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# PERSISTENCE AND FATE OF CARBOSULFAN AND IMIDACLOPRID RESIDUES IN POTATO PLANTS

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## ABSTRACT

**The present study was carried out to investigate the residual behaviour of carbosulfan and imidacloprid insecticides on potato plants (leaves and tubers). The initial deposits of carbosulfan and imidacloprid on the leaves of ditta variety (53.40 and 10.39 ppm) were higher than on the leaves of diamont variety (28.30 and 2.76 ppm), but the calculated residue half-life values ($RL_{50}$) for carbosulfan and imidacloprid on the leaves of ditta variety (18 and 17.75 hours) were shorter than the corresponding values on leaves of diamont variety (22.5 and 22.75 hours). The results also indicated that the initial concentrations of carbosulfan on potato leaves of diamont and ditta varieties (28.30 and 53.40 ppm) were much higher than the initial concentrations of imidacloprid on potato leaves of diamont and ditta varieties (2.76 and 10.39 ppm). Data revealed that the first day following application is critical in the sense of sharply decreases to reach 53.36% and 65.73% for carbosulfan and 52.54% and 66.99% for imidacloprid on potato leaves of diamont and ditta varieties, respectively.**

**Residues of carbosulfan and imidacloprid detected in potato tubers at 1 day or 3 days following application on the vegetative parts of potato plants, are evidence that penetrate, movement and translocated these insecticides through plant tissues to tubers.**

**According to the maximum residue limits (MRLs ), and the determined residues of these insecticides in potato tubers, the approximate waiting time value ( preharvest interval ) for carbosulfan residues in diamont variety could not be determined because the insecticide residues through the**

**sampling period were all exceeded the Codex MRL value, but it was determined as 14 days in case of potato tubers of ditta variety. As for imidacloprid an approximate waiting time of 5 days was determined for potato tubers of the two varieties .**

**Key words** : Persistence, Residues, Carbosulfan, Imidacloprid, Potato .

## INTRODUCTION

Carbosulfan [2,3-dihydro-2 2-dimethylbenzofuran-7-yl (dibutylaminothio) methylcarbamate] is a broad spectrum carbamate pesticides that acts by inhibiting the activity of acetylcholinesterase. It used to control insects, mites and nematodes by soil, foliar and seed treatment applications, foliar pests may be controlled by soil applications via systemic action, and is said to be effective through direct contact or stomach ingestion (FAO/WHO, 1984). Imidacloprid [1-(6-chloro-3-pyridyl methyl)-*N*-nitroimidazolidin-2-ylideneamine] is a systemic chloronicotinyl nitroguanidine insecticide that enters the target pest via ingestion or direct contact. It acts by disrupting nicotinic acetylcholine receptors in the insect central nervous system. Imidacloprid is used for controlling sucking insects, soil insects, termites, and some chewing insects. It is applied to seeds, soil, crops, and structures and is used as topical flea control treatment on domestic pests (Fossen, 2006).

Potato crop is considered one of the most important vegetable crops, and plays an important role in the Egyptian diet, also as an export vegetable crop. Potato plants *Solanum tuberosum* are attacked by many insect species which cause serious injury and thus the final yield is reduced. Aphids are among the most serious pests in cultivation of potatoes. Carbosulfan and imidacloprid are a broad spectrum systemic insecticides and effective by contact action, that has been found useful for the control of aphids.

Extensively use of pesticides in modern agriculture to combat plant pests has begun to receive much attention because pesticide residues in food commodities may be hazardous to human health. It is

well known that the intervals between application of pesticides and harvest for human consumption are critical period. The residues of such pesticides on and in crop should be estimated to give recommendation about the safety consumable time to avoid such hazards. Therefore the objectives of this study were to determine the deposit and residue levels and their rates of dissipation of carbosulfan and imidacloprid insecticides on a common vegetable crop, potato under field conditions. Also the time intervals between application and harvest (waiting time) for human consumption were estimated. A subsidiary comparison of the residues between two famous potato varieties diamont and ditta ( leaves and tubers ) were achieved.

## MATERIALS AND METHODS

**1. Experimental and insecticide treatments**

The experiment was conducted from October 2002 to February 2003 in Kaha-Kalubia Governorate. Diamont and ditta varieties of potato plants were planted on October 29, 2002 under the normal field conditions and agricultural practice. Carbosulfan (Marshal 25% WP) and imidacloprid (Admire 20% SC) were applied on January 26, 2003, at rates of 150 g/100 L water and 50 ml/100 L water (recommended dose), respectively using a knapsack sprayer equipped with one nozzle. Replicate samples, 200 g of potato leaves and 500 g of potato tubers were taken at intervals of one hour after application (zero time), 1, 2, 3, 5, 7, 10 and 14 days and was repeated 21 days (at harvest time) for potato tubers only. Then sub-samples, 50 g each of leaves and tubers were taken and kept in polyethylene bags under deep freezing until analysis.

**2. Analytical procedures**

**A. Extraction**

**A.1. Carbosulfan insecticide:-** The method of Mollhoff (1975) was adopted for extraction of carbosulfan from potato leaves and potato tubers, methanol was used instead of acetone. Fifty gram samples, each of leaves and tubers were placed in the blender cup and a constant volume of methanol (3 ml/gram leaf) and (2 ml/gram tuber) were added, then blended for three minutes and filtered. Extracts were shaken successively with 100, 50 and 50 ml of methylene chloride in separatory funnel after adding 40 ml of sodium chloride solution

(20%); then the water phase was discarded. The combined methylene chloride phases were dried by filtration through anhydrous sodium sulphate. Then, it was evaporated just to dryness using a rotary evaporator at 40°C.

**A.2. Imidacloprid insecticide:-** Method of Blass (1990) was used for extraction of imidacloprid from potato leaves and potato tubers with slight modification of substituting methanol instead of acetonitrile in extraction. Fifty gram samples each of leaves and tubers were blended at high speed for about 3 minutes with 200 ml methanol and filtered. After evaporation of 100 ml of the methanol extract by means of a vacuum rotary evaporator, the aqueous reminder was treated with 50 ml saturated sodium chloride solution and transferred into a separatory funnel and shaken vigorously with 100 ml n-hexane. Then the organic phase was discarded and the aqueous phase was shaken with 100, 50 and 50 ml methylene chloride in separatory funnel. The lower methylene chloride phases were collected and subsequently dried over anhydrous sodium sulfate. Then, it was evaporated just to dryness using a rotary evaporator at 40°C.

**B. Clean-up of extracts.**

**B.1. Carbosulfan extract:-** The clean-up procedure done according to the method of Leppert et al. (1983). Glass chromatographic columns 1.5 cm o.d x 22 cm were plugged with glass wool and washed with HPLC-grade ethyl acetate. Ten grams of 3.5% water deactivated florisil (60-100 mesh) was packed dry and capped with a 2-cm layer of anhydrous sodium sulfate. The sample was added and rinsed on with 5 ml of HPLC-grade hexane. Carbosulfan was eluted from the column with 100 ml of 9:1 (v/v) hexane-ethyl acetate. The eluants were evaporated just to dryness as previously described and redissolved in an appropriate volume of HPLC-grade acetonitrile. Finally, the extracts were filtered through a 13 mm, 0.45 μm nylon filter into a glass stopper test tube, then the residues were ready for analysis by HPLC.

**B.2. Imidacloprid extract:-** The method of Blass (1990) was followed for cleaning-up of the extracted samples. The chromatographic column was prepared by adding 20 ml of HPLC-grade ethyl acetate to a topped glass column (with a plug of glass

wool), followed by 4.5 g of 5% deactivated florisil (60-100 mesh) as a slurry in HPLC-grade ethyl acetate. The dry extract was dissolved in 5 ml of HPLC-grade ethyl acetate and 1 ml was pipetted on the top of column (in case of tuber samples all extracted sample was quantitatively transferred to the top of column). The column was eluted with 20 ml of HPLC-grade ethyl acetate which was discarded. The imidocloprid was eluted from the column with 25 ml of HPLC-grade acetonitrile which was evaporated just to dryness as previously described and redissolved in an appropriate volume of acetonitrile. Finally, the extract was filtered through a 13 mm, 0.45 μm nylon filter into a glass stopper test tube, then the residues were ready for analysis by HPLC.

**C. High pressure liquid chromatography determination.**

Carbosulfan and imidacloprid were detected and determined using an Agilent Technologies Series 1100 HPLC system equipped with workstation. UV-Photodiode array detector set at 220 nm for carbosulfan and 270 nm for imidacloprid, and the analytical column Nucleosil-C18, 5 μm (4 x 250 mm) was used. The mobile phases were acetonitrile-water v/v (9:1) at flow rate 1 ml/min isocratic elution for 15 min for carbosulfan and at flow rate 0.8 ml/min gradient elution for imidacloprid as follows:-

| Time in min | Acetonitrile | Water |
|---|---|---|
| 0 | 35 | 65 |
| 10 | 40 | 60 |
| 15 | 40 | 60 |
| 20 | 35 | 65 |

The injection volume was 20 μl.

Results were corrected according to the rates of recovery which were determined in fortified untreated samples at levels ranged from 0.1 ppm to 1 ppm. Following the techniques previously mentioned, the rates of recovery for carbosulfan were 91% and 97.3% and for imidacloprid were 92% and 73% in potato leaves and potato tubers, respectively.

## RESULTS AND DISCUSSION

The amount of carbosulfan residues detected on and in leaves and tubers of potato plants and their percent loss at different intervals after application are tabulated in Table (1). The initial deposit of carbosulfan on and in potato leaves as determined one hour after treatment were 28.30 and 53.40 ppm for two potato varieties diamont and ditta, respectively. These amounts dropped to 13.20 and 18.30 ppm indicating 53.36% and 65.73% loss after one day farom application. Then the residues of carbosulfan on and in potato leaves had slight decreased gradually until reached 1.32 and 0.31 ppm with percentages of loss 95.34% and 99.42% after 14 days from spraying on diamont and ditta varieties, respectively.

No residues of carbosulfan were detected in potato tubers at zero time (one hour after application), 1 and 2 days after spraying for diamont variety and at zero time and one day after treatment for ditta variety. Slight amount of carbosulfan residue (0.07 ppm) was detected in potato tubers of ditta variety, 2 days after spraying and increased to 0.14 ppm within 3 days after application. Between days 5 and 21 after treatment, the residue was gradually decreased to reach 0.03 ppm at the end of the experiment (21 days after spraying). However, the amount of carbosulfan residue (0.18 ppm) was detected in potato tubers of diamont variety, 3 days after treatment. From day 7 following application a graduate decrease was observed in residue which reached 0.10 ppm after 21 days (harvest time).

The concentrations of imidacloprid residues detected on and in leaves and tubers of potato plants at different intervals after application, and their percentages of loss related to the initial deposits of imidacloprid are tabulated in Table (2). The initial deposits of imidacloprid on and in potato leaves of diamont and ditta varieties were 2.76 and 10.39 ppm, respectively. These values decreased to 1.31 and 3.43 ppm within 1 day after application, showing 52.54% and 66.99% loss, respectively. Residues were gradually decreased and the amounts estimated were 0.05 ppm with the calculated rate of loss 98.18% on potato leaves of diamont variety and undetectable on potato leaves of ditta variety, after 14 days following treatments.

No residues of imidacloprid were detected in potato tubers at zero time (one hour after application) and one day after spraying, for diamont variety and at zero time for ditta variety. Residue of imidacloprid (0.22 ppm) was detected in potato tubers of ditta variety, one day after treatment. This residue increased to 0.39 ppm, 2 days after application, after that the residue was gradually decreased to reach 0.12 ppm, 7 days following treatment. Also the amount of imidacloprid residue (0.10 ppm) was detected in potato tubers of diamond variety, 2 days after spraying. This residue was decreased to reach 0.02 ppm after 7 days following application. No residues of imidacloprid could be detected in potato tubers of diamont and ditta varieties after 10 days from application.

According to Bates (1979) data on residues of pesticides in treated crops are required for the premarket registration of pesticides and for setting maximum residues limits (toxicologically acceptable level) to protect the consumer against the possible health hazards of exposure to pesticides. The maximum residue limits (MRLs) for carbosulfan and imidacloprid in the potato are 0.05 and 0.5 ppm (Codex Alimentarius Commission 2004). According to the maximum residue limits (MRLs ), and the determined residues of these insecticides in potato tubers, the approximate waiting time value ( preharvest interval ) for carbosulfan residues in diamont variety could not be determined because the insecticide residues through the sampling period were all exceeded the Codex MRL value, but it was determined as 14 days in case of potato tubers of ditta variety. As for imidacloprid an approximate waiting time of 5 days was determined for potato tubers of the two varieties.

It is clear from the present study that the initial deposits of carbosulfan and imidacloprid on the leaves of ditta variety were higher than on the leaves of diamont variety, but the calculated residue half-life values ($RL_{50}$) for carbosulfan and imidacloprid on leaves of ditta variety (18 and 17.75 hours) were shorter than the corresponding values on leaves of diamont variety (22.5 and 22.75 hours). This variation could be attributed to the vegetative parts of ditta variety is bigger than the vegetative part of diamont variety or probably relates to their different surface to weight ratios and perhaps, different surface properties. In addition, the ratio of the leaf surface to its weight is

high enough to result in receiving higher initial deposits of the applied pesticide (Ahmed *et al*. 1991 and Dogheim 1966). The influence of plant varieties on the deposition and dissipation of pesticides was discussed by Lee and Cheng (1983).

The present study confirmed that initial concentrations of carbosulfan on potato leaves were much higher than the initial concentrations for imidacloprid on potato leaves. Such difference could be attributed to the higher rate of application of carbosulfan 150 g (i.e. 37.5 g a.i.)/100 L water than imidacloprid 50 ml (i.e. 10 g a.i.)/100 L water, also different types of formulation applied which was Marshal 25% WP for carbosulfan and Admire 20% SC for imidacloprid. In this respect, El-Sayed *et al*. (1976) stated that the amounts of deposits depended on the rate of application, the nature of the treated surface and the relation between the treated surface and its weight.

The degradation and disappearance of carbosulfan and imidacloprid may be due to many factors such as weathering, metabolic conversions or other degradation processes. However, the first day following application is critical in the sense of sharply decreases to reach 53.36% and 65.73% for carbosulfan and 52.54% and 66.99% for imidacloprid from the initial deposit on potato leaves of diamont and ditta varieties, respectively. There were many factors, including plants, pesticides, and environments, which affected the dissipation of pesticides on crops. Christensen (2004) reported that the decline of pesticides may be due to biological, chemical or physical processes, or if still in the field, due to dilution by growth of the crop. Besides, plant growth is also responsible to certain extent for decreasing the pesticide residue concentrations due to growth dilution effect (Walgenbah *et al*., 1991). In addition, the rapid dissipation of originally applied pesticide are dependent on a variety of environmental factors such as sunlight and temperature (Lichtenstein, 1972). However, high temperature is reported to the major factor in reducing the pesticides from plant surfaces (Awad *et al*., 1967). Light plays an important role in the behaviour of pesticide in the environment (Zepp and Cline, 1977). Furthermore, the main breakdown products of imidacloprid in plants are a monohydroxy metabolite, imidacloprid guanidine, imidacloprid olefin and

monoglucoside of 6-chloropicolyl alcohol (Miles, Inc., 1993). The majority of toxicity studies have focused on the parent compound, imidacloprid. It should noted that two imidacloprid derivatives (olefin and nitrosimine) occur as metabolites in treated plants and have greater insecticidal activity than the parent compound (Nauen et al., 1998). The guanidine metabolite of imidacloprid does not possess insecticidal properties, but has a higher mammalian toxicity than the parent compound (Tomizawa and Casida, 1999). The fate of carbosulfan has been investigated in plants and reported by FAO/WHO (1984). In general carbosulfan, carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran are the principle carbamate residues in plants with relative amounts varying from crop to crop and with time. The persistence of carbosulfan and its cholinesterase-inhibiting metabolites on apple leaves after three foliar sprays each of 1.1 kg a.i./ha was investigated by Leppert (1982). Residues of 3-hydroxycarbofuran were approximately one-hundredth and one-tenth of the carbosulfan and carbofuran residues respectively after 1 day from application and were at about the same level (0.03 mg/cm$^2$) on day 21, residues of 3-ketocarbofuran were ≤ 0.01 mg/kg throughout the 21- day study. In plants carbosulfan is typically the same parent compound at or near the last day of application, while thereafter carbofuran and/or 3-hydroxy-carbofuran tend to be the predominant carbamate residues in many commodities, the latter primarily as a conjugate, conjugation increases with time (FAO/WHO, 1984).

Residues of carbosulfan and imidacloprid detected in potato tubers at one day or three days, when these pesticides were applied on the vegetative parts of potato plants are evidence that penetrate, movement and translocated carbosulfan and imidacloprid, through plant tissues to tubers. Several investigators had studied the absorption and translocation of carbosulfan and imidacloprid through plant tissues after application. Umetsu *et al*., (1979) found that carbonyl-$^{14}$C-labelled carbosulfan residues remained at point of application (up to 9 days) when the carbosulfan was applied near the tips of corn and cotton leaves, but were translocated to the whole leaf within 24 hour when it was applied to the base of a cotton leaf. In whole-plant autoradiography studies (Capps, 1980) carbosulfan and carbofuran showed the same movement with residues concentrating in

leaf tips and roots after soil treatment with $^{14}$C-ring-labelled compounds. Fossen, (2006) reported that imidacloprid is rapidly moved through plant tissues after applications, and can be present in detectable concentrations in tissues such as leaves, vascular fluids, and pollen. In a French study, sunflower plants that were seed-treated at a rate of 1.0 mg/seed produced pollen that contained imidacloprid at a concentration of 13.0 ppb (Laurent and Rathahao, 2003). Detections imidacloprid residues in corn plants that were seed-treated at rate of 0.7 mg/seed ranged from an average of 2.1 ppb in pollen to 6.6 ppb in the flowers (Bonmatin *et al*., 2005). Westwood *et al*. (1998) found that the leaves of sugar beet seedings contained an average of 15.2 ppm three weeks after treatment at a rate of 0.9 mg/seed.

**Table (1): Carbosulfan residues on and in leaves and tubers of potato plants.**

| Time after application (days) | Potato diamont variety | | | Potato ditta variety | | |
|---|---|---|---|---|---|---|
| | Leaves | | Tubers | Leaves | | Tubers |
| | Residues (ppm) | % loss | Residues (ppm) | Residues (ppm) | % loss | Residues (ppm) |
| Zero time* | 28.30 | 00.00 | ND** | 53.40 | 00.00 | ND** |
| 1 | 13.20 | 53.36 | ND | 18.30 | 65.73 | ND |
| 2 | 13.11 | 53.67 | ND | 12.20 | 76.69 | 0.07 |
| 3 | 4.91 | 82.65 | 0.18 | 11.20 | 79.03 | 0.14 |
| 5 | 4.01 | 85.83 | 0.24 | 9.02 | 83.11 | 0.11 |
| 7 | 3.92 | 86.15 | 0.18 | 2.56 | 95.21 | 0.09 |
| 10 | 2.02 | 92.82 | 0.15 | 1.39 | 97.40 | 0.06 |
| 14 | 1.32 | 95.34 | 0.12 | 0.31 | 99.42 | 0.04 |
| 21 | - | - | 0.10 | - | - | 0.03 |
| $RL_{50}$*** in hours | 22.5 | | | 18 | | |
| Preharvest intervals (days) | | | Not determine | | | 14 |

* One hour after application

** Not detectable

*** Calculated from persistence curve

**Table (2): Imidacloprid residues on and in leaves and tubers of potato plants.**

| Time after application (days) | Potato diamont variety | | | Potato ditta variety | | |
|---|---|---|---|---|---|---|
| | Leaves | | Tubers | Leaves | | Tubers |
| | Residues (ppm) | % loss | Residues (ppm) | Residues (ppm) | % loss | Residues (ppm) |
| Zero time* | 2.76 | 00.00 | ND** | 10.39 | 00.00 | ND** |
| 1 | 1.31 | 52.54 | ND | 3.43 | 66.99 | 0.22 |
| 2 | 0.83 | 69.62 | 0.10 | 2.35 | 77.38 | 0.39 |
| 3 | 0.63 | 77.17 | 0.26 | 2.01 | 80.65 | 0.36 |
| 5 | 0.45 | 83.69 | 0.11 | 1.58 | 84.79 | 0.28 |
| 7 | 0.11 | 96.01 | 0.02 | 0.60 | 94.22 | 0.12 |
| 10 | 0.08 | 97.10 | ND | 0.09 | 99.13 | ND |
| 14 | 0.05 | 98.18 | ND | ND | – | ND |
| 21 | – | – | ND | – | – | ND |
| $RL_{50}$ *** in hours | 22.75 | | | 17.75 | | |
| Preharvest intervals (days) | | | 5 | | | 5 |

* One hour after application

** Not detectable

*** Calculated from persistence curve

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## الملخص العربي

# ثبات ومصير متبقيات الكاربوسلفان والايميداكلوبريد في نباتات البطاطس

**شكر عبد السلام علي شكر**
المعمل المركزي للمبيدات ، مركز البحوث الزراعية ، الدقي ، الجيزة ، مصر

يهدف هذا البحث الى تقدير متبقيات مبيدي الكاربوسلفان (مارشال) والايميداكلوبريد (أدمير) في نباتات البطاطس (الأوراق والدرنات) للصنفين ديامونت وديتا . حيث كانت المتبقيات الأولية للكاربوسلفان والايميداكلوبريد على وفي أوراق نباتات البطاطس للصنف ديتا ( 53.40 و 10.39 جزء في المليون) أعلى من المتبقيات الأولية لكلا المبيدين على وفى أوراق البطاطس للصنف ديامونت ( 28.30 و 2.76 جزء في المليون ) ، ولكن كانت فترات نصف العمر للكاربوسلفان والايميداكلوبريد على أوراق البطاطس للصنف ديتا ( 18 و 17.75ساعة ) أقل منها لكلا المبيدين على أوراق البطاطس للصنف ديامونت ( 22.5 و 22.75ساعة ) . أوضحت النتائج أيضاً أن الرواسب الأولية لمبيد الكاربوسلفان على وفي أوراق البطاطس للصنفين ديامونت وديتا (28.30و 53.40 جزء في المليون ) أعلى من الرواسب الأولية لمبيد الايميداكلوبريد على وفي أوراق البطاطس للصنفين ديامونت وديتا ( 2.76 و 10.39 جزء في المليون ) . أظهرت الدراسة أن نسبة الفاقد من مبيد الكاربوسلفان من على أوراق البطاطس للصنفين ديامونت وديتا بعد يوم واحد من المعاملة كانت 53.36 % و 65.73 % ، وكانت نسبة الفاقد أيضاً من على أوراق البطاطس للصنفين ديامونت وديتا لمبيد الأيميداكلوبريد بعد يوم واحد من المعاملة هي 52.54 % و 66.99 % .

لم تكتشف أي متبقيات من مبيد الكاربوسلفان في درنات البطاطس للصنف ديامونت حتى اليوم الثاني من المعاملة ولكن المتبقيات ظهرت عند اليوم الثالث من المعاملة حيث كانت 0.18 جزء في المليون ، أيضاً لم تكتشف أي متبقيات لهذا المبيد في درنات البطاطس للصنف ديتا بعد اليوم الأول من المعاملة ولكن ظهرت المتبقيات عند اليوم الثاني من المعاملة بكمية قدرها 0.07 جزء في المليون ، بالنسبة لمبيد الأيميداكلوبريد لم تكتشف أي متبقيات لهذا المبيد في درنات البطاطس للصنف ديامونت عند اليوم الأول من المعاملة ولكن المتبقيات ظهرت عند اليوم الثاني للمعاملة وكانت بكمية قدرها 0.1 جزء في المليون ، أما بالنسبة لدرنات البطاطس للصنف ديتا لم تكتشف بها اي متبقيات من هذا المبيد بعد ساعة واحدة من المعاملة ولكن ظهرت المتبقيات في اليوم الأول للمعاملة بكمية قدرها 0.22 جزء في المليون .

طبقاً للحد المسموح به من قبل لجنة دستور الأغذية والزراعة (الكودكس) لمبيدي الكاربوسلفان والايميداكلوبريد في درنات البطاطس ، فإن فترة ما قبل الحصاد لمبيد الكاربوسلفان لم يمكن تحديدها خلال فترة التجربة بالنسبة لدرنات البطاطس للصنف ديامونت وكانت 14 يوم بعد المعاملة بالنسبة لدرنات البطاطس للصنف ديتا . وكذلك كانت فترة ماقبل الحصاد لمبيد الايميداكلوبريد تقريباً هي خمسة ايام بعد المعاملة لكلا الصنفين .