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Relative Efficacy of Some Chitin Synthesis Inhibitors on the Mortality of Okra Jassid, *Amrasca biguttula biguttula* (Ishida)

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Abstract: In this laboratory study, three chitin synthesis inhibitors (CSIs) *viz.* Tacoma 40SC (Buprofezin), Heron 5EC (Lufenuron) and Pyrifen 10.8EC (Pyriproxifen) were evaluated with three concentrations of each through different application methods *viz*. direct or topical, indirect or leaf-dip and combined (Direct + Indirect). Data on jassids mortality was collected at 3 and 7 days after treatment (DAT) application. Our present study showed that all of the CSIs had significant effect on the mortality of jassid. In all cases, mortality was clearly dose, time and application method dependent. It has been seen that higher concentrations were found to be more effective than lower concentrations. Moreover, significant level of mortality was found at 3DAT but reached to the peak level at 7DAT in case of all selected CSIs. Bioassay study has showed that all the selected CSIs became able to enter the insect body through contact as well as stomach action to disrupt moulting process by inhibiting chitin synthesis that confirmed the contact and systemic actions of the selected CSIs. Moreover, protocols developed in this study for jassids collection and their safe transferring inside the petridishes would be a useful and convenient approach for the researchers.

Keywords: Chitin Synthesis Inhibitor, Elucidation, Mortality, Okra Jassids, Efficacy

1. Introduction

Okra, *Abelmoschus esculentus* L is an annual vegetable crop under the family Malvaceae which is grown for its green tender fruits or pods. This is the most common vegetable crops grown in Bangladesh and highly popular to all classes of peopels [1, 2]. Farmers of Bangladesh faces significant yield loss of okra in every year due to severe attack of various insect pests. Okra is subjected to attack by almost 72 insects species from seedling to fruiting stage [3-5]. Okra is highly susceptible to a variety of insect pests like jassids, whitefly, aphids, shoot and fruit borer etc. Among the sucking insect pests, jassid is the most notorious one that feed the okra crop and remains active throughout the year with high temperature and high humidity condition [6-8]. Both nymphs and adult stages cause huge loss by feeding on ventral sides of leaves and suck the sap from leaves. During feeding, the *A. biguttula biguttula* also inject the toxic material inside the leaf that seriously damages the leaves which looks like as burning. Infested leaves curl upward from edges, dry up and finally drop down [9, 10]. Ultimately, photosynthesis reduction occurs, no or less setting of fruits and causes loss up to 50 to 63% [11].

Various control strategies such as biological, chemical, cultural methods have been evaluated against this insect pest [12]. Conventionally, farmers are using various types of synthetic chemical insecticides to control okra jassids that creates several problems in agro-ecosystem and develop high level of resistance. Conventional insecticides are fast-acting, convenient and economical, making them the most powerful tools in pest management [13]. But injudicious and indiscriminate use of insecticides in many cases causes or accelerates insecticide resistance, pest resurgence, secondary pest outbreak, environmental

contamination, persistent residual toxicity and destruction of beneficial insects [14].

Attempts are therefore being made to develop more specific agents that will do work without leading further environmental degradation, safe for bio-control agents and human health and will not develop resistance or develop slower resistance. The recent discovery of a new class of chemicals, the chitin synthesis inhibitor (CSI), an specific analogue of insect growth regulators (IGRs) may be a step towards achieving this goal [15]. Chitin, a polysaccharide, is a major component of insect cuticle. As insects develop from immature stage to adults, they undergo several molts during which they shed their old cuticle and form new one [16]. Diflubenzuron, the main chemical of CSIs, disrupt moulting process by interfering chitin synthesis and kill insects before attaining maturity [17, 18]. This chemical compound is also highly effective to check the populations of insect pests by interfering spermatogenesis or oogenesis process [19]. Higher animals that do not produce chitin might not be affected by this chemical [20]. Furthermore, it is still not investigated that how CSIs enters inside the insect body. To explore this issue, okra jassids were exposed to different CSIs through various bioassay methods like topical, leaf-dip and their combination. Therefore, the present study was designed to determine the mode of entry of selected CSIs in insect body and also their relative efficacy in controlling okra jassid in laboratory condition.

2. Materials and Methods

2.1. Experimental Site

The effect of some chitin synthesis inhibitors (CSIs) like Tacoma 40SC, Heron 5EC and Pyrifen 10.8EC were evaluated against okra jassid (*A. biguttula biguttula)* under laboratory condition to find out their relative efficacy as well as route of entry in insect body. Experiments were conducted in the laboratory of the Department of Entomology, Bangladesh Agricultural University, Mymensingh from the period of July, 2017 to March, 2018.

2.2. Collection of Okra Jassid from Field

For laboratory bioassay, okra jassids were collected from the entomology field laboratory, Bangladesh Agricultural University, Mymensingh. Okra plants were raised in the field for mass rearing of okra jassid and much attention was given to the okra plants to keep them uncontaminated from any kinds of pesticides or drift residues of pesticides from nearby field. Jassids collections were quite difficult because of small and delicate size of the insect and jumping characteristics. Jassids were collected by self-made aspirator to keep them alive for further use in the laboratory. "Small insect aspirator" is an affordable device comprised of small jar or vial, the lid or stopper of which is penetrated by two tubes (Figure 1). It was developed to gently collect the okra jassid from the rearing colony for use in CSIs efficacy trials. The aspirator is portable and designed to allow jassids on the vial or jar by blowing off to the other side of tubing. After collecting jassids from the field, the aspirator was carried to the laboratory. For transferring jassids to the petridish a convenient technique has been developed. A mosquito net was placed on the top of the aspirator and then opened the cover of the aspirator. As a result, nymphs and adults of jassids have begun to jump into the mosquito net. Then gently squeeze the net and cover the aspirator with cardboard. After that mosquito net was placed on the petridish and covered the lid. Then smoothly pulled out the mosquito net as jassids were forced to move to the petridish (Figure 1).

Figure 1. *Collection procedures of jassids from the field using handmade aspirator. (A) Handmade small aspirator (B) Collection of jassids from the ventral side of the okra leaves through suctioning (C) Collected jassids inside the aspirator (D) Jassids were transferred inside the mosquito net (E) Transferring jassids inside the petridish and removal of mosquito net gently (F) Finally, jassids were placed inside the petridish along with fresh okra leaves and wet tissue to avoid desiccation.*

2.3. Insect Growth Regulators and Their Concentrations

The selected chitin synthesis inhibitors (CSIs) and their specific concentrations have been shown in table 1. The CSIs were purchased from local pesticide dealer shop of Mymensingh town, Bangladesh.

Table 1. *Specifications of selected chitin synthesis inhibitors tested against okra jassids.*

Trade name of CSIs	Chemical name	Doses	Group/Family
Tacoma 40 SC	Buprofezin	0.6, 0.8 & 1.0 ml/L	Chitin Synthesis Inhibitor
Heron 5 EC	Lufenuron	0.5, 1.0 & 1.5 ml/L	Chitin Synthesis Inhibitor
Pyrifen 10.8 EC	Pyriproxyfen	0.5, 1.0 & 1.5 ml/L	Chitin Synthesis Inhibitor

2.4. Bioassay Methods

Treatments were applied through three application methods *viz* topical or direct, leaf-dip or indirect and combine (topical + leaf-dip) method.

2.4.1. Direct or Topical Application Method

In this method, jassids were directly treated with different concentrations of CSIs products. Direct treatment on jassids was quite troublesome because jassids are fast-moving insects when felt disturbed. At first, jassids were trapped in mosquito net from the aspirator. Then a mosquito net with trapped jassids directly dipped into the CSIs solutions for 10 seconds. Then the mosquito net along with jassids has transferred to the Petridishes where fresh, green okra leave was placed previously. Untreated okra leaves were collected from field, washed and dried on tissues. Placing the mosquito net on Petridishes, the lid covered on it and gently pulled out the net. Moist cotton is placed at the base of twig to avoid desiccation. Concurrently, untreated insects were placed on fresh untreated okra leaves as control treatment.

2.4.2. Indirect or Leaf-Dip Method

In this method, unsprayed okra leaves were collected from the field, carefully washed and dipped into different concentrations of selected CSIs solution for a few seconds. Then dipped leaves were taken out from the solution and dried on tissues. Then untreated insects were transferred gently from the mosquito net to petridishes avoiding any injury. Moist cotton is placed at the base of the twig to avoid desiccation. Concurrently, untreated insects were placed on fresh untreated okra leaves as control treatment.

2.4.3. Combined (Direct + Indirect) Method

In this method, both jassids and okra leaves were treated with different concentrations of selected CSIs. After that treated leaves were air dried and then transferred in petridishes. Then, treated jassids were carefully transferred from the mosquito net to petridishes where insects forced to jump into petridish avoiding any injury. Moist cotton is placed at the base of twig to avoid desiccation. At the same time untreated insects were placed on fresh untreated okra leaves as control treatment.

2.5. Data Collection

%mortality of jassids

Regular inspection was done but mortality data was recorded at 3 and 7 DAT. The percentage of mortality of jassid was calculated using the following formula:

$$\% \text{ Mortality} = Po/Pr \times 100$$

Where,

Po=Number of died insects

Pr=Number of treated or untreated insects provided

%mortality of jassids over control through different bioassay methods

$$\%\text{mortality of jassids over control} = \frac{Mt-Mc}{Mt} \times 100$$

Where,

Mt = Mortality through treated condition

Mc = Mortality through control condition

2.6. Statistical Analysis

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance (ANOVA) was done R Statistics Software version 3.5.3. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (DMRT) or Least Significant Difference (LSD) whenever necessary.

3. Results

In this study, three chitin synthesis inhibitors (CSI) were evaluated against okra jassids to elucidate their mode of entry in the insect body as well as relative efficacy for controlling jassids. Our results clearly showed that all the selected CSIs were found very effective against jassids and all had both contact and systemic (translaminar) action. Results are described below based on the effectiveness of each selected CSIs.

3.1. Efficacy of Tacoma 40 SC on the Mortality of Okra Jassid

Mortality percentages through different application methods have been shown in table 2. Data clearly showed that Tacoma 40 SC (Buprofezin) had significant on the mortality of jassids compared to untreated control. Results showed that mortality trend was found clearly dose, time and method dependent. At 3 DAT, 61.66% mortality was recorded when jassids were directly treated with 1.0 ml/L followed by 0.8 ml/L (56.50%) and 0.6 ml/L (48.60%) respectively. The highest mortality was found from 1.0 ml/L of Tacoma 40SC (81.51%) at 7 DAT which was followed by 0.8 ml/L (70.63%) and 0.6 ml/L (59.33%) respectively. Like direct application method, the trend was found to be similar with leaf-dip application method although percent mortality was slightly lower than that of direct application method. At 3 DAT, 56.0% mortality was recorded when jassids were directly treated with 1.0 ml/L which was followed by 0.8 ml/L (49.0%) and 0.6 ml/L (42.0%). The highest mortality was found from 1.0 ml/L of Tacoma 40SC (67.63%) at 7 DAT which was followed by 0.8 ml/L (60.63%) and 0.6 ml/L (48.43%). The highest mortality percentage was found from combined application method i.e. when both leaf and insects were treated with different concentrations of Tacoma 40 SC. Approximately, 76.87% mortality was recorded @ 1.0 ml/L which was followed by 0.8 ml/L (67.61%) and 0.6 ml/L (64.26%) respectively at 3 DAT. The highest mortality was found from 1.0 ml/L of Tacoma 40SC (90.10%) at 7 DAT which was followed by 0.8 ml/L (82.53%) and 0.6 ml/L (78.70%) respectively. This finding has confirmed that

Buprofezin molecule is able to enter in insect body through contact and stomach action.

Table 2. *Mean percent mortality of jassids at different time interval following treated with different concentrations of Tacoma 40 SC through different bioassay methods.*

Treatments	% mortality in different bioassay methods					
	Direct or topical		Leaf-dip		Combined	
	3 DAT	7 DAT	3 DAT	7 DAT	3 DAT	7 DAT
Tacoma 40 SC @ 0.6 ml/L	48.60c	59.33c	42.0c	48.43c	64.26c	78.76c
Tacoma 40 SC@ 0.8 ml/L	56.50b	70.63b	49.0b	60.63b	67.61b	82.53b
Tacoma 40 SC@ 1.0 ml/L	61.66a	81.51a	56.0a	67.63a	76.87a	90.10a
Control	6.36d	8.76d	6.36d	8.76d	6.36d	8.76d
CV (%)	7.67	9.17	8.32	6.36	7.80	5.86
LSD0.05	1.32	1.19	1.63	1.18	0.79	2.25

In a column, means followed by different letters are significantly different 5% level of probability. DAT = Days After Treatment.

3.2. Efficacy of Heron 5EC on the Mortality of Okra Jassid

Mortality of okra jassid following treated with different concentrations of Heron 5EC through different application methods has been shown in table 3. Like as Buprofezin, similar trend was found when insects or leaves were treated with Heron 5EC. The mortality was clearly dose, time and application method dependent. The significant ($P<0.05$) effect was found at 3 DAT which reached the peak level by 7 DAT. At 3 DAT, all the treatments significantly increased mortality compared to control but highest mortality was found at 7 DAT from 1.5 ml/L of Heron 5 EC (81.03%) which was followed by 1.0 ml/L (71.26%) and 0.5 ml/L (59.86%) respectively. Leaf-dip method was found to be less effective than topical or direct application method. At 3 DAT, 54.45% mortality was recorded from 1.5 ml/L that was followed by 1.0 ml/L (43.37%) and 0.5 ml/L (30.38%) respectively. At 7 DAT, about 75.42% mortality was found @ 1.5 ml/L of Heron 5EC which was followed by 1.0 ml/L (57.40%) and 0.5 ml/L (54.65%) respectively. The highest mortality was recorded from the combined application method. At 3 DAT, mortality was found 78.51% @ 1.5 ml/L but highest mortality was found at 7 DAT from 1.5 ml/L of Heron 5EC (88.63%) which was followed by 1.0 ml/L (79.40%) and 0.5 ml/L (62.43%) respectively. From the above results, it was clear that direct application method was found better than indirect application method but combined application method gave the best result as it was more effective for mortality compared to individual application method.

Table 3. *Mean percent mortality of okra jassid at different time interval following treated with different concentrations of Heron 5EC through different bioassay methods.*

Treatments	% mortality in different bioassay methods					
	Direct or topical		Leaf-dip		Combined	
	3 DAT	7 DAT	3 DAT	7 DAT	3 DAT	7 DAT
Heron 5EC @ 0.5 ml/L	46.13c	59.86c	30.38c	54.65c	54.80c	62.43c
Heron 5 EC @ 1.0 ml/L	63.33b	71.26b	43.37b	57.40b	70.79b	79.40b
Heron 5 EC @ 1.5 ml/L	75.06a	81.03a	54.45a	75.42a	78.51a	88.63a
Control	6.53d	8.28d	6.53d	8.28d	6.53d	8.28d
CV (%)	5.45	9.58	8.34	6.18	8.09	7.71
LSD0.05	3.022	1.63	2.05	2.01	2.03	1.92

In a column, means followed by different letters are significantly different at 5% level of probability. DAT = Days After Treatment.

3.3. Efficacy of Pyrifen 10.8EC on the Mortality of Okra Jassid

Like as Tacoma 40 SC and Heron 5 EC, Pyrifen 10.8EC had significant effect on the mortality of jassid but Pyrifen was found slightly less effective than Tacoma or Heron (Table 4, $P<0.01$). Compared to untreated control, the significant level of mortality was found at 3 DAT that reached to the peak level by 7 DAT while 1.5 ml/L was performed the best that was followed by 1.0 and 0.5 ml/L respectively. Among three bioassay methods, combined method has provided the best efficacy that was followed by topical method. Less mortality was recorded from leaf-dip method i.e. when untreated jassids were exposed to treated okra leaves. Approximately, 89.98% mortality was found at 7 DAT when both insects and leaves were treated with Pyrifen 10.8 EC @ 1.5 ml/L (combined) that was followed by topical method (73.66%). The lowest mortality percentage was found in case of leaf-dip method (66.43%). Comparatively lower mortality was recorded from lower concentrations of Pyrifen 10.8EC at 3 and 7DAT.

Table 4. *Mean percent mortality of okra jassid at different time interval following treated with different concentrations of Pyrifen 10.8EC through different bioassay methods.*

Treatments	% mortality in different bioassay methods					
	Direct or topical		Leaf-dip		Combined	
	3 DAT	7 DAT	3 DAT	7 DAT	3 DAT	7 DAT
Pyrifen 10.8 EC @ 0.5 ml/L	25.50c	45.53c	12.85c	41.43c	36.86c	78.46c
Pyrifen 10.8 EC @ 1.0 ml/L	36.56b	61.49b	28.69b	53.00b	46.43b	82.57b
Pyrifen 10.8 EC @ 1.5 ml/L	43.52a	73.66a	37.44a	66.43a	61.85a	89.98a
Control	5.90d	7.53d	5.90d	7.53d	5.90d	7.53d
CV (%)	9.29	7.54	10.45	6.49	7.27	5.98
LSD0.05	1.17	1.36	1.32	1.18	0.88	1.21

In a column, means followed by different letters are significantly different at 5% level of probability. DAT = Days After Treatment.

3.4. Mortality over Control in Different Bioassay Methods

Mortality of jassids over control has been calculated to know the more clear performance of different bioassay methods (Figure 2). Approximately, 88-93% mortality over control was recorded at 7 DAT when both jassids and okra leaves were treated with highest concentrations of selected CSIs that was significantly lowered (70-82%) in case of topical method i.e. when only jassids were directly treated with CSIs. Interestingly, the least mortality was found from leaf-dip method i.e. when untreated jassids were exposed to treated okra leaves (60-70%).

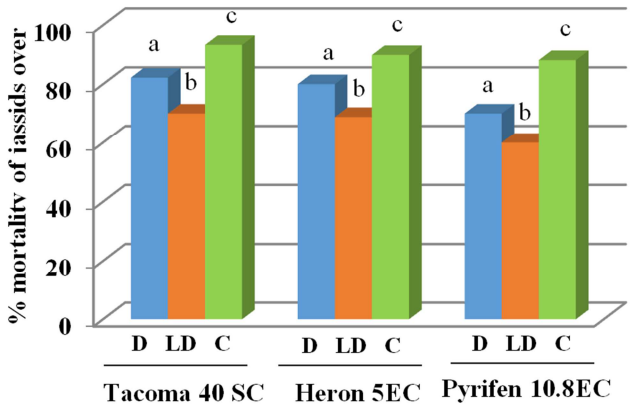

Figure 2. *Percent mortality of jassids over control at 7 DAT following treated with different CSIs using their maximum concentrations through different bioassay methods. Different letters on each bar indicates significant difference from each other at 5% level of probability. D: Direct, LD: Leaf-dip, C: Combined. Different letters on the bar denotes significant difference from each other.*

4. Discussion

Our present laboratory study confirms that Buprofezin, Lufenuron and Pyriproxifen all are highly effective against okra jassids although their relative efficacy depends on concentrations, exposure time and exposure methods. It was also observed that Pyrifen 10.8 EC was comparatively less effective than rest of the two CSIs. Our present findings are in close agreement with others. [21] Vadja and Kalasariya (2015) investigated the effect of Buprofezin on the mortality of bean aphid and they have reported that Buprofezin @ 0.05% caused 69.95% mortality. [22] Reported that Buprofezin was found to be highly effective against brown plant hopper (BPH) and the mortality was clearly dose and method dependent. The highest mortality (90.55%) was recorded from 300 ppm of Buprofezin which was followed by 200 (83.06%) and 100 (43.47%) ppm of Buprofezin respectively. It was observed that Buprofezin 40SC was found to be the most effective against aphids offering the lowest aphid population (1.56/top10cm central twig) at 7 days after treatment (DAT) [23]. A study reported that higher doses of Lufenuron applied on *H. armigera* effectively suppressed the population, resulting significant reduction in crop damage [24]. Moreover, the effectiveness of Heron 5EC (Lufenuron) against 3rd instars larvae of *Spodoptera litura* (Fab.) under laboratory conditions for time-oriented mortality as well as inhibition of growth and development [25, 26]. They showed that 70-80% *S. litura* larvae were died from highest concentrations of Lufenuron and growth and development was significantly arrested as well. [27] Reported that pyriproxyfen was found effective against *Amrasca biguttula biguttula* (Ishida) that reduced 23.61% populations in all sprays. [28] Showed that the performance of pyriproxyfen 10.8EC @ 75, 100 and 125 g a.i. ha^{-1} against sucking insect pests and predatory complex. However, all the three pyriproxyfen dosages were found significant against nymphal population of white fly at 1, 3 and 7 DAS. After 7 days of spray, the percent reduction in white fly nymphs over control in pyriproxyfen 10.8EC @ 100 and 125 g a.i. ha^{-1} was 90.0 and 91.4 percent, respectively. [29] Also found that mortality of mature cotton aphid was increased by higher concentrations of Pyriproxyfen.

Chitin synthesis inhibitors does not works in the central nervous system of insects rather it works as a physiological disruptor i.e. kill insects by stopping moulting process through inhibiting chitin synthesis in cuticle. It was not investigated yet how CSIs molecules enter inside the insect body or enter insect endocrine system. Our present findings report for the first time that Buprofezin, Lufenuron and Pyriproxifen can enters inside the endocrine system through cuticular pores as well as through foods. It was also interestingly observed that all the tested CSIs has translaminar or systemic actions as above 60% jassids were died through leaf-dip method i.e. untreated jassids were released on CSIs treated leaves and finally died when diflubenzuron molecules of CSIs entered inside the jassids body through cell sap.

5. Conclusion

The tested CSIs i.e. Tacoma 40 SC (Buprofezin), Heron 5EC (Lufenuron) and Pyrifen 10.8 EC (Pyriproxifen) may be the potential alternatives of conventional insecticides as they provided 80-90% mortality with their maximum concentrations. It was also observed that CSIs are slower in action and therefore need to wait at least for 7 days after application in the field. Moreover, to get the highest efficacy from the selected CSIs spray coverage should be uniform and complete so that every insects and whole plant parts come in contact with spray droplets.

Conflict of Interest

The authors declare no conflict of interest.

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