

Biochemical effects of the fungicides cyflufenamid and difenoconazole residues on pea fruits

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ABSTRACT: Experiments were conducted on pea to study the residues and dissipation rates of two fungicides cyflufenamid and difenoconazole as well as, their effects on total protein and amino acid patterns on green seeds during the winter season of 2017 at different intervals (2h), 1, 3, 6, 9, 12 and 15 day. Results revealed that, the amounts of residues was higher in difenoconazole than cyflufenamid. Peeling process removed considerable amounts of the residues. Pea green seeds treated with cyflufenamid could be used safely for human consumption after 6 days of spraying. While in case of difenoconazole it was found that no safety intervals was needed for used green pea seeds after spraying according to maximum residues limit (MRL) of EU pesticides database - European Commission. Degradation rate (K) was higher in cyflufenamid than difenoconazole. The half-life ($t_{1/2}$) was 1.67 days in pea pod shells as well as in whole pods treated with cyflufenamid, while in case of difenoconazole the corresponding values were 2.37 and 2.35 days in pea pod shells and whole pods, respectively. The effect of difenoconazole residues was more pronounced in amino acids and protein than cyflufenamid. Protein percentages and amino acid amounts could be recovered after time elapsed of spraying.

Keywords: cyflufenamid, difenoconazole, QuEChERS, pea, residues, Biochemical

1.Introduction

In Egypt, pea crop (*Pisum sativum*) is considered as an important legume for human consumption (either in green stage or in mature stage i.e., the dried seeds as well as frozen). Pea plants are liable to be infested with different plant diseases (*Fusarium solani*, *F. oxysporum*, *F. moniliforme*, *Alternaria solani* and *Rhizoctonia solani*) causing serious injury and reducing the final yield, (Leslie and Summerell, 2006 and Smith, 2007). Abdala *et al.* (1992) reported that *Sclerotium rolfsii* Sacc, *Rhizoctonia solani* Kuhn and *Macrophomina phaseolina*, (Tassi) Goid were the main soil borne pathogens responsible for causing damping-off and root rot diseases in grown pea plants. So, fungicides are necessary and played a significant role in adequate production during the control of these diseases.

Cyflufenamid is used to control of powdery mildew in wheat, top fruit, vegetables and powdery mildew in cereals (Haramoto *et al.*, 2006). Difenoconazole was Systemic fungicide with a novel broad-range activity protecting the yield and crop quality by foliar application or seed treatment. Provides long-lasting preventive and curative activity against Ascomycetes, Basidiomycetes and Deuteromycetes, including *Alternaria*, *Ascochyta*, *Cercospora*, etc. Used against disease complexes in grapes, pome fruit, stone fruit, potatoes and various vegetable crops. (MacBean, 2012).

Different investigators were achieved to determine the residues of pesticides in edible parts of field and vegetable crops to find out the cut off period (Arain *et al.* 2012, Abdallah *et al.* 2014 Hingmire *et al.* 2015 and shalaby, 2016c).

Therefore, the present work was conducted to determine the residues of cyflufenamid and difenoconazole in pea green pods (green pod shell, green seeds as well as the whole green pods). This will help to avoid their hazards to consumers. The effects of their residues on the total protein and the amino acids in green seeds were also studied.

2.MATERIALS AND METHODS

2.1.Field experiment

Experiments were carried out in a private field of pea (*Pisum sativum* ver baladey) located at El-Tahra village, Zagazig district, Sharkia governorate during the winter season of 2017. The experimental area was divided into plots 1/100 (42 m²) of a feddan each arranged in randomized blocks design with three replicates for each treatment and untreated control. The normal agricultural practices were achieved the fungicides used and their rates in gram active ingredients (a.i.) per feddan were:

- 1- Cyflufenamid, Ritreap 5% EW, 1 g a.i.
- 2- Difenoconazole, Curve 25% EC, 12.5 g a.i.

A knapsack hand sprayer fitted with one nozzle boom was used. The two tested fungicides were sprayed at February 2, 2017 at the rate of 20 cm³ 100/L water and 50 cm³ 100/L water for cyflufenamid and difenoconazole, respectively. The untreated control plots were left unsprayed.

2.2.Determination of fungicide residues

2.2.1.Preparation of samples

Representative samples of green pods were taken 2 hrs, 1, 3, 6, 9, 12 and 15 days after spraying for determination of cyflufenamid and difenoconazole residues. Random samples (0.5 kg from each plot) were taken and subsamples of 200 g each of green pods were peeled off to get the green pod shells and green seeds separately, then each component was weighed. Also, after 6, 9 and 15 days samples of treated and untreated green seeds (250 g) were taken to determine the total protein and the amino acid patterns using amino acid analyzer according to the method of Moore *et al.* (1958).

2.2.2.Determination of cyflufenamid residues

Ten g of the homogenized samples (green pod shells and green seeds) were transferred in a 50 ml centrifuge tube. Fifteen milliliters of 1.0% acidified aceton-

trile with acetic acid were added; the screw cap was closed and vigorously shaken for 1 min using a Vortex mixer at maximum speed. Afterwards, 4 g of anhydrous MgSO_4 , 1 g of NaCl, 1 g sodium citrate dehydrate were added, then extracted by shaking vigorously on Vortex for 2 min and centrifuged for 10 min at 5,000 rpm. Five milliliter of the supernatant was transferred to 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, then one milliliter was taken to determine cyflufenamid residues. (Lehotay *et al.* 2010).

Cyflufenamid was determined according to Hirahara *et al.* 2005 Agilent Technologies 7890A gas chromatograph equipped with electron capture detector (ECD) under the following operation conditions: Column: DB-17 (15 m \times 0.32 mm \times 0.52 μm film thickness). Column temperature: 220 $^\circ\text{C}$ for one min, heated to 270 $^\circ\text{C}$ at 10 $^\circ\text{C}/\text{min}$. Injector temperature: 300 $^\circ\text{C}$. Detector temperature: 320 $^\circ\text{C}$. Carrier gas: N_2 . Flow rate: 3.2 ml/min, at these conduction retention time was 4.1 min

2.2.3.Determination of difenoconazole residues

Method of Molhof *et al.* (1975) was adopted for extraction of difenoconazole from pea fruits (green pod shells and green seeds). Samples were placed in the blender cup and a constant amount of methanol 2 ml / gram sample) were added, then blended for 2 minutes. The macerate was filtered through a clean cotton pad into a graduated cylinder. A known volume (100 ml) of the extract was shaken successively with 100, 50 and 50 ml of methylene chloride in a separating funnel after adding 10 ml of saturated sodium chloride solution. The combined organic phases were dried by filtration through anhydrous sodium sulfate. Extraction was evaporated just to dryness using a rotary evaporator operating at 40 $^\circ\text{C}$. The dry extract was then subjected to the clean up procedure suggested by Mills *et al.* (1972) using florisil chromatograph column [40cm 18 \times mm (i.d.) glass column] filled with 6 g of activated florisil (60-100 mesh) and topped with anhydrous sodium sulfate and compacted thoroughly. The column was pre washed using 50 ml n-hexane. The sample extract was dissolved in 10 ml of the same solvent and transferred to the column then eluted with 200 ml of the eluant (50% dichloromethane: 48.5% n-hexane: 1.5% acetonitrile) at a rate of 4.2 ml/min and the residue of difenoconazole were ready for HPLC determination using a UV-detector set at the wavelength 254 nm. A reversed-phase VP-ODS C_{18} column (150mm \times 4 mm i.d., particle size 5 μm) was used and the mobile phase was acetonitrile/water (60 ,40/ v/v) at 0.8 ml/min. These conditions resulted in good separations and high sensitivity was obtained with retention time 2.78 min (Nasr, *et al.*, 2009). The residues of the tested fungicides in the whole green pods were calculated by the following equation (Balinov and shalaby, 1986):

$$\text{ppm (mg/kg) in whole green pods} = A \frac{w_1}{w_3} + B \frac{w_2}{w_3}$$

where: A= ppm in the green pod shells.

B= ppm in the green seeds.

W1= weight in grams of the green pod shells.

W2= weight in grams of the green seeds.

W3= weight in grams of the whole pods.

2.3.Recovery studies

Recovery studies were carried out to define the efficacy of the method used (extraction, clean-up and final determination procedures). The untreated samples of green pod shells (peels) and green seeds of pea were fortified with fungicides used cyflufenamid and difenoconazole active ingredient solutions levels of 0.5 and 1 mg/kg, then the procedures of extraction, clean-up and final determination were performed as previously mentioned. The averages of recovery percentages for spiked samples were 90.24%, 87.64% for cyflufenamid and 91.85%, 86.73% for difenoconazole in green pod shells (peels) and green seeds of pea, respectively. The obtained results were corrected according to their recovery percentages.

2.4.Statistical analysis

Statistical significance of the data was determined by using the analysis of variance with L.S.D method at the probability of 0.05 (Steel and Torrie 1980).

3.Results and discussion

Data summarized in Tables (1 and 2) represent the amounts of cyflufenamid and difenoconazole residues detected in green pod shells, green seeds and whole green pods of pea after different intervals of spraying. It is obvious that the initial deposits of the tested fungicides detected in green pod shells 2 hrs after spraying were 2.534 and 5.243 mg/kg for cyflufenamid and difenoconazole, respectively. These figures indicated that there was a positive correlation between the uptake of the used two fungicides on the recipient surface of pea green pods and their used concentrations. The initial amounts were decreased gradually till reached 0.014 and 0.061 mg/kg after 12 days of spraying of cyflufenamid and 15 days of application of difenoconazole, respectively, recording 99.45 and 98.84% dissipation, respectively. The initial deposits found in green seeds were 0.00 and 0.202 mg/kg for cyflufenamid and difenoconazole, respectively. These figures revealed that 0.00 and 3.85% the deposited fungicides were migrated with 2 hrs from the pod shells to the green seeds, respectively. The amounts of fungicide residues reached the green seeds were increased gradually to reach maximum level where determined after 3 days of treatment being 0.099 and 0.704 mg/kg indicating 3.91 and 13.43% migration of the initial deposits in green pod shells. The migration residues were decreased gradually in green seeds to reach 0.00 and 0.003 mg/kg after 15 days of application, representing 0.00 and 0.06% migration, respectively. Concerning the effect of peeling process, it was found that the removal percentages by peeling ranged between 81.96% - 100.00% for cyflufenamid. While in case of difenoconazole the removal percentages due to the peeling process ranged between 64.14% - 96.14%. Regarding the residues of the two tested fungicide in the whole green pods, it is obvious that the

Table 1: Residues of cyflufenamid detected in pea pods.

| Days after treatment | shell | | Green seeds | | | Whole pods | |
|----------------------|---------------------|---------------|------------------|----------------------|-------------|------------------|---------------|
| | Residues (mg/kg) | Dissipation % | Residues (mg/kg) | Removal % by peeling | Migration % | Residues (mg/kg) | Dissipation % |
| Initial | 2.534 | — | 0.00 | 100 | 0.00 | 1.246 | — |
| 1 | 1.298 | 48.79 | 0.064 | 95.07 | 2.53 | 0.654 | 47.51 |
| 3 | 0.621 | 75.48 | 0.099 | 84.06 | 3.91 | 0.357 | 71.35 |
| 6 | 0.207 | 91.82 | 0.020 | 90.34 | 0.79 | 0.109 | 91.25 |
| 9 | 0.061 | 97.59 | 0.011 | 81.96 | 0.43 | 0.032 | 97.43 |
| 12 | 0.014 | 99.45 | 0.002 | 85.71 | 0.08 | 0.007 | 99.44 |
| 15 | UND | 100 ≈ | UND | 100 ≈ | 0.00 | UND | 100 ≈ |
| K | 0.41454 | | | | | | |
| t _{1/2} | 1.67 | | | | | | |
| Liner equation | y = -0.180x + 0.358 | | | y = -0.180x + 0.074 | | | |

Initial= 2 hours

$$\text{Migration\%} = \frac{\text{Residual amounts in pulp at indicated days}}{\text{Initial deposits on peel}} \times 100 \text{ (shalaby, 2016 c)}$$

UND= undetectable amounts

calculated initial deposits were 1.246 and 2 939.mg/kg for cyflufenamid and difenoconazole, respectively. These amounts were decreased gradually to reach undetectable amounts (UND) and 0.038 mg/kg after 15 days of application recording 100.00 and %98.71 loss of the initial deposits, respectively.

Comparing the maximum residue limits of cyflufenamid (0.02 mg/kg) and difenoconazole (1.0 mg/kg) in green seeds of pea (without shells) presented in EU pesticides database - European Commission with the total residues in Tables (1 and 2). It is clear that the green seeds of pea treated with cyflufenamid could be used

safely for human consumption after 6 days of spraying. While in case of difenoconazole it was found that no safety intervals was needed for used the green pea seeds after spraying.

The residues of cyflufenamid were dissipated in pea pod shells as well as in whole pods with degradation rats (K) of 0.41454 recording half-life values (t_{1/2}) 1.67 days. While in case of difenoconazole its degradation rates were 0.292481 and 0.294784 and the (t_{1/2}) values were 2.37 and 2.35 days in pea pod shells and whole pods, respectively.

Table 2: Residues of difenoconazole detected in pea pods.

| Days after treatment | shell | | Green seeds | | Migration % | Whole pods | |
|----------------------|---------------------|---------------|------------------|----------------------|-------------|------------------|---------------|
| | Residues (mg/kg) | Dissipation % | Residues (mg/kg) | Removal % by peeling | | Residues (mg/kg) | Dissipation % |
| Initial | 5.243 | — | 0.202 | 96.14 | 3.85 | 2.939 | — |
| 1 | 3.524 | 32.79 | 0.424 | 88.30 | 8.09 | 1.966 | 33.11 |
| 3 | 2.263 | 56.84 | 0.704 | 64.14 | 13.43 | 1.322 | 55.02 |
| 6 | 0.825 | 84.26 | 0.203 | 75.39 | 3.87 | 0.442 | 84.96 |
| 9 | 0.403 | 92.31 | 0.054 | 89.26 | 1.03 | 0.238 | 91.90 |
| 12 | 0.146 | 97.22 | 0.007 | 95.21 | 0.13 | 0.064 | 97.82 |
| 15 | 0.061 | 98.84 | 0.003 | 95.08 | 0.06 | 0.038 | 98.71 |
| K | 0.292481 | | | | | | |
| t _{1/2} | 2.37 | | | | | | |
| Liner equation | y = -0.127x + 0.709 | | | y = -0.128x + 0.458 | | | |

Initial = 2 hours,

$$\text{Migration \%} = \frac{\text{Residual amounts in pulp at indicated days}}{\text{Initial deposits on peel}} \times 100 \text{ (shalaby, 2016 c)}$$

Our results are in harmony with those obtained by **Amer *et al* (2007)** studied the residues of tetraconazole (50_{cc} of Domark 10% EC) and diniconazol (35_{cc} of Sumi-eight 5% E.C.) on green beans and found that the initial deposits were 0.296 and 0.027 mg/kg, these amounts gradually degraded to reach 0.00 and 0.001 mg/kg after 14 days of spraying, recording 100.00 and 99.66% loss, respectively. The half-life values were 3 days for diniconazol and from 4.5 to 6.5 days for tetraconazole. No residues could be detected in the plants during the period of 21 days after application. Hence, the plants could be used safely after that period of time. **Nasr *et al* (2009)** recorded that the half-life value was 3.16 days after treated tomatoes with difenoconazole, and pre-harvest intervals (PHI) was determined to be 8 days. **Abd El-Zaher *et al* (2011)** reported that the half-life values were 1.09 days for plantfax in kidney beans and 0.84 days for chlorothate in tomato. The PHI values were 3 and 7 days for plantfax in kidney bean and chlorothate in tomato, respectively. The initial amounts of plantfax in kidney bean was 3.78 mg/kg degraded to reach 0.01 mg/kg after 15 days. The corresponding value for chlorothate in tomato was 3.71 mg/kg and gradually dissipated to reach 0.09 mg/kg after 15 days of spraying. **Abbassy *et al* (2014)** found that the initial deposit of tetraconazol in cucumber fruits was 0.1742 mg/kg and its half-life ($t_{1/2}$) value was 1.4 days. **Abdallah *et al* (2014)** studied the residue behavior of difenoconazole (Scor 25 % EC) in grapes berries, and found that its initial deposit was 1.773 mg/kg, the rate of degradation was 0.294 days, $t_{1/2}$ values was 4.494 days the waiting period was at least 17 days before harvesting. **Hingmire *et al* (2015)** determined the residues of difenoconazole in okra fruits, and reported that this fungicide dissipated with half-life of 2.5 days. The PHI was 6.5 and 19.5 days at single dose and double dose of field application, respectively. Okra samples harvested after the estimated PHIs were found safe for human consumption. **Abdella *et al* (2015)** reported that $t_{1/2}$ values was 1.95 days for penconazol in squash fruits and the PHI was 10 days after application. **Sleem (2015)** reported that the removal percentage of diniconazole residues by peeling was 96.18% after on hour from the application, then reduction was recorded 90.8 and 88.88% after 1, 3 days from the time after application the broad bean with diniconazole (Sumi-eight 50% EC) at the rate of 35cm³ 100/L water as recommended dose, and **Shalaby (2016 c)** studied the residual behavior of Ritreap cyflufenamid) 5% EW at the rate 20 cm³ 100/L water in squash, and found that the removal percentages of Ritreap residues by peeling 100% (after 2 hrs and 9 days) and 88.88, 1944 and 9.68 after 1, 3 and 6 days of spraying respectively. The migration percentages in relation to the initial deposit on squash peel were 0.00, 5.14, 12.42 and 1.2% after 2 hrs, 1, 3 and 6 days, respectively, and squash fruits could be used safely for human consumption after 6 days of squash spraying.

3.1.Effect of cyflufenamid and difenoconazole residues on the total amino acids and protein percentage

The effects of cyflufenamid and difenoconazole residues on total protein and amino acid patterns of pea green seeds are presented in Table (3). It was found that the two tested fungicides significantly reduced the level of the total amino acids during the all 3 tested periods (3, 9 and 15 days after treatments). During the all tested periods, levels the amino acids cystine, phenylalanine, proline, and alanine were insignificant reduced, but the levels of the other determined amino acids were significantly reduced as well as the protein percentage comparing with the control levels.

After 6 days of spraying of pea plants with the two tested fungicides, it was found that the highest reduction percentage was recorded with the amino acid cystine recording 71.43% and 52.38% reduction for difenoconazole and cyflufenamid, respectively. The lowest reduction percentages were recorded with the amino acids proline and arginine indicating 8.31% and 4.39% reduction for difenoconazole and cyflufenamid, respectively (Table, 3). The other amino acids occupied an intermediate position.

After 9 days of spraying, results revealed that (Tables, 3) the amino acid cystine recorded the highest reduction percentages for the two tested fungicides indicating 53.37% and 41.10% reduction for difenoconazole and cyflufenamid, respectively. While the amino acids valine and isoleucine recorded the lowest reduction percentages indicating 8.34% and 6.30% for difenoconazole and cyflufenamid, respectively.

Also, the amino acid cystine recorded the highest reduction percentage after 15 days of spraying the pea plants with the two tested fungicides being 41.11% and 27.78% with difenoconazole and cyflufenamid, respectively, while the lowest reduction percentage was recorded in level of the amino acid isoleucine indicating 7.11% and 5.23% reduction for difenoconazole and cyflufenamid, respectively (Tables, 3).

In conclusion, concerning the effects of the residues for two tested fungicides difenoconazole and cyflufenamid on the levels of the determined amino acids, great interest to note the following two remarks (Tables, 3):

- 1- The effect of the residues of difenoconazole was more pronounced in amino acids than cyflufenamid.
- 2- The recovery of the amino acids was increased with the time elapsed after spraying the pea plants with the two tested fungicides.

The observed decreases of amino acids may be due to the results of a decreased rate of synthesis of amino acids resulting the spraying the pea plants with the two tested fungicides cyflufenamid and difenoconazole. The level of amino acids and the capacity of the levels of treated fungicides to reduce nitrate (through nitrate reductase) and synthesize amino acids (through glutamine and dehydrogenase, transaminase, respiratory enzymes) apparently led **Bidwell *et al* (1964)** to believe that such fungicides reduce efficient synthesis of amino acids.

Our results are in harmony with **Habiba *et al* (1992)** found that profenofos increased the protein content of treated potatoes by 20.0% as compared with control. **Radwan *et al* (1995)** determined protein content on faba bean seeds after spraying with pirimiphos

Table (3). Effects of difenoconazole and cyflufenamid residues on amino acids (mg/100g protein) and total protein % in green pea seeds at different intervals.

| Amino acids (mg/100g protein) | After 6 days | | | After 9 days | | | After 15 days | | |
|----------------------------------|--------------|--------------------|--------------------|--------------|--------------------|--------------------|---------------|--------------------|--------------------|
| | control | D | C | control | D | C | control | D | C |
| Lysine | 8a | 6.286c (21.43) | 6.37b (20.38) | 8.113a | 6.436b (20.67) | 6.583b (18.86) | 8.21a | 6.526c (20.51) | 6.64b (19.12) |
| Methionine | 0.803a | 0.583c (27.39) | 0.623b (22.42) | 0.84a | 0.656c (21.90) | 0.71b (15.48) | 0.863a | 0.733c (15.06) | 0.773b (10.43) |
| Cystine | 0.126a | 0.036a (71.43) | 0.06a (52.38) | 0.163a | 0.076a (53.37) | 0.096a (41.10) | 0.18a | 0.106a (41.11) | 0.13a (27.78) |
| Threonine | 4.083a | 3.49c (14.52) | 3.66b (10.36) | 4.146a | 3.646b (12.06) | 3.746b (9.65) | 4.263a | 3.81c (10.63) | 3.973b (6.80) |
| Isoleucine | 7.416a | 6.683c (9.88) | 6.99b (5.74) | 7.52a | 6.88c (8.51) | 7.046b (6.30) | 7.59a | 7.05c (7.11) | 7.193b (5.23) |
| Leucine | 5.91a | 5.236c (11.40) | 5.386b (8.87) | 6.03a | 5.48b (9.12) | 5.55b (7.96) | 6.143a | 0.646b (8.09) | 5.793b (5.69) |
| phenylalanine | 4.68a | 4.19a (10.47) | 4.266a (8.85) | 4.733a | 4.29a (9.36) | 4.413a (6.76) | 4.816a | 4.21c (12.58) | 4.523b (6.08) |
| Tyrosine | 2.37a | 2.013c (15.06) | 2.08b (12.24) | 2.416a | 2.083c (13.38) | 2.153b (10.89) | 2.51a | 2.196c (12.51) | 2.27b (9.56) |
| Valine | 4.386a | 3.99c (9.03) | 4.04b (7.89) | 4.436a | 4.066c (8.34) | 4.15b (6.45) | 4.533a | 4.156c (8.32) | 4.246b (6.33) |
| Aspartic acid | 12.073a | 10.453b (13.42) | 10.606b (12.15) | 12.46a | 10.973c (11.93) | 11.273b (9.53) | 12.783a | 11.146c (12.81) | 11.386b (10.93) |
| Serine | 3.836a | 3.026c (21.12) | 3.256b (15.12) | 3.943a | 3.186c (19.19) | 3.35b (15.04) | 4.356a | 3.486b (19.97) | 3.523b (19.12) |
| Glutamic acid | 13.283a | 11.05c (16.81) | 11.186b (15.78) | 13.476a | 11.27b (16.37) | 11.316b (16.03) | 13.78a | 11.413b (17.18) | 11.57b (16.04) |
| Proline | 7.616a | 6.983a (8.31) | 7.096a (6.83) | 7.833a | 7.15a (8.72) | 7.216a (7.88) | 7.91a | 7.286a (7.89) | 7.343a (7.17) |
| Glycine | 3.226a | 2.61c (19.09) | 2.99b (7.31) | 3.546a | 2.786c (21.43) | 3.013b (15.03) | 3.613a | 2.953c (18.27) | 3.1b (14.19) |
| Alanine | 3.45a | 2.876a (16.64) | 3.036a (12.00) | 3.58a | 3.056a (14.64) | 3.143a (12.21) | 3.67a | 3.183c (13.27) | 3.276b (10.74) |
| Histidine | 3.856a | 3.05c (20.90) | 3.243b (15.89) | 3.986a | 3.186c (20.07) | 3.383b (15.28) | 4.126a | 3.51b (14.93) | 3.52b (14.68) |
| Arginine | 6.24a | 5.71c (8.49) | 5.966b (4.39) | 6.513a | 5.883c (9.67) | 6.01b (7.72) | 6.7a | 6.083c (9.21) | 6.17b (7.91) |
| T A A | 91.354a | 78.265c (14.33) | 80.854b (11.49) | 93.734a | 81.103b (13.48) | 78.151c (16.62) | 96.046a | 78.493c (18.28) | 85.429b (11.05) |
| Protein % | 19.75a | 13.208c (33.12) | 14.187b (28.17) | 21.083a | 14.687c (30.34) | 16.188b (23.22) | 22.292a | 16.667c (25.23) | 18.687b (16.17) |

In each raw values followed by the same letter are not significantly different at $P \leq 0.05$

D = difenoconazole , C = cyflufenamid , T A A = total amino acid , number between brackets = reduction percentage

-methyl and chlorpyrifos-methyl, and found that pirimiphos-methyl significantly reduced the protein content (g/100g seeds), while chlorpyrifos-methyl caused a significant increase in protein content. **Radwan et al. (2004)** reported that pirimiphos-methyl significantly increased % protein in treated fruits of green pepper and eggplant by 10.19% and 18.09%, respectively. While profenofos was significantly decreased % protein in green pepper fruits. The same author found that pirimiphos-methyl significantly decreased the levels of the amino acids threonine, proline, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine. Also, profenofos significantly decreased alanine, isoleucine, leucine and phenylalanine comparing with untreated pepper fruits. **Shalaby (2016 a)** stated that the residues of thiamethoxam and chlorpyrifos were significantly decreased the protein% of fresh treated okra fruits comparing with the control during 6, 9 and 15 days. **Shalaby (2016 b)** found that profenofos was significantly reduced the mean level of protein in treated tomato as compared with control. **Shalaby (2017)** revealed that lambda - cyhalothrin residues were significantly reduced protein content in pepper fruits during 6 and 15 days.

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التأثيرات البيوكيميائية لمبيدات الفطريات سيفلوفيناميد و ديفينوكونازول على ثمار البسلة

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أجريت التجارب على البسلة لدراسة متبقيات ومعدلات الإختفاء لإثنين من المبيدات الفطرية وهما سيفلوفيناميد و ديفينوكونازول خلال فصل الشتاء حيث تم رش نباتات البسلة يوم 2 فبراير 2017 وتم أخذ العينات على فترات مختلفة من ساعتين ، يوم ، 3 ، 6 ، 9 ، 12 و 15 يوم بعد الرش. وأوضحت النتائج أن كمية متبقيات ديفينوكونازول كانت أعلى من سيفلوفيناميد. كما وجد أن تقشير قرون البسلة أدى إلى إزالة كميات معتبرة من متبقي المبيدين. كما أن بذور البسلة الخضراء المعاملة بالسيفلوفيناميد يمكن استخدامها بأمان للاستهلاك الأدمى بعد 6 أيام من الرش. بينما في حالة ديفينوكونازول وجد أنه لا توجد فترة انتظار للاستهلاك الأدمى لبذور البسلة الخضراء بعد الرش وفقا لقاعدة بيانات الاتحاد الأوروبي لمبيدات الآفات. وكانت معدلات التحطم (K) أعلى في السيفلوفيناميد من ديفينوكونازول. وكانت قيمة نصف العمر ($t_{1/2}$) لقشور قرون البسلة والقرون الكاملة المعاملة بالسيفلوفيناميد 1.67 يوم ، بينما في حالة ديفينوكونازول سجلت 2.37 و 2.35 يوما في قشور قرون البازلاء والقرون الكاملة، على التوالي. كانت بقايا ديفينوكونازول فعالة وأكثر وضوحا على الأحماض الأمينية من سيفلوفيناميد. عند الرش بالمبيدين ، كما أشارت النتائج أنه يمكن أن ترجع الأحماض الأمينية إلى معدلاتها الطبيعية بمرور الوقت.