

Relative efficacy of some chitin synthesis inhibitors in reducing growth and development of okra jassid, *Amrasca biguttula biguttula* (Ishida)

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Abstract

Chitin synthesis inhibitors (CSIs) are chemically diverse compounds that disrupt molting process by interfering chitin synthesis and kill insects before attaining maturity. In this study, some chitin synthesis inhibitors (CSIs) *viz.* tacoma 40SC (Buprofezin), heron 5EC (Lufenuron), pyrifen 10.8EC (Pyriproxifen) and chitosan 75WP were tested against okra jassid, *Amrasca biguttula biguttula* (Ishida) to elucidate their potential effects in arresting body growth and development. The nymphs of jassids was exposed to selected CSIs through different application methods like topical, leaf-dip and the combination of both topical and leaf-dip. Weight data was collected at 7 days after treatment (DAT) application. Results showed that all of the CSIs except chitosan had significant effect on the body weight reduction of okra jassid. Growth reduction was clearly concentrations and application method dependent. It has shown that higher concentrations were found to be more effective than lower concentrations. Bioassay study has showed that all the selected CSIs became able to enter in the insect body through contact as well as stomach action to disrupt molting process by inhibiting chitin synthesis that confirmed the contact and systemic actions of the selected CSIs. This study recommends that tacoma 40SC @ 1.0 ml/L, heron 5EC @ 1.5 ml/L and pyrifen 10.8 EC @ 1.5 ml/L may be the potential alternatives of conventional neurotoxic insecticides in controlling jassids as they reduced 50-60% weight compared to

Received

30th June 2020

Accepted

03rd November 2020

Available online

20th November 2020

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untreated control. Moreover, protocols developed in this study for jassids collection and their safe transferring inside the petri dishes would be a useful and convenient approach for the researchers.

Keywords: Chitin synthesis inhibitor, efficacy, okra jassids, growth, development.

Introduction

Okra, *Abelmoschus esculentus* L is an annual and very popular vegetable crop in Bangladesh that is grown for its green tender fruits^[1]. A significant yield reduction occur every year due to the infestation caused by various types of insects. It has been reported that approximately 72 insect species attacks okra crops from different insect orders like hemiptera, coleoptera, lepidoptera etc. These insect species infests okra plants and fruits from seedling stage to fruiting stage^[2-3]. Okra is highly susceptible to a variety of insect pests like jassids, whitefly, aphids, shoot and fruit borer etc. Among the sucking insect pests, okra jassid, *Amrasca biguttula biguttula* Ishida is the most notorious one that feed the okra crop and remains active throughout the year with high temperature and high humidity condition ^[4-6]. Both nymphs and adult stages cause huge loss by feeding on ventral sides of leaves and sucks the sap from leaves. During feeding, the jassid also inject one kind of toxic material inside the leaf that seriously damage the leaves which looks like as burning. Infested leaves curl upward from edges, dry up and finally drop down^[7-8]. Ultimately, photosynthesis reduction occurs, no or less setting of fruits and causes loss up to 50 to 63% ^[9].

The greatest challenge of today's agriculture is to feed the growing populations and restore the natural enemies. Uncontrolled population growth in developing countries accelerated the imbalance between human needs and agricultural sustainability. Therefore, the production and productivity of crops or vegetables has increased due to the application of synthetic chemical insecticides. Conventional synthetic insecticides are **economically advantageous**, making them the most powerful tools in pest management^[10]. But the increased use of broad-spectrum chemical insecticides has posed many

environmental and health problems. Among these problems, development of insecticide resistance, destruction of beneficial arthropods in crop-ecosystem and persistent residual toxicity in human body are the main concerns^[11].

Attempts are therefore being made to develop more specific agents that will act 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^[12]. Chitin, a polysaccharide, is a major components 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 ^[13]. Diflubenzuron, the main chemical of CSIs, disrupt molting process by interfering chitin synthesis and kill insects before attaining maturity^[14-15]. This chemical compound is also highly effective to inhibit the growth and development of insect by reducing body weight and interfere the new progeny of insect pests by interfering spermatogenesis or oogenesis process^[16]. Underweight or abnormal insects gradually dies and alive insects become unable for further reproduction^[14]. Higher animals that do not produce chitin might not be affected by this chemical^[17]. Furthermore, it is still not investigated that how CSIs enters 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 relative efficacy of some CSIs in reducing growth and development of okra jassid and also to elucidate their mode of entry in insect body.

Materials and Methods

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 in reducing body weight as well as to understand the 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.

Collection of okra jassid from field

For laboratory bioassay, okra jassids were collected from the field in the campus of 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 collection 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 (Fig.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 petri dishes 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 has began to jump into the mosquito net from aspirator. Then gently squeeze the net and cover the aspirator with cardboard. After that mosquito net was placed on the petri dish and covered the lid. Then smoothly pulled out the mosquito net as jassids were forced to move to the petri dish (Fig.1). It is mentioned here that only nymphs were used in all experiments instead of adult jassids.

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Figure 1 - Collection procedures of jassids from the field using hand made aspirator. (A) Hand made 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

Buprofezin, lufenuron, pyriproxifen, chitosan and their concentrations

The selected chitin synthesis inhibitors (CSIs) and their specific concentrations has been shown in table-1. Buprofezin, lufenuron and pyriproxifen were purchased from local pesticide dealer shop of Mymensingh town, Bangladesh. Chitosan 75WP was purchased from Sigma-Aldrich Co., Germany.

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
Chitosan 75 WP	Chitosan	0.50, 0.75 & 1.0 g/L	Chitin Synthesis Inhibitor

Table 1 - Specifications of selected chitin synthesis inhibitors tested against okra jassid, *Amrasca biguttula biguttula* Ishida

Treatments application methods

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

Direct or topical application method

In this method, nymphs of jassids (n = 20) 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 a piece of voile fabric from the aspirator. Then voile fabric with trapped jassids directly dipped into the CSIs solutions for 10 seconds. Then voile fabric along with jassids has transferred to the petri dishes 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 petri dishes, 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. There had three replications for each of the treatment.

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 few seconds. Then dipped leaves were taken out from the solution and dried on tissues. Then untreated nymphs (n =20) were transferred gently from the voile fabric to petri dishes avoiding any injury. 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. There had three replications for each of the treatment.

Combined (direct + indirect) method

In this method, both nymphs (n =20) and okra leaves were treated with different concentrations of selected CSIs. After that treated leaves were air dried and then transferred in petri dishes. Then, treated jassids were carefully transferred from the mosquito net to petri dishes where insects forced to jump into petri dish 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. There had three replications for each of the treatment.

Data collection

Weight of treated and untreated (control) jassids were recorded at 7 days after treatment (DAT) application. Then weight data was converted into percent weight

reduction over control by using following formula:

$$\% \text{weight reduction over control} = \frac{W_t - W_c}{W_t} \times 100$$

W_t = Mean weight of jassid in treated condition

W_c = Mean weight of jassid in untrated or control condition

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.

Results

In this study, four chitin synthesis inhibitors (CSI) were evaluated against okra jassids to elucidate their relative efficacy in reducing weight i.e. growth and development as well as mode of entry in insects body. Our results clearly showed that all the selected CSIs except

chitosan were found effective in reducing growth and development of jassids and all had both contact and systemic (translaminar) action. Results are described below based on the effectiveness of each selected CSIs.

Efficacy of tacoma 40 SC on the weight reduction of *A. biguttula biguttula*

Effect of different concentrations of tacoma 40 SC (Buprofezin) on the weight reduction of jassids over control through different bioassay methods has been shown in table 2. Data clearly showed that tacoma 40 SC had significant effect on the weight reduction of jassids and there had also significant difference among the concentrations. Among the bioassay methods, combined method was found most effective that was followed by indirect or leaf-dip and direct method respectively. 40.24% weight reduction was found when jassids were directly treated with tacoma 40 SC @ 1.0 ml/L but this reduction level further increased to 44.23% through indirect method and reached to 54.05% through combined bioassay method. Comparatively lower weight reduction was found from 0.6 ml/L and 0.8 ml/L considering three application methods.

Treatments	%weight reduction over control at 7 DAT following different bioassay methods		
	Direct/Topical	Indirect/leaf-dip	Combined
Tacoma 40SC@ 0.6 ml/L	20.12c	22.70c	32.14c
Tacoma 40SC@ 0.8 ml/L	26.34b	32.12b	36.10b
Tacoma 40SC@ 1.0 ml/L	40.24a	44.23a	54.05a
LSD _{0.05}	1.45	2.78	4.23
CV (%)	7.80	8.88	10.22

Table 2 - Effect of tacoma 40 SC (Buprofezin) on the weight reduction of okra jassid, *Amrasca biguttula biguttula* Ishida at 7 DAT through different bioassay methods.

Efficacy of heron 5 EC on the weight reduction of okra jassid

Heron 5 EC was found more effective than tacoma 40 SC (Buprofezin) considering weight reduction although trend was similar with previous results (Table-3). 46.67% weight reduction was found when jassids were directly treated with heron 5 EC @ 1.5 ml/L but this reduction level further increased to 53.33% through indirect method and reached to 56.66% through combined bioassay method. Moreover, 50% weight reduction was found from combined method @ 1.0 ml/L that was followed by 43.33 and 36.67% reduction from indirect and direct method respectively. The concentration 0.5 ml/L had comparatively lower effect on weight reduction considering all bioassay methods.

Treatments	%weight reduction over control at 7 DAT following different bioassay methods		
	Direct/Topical	Indirect/leaf-dip	Combined
Heron 5EC @ 0.5 ml/L	30.00c	40.00c	43.33c
Heron 5EC @ 1.0 ml/L	36.67b	43.33b	50.00b
Heron 5EC @ 1.5 ml/L	46.67a	53.33a	56.66a
LSD _{0.05}	3.45	4.34	4.78
CV (%)	5.67	8.23	11.12

Table 3 - Effect of Heron 5 EC (Lufenuron) on the weight reduction of okra jassids, *Amrasca biguttula biguttula* Ishida at 7 DAT through different bioassay methods.

Efficacy of pyrifin 10.8 EC on the weight reduction of okra jassid

Pyriproxifen is an important CSIs that contain diflubenzurin having potential inhibitory effect on growth and development. The present laboratory study clearly showed that pyrifin 10.8EC (Pyriproxifen) has potential effect on the retardation of growth and development of jassids. The data has shown in table 4. Specifically, 63% weight

reduction over control was found when both leaves and jassids were treated (combined method) with pyrifen 10.8EC @ 1.5 ml/L that was followed by 55% and 44% reduction in case of leaf-dip and direct method respectively. On the other hand, 51.56% weight reduction was found from combined bioassay method @ 1.0 ml/L that was followed by 48.44% and 36.66% reduction through leaf-dip and direct method respectively. The concentration 0.5 ml/L had lower effect in arresting growth and development.

Treatments	%weight reduction over control at 7 DAT following different bioassay methods		
	Direct/Topical	Indirect/leaf-dip	Combined
Pyrifen 10.8EC @ 0.5 ml/L	28.33c	41.00c	45.33c
Pyrifen 10.8EC @ 1.0 ml/L	36.66b	48.44b	51.56b
Pyrifen 10.8EC @ 1.5 ml/L	44.00a	55.00a	63.00a
LSD _{0.05}	3.33	4.12	4.50
CV (%)	7.78	8.12	10.23

Table 4 - Effect of Pyrifen 10.8EC (Pyriproxifen) on the weight reduction of okra jassids, *Amrasca biguttula biguttula* Ishida at 7DAT through different bioassay methods.

Efficacy of chitosan 75WP on the weight reduction of okra jassid

Chitosan is a natural polysaccharide that is derived from chitin of shrimp, crab, webstar etc through deacetylation process. It has potential insecticidal and growth inhibitory effect on some lepidopteran insects. In this study, the growth inhibitory effect of chitosan was studied using hemipteran insect like jassid in laboratory condition through different bioassay methods and data has shown in table 5. Unlike buprofezin, lufenuron and pyriproxifen, less efficacy was found from chitosan in arresting growth and development of jassids even from all bioassay methods. Only 18.56% growth reduction over control was observed when both jassids and okra leaves were

treated with 1.0 g/L of chitosan that was followed by 14.45 and 10.12% reduction through leaf-dip and direct method respectively. Comparatively, less weight reduction was found in case of 0.50 and 0.75 g/L of chitosan considering all bioassay methods.

Treatments	%weight reduction over control at 7 DAT following different bioassay methods		
	Direct/ Topical	Indirect/ leaf-dip	Combined
Chitosan 75WP @ 0.50 g/L	5.66a	8.78a	9.67a
Chitosan 75 WP@ 0.75gl/L	6.78a	9.12a	10.12a
Chitosan 75 WP@ 1.0 g/L	10.12b	14.45b	18.56b
LSD _{0.05}	1.56	1.34	1.67
CV (%)	4.56	5.67	5.89

Table 5 - Effect of Chitosan 75WP on the weight reduction of okra jassids, *Amrasca biguttula biguttula* Ishida at 7DAT through different bioassay methods.

Growth reduction through different bioassay methods

In this study, CSIs were applied through topical, leaf-dip and their combined way to elucidate the route of entry of CSIs in jassids body. It was not investigated yet how CSIs are enters in insects body and how does they work. Our topical bioassay methods confirmed that the CSIs molecules can directly enters in insect body through cuticular pores and finally reach endocrine system. Moreover, growth or weight reduction through leaf-dip method further confirmed that all the selected CSIs has translaminar or systemic action because untreated jassids were affected when they sucked cell sap from treated okra leaves.

Discussion

Our present laboratory study suggests that buprofezin, lufenuron and

pyriproxifen all are found effective in reducing growth and development of okra jassids but chitosan 75WP was found very less effective. Similar trend was found in case of tacoma, heron and pyrifen in reducing weight but pyrifen showed slightly better efficacy than rest of the two CSIs. Our present findings are in close agreement with some previous studies. Vadja and others has investigated the effect of buprofezin on the mortality and weight reduction of bean aphid and they have reported that buprofezin @ 0.05% caused 69.95% mortality and 50% weight reduction^[18]. Alam and Das has reported that buprofezin was found to be highly effective against brown plant hopper (BPH) and the mortality and weight reduction was clearly dose and method dependent ^[19]. Das and Islam found that buprofezin is highly effective in reducing growth and development of larvae of brinjal shoot and fruit borer with higher doses ^[20]. About 82% weight was reduced over control when larvae were treated with 800 ppm of buprofezin that was followed by 61.57 and 34.91% reduction @ 400 and 200 ppm respectively. Gogi *et al.* (2006) found that higher dose of lufenuron applied on *H. armigera* effectively suppressed the insect growth and development, resulting significant reduction in crop damage. Some studies have evaluated 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 ^[21-22]. They showed that 70-80% *S. litura* larvae were died from highest concentrations of lufenuron and growth and development was significantly arrested as well. Kumar and others has reported the performance of pyriproxifen 10.8EC @ 75, 100 and 125 g a.i. ha⁻¹ against sucking insect pests and predatory complex^[23]. However, all the three pyriproxifen 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 pyriproxifen 10.8EC @ 100 and 125 g a.i. ha⁻¹ was 90.0 and 91.4 per cent, respectively. Makareminia and his colleagues also found that mortality and weight reduction of mature cotton aphid was increased by higher concentrations of pyriproxifen^[24].

Chitin synthesis inhibitors does not works in central nervous system

of insects rather it works as a physiological disruptor i.e. kill insects by stopping molting process through inhibiting chitin synthesis in cuticle. It was not investigated yet how CSIs molecules enters inside the insect body or enters insect endocrine system. Our present findings report for the first time that buprofezin, lufenuron, pyriproxifen and chitosan has both contact and systemic action and it was supported by the findings from our topical and leaf-dip application methods. 40-45% mortality was found when insects were treated topically with the maximum concentrations of buprofezin, lufenuron and pyriproxifen which suggests that CSIs molecules enters in the insect body through cuticular pores, reached to endocrine system thereafter and finally the affected insects died. On the other hand, 45-55% mortality was found from leaf-dip method which also suggests that the CSI molecules first enters in leaf cell sap, then inside the insect body and in endocrine system through sucking of cell sap and finally died. The possibility that the CSIs might have translaminar or systemic action and therefore growth and development was significantly arrested through molting inhibition and finally insects were died [25].

Conclusion

Tacoma 40 SC (buprofezin), heron 5EC (lufenuron) and pyrifen 10.8 EC (pyriproxifen) may be the potential alternatives of conventional insecticides in controlling jassids as they reduced 50-60% body weight. It has also been noted that the majority of the treated or underweight jassids became abnormal and died shortly. As 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. Considering efficacy, Chitosan 75WP is not recommended for managing jassids in field condition.

Acknowledgements

This study was supported by a research grant from NATP-2/PIU-BARC sub-project (Project ID: 400) to Dr. Gopal Das.

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