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ORIGINAL ARTICLE

Bio-insecticidal Potentiality of Chitinase Extracted from *Amycolatopsis Orientalis A13 sp. nova* Actinomycete Against *Galleria Mellonella*

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Abstract

Chitin is the outermost layer surrounding all arthropod's bodies as the primary protective layer against any adverse conditions. Chitinase is a group of enzymes responsible for degrading the old chitin layer. The current study was conducted under laboratory conditions to evaluate the insecticidal potentiality of chitinase filtrate extracted from *Amycolatopsis orientalis A13 sp*. nova actinomycete against the 3rd and 5th larval instars of the greater wax moth, *Galleria mellonella*. A series of aqueous concentrations have been prepared (25, 50, 100, 150, and 200 µl/g) to carry out the current bio-assay experiment. The cumulative larval death percentages were concentration-dependent, i.e., the highest larval mortality (94.68%) got detected at the highest applied concentration (200 µl/g). LC50, LC90, and LC95 values of chitinase filtrate were recorded at 57.94, 187.82, and 262.14 after 37 days of exposure time, respectively. The obtained data also revealed that the higher the applied chitinase concentration, the longer the treated larvae and pupae duration. The calculated nutritional indices (Relative growth rate (RGR), Relative consumption rate (RCR), efficiency of conversion of ingested food to body substance (ECI), efficiency of digested food conversion (ECD%), and Approximate digestibility (A.D.) showed significant variation at the LC50 value. The results showed that such laboratory trials accredited the chitinase filtrate as a promising bio-agent. However, further studies should be conducted to prove its efficacy under semi-field and field conditions.

Keywords: *Amycolatopsis orientalis A13 sp*. nova, Biocontrol, Chitinase activity, Galleria mellonella, Insecticidal activities

1. Introduction

All arthropods have an exterior coat called chitin that serves as their main barrier against the elements. The insect growth regime coincides with replacing the old chitin layer with a new one to fit the newly emerged insect stage. The old chitin layer is degraded by a group of enzymes that includes chitinase. Accordingly, any disruption in the chitin synthesis process will have negative returns on insect development and can be used in insect pest control. In this approach, a lot of eco-friendly pesticides have been adopted to target chitin synthesis as a novel site of action with minor environmental impacts [1]. The demand for safer alternatives for pest management has become more urgent in order to avoid the negative repercussions of chemical pesticides in terms of resistance and cross-resistance phenomena and to exploit the novelty of such eco-alternatives against pests [2,3] stated that chitinase succeeded in damaging the peritrophic membrane of insect gut inducing nutrient utilization disorders and insect growth retardation. In the same context [4], observed an inhibition growth pattern of the tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae),

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following the nourishment of its larval stage on the chitinase-contaminated diet. Similar output got stated by [5] on the phytophagous ladybird beetles (Coleoptera: Coccinellidae). In general, chitinases are crucial for controlling pathogens and pests. This chemical in the pathogen is an apparent pest management target as chitin production is confined to insects, fungi, and certain algae, many of which are plant pathogens [2].

As microbial chitinases proved to have high potential as biocontrol agents against fungi, nematodes, and insect pests [6], such microorganisms are considered useful reservoirs for chitinolytic enzymes [7]. Out of such microorganisms, actinobacterium is among the most important taxa in the chitinolytic community of soil microbes [8–10]. It stands out as having promising potential for the production of chitinases [11,12], antibiotics, and secondary metabolites [13]. *Amycolatopsis orientalis A13 sp. nova* (Pseudonocardiales: Pseudonocardiaceae) is a Gram-positive actinomycete known for its capability to produce a wide variety of secondary metabolites, including antibiotics, antitumoral agents, insecticides, and hydrolytic enzyme [14,15].

The bioassays need certain insect species to act as laboratory models. In this concern, different insect species have been adopted for the purpose of studying the microbe-host interaction. The larval stage of the greater wax moth, *Galleria mellonella* (*G mellonella*) (Lepidoptera: Pyralidae), got nominated as an effective model. The ease of handling and laboratory production of *G. mellonella* larvae as well as the competitive breeding cost, are the main causes for the adoption of the greater wax moth as an ideal laboratory model [16].

Accordingly, the current study has been designed to examine the insecticidal potentiality of *A. orientalis A13 sp. nova* using *G. mellonella* as a laboratory model.

2. Materials and methods

2.1. Laboratory production of greater wax moth, G mellonella

The greater wax moth, *G. mellonella* was obtained from a colony maintained at the insectaries of the Plant Protection Department, Desert Research Center, Cairo, Egypt. Larvae were reared on an artificial diet described by [17]. *G. mellonella* rearing program was incubated under $30 \pm 2\,^{\circ}C$ and $70 \pm 5\%$ RH conditions.

2.2. Source of crude chitinase enzyme

A. orientalis (*A orientalis*) *A13 sp. nova* was isolated from a soil sample collected from Saini, Egypt [18]. The activity of crude chitinase produced by *A orientalis A13 sp. nova* was found to be 1130 U/mg and used thoroughly this study.

2.3. Bioassay examination

2.3.1. Insecticidal efficacy of the crude chitinase

The insecticidal efficacy of the crude chitinase has been investigated through bio-assaying five aqueous concentrations (25, 50, 100, 150, and 200 μl/g). The artificial diet got more homogenized with each concentration. Such an amount of diet had been equally distributed among 50 glass tubes (2 g diet/per tube and 5 replicates/per concentration). One 1st instar was individually transferred to each tube using a fine hairbrush and then plugged by the cotton stopper. A similar amount of untreated diet was treated with distilled water and distributed on 50 glass tubes to serve as check treatment. Both treated and checked trials were examined daily to determine the death and the longevity of the larval, pupal, and adult stages. The observed death percentages were corrected using Abbott's formula [19].

2.3.2. Effect of crude chitinase on larval feeding indices

In order to carry out the current experiment, 50 newly emerged 3rd and 5th instars of *G. mellonella* larvae got weighed then both instars were subjected to 3 h starvation schedule. At the same time, 2 gm of the artificial diet was mixed with 0.115 ml of the LC_{50} solution of crude chitinase. Thereafter, the chitinase-contaminated diet was equally distributed on 5 petri dishes (90 mm) (5 larvae/per Petri dish). Following the starvation period, 25 larvae from each instar were weighed, and each larval group was separately transferred to one Petri dish. Five replicates were used during the experiments. Each larval group cohort took the chance for 24 h feeding period then the uneaten amount was weighed and replaced by a fresh amount of the artificial diet. Such a procedure was repeated daily for 3 days. Each 24 h, both larval and fecal pellets were weighed using 4 digits electronic balance (Shimadzu, Japan) to determine food consumption and body weight gain. The food ingestion was estimated by subtracting the diet remaining at the end of the experiment from the total weight of the artificial media. Fecal pellets were collected and weighed to estimate the weight of the fecal material. The following calculations were performed according to [20].

Relative consumption rate (RCR) = weight of eaten food/feeding duration (days) x mean larva weight during the feeding period relative growth rate (RGR) = weight gain of the larva during the

feeding period or duration of feeding (days) × mean weight of the larva during the feeding period conversion efficiency of ingested food to body substance (CEI %) = $\frac{RGR}{RCR} \times 100$

Approximate digestibility (A.D.) = 100 × (weight of eaten food - weight of produced feces)/weight of eaten food.

Growth inhabitation = $\frac{GL-TL}{GL} \times 100$, where

GL = Larval weight gain in control and TL = Larval weight gain in treatment [21].

2.4. Statistical analysis

The data were analyzed using a set of analytical techniques, including the independent t-test and Analysis of Variance and Tukey's HSD pairwise comparison using the SPSS (V. 26) statistical program. In addition, all data were graphically represented as the mean ± SE using Microsoft Excel 2010.

3. Results and discussions

3.1. Insecticidal efficacy of the crude chitinase

An aqueous extract of the crude chitinase was subjected to a laboratory bioassay to examine the toxicological efficiency of its prepared concentrations against the 3rd and 5th instars of *G. mellonella* larvae as an eco-alternative to the synthetic insecticide (Table 1). Both larval and pupal durations showed proportional increases with the applied concentrations. That is to say, as the treatment with the highest concentration (200 µl/g) caused the elongation of the larval duration to be ~37 days, the larvae treated with the lowest concentration succeeded in completing their duration in ~21 days. All calculated larval durations fulfilled significant differences at the all-applied concentrations. A similar trend has been noticed for the pupal age. Where the treated pupae got aged about 27 and 9 days following their subjection to 200 and 25 µl/g, respectively (Table 1). Upon lightning the corrected larval death percentage, the obtained data proved the superiority of the highest applied concentration of the crude chitinase (200 µl/g) as larvicide compared with the remaining ones (94.68 corrected larval death percentage at 200 µl/g. The potentiality of the crude chitinase to kill 50, 90 and 95% of the tested larval population (LC50, LC90, and LC95) were 57.94, 187.82, and 262.14, respectively (Table 1), and the data has been graphically represented in Fig. 1 based on Probit analysis. The response of adult emergence to the applied concentrations took a contrary path compared with the immature (larva and pupa) stages. The highest applied concentration met the lowest moth emergence percentage (5.32 adult emergence percent at 200 µl/g).

3.2. Effect of crude chitinase on larval feeding indices

The context of the study illustrated the feeding and digestibility disorders induced by the crude chitinase on the 3rd and 5th instars of *G. mellonella* larvae following their nourishment on an artificial diet contaminated by the LC_{50} concentration of the crude chitinase. The obtained data revealed significant retardation of RCR and RGR values in both treated instars compared with the control trial. On the same approach, the larval instars that experienced the LC_{50} concentration suffered an obvious declination in the calculated percentages of ECI and ECD values upon comparison with the check treatment. Compared to the control, the digestibility of treated 3rd instar larvae increased while decreasing in the 5_{th} instar.

The ECI was greater in the third instar (3.86%) than in the fifth instar (1.91%) (Table 2). This meant that the third instar larvae devoured more food in relation to their body weight. At the same time, the efficiency of digested food conversion (ECD, or the amount of energy allocated to the maintenance of physiological activities of the organism) declined somewhat from the third to the fifth instars (6.59%) (6.34%). The relative growth rate and approximate digestibility were found to be greater in early instars, indicating that dietary energy is being saved for future development.

Table 1. Efficacy of crude chitinase on the larval, pupal, and adult stages of G. mellonella.

Conc. (µl/g)	Larval duration days ± St. Error	Pupal duration days ± St. Error	Obs. mortality%	Corr. mortality%	Adult emergence%	LC (µl/g)	
0	17.20^{a}±0.37	8.60^{a}±0.25	6.00	0.00^{a}	100.00^{a}	LC_{50}	57.94
25	21.20^{b} ± 0.37	8.80^{a}±0.20	24.00	19.15^{b}	84.04^{b}		
50	24.80^{c}±0.37	12.80^{b} ± 0.20	52.00	44.68^{c}	55.32^{c}	LC_{90}	187.82
100	26.40^{d} ± 0.80	13.60^{b} ± 0.25	69.00	67.02^{d}	32.98^{d}		
150	29.60^{e}±0.32	18.00^{c}±0.32	85.00	84.04^{e}	15.96^{e}	LC_{95}	262.14
200	37.00^{f}±0.51	26.80^{d} ± 0.66	95.00	94.68	5.32^{f}		

Means (±standard error) followed by different letters within a column are significantly different at $P < 0.05$ according to Tukey's test.

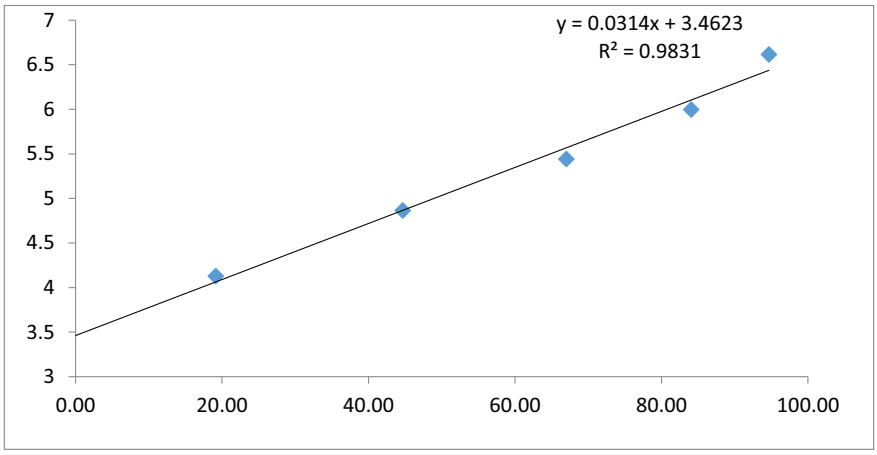

Fig. 1. Toxicity regression line of the G. mellonella larvae treated with crude chitinase.

Based on this interpretation, it was noted that the third-instar-treated larvae's percentage of approximate digestibility (47.43 ± 8.48) was greater than the control larvae's (41.65 ± 0.84). On the contrary, the treated fifth-instar larvae (30.51 ± 2.42) were significantly larger than the control (47.27 ± 0.88).

4. Discussion

The main purpose of this study is to control pests such as *G. mellonella*, which is known for its pest of honeybees and their hives and leads to economic loss. The present work states the in vitro potential of the selected actinomycete strain to control the above pest. Insecticides with a better environmental profile, different modes of action, and lower risks to living systems have resulted from biological and physiological research. Chitinase-producing microbes, for example, may influence development by interfering with the digestive systems of a variety of potentially harmful insects.

The findings of the work described the chitinase isolated from *A. orientalis A13 sp. nova* also found to be very effective against the *G. mellonella because* the chitin molecule in the pathogen is an obvious pest control target. This outcome is consistent with Aggarwal et al. (2015), when *Spodoptera litura* larvae feeding on *Serratia marcescens* experience a substantial influence on proper pupation and adult emergence as a dose-dependent decrease in the larval and pupal time.

Different concentrations of chitinase showed larval mortality percent increased by enzyme filtrate concertation increase to record the highest mortality percent (94.68%) with a concentration of 200 U/ml after 15 days and determination of Lethal concentrations this results from harmony with [6] when observed that insect mortality of The tea mosquito bug (TMB) was 100% after 96 h. When the concentration of *Pseudomonas fluorescens MP-13* chitinase increased from 0.012 to 100% at 0.048 U/ml of purified chitinase.

In our study, we noted an increase in larval and pupal duration by increasing concentrations [22] found that When *S. litura* larvae feed on the chitinolytic bacteria *S. marcescens*, there is a dose-dependent reduction in the larval and pupal period, as well as a significant effect on normal pupation and adult emergence [23] reported that the effect of chitinase isolated from *Aeromonas hydrophila* was evaluated against *G. mellonella* at different concentrations, increased larval and pupal mortality rates in a concentration-dependent manner, and induced a decrease in adults' emergence rate and their fecundity.

Table 2. Efficacy of the LC_{50} of crude chitinase on the feeding indices of G. mellonella larval stage.

Conc. (U/mg protein)	RCR mg/mg/day±SE	RGR mg/mg/day±SE	ECI (%)	ECD (%)	AD (%)
control 3rd instar	3.46 ± 0.006	0.50 ± 0.007	3.864	9.28	41.65 ± 0.84
Treated 3rd instar	2.90 ± 0.009	0.48 ± 0.004	3.12	6.59	47.43 ± 8.48
t-statistic (df)	15.44 (4)	4.80 (4)	–		−1.26 (3)
P value	0.000	0.01	–		0.298
Control 5th instar	2.48 ± 0.010	0.39 ± 0.005	3.14	6.64	47.27 ± 0.88
Treated 5th instar	1.43 ± 0.048	0.17 ± 0.005	1.91	6.34	30.51 ± 2.42
t-statistic (df)	60.04 (4)	84.38 (4)	–	–	11.30 (3)
P value	0.000	0.000	–	–	0.001

Effect of crude chitinase on larval feeding indices found that the third instar larvae devoured more food in relation to their body weight, While the ECD declined somewhat from the third to fifth instars. In addition, the relative growth rate and approximate digestibility were found to be greater in early instars, indicating that dietary energy is being saved for future development. These findings are consistent with [24] who demonstrate that treatment with *Bacillus subtilis* chitinase significantly decreased the nutritional indices (RGR, RCR, ECI, ECD, and AD percent) of third instar *S. litora* larvae compared to controls.

5. Conclusion

A. orientalis strain exhibited insecticidal and biological effects on *G. mellonella*. In laboratory trials, the chitinase filtrate was demonstrated to be a promising bio-agent. Further tests should be conducted to prove its effectiveness in semi-fields and in the field.

Declarations

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Data supporting the conclusions of this article are presented in the main manuscript.

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Conflicts of interest

There are no conflicts of interest.

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