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Bioassay of insecticides against okra leafhopper *Amrasca biguttula biguttula* (Ishida)

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ABSTRACT :

Okra [*Abelmoschus esculentus* L. (Moench)] also known as lady's finger is an important vegetable crop valued for its immature, tender and green fruits in India.One of the major bottlenecks in successful production of okra is the damage caused by early season sucking pests and fruit borers. Among the sucking pests leafhoppers (*Amrasca biguttula biguttula)* is undoubtedly more severe and destructive on okra during early stage of the crop. At present, most of the commonly used insecticides are not able to suppress its population below economic thresholds probably because of development of resistance. Among the different insecticides tested for bioassay under laboratory conditions, thiamethoxam 25 WDG at 0.2 g per litre and flonicamid 50 WG at 0.3 g per litre showed the highest mortality and was followed by fipronil 5 SC at 1 ml per litre, dinotefuran 20 SG at 0.2 g per litre and acetamiprid 20 SP at 1 g per litre which proved to be superior over imidacloprid 17.8 SL at 0.3 ml per litre, diafenthiuron 50 WP at 1 g per litre, lamda-cyhalothrin 5 EC at 0.5 ml per litre, emamectin benzoate 5 SG at 0.2 g per litre, fenpyroximate 5 SC at 1ml per litre and acephate 75 SP at 1 g per litre.The concentration mortality response of nymphs to these chemicals under laboratory was evidenced through leaf dip bioassay and the LC_{50} values for these chemicals were computed. The LC_{50} value of thiamethoxam 25 WDG, flonicamid 50 WG, fipronil 5 SC, dinotefuran 20 SG and acetamiprid 20 SP were 4.03, 4.50, 16.18, 7.60 and 16.40 ppm respectively.The different insecticides which were promising through laboratory were field evaluated and the results revealed that thiamethoxam 25 WDG at 25 g *a. i.* per hectare was found to be effective against the leafhoppers followed by flonicamid 50 WG at 75 g *a. i.* per hectare, fipronil 5 SC at 25 g *a. i.* per hectare, dinotefuran 20 SG at 20 g *a. i.* per hectare and acetamiprid 20 SP at 20 g *a. i.* per hectare. Whereas, acephate 75 SP at 375 g *a. i.* per hectare was least effective against the leafhoppers.

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INTRODUCTION

Okra [*Abelmoschus esculentus* L. (Moench)] also known as lady's finger is an important vegetable crop valued for its immature, tender and green fruits in India. Okra is a warm season vegetable crop and requires a long warm growing season. In India it is grown in summer months and during the rainy season. It is grown over an area of 5.33 lakh hectares with production of 6.34 metric tonnes and productivity of 110 lakh metric tonnes per year in India, whereas in Karnataka it is cultivated over an area of 8,600 hectare with a production of 75.10 thousand tonnes per year (Anonymous, 2014).

One of the major bottlenecks in successful production of okra is the damage caused by early season sucking pests and fruit borers. Among the sucking pests, okra leafhopper, *Amrasca biguttula biguttula* (Ishida); whitefly, *Bemisia tabaci* (Guenn.) and aphid, *Aphis gossypii* (Glover) cause significant damage to the crop.

Among above mentioned insect pests leafhopper (*A. biguttula biguttula)* is undoubtedly more severe and destructive on okra during early stage of the crop. The nymphs and adults suck the plant sap mainly from the lower surface of leaves and cause phytotoxic symptoms known as 'hopper burn' which results in complete desiccation and has become one of the limiting factors in economic productivity reducing the yield to the tune of 54.04 per cent (Chaudhary and Dadeech, 1989). To tackle this menacing sucking pest a number of insecticidal sprays are given, which have led to several problems like presence of residues, elimination of natural enemies, development of resistance and environmental disharmony. To overcome these problems, identification of new molecules is the need of the hour so as to fit them in IPM practices.

MATERIAL AND METHODS

Bioassay of new insecticides against okra leafhopper, *Amrasca biguttula biguttula* (Ishida) under laboratory condition.

Maintenance of stock culture:

Okra seeds of cultivar Arka Anamika were sown in seed bed (2 m×2 m). The seedlings were covered by the nylon cages (45 micron) immediately after germination. Leafhopper nymphs and adults collected from the okra field were released on the caged plants for population build up or multiplication. This stock culture of leafhoppers was used in bioassay studies.

The bioassay method followed for leafhopper was leaf dip bioassay developed and recommended by the Insecticide Resistance Action Committee (IRAC). The experimental set-up consisted of two plastic cups, one serving as the inner test chamber and the other as the outer water reservoir. A small hole was made in the inner cup bottom along the side to insert the petiole of the leaf. The fresh uncontaminated okra leaves (Arka Anamika) were selected and the petiole were cut to a length of approximately four centimeters. The leaves were dipped in the respective insecticidal concentrations for five seconds by holding the petiole end using forceps and then the leaves were shade-dried thoroughly in open air by hanging them vertically.

The petiole of the leaf is passed through the hole in the inner test container and placed inside the outer cup containing water so as to keep it in upright position and to prevent drying. The third instar leafhopper nymphs (or same body sized) were taken from the stock culture and released into the test cup at the rate of 10 per cup using fine camel hair brush and the cup were covered with muslin cloth and secured using a rubber band. Each treatment was replicated thrice with ten insects per replication.

Toxicity of insecticides mentioned in Table A were evaluated against third nymphal instars of *A. biguttula biguttula*.The required concentrations of each insecticide were prepared in distilled water.

Table A : Treatment details of bioassay study

Sr. No.	Treatments	Dosage (per litre)
1.	Flonicamid 50 WG	0.3 g
2.	Acetamiprid 20 SP	1.0 g
3.	Diafenthiuron 50 WP	1.0 g
4.	Fipronil 5 SC	1.0 ml
5.	Lambda-cyhalothrin 5 EC	0.5 ml
6.	Dinotefuran 20 SG	0.2 g
7.	Thiamethoxam 25 WDG	0.2 g
8.	Fenpyroximate 5 SC	1.0 ml
9.	Emamectin benzoate 5 SG	0.2 g
10.	Imidacloprid 17.8 SL	0.3 ml
11.	Acephate 75 SP	1.0 g
12.	Untreated control	-

The observations on the mortality of leafhoppers were recorded at 24, 48 and 72 hours after treatment and the results were expressed as percentage mortality.A control was maintained by dipping the leaf in distilled water alone. The test mortalities were corrected using Abbott's correction (Abbott, 1925) for mortality in the control, if any. The chemicals which showed highest mortality per cents were again considered to carry out bioassay tests. Five concentrations of each effective insecticide was bioassayed with three replications along with untreated control. The data thus generated were subjected to probit analysis to work out the LC_{50} values.

RESULTS AND DISCUSSION

Effective management of insect pest in most of the agricultural ecosystem is dependent on variety of inputs including ready to use safe and highly effective chemical pesticides. Insects because of their abundance and short life cycles and having continuous exposure to same or similar group of insecticidal molecules can readily develop resistance to insecticides. Further, for effective management the search for new molecules in terms of bio efficacy is a continuous and inevitable process. Hence, the Bioassay was carried under laboratory conditions with new insecticidal molecules like flonicamid 50 WG, dinotefuran 20 SG, diafenthurion 50 WP, emamectin benzoate 5 SG, fipronil 5 SC, fenpyroximate 5 SC in comparison with lamda-cyhalothrin 5 EC, thiamethoxam 25 WDG, imidacloprid 17.8 SL, acetamiprid 20 SP, acephate 75 SP against leafhopper. Further, bioassay study was conducted on *A. biguttula biguttula* for the superior or most effective molecules thus its susceptibility status to these insecticides and the results of this study are discussed here under.

In the present study, observations recorded on 24 hours after treatment revealed that thiamethoxam 25

Table 1 : Bioassay of insecticides against leafhopper, *A. biguttula biguttula* in okra

Sr. No.	Treatments	Dosage/ litre	Per cent mortality of leafhoppers (hours after treatment)		
			24 h	48 h	72h
1.	Flonicamid 50 WG	0.3 g	40.00 (39.23)[b]	73.33 (58.91)[b]	100.00 (90.00)[a]
2.	Acetamiprid 20 SP	1.0 g	30.00 (33.21)[e]	40.00 (39.23)[e]	87.00 (68.87)[d]
3.	Diafenthiuron 50 WP	1.0 g	23.33 (28.88)[g]	40.00 (39.23)[e]	53.33 (46.91)[f]
4.	Fipronil 5 SC	1.0 ml	36.67 (37.27)[c]	70.30 (56.98)[c]	93.33 (75.03)[b]
5.	Lambda-cyhalothrin 5 EC	0.5 ml	23.33 (28.88)[g]	36.67 (37.27)[f]	63.33 (52.73)[e]
6.	Dinotefuran 20 SG	0.2 g	33.33 (35.26)[d]	46.67 (43.09)[d]	90.00 (71.57)[c]
7.	Thiamethoxam 25 WDG	0.2 g	70.00 (56.79)[a]	90.00 (71.57)[a]	100.00 (90.00)[a]
8.	Fenpyroximate 5 SC	1.0 ml	20.00 (26.57)[h]	26.67 (31.09)[i]	43.33 (41.17)[h]
9.	Emamectin benzoate 5 SG	0.2 g	23.33 (28.88)[g]	36.67 (37.27)[f]	46.15 (42.79)[g]
10.	Imidacloprid 17.8 SL	0.3 ml	26.67 (31.09)[f]	33.33 (35.26)[g]	43.04 (41.00)[h]
11.	Acephate 75 SP	1.0 g	16.67 (24.10)[i]	20.00 (26.57)[h]	40.67 (39.62)[i]
12.	Untreated control	-	0.00 (0.00)[j]	0.00 (0.00)[i]	0.00 (0.00)[j]
	S.E. ±	-	0.39	0.51	0.44
	C.D. (P = 0.01)	-	1.54	2.02	1.76

Figures in the parentheses are arc sine transformed values
Means followed by same letter (s) in a column not significantly different by DMRT (P = 0.01)

Table 2 : Nymphal susceptibility of okra leafhopper *A. biguttula biguttula* to different insecticides

Sr. No.	Treatments	LC_{50} ppm	Fiducial limit		χ^2 P= 0.05	Regression equation (slope)
			LL	UL		
1.	Dinotefuran 20 SG	7.60	6.16	9.60	2.81	2.63+2.79x
2.	Flonicamid 50 WG	4.50	2.81	15.02	2.08	1.10+4.08x
3.	Fipronil 5 SC	16.18	10.71	24.43	1.83	1.58+3.07x
4.	Thiamethoxam 25 WDG	4.03	2.90	5.58	2.03	2.27+3.60x
5.	Acetamiprid 20 SP	16.40	8.94	30.22	2.81	1.39+3.47x

LL – Lower limit UL – Upper limit

WDG 0.2 g per litre recorded the highest per cent mortality (70.00%) followed by flonicamid 50 WDG 0.3 g per litre (40.00%) fipronil 5 SC at 1 ml per litre (36.67%), dinotefuran 20 SG 1 g per litre (33.33%) and acetamiprid 20 SP 1 g per litre (30.00%) found to be toxic to okra leafhopper nymphs (Table 1).The similar results were also observed in 48 h and 72 h after the treatment wherein thiamethoxam 25 WDG recorded the maximum per cent mortality. From the present investigation it is revealed that thiamethoxam 25 WDG has recorded the highest per cent mortality and similar results were also reported by Shreevani *et al*. (2012) who reported that per cent mortality of leafhopper was found to be maximum (50.67%) in thiamethoxam 25 WDG with non-significant difference with imidacloprid 17.8 SL (46.67%). Clothianidin 50 WDG found to be next best treatment. Similar results were also reported by Shreevani *et al.* (2014). Ghosh and Chakraborty (2015) reported that, imidacloprid provided the best suppression of jassid population (83.24%) closely followed by microbial toxin *Saccharopolyspora spinosa* (74.76% suppression). Similarly Anitha and Nandihalli (2009) reported that, 19 days after sowing (a day before imposition of first spray), plots which received seed treatments, *viz.,* imidacloprid 70 WS and thiamethoxam 70 WS were significantly superior over all other untreated pots, with a population of 0.69 and 0.71 leaf hoppers/ 3 leaves, respectively.

Till recently the synthetic chemical insecticides have been major weapons for controlling this pest. However, the extensive and indiscriminate use of these chemicals has resulted in development of resistance. In order to overcome this complex problem there is a need to monitor susceptibility status of this pest on priority basis which is widely recognized and emphasized. Probit analysis has been routinely used to monitor development of resistance and susceptibility to new insecticides.

In the present study results on nymphal susceptibility of *A. biguttula biguttula* to insecticides revealed that based on LC_{50} values, thiamethoxam 25 WDG observed to be most toxic insecticide to okra leafhopper with LC_{50} value of 4.03 ppm followed by flonicamid 50 WG (4.50 ppm), dinotefuran 20 SG (7.60 ppm), fipronil 5 SC (16.18 ppm) and acetamiprid 20 SP (16.40 ppm) (Table 2). Thus, it can be inferred from these results that among the insecticides thiamethoxam were significantly more toxic to leafhoppers as compared to other insecticides these results are in confirmation with the results of Gavakare *et al*. (2013) who reported that the LC_{50} value of thiamethoxam was 4.1 ppm. Chaudhari *et al*. (2015) reported that, LC_{50} (ppm) for imidacloprid 17.8 SL was 5.76 ppm with fiducial limit of 0.11 to 28.078 ppm. It was 3.99 ppm for thiamethoxam with fiducial limit of 0.23 to 21.571 ppm and 1.43 ppm for acetamiprid 20 SP with fiducial limit of 0.28 to 3.803 ppm in nymphs of leafhopper populations (exposed) at seventy two hours after treatments. Halappa and Patil (2016) reported that, at the very high pesticide usage area (Gulbarga), dinotefuran was found to be highly toxic to leafhopper with the least LC_{50} value of 19.76 ppm followed by clothianidin (68.96 ppm), thiacloprid (96.75 ppm), thiamethoxam (142.00 ppm) and acetamiprid (119.86 ppm). Imidacloprid was found to be the least toxic to the leafhopper with a higher LC_{50} value of 201.36 ppm. Nikita *et al.* (2013) reported that, acetamiprid 20 SP (0.007 ppm) was the most toxic where as spinosad (0.576 ppm) was the least toxic insecticide to okra leafhopper On the basis of LC_{50} values.

However, these results are in contradictory with the studies of Shreevani *et al*. (2014); Ravikumar *et al.* (2003); Mohammad *et al.*(2013); Ghosh *et al.* (2014) and Kalra *et al*. (2001). The LC_{50} values of these chemicals vary with the present investigations. This might be due to the change in the methodology of the experiment, change in the host, change in susceptibility status of insects.

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