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Study on the Development of Resistance in Brinjal Shoot and Fruit Borer against Different Insecticides

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Abstract: A Brinjal Shoot and Fruit Borer (BSFB) population was collected from Jessore and Gazipur district of Bangladesh tested in laboratory against insecticides used intensively in those areas in the department of Entomology, BSMRAU, Gazipur. This stock of BSFB served as the source of reference population. BSFB used in the present study were all 2nd instar larvae. Four bioassay methods such as leaf round, petridish, leaf containing petridish and Leaf disc containing petridish were used under laboratory condition for concentration - mortality test of BSFB. Considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best in estimating the toxicity of Marshal 20 EC, Ripcord 10 EC and Basathrin 10 EC for Jessore and Gazipur region. The concentrations 0.1 were selected discrimination of concentration (DC) of Marshal 20 EC, Ripcord 10 EC and Basathrin 10 EC for Jessore and Gazipur districts. The observed mortalities in those concentrations were 73.33%, 80.00%, 73.33%, 73.33% and 73.33%, 80.00%, respectively for Marshal, Ripcord and Basathrin in Jessore and Gazipur districts. The percentage of resistance was higher (18.19%) to Marshal 20 EC in Jessore BSFB population than in Gazipur (11.52%).

Key word: Egg plant · Insect · Resistance · BSFB · Insecticide

INTRODUCTION

Brinjal Shoot and Fruit Borer (BSFB), *Leucinodes orbonalis* Guen, is the most serious pest of brinjal, an important commercial vegetable crop in Bangladesh [1]. Insect larvae bore into tender shoots and fruits; shoots wilt reducing plant vigor and fruits become unfit for human consumption. Farmers in Asia use large amount of chemical insecticides to control the pest in its larval stage. Without adequate pesticides residue on the plant, however, the newly hatched larvae survive and crawl to the nearest shoot or fruit and once inside, are safe from pesticide spray. This leads farmers to spray chemicals frequently. This excessive use of pesticide has resulted in economic, environmental and human health problems, without resulting in adequate control of the pest.

The yield loss caused by this pest has been estimated up to 67% in Bangladesh [2]. Farmers of Bangladesh particularly of intensive brinjal growing areas like Jessore apply insecticides 84-140 times in a growing season [3]. Pesticide resistance is emerging as one of the key constraints to successful crop protection and public health problems worldwide [4]. Although the resistance is a natural mechanism for survival, its development has been accelerated in recent year due to excessive dependence upon chemical pesticides. Insecticides resistance in BSFB especially to pyrethroids is now widespread in many brinjal-producing countries. High levels of resistance to many organocarbamate and pyrethroids have been reported in field population of BSFB in various regions [5]. Such assumptions are more evident from reports of great variations in the requirements of pesticides application for BSFB control in different areas. No study has been conducted systematically with the fixed populations of different brinjal growing areas.

The timely implementation of a resistance management program may help delay the development of insecticide resistance and provide time for a comprehensive and effective pest management program.

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Resistance detection is the vital components of pesticide resistance management. It aims to identify the initial presence of resistant individuals in a pest population. According to Brent [6] and Dennehy *et al*., [7], the practical resistance detection must give emphasis on the establishment of baseline toxicity using a reliable bioassay method. Ideally, several bioassay methods should be used for establishing the baseline toxicity of a pesticide and for resistance testing, as the methods often serve different aims and the precision of response estimates often varies between methods. Therefore, considerable assessment on the reliability and precision of mortality estimates is required before any particular method is selected. Considering the above perspective the present experiment was designed to evaluate four bioassay methods of different insecticides against BSFB and to detect insecticide resistance in BSFB population collected from major brinjal growing areas of Bangladesh.

MATERIALS AND METHODS

Two Brinjal Shoot and Fruit Borer (BSFB) population, one each from Jessore and Gazipur, were collected from brinjal field. In fact, for the purpose, the infested brinjal fruits from Jessore (23°10'N, 89°10'E) and the most intensively growing areas and from Gazipur (23° 48'N, 90°39'E) the least intensive growing areas were collected. The experiment was conducted in Jessore in the month of May, June and July, 2006 whereas in Gazipur in the month of July, August and September, 2006. The larvae were collected from the farmers' field. Most of the larvae were found in the tender twigs of brinjal and the twigs were cut with the help of sharp scalpel. Fresh excreta on the opening indicate the presence of 2^{nd} or 3^{rd} instars larvae. The brinjal leaves were always collected from unsprayed plot. The stalk of the leaves was cut with a knife and then the leaves were dipped in discriminating concentration (0.3%, 0.2%, 0.1%, .05% and 0.04%) of the tested insecticides and dried. Thus, the leaves of the brinjal were made ready for use.

Procedure of Testing Resistance: The selected discriminating concentrations (0.1% and 0.2%) of three different insecticides (Marshal 20 EC, Ripcord 10 EC and Basathrin 10 EC) were tested against field collected larvae of BSFB of different locations (i.e. Jessore and Gazipur) of Bangladesh.

Test Procedure: Four bioassay methods such as leaf round, petridish, leaf containing petridish and leaf disc containing petridish were used under laboratory condition for concentration - mortality test of BSFB. At least five concentrations for each of the selected insecticides were tested using different methods. In each method and insecticide at least 5 larvae of 2nd instar were released at each concentration in 3 replications.

Leaf Round Method: Fresh brinjal leaves were collected from unsprayed plots and were dipped in required concentration of the tested insecticides and then air-dried. After drying, leaves were cris-crossly placed in the Petridish then five 2^{nd} instar larvae were transferred on the treated surface which were then rounded and closed up at the exposure end with thread. The mortality was observed at 12 hr exposure period.

Petridish Method: Clear glass Petridish of 5 cm diameter was used in this method. The Petridishes were first cleaned with cleaning agent and then air- dried. Again the Petri dishes were sterilized in an oven at 120°C for one hour. Then they were rinsed with 2ml solution of required concentration of tested insecticide with solvent xylene and then air-dried. Five 2^{nd} instar larvae of BSFB were transferred into each Petridish, which were then kept at room temperature on clean bench. The mortality was observed at 12hr exposure.

Leaf Containing Petridish Method: Freshly collected leaves of brinjal were dipped in the required concentration of the tested insecticides and then air-dried. Insecticides treated leaves were cut into a size of 5 cm diameter and placed inside clear glass petridish. Both parts of petridish *were* covered with leaves. Five 2^{nd} instar larvae were then released on the leaves and data on mortality was recorded after 12hr exposure period.

Leaf Disc Containing Petridish Method: Fresh brinjal leaves were collected from unsprayed plots and then dipped into the required concentrations of tested insecticides and then air-dried. The treated leaves were cut into disc (1.5 cm diameter) with the help of sterilized cork borer. The leaf disc was kept in a petridish whose surfaces were also treated by test insecticide. A piece of cloth was soaked in the insecticidal solution and allowed to air dry. Five 2^{nd} instar larvae were then released on the leaves. Then the open top of the petridish was closed with a piece of cloth and its lid on it. Data on mortality were recorded after 12-hr exposure period.

LC_{50} Calculation: The LC_{50} of each insecticide for each of 4 different methods has been calculated by Probit Analysis by using the MSTAT program developed on the basis of D.J. Finney's [8] method. The calculation of LC_{50} by the program required only the doses used, number of insects used in each dose, number of dead insect in each dose, number of insect used in control and the nature of dead insect in control.

Insecticides Treatments: The insecticides used in the present study were all commercial formulations. The following chart shows the brand name, common name and their mode of action.

Brand name	Chemical family	Common name	Mode of action
Marshal® 20 EC	Carbamate	Carbosulfan	Contact, stomach and systemic
Ripcord® 10 EC	Pyrethroid	Cypermethrin	Contact, stomach and antifeedant
Basathrin® 10 EC	Pyrethroid	Cypermethrin	Contact, stomach and antifeedant

Discriminating Concentration for Mortality Test of Field Collected Larvae: Freshly collected brinjal leaves were treated with the selected DC of different insecticides. Two treated leaves of brinjal were placed cris-crossly and five 2nd inster larvae were placed on it. Then the leaves were made round and the open end was closed and tied with thread. They were kept at room temperature. After 12 hrs of exposure, the mortality of the larvae was counted. In each selected location, 100 larvae for a single insecticide in 3 replications were tested. Thus, 300 larvae for three insecticides (Marshal, Ripcord and Basathrin) in each location were tested to find out the resistance severity in the selected area.

The occurrence of resistance in the field population, if any, was determined by the following formula according to Roush and Miller (1986): Resistance in field population (%) = % Mortality of laboratory population at DC - % Mortality of field population at D.C.

RESULTS AND DISCUSSION

Mortalities of Brinjal Shoot and Fruit Borer are presented under the following sub heading for different insecticides such as Marshal 20 EC, Ripcord 10 EC and Basathrin 10 EC for the location of Jessore and Gazipur districts.

Toxicity of Marshal 20 EC Against BSFB Using Different Bioassay Methods at Jessore Region: The estimated LC_{50} values of Marshal 20EC for Jessore region were 0.002%, 0.005%, 0.003%, 0.007% in leaf round, leaf containing petridish, leaf disc + cloth and petridish methods, respectively (Table 1). The χ^2 value was the lowest in case of leaf round method. Marshal® 20EC in Jessore region was found to exert higher toxicity against BSFB larvae through leaf round method (Table 1). Considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value through the leaf round method was found to be the best estimating the toxicity of Marshal® 20EC for Jessore region. Emphasis has been given on the importance and necessity of selecting an appropriate bioassay method for resistance monitoring by several workers [9, 10]. In the present study the different bioassay method were evaluated and among them leaf round method was found to be the best for estimating the toxicity of Marshal® 20EC for Jessore region.

Toxicity of Marshal 20 EC Against BSFB Using Different Bioassay Methods at Gazipur Region: The estimated LC_{50} values of Marshal® 20EC for Gazipur region were 0.002%, 0.004%, 0.006%, 0.007% in leaf round, leaf containing petridish, leaf disc + cloth and petridish methods, respectively (Table 2). The χ^2 value was the lowest in case of leaf round method (2.33). Marshal® 20 EC in Gazipur region was found to exert higher toxicity against BSFB larvae in leaf round method. In Gazipur region considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, by leaf round method was found to be the best for estimating the toxicity of Marshal 20 EC among the other tested methods. Dennehy *et al*. [7] reported that bioassay method having food sources of tested insects estimated mortalities with higher precision than solid media contained bioassay method.

Toxicity of Ripcord 10 EC Against BSFB Using Different Bioassay Methods at Jessore Region: The estimated LC_{50} values of Ripcord® 20 EC for Jessore region were 0.003%, 0.003%, 0.006%, 0.010% by leaf round, leaf containing petridish, leaf disc + cloth and petridish methods, respectively (Table 3). The χ^2 value was the lowest in case of leaf round method. Ripcord® 10 EC in Jessore region was found to exert higher toxicity against BSFB larvae through leaf round method (Fig. 1). Considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best for estimating the toxicity of Ripcord 10EC for Jessore region. Leaf containing petridish method also showed the similar results in respect of estimating the toxicity comparing the leaf round method. Leaf round method did not allow

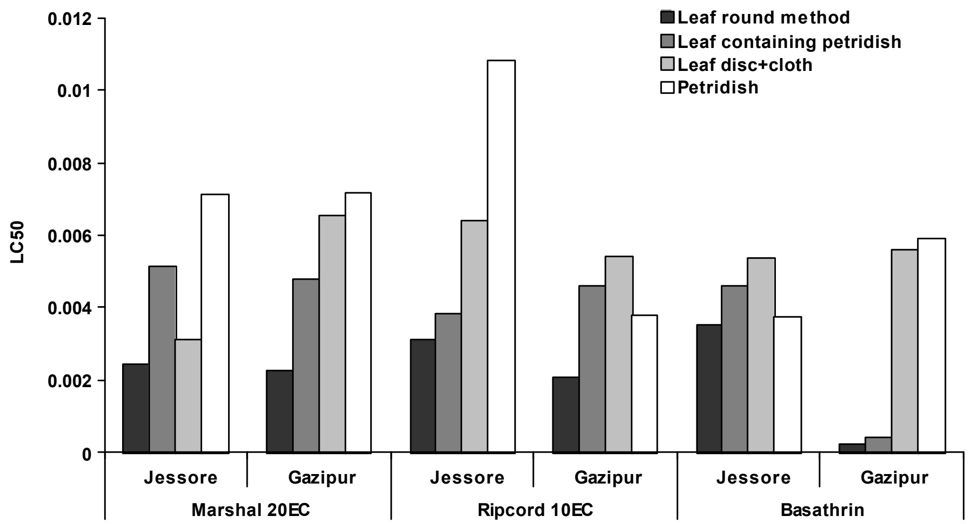

Fig. 2: LC_{50} values for different chemicals estimated through different bioassay methods against BSFB populations from Jessore and Gazipur

Table 1: Estimated LC_{50} values of Marshal® 20 EC against BSFB by using different bioassay method

Method	χ^2	D.F.	Equation	Probability (P)	LC_{50}
Leaf round method	2.12	4	Y=8.12+3.84	0.99	0.002
Leaf containing petridish	3.12	4	Y=3.34+1.12	0.99	0.005
Leaf disc+cloth	2.65	4	Y=5.78+2.03	0.99	0.003
Petridish	4.12	4	Y=11.05+4.61	0.99	0.007

Table 2: Estimated LC_{50} values of Marshal® 20 EC against BSFB by using different bioassay method

Method	χ^2	D.F.	Equation	Probability (P)	LC_{50}
Leaf round method	2.33	4	Y=4.77+1.47	0.99	0.002
Leaf containing petridish	5.51	4	Y=8.15+3.01	0.98	0.004
Leaf disc+cloth	4.84	4	Y=7.54+2.03	0.99	0.006
Petridish	3.45	4	Y=2.96+1.45	0.99	0.007

Table 3: Estimated LC_{50} values of Ripcord® 10EC against BSFB by using different bioassay method

Method	χ^2	D.F.	Equation	Probability (P)	LC_{50}
Leaf round method	2.52	4	Y=2.73+1.01	0.99	0.003
Leaf containing petridish	3.38	4	Y=5.21+2.44	0.99	0.003
Leaf disc+cloth	5.14	4	Y=9.02+4.63	0.99	0.006
Petridish	4.78	4	Y=7.33+3.29	0.99	0.010

Table 4: Estimated LC_{50} values of Ripcord 10EC against BSFB by using different bioassay method

Method	χ^2	D.F.	Equation	Probability (P)	LC_{50}
Leaf round method	4.14	4	Y=2.39+1.01	0.99	0.002
Leaf containing petridish	5.33	4	Y=6.28+3.39	0.99	0.004
Leaf disc+cloth	6.12	4	Y=7.96+3.84	0.99	0.005
Petridish	2.32	4	Y=2.45+1.21	0.99	0.003

BSFB larvae to go away from treated surfaces. Thus, reliable mortality estimates was likely to get from leaf round method.

Toxicity of Ripcord 10 EC Against BSFB Using Different Bioassay Methods at Gazipur Region: The estimated LC_{50} values of Ripcord® 10EC for Gazipur region were 0.002%,

Table 5: Estimated LC_{50} values of Basathrin 10EC against BSFB by using different bioassay method

Method	χ^2	D.F.	Equation	Probability (P)	LC_{50}
Leaf round method	3.15	4	Y=7.66+3.10	0.99	0.003
Leaf containing petridish	6.38	4	Y=2.89+1.84	0.99	0.004
Leaf disc+cloth	4.39	4	Y=6.28+3.52	0.99	0.005
Petridish	3.22	4	Y=9.38+4.52	0.99	0.003

Table 6: Estimated LC_{50} values of Basathrin® 10EC against BSFB by using different bioassay method

Method	χ^2	D.F.	Equation	Probability (P)	LC_{50}
Leaf round method	3.31	4	Y=3.25+1.99	0.99	0.0002
Leaf containing petridish	4.38	4	Y=7.30+3.66	0.99	0.0004
Leaf disc+cloth	3.54	4	Y=4.01+2.70	0.99	0.0055
Petridish	7.11	4	Y=12.19+5.52	0.99	0.0058

0.004%, 0.005%, 0.003% in leaf round, leaf containing petridish, leaf disc + cloth and petridish methods, respectively (Table 4). The χ^2 value was the lowest in case of petidish method. Ripcord 10EC in Gazipur region was found to exert higher toxicity against BSFB larva in leaf round method but the $\chi2$ value was the lowest by petridish method. In Gazipur region considering the variation of mortality estimates, control mortality, the lowest LC_{50} value, the leaf round method was found to be the best for estimating the toxicity of Ripcord 10EC among the petrish methods and than the other tested methods. Tabashink and Cushing (1987) reported that bioassay method having food sources of tested insects estimated mortalities with higher precision than solid media contained bioassay method.

Toxicity of Basathrin® 10 EC Against BSFB Using Different Bioassay Methods at Jessore Region: The estimated LC_{50} values of Basathrin® for Jessore region were 0.003%, 0.004%, 0.005%, 0.003% in leaf round, leaf containing petridish, leaf disc + cloth and petridish methods, respectively (Table 5). The χ^2 value was the lowest in case of leaf round method. Basathrin® 10 EC in Jessore region was found to exert higher toxicity against BSFB larvae in leaf round method among the tested methods (Figure 1). Considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best for estimating the toxicity of Basathrin® 10EC for Jessore region which also closely followed by the petridish method under the present experimental condition. Several workers [9, 10] gave emphasis on the importance and necessity of selecting an appropriate bioassay method for resistance monitoring.

Toxicity of Basathrin® 10 EC Against BSFB Using Different Bioassay Methods at Gazipur Region: The estimated LC_{50} values of Basathrin® for Gazipur region were 0.002%, 0.004%, 0.005%, 0.005% in leaf round, leaf containing petridish, leaf disc + cloth and petridish methods, respectively (Table 6). The χ^2 value was the lowest in case of leaf round method. Basathrin® 10EC in Gazipur region was found to exert higher toxicity against BSFB larvae in leaf round method. In Gazipur region considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best for estimating the toxicity of Basathrin® 10EC among the other tested methods.

Considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best for estimating the toxicity of Marshal® 20EC for Jessore and Gazipur region. Considering the variation in mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best for estimating the toxicity of Basathrin® 10EC for Jessore and Gazipur region. Leaf containing petridish method also showed the similar results in respect of estimating the toxicity comparing the leaf round method. Considering the variation of mortality estimates, control mortality, the lowest LC_{50} value and the lowest χ^2 value, the leaf round method was found to be the best for estimating the toxicity of Basathrin® 10EC for Jessore and Gazipur which also closely followed by the petridish method.

Selection of Discriminating Concentration (DC) for Testing Resistance: The results of the concentration-mortality estimates of BSFB against 3 different

Table 7: Selected discriminating concentrations (DC) of different insecticides for testing resistance in Jessore and Gazipur districts

Insecticide	Concentration (%)	% Mortality ± SE Laboratory	Jessore	Gazipur	Selected DC (%)
Marshal® 20EC	0.1	91.52±10.62	73.33±7.22	80.00±5.78	0.1
	0.2	94.00±11.34	80.00±5.82	86.67±9.29	
Ripcord® 10EC	0.1	89.65±9.87	73.33±8.47	73.33±10.22	0.1
	0.2	92.35±9.95	86.67±9.46	80.00±11.81	
Basathrin® 10EC	0.1	80.61±9.41	73.33±8.22	73.33±5.78	0.1
	0.2	83.65±10.04	80.00±10.35	80.00±9.22	

Table 8: Intensity of insecticide resistance in different population of BSFB

Insecticides	Population	Mortality (%)	Resistance (%)
Marshal® 20EC	Laboratory reared	91.52	--
	Jessore	73.33	18.19
	Gazipur	80.00	11.52
Ripcord® 10EC	Laboratory reared	89.65	--
	Jessore	73.33	16.32
	Gazipur	73.33	16.32
Basathrin® 10EC	Laboratory reared	80.61	--
	Jessore	73.33	7.28
	Gazipur	73.00	7.61

insecticides using 4 bioassay methods clearly indicated the superiority of Leaf round method for lower control mortality, lower SE, higher toxicity of insecticide and better performance in providing homogenous condition of the test. Leaf round method was thus chosen for testing resistance, with discriminating concentration. For selecting discriminating concentration for tested insecticides, two concentrations (0.1 and 0.2%) for each insecticide, which caused 70-95% mortalities in bioassay tests, were selected for DC selection test.

As shown in Table 7, the concentrations of 0.1% for each of Marshal, Ripcord and Basathrin provided lower variability in mortality estimates. Thus, the concentration 0.1% was selected DC for Marshal® 20EC, Ripcord® 10EC and Basathrin® 10EC under laboratory condition, Jessore and Gazipur districts. The observed mortalities at this concentration were 91.52%, 73.33% and 80.00%; 89.65, 73.33% and 73.33%, 80.61% 73.33% and 80.00%, respectively for Marshal, Ripcord and Basathrin in laboratory condition, Jessore and Gazipur districts.

Occurrence and Distribution of Insecticide Resistance in BSFB: Among the population of different district, Jessore population always had higher percentages of resistance (i.e. 18.19%, 16.32% and 7.28%, respectively to Marshal® 20EC, Ripcord® 10EC and Basathrin® 10EC). In case of Gazipur population, the percentage of resistance to Marshal 20EC, Ripcord 10EC and Basathrin 10EC was 11.52%, 16.32% and 7. 61%, respectively. A comparison of resistance among the insecticides showed higher level of resistance to Marshal followed by Ripcord and Basathrin (Table 8).

Of the available techniques, the discriminating test is regarded as one of the most useful and cost-effective tools for monitoring pesticide resistance in pests. Roush and Miller [11] compared DC and multiple concentration techniques and concluded that the DC test was more efficient for resistance monitoring as it accurately distinguished between resistance and susceptible populations. An important consideration for using a DC in monitoring resistance is the interpretation of resistance. The commonly used approach involves classification of the tested strains as resistant and susceptible following some criteria [12] or statistical comparison of mortalities of tested strains with the susceptible [13] or categorization of tested strains simply by percentage survival at a DC [14].

In the present study, 7.28% to 18.19% resistance to three insecticides belonging to carbamate and pyrethroid population was found in populations of BSFB from Jessore and Gazipur. The presence of such cross or multiple resistances in BSFB from Jessore population was likely since farmers of Jessore area sprayed brinjal fields every day or even twice a day with a variety of insecticides [15]. According to the findings of Ramakrishnan *et al*., [16] where they collected *Spodoptera litura* populations from agricultural fields and showed that *S. litura* larvae were 5.73 fold resistant to Malathion, 14.73 fold to Pyrethrum, 16.25 fold to Lindane and 85.91 fold to Endosulfan. Recently, a high degree of resistance to Pyrethroids (Cypermethrin, Fenvelerate and Deltamethrin) and an Organophosphate (Quinalphos) has been reported in this pest from different parts of India [17]. Under the above situation, it is assumed that the development of insecticide resistance in insect pests is likely to be the common happening in Bangladesh. Due to resistance, pest management program is likely to suffer and to some extent would be ineffective resulting serious reduction of crop production. It is therefore, necessary to undertake further studies towards recognition and monitoring of resistance in BSFB and other insects that are exposed very frequently to high selection pressure of insecticides.

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