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Research Article

A biological approach for management of greater wax moth, *Galleria mellonella* L. using *Bacillus thuringiensis*

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ABSTRACT: Bee keeping has taken a shape of promising enterprise and also becoming popular as one of the components in mixed farming systems. *Galleria mellonella* L. damage is the major biological constraint in the beekeeping industry. Microbial agent *Bacillus thuringiensis* and its products have been tried widely against *G. mellonella*. Our previous study has detected six potential bacterial isolates *viz*., C7, A3, A7, N12, F2 and M2 active against *G. mellonella*. The effectiveness of these isolates on the stored honey combs against the *G. mellonella* was evaluated and the safety of these potential bacterial isolates was tested against honey bees and silk worms. The protection range provided by the isolates varied from 89.5–44.3 per cent and the isolate M2 (89.52%) and standard HD-1 (88.89%) rendered very good protection to the combs from wax moth damage. All the six isolates tested were found safe to honey bee adults and also to larvae indicating that the isolates can be safely deployed under field conditions. Two of the six isolates tested *viz*., M2 and N12 were toxic to silk worms. The study demonstrated the potentiality of *Bt* isolates against *G. mellonella* and their safety to the honey bees and silkworms.

KEY WORDS: *Galleria mellonella, Bacillus thuringiensis,* silk worms, honey bees

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INTRODUCTION

For beekeepers worldwide, the greater wax moth, *Galleria mellonella* L. is the most important pest because of the serious losses it can inflict (Smith, 1960; Singh, 1962). They destroy a large number of combs every year, attack the wax foundation and reduces stored combs and weak colonies to a pile of debris. The microbial alternatives to chemical insecticides include a variety of biological agents such as bacteria, viruses and fungi. These agents essentially function as pathogens of target insects causing infections leading to the death of the host insect in the process. Of all the various microbial agents that have been evaluated, the most successful by far, has been the *Bacillus thuringiensis* Berliner, more commonly referred as *Bt*. Since *B. thuringiensis* is being considered as potential control method against greater wax moth *G. mellonella*. Whenever insect pathogens are considered as candidates for biological control, the effects of these organisms on beneficial insects, especially the honeybee and silk worms should be determined (Cantwell, 1964). Therefore, this study was conducted to test the *Bt* isolates in preventing infestation of *G. mellonella* on stored honeycombs and also to evaluate the *Bacillus* isolates for their safety to honey bees and silk worms.

MATERIALS AND METHODS

Preparation of bacterial formulation

Six bacterial cultures those found active on the *G. mellonella* (Taredahalli. *et al*., 2012) were selected for the further evaluation and bio safety studies. Talc based formulation of the six *Bt* cultures namely C7, N12, F2, A3, A7, and M2 was prepared (Ninfa and Rosas-garcia, 2009) and kept in the polythene covers for further use.

Testing the *Bt* isolates in preventing infestation of *G. mellonella* on stored honeycombs

Deserted rock bee combs were fumigated with EDB to prevent the combs from infestation before testing. Each sample formulated was mixed with water (1: 20, wt/wt) and the emulsion was subsequently sprayed on pieces of stored combs with the hand sprayer. Known weight of comb pieces (2.5" x 2.5") were treated the *Bt* formulation. After spraying, the comb was allowed to dry at room

temperature and inoculated with 20 third instar larvae per treatment and treatments were replicated thrice. The controls consist of water sprayed combs. After 20 days, combs were observed for the wax moth damage and the final weight of comb was observed (Cantwell and Shieh, 1981).

Testing the effect of *Bt* isolates on honeybee larvae and adults

The potential bacterial isolates active against *G. mellonella* were further tested on honey bee brood and adults to check their safety. Brood safety evaluation can be done by adding five micro liter of bacterial culture to the two day old brood cell. Graph sheet print taken on the transparent film A4 (210 x 297 mm) was used for marking the brood cell. Treated brood cells were observed for sealing at fourth day of treatment. Mortality was recorded by observing the unsealed brood and corrected according to Abbott (1925).

Adult bees were tested by the food contamination method. Fifty percent honey solution was contaminated with 1 ml of *Bt* isolates. The group of ten acclimatized bees per replication was placed into the wire mesh cages 12.5 x 12.5 x 12.5 cm and the cages were kept in incubator at 35°C and 50% RH in order to imitate the natural habitat of the bees. Experiment was carried out for four days, each day fresh feed with *Bt* isolates was given and mortality was recorded.

Toxicity of *Bt* isolates to silk worm

One ml of five day old *Bt* spore culture with one drop of Teepol® (an adhesive agent), was spread uniformly on the mulberry leaves using a camel hair brush. Treated leaves were air dried in shade and fed to the silk worms. Ten early first instar larvae per each replication were released in petri-plates and each treatment was replicated three times. Mortality rate was recorded on each day and experiment was continued for 3 days.

RESULTS AND DISCUSSION

Effect of *Bt* isolates in preventing infestation of *G. mellonella* on stored honey combs

The results of the present study clearly showed the protection range varied from 89.5 – 44.3 per cent. Among the six isolates obtained, isolate M2 could reduce the damage to a maximum of 89.5 per cent to the stored honeycombs. Minimum protection of 44.3 per cent was observed in F2. On the other hand, control combs were totally destroyed and showed heavy wax moth damage. There was significant difference between the per cent mortality of larvae and weight of the larvae in treated combs compared to the control. These results substantiate the possibility of deploying *B. thuringiensis* isolates for obtaining protection against *G. melonella*, as has been suggested earlier. Some serotypes of *B. thurigniensis* when treated were also observed to give protection up to 2 years at 0.5 per cent in wax and partial protection for 6 years at 2 per cent (McKillup and Brown, 1991; Burges, 1981). Wax combs sprayed with the Certan recorded less damage by *G. mellonella* upto three months in storage (McKillinup and Brown, 1991). The application of 3g spore-crystal complex of *B. thuringiensis* subspecies *thuringiensis* by spray inside bee hive gave protection against the wax moth for minimum period of 52 days (Burges *et al.,* 1976).

Safety to honey bees

Since the *Bacillus* cultures active against *G. mellonella* should be safe to bees for deploying them in the bee hives, the six identified isolates from our previous report were tested for their activity against honey bees, both larvae and the adults. Analysis of variance indicated no significant treatment effect with respect to the mortality both in case of honey bee larvae and adults. The mortality of the honeybee larvae fed with the *Bt* crystal protein from isolates C7 (6.67%), A3 (6.67%), N12 (6.67%), F2 (10%), A7 (13.33%), M2 (10%) and HD-1(10%) did not show any significant difference as compared to the mortality observed in control (13.33%). In case of adult bees also, the average mortality per cent in the control (43.33%) was on par with the other treatments, which indicates that there was no difference in the mortality rate due to the various isolates tested including HD1. Since the observed bee mortality was more in case of adults even though the larval stages were the most susceptible, the results obtained were not clearly conclusive. Studies by Maruthi Prasanna (2003) showed that the honey bees fed with the toxin protein extracted from 50E local isolate of *Bt* did not show significant difference in the mortality of bees fed with the toxin protein (73.33%) as compared to the mortality observed in the control (73.83%) fed with only honey without toxin. This is in total agreement with other investigators who exposed bees to various *Bacillus* species and varieties of *B. thuringiensis* now in commercial use or those being considered for use as microbial control agents (Cantwell *et al*, 1966 and Krieg, 1973) against pests of honeybee. *Bt* formulations based on ä-endotoxins (Cry proteins) are assumed to be highly specific and have negligible effects on non-target organisms because of their limited bioactivity under field conditions (Ignoffo and Garcia, 1978). Bees fed

Table 1. Testing the *Bt* isolates in preventing infestation of *Galleria mellonella* on stored comb

Bt isolates	Average weight before treatment (Grams)	Average weight at the end of experiment (Grams)	Wt loss (Grams)	% comb damage
C7	3.32	2.73	0.58	17.59
N12	3.53	2.60	0.93	26.42
A7	3.87	1.80	2.07	53.45
M2	3.47	3.10	0.37	10.58
A3	3.23	2.67	0.57	17.52
F2	3.47	1.50	1.97	56.73
HD1	3.90	3.46	0.43	11.11
Control	3.93	0.00	3.93	100.0

Table 2. Bioassay studies on *Bacillus* isolates for their safety to two day old honey bee larvae and adults

Sl. No.	Isolate No.	Cumulative percent larval mortality	No. of test insects per replication	Adult Mortality at DAT		
				2 day	3 day	4 day
1	C7	6.67	10	10 .00	23.33	36.66
2	A3	6.67	10	13.33	23.33	46.66
3	N12	6.67	10	6.67	20.00	46.66
4	F2	10.00	10	10.00	23.33	43.33
5	A7	13.33	10	3.34	16.66	43.33
6	M2	10.00	10	10.00	26.66	40.00
7	HD1	10.00	10	13.33	20.00	46.66
8	Control	13.33	10	13.33	20.00	43.33
	F value	1.41	NS	NS	NS	NS

Table 3. Bioassay studies on *Bacillus* isolates for their safety to neonate larvae of silkworm

Sl. No.	Isolate No	% mortality of silkworm larvae		
		After 24 hours	After 48 hours	After 72 hours
1	C7	0.00	0.00	0.00
2	A3	3.33	10.00	10.00
3	N12	46.66	46.66	46.66
4	F2	10.00	10.00	10.00
5	A7	6.00	10.00	10.00
6	M2	56.7	56.7	56.7
7	HD-1	80.0	100.0	100.0
8	control	0.00	0.00	0.00
F value	6.90*	11.4*	11.4*	
SEm	1.14	0.66	0.66	
CD @ 5%	3.14	1.99	1.99	

with purified Cry1Ba toxin at a rate approximating expression in pollen of 0.25 per cent (of total soluble protein) had similar longevity and flight activity to the control bees. This confirms previous studies showing that bees were unaffected when fed on purified *Bt* gene products (Malone *et al*., 1999).

Safety to silkworms

Experiments were also carried out in this study to evaluate the safety of the effective *Bt* isolates to the silkworm, since this would be a pre requisite for any move towards exploitation of the promising isolates in the State of Karnataka. The results of this experiment showed that two (M2 and N12) isolates among the six tested were toxic to the silkworms. Similar results were also reported by van Frankenhuyzen *et al*. (1993) which indicated the susceptibility of silkworm to Lepidoptera specific *Bt* isolates in general. The four isolates namely C7, F2, A7 and A3 did not show any mortality which is explained by the lack of broad-spectrum cross infectivity among Lepidoptera specific *Bt* isolates (Bernhard *et al*., 1997). The results clearly illustrate two different patterns in activity spectrum among Lepidopteran species. Nevertheless, the study clearly point to the possibility of developing a *B. thuringiensis* isolate that is both effective against *G. mellonella* and also being safe to honey bees, both larvae and adults in the first place and then to the silk worms, the other most important insect resource for the farmers of the State.

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