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Laboratory evaluation of insect growth regulators and bacterial fermented insecticides on the mortality of papaya mealy bug

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Abstract
The papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) is a serious insect pest causing severe damage to papaya plants. Effectiveness of two insect growth regulators *viz*. Tacoma 40 SC (Buprofezin) and Heron 5 EC (Lufenuron), two bacterial fermented insecticides *viz.* Libsen 45 SC (Spinosad) and Suspend 5 SG (Emamectin benzoate) and one mixed formulation *viz*. Lumectin 10 WDG (Lufenuron + Emamectin benzoate) were evaluated against papaya mealy bug. The experiment was conducted under laboratory condition to investigate the time-oriented mortality of papaya mealybug after exposure with selected treatments. Results clearly showed that about 100% and 90% mortality were found from Tacoma 40 SC and Heron 5 EC at 7 DAT with their highest concentrations @ 1.0 ml/L and 2.0 ml/L respectively. Among bacterial fermented insecticides, Libsen 45 SC was found better than Suspend 5 SG regarding mortality. About 90% and 75% mortality was found from Libsen 45 SC @ 0.75 ml/L and Suspend 5 SG @ 1.25 g/L at 7 DAT respectively. Moreover, Lumectin 10 WDG, a combination of Lumectin and Emamectin Benzoate performed excellent efficacy while 100% mortality was recorded @ 1.25 g/L. The present study showed that each of the insect growth regulator and microbial biopesticides had significant effect against papaya mealybug. The mortality of papaya mealy bugs was gradually increased when insects and papaya plants both were directly treated with selected IGRs and biopesticides with increasing concentration level and time. Finally, it can be concluded that Tacoma 40 SC, Heron 5 EC, Libsen 45 SC and Lumectin 10 WDG would be alternate and promising options of conventional insecticides to the farmers to control papaya mealybug effectively.

Keywords: papaya mealybug, laboratory, IGRs, insecticides, mortality

1. Introduction
Papaya *(Carica papaya* L.) is a soft-wooded perennial plant and belongs to the family Caricaceae. It is one of the most important fruits cultivated throughout the tropical and subtropical regions of the world [1]. Papaya fruits are tasty, sweet, juicy and very nutritious. It is regarded as an excellent source of ascorbic acid, a good source of carotene, riboflavin and a fair source of iron, calcium. Each and every part of papaya plant from root to shoot is used for medicament purposes. Seeds are also rich source of amino acids; scented oil was extracted, used in treatment of sickle cell disease and poisoning related disorders [11]. But insect infestation is a limiting factor in growing papayas, especially from fruit setting stage until harvest of papaya [3]. The papaya mealybug is a polyphagous sucking insect and is considered a significant pest of many tropical and subtropical fruits, vegetables, and ornamental plants [7]. Although specimens of papaya mealybug were first collected in Mexico in 1955, the species was only described in 1992 in the Neo-tropical region occupying Belize, Costa Rica, Guatemala, and Mexico [17]. This pest got introduced without their native natural enemies and posed a potential threat to papaya in India and Bangladesh [9].
Heavy infestation of papaya mealybug causes deformation of fruit, buildup of honeydew and aggregation of thick white waxy appearance on the fruit surface. For these reasons, market value of papaya fruits decreases markedly. Papaya growers reported that they destroy infested papaya fruit in the field instead of brings them to the market because the transporting cost did not meet from the sale price. As a result farmers faced great economic loss due to infestation of papaya mealybug and the economic loss become much higher [6].
A recent survey in Bangladesh found that about 40% papaya plants are attacked with mealybug in the orchards and farmers are applying different chemical insecticides indiscriminately which do not provide effective control [6].

Therefore, it is urgently needed to search for an alternative means of pest control which can minimize the use of these synthetic chemicals. Along with synthetic insecticides, increasing use of biopesticides like insect growth regulators (IGRs), bacterial fermented insecticides in an agro ecosystem is now emerging as one of the prime means to protect crops and the environment from pesticidal pollution.

The mode of action of IGRs is not central nervous system-oriented but kills insects potentially through cessation of moulting process [2]. Tacoma 40SC (Buprofezin) and Heron 5 EC (Lufenuron) are the potent chitin synthesis inhibitor (CSI). They are potentially inhibited chitin bio-synthesis and deposition in insect body. Consequently, no formations of new cuticle and old cuticle become fractured and insect finally die [4]. Both were found to be very effective against papaya mealybug. It was reported that Tacoma 40 SC and Heron 5 EC are an eco-friendly biorational pesticides that are safe for non-target organisms, highly biodegradable and action is target pest specific [15]. In addition, research work with bacterial fermented insecticides viz., Libsen 45 SC and Suspend 5 SG to determine mortality is also noticeable. Therefore, the present research work was planned to evaluate the efficacy of two IGRs such as Tacoma 40 SC and Heron 5 EC, two bacterial fermented insecticides Libsen 45 SC and Suspend 5 SG and one mixed formulation Lumectin 10 WDG on the mortality of papaya mealybug under laboratory conditions.

2. Materials and methods

2.1 Experimental site: Experiments were conducted in the laboratory of Department of Entomology, BAU, from the period of February to May, 2018.

2.2 Host Plant

Thirty days old healthy and uninfested papaya seedlings along with polythene bag (*Carica papaya*) (variety: Sinta, Lal Teer Seed Limited, Bangladesh) were collected from local nursery and used as experimental host plant. After collection, the seedlings were kept in laboratory for about 10 days for settlement or adaptation and thereafter seedlings were used for experimental purposes.

2.3 Collection of papaya mealybug

Papaya mealybugs were collected from untreated papaya plant with leaves. Then insects were released on previously grown papaya seedlings until their settlement or adaptation in the laboratory condition.

Table 1: Specification of bacterial fermented insecticides and insect growth regulators

Trade name	Chemical name	Doses	Group/ Family
Tacoma 40 SC	Buprofezin	0.5 and 1.0 ml/L	Insect Growth Regulator (IGR)
Heron 5 EC	Lufenuron	1.0 and 1.5 ml/L	Insect Growth Regulator
Suspend 5SG	Emamectin benzoate	1.0 and 1.5 g/L	Avermectin
Libsen 45 SC	Spinosad	0.50 and 0.75 ml/L	Spynosin
Lumectin 10 WDG	Lufenuron + Emamectin benzoate	1.0 and 1.5 g/L	IGR + Avermectin

2.4 Experimental procedure

To test the efficacy, both plants and insects were treated with selected IGRs or microbial biopesticides using micropipette or microsprayer. At first, papaya seedlings were treated with selected insecticides with specific concentrations using microsprayer. The spraying was done in such a way that the whole plant was thoroughly covered by spray material. After that, the treated plants were air-dried thoroughly using electric fan. Secondly, ten mealy bugs were directly treated with selected insecticides using micropipette. Then, treated insects were carefully transferred on treated papaya plants using fine camel hair brush and special care was taken during transferring mealy bugs to avoid any injury. After the release of mealy bugs, each plant was placed individually on a special plastic tray with an inner and outer ring. The plant was placed on inner ring and the outer ring was filled with detergent water to prevent mealy bugs escaping. Laboratory experiments were consisted of five treatment combinations. Four doses of each insecticide were provided as treatments. Each treatment was replicated thrice and ten mealy bugs were used for each replication. Care was taken to avoid spray drift on adjacent plants. The control plants were sprayed only with water. In this experiment, weight changes of papaya mealy bugs were also observed. For that, pre-treated and after-treated (3 and 7 DAT) weights of mealybugs were measured using precision balance (Mettler, Toledo, Germany).

2.5 Data collection

The data on the number of adult survived per plant under individual treatment were recorded after 1, 3 and 7 DAT (Days after treatment) application. Finally, the mortality of papaya mealybug was calculated with the following formula:

% mortality = Po/Pr × 100

Where,

Po = Number of mealy bugs died

Pr = Number of treated mealy bugs provided

2.6 Statistical analysis

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance (ANOVA) was done with the help of computer package MSTAT. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD).

3. Results

3.1 Efficacy of different doses of Tacoma 40 SC (Buprofezin) on the mortality of papaya mealybug

The effect of different doses of Tacoma 40 SC on the mortality of papaya mealybug is shown in the Table 2. The mortality was found to be dose and time dependent. Significant level of mortality was found at 1 DAT which was further increased at 3 DAT and reached to the peak level by 7 DAT. The maximum, 100% mortality was recorded from the dose 1.0 ml/L which was followed by 0.75 ml/L (96.67%), 0.50 ml/L (80.83%) and 0.25 ml/L (63.0%) of Tacoma 40 SC respectively at 7 DAT.

Table 2: Mean percent mortality of papaya mealybug, *P marginatus* at different timeinterval after treating with different concentrations of Buprofezin (Tacoma 40 SC)

Treatments	Mean percent mortality of *P. marginatus* at different DAT		
	1	3	7
Tacoma 40 SC @ 0.25 ml/L	27.50b	37.50b	63.00c
Tacoma 40 SC @ 0.50 ml/L	37.50ab	69.17a	80.83b
Tacoma 40 SC @ 0.75 ml/L	44.16a	71.67a	96.67a
Tacoma 40 SC @ 1.0 ml/L	45.83a	76.50a	100.00a
Control	4.17c	8.33c	12.50d
CV (%)	14.87	12.14	15.35
SD	0.15	1.08	0.89
Significance level	**	**	**

[In a column, means followed by different letters are significantly different. **means at 1% level of probability, DAT: Days after treatment.]

3.2 Efficacy of different doses of Heron 5 EC (Lufenuron) on the mortality of papaya mealybug

The direct effect of different doses of Heron 5 EC on the mortality of *P. marginatus* has been shown in the table 3. The insect mortality reached to the 75.83% by 3 DAT and 87.66% by 7 DAT when papaya mealybugs were directly treated with 2.0 ml/L of Heron 5 EC while about 43.33% and 61.67% mortality was found in 3 DAT and 7 DAT respectively at 0.5 ml/L of Heron 5 EC.

Table 3: Mean percent mortality of *P. marginatus* at different time interval after treating with different concentrations of Lufenuron (Heron 5 EC)

Treatments	Mean percent mortality of *P. marginatus* at different DAT		
	1	3	7
Heron 5 EC @ 0.5 ml/L	30.83 c	43.33 c	61.67 c
Heron 5 EC @ 1.0 ml/L	40.83 b	60.67 b	71.66 b
Heron 5 EC @ 1.5 ml/L	56.67 a	73.33 a	84.17 a
Heron 5 EC @ 2.0 ml/L	57.50 a	75.83 a	87.66 a
Control	4.17d	4.17 d	8.33 d
CV (%)	10.92	12.50	12.14
SD	2.24	1.78	1.68
Significance level	**	**	**

[In a column, means followed by different letters are significantly different. **means at 1% level of probability, DAT: Days after treatment]

3.3 Efficacy of different bacterial fermented insecticides on the mortality of *P. marginatus*

3.3.1 Effects of Suspend 5 SG on the mortality of *P. marginatus*

The mortality of papaya mealybug was significantly increased when papaya mealybugs were directly treated with different concentrations of Suspend 5 SG ($P<0.01$; Table 4). The highest mortality was found at 7 DAT. About 76.66% mortality was recorded @ 1.25 g/L of Suspend 5 SG which was followed by 1.0 g/L (71.66%), 0.75 g/L (63.33%) and 0.50 g/L (53.33%) respectively. It was clearly observed that the mortality level was not reached to >80-90% even with maximum doses at 7 DAT which confirms that Emamectin benzoate (Suspend 5 SG) is moderately effective against papaya mealybug. Papaya mealybug is absolutely a sucking insect which suck the cell sap from the plant/leaf. On the other hand, Suspend 5 SG is basically a contact insecticide which might be the possibility of getting moderate mortality from Suspend 5 SG.

Table 4: Mean percent mortality of *P. marginatus* at different time interval after treating with different concentrations of Emamectin benzoate (Suspend 5 SG)

Treatments	Mean percent mortality of *P. marginatus* at different DAT		
	1	3	7
Suspend 5 SG @ 0.50 g/L	46.67 b	50.00 b	53.33 c
Suspend 5 SG @ 0.75 g/L	53.33 ab	55.33 b	63.33 b
Suspend 5 SG @ 1.00 g/L	60.00 ab	70.00 a	71.66 a
Suspend 5 SG @ 1.25 g/L	66.67 a	73.33 a	76.66 a
Control	3.33 c	3.33 c	10.00 d
CV (%)	12.30	13.39	12.04
SD	2.04	1.64	1.57
Significance level	**	**	**

[In a column, means followed by different letters are significantly different. **means at 1% level of probability, DAT: Days after treatment]

3.3.2 Effects of Libsen 45 SC on the mortality of *P. marginatus*

The direct effect of different doses of Libsen 45 SC on the mortality of *P. marginatus* has been shown in the table 5. The results clearly revealed that Libsen 45 SC had significant effect ($P<0.01$) on the mortality of *P. marginatus* and the effect was clearly dose and time dependent. About 90% mortality was found at 7 DAT with the concentration of 0.75 ml/L of Libsen 45 SC which was followed by 0.50 ml/L (85.00%), 0.25 ml/L (75.00%) and 0.125 ml/L (68.33%) respectively.

Table 5: Mean percent mortality of *P. marginatus* at different time interval after treating with different concentrations of Spinosad (Libsen 45 SC)

Treatments	Mean percent mortality of *P. marginatus* at different DAT		
	1	3	7
Libsen 45 SC@ 0.125 ml/L	53.33b	63.33c	68.33c
Libsen 45 SC@ 0.25 ml/L	60.00b	69.33bc	75.00bc
Libsen 45 SC@ 0.50 ml/L	76.67a	78.33ab	85.00ab
Libsen 45 SC@ 0.75 ml/L	76.67a	86.66a	89.41a
Control	3.33c	6.67d	6.67d
CV (%)	10.056	11.65	13.47
SD	2.03	1.47	1.07
Significance level	**	**	**

[In a column, means followed by different letters are significantly different. **means at 1% level of probability, DAT: Days after treatment]

3.3.3 Effects of Lumectin 10 WDG on the mortality of *P. marginatus*

The mortality of papaya mealybug was significantly increased when papaya mealybugs were directly treated with different concentrations of Lumectin (P<0.01; Table 6). The mortality of papaya mealybug was gradually increased with increasing concentration level. Significant instant mortality was achieved within 1 day for the application of Lumectin where the mortality was 76.67% at 1.25 g/L. Lumectin 10 WDG provided 100% mortality of mealybug in 3 DAT and 7 DAT at 1.25 g/L and no mealybugs were recorded in the treated plants. Lumectin 10 WDG showed highest efficacy against papaya mealybug and this insecticide was able to kill 100% of papaya mealybug in 3 DAT and 7 DAT. Lumectin is the commercial mixture of Lufenuron (an IGR) and Emamectin benzoate. Emamectin benzoate has provided moderate mortality against papaya mealybug but this mortality level reached to 100% when Emamectin benzoate was added with Lufenuron. It was also confirmed that there has a synergistic effect between Emamectin benzoate and Lufenuron and this combination potentially increased mortality level of *P. marginatus*.

Table 6: Mean percent mortality of *P. marginatus* at different time interval after treating with different concentrations of Lumectin 10 WDG

Treatments	Mean percent mortality of *P. Marginatus* at different DAT		
	1	3	7
Lumectin 10 WDG @0.5 g/L	43.33c	60.00c	63.33c
Lumectin 10 WDG @0.75 g/L	56.67b	76.67b	80.00b
Lumectin 10 WDG @1.0 g/L	63.33b	93.33a	96.67a
Lumectin 10 WDG @1.25 g/L	76.67a	100.00a	100.00a
Control	3.33d	3.33 d	6.67d
CV (%)	8.91	4.24	5.25
SD	3.04	1.50	1.41
Significance level	**	**	**

[In a column, means followed by different letters are significantly different. **means at 1% level of probability, DAT: Days after treatment]

4. Discussion

In the present study it has been observed that Tacoma 40 SC as well as Heron 5 EC worked as potent insect growth regulator (IGR) against papaya mealybug (*P. marginatus*). Tacoma 40 SC and Heron 5 EC were applied directly on the papaya mealybug and it has been clearly investigated that Tacoma 40 SC worked more potently than Heron 5 EC. The mortality was significantly increased at 3 days after treatment (DAT) application that further increased at 7 DAT which suggests that it takes longer time to inhibit cuticle synthesis through the interaction of IGR and insects intercellular molecules. Unlike traditional chemical insecticides, Tacoma 40 SC (Buprofezin) reduces pest population by preventing moulting through the inhibition of chitin bio-synthesis. Chitin is a major component of the insect exoskeleton. Insects poisoned with IGR are unable to synthesize new cuticle, thereby preventing them from moulting successfully to the next stage and ultimately leading to death by fracturing the cuticle [16]. From previous findings it has been found that the effect of Buprofezin on the mortality is dose and method dependent and Award 40 SC (Buprofezin) is highly potential against papaya mealybug [6]. The mortality of papaya mealybugs was approximately 82 and 96% after given first and second spray in the laboratory. Significant and profound effect of Buprofezin was also found from the field study [13]. From the laboratory work, Lumectin 10 WDG showed maximum mortality compared to Libsen 45 SC or Suspend 5 SG. Suspend 5 SG and Libsen 45 SC are bacterial fermented biopesticides while Lumectin 10 WDG is the mixture of Lufenuron and Emamectin benzoate. Suspend and Libsen are ideally contact insecticides and therefore, the mortality level was found to be between 70 to 90% against papaya mealybug. In contrast, mortality level reached to 100% at 7 DAT when papaya mealybugs were treated with Lumectin 10 WDG @ 1.25 g/L. This raises the possibility that both Suspend and Lufenuron synergistically worked against papaya mealybugs and therefore mortality level reached to 100%. Emamectin benzoate has provided moderate mortality against papaya mealybug but this mortality level reached to 100% when Emamectin benzoate was added with Lufenuron. It was also confirmed that there has a synergistic effect between Emamectin benzoate and Lufenuron and this combination potentially increased mortality level of *P. marginatus*. From previous findings it has been found that the combination of deltamethrin with Emamectin benzoate showed synergistic effect than individual application of Emamectin benzoate on the cotton mealybug, *Phenacoccuss olenopsis* [10, 14].

Libsen 45 SC (Spinosad) is a microbial derived insecticide which activates the central nervous system of insects resulting in paralysis and death of Hemipterans such as *Aphis craccivora*. High concentration of Libsen (Spinosad) moderately reduced the percentage of adult emergence of

Aphis craccivora in cowpea. In direct spray method, the adult mortality of *Aphis craccivora* was moderate in Spinosad treated leaves after 48 hours which is in agreement with our present findings [8]. From previous findings it has been found that Spinosad provided moderate control in sucking pests of okra with mean population of 6.33 whitefly/15 leaves [12].

5. Conclusion

Considering the percent mortality, Tacoma 40 SC and Lumectin 10 WDG were found to be the best where 100% mortality was recorded. Next, Libsen 45 SC, Heron 5 EC and Suspend 5 SG were found to be moderately effective regarding the mortality of *P. marginatus*. Therefore, it can be concluded from the present study that the selected IGRs, Libsen 45 SC and Lumectin 10 WDG would be the alternate option of conventional insecticides to control papaya mealybug effectively. Suspend 5 SG (Emamectin benzoate) can be applied as an integral component of IPM program rather than its individual application. Field trials are needed to verify these laboratory findings.

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