Determination of the residues of azoxystrobin and epoxiconazole fungicide mixture on beans and zucchini using high-performance liquid chromatography (HPLC)

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Abstract: The purpose of this study is to analyze how the systemic fungicide of azoxystrobin and epoxiconazole combination in zucchini and beans break down after being applied at the recommended rate in open Egyptian fields. This will be done using the QuEChERS technique along with high-performance liquid chromatography (HPLC). When validating a method for analyzing fungicides in commodities. These include the linearity range, the limit of quantitation (LOQ), and the accuracy in terms of precision and trueness. In our testing, we found that the recovery rates of the method at different fortification levels (0.01, 0.1, and 1.0 mg/kg) ranged from 96% to 102% for the two tested fungicides in both commodities. Additionally, the relative standard deviations (RSDs) of the two tested fungicides in both commodities ranged from 0.98% to 1.52%. Furthermore, the precision of the method in terms of repeatability at one day (RSDr) ranged from 0.88% to 1.492%. This indicates that the method is reliable and accurate for analyzing fungicides in commodities. Good linearity was obtained over the concentration ranges (0.01 -5 μg mL^{-1}) with correlation coefficient R^2 = 0.999. The limit of quantification (LOQ) for azoxystrobin and epoxiconazole was estimated to be 0.01 mg/kg. A premixed formulation (35% suspension concentrate (SC)) containing 5% azoxystrobin and 30% epoxico nazole was applied to zucchini and beans at the recommended rate of 168 grams of active ingredient per hectare. Samples were taken randomly at zero, 1, 3, 7, 10, and 15 days after the application. The fungicide residues declined following first-order kinetics rate with a half-life of 1.85 and 1.21 days for azoxystrobin in beans and zucchini, respectively, and in the case of epoxiconazole, a half-life was 3.39 and 2.89 days in beans and zucchini, respectively. The fungicide pre-harvest interval (PHI) after treatment at the recommended dose on zucchini and beans was 14 days based on the European Union maximum residue levels (MRL) (0.15, 0.1 mg/kg) for azoxystrobin in beans and zucchini, respectively, and (0.05 mg/kg) for epoxiconazole in both commodities.

Keywords: Residues, QuEChERS, Azoxystrobin, Epoxiconazole, zucchini, beans.

1. INTRODUCTION:

The common bean, *Phaseolus vulgaris L.*, is a highly valued crop species with countless applications. There are many different sorts of beans worldwide, categorized into two main groups: bush and pole beans. Open-field beans are usually harvested between May and October, while greenhouse-grown beans are available from April to December. In Egypt, it is cultivated for local market and exportation with an estimated harvested area of about 36.7 thousand hectares that produce about 144.8 thousand tons. In addition, it is widely cultivated in many countries with a total estimated harvested area of about 34.8 million hectares that produce about 27.5 million tons **(FAOSTAT, 2020).** To ensure the shelter of consumers, the beans are tested regularly for pesticide residues, which is a positive step towards securing their quality.

Zucchini is a nutrient-rich summer squash that belongs to the Cucurbitaceae family **Lucera *et al.* (2010).** it is rich in several essential nutrients, such as carbohydrates, vitamins, and minerals. and is widely cultivated in Egypt year-round ***J.-Y. Park et al*. (2011).** Zucchini is second only to watermelon in terms of popularity among cucurbits, as reported by **Ghobary and Ibrahim (2010).** Despite its popularity, this crop is vulnerable to fungal attacks that can significantly reduce its yield. To address this issue, the Agricultural Pesticides Committee of the Ministry of Agriculture and Land Reclamation highly recommended azoxystrobin and epoxiconazole for effective pest control on zucchini crops.

Fungicides play a crucial role in managing agricultural crops to achieve better yields. Fungicides can be classified into two types - protectants and specific sorts. Protectants like copper and sulfur-based products have been in use for a long time **Tentu *et al* (2013)**. This product effectively forms a protective film on the plant surface, actively preventing the germination of fungal spores, making it an essential solution for plant protection. furthermore, specific types of fungicides work by targeting a particular chemical reaction in the fungus. However, these fungicides can leave residues in crops, which may potentially cause damage to the environment and human health **Zixi *et al* (2021).** Strobilurins is one such specific type of fungicide, which is currently the world's biggest-selling fungicide namely azoxystrobin **(Rania, 2013).**

Azoxystrobin IUPAC name [methyl (*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-

methoxyacrylate] **(Fig. 1)** is a powerful fungicide that is widely used on a variety of crops. It belongs to the group of methoxyacrylates, which are derived from natural strobilurins. This broad-spectrum fungicide has many unique properties, such as curative, protectant, eradicate, and translaminar properties, making it effective against a wide range of fungal infections caused by Basidiomycota, Ascomycota, Oomycota, and Deuteromycota **(Kondo *et al.*, 2012).** Azoxystrobin is particularly effective against foliar and soil-borne diseases like downy and powdery mildew, early and late blight, and the pathogens Sclerotinia, Alternaria, Ascochyta, Pythium, and Rhizoctonia. In addition, it can be combined with other fungicides like Thiram, Metalaxyl, Difenoconazole, Chlorothalonil, Tebuconazole, and Epoxiconazole to achieve even better results against fungal growth in crops.

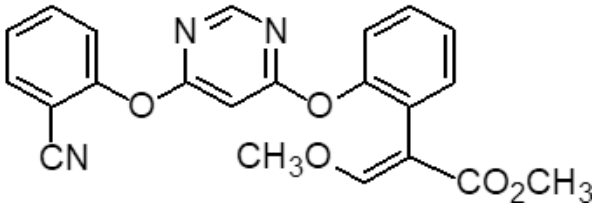

Fig. 1. Chemical structure of Azoxystrobin

Epoxiconazole chemical abstracts name is-1-[[3-(2-chlorophenyl)-2-(4-fluorophenyl) oxiranyl] methyl]-1H-1,2,4-triazole **(Fig. 2)**, is a triazole fungicide that was first produced by BASF Corporation **(Hongwu.*et al.*, 2012).** It works by inhibiting ergosterol biosynthesis, which interferes with fungal cell membrane synthesis. As a result, it is a broad-spectrum fungicide that can be used preventively and curatively to control diseases caused by Ascomycetes, Basidiomycetes, and Deuteromycetes in cereals, sugar beet, peanuts, oilseed rape, apples, and ornamentals **(Bertelsen *et al.*, 2001).**

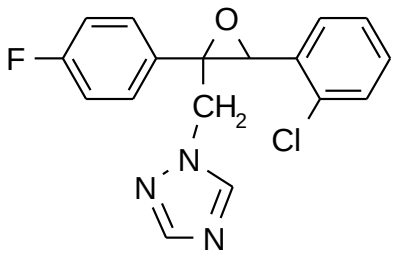

Fig. 2. Chemical structure of Epoxiconazole

In pest management programs, using a combination of fungicides is crucial. To combat fungal infections in crops, both azoxystrobin and epoxiconazole have been used individually and in combination. Many farmers worldwide use this product to manage fungal growth in their crops. It contains different components with distinct chemical molecules that work in various ways to control the fungus. As a result, it offers a broad range of effectiveness. Its combination has been proven successful in the field of plant cultivation **(Kaliyan and Tamilselvan, 2018).**

When a new pesticide is proposed for approval and maximum residue limits (MRLs) are set, gathering detailed information about pesticide residues in crops and evaluating pre-harvest intervals (PHIs) is essential. To ensure consumer safety and well-being while minimizing potential health risks associated with pesticide exposure. The Central Agricultural Pesticide Laboratory, (CAPL) located in Giza, Egypt, plays a critical role in determining PHIs when a new chemical is added to the regulatory list. This helps avoid the distribution of agricultural products that contain residues beyond the allowable limits, as stated by **Rania (2013).** As a result, the setting up of PHIs and risk assessment of pesticide residues in crops has increased the demand for the development of analytical techniques.

HPLC is a commonly used tool in analytical work that allows for both qualitative and quantitative analysis in a cost-effective, timely, and simple manner. This study utilized an HPLC method to analyze a mixture of azoxystrobin and epoxiconazole pesticides for regulatory validation purposes, including precision, linearity, repeatability, and recovery. In this study, we conducted an HPLC analysis to examine how the fungicide combination of azoxystrobin and epoxiconazole dissipates in beans and zucchini.

2.Material and Methods:

2.1.Chemicals and Reagents:

Authenticated reference analytical standards of azoxystrobin (>99% purity) and epoxiconazole (purity 99.9%) from Dr. Ehrenstorfer GmbH (Augsburg, Germany). (Ultra mil 35% suspension concentrate (SC)), containing 5% azoxystrobin and 30% epoxiconazole, was from Starchem Industrial Chemicals. HPLC-grade methanol and acetonitrile were purchased from Sigma (Sigma GmbH,

Darmstadt, Germany). Primary secondary amine (PSA, 40 µm Bondesil) and graphitized carbon black sorbent were bought from Supelco (Bellefonte, Pennsylvania, USA). Analytical grade of anhydrous magnesium sulfate and sodium chloride was obtained from CARLO ERBA Reagents S.A.S.

2.3.Preparation of standard solutions:

To conduct an HPLC analysis, a 100 ml/L mixed stock solution of azoxystrobin and epoxiconazole was prepared in acetonitrile. To set up consecutive working dilution and spike standard solution, the stock solution was diluted accordingly. All standard and working solutions were stored at 4 °C.

2.4.Field trials:

A field experiment was executed in El-Monofia Governorate, Egypt to determine the degradation of azoxystrobin and epoxiconazole fungicides on beans and zucchini. Both plants were sprayed with Ultra mil® 35% SC - a commercially available suspension concentrate formulation, at a recommended dose of 50 cm^3 / 100 L water for each plant. The treatment was carried out in 2020 as per the pest control program of the **Ministry of Agriculture and Land Reclamation (2023)**. The experiment consisted of both treated and untreated (control) plots, replicated thrice in a complete randomized block design in an open field. A knapsack hand sprayer fitted with one nozzle boom was used for pesticide treatment.

Samples of one kg of beans and two kg zucchini were collected at random from the sampling plots at intervals of one hour after application (zero time), 1, 3, 7, 10, and 14 days. The collected samples were immediately placed in polyethylene bags and transferred to the laboratory in an ice box. The samples were then roughly cut into small portions and homogenized in a food processor (HOBART). The homogeneous matrix was stored in a sealable plastic bag at -20°C until the preparation day.

2.5.Sample extraction and clean-up:

During the extraction and clean-up process, the unique virgin QuEChERS method developed by **Anastassiades *et al*. (2003)** was used with full authority. To start, 10g of homogenized beans and zucchini were weighed and placed in 50ml Teflon tubes. Next, 10 ml of acetonitrile was added and the mixture was shaken vigorously for 1 minute. After that, 4.0 g of anhydrous magnesium sulfate and 1.0 g of sodium chloride were added and shaken again for 1 minute. The tube was then immediately centrifuged at 4000 rpm for 5 minutes in a 5°C refrigerated centrifuge. Then, 1ml of the supernatant was subjected to a clean-up process using 25 mg primary secondary amine, 150 mg anhydrous magnesium sulfate, and 10 mg GCB. The tube was shaken vigorously for 1 minute and then centrifuged at 4000 rpm for another 5 minutes. Finally, 0.5 ml of the supernatant was transferred to a vial after being filtered through a 0.22µm PTFE filter (Millipore, Billerica, MA). The filtered liquid was then injected into a High-Performance Liquid Chromatography system.

2.6.Method validation:

In this experiment, we aimed to expound that our method is strongly suitable for extracting and quantitatively determining the levels of azoxystrobin and epoxiconazole in beans and zucchini. We validated our analytical method according to **SANTE/12682/2019** guidance, which included assessing matrix effects, accuracy, LOQ, precision, linearity, and trueness (bias).

To define the effectiveness of our technique, we established its linearity constructed on the concentration of azoxystrobin and epoxiconazole, which we diluted in a solvent. We evaluated the resulting correlation coefficient (R2) using a 5-point calibration curve series (0.01, 0.1, 0.5, 1.25, 2.5, and 5) µg/ml for HPLC analysis.

To confirm the matrix match effect, we compared the response from the azoxystrobin and epoxiconazole in a pure solvent with the spiked from azoxystrobin and epoxiconazole in the blank matrix (beans and zucchini) samples in the same solvent following extraction at the same concentration points (0.01, 0.1, 0.5, 1.25, 2.5, and 5) mg/kg for HPLC analysis. This step was crucial to ensure the accuracy and reliability of the results obtained.

The LOQ is a vital tool in accurately measuring the lowest concentration of azoxystrobin and epoxiconazole in beans and zucchini with high levels of precision and trueness. By following the defined analytical technique of S/N ratio of 10:1, we can confidently determine the LOQ, which guarantees the accuracy and reliability of our measurements. This information is crucial to ensure the quality of our research and helps us make informed decisions with confidence. On the authority of the document **SANTE/12682 (2019).** The limit of quantification must be less than or equal to the maximum residue limit (MRL) (0.15, 0.1 mg/kg) for azoxystrobin in beans and zucchini, respectively, and (0.05 mg/kg) for epoxiconazole in the two commodities, according to the **(European Union's regulations of 2016)**.

As part of the testing process, we conducted a recovery experiment to determine the accuracy of our results. We analyzed five replicates of a blank sample spiked with azoxystrobin and epoxiconazole at three different levels (0.01, 0.1, and 1 mg/kg) in beans and zucchini. The SANTE/11813/2017 document specifies that acceptable mean recoveries should fall between 70% and 120%.

To evaluate our precision, we used the standard deviation (RSD) for repeatability (r). We performed a similar method on similar samples in the lab over a short period. The maximum allowable limit for the relative standard deviation of repeatability (RSDr) was set at $\leq$20%. We tested five replicates at three different recovery levels (0.01, 0.1, and 1 mg/kg) for a day and

repeated the process over three different days to ensure precision.

2.7.HPLC determination:

The HPLC (Agilent 1260 infinity series) system was used to perform chromatographic analyses. This system was equipped with a quaternary pump, variable wavelength diode array detector (DAD), and an analytical column: Nucleosil C_{18} (30 × 4.6 mm (i.d.) × 5 um film thickness) with an auto sample valve. Chromatographic separation was achieved using a mobile phase of acetonitrile: water (70:30 v/v) at a flow rate of 1 ml/min. The injection volume was 20 µl and the wavelength used was 230 nm. The retention time obtained was 5.52 min for azoxystrobin and 7.21 min for epoxiconazole.

2.8.Decomposition rate:

The dissipation kinetics of azoxystrobin and epoxiconazole residues in beans and zucchini were determined by plotting residue concentration versus pass time after application and equations of the best curve fit with maximum coefficients (R^2) were determined. For the dissipation of azoxystrobin and epoxiconazole in beans and zucchini, exponential relationships were found to be applicable corresponding to the general first-order kinetics equation: $Ct=C0^{e-kt}$

Where Ct symbolized the concentration of the pesticide residue at the time of t, C0 symbolized the initial deposits after application and k is the constant rate of pesticide dissipation per day **(Wang and Hoffman, 1991).**

The half-life periods (RL50) of azoxystrobin and epoxiconazole were calculated as follows: (ln 2/k) **Moye *et al.* (1987).** The following equation calculated the degradation percentage:

%degradation = C0 - Ct/C0 × 100

where C0 is the concentration of the pesticide (ppm) at 0 time and Ct is the concentration of the pesticide (mg/kg/) during time t

3. RESULTS:

3.1. Method Validation:

In the present study, we successfully developed an HPLC-DAD method to analyze azoxystrobin and epoxiconazole residues in beans and zucchini samples.

3.1.1. Linearity: To assess the method's sensitivity, we used serial standard calibration curves. However, to improve its accuracy, we implemented matrix standard calibration curves in the quantitative calculation instead of solvent standard calibration curves. We plotted standard concentrations against quantitative ion chromatographic peaks' responses to construct calibration curves. Both fungicides showed excellent linearity of the calibration curves, as evidenced by a correlation coefficient (R2) higher than 0.999. Linear regression equations were obtained using peak areas from varying concentrations of fungicides. The equation for azoxystrobin was $y = 83.292x + 2.5097$, and for epoxiconazole, it was $y = 113.55x + 16.866$, where y denotes the peak area, and x was the concentration (mg/kg). Figure 1,2 presents calibration curves of both azoxystrobin and epoxiconazole.

3.1.2. Matrix effect (ME%) and Limit of quantification LOQ:

To ensure that the method is accurate and can be repeated, it is important to take into account the matrix effects. In this study, calibration was carried out for azoxystrobin and epoxiconazole by adding external standards to extracts of beans and zucchini. The impact of the matrix on the HPLC response was determined by comparing the slope of the calibration curve for the compounds prepared in pure solvent (acetonitrile) with those prepared in the beans and zucchini matrix. The matrix effect is a measure of how different matrices affect the response of a compound. A zero value indicates no effect, a positive value indicates enhancement and a negative value indicates inhibition **(Yanbing et al., 2016).** In this study, the matrix effect for HPLC analysis of azoxystrobin in beans and zucchini was -10.8% and -17.4%, respectively. For epoxiconazole in beans and zucchini, the matrix effect was -7.35% and -16.49%, respectively. The negative values of ME% of beans and zucchini HPLC-UV analysis reflect matrix-induced suppression.

The limit of quantification (LOQ) is a measure used to determine the accuracy and precision of a method. It represents the minimum concentration of target analyses in a given matrix with a signal-to-noise ratio of 10 **(Su et al., 2020).** In the experiment previously mentioned, the LOQs for azoxystrobin and epoxiconazole in beans and zucchini matrices were found to be 0.01 mg/kg. According to **SANTE/12682 (2019)**, LOQ values are acceptable as long as they are less than or equal to the maximum residue limit (MRL) (0.15, 0.1 mg/kg) for azoxystrobin in beans and zucchini, respectively and (0.05 mg/kg) for epoxiconazole in the two commodities confirmed by the European Union (**European Union 2016**).

3.1.3. Trueness and precision:

As a part of the study, various concentrations of azoxystrobin and epoxiconazole standard solutions were added to blank beans and zucchini samples. The recovery mean was determined in five replicates at three fortification levels (1, 0.1, 0.01 mg/kg) by spiking ten g of blank samples with the standard solution, all done by a single analyst in a day.

To evaluate the accuracy of the technique, the relative standard deviation (RSD) was examined, and the observed area had an RSD of less than 20%. To assess the intra-day precision, RSDr was tested by analyzing five replicates at the LOQ level of 0.01 mg/kg on the same day. This demonstrated that azoxystrobin and epoxiconazole can be identified with acceptable precision, as long as the extraction technique used has good recoveries.

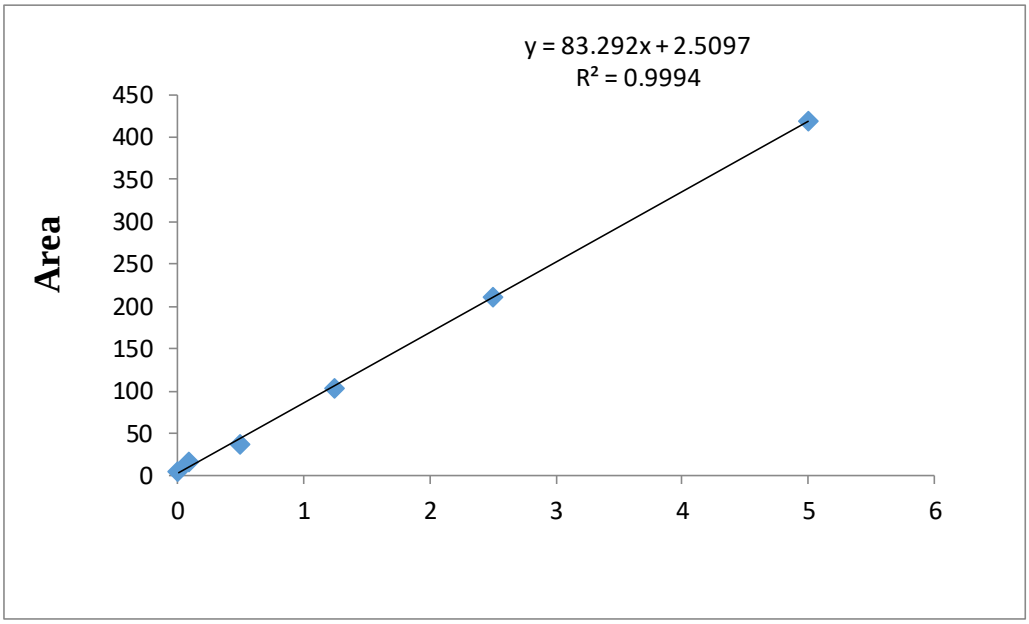

Fig. 3.Calibration curve of azoxystrobin with HPLC-DAD analysis

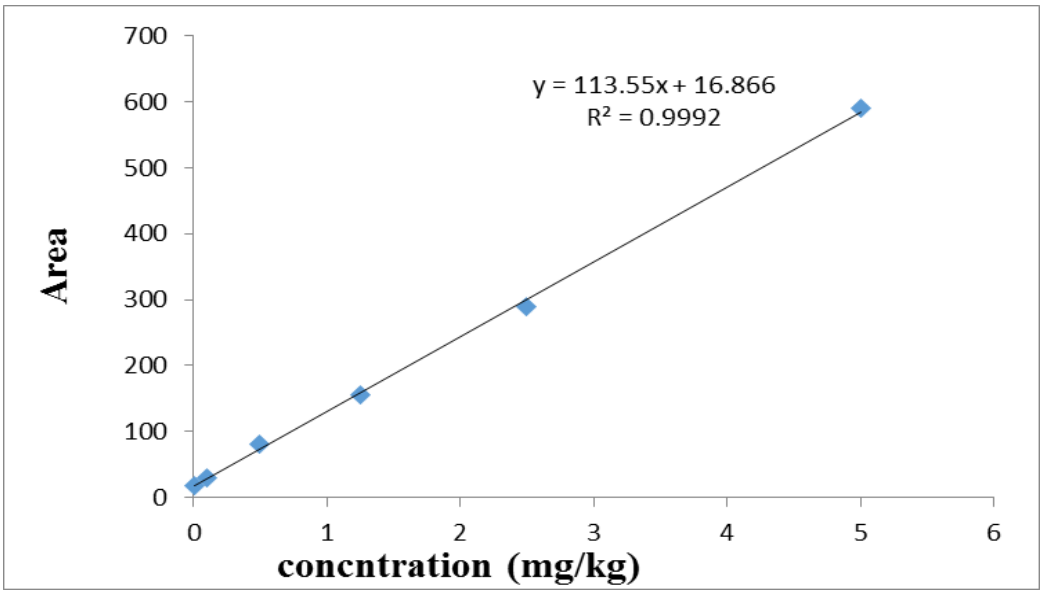

Fig. 4. Calibration curve of epoxiconazole with HPLC-DAD analysis

Lastly, the samples were analyzed and tested for pesticide residue according to well-established guidelines.

The average fortified mean recoveries of Azoxystrobin in beans ranged from 98.09-100.68% with a relative standard deviation (RSD) of 1.22-0.98%, and the recoveries of Azoxystrobin in zucchini ranged from 97.29-101.48% with the relative standard deviation (RSD) of 1.35–1.14% Table 1,2. On the other hand, the obtained mean recoveries ranged from 96.89% to 99.68%, RSD ranging from 1.84 to 1.34 for Epoxiconazole in beans, and Epoxiconazole ranged from 96.69-100.68% in zucchini with the relative standard deviation (RSD) of 0.85–0.98% Table.3,4, according to (**SANTE/12682/2019**),. The results agree with the SANTE guide for pesticide residues, which states that the recovery values for each spiking should be between 70 and 168%, with an RSD of less than 20% [**SANTE/12682/2019**].

Table (1): Fortification level and recovery percentage (±RSD) of Azoxystrobin in Beans for HPLC-DAD analysis

Spiking level (mg/kg) (n*=5)	Mean recovery (%RSD)	RSDr%
0.01	98.09±1.22	1.24
0.1	101.26±1.07	1.06
1	100.68±0.98	0.97

Table (2): Fortification level and recovery percentage (±RSD) of Azoxystrobin in zucchini for HPLC-DAD analysis

Spiking level (mg/kg) (n*=5)	Mean recovery (%RSD)	RSDr%
0.01	97.29±1.35	1.38
0.1	102.46±1.52	1.49
1	101.48±1.14	1.12

Table (3): Fortification level and recovery percentage (±RSD) of Epoxiconazole in Beans for HPLC-DAD analysis

Spiking level (mg/kg) (n*=5)	Mean recovery (%RSD)	RSDr%
0.01	96.89±1.84	1.9
0.1	101.46±1.46	1.44
1	99.68±1.34	1.34

Table (4): Fortification level and recovery percentage (±RSD) of Epoxiconazole in zucchini for HPLC-DAD analysis

Spiking level (mg/kg) (n*=5)	Mean recovery (%RSD)	RSDr%
0.01	96.69±0.85	0.88
0.1	100.26±1.22	1.22
1	100.68±0.98	0.97

3.2.Dissipation behaviors of Azoxystrobin and Epoxiconazole in beans and zucchini:

Table 5,6 indicates the residues, pattern of percentage dissipation, regression equations, half-life value, PHI, and dissipation rate constant for Azoxystrobin and epoxiconazole in ready premix formulation in beans and zucchini. Additionally, Figure 3 illustrates the dissipation kinetics curve based on the experimental data.

After applying the authorized dose of 168 g a.i/ha of fungicide to both beans and zucchini, monitoring the levels of residues for up to 10 days after the application. Our analysis revealed that the residues of Azoxystrobin and epoxiconazole in both beans and zucchini declined significantly over time, and the dissipation rates followed a first-order kinetics model. The equations for the degradation dynamics and dissipation rates after the authorized dose application were as follows $Ct = = 0.7247e^{-0.344x}$ for beans and $Ct = 0.6517e^{-0.439x}$ for zucchini. in the case of Azoxystrobin, and $Ct = 6.0207e^{-0.328x}$ for beans and $Ct = 7.9851e^{-0.29x}$ for zucchini in the case of epoxiconazole.

After applying a ready premix formulation containing Azoxystrobin and epoxiconazole on zucchini and beans, the dissipation residue amount decreased by elapsing time. after one hour of application, the initial deposits of Azoxystrobin at the authorized dose on beans and zucchini were 0.83 and 0.76 mg·kg−1, respectively. After 7 days from application, residue levels decreased to 0.08 and 0.04 mg/kg mentioning that 90.36% and 94.73% of Azoxystrobin residue had dissipated in beans and zucchini, respectively. Residues for beans were beneath the MRL of 0.51 mg/kg (EU-MRL database). For zucchini, residues were beneath the MRL of 1 mg/kg after application and undetectable after ten days. After the application of epoxiconazole in beans and zucchini, the initial residues showed 6.62 and 5.41 mg/kg, respectively. The residues decreased to 0.37 and 0.17 mg/kg after 10 days of spraying, indicating a dissipation of more than 96%. By the 15th day, a

complete dissipation of residues below the detectable level at the recommended dose on beans and zucchini was recorded.

Based on the breakdown curve, we calculated the degradation half-lives of Azoxystrobin and epoxiconazole in beans and zucchini province as 1.85 and 1.21 days for azoxystrobin in beans and zucchini, respectively, and in case of epoxiconazole a half-life were 3.39 and 2.89 days in beans and zucchini, respectively, using the method of **Hoskins (1961)** (See Table 5.6). The fungicide pre-harvest interval (PHI) on beans and zucchini was 14 days constructed on the European Union maximum residue levels (MRL) after treatment at the mentioned dose and the residues were observed under the detectable level of maximum residue levels (0.15, 0.1 mg/kg) for azoxystrobin in beans and zucchini, respectively and (0.05 mg/kg) for epoxiconazole in the two commodities.

Table (5): Residue levels and dissipation behavior of azoxystrobin in zucchini and Beanss under field conditions

intervals (days)	Residues (ppm)	% Loss	% Persistence	Residues (ppm)	% Loss	% Persistence
	zucchini			Beans		
initial*	0.76±0.007	0.00	100.00	0.83±0.035	0.00	100.00
1	0.59±0.007	22.36	77.64	0.63±0.07	24.09	75.91
3	0.08±0.007	89.47	10.53	0.15±0.02	81.92	18.08
7	0.04±0.02	94.73	5.27	0.08±0.04	90.36	9.64
10	ND	100.00	0.00	ND	100.00	0.00
14	ND	100.00	0.00	ND	100.00	0.00
EU MRL		1			0.15	
PHI (days)		1			7	
RL50 (days)		1.21			1.85	

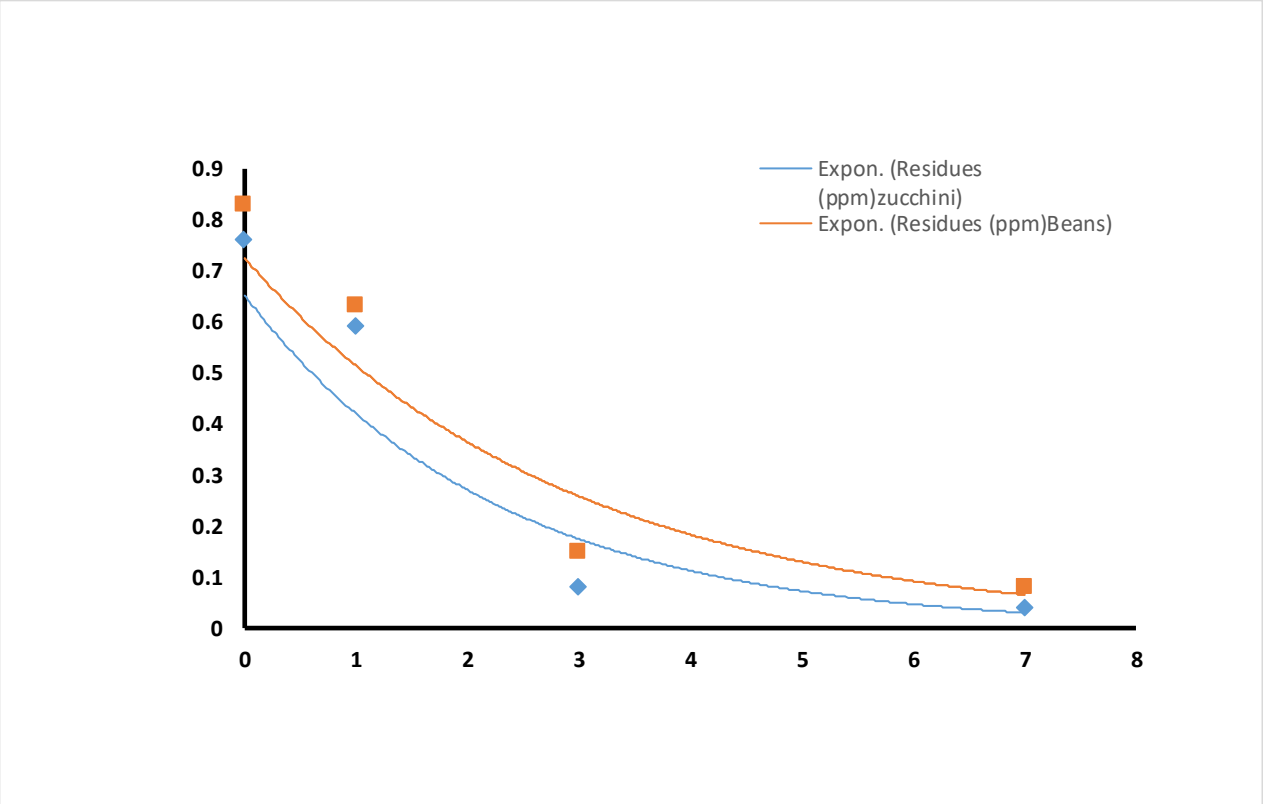

Fig. 5. Dissipation behavior of azoxystrobin in zucchini and Beans under field conditions

Table (6): Residue levels and dissipation behavior of epoxiconazole in zucchini and Beanss under field conditions

intervals (days)	Residues (ppm)	% Loss	% Persistence	Residues (ppm)	% Loss	% Persistence
	zucchini			Beans		
initial*	5.41±0.48	0.00	100.00	6.62±0.33	0.00	100.00
1	4.35±0.32	19.95	80.05	5.83±0.35	11.93	88.07
3	2.18±0.16	59.7	40.3	4.23±0.09	36.1	63.9
7	0.92±0.02	82.99	17.01	1.23±0.21	81.41	18.59
10	0.17±0.03	96.85	3.15	0.37±0.07	94.41	5.59
14	ND	100.00	0.00	ND	100.00	0.00
EU MRL		0.05			0.05	
PHI (days)		14			14	
RL50 (days)		2.89			3.39	

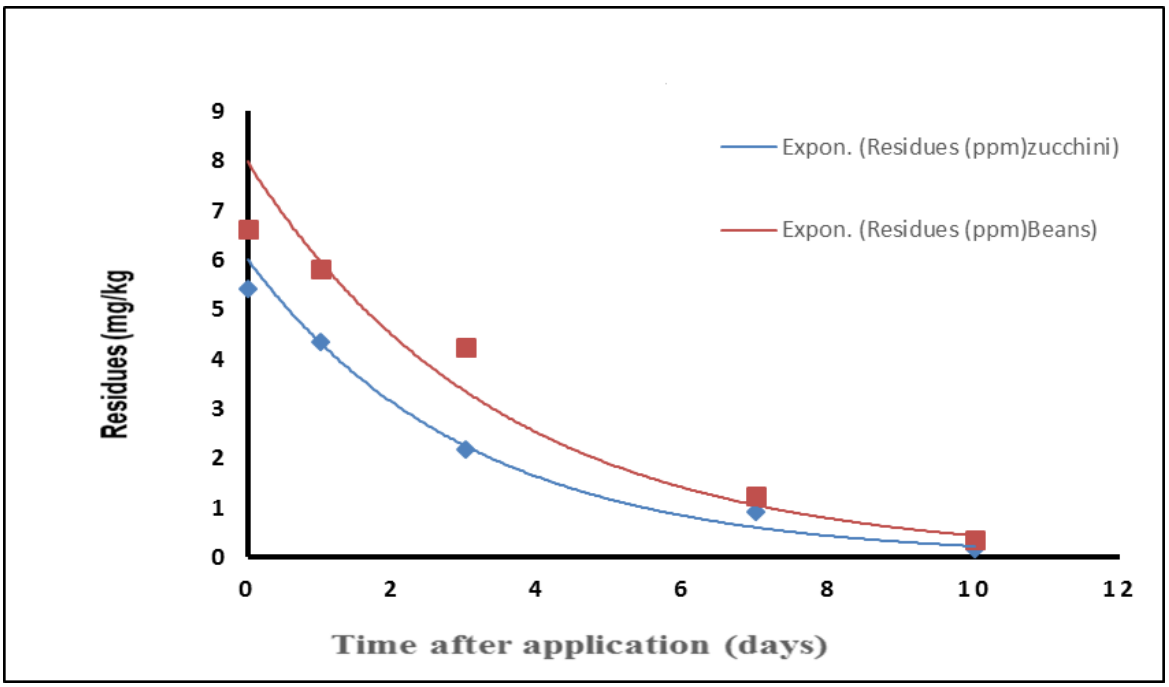

Fig. 6 Dissipation behavior of epoxiconazole in zucchini and Beans under field conditions

4.DISCUSSION:

The results of method validation demonstrated that the QuEChERS sample technique, coupled by HPLC-DAD analysis was a valid method and fitted to detect and quantified azoxystrobin and epoxiconazole in beans and zucchini samples. The results of validation are within agreeable criteria for pesticide residue analysis according to **SANTE/12682 (2019).**

The data show that beans and zucchini could be safely consumed after 14 days of premixed formulation application according to the recommended maximum residue limit (MRL). However, the fungicide combination generally dissipated faster in zucchini compared to beans. Several factors can affect how pesticide residue breaks down in plants under field conditions. These factors include natural processes, such as volatilization, wash-off, and photodegradation, as well as climatic conditions like sunlight, temperature, humidity, and wind (**Tripathi *et al.*, 2015**). Other factors that can have an impact include the stability of the exposure, the amount of pesticide used, the duration of pesticide application, the characteristics of the pesticide, such as its total stability, volatility, solubility, and formulation, the sort of crops being treated, and the timing and location of pesticide application **(Malhat *et al.*, 2016 and Malhat *et al.*, 2017).** Furthermore, the growth dilution factor can also impact pesticide residue concentrations while still in the field. Plant growth can decrease pesticide residue concentrations due to growth dilution effects**. (Dalia and Rania, 2017).** As maintained by the results

of a literature study, Pyraclostrobin and epoxiconazole residues on groundnut plants disappear within 10 days. Their DT_{50} (Half-Life) was calculated as 2.84 days using dissipation data regression analysis **(Tentu *et al*., 2013).** A recent research was carried out on the residues of a commercial (20% epoxiconazole + 20% pyraclostrobin) in wheat grain. The study revealed that epoxiconazole and pyraclostrobin have different dissipation behaviors in grain and that the recommended dosage is safe. **Zixi *et al*. (2021).**

CONCLUSION:

The study successfully extracted residues of Azoxystrobin and epoxiconazole from beans and zucchini samples using the QuEChERS method combined with HPLC. The results showed acceptable accuracy and precision. The proposed method was verified according to the guidelines of SANTE/12682/2019. The developed method satisfied the criteria related to linearity, LOQs, recoveries, accuracy, and matrix effect, and was effectively used to study the dissipation kinetics of Azoxystrobin and epoxiconazole in beans and zucchini from the field. The degradation rates of two fungicides tested on beans and zucchini were evaluated under field conditions in Egypt. The results showed that the degradation rate of both fungicides was faster on zucchini than on beans, with a calculated half-life ($t_{0.5}$) of [1.85 and 1.21 days for azoxystrobin in beans and zucchini, respectively and in case of epoxiconazole a half-life were 3.39 and 2.89 days in beans and zucchini, respectively,]. Complete dissipation of residues was observed below the detectable level at the recommended rate of app;ication on both beans and zucchini by the 15th day. The pre-harvest interval (PHI) for fungicide on beans and zucchini was determined to be 14 days based on the European Union maximum residue levels (MRL) after treatment at the recommended dose. The residues were found to be under the detectable level of maximum residue levels (0.15, 0.1 mg/kg) for azoxystrobin in beans and zucchini, respectively, and (0.05 mg/kg) for epoxiconazole in both commodities.

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تقدير متبقيات مخلوط المبيد الفطرى الآزوكسي استروبين والإيبوكسي كونازول على ثمار الفاصوليا والكوسا باستخدام جهاز الكروماتوجرافي السائل عالي الأداء (HPLC)

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الملخص العربى:

الغرض من هذه الدراسة هو كيفية تحليل مخلوط مبيد الفطريات الجهازي المكون من الازوكسى استروبين وإيبوكسي كونازول في الكوسة والفاصوليا بعد تطبيقه بالمعدل الموصي به في الحقول المصرية المفتوحة. وذلك باستخدام تقنية QuEChERS مع التحليل السائل عالي الأداء . (HPLC) للتحقق من صحة طريقة التحليل هناك معايير وهى الخطية، والحد الكمي(LOQ) ، والدقة و معدل الاسترجاع. ، وجدنا أن متوسط معدلات الاسترجاع للطريقة عند مستويات مختلفة (٠,٠١، ٠,١، ١,٠ مجم/كجم) تراوحت من ٩٦% إلى ١٠٢% لمبيدي الفطريات المختبرين في كلا من الثمار المعاملة. بالإضافة إلى ذلك، تراوحت الانحرافات القياسية النسبية (RSDs) لمبيدي الفطريات المختبرين في كلا من الثمار المعاملة من ٠,٩٨% إلى ١,٥٢%. علاوة على ذلك، تراوحت دقة الطريقة من حيث التكرار في يوم واحد (RSDr) من ٠,٨٨% إلى ١,٤٩٢%. يشير هذا إلى أن الطريقة موثقة ودقيقة لتحليل مبيدات الفطريات في ثمار الخضر المعاملة. تم الحصول على خطية جيدة على مدى تركيز (٠,٠١-٥ ميكروجرام/مل) مع معامل ارتباط .R2 = 0.999 تم تقدير الحد الكمي(LOQ) للطريقة وكان للأزوكسيستروبين والإيبوكسيكونازول بـ ٠,٠١ مجم / كجم. تم رش محصول الكوسة والفاصوليا بمستحضر الترا ميل ٣٥% مركز معلق يحتوي على ٥% أزوكسيستروبين و٣٠% إيبوكسيكونازول بالمعدل الموصى به وهو ١٦٨ جم (مادة فعالة/ هكتار). وقد تم أخذ العينات عشوائياً بعد ساعة من الرش (صفر) ، ١، ٣، ٧، ١٠، و١٤ يوماً بعد التطبيق. تم استخلاص العينات وتنقيتها باستخدام طريقة QuEChERS . وتشير النتائج ان منحنى اختفاء مبيد الازوكسى استروبين وإيبوكسي كونازول يتبع معادلة الخط المستقيم من الدرجة الأولى وكانت فترة نصف العمر ١,٨٥ و ١,٢١ يومًا الازوكسى استروبين في الفاصوليا والكوسا، على التوالي، وفي حالة وإيبوكسي كونازول كانت فترة نصف العمر ٣,٣٩ و ٢,٨٩ يومًا في الفاصوليا والكوسة على التوالي. وكانت فترة ما قبل الحصاد للمبيد الفطرى (PHI) بعد التطبيق بالجرعة الموصى بها على الكوسة والفاصوليا ١٤ يومًا بناءً على مستويات المتبقيات القصوى المسموح بها للاتحاد الأوروبي (MRL) (٠,١٥-٠,١ مجم / كجم) الازوكسى استروبين في الفاصوليا والكوسة، على التوالي. و(٠,٠٥ ملغم/كغم) إيبوكسي كونازول في كلا المحصولين.

الكلمات الدالة: متبقيات – QuEChERS – مبيد الازوكسى استروبين– إيبوكسي كونازول - الفاصوليا -الكوسة.