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QUANTIFYING RESIDUES OF INSECTICIDE APPLIED FOR MANAGEMENT OF BRINJAL SHOOT AND FRUIT BORER

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Abstract
The experiment was undertaken to detect and quantify carbosulfan residues on brinjal fruit with comparison with maximum residue level (MRL) recommended by Food and Agricultural Organization (FAO). Carbosulfan was applied at the recommended rate of 1.5 ml·L^{-1} and the elevated rate of 3.0 ml·L^{-1} of water under tropical conditions. Samples were collected at different days after treatment (DAT) to determine presence, and degradation of, residues. A residue above the MRL was detected up to 3 DAT for the 1.5 ml·L^{-1} rate and 10 DAT for 3.0 ml·L^{-1} rate. Degradation shortly after application proceeded more slowly, but the rate of degradation increased over time. Carbosulfan is still legal for use on brinjal and it should not be applied above 1.5 ml·L^{-1} and the pre-harvest interval should not be less than 7 days.

Key Words: Carbosulfan; Insecticide; *Solanum melongena*; Residual effect.

Introduction

Brinjal (*Solanum melongena* L.) is grown under tropical conditions, but it is susceptible to attack by insect pests requiring frequent application of insecticides [1]. Insecticides benefited agricultural development [2]. Overuse of pesticides can lead to short, or long, term contamination of air, water and food.

Pesticide residue in food has become a safety issue and consumers have the right to know how much pesticide is present in, or on, food. Detection, identification and quantification of pesticides in food are necessary. There is little information are available on presence of pesticides in vegetables in Bangladesh [3]. Growers of brinjal in Bangladesh mostly depend on chemical insecticides for crop production. Producers use a wide range of carbamate, organophosphate and synthetic pyrethroid in various formulations against brinjal shoot and fruit borer (SFB) [4,5]. Carbamate insecticides have been reported effective in reducing incidence of the pest at all stages of crop growth [6].

In the previous experiments, the potential of carbosulfan has been proven for SFB management and has been incorporated into the effective IPM practice [7]. Although the practice is to use carbosulfan based on SFB monitoring results, its use may leave substantial amounts of residue on the crop. Insecticides can be toxic to human, and the possibility exists for residue to accumulate in the environment [8]. There are recommended Maximum Residues Limit (MRL) based on the Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI) of almost all pesticides [9, 10] that should not be exceeded on food items. This experiment was undertaken to determine residue of carbosulfan in brinjal based on dose over time and to recommend the pre-harvest interval for carbosulfan application in brinjal.

Materials and Methods

Experimental location

The experiment was conducted from July to December, 2007 at the experimental farm of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh.

Experimental materials

The small fruited cv. Chega of brinjal was used. The seeds of brinjal were collected from the Horticultural Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.

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Production procedures

The collected seeds were directly sown in the nursery seed bed of the experimental farm of BSMRAU. Cow dung and other chemical fertilizers were applied as recommended by Rashid [11] for brinjal cultivation @ 15 tons of cow dung, and 115, 60 & 75 kg of N, P_2O_5 & K_2O, respectively per hectare. The half of cow dung and P_2O_5 were applied as basal dose during land preparation. The remaining cow dung, P_2O_5 and one-third N and one-third of K_2O were applied in the pits of main field at transplanting of brinjal seedlings. The entire dose of N and the rest of K_2O were applied as top dressing. Thirty-days-old healthy seedlings were transplanted in the pits of experimental plots considering 3.0 m × 2.0 m plot size maintaining 75 cm × 60 cm plant spacing. The plots were lightly irrigated regularly for ensuring proper growth and development of the seedlings. Subsequent irrigation and other cultural operations were also done.

Experimental treatments

No chemical was applied other than selected carbosulfan insecticide. Plots consisting three rows, of which one row treated with carbosulfan @ 1.5 ml·L^{-1}, another with carbosulfan @ 3.0 ml·L^{-1}, and the remainder one considered as untreated control which was treated only with water. Carbosulfan (commercially available as Marshal® 20 EC formulation) obtained locally was applied with a knapsack sprayer at fruiting stage to wet the fruits completely. Carbosulfan was applied three times at 15 days interval.

Sample collection

Brinjal fruit weighing more than 100 g were harvested at 0, 1, 2, 3, 4, 5, 6 and 7 DAT from the row receiving carbosulfan @ 1.5 ml·L^{-1} and at 0, 2, 5, 7, 10 and 12 DAT from the row receiving carbosulfan @ 3.0 ml·L^{-1} and placed in separate labeled bags. Samples were stored at 4°C until testing.

Extraction and separation of Carbosulfan

From each sample a 100 g sub-sample was ground separately with a meat grinder (Handmixer M-122, Bamix, Switzerland), and 20 g was placed in a wide mouth jar with 100 mL of hexane. Sodium sulphate (Na_2SO_4) was added until water was removed from the sample. The mixture was macerated with a high-speed homogenizer (model IKA T18, Ultraturax, Belgique, Germany) for 2 min. The homogenized material was then poured into a 250 ml conical flask and placed on a shaker (Orbital Shaking Incubator, Rexmed, Sweden) for 12 hrs. After shaking, the slurry was filtered through a Buchner funnel with suction with filter paper. The flask and filter cakes were rinsed with 25 ml of hexane. The filtrate was then transferred into 250 ml round bottom flask and dried to 3-5 ml by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). Ten-ml of the concentrated filtrate was transferred to a 500 ml separatory funnel. Twenty-ml of methane was added to and the mix shaken magnetic stirrer vigorously for 3-5 min. After shaking, the separatory funnel was set on 'A' stand and left undisturbed for 3-5 min. The clear part of the solution from the bottom of the separatory funnel was collected in vial, which was centrifuged at 1200 rpm for 5 min (Laboratory Centrifuges, Sigma-3K30, Germany). The supernatant was collected, labeled, and preserved for injection.

Recovery Test

A GC-MS QP2010 (Shimadzu, Kyoto, Japan) was used for analysis of carbosulfan. Prior to injection of the sample extract, a recovery test was carried-out using a standard of carbosulfan obtained from Sigma-Aldrich Laborchemikalien (Seelze, Germany). The purity of the standard was >99.6%. Standard solutions of concentrations of carbosulfan were prepared and injected into the GC-MS. Samples were calibrated against standard solutions of carbosulfan. Peaks were characterized by retention time. The electron ionization mode was used. Parameters of the capillary column used were: AT-1, length 30m, ID 0.25mm and film thickness 0.25µm. Helium was used as carrier the instrument parameters for detecting carbosulfan were provided by the manufacturer.

Detection and Quantification of pesticide residue in samples: For detection and quantification of carbosulfan the supernatant collected for each sample was injected @ 2 ml per injection. Each sample was compared with the calibration curve and the concentration in each sample obtained by back calculation.

The rate of degradation was calculated with the following formula:

$$\% \text{ degradation} = \frac{\text{Residue at 0 DAT - Residue at sampling DAT}}{\text{Residue at 0 DAT}} \times 100$$

Data analysis: The recorded data were analyzed using the MSTAT-C [12] software and the means were separated by Duncan's Multiple Range Test to determine the level of significance.

Results and Discussion

Residues of carbosulfan at single dose and double doseThe estimated residue level of carbosulfan obtained from samples treated with levels of carbosulfan decreased significantly over time (Table 1 and 2). The fruit treated with 1.5 ml L^{-1} as single dose, the residues of carbosulfan were detected from 0 to 7 days after

treatment (DAT) and quantities ranged from 0.882 to 0.076 mg kg^{-1} (Table 1). Among which, the quantities from 0 to 3 DAT ranged from 0.882 to 0.299 mg kg^{-1}, which were over MRL, where FAO recommended MRL (Maximum Residue Limit) of Carbosulfan was 0.20 mg kg^{-1} crop. The residue of 0.882 mg kg^{-1} found at 0 DAT was degraded to 0.799 mg kg^{-1} at 1 DAT and up to 0.076 at 7 DAT. The quantities from 4 to 7 DAT ranged from 0.104 to 0.076 mg kg^{-1} were below the MRL.

Table 1. Carbosulfan residue from brinjal treated with 1.5 mL ha^{-1}

Days after treatment	Residue of carbosulfan (mg kg^{-1})
0	0.882 a
1	0.799 b
2	0.639 c
3	0.299 d
4	0.104 e
5	0.083 ef
6	0.078 f
7	0.076 f
$LSD_{0.01}$	0.024
CV (%)	3.00

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

Table 2. Carbosulfan residue from brinjal treated with 3 mL ha^{-1}

Days after treatment	Residue of carbosulfan (ppm)
0	5.07 a
2	2.44 b
5	2.06 c
7	1.60 d
10	0.28 e
12	0.004 f
$LSD_{0.01}$	0.025
CV (%)	0.77

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.01 level of probability

The fruit treated with 3 ml L^{-1} as double dose, the residue level was highest (5.07 mg kg^{-1}) at 0 DAT that was much higher than that of FAO recommended MRL and gradually decreased until 12 DAT (0.004 mg-kg^{-1}), which was below MRL. At 10 DAT, the residue (0.28 mg kg^{-1}) was higher than MRL (Table 2).

Degradation of carbosulfan over time

The carbosulfan used in the trial was degraded significantly over time (Fig. 1 and 2). The fruit treated with 1.5 ml L^{-1}, the rate of degradation detected from 0 to 7 DAT ranged from 0.00 to 91.37% (Fig. 1). Among which, rate of degradation was slower from 0 to 2 DAT, but increased later gradually. In case of fruit treated with 3.0 ml L^{-1}, the rate of degradation detected from 0 to 12 DAT ranged from 0.00 to 99.90% (Fig. 2). Among which, the rate of degradation (0.00 to 51.73%) was rapid from 0 to 2 DAT and increased later gradually.

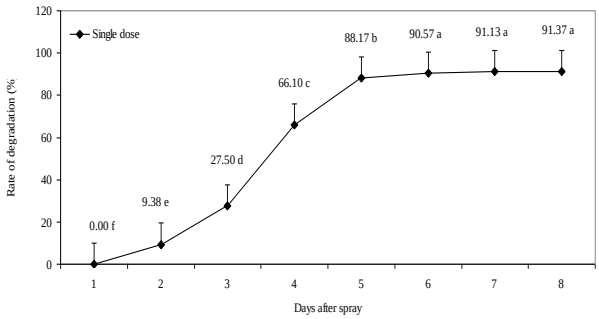

Figure 1. Degradation rate of carbosulfan 20 EC @ 1.5 $mL.^{-1}$ of water at different days after spray

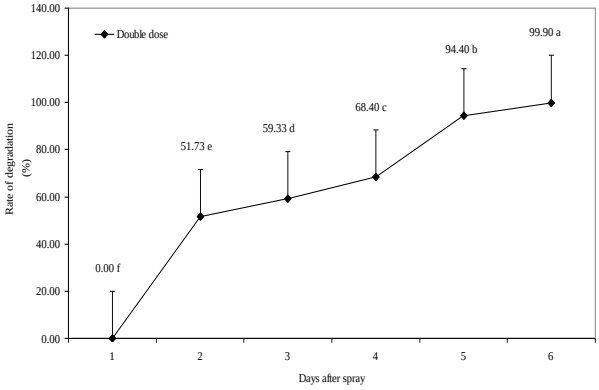

Figure 2. Degradation rate of carbosulfan 20 EC @ 3.0 $mL.^{-1}$ of water at different days after spray

About similar trend of carbosulfan degradation was reported by others. Trevisan *et al.* [1] reported that the residue level of carbosulfan in citrus decreased rapidly and could not be detected in samples after 7 DAT. Varca *et al.* [13] reported that in rice leaves carbosulfan residue was not found after 7 DAT. Rajeswaran *et al.* [14] reported that the residue of carbosulfan in brinjal was below the MRL at the first harvest when it was sprayed at the recommended dose of 250 g a.i. ha^{-1}. From this study it is revealed that carbosulfan is still legal for use on brinjal and it should not be applied above 1.5 ml L^{-1} and the pre-harvest interval should not be less than 7 days.

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