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Nithin Vijayakumar
Department of Biotechnology,
Karpagam University,
Coimbatore, Tamil Nadu-620421

Sangilimuthu Alagar
Department of Biotechnology,
Karpagam University,
Coimbatore, Tamil Nadu, India.

Nalini Madanagopal
Department of Zoology,
Poompuzhar College, Melaiyur,
Tamil Nadu, India.

Correspondence
Sangilimuthu Alagar
Department of Biotechnology,
Karpagam University,
Coimbatore, Tamil Nadu, India.

Effects of chitinase from *Trichoderma viride* on feeding, growth and biochemical parameters of the rice moth, *Corcyra cephalonica* Stainton

Nithin Vijayakumar, Sangilimuthu Alagar and Nalini Madanagopal

Abstract
Investigations on the development of a biocontrol agent against the alarming stored food pest *Corcyra cephalonica* has witnessed noteworthy growth in current years from mainly physiological studies, to tackling fundamental queries concerning the underlying mode of action. Chitinase from *Trichoderma viride*, which has inauspicious effects on feeding behavior, development and biochemical parameters of insects. The effects of oral intake of chitinase integrated feed in IV instar *C. cephalonica* were determined by evaluating their feeding deterrence and changes in its biochemical properties. Feeding deterrence index steadily increased with a dose-dependent mode. Chitinase demonstrated the highest feeding deterrence at 229.24 ppm concentration. Our study exemplifies that treatment of stored food products with chitinase can reduce the larval feeding efficiency, deprived growth, and larval weight reduction, and reduce the number of next generation. This may serve as a powerful biocontrol agent against *C. cephalonica* and substitute to synthetic pesticides such as malathion.

Keywords: Antifeedant, biocontrol, chitinase, *Corcyra cephalonica,* feeding deterrence index

1. Introduction
Rice has shaped the society, diets and economics of thousands of millions of people. World rice production is extending across at least 114 countries [1] and rice is grown-up on 144 million farms universal more than for any other crop. For more than half of civilization rice is life and rice unquestionably counted as the most imperative food grain of India. Even though India is the second largest rice producer after China in the world, we are in front position regarding wastage of rice in the form of post-harvest storage.

Post-harvest losses can be accounted as any considerable qualitative and quantitative food loss along with the supply chain from the time of harvest till its consumption [2]. Quantitative food loss in the drop of weight of edible grain due to spillage, consumption by incidence of insect-pest, mites, rodents and birds, or from handling and physical changes in temperature, moisture content and qualitative losses come about due to, physical changes or chemical changes in fat, carbohydrates, protein, mycotoxins, pesticide residues and dead bodies [3].

Synthetic pesticides are used to control the pre/post-harvest losses. Due to the incessant usage of synthetic pesticides show the way to resistance development and most of them have been disqualified due to elevated toxicity, ozone depletion and non-biodegradable properties. Consequently, it is intelligent to opt for an eco-friendly substitute for pest management. Due to the increase in above stated ill effects, agriculturists are in hunt of pesticides with no residue deposition and no effect on non-target organisms. The development of such insecticides can be accomplished only by recognizing naturally occurring compounds having insecticidal effect. Biopesticides are less toxic than that of synthetic pesticides, often target specific, cause negligible impairment to birds and mammals. In addition, even if used in an open field, they are having the capability to degrade rapidly, in that way reduce the threat of environmental pollution.

Chitin, which is the second abundant biopolymer present in earth next to cellulose. It is predominantly present in nature and as a structural polysaccharide in fungal cell wall, the exoskeleton of arthropods and insect gut lining. Chitin which can be degrade to low molecular weight chito-oligomers by chitinases, which serve up a wide variety of biotechnological applications, agricultural, industrial and as therapeutics.

Chitinases (E.C.3.2.2.14) are hydrolytic enzymes with a wide berth of size varying from 20 kDa to about 90 kDa. Particularly chitinases have been getting an increased consideration in agricultural fields as a powerful biocontrol agent against fungal phytopathogens and dreadful insect pest. Chitinases which can be classified into two foremost categories such as endochitinases (cleave the chitin polymer at any point inside the fiber) and exochitinases (cleave from the non-reducing end of the polymer).

Insect growth and development are sturdily dependent on the construction and remodeling of chitinous structures. Chitinase bring damage to the peritrophic membrane in the insect gut causes a remarkable decrease in nutrient utilization and as a result in insect growth. Due to this, chitinase present in the insect diet can reduce insect growth. The damage persuades by chitinase to the peritrophic membrane of insect gut lining will assist pathogenic microbes to enter the hemocoel and destroys the commencement of immune responses.

Corcyra cephalonica. Stainton (Lepidoptera: Pyralidae), otherwise known as "rice moth", is one of the most widespread and serious insect pest of stored products causes severe damage. The significance of *C. cephalonica* is that they develop more rapidly on whole grains and on powdered forms. The whole developmental stages of *C. cephalonica* ranges to 47-59 days depending on the product it infests. Within this short duration of life *C. cephalonica* makes a high economic loss. Synthetic pesticides such as methyl bromide and phosphine fumigants are considered as the most common treatment to control stored pests for decades.

In the present study chitinase from *Trichoderma viride* (SIGMA-ALDRICH) was obtained and treated against *C. cephalonica.*

2. Materials and methods

2.1 Insect Rearing

The rice moth (*C. cephalonica*) eggs were acquired from Department of Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. *C. cephalonica* were raised under laboratory circumstances at 28±1 °C and more or less 65% relative humidity under 12 h light/12 h dark cycle and established on a medium together with crushed pearl millet (2.5 kg), broken up groundnut kernel (100 g), yeast (5 g), sulphur (5 g) and antibiotic[4] with slight alteration. The food media were subjected to UV-exposure in a laminar air flow chamber prior than inoculating egg. The feed (=2.5 kg) in a hygienic plastic tray (5 L), eggs (=16,000) were introduced; it was enclosed with fine cloth for aeration. All the cultures in the plastic trays were detained inside a wired mesh cupboard to avert the rodents and other insects from crawling into them.

2.2 Preparation of flour disks

Pearl millet flour (1.5 g) was placed in a 10 mL glass beaker (Borosil) with a magnetic bead. Five milliliter of an aqueous solution (Milli Q water) containing the experimental material (Chitinase from *T. viride*; SIGMA-ALDRICH) and the combination stirred magnetically. Four diverse concentrations of chitinase (62.5, 125, 250 and 500 ppm) were prepared as treatments and the aqueous solution devoid of chitinase served as control. Aliquots of 250 µl of the stirred suspension were placed on a polystyrene petri dish to form disks and left in laminar air flow chamber overnight to air dry. The disks were then moved to an incubator to equilibrate at 30±1 °C for 48 h. Individual weighed disks (range from 37-41 mg) were relocated to Petri dishes for additional bioassays.

2.3 Feeding deterrence bioassay

Bioassays were carried out in 12 h dark/12 h light at 30±1 °C. Three larvae (IV instar) (six replicates) were weighed and then set aside for three days on petri dish containing the flour disks of varied concentrations of chitinase. All the larvae were starved for 24 h prior to use. The investigational setup was left in the incubator for 3 days, after which the weights of the live larvae and disks were taken and the mortality if it occurred were recorded. The following calculations were made for the study of nutritional indices.

$$\text{Relative growth rate (RGR)} = \frac{(A - B)}{(B \times 3)}$$

Where A is the weight of larvae on the third day (mg)/ number of the live larvae on the third day; B is original weight of the live larvae (mg)/original number of larvae.

$$\text{Relative consumption rate (RCR)} = \frac{D}{(B \times 3)}$$

Where D is the biomass ingested (mg)/number of live larvae on the third day.

$$\text{Efficiency of conversion of ingested food (ECI)}(\%) = \left(\frac{RGR}{RCR}\right) \times 100$$

$$\text{Feeding deterrence index (FDI)}(\%) = (C - T)X\frac{100}{C}$$

Where C is the consumption of the control pearl millet disks and T is the consumption of the treated disk [5].

2.4 Evaluation of effect of chitinase (total protein, glucose and glycogen)

The larvae prior to pupation were used for evaluation of total proteins [6], glucose and glycogen (Anthrone reagent method [7]) in the whole body homogenate. Six replicates were maintained for each concentration.

3. Statistical analysis

Data from nutritional indices and biochemical parameters were expressed as the mean of six replicates. The lethal concentration (LC_{50}) was calculated using ED50PlusV1.0.

4. Results

4.1. Antifeedant activity of chitinase on *C. cephalonica*

Feeding deterrence indices (FDI) demonstrated that the chitinase from *T. viride* gives significant feeding deterrence on *C. cephalonica* larvae at the uppermost concentration (500 ppm) with an antifeedant index percentage of 99.13 (Table 1). Chitinase appreciably abridged both the growth rate (RGR) and food consumption (RCR) of *C. cephalonica* larvae at concentrations of 125 ppm and above in a dose-dependent manner. Efficiency of conversion of ingested food (ECI) was also considerably decreased from that of the controls at concentrations of 125 ppm and above.

Table 1: Nutritional and feeding deterrence indices of *Corcyra cephalonica* IV instar larvae fed on flour disks treated with Chitinase from *Trichoderma viride*

Concentration (ppm)	RGR (µg/mg/day)	RCR (µg/mg/day)	ECI (%)	FDI (%)	Mortality (%)	
					Day 3	Day 7
0	55.80±1.60	248.32±1.41	22.47	-	0	0
62.5	45.51±1.04	238.82±1.52	19.05	14.64	0	0
125	43.81±1.70	237.83±1.73	18.42	15.5	0	0
250	9.04±1.23	72.78±1.11	12.42	74.99	0	22
500	0.133±0.006	3.90±1.15	2.89	99.13	0	38

*Each data represents the mean of six replicates. RGR-Relative growth rate; RCR-Relative consumption rate; ECI-Efficiency of conversion of ingested food; FDI-Feeding deterrence index [8]

4.2. Effect on total protein, glucose and glycogen

The results of the total protein, glucose and glycogen in IV instar larvae of *C. cephalonica* following treatment with chitinase from *T. viride* were shown in Table 2. The chitinase were induced the abnormal growth changes and alteration of biochemical parameters such as carbohydrates and proteins. The amount of the total protein of the larvae treated with higher concentration of 500 ppm (571.83±1.12 µg/ml) of chitinase demonstrated differences in the treated larva in contrast with the control (1235.91±1.14 µg/ml). In the same way glucose level in treated larvae (65.41±1.20 µg/ml) also reduced, compared with the control (227.18±0.736 µg/ml) and it was also in a dose-dependent manner. Similarly the glycogen level showed a prominent turn down (47.86±1.80 µg/ml) with the treatment of chitinase from *T. viride* at the same time as in contrast with that of the control larvae (197.46±1.26 µg/ml).

Table 2: Biochemical parameters (Total protein, glucose and glycogen) of *Corcyra cephalonica* IV instar larvae fed on flour disks treated with Chitinase from *Trichoderma viride*

Concentration (ppm)	Total protein (µg/ml)	Glucose (µg/ml)	Glycogen (µg/ml)
0	1235.91±1.14	227.18±0.736	197.46±1.26
62.5	1214.51±1.5	170.30±1.06	157.04±1.70
125	1119.70±1.007	132.98±1.34	121.65±1.33
250	888.82±0.838	84.46±1.01	77.14± 1.16
500	571.83±1.12	65.41±1.20	47.86±1.80

*Each data represents the mean of six replicates

5. Discussion

In past, chitinase has been used as an insecticide and fungicide [9]. Mortality of *Lymantria dispar* larvae was improved when chitinase was integrated with *Bacillus thuringiensis* compared with treatment with the bacteria alone, and this result was interrelated with enzyme levels. The larvicidal action of a nuclear polyhedrosis virus (NPV) toward *L. dispar* larvae was higher when it was co-administered with bacterial chitinase [10]. From the earlier investigations, it was clear that chitinases cause perforations in the insect gut peritrophic membrane, aiding entry of the pathogens into the hemocoel of susceptible insects [11].

The outcome of current analysis portrayed an elevated deterrence effect of chitinase from *T. viride.* It also depicted that chitinase sternly have an effect on larval feeding behavior and mainly act as an antifeedant. By increasing concentration, deterrence index increases and an excellent dose-response relationship is attained. Deterrence index in maximum concentration reaches 99.13% (500 ppm). In our study it was observed that the relative growth rate (RGR) and the relative consumption rate (RCR) of *C. cephalonica* were significantly decreased when treated with chitinase. Thereby it leads to the extended larval developmental period. Our results were similar to Senthil-Nathan *et al.* [12, 13, 14]. Reduced larval growth was coupled with decreased RGR, since the food was hold on to the gut of the larvae for maximum of estimated digestibility to boost the nourishment. In the way of development, insects have made admirable use of the firmness and chemical constancy of the polymeric chitin to bring together extracellular associations such as the exoskeleton and gut lining (PM), in together facilitate insects to be sheltered from the surroundings while permitting development, mobility, respiration and communication [15]. Chitinase persuaded damage to the PM in the insect gut grounds a major decrease in nutrient utilization and accordingly in insect growth [16].

In physiological studies, the estimation of the total protein, glucose and glycogen level is vital. Proteins are most important biochemical components indispensable for an organism to nurture, develop and carry out its crucial activities. The mean of protein content values were estimated in the IV instars treated with 62.5, 125, 250 and 500 ppm concentrations of chitinase from *T. viride*. From the data recorded in Table 2, it is obvious that total protein was appreciably decreased with a dose-dependent manner. The reduced level of protein content in the larvae was observed in plant extract treated *Crocidolomia binotalis* [17]. Treatment of *Spodoptera littoralis* and *Agrotis ipsilon* with the methanolic extract of *Melia azedarach* L. on the hemolymph protein reported comparable patterns of result as shown in the present study [18].

In the present study it was observed that reduced levels of glucose, glycogen and protein compared with control in chitinase treated *C. cephalonica* IV instar larvae in a concentration-dependent manner. Similarly Ansari *et al.* [19], has reported with a reduced glucose and glycogen level with increased protein concentration among the chitinase treated groups. There will be a high reduction in the carbohydrate level due to its exploitation during high energy demand due to distorted metabolism [20]. Unstable protein synthesis might be due to the exhaustion of total carbohydrate level [21]. From our findings, it can be implicit that malnourishment of larvae possibly will be dependable for the distorted metabolism which could pave way to diminish in total glucose and glycogen levels. In addition, stress might show the way to increase translation, causative to the increased concentration of protein in order to meet energy demand.

6. Conclusion

Our present study affirms that chitinase from *T. viride* is efficient against the *C. cephaloica* through poor feeding, decreasing the growth rate Biochemical parameters such as total protein, glucose and glycogen level also decreased with a dose-dependent manner. This can serve as an alternative to the synthetic pesticides with a lesser production cost. We are continuing to develop fungal chitinase as a potent biocontrol agent against the stored food pests, by inspecting the feeding deterrence, growth rate, and major biochemical levels. In

future the mode of action, mechanism it's cytotoxic and genotoxic effects need to be evaluated.

7. Conflict of interest

The authors declare no conflict of interest with respect to this article.

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