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DISSIPATION OF BENSULTAP AND IMIDACLOPRID IN BROAD BEAN (Vicia faba) PLANT AND ITS SOIL

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ABSTRACT

Field experiments were carried out to study the residual behavior of foliar application of bensultap and imidacloprid in broad bean plants and their runoff persistence in soil under plants. The residues amount decreased to be 0.29 mg/kg after 22 days of treatment with 97.7 % loss in the leaves of broad bean after bensultap application. While residues disappeared in leaves after 22days of imidacoprid application.

The half-life time ($t_{1/2}$) values for bensultap in green pods of faba bean was calculated to be 2.9 days for peels. Whereas ,in the case of imidacloprid, the RL_{50} value was 0.93 days in faba bean green peels but, residues could not be detected in green seeds one hour after treatment Data concluded that. the green pods can be safely harvested for human consumption after the third day of application based on the PHI value obtained. In soil the RL_{50} of bensultap was 4 days, while the persistence of the runoff of imidacoprid on the soil under the plants was a little longer than that in bensultap which was 5 days.

INTRODUCTION

Broad bean (Vicia faba) has a great role in human nutrition as a major source of plant protein and is considered an important component of Egyptian farming systems. In Egypt, the faba bean is the most important food legume crop that has the potential to meet the increasing demand for food. Faba bean is subjected to several sucking insects e.g. aphids, whitefly, leaf miners, that can cause serious economic loss of the crop yield.

Therefore, in the search for non-traditional active ingredient to control such sucking insect pests, a group of insecticides; nereistoxin analogue is being applied include cartap, bensultap and thiocyclam. Bensultap is an antagonist, blocking cholinergic transmission resulting in paralysis and insect death. Once eaten by the insect, bensultap is metabolically converted to nereistoxin, which interacts with nicotinic acetylcholine receptor (Perry et al 1997).The second tested insecticide; imidacloprid belongs to a new chemical class of active ingredients, the chloronicotinyls, (neonicotinoids). It has a new mode of action, as an agonist on the postsynaptic nicotinic acetylcholine receptors of motor neurons in insects. This causes an over-stimulation of the nervous system, and ultimately kills the insect (National Pesticide Information Center 2010). Because of its excellent systemic properties imidacloprid is used as a seed dressing as well as foliar and soil particularly for controlling sucking insects.

Recently there has been increased awareness about insecticide residues in crops and environmental matrices to minimize the risk they may pose for human and the environment.

The objective of this study was to study the behavioral residues of bensultap and imidacloprid as new classes of insecticides in broad bean plant (leaves and pods) to determine the period required for safe human consumption. The contamination of the soil under the plants with the both insecticides was also investigated.

MATERIALS AND METHODS

Insecticides used:

The two insecticides used throughout the study were commercial formulations of imidacloprid (Admire 20% SC) Bayer Crops Science at the rate 50ml/100 L water and bensultap (Bancol 50%WP) Takeda Chemical Industries, Ltd at rate 150 g/100 L water as recommended by the Ministry of Agriculture

Plant used:

Broad bean (Vicia faba) seeds variety "balady" 843 was obtained from the Agricultural research center and experiments were carried out in 2011 growing season

Broad bean plants were planted in agricultural experimental station, Faculty of agriculture, Cairo University. Broad bean (vicia faba) seeds were sown, cultivated, irrigated and fertilized according to local agricultural practices. Tested insecticides at recommended rate (as mentioned above) were applied as foliar application using knapsack sprayer.

1- Analytical procedure of bensultap residues in broad bean plants and the soil:

Leaves, and green pods were collected randomly one hour as well as 1, 3, 7, 11, 15 and 22 days after insecticide application. On the other hand, representative samples of treated soil were collected from the surface of the soil to the depth of 10 cm after one hour, and 3, 7, 11, 15 and 22 days of bancol treatment

1-1 -Bensultap Extraction and clean -up:

Bensultap was extracted from plants according to Johnson and Stansbury (1966) with some modification as follows: Fifty gram from each broad bean leaves and pods was macerated with 20 g activated anhydrous sodium sulfate till complete mixing. Dichloromethane (150 ml HPLC grade) was added and mixed in a warring blender on high speed for 3 min. The macerate was filtered through a clean pad cotton and anhydrous sodium sulfate. Known volume of crude extract was evaporated until dryness at 40 ^{0}C.

Soil samples (clay) at 5-10 cm depth were extracted according to Nelsen and Cook (1979). Soil sample (100 g) was placed in a 500 ml conical flask, then shaken mechanically with 200 ml of dichloromethane for 30 min and 15 g of anhydrous sodium sulfate was added during shaking. The extract was left for 10 min and carefully filtered through a clean pad of cotton, then evaporated to dryness at 40 ^{0}C. The extracts were cleaned up according to the method of Krause (1980). The dry extract was re-dissolved in 20 ml ethylacetate and mixed with 2 g of absorbent mixture (activated

charcoal/celite 545= 1/4, w/w) then shaken for two minutes and filtered through a clean pad cotton and anhydrous sodium sulfate. The filtrate was rinsed carefully with additional 20 ml ethylacetate and evaporated by rotary evaporator till dryness at 40 ^{0}C.

1-2 Instrumentation:

HPLC analysis was performed with an Agilent 1100 HPLC system, with photodiode array detector. The chromatographic column was C_8 and mobile phase (acetonitrile /water 60/40 v/v) and the flow rate was 1 ml/min. Detection wavelength was 230 nm and the retention time of bensultap was 3.2 min**.**

2-Analytical procedure of imidacloprid residues in broad bean plants and the soil:

Mature plants were sprayed as foliar application with imidacloprid as mentioned above with the same intervals.

2-1-Imidacloprid extraction and cleanup:

Imidacloprid was extracted from plants according to the method by Mollhof (1975). Different plant parts (leaves and pods collected randomly), were taken one hour, and 1, 3, 7, 12, 16, 22 days after insecticide application. Plant material (50g) was cut into small pieces in a warning blender, then 200ml of methanol was added to the samples and blended for 3min. at a higher speed and filtered through a clean dry pad cotton into a graduated cylinder and a known volume of extract was taken. To separate aqueous layer from methanol, NaCl saturated solution (40ml) was added and the extract was portioned successively with 100, 50 and 50 ml of dichloromethane. The combined methylene chloride portions were dried by filtration through a pad of cotton and anhydrous sodium sulfate, and evaporated to dryness under vacuum at 40^{0}C.

Representative soil samples were collected at 1, 3, 7,12,16,22 days after imidacloprid application and extraction was done according to Bachlechner (1989).

Representative soil samples (30 g) at the same depth as mentioned before were taken and 200ml mixture of acetonitrile: water 8:2 (v/v) was added, shaked thoroughly in a shaker for 30min. and left overnight. Soil extract was filtered then 100ml of the clear acetonitrile phase was transfer to a spherical flask and evaporated to dryness at 40 ^{0}C. The residue in the flask was portioned with 100, 50, and 50ml dichloromethane. The combined dichloromethane portions were dried by filtration through a pad of cotton and anhydrous sodium sulfate, and then evaporated to dryness at 40^{0}C.

Clean up for either broad bean plant or soil of imidacloprid extracts, was done according to the method of Blass(1990) as follow. A chromatographic column was prepared and preconditioned by adding 20ml of distilled ethylacetate to a tapped glass column contained 4.5 g deactivated florisil as a slurry in ethylacetate then 2g anhydrous sodium sulfate were added and washed with 20ml ethyl acetate. The residue was re-dissolved in 5ml of ethyl acetate and added to the top of chromatographic column. The column then was eluted with 20ml ethyl acetate, which was discarded. The

imidacloprid residues were eluted with 25ml acetonitrile, which was taken to evaporate at 40 ^{0}C and prepared for the determination.

2-2-Instrumentation:

PLC analysis was performed with an Agilent 1100 HPLC system, with photodiode array detector. The chromatographic Column was C18, mobile phase (acetonitrile/water = 65/35 v/v) and the flow rate was 0.8 ml/min. Detection wavelength was 270 mm and the retention time of imidacloprid was 2.7min.

The half-life calculation: Residue half life of the two insecticides was calculated according to Moye et al

(1987) equation Half- life ($t_{1/2}$) = 0.693/K where K is the rate of degradation

Recovery Test:

Samples of untreated soil and broad bean leaves and pods were fortified with acetone solutions of known amounts of bensultap and imidacloprid standard. Fortified samples were processed according to extraction and clean up procedures mentioned earlier. Recovery percentages are shown in Table (1).

RESULTS AND DISCUSSION

Table (1) indicated the percentage recovery of the tested insecticides from the spiked samples. Data showed that the average recovery percent ranged between 87.5 to 100% for bensultap while the mean recovery percent ranged between 92 to 100% for imidacloprid

Data also indicated that the recovery from the soil samples was higher for both insecticides than those from plant parts

Table (1): Recovery rate for bensultap and imidacloprid in broad bean leaves, pods and soil.

insecticide used	*Recovery %			
		Pods		
	leaves	peels	seeds	soil
Bensultap	87.5±0.9	85±0.9	91±1.9	100
Imidacloprid	92±2.1	77.4±0.5	96±0.3	100

*** Mean recovery ± SD**
Number of replicates= 3

Bensultap residues in broad bean plants:

Data shown in Table (2) indicated that the concentration of bensultap after one hour of application at the recommended rate was 12.5 mg/kg in the whole broad bean leaves. The insecticide dissipated rapidly one day after application to reach 5.88 mg/kg which in turns indicated that almost 53% loss of the insecticide occurred. Following that period, the residues amount decreased to be 0.29 mg/kg after 22 days of treatment with 97.7 % loss.

Results showed that the RL_{50} in broad bean leaves treated at the recommended rate was 0.9 days. Whereas the dissipation of the insecticide

was much slower in the peels of the broad bean pods one day after application as persistence was 95.5%. Following this intervals, the insecticide started to decline gradually as residues amount was 0.08 mg/kg after 22 days from application and the half-life value was 2.9 days as shown in Table 2.

With regards to the persistence of the bensultap in green seeds of broad beans, after the first 24 h of application the initial deposit was not detectable. However, the concentration was 0.63 mg/kg after the third day of application and decreased until residue amounts were undetectable 22 days after application.

The residue half life value could not be detected due to undetectable amounts of bensultap in the initial time.

Generally the dissipation of the insecticide residues in/on crops depend on environmental conditions, type of application, plant species, dosage, interval between application, the relation between the treated surface and its weight and the living state of the plant surface, in addition to the harvest time (Khay et al.2008). That might explain our obtained data as different dissipation rate of bensultap based on the type of the plant parts. Also it could be attributed to other factors such as migration of residues of such systemic insecticides and /or active metabolic conversion in the plant leaves (Barakat et al 1994)

According to The Japan Food Chemical Research Foundation (2008), maximum residue limit (MRLs) for bensultap in broad bean seeds is 3 mg/kg.

Also obtained results agreed with those reported by Mojaševic et a (1996) since maximum residue limits approached ≤ 0.1 mg/kg in Lucerne in less than 14 days following the application of the recommended bancol rate. The dissipation rate of bensultap was found to be slower in grapes, but reached detection limits (0.02 mg/kg) in about 30 days.

Our data pointed out those broad beans green seeds could be consumed safely after 3 days of the insecticide application as determined pre-harvest interval value was 3 days.

Table (2): Bensultap residues in broad bean plants at different time intervals.

Time after application (days)	Leaves			Pods			
				Peels			Green seeds
	ppm	Loss %	Persistence %	ppm	Loss %	Persistence%	ppm
Initial*	12.5	0.0	100	2.21	0.0	100	ND
1	5.88	**52.9**	47.1	2.11	**4.5**	95.5	0.63
3	2.93	76.5	23.5	1.01	54.3	45.7	0.21
7	1.66	86.7	13.3	0.59	73.3	26.7	0.15
11	1.46	88.30	11.7	0.45	79.6	20.4	0.08
16	0.34	97.3	2.7	0.38	82.8	17.2	0.01
22	0.29	97.7	2.3	0.08	96.4	3.6	ND
RL_{50} days	0.9			2.9			

- ***One hour after application**
- **ND : Not Detectable**
- **MRL: 3 ppm according to the Japan Food Chemical Research Foundation (2008)**
- **PHI : 3 days**

Bensultap residues in soil

As shown in Table (3) persistence of bensultap in contaminated soil under the treated plants is contrasting to that in broad bean plant. The initial deposit was much lower than that in leaves or peels with a value 0.54 mg/kg. This may be due to the lower amount of the insecticide dropped out of the leaf surface as the soil was not the target of application. The rate of disappearance gradually decreased within all sampling dates to reach 85.2%, after 22 days post application. The half life value of bensultap calculated in soil under the treated plants which was 4 days, indicating that bensultap remains relatively longer in the soil compared with its persistence in the plants. On the contrary to bensultap, organophosphate insecticide pirimiphos-methyl have relatively longer residue half life value as it was 6 days as reported by Shokr et al (2000)**.** DT_{50} of bensultap is 3-35 days based on soil type. (Pesticide Manual 2005)

Table(3): Bensultap runoff residues in soil at different time intervals

Time after application (days)	ppm	Loss %	Persistence %
Initial*	0.54	0.0	100
3	0.31	42.6	57.4
7	0.18	66.7	33.3
11	0.17	68.5	31.5
16	0.16	70.4	29.6
22	0.08	85.2	14.8
RL_{50} days	4		

- ***One hour after application**

Residues of imidacloprid in broad bean plants:

Data in Table (4) revealed that the initial concentration of imidacloprid determined in broad bean leaves was much less than that found in leaves treated with bensultap which was 2.78 mg/kg. This may be due to the lower rate of application and less active ingredient in the formulation used. As the concentration of bensultap is 50% with a rate of 150 g/ L water whereas , imidacloprid was 20% with a rate of 50 ml/100 L water. The dissipation of imidacloprid in the leaves was generally slower particularly one day after application which was only 27.7%.. At 3 days sampling time, the insecticide residues start to decrease gradually as it was 0.93 mg/kg. Imidacloprid relatively slowly declined to reach 0.23 mg/kg 16 days after application. Three weeks after application imidacloprid residues were not detected.

Residue half life of imidacloprid in broad bean leaves was 2.25 days. These results agreed with those of Jian-Qing et al (2004) who determined the half-life value of imidacloprid in cabbage which was 2-4 days depending on the concentration used.

Foliar penetration and short-term translocation patterns of imidacloprid were similar in cabbage and cotton leaves after foliar application. Nevertheless imidacloprid penetrated twice as much into cabbage leaves as it did into cotton leaves. It showed a comparable translaminar behavior and

was entirely translocated acropetally, indicating its well-known xylem mobility Buchholz and Nauen (2002)

With regards to the imidacloprid residual behavior in broad bean peel of the pod, initial amount was 2 mg/kg, however, the insecticide started to greatly decrease one day after application with a value of 53.5%.loss.

The persistence of imidacloprid gradually reduced through the time elapsed of the experiment to reach 0.02 mg/kg at the end of the sampling (22 days) as shown in Table (4).In the case of persistence of imidacloprid in broad bean seeds, residue amounts could not be detected one hour after application. Indicating that both imidacloprid and bensultap are mainly retained in the peels and slowly migrate to the internal seeds during the first hour after treatment. Data revealed that concentration of imidacloprid after one day of application was less than 1 ppm which was 0.78 mg/kg. According the European Union (2010**)** as stated that MRL of imidacloprid is 2 mg/kg. Amount of detected insecticide started to decrease gradually to reach 0.05 ppm at 16 days after application. Our data is in consistent with these data since the PHI is 3 day in broad bean seeds.

Imidacloprid half-lives on plant surfaces found in the literature ranged from 3 days (Mukherjee and Gopal, 2000) up to 32 days as reported by Vogeler et al. (1992). Juraske (2007) estimated measurements and models to conclude that imidacloprid as spray deposition on the plant surface does not tend to cross through the cuticle of tomato fruits and suggest a low potential for bioaccumulation.

Furthermore, the permeation through plant cuticles depends on the solute mobility in the limiting skin, the path length of the limiting skin and the partition coefficient between cuticle and deposited surface residue (Schreiber, 2005; Trapp, 2004).

According to our experimental results, in similar manner, imidacloprid may maintain to some extent in the skin of broad bean seeds. In contrast to the present data, average residue concentrations of imidacloprid on tomato fruit ranged from 1.60 mg /kg at zero time to 0.18 mg/ kg 28 days after the spray application. The half-life of imidacloprid is 8.2 days if applied on tomato fruits Juraske (2007).

Table (4): Residues of imidacloprid insecticide in broad bean plants

Time after treatment (days)	Leaves			Pods			
				Peels			Green seeds
	ppm	Loss%	Persistence	ppm	Loss%	Persistence	ppm
Initial*	2.78	0.0	100	2.00	0.0	100	ND
1	2.01	27.7	72.3	0.93	53.5	46.5	0.78
3	0.93	66.6	33.4	0.76	62.0	38.0	0.61
7	0.81	70.9	29.1	0.53	73.5	26.5	0.46
12	0.49	82.4	17.6	0.25	87.5	12.5	0.19
16	0.23	91.7	8.3	0.09	95.5	4.5	0.05
22	ND	-		0.02	99.0	1.0	-
RL_{50} (days)	2.25			0.93			-

- ***One hour after application**
- **ND : Not detectable**
- **MRL: 2 ppm EU 2010**
- **PHI :3 day**

Residues of imidacloprid insecticide in soil

Data in Table (5) reveal the behavioral persistence of imidacloprid runoff in the soil under treated plants.

Initial deposits of runoff of imidacloprid in the soil was less than that detected in leaves or pods which was 0.98 ppm. Three days after the application, the concentration declined to 0.73 mg/kg. At the end of the sampling date the residues reach 0.004 mg/kg after 22 days from application.

In general, imidacloprid disappeared relatively rapidly in soil particularly at the seventh day as the residue amount was 0.06 ppm.

In the present studies we estimated the residue half life of imidacloprid in the soil to be 5 days. Mohamed and Mahmoud (2009) calculated the residue half-life value of the insecticide in the soil which was as 7.66 days .This value might be due to the higher field rate used in that study (125 ml), while in our study only 50 ml were applied.

The present results is in agreement with Jian-Qing et al (2004) who determined the half life of imidacloprid in the soil to be 5-8 days

Nevertheless, data obtained is in contrast with Sarkar *et al.* (2001) who found slower degradation of imidacloprid in soil compared to metabolism in plant tissue.The insecticide is quite water soluble even at the lowest solubility value reported (510 mg/L) and could potentially leach to groundwater (Mulye 1995) .Thus, imidacloprid can persist in soil depending on soil type, pH, temperature , use of organic fertilizers, and presence or absence of ground cover (Baig *et al.,* 2012).

Table (5): Residues of imidacloprid insecticide in soil

Time after treatment(days)	ppm	Loss%	Persistence%
Initial*	0.89	0.0	100
3	0.73	18.0	82.0
7	0.06	93.3	6.7
12	0.02	97.8	2.3
16	0.01	98.9	1.1
22	0.004	99.6	0.45
RL_{50} (days)	5 days		

- ***One hour after application**

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تقدير متبقيات مبيدى الايميداكلوبريد و بنسالتاب فى نبات الفول البلدى و التربة

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الهدف من البحث هو دراسة سلوك متبقيات مبيدى الايميداكلوبريد و بنسالتاب فى نبات الفول البلدى تحت الظروف الحقليه و كذلك فى التربة اسفل النبات

انخفضت متبقيات مبيد البنسالتاب الى 0.29 جزء فى المليون بعد 22يوم من المعاملة بنسبة فقد 97.7% فى اوراق نبات الفول البلدى. بينما لم تظهر متبقيات مبيد الايميداكلوبريد بعد نفس المدة فى اوراق نبات الفول البلدى. سجلت فترة نصف العمر لمبيد البنسالتاب فى قشور قرون الفول البلدى 2.9 يوم.كما سجلت فترة نصف العمر لمبيد الايميداكلوبريد فى قشور قرون الفول البلدى 0.93 يوما . بينما لم تظهر متبقيات المبيد بعد ساعة من المعاملة.

يمكن استخدام قرون الفول الخضراء بامان بعد 3 ايام من المعاملة لكلا المبيدين . كانت فترة نصف العمر لمبيد البنسالتاب فى التربة 4 ايام بينما لليميداكلوبريد 5 ايام

قام بتحكيم البحث

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