acetonitrile to create the working standard solutions. Meanwhile, a matrix-matched standard solution was similarly prepared by applying eggplant matrix blank instead of solvent at the same concentrations. The standard calibration curve for each was constructed by graphing the concentration of the analyte against the peak area.

Table 1. Common names, trade names, empirical formulas, insecticide groups, and application rates of tested insecticides.

Common names	Trade names	Empirical Formulas	Insecticide groups (IRAC)	Field-recommended rates
Pymetrozine	Chess 50 % W.G.	$C_{10}H_{11}N_5O$	9B; selective feeding blocker 	240 g./100 L
Pyriproxyfen	Pyrufix 10 % E.C.	$C_{20}H_{19}NO_3$	7C; juvenile hormone mimic 	50 cm^3/100 L
Acetamiprid	Tolan 20% S.P.	$C_{10}H_{11}ClN_4$	4A; neonicotinoid 	25 g /100 L

Field experiment design and Sampling

Eggplant 'White Long' (*Solanum melongena* L.) was planted under greenhouse conditions at the Faculty of Agriculture at Mansoura University, Egypt. Greenhouse was exposed to normal agricultural services. Three treatment plots and one control 42 m^2 each. At the fruiting stage and taking into consideration pests infection period pymetrozine, pyriproxyfen, and acetamiprid were sprayed with dose using a knapsack sprayer. Treatments were coordinated in a randomized block design. Samples each 500 g were randomly selected from every plot including control at one hour, 1, 3, 5, 7, 10, 15, and 21 days after spraying. Samples were transported to the lab under cooling conditions. Samples were chopped, homogenized, and stored at -20°C till extraction.

Extraction and clean-up:

The QuEChERS approach was used for extraction and cleanup as described by (*Anastassiades et al.*2003). 10 ml of acetonitrile was added to 10 g of homogenized eggplant samples in a 50 ml Teflon tube, and the mixture was shaken vigorously by a vortex mixer. After that, 1 g of sodium chloride (NaCl) and 4 g of anhydrous magnesium sulfate (MgSO4), 0.5 g disodium hydrogen citrate sesquihydrate, and 1 g sodium citrate dehydrate were added, and the mixture was strongly shaken by hand for 1 min and vortex for 1 min too before being centrifuged for 5 min at 5000 rpm. An aliquot of 3 ml was taken from the supernatant and transferred to a new 15 ml centrifuge tube. Clean-up was fulfilled using dispersive solid-phase extraction with 75 mg of primary secondary amine (PSA) and 450 mg of magnesium sulfate. The resulting solution was centrifuged for 5 min at 6000 rpm. Two milliliters of the supernatant were filtered over a 0.2 µm PTFE Filter (Millipore, USA) and then analyzed using Agilent 1260 HPLC-DAD.

Validation parameters:

The European Commission (2019) published guidelines for the validation of analytical procedures and quality control techniques for pesticide residues in feed and food. The validation parameters evaluated for the employed method were linearity, accuracy, and precision. Calibration curves for pymetrozine, pyriproxyfen, and acetamiprid with concentrations of 0.05, 0.1, 0.5, and 1 mg/kg were used to measure linearity, and the correlation coefficient (R^2) requirement was fulfilled. To assure accuracy and precision, a recovery test was conducted by weighing 10 g of control samples, then spiked by adding an exact amount of every standard solution. Achieving levels (1, 0.1, and 0.05 mg/kg) for pymetrozine, pyriproxifen, and acetamiprid. Fortified samples were kept at room temperature for 20 minutes to permit pesticides to penetrate the matrix before extraction and solvent evaporation.

Chromatographic Analysis and Determination:

Agilent HPLC 1260 (Agilent Technologies) with an auto-sampler, diode array detector (DAD), and quaternary pump. HPLC conditions are displayed in Table (2).

Table 2. High-performance liquid chromatography (HPLC) conditions for pymetrozine, pyriproxyfen, and acetamiprid

Analytical parameter	Technical material		
	Pymetrozine	Pyriproxyfen	Acetamiprid
UV wavelength	205 nm	260 nm	246nm
Mobile phase	90% acetonitrile: 10% water	90% acetonitrile: 10% water	60% acetonitrile: 40% water
Flow rate	1 mL/min.	1 mL/min.	0.8 mL/min.
Absolute retention time	8.26 min.	4.9 min.	5.98 min.
Column	C_{18} Zorbax XDE (250 mm x 4.6 mm, 5 µm). (The column was reserved at room temperature)		

Dietary exposure risk assessment

To assure human health risk safety, the risk quotient (RQ) was calculated for long-term intake risk. According to Wang et al. (2016), Qian et al. (2017) and Mostafa et al. (2024) (RQ) equation was used (RQ) =NEDI/ADI. National estimated daily intake (NEDI, mg/kg/day) = STMR*Fi/b. w. Equations abbreviations are as follows STMR is median residue data from controlled trials, Fi is food intake(kg/day), bw is body weight (kg) and ADI is the acceptable daily intake.

RQ calculations included adult weight in Egypt which is 60 kg, RQ values are accepted if the value is less than 1, which indicates consumer safety, in contrast if RQ value is higher than 1. (Oliva et al. 2017, Zhang et al. 2021).

Kinetic calculations:

The decline was appraised according to Timme and Frehse (1980) and Soliman *et al.,* (2017) which conducted depletion behavior of pesticide remainder as the rate of dissipation (K) as follows:

$$\text{Log } R = \log R_0 - 0.434K_t$$

Where R_0 and R express residues at zero time and gap days consecutively, K_t is the decline rate at t day. The rate of dissipation (K) was assessed by a first-order kinetic equation and therefore half-life ($t_{1/2}$) was served by K value, the equation is ($t_{1/2}$=Ln2/k) Moye *et al.,* (1987).

Effect of pesticides residues on some quality parameters of eggplant

On leaves:

Representative samples were randomly selected after application at 7, and 14 days to estimate the Photosynthetic pigments (Chlorophyll a and b) content following Sadasivam and Manickam, (1996) method. Chemical constituents (Macronutrients) including total nitrogen content which was estimated using the modified Micro-Kjeldahl device as Jones *et al.,* (1991) described. Total Phosphorus content was determined by Milten Roy Spectronic 120 spectrophotometer at wavelength 725 nm, and total potassium content was assessed by Jenway Flame photometer, Model corning 400 as described by Peters *et al.,* (2003).

On fruits:

The chemical quality parameters were tested for untreated and treated eggplant fruits 10 days after application. These chemical parameters included total soluble solids (T.S.S.), % acidity, and ascorbic acid (vitamin C) were estimated according to (A.O.A.C. 2000) method, total nitrogen content was employed as stated by Jones *et al.,* (1991) by the modified Micro-Kjeldahl apparatus, and total carbohydrates were measured by spectrophotometer at 630 nm (Sadasivam and Manickam, 1996). β-carotene was estimated by the technique of Goodwine (1965) and calculated as mg/g fresh weight. Total soluble sugars (T. Sugar) were evaluated as the method mentioned by Sadasivam and Manickam, (1996), also the amount of Fe was determined as stated by Kumpulainen *et al.,* (1983).

Statistical analysis: -

Costat, (1990) was used to analyze the data. A one-way analysis of variance (ANOVA) was used to determine the significance of differences; with $P < 0.05$ being considered statistically significant. (values sharing the same letter are considered not significantly different).

RESULTS AND DISCUSSION

Validation parameters:

The data presented in Table (3) revealed that the typical recovery percentages varied from 86 to 95% for pymetrozine, 91 to 105 % for pyriproxifen, and 93 to 104% for acetamiprid. According European Commission accepted range for recovery is from 80 – 110 %, and RSD must be below 20% (Sanco 2021). RSD fluctuated from 1.52 to 5.29, 5.56 to 7.5, and 5.85 to 10.26% for pymetrozine, pyriproxyfen, and acetamiprid, consecutively which is satisfactory. By comparing the peak area of the standards with that of the unidentified or spiked samples run under the same conditions, the lowest observed concentration residues were determined. Linearity is achieved as the regression coefficient (R^2) exceeded 0.987.

Table 3. The percentage of recovery and the relative standard deviation (RSD) of pymetrozine, pyriproxyfen, and acetamiprid at spiked levels 1.00, 0.10, and 0.05 mg/kg.

Spiked level ($mg.kg^{-1}$) r=6	pymetrozine		pyriproxyfen		acetamiprid	
	% of Recovery	% RSD	% of Recovery	RSD %	% of Recovery	RSD %
1	86	1.52	103	5.56	104	7.76
0.1	95	2.64	105	7.5	100	5.85
0.05	92	5.29	91	6.55	93	10.26

(r) replicates number.

Depreciation study of insecticides used on and in eggplant fruits:

Pymetrozine:

Table (4) displays the residues, half-life of pymetrozine, rate of degradation, and pre-harvest interval (PHI) in eggplant fruits.

After one hour of application, the initial deposits of the pymetrozine residue in eggplant fruits was 0.979 mg/kg which was subsequently reduced to 0.397, 0.112, 0.052, 0.039, and 0.034 mg/kg indicating that the rates of loss were 59.45, 88.56, 94.69, 96.02 and 96.53%, after 1, 3, 5, 7, and 10 days of application, in that order. After 15 days of treatment, pymetrozine was not detected in the eggplant fruits. Also, the half-life (RL50) time of pymetrozine in eggplant fruits value was 1.152 days, and a rate of degradation (K) of 0.6018 day^{-1}. According to the maximum residue limit (MRL) of pymetrozine in eggplant (0.5 ppm) (EU 2015), data indicated that eggplant could be safely consumed under greenhouse settings after three days of treatment. The outcomes demonstrated that the rate of pymetrozine degradation on eggplant fruits was comparatively quicker within the first three days after treatment. This quick degradation of pymetrozine in eggplant fruits may be explained by the eggplant's natural surface or the ripening of the eggplant which might reduce the content of pymetrozine which agrees with Abd-Alrahman and Kotb's (2020) findings, average recovery of pymetrozine was 90.75% (88.5 - 93%) in tomatoes, residue half-live was 1.31 days, and the PHI was 3 days. Talebi (2006) found no pymetrozin residues in cucumber samples which were collected four days after spraying. Li *et al.*, (2011) determined the pymetrozine residues in the rice paddies and discovered that 0.89 days was the half-life, while the average recovery was 82.9–85.3%. Another study by Zhang *et al.*, (2015) found that the rice's pymetrozine half-life (RL50) varied from 2.3 to 2.6 days. Wang, *et al.*, (2022) investigated the impact of temperature on the degradation of insecticides and observed that Pymetrozine half-lives in cabbage were 1.89, and 2.80 days in May–June season and the October–November season, respectively.

As opposed to that, Shen *et al.*, (2009) reported that the half-life of pymetrozine in broccoli was 3.5 days, with an average recovery of 87–93%. This indicated that the vegetable harvest interval was 23 days.

Pyriproxyfen:

Table (4) displays the residues, half-life of pyriproxyfen, rate of degradation, and pre-harvest interval (PHI) in eggplant fruits.

The initial pyriproxyfen residue in eggplant fruits was 1.41 mg/kg one hour after application; this later dropped to 1.264, 1.053, 0.758, 0.308, and 0.204 mg/kg after 1, 3, 5, 7, and 10 days of treatment, the rates of loss were, correspondingly, 10.61, 23.53, 46.39, 78.22, and 85.57%. After 15 days of treatment, no pyriproxyfen was detected in eggplant fruits. Pyriproxyfen in eggplant fruits had a half-life (RL50) time value of 4.643 days and a rate of degradation (K) of 0.1493 day-1. The study revealed that depending on the maximum residue limit (MRL) of pyriproxyfen in eggplant (0.6 ppm) (EU 2023), fruits of the eggplant could be safely consumed under greenhouse settings after seven days of treatment.

No more research related to the dissipation of pyriproxyfen than those done by Fenoll *et al.*, (2009) who investigated pyriproxyfen's dissipation in peppers grown in the greenhouse and found the half-lives were, respectively, 18.57 and 21.47 days following both the first and second application. The PHI was 3 days. while, Dong *et al.*, (2018) suggested that the half-life of pyriproxyfen in citrus was 13.3 days, and its PHI was 14 days. These variations may be due to the type and growth rate of crops, environmental conditions, and application doses.

Acetamiprid:

Table (4) displays the residues, half-life of acetamiprid, rate of degradation, and pre-harvest interval (PHI) in eggplant fruits. After one hour of application, the initial acetamiprid residue in eggplant fruits was 1.258 mg/kg which was reduced to 0.964, 0.454, 0.152, and 0.011 mg/kg, after 1, 3, 5, 7, and 10 days of treatment, the loss rate values were 23.37, 63.91, 87.92, and 99.13%, respectively. After ten days of application, acetamiprid was dissipated in eggplant fruits. In eggplant fruits, acetamiprid had a half-life (RL50) of 1.62 days and a rate of degradation (K) of 0.4264 day-1. According to the maximum residue limit (MRL) of acetamiprid in eggplant (0.2 ppm) (EU 2019), data indicated that eggplant could be safely consumed under greenhouse settings after five days of treatment.

The current findings are in the same as many investigations, Cara *et al.*, (2011) discovered that the PHI of acetamiprid in cucumber in a greenhouse was 7 days. Also, Varghese *et al.*, (2015) found that the half-life in chili pepper fruits was 2.27 days while the PHI was 7.18 days. Abdel-Ghany *et al.*, (2016) found that the PHI of acetamiprid in cucumber was 5 days. Lee *et al.*, (2019) estimated acetamiprid residue in kimchi cabbages planted in open fields at 2 different locations in Korea and found that the half-lives were 6.3 and 5.2 days and it could be safely consumed after three and seven days. The recovery of acetamiprid was in the range of 103.6–113.9% at fortification levels of 0.05 and 0.25 mg.kg^{-1}. Barakat *et al.*, (2023) determined acetamiprid residues in fennel and found that a recovery percentage was 99.7%. The initial amount of acetamiprid was 0.95 mg. kg^{-1} while it was not detected in 10 days. Gaber *et al.*, (2022) estimated acetamiprid residues in tomatoes planted in fields and greenhouses. The recovery rate for acetamiprid was 87.71 ± 1.33 %, the half-life values were 1.80, and 1.48 days while, the pre-harvest intervals were 5, and 7 days in open fields and greenhouses respectively. Also, these findings are in agreement with Norouzi *et al.*, (2023).

On the other hand, Abdallah *et al.*, (2017) investigated the acetamiprid's dissipation in rocket and parsley and then reported that the half-lives (t1/2) were 4.24 and 2.68 days, while the (PHI) values were 1.42 and 5.53 days in rocket and parsley, respectively. Badawy *et al.*, (2019) determined acetamiprid residue in tomatoes under greenhouse conditions and found that the average recovery percentage was 91.91%, and the half-life period was 2.07 days, whereas the PHI was 3 days. Also, Abdelfatah *et al.*, (2020) reported that tomato fruits had a half-life time of 1.19 days and average recovery percentages of acetamipride ranging from 92.85 to 97.86%, and the PHI was 1 day after the treatment. While Lazićet *et al.*, (2016) studied the dissipation of acetamiprid residue under greenhouse conditions in pepper and tomato. Acetamiprid's half-lives in pepper and tomato were 3.9 and 4.3 days, respectively, while the PHI was 14 days.

Table 4. Half-life (RL_{50}), Pre-harvest interval (PHI), Decomposition rate (k), and Residues of pymetrozine, pyriproxyfen, and acetamiprid in eggplant fruits.

Days following application	pymetrozine		pyriproxyfen		Acetamiprid	
	Residues (mg/kg)**	% of loss	Residues (mg/kg)**	% of loss	Residues (mg/kg)**	% of loss
Initial*(1 h.)	0.979	00.00	1.414	00.00	1.258	00.00
1	0.397	59.45	1.264	10.61	0.964	23.37
3	0.112	88.56	1.053	23.53	0.454	63.91
5	0.052	94.69	0.758	46.39	0.152	87.92
7	0.039	96.02	0.308	78.22	0.011	99.13
10	0.034	96.53	0.204	85.57	N.D.	100
15	N.D.	100	N.D.	100	N.D.	100
21	N.D.	100	N.D.	100	N.D.	100
K	0.6018		0.1493		0.4264	
RL_{50} (days)	1.152		4.643		1.62	
MRL (mg/kg)	0.5 (EU 2015)		0.6 (EU 2023)		0.2 (EU 2019)	
PHI (days)	3		7		5	

***: Samples were collected 1 h. after application. N.D. = Not detectable.**
****: Average of 3 replicates MRL = The maximum residue limit.**
EU = (European Union).

Under greenhouse conditions, it was discovered that the half-life of acetamiprid in zucchini fruits was 1.9 days (Park *et al.*, 2011) and 2.24 days in Chili (Sanyal *et al.*, 2008) recommended a waiting period of one day. Also, Romeh and Hendawi (2013) found that the values of acetamiprid half-life in eggplant leaves and fruits were 2.31 and 1.96 days, respectively, and recommended that eggplant be consumed after one day of application. Wu and Zhang (2012) showed that the fortified recoveries of acetamiprid in watermelon samples ranged from 73.7% to 107.5%, and the half-life was 1.18 and

3.12 days in Shandong and Beijing in the respective. Majumder *et al.*, (2022) found that the recovery of acetamiprid in paddy grain, and straw ranged between 79.67-and 98.33 %.

Overall different results indicate that the degradation of insecticides applied to crops is usually controlled by numerous factors like physicochemical characteristics of the pesticide (hydrolysis, water solubility, vapor pressure), the crop characteristics (translocation, growth rate, excretion, ease of penetration), environmental factors (humidity, temperature, rainfall, sunlight), microbial activity, application technique, and the interval between applications Edwards (1975); Tewary *et al.*, (2005) and Fujita *et al.*, (2012).

Dietary exposure risk assessment:

RQ calculated values less than 1 indicate a low risk for human health, in contrast, RQ values higher than 1 lead to high risk and low safety. Our work indicated in Table (5) that pymetrozine effect was high risk until almost 2 days after application on eggplant, while pyriproxyfen until 7 days and acetamiprid till 3 days. 5,7 and 10 days are low risk in both pymetrozine and acetamiprid 7 and 10 days for pyriproxyfen are low risk for human health.

Table 5. Dietary intake risk assessment, risk quotient (RQ), and National estimated daily intake (NEDI) for pymetrozine , pyriproxyfen, and acetamiprid in eggplant.

Time	Pymetrozine		pyriproxyfen		Acetamiprid	
(days)	NEDI	RQ	NEDI	RQ	NEDI	RQ
0	0.210	7.016	0.304	3.040	0.270	3.864
1	0.085	2.845	0.272	2.718	0.207	2.961
3	0.024	0.803	0.226	2.264	0.098	1.394
5	0.011	0.373	0.163	1.630	0.033	0.467
7	0.008	0.280	0.066	0.662	0.002	0.034
10	0.007	0.244	0.044	0.439	ND*	ND*

ND*= not detectable

Side effects of insecticides used on some physiological and biochemical processes in eggplant:

Pesticide residues can affect physiological and biochemical processes in plants, decreasing their productivity. Also, they may lower the quality of food and even prevent it from being used as a food source (Othman, *et al.*, 1987). So, the effect of pymetrozine, pyriproxyfen, and acetamiprid residues on the chemical components of eggplant was estimated.

The effect of pymetrozine, pyriproxyfen, and acetamiprid residues on photosynthetic pigments (Chlorophyll a and b) and macronutrients (N, P, K) contents on eggplant leaves after 7 and 14 days of treatment are presented in Table (6) and (7). Concerning the mean values all tested insecticide residues decreased significantly the levels of all parameters in leaves of eggplant throughout the experiment periods in comparison with the control group. The pyriproxyfen treatment showed the most significant decline (5.69, 8.03, 5.43, 5.77, and 7.22 %) for the contents of chlorophyll a, chlorophyll b, N, P, and K, in that order. While there was no significant difference between pymetrozine, and acetamiprid.

Table (8) data indicated that pymetrozine and acetamiprid treatments significantly decreased the total soluble solids (T.S.S.), ascorbic acid (vitamin C), carbohydrates, and total soluble sugars content in eggplant fruits compared with control. However, they did not significantly increase acidity percentage and did not significantly decrease total nitrogen, crude protein, and Fe content. The Pyriproxyfen treatment significantly increased acidity percentage and significantly decreased all tested parameters. The highest decrease compared with the control was observed following the application of Pyriproxyfen .

Table 6. Effect of pymetrozine, pyriproxyfen, and acetamiprid on Photosynthetic pigments (Chlorophyll a and b) of treated leaves of eggplant:

Treatments	Chlorophyll a mg/g				Chlorophyll b mg/g			
	7 days	14 days	Mean	Mean Red. %	7 days	14 days	Mean	Mean Red. %
Pymetrozine	0.636	0.672	0.654^{b}	2.09	0.443	0.473	0.458^{b}	3.17
Pyriproxyfen	0.615	0.646	0.630^{c}	5.69	0.423	0.447	0.435^{c}	8.03
Acetamiprid	0.642	0.668	0.655^{b}	1.94	0.448	0.467	0.457^{b}	3.38
Control	0.653	0.684	0.668^{a}	——	0.461	0.486	0.473^{a}	——
LSD 5 %			0.0052			0.0055		

The means in each column that are followed by the same letter (s) do not differ at P < 0.05, as per the results of Duncan's multiple range test. Each value represents the average of three replicates.

Table 7. Effect of pymetrozine, pyriproxyfen, and acetamiprid on macronutrients (N, p, K) of treated eggplant leaves:

Treatments	N%				P%				K%			
	7 days	14 days	Mean	Mean Red. %	7 days	14 days	Mean	Mean Red. %	7 days	14 days	Mean	Mean Red.%
Pymetrozine	3.63	3.78	3.705^{b}	1.85	0.361	0.383	0.372^{b}	2.36	3.01	3.21	3.11^{b}	2.35
Pyriproxyfen	3.48	3.66	3.57^{c}	5.43	0.348	0.370	0.359^{c}	5.77	2.87	3.04	2.955^{d}	7.22
Acetamiprid	3.61	3.81	3.71^{b}	1.72	0.363	0.386	0.374^{b}	1.84	2.98	3.17	3.075^{c}	3.45
Control	3.68	3.87	3.775^{a}	—	0.368	0.394	0.381^{a}	—	3.08	3.29	3.185^{a}	——
LSD 5 %			0.0133				0.004				0.0244	

The means in each column that are followed by the same letter (s) do not differ at P < 0.05, as per the results of Duncan's multiple range test. Each value represents the average of three replicates.

Table 8. Effect of pymetrozine, pyriproxyfen, and acetamiprid on some quality parameters of treated eggplant fruits:

Treatments	Parameters							
	TSS%	VC mg/100g	Acidity%	N%	Carbohydrates %	β-carotene mg/100g	T. Sugar %	Fe (ppm)
Pymetrozine	5.92^{b}	3.83^{b}	0.380^{ab}	2.30^{ab}	26.45^{b}	0.45^{b}	8.23^{b}	29.76^{ab}
Pyriproxyfen	5.16^{c}	3.24^{c}	0.401^{a}	2.16^{b}	24.76^{c}	0.35^{c}	7.38^{c}	28.91^{b}
Acetamiprid	5.77^{b}	3.96^{b}	0.384^{ab}	2.33^{a}	26.12^{b}	0.47^{ab}	8.47^{b}	29.92^{a}
Control	6.28^{a}	4.22^{a}	0.372^{b}	2.40^{a}	27.23^{a}	0.51^{a}	8.91^{a}	30.42^{a}
LSD 5 %	0.3123	0.1377	0.02543	0.1639	0.4815	0.0564	0.2796	0.8648

The means in each column that are followed by the same letter (s) do not differ at P < 0.05, as per the results of Duncan's multiple range test. Each value represents the average of three replicates.

The results obtained in the current study agree with those achieved by Chauhan, *et al.*, (2013) who discovered that imidacloprid (neonicotinoid pesticide) reduced the ascorbic acid, reducing sugar, and total phenols contents of potatoes.

Also, Radwan, *et al.,* (2004), and AbdEl-Fatah, *et al.,* (2015) reported that some organophosphorus insecticides decrease T.S.S., total nitrogen, total soluble sugars, and β-carotene while increased ascorbic acid (vitamin C) in eggplant, and green bean and squash respectively.

According to Shalaby (2016 a), residues of chlorpyrifos, and thiamethoxam (neonicotinoid insecticide) considerably decreased the okra fruits' N%, P%, K%, T.S.S.%, acidity%, ascorbic acid mg/100g, dry matter percentage, beta-carotene mg/100g, and T. sugar%. Also, Shalaby (2016 b) discovered that the average levels of N, P, K, Fe, ascorbic acid, T.S.S., acidity, and beta-carotene decreased following six, nine, and fifteen days of profenofos tomato spraying. Shalaby (2017) discovered that, except for dry matter, lambda-cyhalothrin residues dramatically reduced the levels of all evaluated quality indices (N%, P%, K%, ascorbic acid, Fe mg/kg, TTS, β-carotene, acidity, and total soluble sugar) in pepper.

On the other hand, Shalaby and Gad (2016) found that acetamiprid, chlorpyrifos, and carbosulfan caused a significant increase in average values of tomato fruit quality parameters (N, P, K, Fe, T.S.S., ascorbic acid, total protein, and carotene content)

It seems obvious that these biochemical alterations in eggplant fruits treated with pymetrozine, pyriproxyfen, and acetamiprid may be caused by the systemic effects of these insecticides which penetrate the fruits more deeply and work on the biochemical system.

This study could be used as a guide for health risk safety use of pymetrozine, pyriproxyfen, and acetamiprid in eggplant under greenhouse conditions, also provides instructions on the safe consumption of eggplant and their impact on some physiological and biological processes in eggplant. Tested insecticides had different effects on plant components and quality which require more in-depth research in the future.

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دراسة الاختفاء و تقييم المخاطر لمتبقيات البيميتروزين والبيريبروكسيفين والأسيتاميبريد باستخدام طريقة QuECHERS وجهاز(HPLC-DAD) وتأثيراتها البيوكيميائية على الباذنجان تحت ظروف الصوب

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الملخص

الباذنجان من الخضروات ذات الاهمية الاقتصادية الكبيرة في كل من آسيا وأفريقيا بسبب غناه بالفيتامينات ومضادات الأكسدة. وتهدف الدراسة لتقدير فترة الامان وتقييم المخاطر الغذائية، وتأثير البيميتروزين، والبيريبروكسيفين، والأسيتاميبريد على بعض معايير الجودة على الباذنجان تحت ظروف الصوب. تم استخدام طريقة QuEChERS لاستخراج متبقيات المبيدات محل الدراسة. كما تم استخدام اللوني جهاز الكروماتوجرافى السائل عالي الأداء للتحليل (HPLC-DAD). وتراوحت نسبة الاسترجاع من 86 إلى 105% مع انحراف معياري نسبي (RSD) يتراوح من 1,52 إلى 10,26%. بلغت فترة نصف العمر للبيميتروزين والأسيتاميبريد والبيريبروكسيفين بمقدار 1,62، 1,152 و4,643 يومًا على التوالي. وكانت فترة ماقبل الحصاد هى 3 و 5 و 5 أيام للمبيدات الحشرية الثلاثة على التوالي. أدت جميع المبيدات الحشرية المختبرة إلى انخفاض معنوي في مستويات الكلوروفيل أ، والكلوروفيل ب، والنيتروجين، والفسفور، والبوتاسيوم في أوراق الباذنجان مقارنة بالكنترول كما سجل البيريبروكسيفين أعلى انخفاض. أدى البيميتروزين والأسيتاميبريد إلى انخفاض معنوي في محتوى المواد الصلبة الذائبة الكلية (T.S.S.) وحمض الأسكوربيك (فيتامين ج) والكربوهيدرات والسكريات الذائبة الكلية في ثمار الباذنجان مقارنة مع الكنترول، ولم يحدث انخفاض معنوي في محتوى النتروجين والبروتين والحديد الكلي. كما أدى البيريبروكسيفين إلى انخفاض معنوي في جميع القياسات باستثناء نسبة الحموضة حيث سجل أعلى انخفاض مقارنة مع الكنترول. ومما سبق نستنتج انه يمكن حصاد الباذنجان بأمان بعد 3 إلى 7 أيام من رشه بالمبيدات المختبرة. حيث كان معدل الخطر (RQ) أعلى من 1 حتى اليوم الخامس والسابع بعد التطبيق، ثم أقل من 1 لبقية الأيام، مما يدل على سلامة عالية على صحة الإنسان وانخفاض المخاطر على المدى الطويل. لذلك يجب دراسة التأثير على مكونات النبات وجودته في الابحاث المستقبلية.

الكلمات الدالة: سلوك المتبقي، الباذنجان، تقييم المخاطر، الجودة.