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Determination of chlorofenapyr residues in squash during crop production cycle.

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ABSTRACT

A method for determination of chlorofenapyr residues in squash by HPLC is described. Samples were extracted with methanol/water, followed by liquid- liquid partitioning and clean-up in chromatographic column, concentrated to a small volume. Analysis was then determined by HPLC equipped with UV-vis detector at 260 nm. The degradation rate of chlorfenapyr SC was studied and the results indicated that final residue in squash leaves reached 0.1 mg/kg after 14 day and was undetected for the fruits, which was considered safe for human beings and animal consumption.

Keywords: Chlorfenapyr; insecticide residue; HPLC; squash

INTRODUCTION

Squash (Cucurbita pepo L.) is one of the most popular vegetable crops grown in Egypt that is grown in more than one season (Shehata *et al*., 2009). It is ranked second among the popular cucurbits preceded by watermelon (Ghobary and Ibrahim, 2010). This crop is usually attacked by two-spotted spider mites, aphids and white flies. The Agricultural Pesticides Committee, Ministry of Agriculture and land Reclamation have recommended the use of Chlorfenapyr on this crop for pest control.

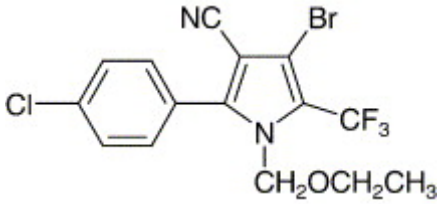
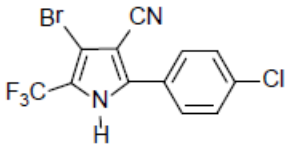


Fig.1: The structure of Chlorfenapyr



CL303268

Fig. 2: Structure of the activated form of Chlorfenapyr (CL303268)

Chlorfenapyr [4- Bromo -2- (P-chlorophenyl) - (ethoxymethyl)-5-(trifluoromethyl) pyrrole-3-carbonitril] (Fig. 1) is a pesticide derived from a class of microbial produced compounds known as halogenated pyrolles (FAO/WHO, 2012). Its biological activity depends upon its activation to another chemical (CL303268), (Fig. 2), (Lunn *et al*., 2010). It is a pro-insecticide-miticide which is metabolized into an active insecticide after being ingested by the host. Once formed, the metabolite uncouples oxidative

phosphorylation at the mitochondria, resulting in the disruption of ATP production, cellular death, and ultimately organism mortality (Lunn *et al*., 2010). Seal (1994) studied the effect of chlorfenapyr application on squash leaves and concluded that it was effective in reducing thrips adults and larvae. However, there were no studies concerning chlorfenapyr residues on this plant (leaves and fruit) (Rust and Saran 2008). Thus, the aim of the current study is to evaluate the residue of chlorfenapyr formulation in squash leaves and fruits so as to determine the interval between spraying and harvest required for safe use of this crop to reduce any health problem referred to consumers.

MATERIALS AND METHODS

Insecticide: Chlorfenapyr standard: Super Challinger (24% Suspension Concentrate -SC) (purity 99.2 %), BASF Chemical Ag.

Solvents: analytical grade acetone (99.5%), methanol (99.8%), dichloromethane and n-hexane were used without further purification. Chemicals: sodium chloride (4%) and anhydrous sodium sulfate.

Apparatus: Shimadzu HPLC system Model LC -10 AT with pump associated with a 7125 Rheodyne, six–port valve with a 20-µl loop and a UV-Vis detector connected to a Shimadzu Model C-R6A integrator for data acquisition (auto -sampler, computer integrating software). The analytical column used was Kromasil C18 (150 µm × 4.5 mm i.d.) and precolumn with same material.

Field trails: The experiment was carried out during summer season 2012 at fields near Benha, Quliobya Governorate. Squash was planted in field plots in areas of 600 m. Each plot contained around 150 plants. The plots were distributed in a completely randomized pattern. The growing plants were treated with the chlorfenapyr at the recommended dose of 60 cm^3/100 liter water. Field treatments were arranged in randomized complete bloke design (RCBD) to facilitate replications. For each treatment, four replications were used: one for standard and the other three for analytical determination of pesticides residues. Squash leaves were taken randomly: 0, 1, 3, 5, 7, 9, 14 and 21 days after spraying but fruit samples were taken after 21 days from spraying. The samples were collected in plastic bags. Control samples were also collected concurrently. All collected samples were placed in a deep freezer at –20 °C and were transferred to lab for analysis by HPLC.

Sample extraction and clean –up

In analytical applications, an important consideration in extraction is the polarity of the analyte and the solubility ranges of the different pesticide families in water (Ahmed, 2001). The basic approach for plant matrices employs extraction by homogenization with methanol: water, and column clean-up using SPE (Lunn *et al*., 2010). The extraction and clean up method applied were according to Cao *et al*. (2005) and were as follows:

Extraction:

A vegetable sample (50g) was cut and placed in a conical flask and shaken with 150 ml acetone (3 times) for 1 hour. The extracts were filtered with a filter-paper and followed by evaporation using a water bath until the final volume reached 10 ml. The sample was transferred to a separator funnel containing 100 ml of 4% sodium chloride, and then followed by liquid-liquid partitioning with dichloromethane for three times at the volume of 50, 30 and 30 mL, respectively. The organic phase was combined, dehydrated by passing through a bed containing anhydrous sodium sulfate and was further concentrated using a water bath to a final sample volume of 2 ml for column chromatography.

Clean-up:

The concentrated extract was transferred quantitatively to a glass beaker with 20 ml of n-hexane and mixed with 2 g activated charcoal and 2 g anhydrous sodium sulfate and the slurry was allowed to settle. The clear layer of the slurry was transferred to suitable chromatographic column (200 x 9mm id), fitted with stopcock and packed with silica gel and allowed to pass slowly through the column (30 drops/min). The charcoal was washed 6 times with 20 ml n-hexane each and passed through the column. The combined extract was evaporated to dryness and transferred quantitatively with methanol to a 10 mL volumetric flask for injection in the HPLC.

HPLC conditions: The mobile phase used was methanol - water (80:20 v/v) at flow rate of 1 mL /min flow. The column oven was kept at 30°C and the best detection was attained at wavelength of 260 nm.

Statistical analysis: The degradation kinetics of the chlorfenapyr in squash leaves were determined by plotting residue concentration against time and R^2 was used to determine the best fit and the rate equation was determined.

RESULTS AND DISCUSSION

HPLC chromatogram for chlorfenapyr

Reversed – phase HPLC, with UV detection, has proven to be a good alternative for chlorofenapyr determination because no derivation step is needed and the use of C18 columns provides good results (Cao *et al*., 2005, Kandil *et al*., 2011). The detection at 260 nm offers suitable chromatograms for the quantification of chlorofenapyr in real samples. Under the chosen conditions, chlorofenapyr showed a retention time of 24.546 min, allowing a complete separation of its signal from those of foreign substances present in the samples (Fig. 3). This time is 10 min above the time reported by Cao *et al*. (2005) which may be attributed to the difference in dimensions of the HPLC separation column used as well as the difference in plant studied.

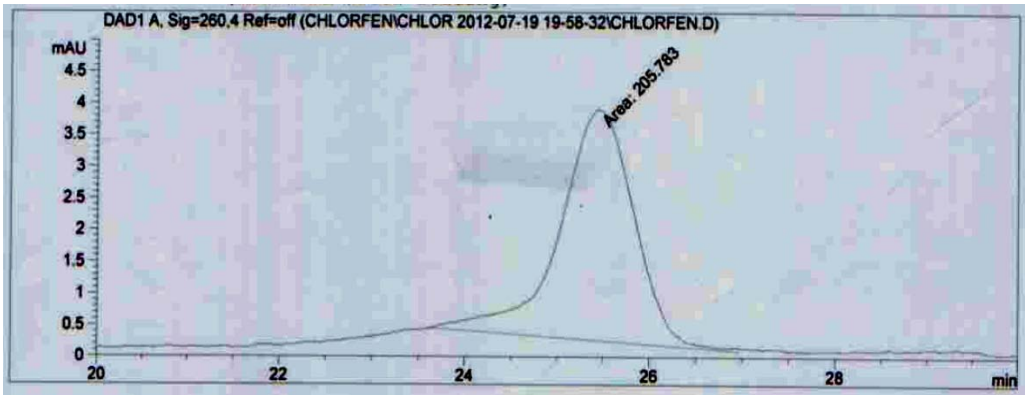


Fig. 3: HPLC chromatogram of Chlorfenapyr (99%) solution 40%.

Testing the stability of the prepared chlorfenapyr Solution

The stability of chlorfenapyr was studied using HPLC and mobile phase methanol: water (80: 20 v/v) with no change in pH or buffering. The data obtained (Fig. 4) indicated that the concentration of pesticide decreased gradually during the first 3 days after which an increase in peak area was observed at the 5th and 19th day of solution preparation.

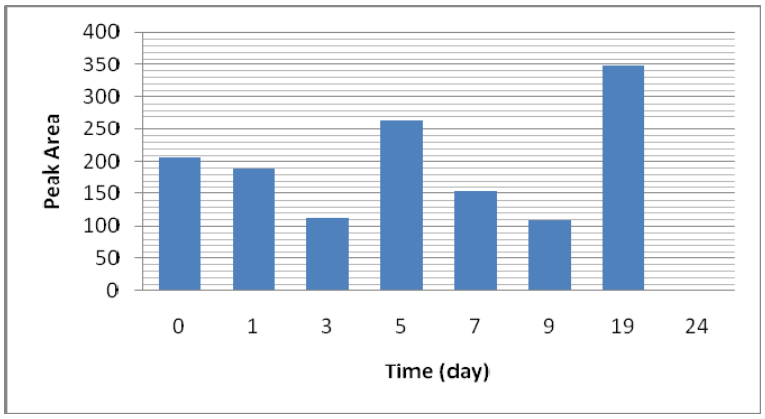


Fig. 4: The stability of Chlorfenapyr solution (60%) with time

This was attributed to the formation of metabolites. Lunn *et al*. (2010) concluded that chlorfenapyr was the only significant residue on plants while other metabolites were present in lower levels. They also provided a scheme explaining the possible pathways for the transformation of the chemical into its metabolites plant leaves (Fig. 5).

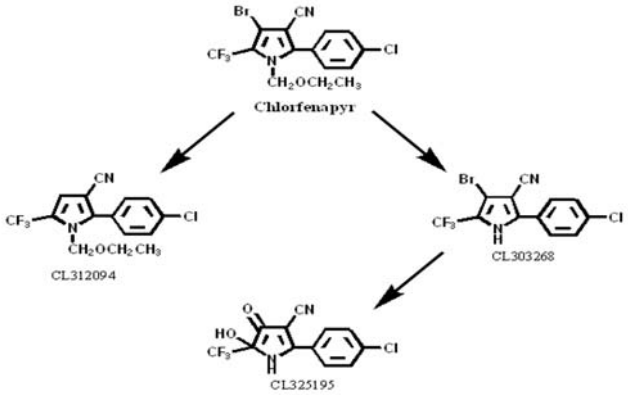


Fig. 5: The proposed pathway for the degradation of chlorfenapyr in head lettuce leaves (Lunn *et al*., 2010)

Degradation Kinetics of Chlorfenapyr on squash.

Table 1 and Fig. 6 show the amount of chlorfenapyr residue determined in squash leaves over the testing time period. It has to be noted that after 21 day of pesticide application, the amount of residue was undetectable. This is in accordance with the findings of Cao *et al*. (2005) which indicated that both SC and nanoformualtions of these chemical reached undetectable levels after application on cabbage leaves.

Table 1: The amount of pesticides residue (mg/kg) on squash leaves after plant sparing

Time (day)	Residue (mg/kg)
0	4.5
1	4.0
3	3.4
5	3.0
7	1.0
9	0.2
14	0.1
21	0.0

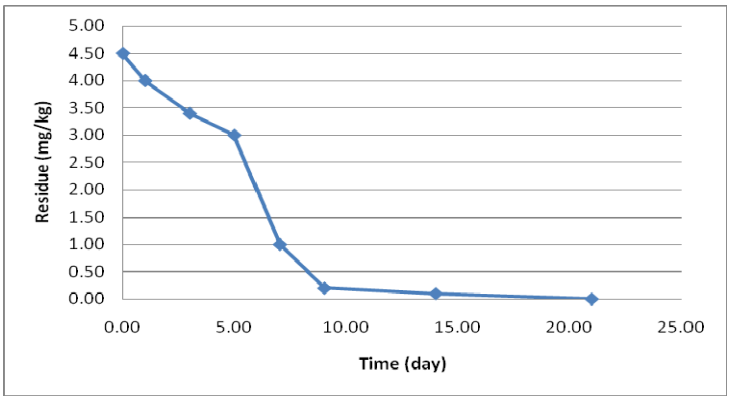


Fig. 6: The degradation curve for Chlorfenapyr on squash leaves

As expected gradual and continuous deterioration of the pesticide residues in and on the treated plants was observed as a function of time after application. In this respect, the magnitude of loss was recorded to reach 99.7% after 14 day.

In order to determine the rate of degradation of chlorfenapyr on squash, the values of ln Ct was plotted against t and the resultant is shown in Fig. 7. From this curve, the dynamics could be described by the equation ($C=1.869\ e^{-.3021\ t}$) with square of coefficient $R^2=0.9022$.

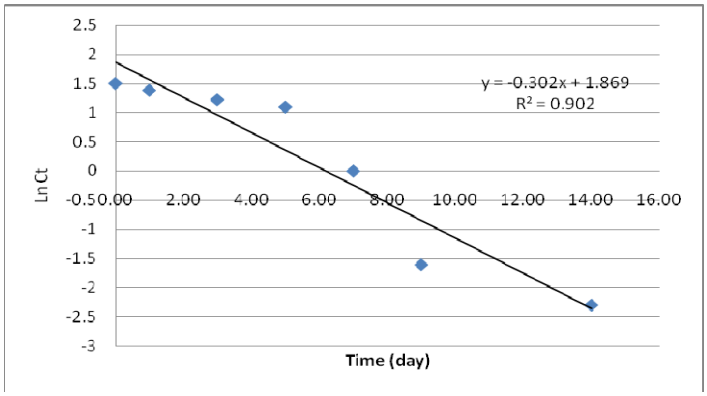


Fig. 7: Ln Ct vs time for Chlorfenapyr degradation on squash leaves.

According to this equation, the half life of chlorfenapyr suspension concentration in squash leaves was found to be 3.5 days which is longer that the value obtained by Cao *et al*. (2005) of in cabbage which was 2.6 d. However, the rate of chlorfenapyr degradation obtained in this study was k= 0.3021 which is comparable to the rate Cao *et al*., (2005) obtained for the chlorfenapyr nanoformualtion of this pesticide (k= 0.3103). As well, the current results also affirmed that the chemical degradation rate followed first order kinetics. Finally, analysis of the fruit revealed that chlorfenapyr residues were undetectable after 21 days of pesticide application which indicates that no translocation of chemical from foliar into the fruits occurred which is in agreement with the findings of Lunn *et al*. (2010).

CONCLUSION

The present work investigated the presence of chlorfenapyr residues on summer squash. The results indicated the stability of the applied chemical formulation under the recommended dose ($60cm^3$/ 100 liter). As well, it revealed that the degradation kinetics of the chemical followed first order equation and the rate obtained was comparable to chlorfenapyr nanformulation. In addition, the half life of the chemical on squash was 3.5 days. The results also indicated that the final after 14 days reached the safe limit of

under (0.5 mg/kg) and was undetectable after 21 days in the fruit which makes its safe for human consumption.

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ARABIC SUMMARY

تقدير الأثار المتبقية من مبيد الكلورفينبير فى الكوسة أثناء فترة الأنتاج

فريدة محمد سعد الدين الدرس[1]، مارجريت عدلى رزق[2] ، شيرى صبحى تكلا[2]

1- قسم الكمياء - كلية العلوم جامعة حلوان

2- معهد بحوث وقاية النباتات – مركز البحوث الزراعية – بالدقى - مصر

تهدف هذه الدراسة الى تقدير ومتابعة سلوك متبقيات المبيد الحشرى الكلورفينبير فى أوراق الكوسة عن طريق جهاز التحليلى الكروماتوجرافى HPLC و UV وذلك عن طريق أستخدام عامل الفصل الميثانول مع الماء بنسبة 80 : 20 ثم يتبع ذلك الأستخراج و التحليل الجزئى المائى ويليه مرحلة التنظيف .هذه التحاليل قد قيست على جهاز HPLC و UV عند طول موجى 260 نانوميتر. قد وجد أن معدل التكسير لمادة الكلورفينبير الموجود فى المبيد الحشرى السوبر شالنجر على أوراق الكوسة تصل الى 0,1 ملى جرام / كليو جرام بعد 14 يوم من الرش وهذا المعدل يعتبر الأكثر أمان للأستهلاك الأدامى والحيوانى .

تضمنت خطة الدرسة النقاط الاتية :

1 - تحديد المبيد المستخدم والمحصول.

2 - دراسة الطرق العملية التحليلية المناسبة لأستخراج بقايا المبيدات من المنتجات الزرعية .

3 - تقيم بقايا المبيدات فى العينات المجمعة بطرق الفصل الكروماتوجرافى وعملية التجهيز والتنظيفHPLC.

ويمكن تلخيص النتائج المتحصل عليها فيما يلى:

أولا : تقيم طرق التحليل وجد أن مبيد الكلورفينبيرتم تقديرة بواسطة جهاز وأعطت أحسن النتائج لانها لاتحتاج الى آى خطوات جزئية اثناء عملية التحليل HPLC و UV.

ثانيا : تحليل متبقيات الكلورفينبير قد أوضحت النتائج أن كميات الكلورفينبير المقاسة فى أوراق الكوسة تحتاج الى وقت يعادل 24.546 دقيقة لحدوث الفصل الكامل لجميع المواد الغريبة وهذا المعدل يصل الى 0,1 ملى جرام / كليو جرام بعد 14 يوم من الرش وهذا المعدل يعتبر الأكثر أمان للأستهلاك الأدامى والحيوانى .