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Research Article

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ANALYSIS OF SELECTED PESTICIDES (I.E. IMIDACLOPRID AND DIMETHOATE) RESIDUE IN YARD LONG BEANS (*VIGNA UNGUICULATA* SUBSP. *SESQUIPEDALIS)* AND OKRA (*ABELMOSCHUS ESCULENTUS* L. (MOENCH).

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Abstract

A study was conducted to analyse the residues of some selected pesticides (i.e. Imidacloprid and Dimethoate) on the okra fruits (*Abelmoschus esculentum* L. (Moench)) and yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) with the help of HPLC. The purpose of the experiment was to determine the interval between spraying and harvest required for the safe use of these crops to prevent any health problems to the consumers. During the month of October and November 2014, yard long bean samples were taken at 3, 7, 10, 15 and 21 days after spraying with the pesticide and okra/lady's finger were plucked at 3, 8 and 14 days after spraying. The half life of imidacloprid on yard long beans and okra fruits was calculated to be 2.08 and 2.66 days respectively. The half life of dimethoate was calculated at 3.7 days on yard long beans and 2.45 days on okra fruits. The concentration of pesticide residues declined gradually. The degradation of the pesticides on yard long beans followed second order kinetics while that on lady's finger followed first order kinetics.

Keywords: Dimethoate , HPLC , Imidacloprid, Residue Analysis.

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Introduction

Although the damage of pests and diseases are cause of significant losses in potential production, the use of chemical pesticides is a major concern in agriculture as it not only leads to the chemical build up of the residues but also disrupts the biological parameters of the plant (Chauhan *et al.*, 2013). The persistent and indiscriminate use of these chemicals leads to build up of residues on crop produce. After application, pesticides may interact with the plant surfaces or be exposed to environmental factors like sun, wind or be washed off during rainfall. Food safety issues in developing countries are widely recognized and WHO has placed it among its top 11 priorities and is making tremendous efforts to address the issue, especially in developing countries (WHO, 2002). Dinham (1993) made a comparative study of pesticide use and exposure patterns in different countries including India and concluded that a general lack of hazard awareness, lack of protective clothing or difficulty of wearing protective clothing in tropical climates, shortage of facilities for washing after use, reusing the containers for storing food and drink, illiteracy, lack of regulatory authorities and lack of enforcement play a major role in magnifying its health and safety issues. Pesticide residue levels in food commodities besides their direct implication on human health also affect international trade (Sanborn *et al*., 2004). To ensure the safety of food for consumers and to protect consumer's health, the monitoring of pesticide residues in food products is necessary. The results from these monitoring programmes are also used by regulatory bodies for future developments in setting MRLs and risk assessment exercises for public health (Keikotlhaile and Spanoghe, 2011).

Imidacloprid (1-(6-chloro-3-pyridyl-methyl) N-nitro-imidazolidin-2-ylideneamine) is a highly systemic chloronicotinoid which is effective for controlling aphids, whiteflies, thrips, scales, psyllids, plant bugs, leafhoppers and other various harmful pest species including resistant strains (Ishii *et al*., 1994). Dimethoate is an organophosphorous insecticide which is widely used against insect pests like mites and aphids systemically and on contact. Keeping the above points in view, the following study was conducted in Nayabeel, Cachar District, Assam to analyse the residues of some selected pesticides on okra fruits (*Abelmoschus esculentum*) and yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) in farmer's field.

MATERIALS AND METHODS

Yard long beans and okra fruit samples were collected from a farmer's field in Nayabeel during the month of October and November 2014. Cow pea samples were taken at 3, 7, 10, 15 and 21 days after spraying with the pesticide and lady fingers were plucked at 3, 8 and 14 days after spraying. The samples were collected in plastic bags and transferred to the lab for analysis. These were then washed, and 100 gm was weighed and grinded. After grinding, the sample was treated with 100 ml of methanol with occasional shaking. After 48 hrs, the solution was filtered and the residue was treated with 50 ml of methanol and filtered again. Both the filtrates were mixed together and the solution was concentrated to 30 ml by distillation process and filtered again. It was then injected in the HPLC (HPLC Agilent Infinity 1260) for analysis.

RESULTS AND DISCUSSION

As shown in Table 1, the residues of both dimethoate and imidacloprid decreased in time. The degradation of imidacloprid was faster than dimethoate with time. The initial concentration of imidacloprid three days after spraying was 8.13 mg/kg which decreased to 0.78mg/kg in the last observation 21 days after spraying (Table 1). By the second sampling, which was seven days after spraying, 68 % of imidacloprid had degraded by the third and fourth sampling ten and fifteen days after the spraying, 83 % and 87 % of total degradation had occurred respectively. 90.4% of the total imidacloprid had degraded by the end of the experimental period of twenty one days. The initial concentration of imidacloprid (8.13mg/kg) on yard long bean was higher than that of dimethoate (5.50 mg/kg).

In case of dimethoate, the initial concentration detected was 5.50 mg/kg which decreased to 1.45 mg/kg after 21 days of observation. The degradation rate was at a constant with 73.6 % of initial concentration of the applied dimethoate degraded by the last observation i.e. after 21 days of spraying.

The residues of the pesticides in okra are shown in Table 2. The residue of dimethoate and imidacloprid on the vegetable samples showed a continuous rate of decline. The initial concentration of dimethoate was 5.92 mg/kg in the okra fruits three days after the spraying which reduced to 1.05 mg/kg in the second observation 8 days after the spraying of dimethoate. The last sampling showed a concentration of 0.31 mg/kg. By the time of harvest, i.e. after 14 days of spraying, 95% of initial concentration dimethoate had degraded in the okra fruits.

In the analysis of the pesticide residues of imidacloprid from okra fruits, the initial concentration was found to be 3.81mg/kg which declined to 0.19 mg/kg in the last observation 14 days after spraying (Table 1 & Fig 4) which was loss of 94.7% of the initial concentration of dimethoate.

The residue of imidacloprid on the yard long beans and lady's finger/ okra in the last observation (0.78 mg/kg and 0.19mg/kg respectively) is below the MRL (1 mg/kg) set by the WHO (1988). In case of dimethoate also, the concentration of the pesticide in the last observation is well below the set MRL (2mg/kg) in both the vegetable samples.

Table 1: Imidacloprid and Dimethoate residues in yard long beans.

Days	Conc.of Imidacloprid (mg/kg)	Conc. of Dimethoate (mg/kg)
3	8.13	5.50
7	2.53	3.94
10	1.38	2.16
15	0.98	1.75
21	0.78	1.45
Regression equation	y= 0.0656x-0.0253	y= 0.0296x + 0.1009
Determination coefficient (R^2)	0.975	0.949
Degradation Constant (day $^{-1}$)	0.06	0.07
Half life $t^{1/2}$ days	2.08	3.7

Table 2: Imidacloprid and Dimethoate residues in okra fruits.

Days	Conc. of Imidacloprid (mg/kg)	Conc. of Dimethoate (mg/kg)
3	3.81	5.92
8	0.67	1.05
14	0.19	0.31
Regression equation	Y= -0.269x + 2.004	y = -0.265x + 2.425
Determination coefficient (R^2)	0.979	0.977
Degradation Constant (day $^{-1}$)	0.26	0.28
Half life $t^{1/2}$ days	2.66	2.45

Degradation kinetics of the pesticides

In order to model the degradation rate of the pesticides on the vegetable sample, the order of transformation reaction is studied through the establishment of corresponding rate equation as it links the reaction rate co-efficient and constant parameters. The rate equation being a differential equation can be integrated to obtain an integrated rate equation by linking the concentrations of the reactants with time. Plotting the logarithm residue concentration (lnqt) of the pesticides against time (t) resulted in best fitting R^2 values (0.979 and 0.977) for imidacloprid and dimethoate respectively on okra fruits, hence the degradation follows first order reaction kinetics (Fig. 1 & 2). The first order rate constant (k) was calculated from the following equation:

$$\text{Ln}\,[qt] = -kt + \ln\,[qt]_0$$

Where, qt= concentration of pesticide at time t; qt_0 = initial concentration of pesticide

k = degradation rate constant (s^{-1}) ; t= time

The half life of the pesticides was calculated by the following formula:

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

The plot of 1/qt (1/concentration) versus t (time) for both the pesticides on yard long beans and showed a straight line with appreciable R^2 values (0.975 for imidacloprid and 0.949 for dimethoate) which was more linear than the logarithm of residue (Lnqt) versus time (t) graph which indicate that the reaction follows a second order kinetics (Fig. 3 & 4). The second order rate constant (k_2) was calculated using the following equation:

$$1/[q_t] = k_2 t + 1/[q_{t0}]$$

Where, q_t = concentration of pesticide at time t ; q_{t0} = initial concentration of the pesticide

k_2 = second order rate constant k_2

The half life of the pesticides was calculated by the following formula:

$$t_{1/2} = 1/k_2\,[qt_0]$$

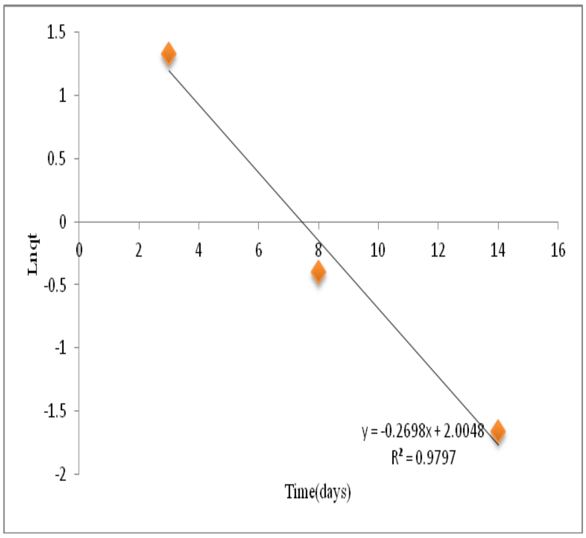

Fig.1: Graph showing the first order plot for imidacloprid on okra fruits

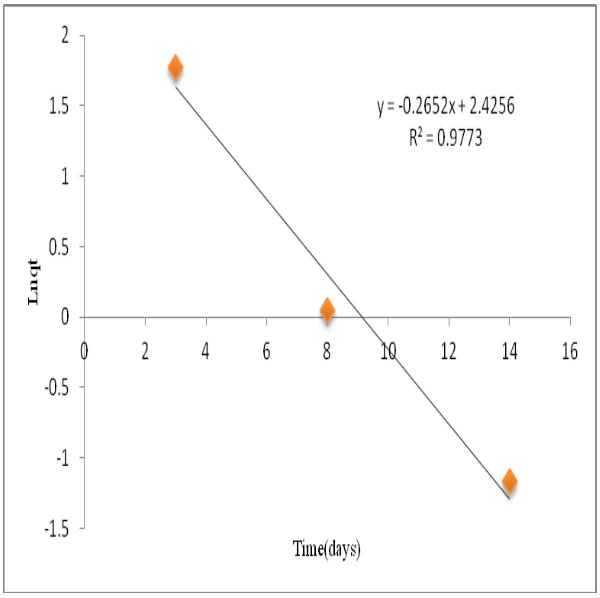

Fig.2: Graph showing the second order plot for dimethoate on okra fruits

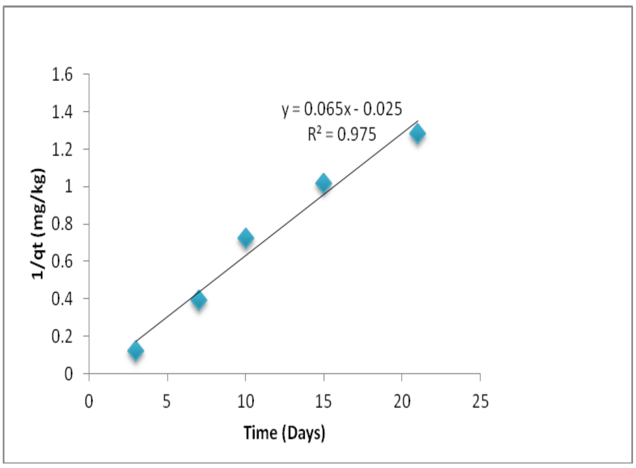

Fig. 3: Graph showing the second order plot for imidacloprid residues on yard long beans

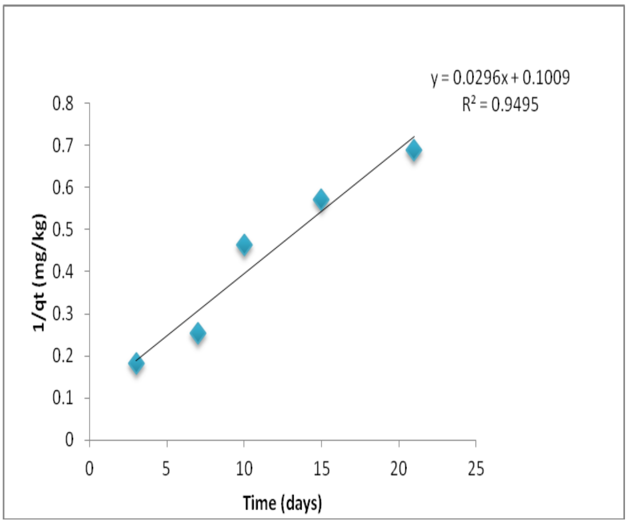

Fig. 4: Graph showing the second order plot for dimethoate on yard long beans

Honnappagouda *et al.* (2014) also studied the residues of imidacloprid formulations in okra fruits from seed treatment and foliar application. In the seed treatment, no detectable residues of imidacloprid were found in the fruits harvested. But the residue persisted upto five days in the fruits in the foliar spray treated plants although the residue became non detectable on the 7th day after application.

A study was done by Akbar *et al.* (2012) to find out the degradation of three conventional pesticides (Imidacloprid, Endosulfan and Profenofos) and two bio-insecticides (biosal and spinosad) sprayed on okra crop. The insecticide residues were analyzed in the leaf and fruit after 0, 1, 3 and 7 days using high performance liquid chromatography. First order degradation kinetics was fitted on this data and degradation rate constants and half life were also calculated. Conventional insecticides were found to be more persistent in the crop (Average half life: 1.95, 2.42 and 1.57 days for imidacloprid, endosulfan and profenofos respectively) than bioinsecticides (Average half life 1.25 and 0.27 days for spinosad and biosal respectively). Residues of all the tested insecticides were compared with codex and EU MRLs and found that both the bio-insecticides treated crops are safe for human consumption even after few hours of spray.

Banerjee *et al.* (2012) observed that imidacloprid followed concentration-dependent first-order kinetics, when its persistence was studied in okra, brinjal and tomato fruits, where the degradation constant varied between –0.21 and –0.34 day^{-1} and the half-life varied between 1.98 and 3.3 days.

In another study carried out by Abassy *et al.* (2014) the residues of imidacloprid and tetraconazole was analysed in cucumber fruits. The estimation was done by High Performance Liquid Chromatography equipped with diode array UV detector. The initial deposit was 0.943 mg/kg after one hour of application, while the detected residue amounts were 0.365, 0.271, 0.226, 0.086. 0.049, 0.028 and 0.013 mg/kg of imidacloprid after 1, 3, 5, 8, 11, 15, and 21 days of treatment respectively. Data also revealed that the first day following application is critical in the sense of sharply decrease that reach 62.29 % from the initial deposit of imidacloprid on cucumber fruits but the loss of tetraconazole at first day was 40.41% from the initial residue.

In a study by Mukherjee and Gopal (2000), imidacloprid was applied to three vegetable crops (eggplant, cabbage leaves and mustard leaves) at 20 and 40 g AI ha^{-1} . The persistence of the parent insecticide and its translocation along with the quantification of the metabolites formed on these crops were observed. The parent insecticide dissipated with a half life of 3-5 days and persisted longer on the mustard leaves. The detectable limit of the HPLC method was 0.01 µg g^{-1} .

In the study conducted, the level of residue of the pesticides was higher in the yard long beans compared to okra. As the vegetable crops are grown in similar environmental conditions simultaneously, the physical factors affecting the persistence of these pesticides in the vegetables are the same. The persistence of either imidacloprid or dimethoate with respect to different vegetables provide a clue to the involvement of non enigmatic antioxidants rather than enzyme mediated reactions that pesticides undergo in plants, which is in accordance with the study by Pico and Kozmutza (2007) which concluded that persistence is enhanced by the presence of anti oxidants. The systemic or non systemic character of the different chemicals studied or their hydrophilic- lipophilic nature may also control the penetrability of applied agrochemicals in the fruit tissues (Abassy *et al*., 2014).

The degradation and residues of imidacloprid and dimethoate from the vegetable samples occurred as the initial deposits and residues at different intervals are influenced by the evaporation of surface residue which is dependent on temperature conditions, biological dilution, which is dependent on the increased mass of fruits, chemical or biochemical decomposition, metabolism and photolysis as suggested by Abassy *et al.* (2014). Rapid dissipation of applied pesticides is dependent on a variety of environmental factors such as sunlight and temperature. Light is a major factor and it plays an important role in the behaviour of pesticides in environment (Lichtenstein, 1972; Zepp *et al*., 1977).

The difference in the initial deposits of both pesticides on the vegetable samples may be mainly due to the ratio of surface to mass area and character of the treated surface, whether it is smooth or rough and waxy or non waxy as suggested by El-Ghar and Ramadan (2004).

The present study shows that the degradation of the pesticides on yard long beans follows a second order kinetics while that on the okra fruits follow first order kinetics. The residue of pesticides in food commodities has an adverse effect on the human health, therefore it is necessary that strict guidelines and instructions should be followed by the farmers while applying the chemicals on the crops.

Therefore, it can be concluded that the after the experimental period, the concentration of pesticide residue was below the maximum residue limit and the acceptable daily intake values as previously set. Thus the period of twenty one days for yard long beans and 14 days for okra is a safe interval between the spraying of pesticides (i.e imidacloprid and dimethoate) and harvest of the fruits for human as well as animal consumption.

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