Pest Management in Horticultural Ecosystems
Vol. 28, No.1 pp 107-116 (2022)
DOI number : 10.5958/0974-4541.2022.00026.1

Isolation and characterization of beewax degrading enzymes from the digestive guts of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae)

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ABSTRACT: The greater wax moth, *Galleria mellonella* (L.) is one of the most destructive honeycomb pests in the world. The digestive gut enzymes, esterase and lipase are a group of enzymes that hydrolytically catalyzes the esters and long-chain fatty acid linkage of wax components of beeswax. Final instar stage larvae were selected and homogenized for enzymes activity assay and subsequent analysis. The enzymes were purified by combination of ammonium sulfate precipitation and gel filtration using Sephadex G-100. The amount of protein and specific activity of lipase were 0.01 ± 0.002 mg/mL and 1.63 ± 0.88 mol/min/mg protein, respectively, with a 163-fold purity and 33.3 percent recovery, while for esterase14.71 ± 1.46 and 11.23 ± 1.41 mol/min/mg protein, respectively, with a 1.97-fold purity and 34.383 percent recovery. Furthermore, biochemical characterization esterase and lipase were carried out through testing its activities against factors, such as different temperatures and pH ranges with the use of *p*-nitrophenyl butyrate (PNPB) *p*-nitrophenyl palmitate substrates. The highest activities of enzymes were determined at the temperature ranges of 35–40 °C and pH ranges of 7–9.Insect pest damage to agricultural products can be reduced by using inhibitors against digestive enzymes. The findings of this research establish the role of esterase and lipase in the physiological function of *G. mellonella* in the degradation of beeswax and developing novel inhibitors against these enzymes can be implemented to control *G. melonella*. Isolation, purification, and characterization of esterases and lipases from the guts of *G. mellonella* could be a crucial step toward a better understanding of their action and the establishment of a safe and effective control method for *G. mellonella* as well as other insect pests.

Keywords: *Galleria mellonella*, Isolation, Characterization, Beeswax, Esterase and Lipases.

INTRODUCTION

Beeswax honeycomb is a hexagonal prismatic wax cell structure constructed by honey bees and made up of long fatty acids, esters and hydrocarbons (Blomquist *et al.,* 1980). It is a complex substance made up of wax esters, fatty acids and hydrocarbons (Piek, 1964; Tulloch, 1970). The composition of beeswax varies depending on place of production (Europe, Asia, or Africa), species of honeybee, and age of wax (Tinto *et al.,* 2017). Generally, unhydrolyzed & unrefined beeswax contains hydrocarbons (15%), esters (71%), free acids (8%), and other compounds (6%) (Tulloch, 1970; Parish *et al.,* 2002). The beeswax comprises ~70% esters in its composition mainly monoesters (35%), diesters (14%), triesters (3%), hydroxymonoesters (4%), hydroxypolyesters (8%), acid esters (1%) and acid polyesters (2%). In addition, beeswax also contains hydrocarbons (14%), free fatty acids (12%), free alcohols (1%) and miscellaneous groups (6%). This wide range of biochemical composition offers beeswax many distinctive features (Goodman, 2003) and also prevents us from fully comprehending the production and secretion process.

The greater wax moth (GWM)*, Galleria mellonella* (L.) (Lepidoptera: Pyralidae) is one of the most damaging pests of honey bee colonies around the world (Oh *et al.,* 1995). Beekeepers suffer significant financial losses as a result of the damage to wax combs. The devastation of the comb by *G. mellonella* often considered to be the cause of honey leaking/ contamination, bee larvae death and the spread of honey bee diseases (Caron, 1992). Wax moth larvae do not harm bees directly (i.e., they do not feed on any life stages of the), but they do attack beeswax combs, which are an important component of the honey bee colony (Abou El-Ela, 2014; Berry and Delaplane, 2001; Hamby, 2006; Swamy, 2008a). Greater wax moth infestations can drive weak colonies to abscond, or quit the nest entirely, and can also overrun seemingly robust colonies, but this is rare (Swamy, 2008b). Greater wax moth infestation is also more common in stored combs and weak colonies, resulting in huge financial losses for

Table 1. Purification process of lipase from the guts of *G. mellonella* larvae

Purification step	Protein[a] (mg/ml)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	2.33 ± 0.19	0.03 ± 0.004	0.01 ± 0.0008	1	100.00
40 % SAS*	1.21 ± 0.17	0.02 ± 0.009	0.02 ± 0.009	2	66.66
80 % SAS*	0.21 ± 0.01	0.01 ± 0.011	0.11 ± 0.04	11	33.33
Sephadex G-100	0.01 ± 0.002	0.01 ± 0.007	1.63 ± 0.88	163	33.33

[a] Protein content was measured by the Bradford method (1976); *SAS= Saturated Ammonium Sulfate; [b]one unit of activity corresponds to 1µmol of pNP released per min using 50mM *p*-NPP as the substrate at 37 °C; Data are showed as (mean ± SE), n = 3 replicates.

beekeeping industry (Swamy *et al.,* 2010). However, no comprehensive control methods for this pest have been developed to date. Currently practiced physical and chemical techniques are highly ineffective (Burges, 1978). As a result, numerous studies have been conducted in order to find alternate ways to regulate this pest.

In recent past, the digestive enzymes of several insect pests have been studied as target molecules for pest management. Particularly studies on the use of enzyme inhibitors are indeed a priority to develop optimal and effective control measures involving advanced biotechnological methods (Oh *et al.,* 1995; Pereira *et al.*, 2005). As the beeswax that serves as major food source for the larvae of GWM comprises a high concentration of esters, long-chain fatty acids and lipids as mentioned earlier, it is crucial to understand the gut enzymes of the greater wax mothlarvae that can depolymerize the complex substrate (= beeswax). In general, the specific enzymes which act on these substrates are usually esterases and lipases which hydrolyzes short-chain and long chain esters respectively at different reaction rates (Chahinian and Sarada, 2009). Esterases are hydrolytic enzymes that break down the ester bonds in a wide range of biomolecules, are widely present across the animals, plants, and microorganisms (Oakeshott *et al.,* 2005; Upadhya *et al.,* 1985). Esterases present in the larval gut hydrolyzes the esters to an alcohol moiety and fatty acids which are further broken down (Niemierko and Wlodawer, 1950). Insect lipases, on other hand are classified into phospholipases, triacylglycerol lipases (TAG-lipases), alkaline and acid phosphatases (Terra and Ferreira, 2012) that are divided into two categories: lysosomal (intracellular) and digestive lipases (Miled *et al.,* 2000). Usually, triacylglycerols (TAG) are hydrolyzed by intracellular lipases and are preserved as lipid droplets, while digestive lipases hydrolyze TAGs in the diet. Both esterases and lipases can attack the same substrate depending on the physical state of the substrate.

The extraction of digestive esterase from the guts of *G. mellonella* larvae was attempted earlier (Juraimi, 1986). Esterases of the GWM larvae are categorized as carboxylesterases that converts the beeswax esters into various acids and alcohols (Jacobson et al., 1968; Niermerko, 1959). However, only about 50% of the ingested wax is utilized and metabolized by theGWM larvae and the excess acid resulting from the high lipid diet is excreted (Vonk *et al.,* 1984).While, the lipases of GWM larvae are mostly triacylglycerol lipase, that perhaps hydrolyses bees wax TAGs into various fatty acids and glycerol (Mahdy *et al.*, 2020).Similarly, β-glucosidase, a type of digestive enzyme that hydrolytically catalyzes the beta-glycosidic linkage of glycosides was also extracted earlier from the guts of *G. mellonella* (Kara *et al.,*2014). They found that β-glucosidase degrades the β-glycosides components of beeswax and comprehended that establishing its role could be a prospective control procedure for *G. mellonella.* As a result, very limited information is available on the nature of the enzymes that are responsible for the hydrolysis of carboxylic acid esters and fatty acids of beeswax in *G. mellonella.* Therefore, the present study was initiated as an attempt to isolate, identify, characterize and measure the activity of esterase and lipase enzymes from the guts of *G. mellonella* larvae.

MATERIALS AND METHODS

Insects rearing

Galleria mellonella larvae (7–8thinstar) were obtained from the Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru, India and maintained at the Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru continuously as per the procedures described earlier (Li *et al.*, 2019; Cassone *et al.*, 2020). Larvae of *G. mellonella* were reared on honeycombs of Indian honey bee, *Apis cerana indica* Fab. in a dark plastic container (20 × 20 ×

Table 2. Purification process of esterase from the guts of *G. mellonella* larvae

Purification step	Protein[a] (mg/ml)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	81.24 ± 2.62	464.20 ± 11.26	5.71 ± 0.04	1	100
50 % SAS	45.17 ± 2.87	349.30 ± 8.32	7.81 ± 0.68	1.37	75.24
80 % SAS	31.53 ± 2.58	268.43 ± 8.30	8.60 ± 0.59	1.50	57.82
Sephadex G-100	14.71 ± 1.46	161.71 ± 1.39	11.23 ± 1.41	1.97	34.83

[a] Protein content was measured by the Bradford method (1976); *SAS= Saturated Ammonium sulfate; [b]one unit of activity corresponds to 1µmol of pNP released per min using 50mM *p*-NPB as the substrate at 37 °C. Data are showed as (mean ± SE), n = 3 replicates

30.5 cm; length × width × height), at ambient conditions (27 ± 1°C, 75 ± 2% RH and 14L: 10D h photoperiod). The larvae during their final instar stage (7^{th}-8^{th}) were collected for further experiments.

Chemicals

The substrates for enzyme activity studies [*p*-nitrophenyl acetate (CAS No. 830-03-5), *p*-nitrophenyl palmitate (CAS No. 1492-30-4)] and protein assay reagents were purchased from Sigma-Aldrich, India.

Enzyme Preparation

A total of 20 larvae of *G. mellonella* (58 g weight) were rinsed in cold distilled water and blotted with filter paper, homogenized in a pre-cooled pestle and mortar in 25 mL of 50 mMTris-HCl buffer at pH 7.0. The homogenate (~25 mL) was centrifuged at 4°C for 15 min at 10,000 g and the supernatant collected (~20 mL) was used directly as enzyme source and the pellet discarded. The activity of the enzymes was tested in triplicates. Crude enzymes were stored at -80 °C until further use.

Estimation of protein concentration

Protein concentration of crude enzymes was determined as described by Bradford (1976) with bovine serum albumin (BSA) as standard protein.

Determination of lipase activity

Lipase activity was measured based on procedures described by Tsujita *et al.* (1989) and Zuo *et al.* (2010) with slight modifications. The reaction mixture that consisted 250 µL of 100 mM sodium phosphate buffer (pH 7.0), 30 µL of 5 mM *p*-nitrophenyl palmitate as a substrate (PNPP)and 30 µL of crude enzyme were thoroughly mixed and incubated at 37°C for 30 minutes. Later, 100 µl of distilled water were added to each tube (control and treatment). The reaction was stopped after incubation with 50 µL of 3.5% SDS solution and the color that developed due to liberation of the *p*-nitrophenols were immediately measured using a $T80^{+}$ UV/VIS spectrometer (PG Instruments, UK), at 410 nm approximately for 5 min. For negative control tubes, samples were placed in a boiling water bath for 15 min to destroy the enzyme activity then cooled. One unit of esterase activity was defined as the rate at which 1 µmol of *p*-nitrophenol is liberated per min under assay conditions (Rúa *et al.,* 1997).

Determination of esterase activity

Esterase activity was measured based on the procedures described by Zuo *et al.* (2010) and Wheeler *et al.* (2010) with slight modifications. The reaction mixture contained 250µL of 100 mM PBS buffer (pH 7.0), 30 µL of 5 mM of *p*-nitrophenyl butyrate (PNPA) to which 30 µL of crude enzyme was added. The mixture was thoroughly mixed and incubated at 37°C for 30 minutes and a total of 100 µl of distilled water were added to each tube (control and treatment). For negative control tubes, samples were placed in a boiling water bath for 15 min to destroy the enzyme activity then cooled. Spectrophotometer reading at 410 nm was measured immediately after incubation of the reaction mixture. One unit of esterase activityas defined as the amount of enzyme that produced one micromole of product, *p*-nitrophenol per minute in a substrate (5mM of PNPB) solution at 37°C and pH 7.5. Specific activity was expressedas the ratio of units of enzyme activity per milligramof protein in the solution (Juraimi,1986; Rúa *et al.,* 1997).

Purification of lipase and esterase

The purification of gut lipase and esterase was done based on the procedure described by Zibaee *et al.,* (2012) and Orscelk *et al.,* (2007) with minor modifications. All the purification steps were performed at 4 °C as mentioned below and in each step the activity of enzyme and content of protein were determined.

Ammonium sulfate precipitation

Samples were first exposed to ammonium sulfate

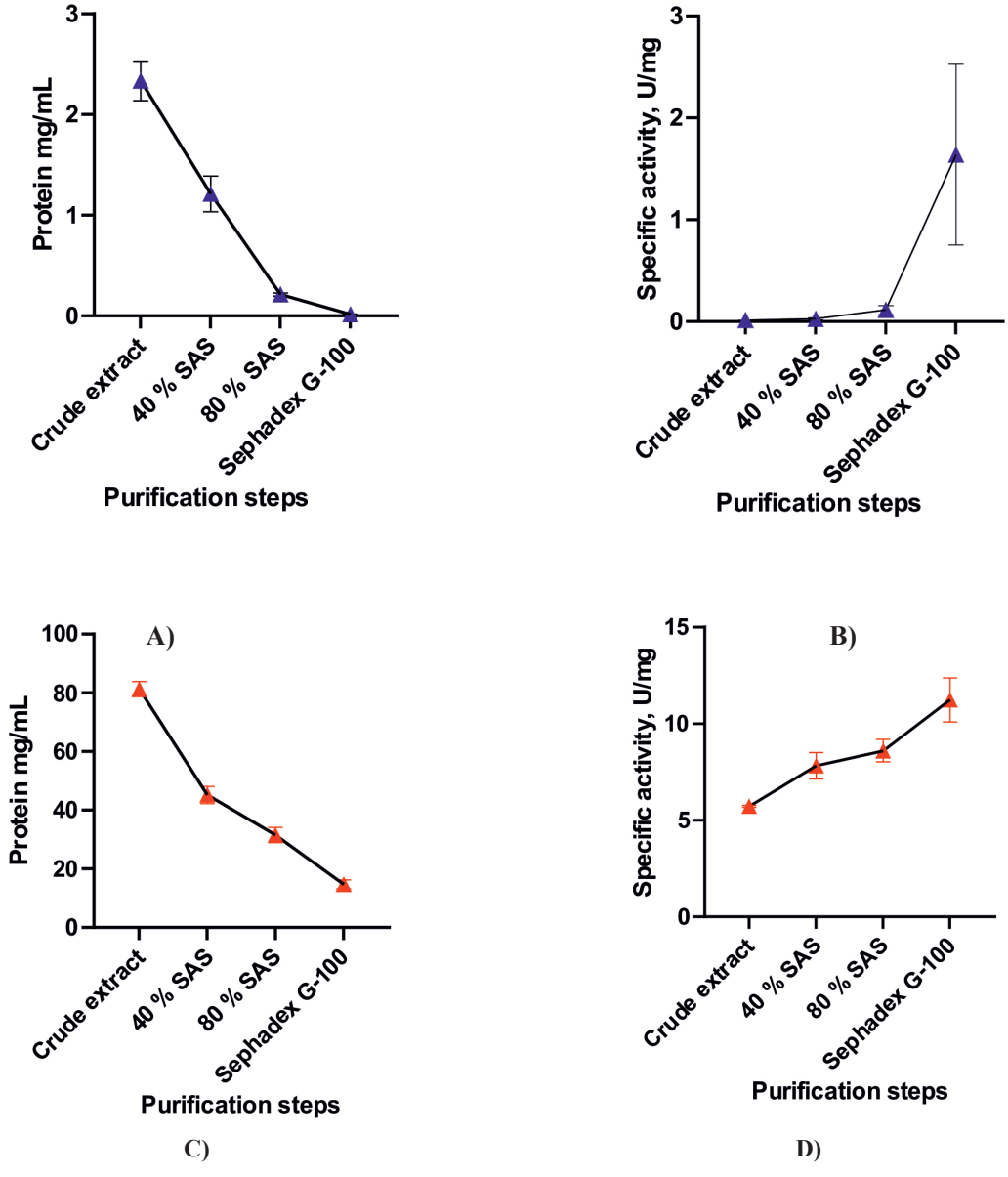

Fig 1. The purification of the lipase (A and B) and esterase (C and D) of *G. mellonella* extracted from the different fractions. *SAS= Saturated Ammonium sulfate.

precipitation by making use of 40% and 80% of saturated ammonium sulfate solution (SAS) fraction was then gathered and centrifuged at 12,000 rpm for 10 min and further subjected for gel filtration as mentioned below.

Sephadex G-100 gel filtration chromatography

The final fraction of ammonium sulphate was gel filteredon a dried Sephadex G-100 column. The resultant dried gel was incubated at 70°C for 5 hours in distilled water. After cooling and removing air, the gel was put into the column [12 × 2 cm, equilibrated with 50mM universal buffer (pH 10) and 50mM $(NH_4)_2SO_4$ at 30°C. With the same buffer, five milliliters of enzyme fractions were collected at a flow rate of 20 mL/h. Protein content and enzyme activity were determined for each fraction.

Determination of biochemical characteristics of the purified esterase and lipases

Determination of Optimal pH

The pH effect on the activity of purified lipase and esterase was measured using purified enzyme diluted in 25µL universal buffer with the pH range from 2 to 13 (Mahdy *et al.,* 2020; Stauffer, 1989). Lipase & esterase activity was assayed as described above, after incubation for 1 h at each pH value.

Determination of Temperature

Esterase and lipase were diluted in 25 µL of buffer (50 mM Tris–HCl pH 7–7.5) and incubated for 1 hour at temperatures ranging from 20 to 70°C. The activity of lipase and esterase was evaluated immediately after incubation using PNPP and PNPA as substrates, as described before.

Statistical analyses

Data were analysed using the Graph Pad Prism software, V.9.0 and expressed as mean ± standard error (SE) of three replicates for each determination. Before conducting the analysis, all the data were subjected for normality distribution test to confirm the normality. As the data confirms normal distribution, the difference between means was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, when $P \leq 0.05$.

RESULTS

Purification of esterase and lipase from the *G. melonella* larvae

Partial purification of the esterase and lipase activity of the *G. melonella* larvae was attempted through ammonium sulfate fractionation. In case of lipase, the amount of protein decreased from 2.33 ± 0.19 mg in crude extract to 1.21 ± 0.17 in 40% ammonium sulfate fraction with the specific activity of 0.02 ± 0.009 µmol/min/mg protein, 66.66% recovery and 2-fold purification. While with 80% ammonium sulfate fraction, the quantity of lipase decreased to 0.21 ± 0.01 mg, with 0.11 ± 0.04 µmol/min/mg protein, 33.33% recovery and 11-fold purification. The protein amount and the specific activity of digestive lipase from the fractions of Sephadex G-100 column were 0.01 ± 0.002 mg/mL and 1.63 ± 0.88 µmol/min/mg protein, respectively with 33.33% recovery and 163-fold purification. Statistically, One-way ANOVA with Tukey's multiple comparison test revealed that, there was a significant difference in protein concentration between crude sample and sample after ammonium sulfate precipitation steps[40% and 80%] and Sephadex G-100 (One-way ANOVA; $P<0.0001$, $df=3$, $F_{(3,8)} = 64.63$,Fig. 1A). The specific activity of lipase in Sephadex G-100 was found to be significant over40% SAS, 80% SAS fractions and crude extract (One-way ANOVA; $P = 0.008$, $df = 3$, $F_{(3,8)} = 8.19$, Fig. 1B). This clearly exhibits that Sephadex G-100 step was more effective in the purification process and the specific activity of lipase was more with the substrate, *p*-PNPP (Table 1).

Similarly, in case of esterase, the amount of protein decreased from 81.24 ± 2.62 mg in crude extract to 45.17 ± 2.87 in 40% ammonium sulfate fraction with the specific activity of 7.81 ± 0.68 µmol/min/mg protein, 75.24% recovery and 1.37-fold purification, while with 80% ammonium sulfate fraction, the quantity of esterase decreased to 31.53 ± 2.58 mg, with 8.60 ± 0.59 µmol/min/mg protein, 57.82% recovery and 1.50-fold purification. The protein amount and the specific activity of digestive esterase of fractions from Sephadex G-100 column were14.71 ± 1.46 mg/ml and 11.23 ± 1.41 µmol/min/mg protein, respectively with 34.83% recovery and 1.97-fold purification. Significant difference was observed in protein concentration between crude sample, sample after ammonium sulfate [40% and 80%] precipitation steps and Sephadex G-100 (One-way ANOVA; $P<0.0001$, $df = 3$, $F_{(3,8)} = 133.5$, Fig. 1C). Whereas, the specific activity of esterase in Sephadex G-100 was found significantly higher than40% SAS and crude extract (One-way ANOVA; $P = 0.004$, $df = 3$, $F_{(3,8)} = 9.79$, Fig. 1D). However, no significant difference was observed between the Sephadex G-100 and 40% SAS ($P=0.12$) indicating the Sephadex G-100 step was more effective in the purification process of protein and the specific activity of esterase was more with the substrate, *p*-PNPB (Table 2).

Biochemical characterization of purified lipase and esterase

The effect of pH on lipase and esterase activity

The effect of pH on lipase and esterase activity is depicted in (Fig. 2A and B). The activity of lipase gradually increased from 5 to 8 and then reduced until pH 14. Activity was high when assayed at pH 7–10, with the maximal activity at pH 8. Lipase activity was decreased at pH 13. While in case of esterase, the activity gradually increased from 6 to 8 and then reduced until pH 13. The specific activity of esterase was found high at pH 8-8.5.

The effect of temperature on lipase and esterase activity

The effect of temperature on the activity of pure lipase and esterase was investigated throughout a temperature range of 20 to 70 °C (Fig. 2 C and D). Purified lipase activity increased gradually as incubation temperature was raised from 20 to 40 °C, then declined till 70 °C. The Maximum activity under these conditions was found at 37 °C. Similarly, esterase activity was also increased gradually with raise in incubation temperature from 20 to 40 °C, then declined till 70 °C. The Maximum activity under these conditions was at both 35 °C and 40 °C.

DISCUSSION

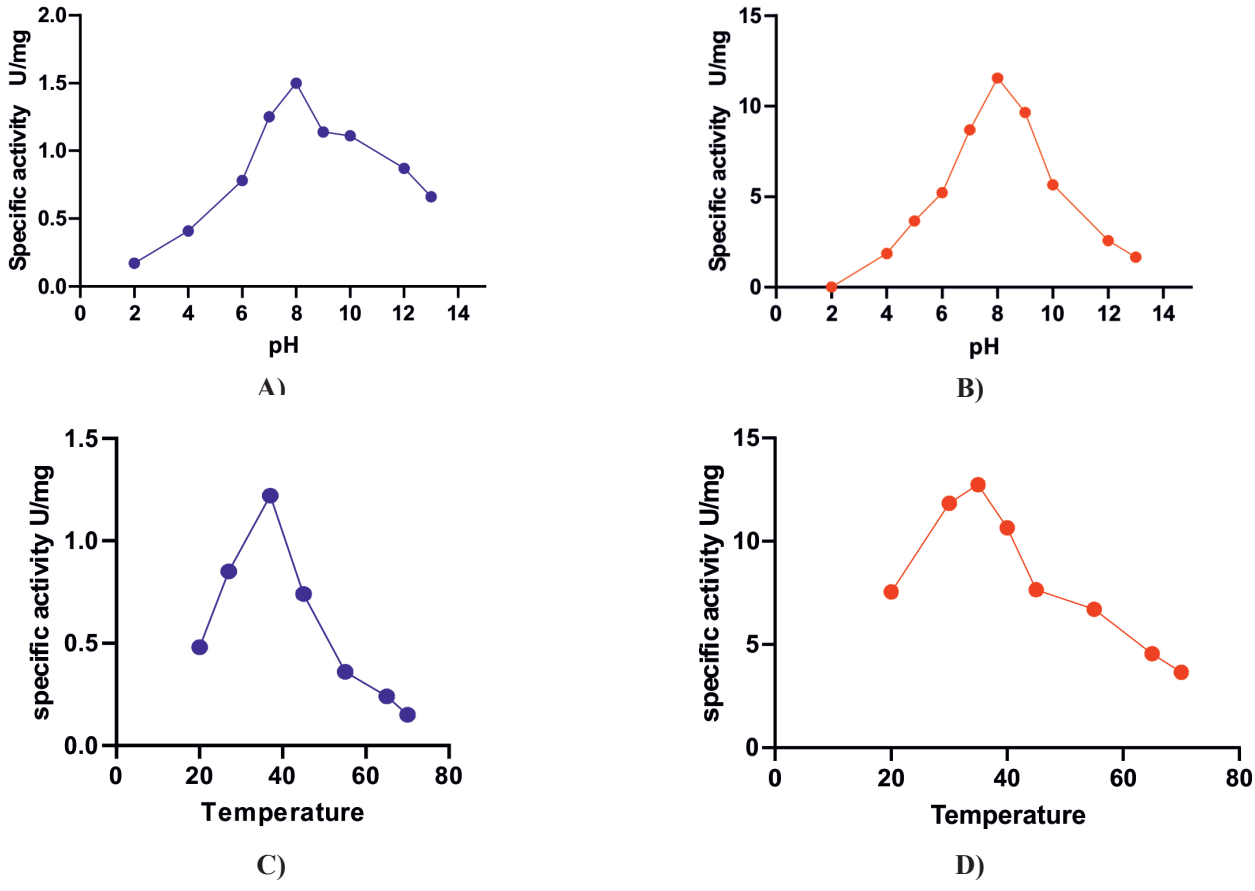

Fig 2. Effect of pH and temperature on the lipase and esterase from larvae of *G. mellonella*. Effect of pH on activity of lipase (A) and esterase (B) using different pH ranges. Effect of temperature (° C) on activity of lipase (C) and esterase (D) using different temperature ranges.

The greater wax moth is considered as a global challenge to the bee health and the beekeeping industry, however received very less attention in terms of research to control its damage. Several management approaches are developed to control *G. melonella* damage such as temperature, insecticides, entomopathogens (*Bacillus thuringiensis*), natural enemies (employing larval (*Bracon hebetor* Sayand *Apanteles galleriae* Wilkinson) and egg (*Trichogramma pretiosum* Riley, *Trichogramma evanescens* Westwood and *Trichogramma minutum* Riley) parasitioids, Sterile Insect Technique (SIT) and semiochemicals (Kwadha *et al.,* 2017), which are found to be ineffective and unsatisfactory. Greater wax moths are voracious wax feeders, and their guts may contain enzymes that break down wax containing long-chain fatty acids and carboxyl esters. Degrading these complicated long chain esters and fatty acids is usually a difficult and time-consuming task, and it's still unclear why and how these tiny larvae of GWM use beeswax as their regular dietary substance.

The present study describes the activity of gut digestive enzymes of *G. mellonella* namely esterase and lipases. The extracted lipase and esterase from *G. mellonella* larval guts were purified by using two steps involving SAS precipitation and gel filtration. These two procedures are generally employed to isolate and purify various enzymes including lipase and esterase (Mahdy *et al.,* 2020).In the present study, salting-out using SAS helped to remove the non-protein components from proteins, as addingSAS reduced the number of solvent molecules that interact with proteins resulting in coagulation and precipitation of proteins (Robinson, 2015). Earlier studies mentioned that this process can also be employed to separate a particular enzyme from other proteins (Mahdy *et al.,* 2020).

In the present study, we were able to isolate and purify specific gut enzymes namely esterase and lipase from the larval guts of *G. mellonella*. The enzyme activity of crude extracted lipase and esterase was assayed with the substrates *p*-nitrophenyl butyrate and *p*-nitrophenyl acetate. For both substrates, the total enzyme activity for lipase and esterase was found to be significantly high, with a 100% yield of protein. The enzyme activity was gradually decreased after precipitation with ammonium

sulphate saturation and purification with gel filtration. However, the specific activity of lipase and esterase was gradually enhanced with the purifying forms of the enzymes. The highest specific activity was observed with the gel filtered purified enzyme for both lipase and esterase. Similar results were observed by Zibaee *et al.* 2012 on rice green caterpillar, *Naranga aenescen* Moore, where they found the highest specific activity of lipase in the 3rd instar stage larva on substrate *p*- nitrophenyl butyrate. In 1986, Juraimi demonstrated the extraction of digestive esterase from the guts of *G. mellonella* larvae and reported its activity on substrates namely acetylsalicylic acid, and acetyl-p-hydroxybenzoic acid and tentatively classified esterase of *G. mellonella* as carboxylesterase. He reported highest specific activity of the purified esterase on these substratesat 70 % SAS. Mahdy *et al.* (2020)for the first time extracted, purified and characterized the fat body lipase from the guts of *G. mellonella* larvae and reported highest specific activity of purified lipase with the substrates, trioleoylglycerol, triton-X100 and *p*-nitrophenol butyrate. Similar procedures were employed to extract and purify the gut lipases from *Ectomyelois ceratoniae* Zeller (Ranjbar *et al.,* 2015) and *Antheraea mylitta* Drury (Marepally and Benarjee, 2016) larvae where they reported the highest specific activity of lipase on selective substrate, *p*-nitrophenol butyrate. Wheeler *et al.*(2010) identified and purified four esterase genes and quantified esterase activity from the guts of termites, *Reticulitermes flavipes* Kollar to understand its metabolism.

The pH is one of the most important variables in biochemical reactions. In our study, the activity of digestive lipase of *G. mellonella* was found to increase with pH from 6.0 to 8.5 with the highest activity at pH 8.0. While the most activityof lipase was lost at pH 12.0-13.0. This shows that lipases from *G. mellonella* have a slightly alkaline optimum pH similar to studies reported in the kissing bug *Rhodnius prolixus* Stal which have high lipase activity at pH 7.0 - 7.5 (Grillo *et al.,* 2007) and *Manduca sexta* (L.) at pH 7.9 (Arrese and Wells, 1994).In case of esterase, the highest activity was recorded at pH from 8.0 to 9.5, while most activity decreased gradually from thereon (pH 10.0-13.0). This indicates that the esterase of *G. mellonella* is active in the mild alkaline optimal pH range (8.5 to 10.0; 7.5 to 8.0) which is observed similarly in rice green caterpillar *Naranga aenescens* Moore and *G. mellonella* (Zibaee *et al.*, 2012; Nemec and Zenka, 1996).

Temperature is another variable that affects biological responses. In the current study, the temperature effect on lipase and esterase activity was assessed over a range from 20 to 70 °C. The maximum activity of lipase was at 37 °C while the minimum activity was at 70 °C, whereas esterase activity increased gradually with increase in the temperature from40 - 60°C and then dropped down after 70 °C to till 100 °C. This revealed that the enzyme activity for lipase of *G. mellonella peaked* at the most optimal temperature range (37 °C), then reduced as the temperature was raised until the enzyme denatured and the activity dropped significantly. Similar results were reported in other insect gut lipases, as in gypsy moth *Lymantria dispar* (L.) (Mrdaković *et al.,* 2008) and *Rhynchophorus palmarum* (L.) (Santana *et al.,* 2017). Our results indicated that the enzyme activity for esterase was eventually high with the raise in the temperature and dropped at the denaturation point of enzyme at higher temperature. Parallel results were observed from the guts of *Triatoma infestans* Klug (De Malkenson *et al.* (1984) and eastern subterranean termites, *Reticulitermes flavipes* Kollar (Davis *et al.,* 1995). In contrast, Zhu *et al.* (1990) observed a decrease in the esterase activity from the guts of *Lygus hesperus* Knight after 40 °C of temperature. This suggests that the esterase activity perhaps is temperature independent and varies between the species, particularly in *G. mellonella*, due to its nature of feeding beeswax.

The use of enzyme inhibitors was indeed a priority segment in integrated pest management programs due to the widespread use of synthetic pesticides and the emergence of environmental concerns such as pest recurrence, pesticide resistance, and harmful effects on non-target organisms. Detailed investigations on the identification, characterization, and inhibition of specific digestive enzymes across a wide range of economically significant insect species will aid our understanding of insect nutrition, growth/development, and mortality. Therefore, employing the inhibitors that target the specific enzymes is likely to be more effective provided the holistic knowledge about the insect digestive enzymes is available. Our finding is the first step in this direction, and it will aid future research into understanding the physiological function of the identified esterase and lipase of *G. mellonella* in the degradation of beeswax and envisages identifying novel enzyme inhibitors to control the pest damage.

ACKNOWLEDGEMENTS

Authors thank the Director, Indian Institute of Horticultural Research, Bangalore for providing research facilities. This research has been supported by the National Fellow Project (Award no. 27(3)/2010-HRD), Indian Council of Agricultural Research, New Delhi.

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MS Received: 28 April 2022
MS Accepted: 28 May 2022