Deterioration of Some Pesticide Residues in Strawberry Fruits and Their Health Risk Assessment

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Abstract: The objective of the present investigation was to evaluate the risk associated using five pesticides and examine the residue dynamics of those treatments on strawberry plants. In the mature stage, different applications of cyazofamid, azoxystrobin, tricyclazole, prochloraz, and pyrimethanil were applied. High performance liquid chromatography (HPLC) was used to examine fruit samples that were taken at various intervals between two hours and fifteen days following application. Cyazofamid with azoxystrobin, prochloraz, tricyclazole, and pyrimethanil had respective half-lives (t½) of 2.88, 2.97, 2.02, 2.94, and 3.10 days. The corresponding t_{90} values were 4.49, 4.58, 3.63, 4.55, and 4.71 days. Strawberry fruits might be safely consumed after 10 days of all treatments except for pyrimethanil after 15 days, according to MRLs and hazards. The dissipation pattern highlights how important it is to have appropriate pre-harvest intervals in order to minimize residue levels before consumption, and even with considerable residues, the risk quotient (RQ) levels were still below 1, indicating a very insignificant risk to the adults.

Keywords: Strawberry, residue, risk, cyazofamid, azoxystrobin, tricyclazole, prochloraz, pyrimethanil.

1.Introduction:

Strawberries are renowned around the world for their rich nutritious content and mouthwatering flavor. Due to their importance in our diet, strawberry fruits are widely recognized for their positive effects on human health. Only 32 calories are found in 100 grams of fresh strawberries. They boost the human immune system and provide vitamins and minerals to diet. (**Shalaby *et al*., 2022)** and **(El-Dawlatly *et al*., 2025).**

A major horticultural crop in Egypt, strawberries make up around 5% of global production and 75% of African production, respectively **(FAO, 2018)**. strawberry is a vital crop for Egyptian exporters, on count of richness in protein, fiber, organic acids, polyphenols, vitamins, minerals, and carbohydrates (**Salim *et al*., 2019)**. Accordingly, strawberries provide a number of health benefits to people, including protection against cancer, obesity, aging, neurological disorders, and cardiovascular disease (**Saber *et al*., 2016)**

Significant problems with pests and diseases have been found in the methods used for planting and manufacturing, as well as in the use of pesticides to manage a range of diseases caused by insects, mites, fungi, and bacteria.

These diseases must be prevented and treated with fungicides. However, since such treatments leave pesticide residues in raw strawberries, it may be hard to get the fruit to consumer within the duration that is limited whereas residual levels are existed. Strawberries are usually eaten fresh without further processing; the dietary risks connected with them have admiring a lot of thought recently and turn into a major concern in food safety and global exchange. Meanwhile, unresponsible management of pesticides has led to the appearance of pesticide resistance **(Cosseboom *et al*., 2019), (Brevik *et a*l., 2018)**. In addition to decreasing the efficacy of these pesticides, this compromises the quality and safety of agricultural goods by leaving pesticide residues in them **(Malalgoda *et al*., 2021), (Narenderan et al., 2020)**.

Investigating the dissipation of cyazofamid, azoxystrobin, tricyclazole, prochloraz, and pyrimethanil and their long-term health risk in strawberries were goals of this study. This would offer fundamental data for formulating rules pertaining to the safe application in environmental protection, pest management plans and health risk evaluation of the five fungicides tested on strawberry fruit helping conducting safety for human health.

2.Materials and methods:

2.1.Standards and reagents:

Cyazofamid, azoxystrobin, tricyclazole, prochloraz and pyrimethanil certified reference standards, > 98% pure, were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Merck provided the anhydrous magnesium sulfate and sodium chloride as extraction salts. Solid phase extraction (SPE) clean- up kits (Agilent Technologies), and organic solvents of HPLC quality (acetonitrile and methanol) were obtained from Sigma (Sigma GmbH Darmstadt, Germany).

Stock solutions prepared in acetonitrile and stored at -20 ºC until needed. Calibration standard solutions were adopted at concentrations of 10, 5, 1, 0.5, 0.1, 0.05, and 0.01 mg/L to get them ready for HPLC detection.

2.2.Field experiments:

The experiment was carried out in a strawberry field in Abu Zaabal village, Qalyubia Governorate, Egypt. A randomized full design was used for the studies. Determined 50 m^2 plot was used for each pesticide, with a 2 m gap between plots. A motor sprayer was utilized to apply the tested pesticides rates. Mystic Pro 50% EC was sprayed on the mature strawberry fruits at a rate of 25 cm^3/100 L of water, Zemac Seif 30% SC was sprayed at a rate of 120 cm^3/100 L of water, and Gesarol 30% SC was sprayed at a rate of 100 cm^3/100 L of water. The control plot was away from treated plots for at least 40 m and left unsprayed. Fruit samples were taken 2 h, 1, 3, 5, 7, 10, and 15 days after pesticides applications The collected samples (2 kg collected randomly) were transferred to the lab.

2.3.Samples preparation:

Extraction and cleanup were proficient following a modified method of **(Anastassiades *et al*., 2003)**. 10 g of well-grounded and homogenized strawberry samples put into 50 mL Falcon tube. Then 10 mL of 0.1% acidified MeCN were added. Tubes were stirred vigorously for one min by using vortex mixer at maximal speed then extraction salts added for each, mixed for 30 seconds, then centrifuged for 4 min 5000 rpm. Approximately, 1mL of the upper MeCN transferred to 15 mL Falcon tubes containing clean-up salts, 25 mg PSA sorbent and 150 mg anhydrous $MgSO_4$, capped tightly and shaken by hand for 1 min. Samples were recentrifuged. Extraction and cleaned up conducted in triplicates. About 0.5 mL of extracts was transferred into amber HPLC vials for analysis.

2.4.Method Validation:

To assess extraction efficiency, several important aspects of the extraction procedure were modified. The guidelines in Document **(SANTE, 2021)**, **(European Commission, 2021)** were followed for analysis validation. The validation parameters of linearity, accuracy, precision, limit of quantification (LOQ) were assessed to confirm viability. Three concentrations 0.05, 0.5 and 5 mg/kg of cyazofamid, azoxystrobin, tricyclazole, prochloraz, and pyrimethanil were applied to strawberry blank samples in order to assess accuracy and precision. Each treatment was replicated five times. The recovery and relative standard deviation (RSD) percentages were computed in order to identify best effective combination of purifying agents. The spiked samples were extracted, cleaned-up, and analyzed as described above after an hour to allow for pesticide absorption. LOQ were used to test the method's sensitivity. The lowest spiked concentration quantification was used to determine the limit of quantification (LOQ) for the suggested approach. The accuracy (average recovery) was determined by splitting the spike measured concentration by the matrix recovered, and the precision (relative standard deviation of repeatability analyses, (RSD) was determined by partitioning the standard deviation by the mean concentration. To detect linearity and pesticide content in samples, calibration curve was achieved.

2.5.Instrumentation:

An Agilent HPLC 1260 infinite series (Agilent Technologies) HPLC system equipped with an autosampler, electric sample valve, diode array detector (DAD), and a quaternary pump were used. An ODS analytical column of 150 mm x 4.6 mm x 5 m was used in the HPLC system. table 1 displayed mobile phases, flow rates, detection wavelengths, and retention time.

Table 1. Mobile phase, flow rate, wave length and retention time for tested pesticides

Pesticide	Mobile phase	Flow rate ml/min	Wave length (nm)	Retention time (min.)
Cyazofamid	methanol 80% + water 20 %	1	254	6.21
Azoxystrobin	methanol 80% + water 20 %	1	210	4.05
Tricyclazole,	acetonitrile 70% + water 30 %	1	205	5.28
Prochloraz	methanol 80% + water 20 %	1.3	210	4.53
Pyrimethanil	methanol 80% + water 20 %	1	270	5.74

2.6.Statistical analysis:

Depreciation performance and half-lives for cyazofamid, azoxystrobin, tricyclazole, prochloraz, and pyrimethanil in/on strawberry calculated by first-order kinetic model, were represented by Equations. (1,2, 3):

(1) $$C_t = C_0 e^{-kt}$$

(2) $$t_{1/2} = \ln 2/k$$

(3) $$t_{90} = \ln 10/k$$

where C_0 is the residue concentration at zero time (mg kg^{-1}); Ct is the residue level (mg kg^{-1}) at time t (day) after application; and k is the degradation rate constant (day^{-1}).

For appraising chronic risk in contaminated samples, national estimated daily intake (NEDI, mg/kg bw) to detect long term intake risk and risk quotient (RQ) were figured using the following equations (4, 5).

(4) $$NEDI = \Sigma(Fi \times STMR / bw)$$

(5) $$RQ = NEDI / ADI$$

Where (NEDI) is National Estimated Daily Intake (mg/kg/bw), STMR is the median residue, Fi is the food consumption strawberry in Egypt (0.0589 kg/day), bw is the average body weight of an Egyptian adult (60 kg), and ADI is the acceptable daily intake of cyazofamid, azoxystrobin, tricyclazole, prochloraz, and pyrimethanil (0.2, 0.2, 0.01, 0.03 and 0.2 mg/kg bw) respectively.

3.Results and discussion:

3.1.Method Validation:

Among the validation factors were the analytical method's precision, accuracy, linearity, and limit of quantitation. The accuracy and precision of the approach were assessed using the recovery test and relative standard deviation (RSD). The cyazofamid, azoxystrobin, tricyclazole, prochloraz and pyrimethanil recovery ranges and corresponding relative standard deviations for the three spike levels in strawberry were calculated. The mean recovery of cyazofamid ranged from 88.14 to 105.87% with RSD values were ranged 1.5% to 5.66%, while the mean recovery of azoxystrobin ranged from 92.45 to 110.23% with RSD ranged from 0.91% to 3.11%, on the other hand the mean recovery of prochloraz ranged from 90.76 to 98.42% with RSD values ranged from 2.41% to 5.98% and the mean recovery of tricyclazole ranged from 91.10 to 100.63% with RSD values were ranged 1.21% to 3.74%, also, the mean recovery of pyrimethanil ranged from 87.33 to 96.85% with RSD values were ranged 3.01% to 6.25%. The results show how precise and accurate the recommended approach is. The suggested method shows the assessment of linearity, limit of quantitation, precision, and accuracy and can be used for the quantitative measurement of cyazofamid, azoxystrobin, tricyclazole, prochloraz and pyrimethanil in strawberry. Automation reduces amount of organic solvent used and increases environmental safety than old methods. The method defined by a high detection efficiency and is acceptable priced. Also had exceptional stability, high reliability, and remarkable sensitivity are further benefits.

The limit of quantification (LOQ) expressed as the minimum concentration that gives a signal-to-noise of 10 in the chromatographic signal. Good linearity with R^2 values ranged between 0.979 and 0.996 was obtained for all tested pesticides.

3.2.Deterioration studies:

Residues and deterioration rates and RSD of cyazofamid and azoxystrobin, prochloraz, tricyclazole and pyrimethanil in and on strawberry fruit are illustrated in Fig. 1

The initial time in strawberry fruit after 2 h of treatment were 1.65, 2.21, 2.38, 157 and 1.85 mg kg^{-1} for cyazofamid and azoxystrobin, prochloraz, tricyclazole and pyrimethanil respectively. These amounts dropped to 0.04 and 0.1, 0.1, 0.05 and 0.1 mg kg-1 after 10 days of the application for cyazofamid and azoxystrobin, prochloraz, tricyclazole and pyrimethanil respectively. The residues for all the pesticides were below the detection limits at 15 days after treatments except prochloraz, residue was 0.04 mg kg^{-1}. The t½ values were 2.88, 2.97, 2.02, 2.94 and 3.10 days, respectively from the time of treatment. While the t_{90} values were 4.49, 4.58, 3.63, 4.55 and 4.71 days for cyazofamid and azoxystrobin, prochloraz, tricyclazole and pyrimethanil respectively from the time of treatment. Apparently, strawberry fruits could be consumed safely after 10 days of all treatments and after 15 days of pyrimethanil application.

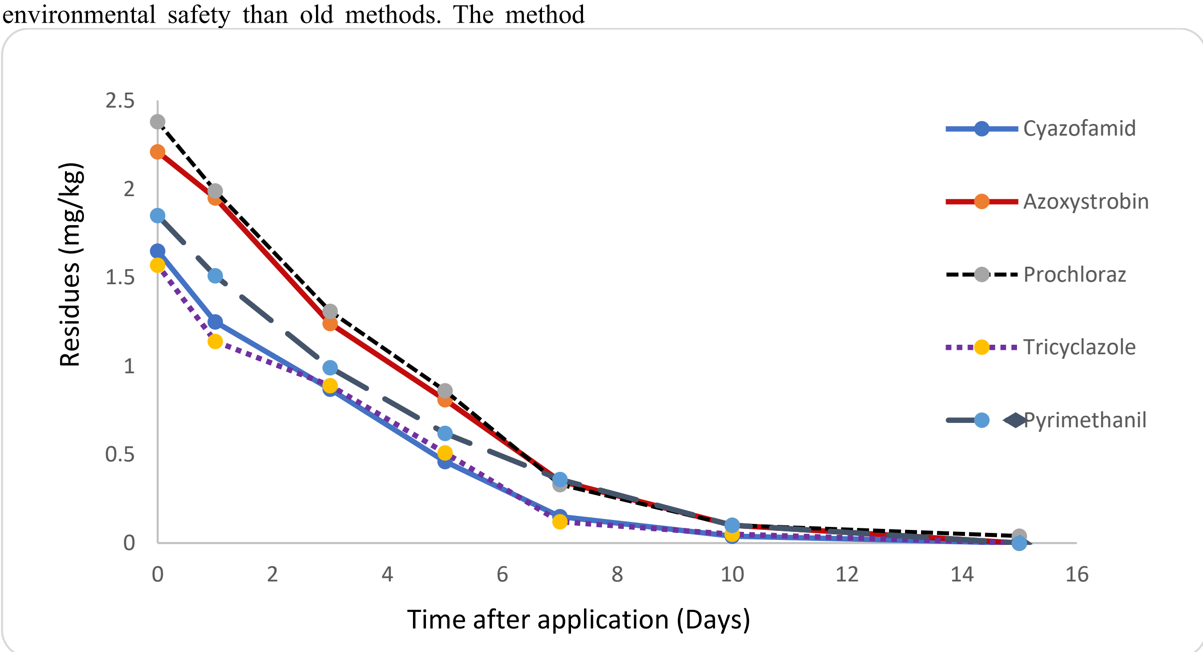

Fig. 1 Deterioration pattern of tested pesticides in strawberry fruit

Our results are in agreement with (**Salim *et al.*, 2019**) who evaluated the residues of propiconazole in strawberry samples that collected from Bader city and El-Minofyia city. The Residue levels were 0.048 and

0.038 mgKg^{-1} in both cities, respectively. On other hand residues of detected pesticide were lower than their reciprocal maximum residue limits of European Union (EU-MRLs) that focused the safety of strawberry retailed for Egyptian consumers. (studied the residue dynamics of hexythiazox and spinosad on strawberry plants, The results showed that the half-life (t½) values of hexythiazox and spinosad were 2.23 and 3.24 days in strawberry fruit, respectively. Regarding MRLs and risks, strawberry fruits could be consumed safely at 6 days of hexythiazox or spinosad application **(Angioni, *et al*., 2004)** Identified the pyrimethanil and azoxystrobin residues in strawberry samples. which, respectively, averaged 0.55 and 2.98 mg/kg. With a half-life of roughly 8 days for azoxystrobin and 4.8 days for pyrimethanil, all residues decreased below the maximum residue level (MRL) set by the European Union (2.0 and 5 mg/kg, respectively). The characteristics of the pesticide, such as its overall stability, volatility, solubility, formulation, and method and site of application, as well as environmental factors, specifically temperature, precipitation, and air movement, are among the factors that affect a pesticide's persistence. The plant species, the type of crop that was harvested, the cuticle structure, the growth stage and rate, and the overall health of the plant are other factors that affect the persistence of pesticides in plants (**Edwards, 1975)**.

3.3.Risk assessment calculation:

The national estimated daily intakes (NEDIs), and health risk assessment for cyazofamid, azoxystrobin, prochloraz, tricyclazole and pyrimethanil in the treated strawberry fruit are summarized in Table 2.

The associated risk was assessed using median residue analysis at different intervals. The national estimated daily intake (NEDI) values for cyazofamid, ranging from 2.9×10^{-5} to 1.2×10^{-3} mg kg-1 body weight. While NEDI) values for azoxystrobin, ranged from 7.0×10^{-5} to 1.6×10^{-3} mg kg^{-1} body weight. On other hand, the NEDI values of prochloraz, tricyclazole and pyrimethanil were raged from $3.0\text{x}10^{-5}$ to $1.7\text{x}10^{-3}$, $4.0\text{x}10^{-5}$ to $1.1\text{x}10^{-3}$ and $7.0\text{x}10^{-5}$ to $1.3\text{x}10^{-3}$ mg kg^{-1} body weight. and risk quotient (RQ) values, all well below 1 threshold, provide reassurance of the minimal health risk from tested pesticide residues in strawberry fruits. The amounts of pyrimethanil, prochloraz, tricyclazole, azoxystrobin, and cyazofamid residues steadily dropped over time. Even with significant residues, the RQ levels were still below 1, indicating a very small risk to the adults. The dissipation pattern emphasizes how crucial to have suitable pre-harvest intervals in order to lower residue levels prior to consumption. Our findings offer crucial information for applying these pesticides in strawberry farming in a safe manner.

Our results were in agreement with (**El-Dawlatly *et al*., 2025)** who stated that the estimated daily intake (EDI) of spinosad did not exceed the acceptable daily intake (ADI= 0.02 mg/kg according to EU-Codex). Therefore, there was no risk associated with eating these fruits **(Brancato *et al*., 2018)**. The health index (HI) amount was less than 100; in strawberry fruits collected immediately after spray, its value was 18 and decreased with time elapsed to reach 3.5 after 5 days. Additionally, (***Shalaby et al*., 2022)** reported that the risk of pesticides was very low six days after application, and that this risk may be shortened by processing processes. When strawberry collected has low quality or any defects, they were turned into jam as consequences the six-day safety period after treatment may be decreased to just two hours after spraying.

Conclusion:

For the analysis of cyazofamid with azoxystrobin, prochloraz, tricyclazole, and pyrimethanil residues in strawberries, the HPLC-DAD method was verified and refined. 2.88, 2.97, 2.02, 2.94, and 3.10 days are the half-lives (t½). The matching t_{90} values, on the other hand, were 4.49, 4.58, 3.63, 4.55, and 4.71 days. According to residual data, strawberry fruits can be safely consumed after 10 days, with the exception of pyrimethanil, which should be consumed after 15 days. Risk was evaluated, and the findings showed that eating strawberries did not pose any major health risks.

Table 2. NEDI and RQ values though consumption of strawberry fruits treated with cyazofamid, azoxystrobin, prochloraz, tricyclazole and pyrimethanil

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Time after application (days)	Cyazofamid			Azoxystrobin			prochloraz			tricyclazole,			pyrimethanil		
	Residues	NEDI (mg/kg))	RQ	Residues	NEDI (mg/kg))	RQ	Residues	NEDI (mg bw)	RQ	Residues	NEDI (mg/kg)w	RQ	Residues	NEDI (mg/k	RQ
0	1.65	$1.2x10^{-3}$	$6.1x10^{-3}$	2.21	$1.6x10^{-3}$	$8.1x10^{-3}$	2.38	$1.7x10^{-3}$	$1.7x10^{-1}$	1.57	$1.1x10^{-3}$	$3.8x10^{-2}$	1.85	$1.3x10^{-3}$	$6.8x10^{-3}$
1	1.25	$9.2x10^{-4}$	$4.6x10^{-3}$	1.95	$1.4x10^{-3}$	$7.1x10^{-3}$	1.99	$1.4x10^{-3}$	$1.4x10^{-1}$	1.14	$8.4x10^{-4}$	$2.7x10^{-2}$	1.51	$1.1x10^{-3}$	$5.5x10^{-3}$
3	0.87	$6.4x10^{-4}$	$3.2x10^{-3}$	1.24	$9.1x10^{-4}$	$4.5x10^{-3}$	1.31	$9.6x10^{-4}$	$9.6x10^{-2}$	0.89	$6.6x10^{-4}$	$2.1x10^{-2}$	0.99	$7.3x10^{-4}$	$3.6x10^{-3}$
5	0.46	$3.3x10^{-4}$	$1.6x10^{-3}$	0.81	$6.0x10^{-4}$	$2.9x10^{-3}$	0.86	$6.3x10^{-4}$	$6.3x10^{-2}$	0.51	$3.8x10^{-4}$	$1.2x10^{-2}$	0.62	$4.6x10^{-4}$	$2.2x10^{-3}$
7	0.15	$1.1x10^{-4}$	$5.5x10^{-4}$	0.35	$2.6x10^{-4}$	$1.2x10^{-3}$	0.33	$2.4x10^{-4}$	$2.4x10^{-2}$	0.12	$9.0x10^{-5}$	$2.9x10^{-3}$	0.36	$2.7x10^{-4}$	$1.3x10^{-3}$
10	0.04	$2.9x10^{-5}$	$1.4x10^{-4}$	0.10	$7.0x10^{-5}$	$3.7x10^{-4}$	0.10	$7.0x10^{-5}$	$7.0x10^{-3}$	0.05	$4.0x10^{-5}$	$1.2x10^{-3}$	0.10	$7.0x10^{-5}$	$3.7x10^{-4}$
15	ND	---	---	ND	---	---	0.04	$3.0x10^{-5}$	$2.9x10^{-3}$	ND	---	---	ND	---	---

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تدهور متبقيات بعض المبيدات في ثمار الفراولة وتقييم المخاطر الصحية المترتبة عليها

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الملخص العربى:

هدفت الدراسة لتقييم المخاطر وكذاك دراسة ديناميكيات بقايا خمس مبيدات على نباتات الفراولة. تم تطبيق كلا من مبيد سيازوفاميد، وأزوكسيستروبين، وتريسيكلازول، وبروكلوراز، وبيريميثانيل علي الثمار خلال مرحلة النضج. تم استخدام كروماتوجرافيا السائل عالي الأداء (HPLC) لفحص عينات الفاكهة المأخوذة على فترات زمنية متباينة تتراوح بين ساعتين وخمسة عشر يومًا بعد الرش. كانت فترات نصف العمر ($t_{½}$) لكلا من السيازوفاميد و الأزوكسيستروبين، والبروكلوراز، والتريسيكلازول، والبيريميثانيل 2.88، و2.97، و2.02، و2.94، و3.10 يومًا على التوالي. وكانت قيم t_{90} المقابلة 4.49، و4.58، و3.63، و4.55، و4.71 يومًا على التوالي. وفقا للنتائج المتحصل عليها فانه يُمكن استهلاك ثمار الفراولة بأمان بعد 10 أيام من جميع المعاملات، باستثناء بيريميثانيل بعد 15 يومًا، وفقًا لحدود المتبقيات المسموح بها والمخاطر. يُبرز نمط التبديد أهمية وجود فترات زمنية مناسبة قبل الحصاد لتقليل مستويات المتبقيات قبل الاستهلاك، وبالرغم من وجود مستويات عالية من المتبقيات، الا انه ظلت مستويات معامل الخطورة أقل من 1، مما يُشير إلى أن هناك خطر ضئيل جدًا على البالغين.