Evaluation of Three Bioassay Techniques for Insecticide Resistance Monitoring in Cotton Aphid (Homoptera:Aphididae)

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Abstract : Results of three laboratory bioassay techniques viz. leaf spray, dipping, and hole slide dip methods were compared with those of a field trial to assess the toxicity of dichlorvos 50EC and permethrin 20% to apterous adult females of cotton aphid, *Aphis gossypii* Glover (Homoptera:Aphididae) infesting eggplant and okra respectively. The objective was to look for an inexpensive, easy to handle but effective time-variable method for assay of cotton aphid, meant to assist in the monitoring of field resistance, as part of an integrated pest management strategy. Resistant ratios did not vary much between the techniques but percentage exaggerations depicted differences in terms of both under- and over-estimations of resistance. Even though the leaf spray method over-estimated resistance to permethrin in some cases, it generally compared most favourably with field control and could detect better the relative susceptibility to both insecticides by cotton aphid.

Key Words : Cotton aphid, Bioassay, Insecticide susceptibility, Resistance monitoring

Introduction

Laboratory techniques for monitoring insecticide resistance in arthropod pests have failed to produce results that are desirable enough for field resistance monitoring. This is because results of laboratory bioassay techniques employed for such purpose do not correlate well with those of field. In most cases, such methods tend to underestimate resistance, thus making decisions from results projected from laboratory to the field less rewarding. It is therefore necessary to evaluate a number of bioassay techniques before embarking on studies concerning insecticide resistance. The purpose of such an evaluation is to come out with a method that lessens the deviation between laboratory and field control estimates.

In general the choice of a particular bioassay technique can be of great importance in studies concerning mechanisms of insecticide resistance. Different techniques may alter the level of resistance observed in a particular insect and limit one's ability to identify the mechanism involved[1]. In cotton aphid a number of laboratory bioassay techniques have been described for insecticide susceptibility[2-6]. However, in none of the techniques were results compared to those obtained under field conditions. In view of the need to relate results of laboratory bioassay to field control, three *in vivo* toxicological techniques were evaluated and results compared to those obtained from a field trial, using dichlorvos 50EC and permethrin 20% as test insecticides. The objective was to look for an inexpensive, easy

to handle but effective time-variable technique. It is expected that such a technique will help in an on-farm resistance monitoring strategy which is an indispensable part of an integrated pest management/control programme.

Materials and Methods

Insecticides Permethrin (20%) a synthetic pyrethroid insecticide was obtained from Sankyo Chemical Company Ltd., while dichlorvos (50EC), a systemic organophosphorus insecticide was obtained from Tomono Chemical Industry.

Field evaluation An adult apterous female cotton aphid picked from either eggplant or okra in the same field where both crops were inter-cropped, was isolated onto a seedling of its respective host kept in an insectary (25℃; 16L/8D). The insects were allowed to multiply before field infestations were carried out.

Both eggplant and okra seedlings were transplanted onto previously prepared beds in a randomized complete-block design. Each block of either eggplant or okra as test crop, was demarcated into two subplots of four rows each. Each row consisted of plants spaced at 60 cm × 60 cm intra-and inter-row respectively. Each row in a subplot was labelled for a particular concentration of the respective insecticide used. All plants were infested with cotton aphid from the insectary kept clones three weeks after transplanting, and the insects allowed to multiply. Spraying commenced six weeks after infestation against a control. Before and 24 h after spraying, three randomly selected and tagged leaves of each treatment plant were thoroughly surveyed with the aid of a hand lens and counts on all live insects taken. To check whether contamination by migrant aphids was a problem or not, two separate plots each spaced at about 10 m from the main experimental plot, were also planted with four plants each of the two test crops. Plants on these plots were not infested with cotton aphid and thus used to check the presence of migrant aphids. Since no aphid was noticed on any of the plants of the two test crops on these plots throughout the period of the experiment, the possibilities of contamination and clonal differentiation were neglected. Thus results were attributed solely to differences in experimental treatments.

Bioassay Insects raised from a single apterous female as described above were used for the tests. A leaf spray technique was as follows: Female adult aphids were confined to leaves of seedlings of their respective host, which had been sprayed with a particular concentration of the test insecticide prior to infestation. Five minutes after spraying, the seedlings were infested with ten wingless female adult aphids and kept in cages under a temperature of 25℃. Each seedling with ten insects, represented a treatment for a particular insecticide concentration, and was replicated three times.

A dipping technique similar to that of HAMA[2] was modified and executed as follows: One end of a glass tube (30×20 mm L/ID) was sealed with a piece of nylon cloth. Female adult aphids were then introduced into the tube and the other end sealed with parafilm to prevent escape. The bottom end with nylon cloth which harboured the aphids, was then soaked with shaking in the various concentrations of the test insecticide dissolved in water.

Excess liquid was removed by blotting on a piece of tissue paper. Aphids were then picked with a soft brush and placed in plastic cups containing fresh excised leaves of their respective host, and left under a temperature of 25℃.

A hole slide dip bioassay similar to the microscope slide dip technique described by Kerns and Gaylor[6], was modified as follows: adult female aphids were placed venter side on small spots of glue, which were applied to stripes of a transparent tape attached to the back of a hole slide glass. Ten glue spots corresponding to 10 aphids on each slide glass were replicated three times for each concentration of insecticide used. After 10 aphids were glued to a slide glass, it was placed horizontally in a petri dish and 2 ml of insecticide pipetted onto the aphids, ensuring complete submergence of the insects. Slides were then hung vertically under a temperature of 25℃.

In all the three techniques used, each treatment was replicated three times, with water as control. The numbers of dead insects were counted 24 h after exposure to insecticides, and fifty as well as ninety-five percent lethal concentrations calculated using an NEC computer with a basic programme which corrected for mortality in control by the formula of Abbot[7].

Results and Discussion

In general, dichlorvos proved to be a better aphicide than permethrin both in the field and laboratory trials. Comparing results of the three laboratory bioassay techniques with those of field, it was realized that percentage exaggerations with respect to both LC_{50} and LC_{95} were erratic.

Table 1. shows the susceptibility of eggplant host associated cotton aphid to the two

Table 1. Susceptibility of eggplant host associated cotton aphid to dichlorvos and permethrin

Insecticide	Technique	b±SE	r^2	LC_{50} (ppm)	LC_{95} (ppm)	%EX LC_{50}	%EX LC_{95}
Dichlorvos	Field	0.38	0.96	134.62	253.34	–	
	Leaf spray	6.85±1.24	0.97	113.35	197.02	15.80	22.23
	Dipping	6.02±1.23	0.96	119.04	223.24	11.57	11.88
	Slide dip	3.78±0.63	0.94	92.12	251.00	31.57	0.92
Permethrin	Field	0.12	0.92	483.35	865.09	–	
	Leaf spray	4.64±0.78	0.94	496.15	1112.52	-2.65	-28.60
	Dipping	6.78±1.21	0.96	439.24	767.73	9.13	11.25
	Slide dip	6.19±1.18	0.98	348.89	643.21	27.82	25.65

EX=exaggeration
%EX={(LC_xField - LC_xLab.Tech.)/LC_xField}x100

insecticides. Less than 40% exaggeration was obtained at both levels of lethal

concentration for the two insecticides in all techniques. On the part of dichlorvos, the slide dip method recorded 31.57% exaggeration at the LC_{50} level, thus depicting an almost two fold exaggeration percentage when compared with the other techniques. On the contrary, it showed 24-and 13-fold less exaggerations at the LC_{95} level, than the leaf spray and dipping methods respectively. Using permethrin however, the leaf spray method over-estimated resistance, depicting lethal concentration values which were greater than those estimated from the field trial. In this treatment too, the slide dip method however gave the highest exaggeration percentage of 27.82 and 25.65 for LC_{50} and LC_{95} respectively.

Table 2. also shows the response of okra host associated cotton aphid to the two

Table 2. Susceptibility of okra host associated cotton aphid to dichlorvos and permethrin

Insecticide	Technique	b±SE	r^2	LC_{50} (ppm)	LC_{95} (ppm)	%EX* LC_{50}	%EX* LC_{95}
Dichlorvos	Field	1.25	0.92	115.25	195.78		–
	Leaf spray	3.58±0.52	0.94	87.72	168.91	23.89	13.72
	Dipping	6.32±1.54	0.96	75.29	155.33	34.67	20.66
	Slide dip	0.99±0.12	0.93	65.28	105.32	43.36	46.20
Permethrin	Field	0.13	0.92	507.20	852.37		–
	Leaf spray	7.39±1.33	0.97	411.44	686.85	23.27	24.10
	Dipping	3.87±0.79	0.89	364.79	970.24	39.04	-12.15
	Slide dip	4.27±0.84	0.93	309.08	750.40	64.10	13.59

* See Table 1

insecticides. Generally, percentage exaggerations here were higher than those of eggplant. With both insecticides, the slide dip again gave an almost two fold exaggeration percentage when compared with the other methods.

Specific toxicological studies at the recommended rates of both insecticides (Table 3.),

Table 3. Susceptibility of eggplant and okra host associated cotton aphid to selected doses of dichlorvos and permethrin

Crop	Insecticide	Conc. (ppm)	Technique			
			Field	Leaf spray	Dipping	Slide dip
			%mortality			
Eggplant	Dichlorvos	500	85.27	96.52	100.00	100.00
	Permethrin	500	23.76	40.00	50.72	83.33
Okra	Dichlorvos	500	92.31	100.00	100.00	100.00
	Permethrin	500	33.33	56.66	73.33	76.67

revealed that the leaf spray method gave results comparable with those of field. On the whole, a resistance under-estimation ratio of less than two by all three techniques, was obtained for both insecticides. Though this is less than expected considering the remarks of field workers, it nevertheless underlies the fact that underestimation of resistance cannot be neglected entirely in resistance studies when laboratory assay techniques are employed in monitoring. Results of laboratory bioassay in most cases are suggestive of the fact that at a particular concentration of an insecticide, effective field control should be a foregone conclusion. These results however do not express positive bearings on real field situations thus prompting researchers to question the technical abilities of farmers in the area of insecticide application.

In the midst of such conflicting results the most important problem however will be to define what an effective cotton aphid control actually is. Ball[8], notes that when it comes to the question of what constitutes an effective insect control in the field for various crops and different insecticides, considerable differences in opinions exist. Nevertheless, it is an important factor we must consider if we are to devise collectively a workable technique to assess field resistance and to devise an effective integrated pest management programme.

The inability of an insecticide to effectively control insect pests in the field is an interplay of a series of factors and does not always link itself to the development of resistance by the target insect. Ecophysiology, method, and effectiveness of insecticide application, as well as the kind of management practiced on the farm are some of the factors that affect pest control. In some cases, eradication of the pest's natural enemies is the main cause of a seemingly ineffective control by insecticides, because of continuous crop infestation and/or higher resurgence rate by the pest. In the course of work, it was observed that detection of insecticides by alate forms of cotton aphid leads to an escape response, whereby they fly out of the area and resettle after some time, thus ensuring a relatively high reinfestation rate. This kind of behaviour by the insect is likely to be mistaken as a problem of pesticide ineffectiveness in certain situations.

All techniques studied led to comparable resistance ratios and discriminating concentrations for dichlorvos and permethrin. The leaf spray technique gave comparable results regarding the relative susceptibility of cotton aphid to insecticides, implying that it could be used for resistance monitoring on the farm. However, the tediousness involved in the raising of seedlings as well as time factor, do not auger well for a continuous assay programme. These then pave the way for the less cumbersome and easy to handle dipping technique, since it requires less time and plant materials.

In terms of developing an on-farm insecticide and resistance monitoring technique for cotton aphid, bioassay techniques that will minimize exaggeration of resistance in the field will be of importance in cutting down the frequency of insecticide applications and facilitating management of resistance.

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