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# SOME BIOCHEMICAL AND TOXICOLOGICAL STUDIES WITH IMIDACLOPRID INSECTICIDE ON BROAD BEAN PLANTS

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## ABSTRACT

Broad bean was planted at El-Badrachin, Giza Governorate in the season of 2001. The experimental area was divided into two parts; one part was treated with a single spray of imidacloprid insecticide and the other was treated twice. The effect of the single and double treatment on the chemical composition of broad bean leaves and seeds was studied. The data showed that imidacloprid did not have any obvious changes in the chemical composition of broad bean leaves or seeds, either in the single or double treatments. The pesticide residual study was carried out on the plant area that received single spray. The half- life values of imidacloprid in broad bean leaves and peels of the green pods were 1.6 and 1.2 days, respectively. One hour after plant treatment Imidacloprid residues in green seeds were below the limit of determination (<0.01 ppm). One day after treatment, it was 0.96 ppm, then decreased gradually to 0.05 ppm after 20 days. The pre- harvest interval (PHI) in green seeds was 15 days. No residues were detected in dry seed samples at harvest, (27 days after treatment). When male rats were fed on broad bean crushed dry seeds that resulted from plants received single treatment of imidacloprid, there were significant decreases in plasma urea , total protein and albumin by 39.09, 36.48 and 33.48% relative to the control, respectively.While significant decreases were found in urea, creatinine, triglycrides cholinesterase (ChE) and total protein of male rat`s plasma after feeding on broad

bean dry seeds resulted from plants treated twice with imidacloprid. The percentage decreases were 40.25, 15.31, 37.13, 43.39 and 44.69% relative to the control, respectively.

*Key words:broad bean, imidacloprid, residues.*

## 1.INTRODUCTION

If a pesticide is to be accepted for use in controlling pests on plants, it must be effective against these pests with low persistence to avoid residue problems in harvested crop. It must has no adverse effect on the treated plant in addition to low mammalian toxicity.

Imidacloprid insecticide (1-(6chloro 3- pyridyl methyl- N-nitro imidazolidin- 2- yludene- amine) had a superior performance on sucking pests such as leaf hoppers, white flies and aphids (Elbert *et al*., 1990). A comparison with conventional aphicides demonstrates the superiority of imidacloprid in its selective action. Imidacloprid with its much superior insecticidal efficacy is much less toxic to mammals than nicotine, (Leicht 1996). Imidacloprid is recommended to use on broad bean for controlling aphids, white flies and leaf miners.

The aim of the present study was to investigate the effect of imidacloprid insecticide on the chemical constituents of broad bean leaves and seeds (green and dry), the residual behavior of imidacloprid in treated plants and to check the effect of feeding rats on the resulted mature dry seeds on the biochemical parameters of the plasma.

## 2.MATERIALS AND METHODS

A field experiment was conducted at EL-Badrachin, Giza Governorate during the growing season of 2001, where broad bean (*Vicia faba)* Giza 427 variety was planted on the 11$^{th}$ of November 2000. The experimental area was divided into two parts, each part was divided into 12 plots of 1/200 feddan. The first part was treated with a single treatment of imidaclo of prid (Confidor 35% S.C.) at the rate of 75ml/100L of water on the 18$^{th}$ of March 2001. The second part was treated twice with imidacloprid in the same rate; the first was on January the 22$^{nd}$, 2001 and the second was on March the 18$^{th}$ 2001. Inside each part three plots were left without treatment for comparison and considered as control.

**2.1.Determination of biochemical constituents of broad bean samples**

Representative plant samples (leaves and green pods) were taken from each of the two parts of the single and double treatment after 6, 20 days from treatment and then at harvest (27 days after treatment). Total carbohydrates, total proteins, and lipids, fiber and ash contents were determined according to the method outlined in the A.O.A.C. (2000). While chlorophyll and (a), (b) and carotenes were determined according to Arnon(1949).

**2.2. Imidacloprid residue analysis**

Another part of the representative samples were taken from the single treatment; part only from leaves, green pods one hour after treatment followed by the intervals of 1, 3, 6, 10, 15, 20 and at harvest (27 days after treatment).

The method of (Blass, 1990) was adopted for extraction and cleaning-up of imidacloprid residue analysis where acetonitrile used in the method was replaced by methanol for extraction. This modification was done by Mahmoed (2000). Deactivated florisil 5% chromatographic column was used for clean- up. Agilent 1100 series HPLC equipped with photodiod array UV detector was used at the wavelength of 270 nm. The column used was Nucleosil 100-5 reversed phase (C18) 5 µm, 250 x 4 mm. The injection volume was 20 µl. The mobile solvent was acetontril: water gradient as mentioned in the method of Blass (1990). The retention time of imidacloprid under the mentioned conditions was 2.65 minutes.By using this method, the average rates of recovery were 65.50, 88.74, 86.00 and 100% for green seeds, leaves, green peels and dry seeds.

**2.3.Toxicological studies**

The present investigation was carried out on male albino rats with an average weight of120-140gm purchased from a specialized animal colony , Giza Governorate , Egypt . Such animals were kept in cages under appropriate conditions with free running tap water and room temperature. The offered food to the experimental rats was crushed dry seeds of broad bean which was daily provided to avoid any contamination of any sort except imidacloprid residues. Acclimation of all animals was set for one week after that time. Such adapted animals were subdivided into 3 groups, each group contained 5 rats.

The first group was fed on untreated dry seeds (control). The second group was fed on dry seeds resulted from plants received single treatment. The third group was fed on dry seeds, resulted from plants that were treated twice. It is well known that the rat feeds on 10% of its weight (dry seeds) daily. At the end of experimental period (28 days), blood samples were taken from four rats of each group which were sacrificed. Blood samples were collected from orbital veins technique by heparinized capillary tubes at the end of experiment into clean, dry and labeled eppendorf tubes (1.5ml). Samples were centrifuged in a refrigerated centrifuge at 3500 rpm for 15 min. to separate plasma.

Liver function, kidney function, total protein, albumin, alkaline phosphatase (ALP), cholinesterase (ChE), total cholesterol, triglycrides and glucose were determined in blood plasma. The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the method adopted by Reitman and Frankel (1957). Urea concentration was measured by the method adopted by Fawcett and Scott (1960). Creatinine concentration was measured by the method of Siest *et al.*, (1985). Total protein was determined by the method adopted by Weichsebaum (1946). Albumin was determined by the method adopted by Doumas *et al.*,(1971). Plasma alkaline phosphatase activity (ALP) was determined according to Kaplan and Righett (1955). The activity of plasma cholinesterase was determined by the method adopted by Ellman *et al.*(1961). Plasma triglycrid concentration was measured by the method adopted by Jacobs and Van Demark(1960). Plasma cholesterol concentration was measured as described by Allain *et al.* (1974). Glucose concentration was determined by the method adopted by Barham and Trinder (1972).

### 2.4. Statistical analysis

Statistical analysis was done by using the analysis of variance (t.test) as described by Miller and Miller (1992).

## 3. RESULTS AND DISCUSSION

### 3.1.Effect of imidacloprid on some chemical constituents of broad bean leaves and seeds

Regarding the average values in Tables (1) and (2), the results show that the total protein and nitrogen in broad bean leaves and green seeds were not affected by imidacloprid treatment compared

with the untreated control. These results are in agreement with Salem and El-Sherief (1998), who reported that the total protein content of broad bean leaves was not affected by any insecticide they used. With regard to the general mean in Tables (1) and (2), the results indicate that no clear difference between the plants received single treatment or that treated twice. In general, the total carbohydrate and lipid contents had not any changes by imidacloprid treatments. Also ash and fiber contents were not effected by imidacloprid. Although it could be noticed that there was a fluctuation in all values during the study intervals, those values were around the check treatment range. The pigments reflect the condition of the plants, therefore it was important to study the effect of imidacloprid on leaves of broad bean. It was clear from Table (3) that imidacloprid had no significant effect on chlorophyll and carotine contents and these results are in agreement with Rouchoud *et al.*( 1984).

### 3.2. Residual behavior of imidacloprid on and in broad bean leaves and seeds

The results in Table (4) represent the residues of imidacloprid on and in leaves, green seeds and peels of green pods. The initial deposits determined on the leaves and green pods peels were 2.36 and 1.23 ppm, respectively. One day after treatment, the amount of residues decreased to 1.53 and 0.63 ppm for leaves and peels, respectively. This amount decreased to 0.24 and 0.30 ppm after 15 days from application. The percentage loss amounted to 89.88 and 75.95% after 15 days from treatment for leaves and peels, respectively. The residue half-life values in leaves and peels were 1.6 days and 1.2 days, respectively. By analyzing the green seeds of broad bean one hour after application, no amount of imidacloprid residues was detected (LOD was 0.05ppm in green seeds). Following this period, the residue of imidacloprid began to appear; it was 0.96 ppm and then decreased gradually. It was 0.73, 0.54, 0.23, 0.11 and 0.05 after ppm 1, 3, 6, 10, 15, and 20 days after treatment, respectively. No imidacloprid residues were detected in dry seeds after 27 days of treatment. Concerning food safety measures, the results indicate that the pre-harvest interval (PHI) for green seeds was 15 days after treatment . According to the Canadian Maximum Residue limits for agricultural chemicals, 2003, the maximum residue limits of imidacloprid on vegetables is 0.2ppm.

Table (1):Effect of imidacloprid on some chemical constituents (%) of broad bean leaves (dry weight basis).

| Constitu-ents | 6days | | | 20 days | | | Mean ± SD | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Single | Twice | Control | Single | Twice | Control | Single | Twice |
| T.C. | 54.45 | 56.14 | 55.91 | 60.46 | 60.21 | 58.54 | 57.46±4.25 | 58.18±2.88 | 57.23± 1.86 |
| T.P. | 25.48 | 24.19 | 25.50 | 20.53 | 20.36 | 23.45 | 23.01±3.50 | 22.28±2.71 | 24.48±1.45 |
| T.L. | 0.82 | 0.78 | 0.89 | 0.69 | 0.65 | 0.57 | 0.76±0.09 | 0.72±0.09 | 0.73±0.23 |
| T.N. | 4.08 | 4.21 | 4.10 | 3.28 | 3.26 | 3.75 | 3.86±0.57 | 3.76±0.64 | 3.93±0.25 |
| Fiber | 11.29 | 11.96 | 10.25 | 10.08 | 9.49 | 9.13 | 10.69±0.85 | 10.73±1.75 | 9.69±0.79 |
| Ash | 7.98 | 7.26 | 7.31 | 8.24 | 9.28 | 8.31 | 8.11±0.18 | 7.77±0.82 | 7.81±0.71 |

Table (2):Effect of imidacloprid on some chemical constituents (%) of broad bean green and mature seeds (dry weight basis).

| Constitu ents | 6days | | | 20 days | | | 27days | | | Mean ± SD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Single | Twice | Control | Single | Twice | Control | Single | Twice | Control | Single | Twice |
| T.C. | 57.90 | 54.29 | 52.66 | 56.90 | 57.42 | 56.66 | 56.22 | 57.19 | 61.07 | 57.01±0.85 | 56.30±1.74 | 56.79±4.21 |
| T.P. | 22.86 | 27.00 | 27.51 | 25.18 | 25.47 | 25.50 | 27.01 | 25.88 | 22.14 | 25.02±2.02 | 26.12±0.79 | 25.05±2.71 |
| T.L. | 1.03 | 1.53 | 1.34 | 0.83 | 0.83 | 0.85 | 0.32 | 0.37 | 0.37 | 0.73±0.366 | 0.91±0.58 | 0.85±0.49 |
| T.N. | 3.66 | 4.32 | 4.41 | 4.03 | 4.07 | 4.08 | 4.32 | 4.14 | 3.54 | 4.00±0.33 | 4.18±0.13 | 4.01±0.44 |
| Fiber | 12.78 | 12.17 | 13.25 | 12.60 | 11.74 | 12.57 | 12.49 | 12.67 | 12.56 | 12.62±0.15 | 12.19±0.47 | 12.82±0.37 |
| Ash | 5.42 | 5.01 | 5.14 | 4.49 | 4.55 | 4.42 | 3.96 | 3.88 | 3.86 | 4.62±0.74 | 4.48±0.57 | 4.47±0.64 |

T.C. : Total Carbohydrates
T.P.: Total Protein
T.L. : Total Lipids
T.N.: Total Nitrogen
Single : Single treatment
Twice: Twice treatments

**Table (3): Effect of imidacloprid on chlorophyll (a), chlorophyll (b), total chlorophyll and carotine concentration(mg/100g) in broad bean leaves.**

| Pigments | 6 days | | | 20days | | | Mean ± SD | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | (1) | (2) | Control | (1) | (2) | Control | (1) | (2) |
| Chl(a) | 1.23 | 1.53 | 1.16 | 1.00 | 1.00 | 1.18 | 1.12± 0.16 | 1.27± 0.38 | 1.17± 0.01 |
| Chl(b) | 0.79 | 0.48 | 0.39 | 1.04 | 1.04 | 0.67 | 0.92± 0.18 | 0.76± 0.40 | 0.53± 0.20 |
| T.Chl | 2.00 | 2.01 | 1.55 | 2.04 | 2.04 | 1.85 | 2.02± 0.28 | 2.02± 0.21 | 1.71± 0.20 |
| Carotines | 0.65 | 0.91 | 0.43 | 0.63 | 0.63 | 0.66 | 0.64± 0.01 | 0.77± 0.20 | 0.55± 0.16 |

(1): Single treatment (2): Twice treatment
Ch(a): Chlorophyll(a) Ch(b): Chlorophyll(b) T.Ch: Total Chlorophyll

**Table (4): Residues of imidacloprid in leaves, green seeds and green peels of broad bean.**

| Days after treatment | Leaves | | Green seeds | | Green peels | |
|---|---|---|---|---|---|---|
| | Residues (ppm) | Loss % | Residues (ppm) | Loss % | Residue (ppm) | Loss % |
| Initial** | 2.36 | -- | ND | -- | 1.23 | -- |
| 1 | 1.53 | 46.58 | 0.96 | -- | 0.63 | 48.67 |
| 3 | 0.98 | 58.31 | 0.73 | 24.03 | 0.44 | 64.04 |
| 6 | 0.87 | 63.03 | 0.54 | 43.39 | 0.37 | 69.89 |
| 10 | 0.52 | 78.18 | 0.23 | 76.13 | 0.32 | 74.34 |
| 15 | 0.24 | 89.88 | 0.11 | 88.43 | 0.30 | 75.95 |
| 20 | ND | -- | 0.05 | 94.34 | 0.22 | 82.05 |
| **RL50** | **1.6 days** | | | | **1.2 days** | |

ND: Not detected

Lichtenstein (1972) reported that residue dissipation could be attributed to a variety of environmental factors such as sunlight and temperature. Besides, plant growth, particularly for fruits, is also responsible to a great extent for decreasing pesticide residue concentrations due to growth dilution effects. Walgenbach *et al.* (1991) mentioned that the systemic properties of imidacloprid allow it to become evenly distributed in the young and growing plant. After spraying application, most of residue on the leaf surface consists of unchanged parent compound.

### 3.3.Effect of imidacloprid on some parameters of male rat's plasma

The results in Table (5) indicate that urea concentration decreased significantly in plasma male rat fed on dry mature seeds of

broad bean that resulted from treated plants with either single or double treatment of imidacloprid. This result is in agreement with that reported by El- Kashoury (1999). Creatinine concentration in the plasma of male rat did not alter by feeding rats on dry mature seeds resulted from plants received a single treatment. While feeding on dry

**Table (5): Effect of dry broad bean seeds feeding (resulted from treated plants by imidacloprid) on some parameters of male rat's plasma.**

| Treatment / Parameters (Mean ± SE) | Control (untreated) (Mean ± SE) | Single treatment (Mean ± SE) | Twice treatment |
|---|---|---|---|
| **Urea (mg/dl)** | 58.68 ± 7.608 | 35.74 * ± 5.467 | 35.06 * ± 3.796 |
| **Creatinine (mg/dl)** | 0.196 ± 0.000 | 0.170 ± 0.021 | 0.166 * ± 0.006 |
| **Triglycrid(mg/dl)** | 24.56 ± 1.719 | 24.09 ± 2.171 | 15.44* ± 2.004 |
| **Cholesterol(mg/dl)** | 70.49 ±7.870 | 69.01±7.361 | 52.39 ±4.196 |
| **ChE (U/L)** | 1243.38 ± 179.44 | 641.24** ± 67.27 | 703.80 *± 34.52 |
| **AST (U/L)** | 32.35 ± 10.86 | 44.59 ± 3.13 | 40.34 ± 2.73 |
| **ALT (U/L)** | 3.414 ± 0.92 | 7.404 * ± 1.28 | 5.372 ± 1.03 |
| **ALP (U/L)** | 137.85 ± 29.57 | 312.12 ±23.11 | 283.07 ± 15.49 |
| **Glucose(mg/dl)** | 70.79 ± 9.773 | 78.08 ± 2.059 | 81.55±5.033 |
| **Total protein( g/dl)** | 5.93 ± 0.511 | 3.77* ±0.652 | 3.28*± 0.655 |
| **Albumin(g/dl)** | 3.18 ± 0.142 | 2.11* ±0.288 | 2.66 ± 0.173 |

* : $P < 0.05$ ** : $P < 0.01$

ChE: Cholinesterase AST : Aspartate aminotransferase

ALT: Alanine aminotransferase ALP:Alkaline phosphatase

seeds of treated plants that received two doses induced a significant decrease in Creatinine concentration. The results are in agreement with the findings of Barlas (1996) and El- Said (1997). Triglycrides and cholesterol did not alter significantly by feeding rats on dry seeds, that resulted from plants that received a single treatment. While feeding on dry seeds resulted from plants treated twice caused a significant decrease in triglycrides content. The data also showed that imidacloprid had a significant decrease in the activity of cholinesterase (ChE) in plasma, either in single or double treatments. These findings are in good agreement with those obtained by Vodela and Dalvi (1995) and Bayoumi *et al.*( 1997). Imidacloprid had no significant effect on the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) either in single or double doses. The data did not show any significant increase in alkaline phosphatase (ALP)

activity in plasma male rats which were fed on dry seeds resulted from plants received a single treatment. The results obtained are in agreement with those of Barlas (1996) and El- Said (1997), who noted that no alteration occurred in the activity of aspartate amino transferase (AST) in rats treated with several toxicant substances at low level, *i.e.*, imidacloprid at 14.8 mg/kg b. wt. Plasma glucose level did not change in rats which were fed on dry seeds that resulted from treated plants.Feeding dry seeds resulted from plants treated with imidacloprid had a significant decrease in total protein or albumin in plasma, either in single or double treatments.

**Conclusions**

Imidacloprid has no adverse effect on broad bean plant constituents so it could be used safely at the examined rate of application, either for one or two treatments for controlling of sucking insects. Considering the MRL of imidacloprid, the pre- harvest interval (PHI) for imidacloprid on broad bean is 15 days to use the green seeds for human consumption. Further studies on imidacloprid metabolites in male rats feeding is needed in order to interpret the data of toxicological studies as the level of the parent compound in the dry seeds is below the detection limit (0.01 ppm). The use of pesticides leads to residues on crop at harvest .The term pesticide residues includes any derivative such as conversion products, metabolites, reaction products and impurities to be of toxicological significance Handa, (1999). But this study was restricted in laboratory hindered by the difficulty of getting the pure active ingredient of imidacloprid metabolites.

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# بعض الدراسات الكيماوية الحيوية ودراسات السمية لمبيد الايميداكلوبريد على نبات الفول البلدى

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## ملخص

تم زراعة محصول الفول البلدي بمنطقة البدرشين بمحافظة الجيزة فى موسم ٢٠٠١ . قسمت المساحة التجريبية إلي قطعتين عوملت إحداهما بالرش مرة واحدة من الايميداكلوبريد بينما عوملت الثانية بالرش مرتين . ودرس تأثير المعاملة سواء مرة واحدة أو مرتين على التركيب الكيماوي لاوراق و بذور نبات الفول البلدي . أظهرت النتائج انه لم يحدث أي تغيرات معنوية في التركيب الكيماوي لأوراق وبذور نبات الفول الخضراء بعد معاملتها بالمبيد محل الدراسة.

كما درس سلوك متبقيات مبيد الايميداكلوبريد على نبات الفول البلدي التي عوملت بالرش مرة واحدة فقط. وجد أن قيمة فترة نصف العمر للمبيد علي أوراق النبات كانت ١,٦ يوما بينما كانت ١,٢ يوما على قشور القرون الخضواء. لم تظهر في البذور الخضراء متبقيات المبيد بعد ساعة واحدة من المعاملة حيث كانت اقل من حدود الطريقة المتبعة في التقدير، وبدا ظهور متبقيات المبيد بعد يوم من المعاملة حيث قدر ٠,٩٦ جزء في المليون ثم تناقصت هذه الكمية فوصلت إلى ٠,٠٥ جزء في المليون بعد عشرين يوما من المعاملة و قد قدرت فترة ما قبل الحصاد ب ١٥ يوما بعد المعاملة. بينما لم يمكن تقدير إي كمية من متبقيات المبيد في البذور الجافة .

وجد عند تغذية ذكور الفئران على البذور الجافة الناتجة من النباتات المعاملة مرة واحدة بالمبيد ان هناك نقصا معنويا في كل من مستوى اليوريا , محتوى البروتين الكلى و الالبيومين في بلازما الدم حيث كانت نسب الانخفاض ٣٩,٠٩ ، ٣٦,٤٨، ٣٣,٤٨ % مقارنة بالكونترول على التوالى. بينما نجد عند التغذية على البذور التي نتجت من النباتات التى عوملت مرتين حدوث نقص معنوي في اليوريا, الكرياتينين, الجلسريدات الثلاثية ,نشاط الكولين استيراز و محتوى البروتين الكلى في بلازما ذكور الفئران. وكانت نسب الانخفاض ٤٠,٢٥ ، ١٥,٣١ ، ٣٧,١٣ ، ٤٣,٣٩، ٤٤,٦٩ % مقارنة بالكونترول على التوالى.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٥) العدد الرابع (أكتوبر ٢٠٠٤):٥٥٧-٥٦٨.