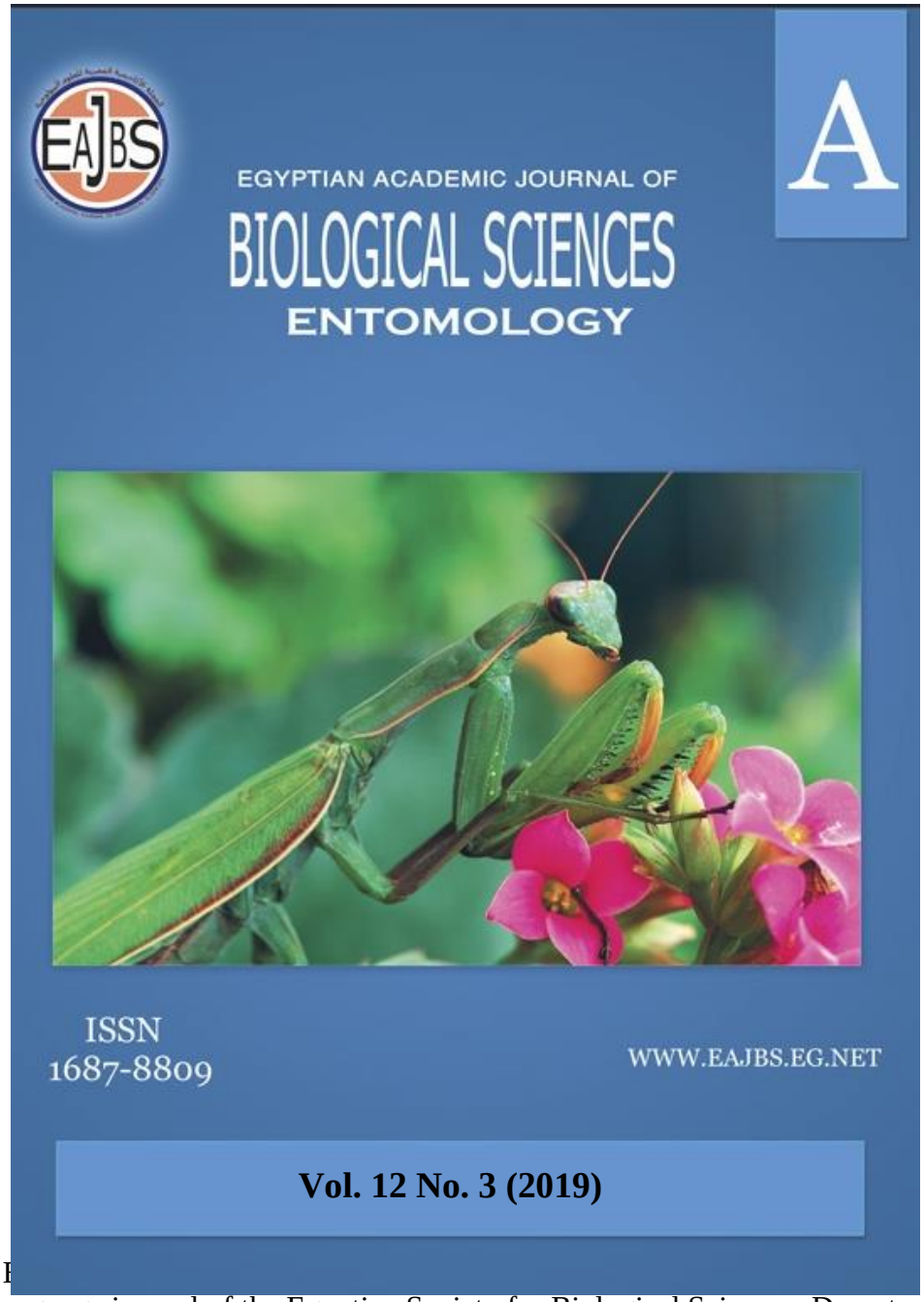
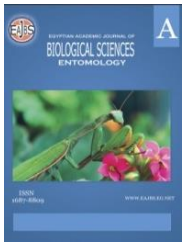


language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.
Entomology Journal publishes original research papers and reviews from any entomological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution, control of insects, arachnids, and general entomology.
www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 12(3):49– 61(2019)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



The Differences in the Digestive System and Enzymes between Soldiers and Workers of the Subterranean Termite, *Psammotermes hypostoma* Desneux (Rhinotermitidae: Isoptera)

Huda R. K. Ali[1] , Alaa Ropy[2]& M.A. Batt[1]
[1]Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.
[2]Department of Chemistry, Faculty of Science, Fayoum University, Fayoum 63514, Egypt
Email: huda.r.k.ali@gmail.com - arm01@fayoum.edu.eg - d.mohammed_batt@yahoo.com

ARTICLE INFO
Article History
Received:28/4/2019
Accepted:12/5/2019

Keywords:
Subterranean termite, *Psammotermes hypostoma*, digestive system, Enzymes, Endo-β-1,4-glucanase, Cellobiase (β -glucosidase) , Amylases. Alimentary canal.

ABSTRACT

Of eight termite species found in Egypt, the subterranean termite *Psammotermes hypostoma* Desneux is considered as the most important serious pest, which destroy the wooden structures in buildings and other wood products or any material containing cellulose. Therefore, cellulases enzymes are considered the main enzymes in the digestive system of termite. The termite digestive system is considered a target for best control.

This report discusses the descriptive variations of alimentary canals for the worker and the soldier, also, the detection of three digestive enzymes; two of them belonging to cellulases enzymes (endo-β-1,4-glucanase, and cellobiase (β -glucosidase) and the third is amylase in guts extracts from the termite workers and soldiers. The workers had higher endo-β-1,4-glucanase and amylase activities, while soldiers had higher cellobiase (β-gl`ucosidase) activity. It is clear that the presence of α-amylase in the digestive system of *P. hypostoma* workers and soldiers means that this species of termite is able to decompose starch, which explains why termites attack grain silos.

Effect of temperature and PH on enzyme activity showed that the optimum temperature /PH for workers were 70 °C/ PH6 for endo-β-1,4-glucanase, 50 °C/PH6 for cellobiase (β -glucosidase) and 70 °C/pH 7 for amylase, while, in case of soldiers, optimum temperature/pH were 70 °C/PH6 for endo-β-1,4-glucanase, 40 °C/PH6 for cellobiase (β-glucosidase) and 60 °C/PH 7 for amylase.

This work is the first in Egypt concerning the study of the enzymes activities of the digestive system of termite and may be considered an initial step to contribute to control this species of termite by the development of new termiticides.

INTRODUCTION

The subterranean termites are considered the most serious pests all over the world (Su and Scheffrahn, 1990). The worldwide annual control and repair cost was estimated and data showed that the global economic impact of termite pests has increased to $40 billion.

Subterranean termites accounted for≈80% of the costs ($35 billion) (Rust and Su, 2012).

In Egypt, eight species of subterranean termites were recorded. The sand termite, *Psammotermes hypostoma* Desneux is the most serious, voracious, and destructive termite, causing damage to any materials containing cellulose, the annual control and repairing of damages costs tens of millions of pounds essentially in Upper Egypt, Middle Egypt and the New Valley (Ahmed *et al.,* 2014& El-Sebay and Ahmed, 2006).

The termite soldiers are unable to feed themselves due to their elongated jaws and must rely on the workers to feed them and play an important role in colony defense (Koshikawa *et al.***,** 2003). Results by Huang *et al*., 2008 indicated that the transfer of nutritional material from foraging workers to non-foraging workers, soldiers, and fifth and sixth instars by trophallaxis, soldiers rely entirely on workers for nutrition.

Termites can be divided into two groups: higher termites (Termitidae) and lower (all families except Termitidae) based on absence or presence of flagellate protists in their hindgut, respectively, and also on different feeding and nesting habits (Varma *et al*., 1994 and Eggleton, 2011). It is also known that the higher termite (family, Termitidae) decompose cellulose by using their own enzymes (endogenous enzymes) which were secreted by their gut and salivary glands (Breznak and Brune, 1994; Ohkuma, 2003 and Ramin *et al.,* 2008) and by their gut symbiotic bacteria, such as fibrobacters (Warnecke *et al*., 2007). While the lower termites depend on their own digestive enzymes, symbiotic flagellates and symbiotic bacteria (Yamin and Trager, 1979; Brune, 2011; Wenzel *et al*., 2002; Husseneder, 2010; Hongoh, 2011; Brune, 2014 & Peterson and Scharf, 2016). The cellulose is usually decomposed by endo-β-1,4-glucanase, exo-l,4- β-glucanase and β-glucosidases (Breznak and Brune, 1994). Molecular biological techniques revealed that the lower termite *Reticulitermes speratus* (Rhinotermitidae) secretes endogenous cellulase from their salivary glands or midguts (Watanabe *et al.*, 1998), moreover, in the lower termite, the symbiotic protozoa play an important role in digesting cellulosic materials (Yoshimura *et aI.,* 1993a, 1993b, 1993c), because most of the species of the protozoa have endo-β-1,4-glucanase, exo-l,4- β-glucanase, and β-glucosidase (Yamin and Trager, 1979& Odelson and Breznak, 1985a). Cellulases enzymes, the major digestive enzymes in termites, can be an attractive candidate for controlling termite by inhibiting the enzyme through cellulase inhibitors.

In the lower termite, the hydrolysis of cellulose is initiated by endogenous enzymes (endo-β-1,4-glucanase) that are secreted by the salivary glands into their foregut or by the midgut (Watanab *et al*., 1998; Tokuda *et al*., 2004; Li *et al.,* 2006 and Fujita *et al*., 2010). Any glucose that is released in the midgut is resorbed through the epithelium, whereas the partially digested wood particles pass through the enteric valve into the large hindgut paunch. Then they are immediately phagocytized by cellulolytic flagellates, which hydrolyze the remaining polysaccharides using powerful cellulases (such as endoglucanases, exoglucanases and β glucosidases) and hemicellulases such as xylanases, arabinosidases, mannosidases and arabinofuranosidases that are secreted into their digestive vacuoles, as well as enzymes produced by termite bacteria (Inoue *et al*., 1997; Ni and Tokuda, 2013; Hongoh, 2011; Brune, 2014& Talia and Arneodo, 2018). However, the higher termites have only endogenous cellulases enzymes from salivary glands and midguts as well as the secreted enzymes from cellulolytic bacteria which harbor the hindgut, whereas, higher termites digest cellulose efficiently without gut protozoa.

Cellulases Enzymes were estimated in the gut extracts of the higher termite *Nasutitermes corniger* workers and soldiers, it was found that endoglucanases were the most active cellulolytic enzymes followed by exoglucanases, and finally β-glucosidase (Lima *et al*., 2014).

The gut of higher termite *Odontotermes brunneus* contains high levels of protease and cellulase enzymes, the study revealed that the diet of *O. brunneus* from different sources

might affect the enzyme production and stability (Muthusamy *et al*., 2018). In addition to the major importance of cellulases for termite, these enzymes are also being widely used in many industries such as food processing, animal feed, fermentation, agriculture, pulp and paper, bioethanol industry, and textile applications and wine and brewery industry (Kuhad *et al*., 2011)

The amylases are enzymes that hydrolyze α-1,4-glycosidic bonds in amylose chains producing glucose, maltose, and maltotriose units. These enzymes are biotechnologically applied in food, paper, detergent, Textile and pharmaceutical industries (Souza and Magalhães, 2010). In addition to cellulose, termites are able to digest other glucose polymers such as starch and glycogen through the action of amylases (Waller and Fage, 1986 & Azuma *et al*., 1984). The presence of α-amylase has been reported in the digestive systems of insects from the orders Orthoptera, Hymenoptera, Diptera, Lepidoptera, and Coleoptera (Terra and Ferreira, 1994), while few researches have been done to study amylase in termites. Alpha-amylases are major digestive enzymes that act in the first step of maltopolysaccharide digestion. Historically, insect amylases were widely studied early when electrophoresis techniques were developed. In insects, these enzymes have long been studied for applied as well as purely scientific purposes. In many species, multiple gene copies produce amylases. Most insects are strongly dependent on their amylases for development and survival. In most species studied, amylase is secreted at least in the midgut (Da Lage, 2018), the study by Odelson and Breznak, 1985b confirmed that the protist *Trichomitopsis termopsidis,* a symbiotic protozoan possesses hemicellulase and amylase as well as endo-β-1,4-glucanase activities. On the contrary, the saliva of workers of the three subterranean termites species; *Macrotermes gilvus, Coptotermes formosanus* and *Reticulitermes speratus* contains sufficient α-amylase activity to metabolize all ingested starch into maltose without the aid of their symbiotic partners (Subekti and Yoshimura, 2009)

The characterization of digestive enzymes from termites can contribute to the development of new insecticides. Natural insecticides derived from plants, such as enzyme inhibitors, lectins, and secondary metabolites, are known to interfere with the activities of the digestive enzyme (Lima *et al.*, 2014). In this field, Termiticidal lectins from *Myracrodruon urundeuva* influence the production of enzymes, cause severe injuries, oxidative stress and cell death in the midgut of the termite *N. corniger* workers (Lima *et al*., 2016). *Microgramma vaccinifolia* rhizome lectin possessed termiticidal activity and was able to enhance imbalances in the activities of acid phosphatase and trypsin-like protease as well as modulation of cellulase activities in the gut of workers and soldiers, which result in deregulating the entire process of cellulose digestion from the gut of termites. This lectine killed *Nasutitermes corniger* workers and soldiers through termiticidal mechanisms (Albuquerque *et al*., 2012).

Few researchers studied the digestive system in termite, but this is the first study on the digestive system of the subterranean termite *Psammotermes hypostoma* and comparing them between workers and soldiers. In the present study, we focus on the digestive system anatomy and three main digestive enzymes activity (endo-β-1,4-glucanase, cellobiase (β -glucosidase) and amylases) in two castes(workers and soldiers). And we hope this research will provide useful information about enzymes of these insects to use them in complementary studies for the goal of finding new control method, managing these important pests and the development of novel insecticides. Also, the morphology of the alimentary channels was described for the worker and the soldier.

MATERIALS AND METHODS

Termite Collections:

Subterranean termite was collected using traps of cellulose (El-Sebay, 1991) which were located in Al –Hashatra village, Fayoum Governorate, in April 2018, and then traps were collected after one month. The termite samples were then transported to Plant Protection Research Institute, A. R. C. Egypt. The termite was identified based on its morphological characters at Department of Termite and Wood Borers, Agricultural Research Center, Giza, Egypt.

Separation of Workers and Soldiers:

Workers and soldiers were separated depending on its enlarged mandibles and head capsules.

The Description Morphology of Termite Worker and Soldier Alimentary Canals:

To study the descriptive variations of alimentary canals, the worker and soldier termites were dissected in Ringer solution (6.5g sodium chloride, 0.14g potassium chloride, 0.12g calcium chloride, 0.2g sodium carbonate, and diluted with1000 distilled water) under stereomicroscope with syringe needle. Alimentary canals were isolated on glass slide and cleaned from fatty bodies by insulin syringe in sequenced changes of dissecting solution. Specimens were separated, described, and photographed using a computer and video microscope digital.

Isolation of the Digestive Tube to Evaluate Enzymes Activities:

One hundred and fifty termites of both workers and soldiers were surface washed with sterile distilled water. After cutting of their heads, using syringe needle, the entire guts were removed in buffer sodium acetate in ice and then were stored on ice in 0.1 M sodium acetate.

Preparation of Gut Extracts:

About 150 guts from workers and 150 guts from soldiers were separately homogenized using a porcelain mortar and pestle under cooling conditions in 2 ml of 0.1 M acetate buffer (pH 5.5) (Lima *et al*., 2014). Then, both homogenates were centrifuged at 9000 g for 15 min at 4 °C, where extracts were used for determination of protein concentration and digestive enzyme activities.

Protein Assay:

The protein amounts in the prepared extracts were determined using the Bradford assay at 595 nm (Willbur *et al*., 2016). Bovine serum albumin (BSA) was used to set up standard curves for the calculation of protein concentration. The developed color in this assay and the next enzyme assays were measured by spectrophotometer GENESYS 10 UV (Thermo Spectronic, Rochester, NY, USA). All experiments for protein and enzyme assays were repeated three times.

Determination of Endo-β-1,4-glucanase Activity:

To determine the endoglucanase activity on carboxy methylcellulose (CMC), briefly, 100 µL gut extract was mixed with 400 µL 1% CMC in 0.1 M sodium acetate buffer (pH 5.5) and 0.015 M NaCl. The reaction mixture was incubated for 10 min at 50 °C then 500 µL of DNS reagent was added, and finally boiled for 5 min. After the reaction temperature became as room temperature, the developed color was measured at 540 nm **(Lima *et al.*, 2014).** Glucose was used as a standard product for calculation of cellulase activity. One endo-β-1,4-glucanase unit is defined as the enzyme amount that liberates one µmole of glucose per minute.

Determination of Cellobiase (β-glucosidase) Activity:

The assay involved incubation of 100 µL gut extract and 400 µL (1%) cellobiose in 0.1 M sodium acetate buffer pH 5.5 for 10 min at 50 °C. The concentration of the resulting glucose was determined with a glucose kit assay purchased from Biodiagnostic according to the supplier´s instructions (Elnesr *et al*., 2018). This is an enzymatic method depending on

the action of glucose oxidase and peroxidase on glucose resulted from cellobiase activity on cellobiose. The colored quinonimine product in the test and standard assays were measured at 510 nm. One cellobiase (β -glucosidase) unit is the enzyme amount that generates one μmole of glucose per minute under the assay conditions.

Determination of Amylase Activity:

Each reaction contained 100 μL gut extract and 400 μL (1%) soluble starch in 0.1 M sodium acetate buffer pH 5.5 containing 0.02 M CaCl2 and 0.15 M NaCl. Then it was incubated at 50 °C for 10 min, after which the DNS method was continued as described previously to measure the developed color of the resulting reducing sugar (Lima *et al*., 2014). One amylase unit is defined as the enzyme amount that produces one μmole of glucose per minute under the given conditions.

Effect of pH and Temperature on Enzyme Activity:

We studied the effect of different pH (3-12) and temperatures (30-100 °C) on CMCase, cellobiase (β -glucosidase) and amylase activities. For studying the effect of pH on given enzyme activity, each reaction contained 100 μL gut extract mixed with 400 μL (1%) substrate in each of the buffers adjusted to the corresponding pH 3-12, and incubated at 50 °C for 10 min. After that, each reaction was continued for measuring the color product as described above in each enzyme assay. We used the buffers 0.1 M citrate-phosphate for pH 3-6, 0.1 M sodium phosphate for pH 7, 0.1 M tris-HCl for pH 8 and 9, 0.1 M Sodium bicarbonate and sodium hydroxide for pH 10 and 11, and 0.1 M sodium hydroxide solution for pH 12. For studying the effect of temperature on the given enzyme activities, the assays were done as described above with exception of incubation temperature which was changed to be 30, 40, 50, 60, 70, 80, 90 and 100 °C (Lima *et al*., 2014).

Statistical analysis of enzyme activity of each endo-β-1,4-glucanase, cellobiase (β -glucosidase) and amylase in workers and soldiers was estimated by ‘’T’’ test (SAS program, 2001)

RESULTS AND DISCUSSION

The termite was identified based on its morphological characters as the subterranean termite *Psammoterme hypostoma* Deseunex belonging to the lower termite group (family: Rhinotermitidae) This species of termite is considered as the most voracious pest that could cause severe damages for buildings, silos of grain, living trees and crops or any material containing cellulose, When termites attack buildings, they destroy lumber, furniture, wood doors, windows, flooring, carpeting, wood panels, wallpaper, paper products, and fabric made of plant fibers, artwork, books, clothing, and valuable papers.

The Description Morphology of Termite Worker and Soldier Alimentary Canals:

The worker and soldier alimentary canals of *P. hybostoma* are tubular with widened parts. The length of foregut, midgut and hindgut 37.8%, 27.1% and 35.1%for soldiers and 23.8%, 28.6% and 47.6% respectively for workers of the total length of alimentary canal Table (1). In soldiers, foregut is translucent and tubular in the interior part (esophagus) and broadens without a precise limit consisting crop. The crop, which appears as a simple dilatation of the esophagus connects in its base with spherical and translucent part (gizzard). The posterior end of the foregut is buried into the midgut and forms the cardiac valve. The midgut (mesenteron) is translucent, cylindrical with a uniform diameter and does not have a gastric caecum, which is seen in the midguts of most insects. Malpighian tubules are yellowish in color and attached at the mesentero-proctodeal junction. The hindgut (proctodeum) was the most developed portion of the gut; it consists of three segments and has modifications including the 1st segment which is a narrow and short tube at the junction with the midgut. The 2nd proctodeal segment or paunch is voluminous and dilated where the bulk of symbiotic microbiota is harbored. The 3rd segment is the colon which followed by

rectum. Soldier gut lacks particulate food contents, except the hindgut (Fig.1).

No significant differences were observed in morphological structures between workers and soldiers, but workers have internal pigmented colour because of finding particulate of food and symbiotic microbiota. Also, soldier hindgut is less developed than that of workers. Descriptive variations between workers and soldiers (maybe for differences in functional work and food requirements) were observed.

Table (1). Percentages of length for different parts of workers and soldiers gut of *P. hybostoma* termite.

Caste	ages of length for different parts of the gut		
	Foregut	Midgut	Hindgut
Workers	23.8	28.6	47.6
Soldiers	37.8	27.1	35.1

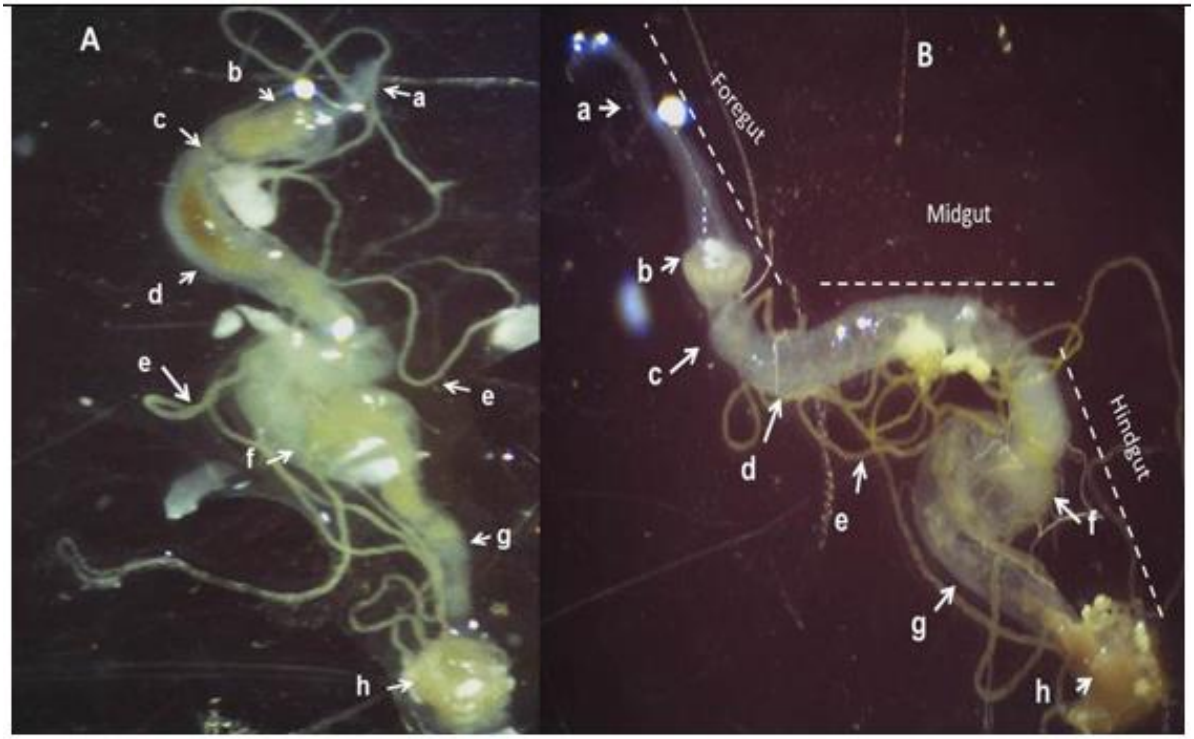


Fig.1. Morphology of *P. hypostoma* alimentary canal. A)Workers; B)Soldiers (a, oesophagus; b, crop; c, gizzard; d, midgut; e, malpighian tubules; f, paunch; g, colon; h, rectum)

Enzyme Activity:

Enzyme activity of each, endoglucanase cellobiase and amylase in both workers and soldiers are clarified in Table (2). After dissecting the termite workers and soldiers and getting their guts, we characterized specific activities of endo-β-1,4-glucanase at pH 5.5 and 50 °C in extracts of workers and soldiers guts as **12.3 ±0.057** and **6.4 ± 0.034** U/mg, respectively. Also, their cellobiase (β–glucosidase) activities were **3.4 ± 0.028** and **5.6 ±0.057**U/mg, respectively. Additionally, their amylase activities were **31.6 ± 0.115** and **16.1±0.88U**/mg, respectively.

Table 2. Enzyme activities of each endo-β-1,4-glucanase, cellobiase (β -glucosidase) and amylase from the gut extracts of workers and soldiers of *Psammotermes hypostoma* termite at pH 5.5 and 50 °C.

Caste / Enzyme	Workers		Soldiers		T – value
	Range	Av. ± SE	Range	Av. ± SE	
Endo-β-1,4-glucanase	12.3 - 12.4	12.3 ±0.057	6.34 – 6.46	6.4 ± 0.034	87.628
Cellobiase (β-glucosidase)	3.35 - 3.45	3.4 ± 0.028	5.5 – 5.7	5.6 ±0.057	-36.333
Amylase	31.4 - 31.8	31.6 ± 0.115	15.9 – 16.2	16.1 ± 0.88	106.908

The results in Table (2) showed highly significant differences in enzyme activity between both workers and soldiers of *Psammotermes hypostoma* termite, the obtained T values was 87.63, -36.33 and 106.91 for each endo-β-1,4-glucanase, cellobiase (β -glucosidase) and amylase, respectively.

Effect of Temperature and PH on the Activity of Different Enzymes:

The effect of different temperatures on (endo-β-1,4-glucanase) activity displayed that both workers and soldiers had maximum specific activities of 15.9 and 8.3 U/mg, respectively, at 70 °C. compared to this optimum temperature, endo-β-1,4-glucanase activity declined to 45%, 62%, 77%, 90%, 39%, 26% and 5.7% in workers, and 43%, 63%, 78%, 87%, 43%, 28% and 6% in soldiers at temperatures 30, 40, 50, 60, 80, 90, and 100 °C, respectively (Fig. 2A). endo-β-1,4-glucanase activity was also affected by pH variation. Where, pH 6 was the optimum for the enzyme activity in both workers and soldiers with corresponding activities 13.2 and 8.1 U/mg. However, endo-β-1,4-glucanase activity decreased to 27%, 59%, 86% 91%, 72%, 63%, 25%, 9% and 2% in workers at pH 3, 4, 5, 7, 8, 9, 10, 11 and 12, respectively. Similarly, endo-β-1,4-glucanase activity declined to 22%, 67%, 73%, 93%, 75%, 49% and 26% in soldiers at pH 3, 4, 5, 7, 8, 9 and 10 (Fig. 2B). Similar to our results, it was stated that *Coptotermes formosanus* endoglucanase had optimum PH 5.8 -6.0 and optimum temperature 70 °C after expression in *E. coli* (Inoue *et al*., 2005). On the other hand, it was found that endo-β-1,4-glucanase displayed the highest activity in *Nasutitermes corniger* workers and soldiers at pH 4 (Lima *et al.,* 2014). It is obvious that endo-β-1,4-glucanase activity in workers was significantly higher than soldiers as found in previous studies on *N. corniger* (Lima *et al.,* 2014) and *N. takasagoensis* (Fujita et al., 2008).

The highest cellobiase **(**β -glucosidase**)** activity was achieved in workers and soldiers at 50 °C and 40 °C, respectively, with corresponding specific activities 3.2 and 5.6 U/mg. Workers cellobiase (β -glucosidase) activity decreased to 73.5%, 60.5%, 5%, 2% and 0.6% at temperatures 60, 70, 80, 90, and 100 °C, respectively. While, soldiers cellobiase (β -glucosidase) activity declined to 85.5%, 68%, 59%, 32%, 16% and 3.5% at temperatures 50, 60, 70, 80, 90, and 100 °C, respectively (Fig. 2C). On the other hand, the maximum cellobiase **(**β -glucosidase) activity was achieved at pH 6 for both of workers and soldiers with corresponding specific activities 6.5 and 9.6 U/mg. However, cellobiase (β -glucosidase) activity declined to 1%, 41%, 54%, 69%, 23%, 14%, 7%, 5% and 1% in workers at pH 3, 4, 5, 7, 8, 9, 10, 11 and 12, respectively. Also, cellobiase **(**β -glucosidase) activity decreased to 0.5%, 44%, 68%, 73%, 21% and 15% in soldiers at pH 3, 4, 5, 7, 8 and 9, respectively, while the activity was totally lost at pH 10-12 (Fig. 2D). These results indicate that cellobiase (β -glucosidase) activity in workers is lower than soldiers and is similar to the previous study which found that β-glucosidase on ρ-nitrophenyl-β-Dglucopyranoside (ρNPG) was maximum at pH 11 in workers and pH 4 in soldiers at 30 °C (Lima *et al.* 2014). They also stated that *N. corniger* cellobiase (β -glucosidase) activity is declined by heating over 30 °C.

Cellulose is initially and partially degraded by endoglucanase which hydrolyzes the

amorphous region of cellulose (Watanab *et al*., 1998; Tokuda *et al.*, 2004; Brune, 2014 and Seneesrisakul *et al*., 2017), the termite soldiers receive the partially digested wood particles which were exposed to endoglucanase from the workers by trophallaxis (Huang *et al*., 2008) which confirms our results that the endoglucanase is higher (twice) in workers than in soldiers. After that the soldiers passé the partially digested cellulose to the midgut and hindgut where cellobiase and the other cellulase enzymes which are responsible for the final digestion of cellulose components complete the digestion process (Fischer., *et al.*, 2013) which confirms our result that cellobiase (β -glucosidase) is higher in soldiers than workers, our results also agrees with (Lima *et al.*,2014) who stated that, the levels of endoglucanase seen in workers, would not be required in the gut of the soldier. Otherwise, the enzymes exoglucanase and β-glucosidase, which are responsible for the final digestion of cellulose components, would be present at higher concentrations in soldiers

The optimum temperatures for amylase activity in workers and soldiers were 70 °C and 60 °C, respectively, with corresponding activities 35.8 and 18.2 U/mg. Increasing temperature to 80, 90 and 100 °C reduced amylase activity in workers to 80%, 53% and 13%, respectively. While soldiers amylase activity was declined to 94%, 77%, 54% and 11% at 70, 80, 90, and 100 °C, respectively (Fig. 2E). On the other hand, the maximum amylase activity was reached at PH7 for workers and soldiers with corresponding activities 41.8 and 22.4 U/mg. pH variation reduced workers amylase activity to 50%, 57%, 64%, 75%, 91%, 68%, 57%, 36% and 12% at pH 3, 4, 5, 6, 8, 9, 10, 11 and 12, respectively. Similarly, soldiers amylase activity was declined to 52%, 56%, 72%, 80%, 96%, 84%, 80%, 36% and 9% at pH 3, 4, 5, 6, 8, 9, 10, 11 and 12, respectively (Fig. 2F). Clearly, the presence of α-amylase in the digestive system of *P. hypostoma* workers and soldiers means that this species of termite is able to decompose starch, which explains why termites attack grain silos.

The results displayed that amylase activity in workers was higher than soldiers, which is in agreement with the previous study, which found that pH 6 displayed maximum amylase activity in *N. corniger* workers and soldiers at 30 °C (Lima *et al.*, 2014). Also, it was found that the highest amylase activity was achieved in workers of *Odontotermes brunneus* at pH 6 and 30 °C (Muthusamy *et al*., 2018). Additionally, the authors found other pH values and increasing temperature over 30 °C lead to a decline in amylase activity. The role of salivary enzymes in the detection of polysaccharides in the lower termite *Reticulitermes flavipes* was studied and found that the enzymes are necessary for termites to detect the presence of polysaccharides, the termites are able to detect starch because the amylase in their saliva hydrolyzes the starch into its monomer components (Cypret and Judd, 2015). Also the activity of amylase was detected in the salivary glands of the lower termite *Mastotermes darwiniensis* and formed 81% of the total amylase activity. The enzyme was of the α-type and produced mainly, maltose and maltotriose during the hydrolysis of amylose (Veivers., *et al*. 1982). Low levels of amylase activity are present in the higher termite *Nasutitermes walker*, it is secreted in the salivary glands and it is only found in the anterior portion of the midgut (Hogan., *et al.* 1988). Furthermore, insect amylases can be considered as targets enzymes of enzyme inhibitors, the α-amylase inhibitors are highly specific for their target enzymes, and their use for insect control especially weevils as these are highly dependent on starch for their energy supply.

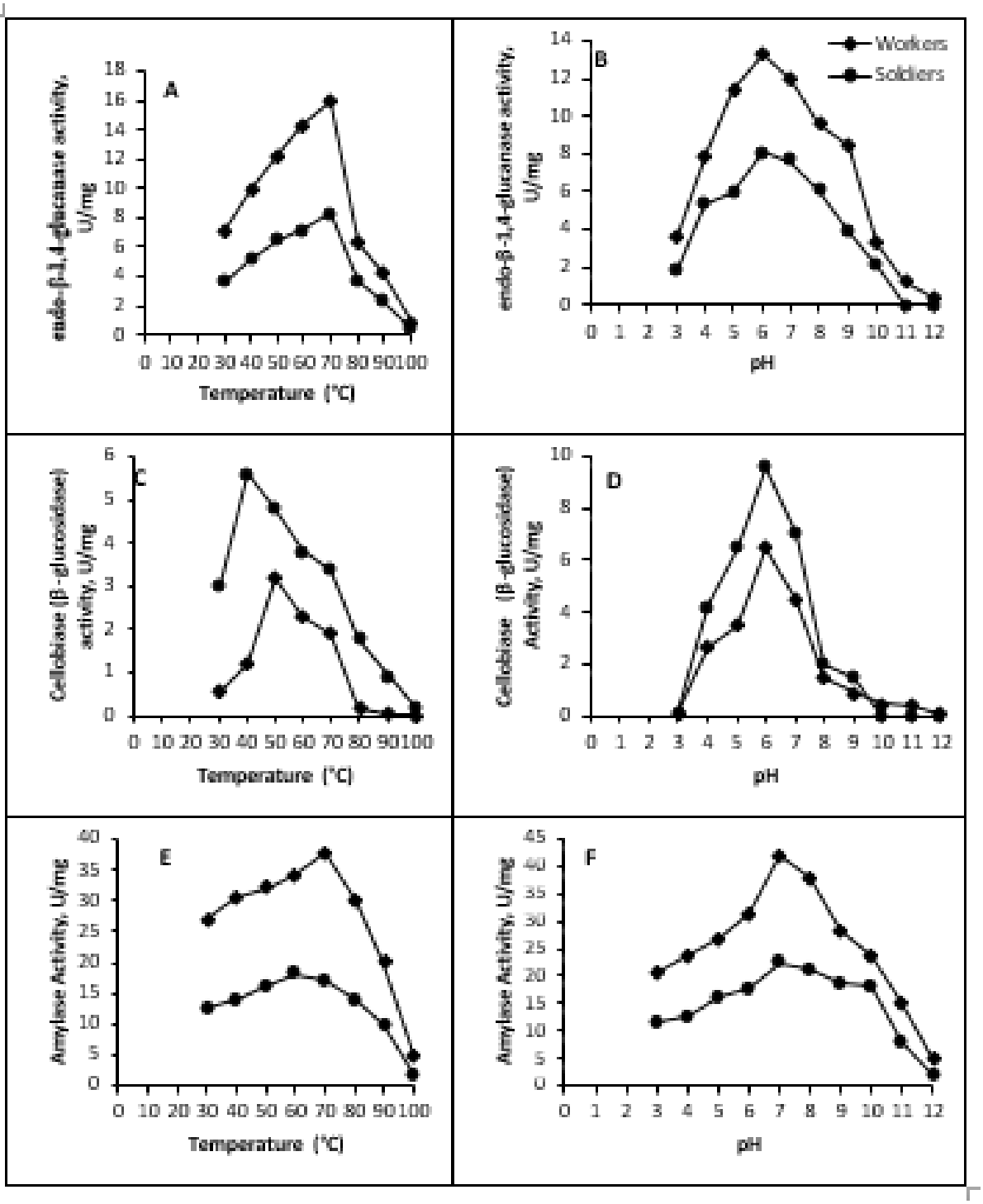


Fig. 2. Effects of temperature variation on endo-β-1,4-glucanase, cellobiase (β -glucosidase) and amylase specific activities are shown in figures A, C and E, respectively. While, the effects of different pH levels on the activities of endo-β-1,4-glucanase, cellobiase (β -glucosidase) and amylase specific are shown in figures B, D and F, respectively.

CONCLUSION

Activities of endo-β-1,4-glucanase and amylase were higher in workers than soldiers while cellobiase (β -glucosidase)) activity was lower in workers than soldiers. pH 6 was optimum for activities of endo-β-1,4-glucanase and cellobiase (β -glucosidase), while pH 7 was optimum for amylase activity in workers and soldiers. Also maximum endo-β-1,4-glucanase in extracts of workers and soldiers activity was achieved at 70 °C. While cellobiase

(β -glucosidase) activity was maximum in workers and soldiers at 50 °C and 40°C, respectively. Additionally, workers amylase activity was maximum at 70 °C, whereas 60 °C was optimum for soldier's amylase.). It was found that the activity of worker and soldier endo-β-1,4-glucanase was lost after heating at 100 °C, also at 11 and 12 pH for soldiers and 12 pH for workers. While the activity of worker and soldier cellobiase (β -glucosidase) was lost after heating at 80 °C for soldiers and 100 for workers, also at 10, 11and 12 pH for soldiers and workers. In the next study, inhibition studies of the cellulase system enzymes may lead to the development of potential pesticides for termite control.

REFERENCES

Ahmed, M. A. I.; S. A. Eraky; M. Fakeer and A. S. S. Desoky (2014): Toxicity assessment of selected neonicotinoid pesticides against the sand termite, *Psammotermes hypostoma* Desneux Workers (Isoptera: Rhinotermitidae) under laboratory conditions. Aust J. Basic Appl. Sci. 8(9):238-240.

Albuquerque, L. P.; G. M. S. Santana; E. V. Pontual; T. H. Napoleão; L. C. B. B. Coelho and P. M. G. Paiva (2012): Effect of *Microgramma vaccinifolia* rhizome lectin on survival and digestive enzymes of *Nasutitermes corniger* (Isoptera, Termitidae). Int. Biodeterior. Biodegrad. 75, 158–166.

Azuma, J.; K. Nishimoto and T. Koshijima (1984): Studies on Digestive System of Termites*II. Properties of Carbohydrolases of Termite *Coptotermes formosanus* Wood Res., 70, 1-16.

Breznak, J. A and A. Brune (1994): Role of microorganisms in the digestion of lignocellulose by termites. Annu. Rev. Entomol. 39: 453–487.

Brune, A. (2011): Microbial Symbioses in the Digestive Tract of Lower Termites. In: Rosenberg, E. and Gophna, U. (eds.). Beneficial Microorganisms in Multicellular Life Forms, DOI 10.1007/978-3-642-21680-0_1, Springer Verlag Berlin Heidelberg, 348pp. (Book)

Brune, A. (2014): Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, 12, 168–180.

Cypret, J. A. and T. M. Judd (2015): The Role of Salivary Enzymes in the Detection of Polysaccharides in the Termite *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitdae), Sociobiology 62(4): 593-597.

Da Lage, J-L (2018): The Amylases of Insects. International Journal of Insect Science Volume 10: 1–14.

Eggleton, P. (2011): An Introduction to Termites: Biology, Taxonomy and Functional morphology. PP. 1-26 in: Bignell, D.E., Y. Roisin & N. Lo (Eds). Biology of Termites: A Modern Synthesis. London, New York: Springer Science +Business Media BV. 576 pp. (Book).

Elnesr, S.S.; A. Ropy and A. H. Abdel-Razik (2018): Effect of dietary sodium butyrate supplementation on growth, blood biochemistry, hematology and histomorphometry of intestine and immune organs of Japanese qua*il.* animal. 1–11 doi:10.1017/S1751731118002732

El-Sebay, Y. (1991): A modified El-Sebay trap for subterranean termite. Proc. 4th Arab Cong Plant Protection: 245-247.

El-Sebay, Y. and H. M. Ahmed (2006): Survey and damage assessment of subterranean termite at Fayoum and North Sinai governorates, in Egypt, Egypt. J. Agric. Res., 84 (5): 1419-1427.

Fischer, R.; R. Ostafe and R. M. Twyman (2013): Cellulases from insects. In: Vilcinskas, A. (Ed.),Yellow Biotechnology II: Insect Biotechnology in Plant Protection and

Industry. Advances in Biochemical Engineering/Biotechnology, vol. 136. Springer, Berlin Heidelberg, Berlin, pp. 51–64.

Fujita, A.; M. Hojo; T. Aoyagi ; Y. Hayashi; G. Arakawa; G. Tokuda; and H. Watanabe (2010): Details of the digestive system in the midgut of *Coptotermes formosanus* Shiraki. J Wood Sci 56:222–226.

Fujita, A.; T. Miura and T. Matsumoto (2008): Differences in cellulose digestive systems among castes in two termite lineages. Physiol. Entomol. 33, 73–82.

Hogan, M.; P. C. Veivers; M. Slaytor and R.T. Czolij (1988): The site of cellulose breakdown in higher termites (*Nasutitermes walkeri* and *Nasutitermes exitiosus*). J. Insect Physiol.34(9), 891–899.

Hongoh, Y. (2011): Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. Cell. Mol. Life Sci. 68:1311–1325.

Huang, Q. Y.; W. P. Wang; R. Y. Mo and C. L. Lei (2008): Studies on feeding and trophallaxis in the subterranean termite *Odontotermes formosanus* using rubidium chloride. Entomologia Experimentalis et Applicata 129: 210–215.

Husseneder, C. (2010): Symbiosis in subterranean termites: A review of insights from molecular studies, Environ. Entomol. 39(2): 378-388.

Inoue, T.; K. Murashima ; J. Azuma; A. Sugimoto and M. Slaytor (1997): Cellulose and xylan utilisation in the lower termite *Reticulitermes speratus*. J Insect Physiol 43(3):235–242.

Inoue, T; S. Moriya; M. Ohkuma;, and T. Kudo (2005): Molecular cloning and characterization of a cellulase gene from a symbiotic protist of the lower termite, *Coptotermes formosanus*. Gene, 349:67–75.

Koshikawa, S.; T. Matsumoto & T. Miura (2003): Mandibular morphogenesis during soldier differentiation in the damp-wood termite *Hodotermopsis sjostedti* (Isoptera: Termopsidae) *Naturwissenschaften*, 90, 180 – 184.

Kuhad, R. C.; R. Gupta and A. Singh (2011) Microbial Cellulases and Their Industrial Applications. Enzyme Research, Article ID 280696, 10 pages.

Li, L.; J. Fröhlich and H. König (2006): Cellulose Digestion in the Termite Gut. pp 221-241 in: König, H and A. Varma (Eds.) Intestinal Microorganisms of Soil Invertebrates, Soil Biology, volume (6), Springer-Verlag Berlin Heidelberg.

Lima, T. A.; E. V. Pontual; L. P. Dornelles; P. K. Amorim; R. A. Sá; L. C. B. B. Coelho; T. H. Napoleão and P. M. G. Paiva (2014): Digestive enzymes from workers and soldiers of termite *Nasutitermes corniger*.. Comp Biochem Physiol B Biochem Mol Biol. Part B 176:1-8.

Lima, T. A.; K. M. Fernandes; A. P. Oliveira; L. Dornelles; G. F Martins; T. Napoleãoa and P. Paiva (2016): Termiticidal lectins from *Myracrodruon urundeuva* (Anacardiaceae) cause midgut damage when ingested by *Nasutitermes corniger* (Isoptera: Termitidae) workers. Pest Manag Sci,73:991–998.

Muthusamy, R.; R. Gayathri; K. Suresh and K. T. Chethan (2018) Effect of diets on digestive enzymes from worker termites of *Odontotermes brunneus* (Termitidae). J Indian Acad Wood Sci, 15(1):61–67.

Ni, J. and G. Tokuda (2013): Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. Biotechnol. Adv. 31(6): 838–850.

Odelson, D. A. and J. A. Breznak (1985a) Nutrition and growth characteristics of *Trichomitopsis termopsidis*, a cellulolytic protozoan from termites. Appl Environ Microbiol 49:614–621

Odelson, D. A. and J. A. Breznak (1985b) Cellulase and other polymer-hydrolyzing activities of *Trichomitopsis termopsidis*, a symbiotic protozoan from termites. Applied and Environmental Microbiology 49(3): 622–626.

Ohkuma, M. (2003) Termite symbiotic systems: efficient bio-recycling of lignocellulose," Appl Microbiol Biotechnol 61(1):1-9.

Peterson, B. F. and M. E. Scharf (2016): Lower termite associations with microbes: synergy, protection, and interplay, Front Microbiol 7: 1-7.

Ramin, M.; A. R. Alimon; N. Abdullah; J .M. Panandam and K. Sijam (2008): Isolation and identification of three species of bacteria from the termite *Coptotermes curvignathus* (Holmgren) present in the vicinity of university. Res J Microbiol 3 (4): 288-292.

Rust, M and N.Y. Su (2012):. Managing social insects of urban importance. Annu. Rev. Entomol. 57: 355-375.

SAS institute (2001). Version 8.02.SAS Institute, Cary, N C.

Seneesrisakul K.; S. A. Guralp; E. Gulari and S. Chavadej (2017): *Escherichia coli* expressing endoglucanase gene from Thai higher termite bacteria for enzymatic and microbial hydrolysis of cellulosic materials. Electronic Journal of Biotechnology 27:70– 79.

Souza, P. M. and P.O. Magalhães (2010): Application of microbial α-amylase in industry — a review. Braz. J. Microbiol. 41: 850–861.

Su, N.-Y. and R. H. Scheffrahn (1990): Economically important termites in the United States and their control. Sociobiology 17: 77–94.

Subekti, N. and T. Yoshimura (2009): α.Amylase activities of saliva from three subterranean termites : *Macrotermes gilvus* Hagen *Coptotermes formosanus* Shiraki, and *Reticulitermes speratus* (Kolbe), Jpn. J. Environ. Entomol. Zool. 20 (41) : 191-194

Talia, P. and J. Arneodo (2018): Lignocellulose Degradation by Termites. PP.101-117, in: M.A. Khan, W. Ahmad (eds.), Termites and Sustainable Management.

Terra, W. R. and C. Ferreira (1994): Insect digestive enzymes: properties compartmentalization and function. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 109(1), 1–62.

Tokuda, G.; N. Lo; H. Watanabe; G. Arakawa; T. Matsumoto and H. Noda (2004): Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. Mol. Ecol. 13: 3219–3228.

Varma, A.; B. K. Kolli; J. Paul; S. Saxena and H. Konig (1994): Lignocellulose degradation by microorganisms from termite hills and termite guts: a survey on the present state of art. FEMS, Microbiol Rev 15(1):9-28.

Veivers, P.C.; A.M. Musca; R.W. O'Brien and M. Slaytor (1982): Digestive enzymes of the salivary glands and gut of *Mastotermes darwiniensis*. Insect Biochemistry 12(1):35–40.

Waller, D. A. and J. P. Fage (1986):Nutritional ecology of termites. In: Slansky Jr., F., Rodriguez, J.G. (Eds.), Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. John Wiley & Sons, New York, pp. 487–532

Warnecke, F.; P. Luginbühl; N. Ivanova; M. Ghassemian; T. H. Richardson; J. T. Stege; M. Cayouette; A. C. McHardy; G. Djordjevic; N. Aboushadi; R. Sorek; S. G. Tringe; M. Podar; H. G. Martin; V. Kunin; D. Dalevi; J. Madejska; E. Kirton; D. Platt; E. Szeto; A. Salamov; K. Barry; N. Mikhailova1; N. C. Kyrpides; E. G. Matson5; E. A. Ottesen; X. Zhang; M. Hernández; C. Murillo; L. G. Acosta; L Rigoutsos; G. Tamayo; B. D. Green; C. Chang; E. M. Rubin; E. J. Mathur; D. E. Robertson; P. Hugenholtz and J. R. Leadbetter (2007): Metagenomic and functional analysis of hindgut microbiota of a wood feeding higher termite. Nature 450, 560–565.

Watanabe, H.; H. Noda; G. Tokuda and N. Lo (1998): A cellulase gene of termite origin. Nature 394: 330–331.

Wenzel, M; I. Schonig; M. Berchtold; P. Kampfer and H. Konig (2002): Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis*

angusticollis, J Appl Microbiol, 92: 32-40.

Willbur, J.F.; J.D. Vail; L.N. Mitchell; D.L. Jakeman and S.C. Timmons (2016): Expression, purification, and characterization of a carbohydrate-active enzyme: A research-inspired methods optimization experiment for the biochemistry laboratory. Biochem. Mol. Biol. Educ. 44: 75–85.

Yamin, M. A. and W. Trager (1979): Cellulolytic activity of an axenically cultivated termite flagellate, *Trichomitopsis termopsidis*. J. Gen. Microbiol. 113, 417–420.

Yoshimura, T.; J.-1. Azuma; K. Tsunoda and M. Takahashi (1993a): Cellulose metabolism of the symbiotic protozoa in termite, *Coptolermes formosanus* Shiraki 75 (lsoptera: Rhinotermitidae) I. Effect of degree of polymerization of cellulose. *Mokuzai Gakkaishi* 39, 221-226.

Yoshimura, T.; J-1. Azuma; K. Tsunoda and M. Takahashi (1993c): Cellulose metabolism of the symbiotic protozoa in termite, *Coptotermes formosanus* Shiraki (Isoptera:Rhinotermitidae) III. Utilization of non-natural celluloses. *Mokuzai Gakkaishi* 39, 1322-1326.

Yoshimura, T.; J-I. Azuma; K. Tsunoda and M. Takahashi (1993b): Cellulose metabolism of the symbiotic protozoa in tennite, *Coptolermes formosanus* Shiraki (Isoptera: Rhinotermitidae) II. Selective defaunation of protozoa and its effect on cellulose metabolism. *Mokuzai Gakkaishi* 39, 227-230.

ARABIC SUMMARY

الاختلافات فى الجهاز الهضمي و الانزيمات بين شغالات وجنود النمل الأبيض التحت أرضى *Psammotermes hypostoma* Desneux (Rhinotermitidae: Isoptera)

هدى رجب خليل على[1] - علاء روبي [2] - محمد عبد الغنى بط[1]

1-معهد بحوث وقاية النباتات-مركز البحوث الزراعية-الدقي – الجيزة-مصر

2- قسم الكيمياء، كلية العلوم / جامعة الفيوم

من بين ثمانية أنواع من النمل الأبيض الموجودة في مصر، يعتبر النمل الأبيض التحت أرضى *Psammotermes hypostom* آفة خطيرة تحطم الهياكل الخشبية في المنازل ومنتجات الأخشاب الأخرى أو أي مادة تحتوي على السليلوز، وبالتالي تعتبر أنزيمات السيلوليز هي الأنزيمات الرئيسية في الجهاز الهضمي للنمل الأبيض، و يعتبر الجهاز الهضمي للنمل الأبيض هدفا لتحقيق أفضل مكافحة لتلك الافة.

يناقش هذا البحث وصف الاختلافات المورفولوجية للقنوات الهضمية للشغالة والجندي وكذلك الكشف عن ثلاثة إنزيمات هضمية: اثنين من انزيمات السيلوليز (Endo-β-1,4-glucanase ، β-glucosidase) والانزيم الثالث أنزيم amylase في مستخلصات الأمعاء من الشغالات والجنود. بالإضافة إلى ذلك، تمت دراسة تأثير التنوع في درجة الحرارة ودرجة الحموضة على أنشطة الإنزيمات. وقد وجد أن الشغالات لديهم نشاط endo-β-1,4-glucanase و amylase أعلى ، بينما كان للجنود نشاط سيلوبيز (β-glucosidase) أعلى. من الواضح أن وجود α-amylase في الجهاز الهضمي للشغالات والجنود لهذا النوع من النمل الابيض، يؤكد أن هذا النوع من النمل الأبيض قادر على تحليل النشا، وهو ما يفسر لماذا يهاجم النمل الأبيض صوامع الحبوب.

تأثير درجة الحرارة و درجة الحموضة على النشاط الانزيمي أوضح أن درجة الحرارة ة و الحموضة المثلى كانت °70 درجة مئوية ،6 درجة حموضة لـ endo-β-1,4-glucanase ، و°50 درجة مئوية، 6 درجة الحموضة بالنسبة للسيلوبيز (β-glucosidase) و °70 درجة مئوية، 7 درجة الحموضة لـ amylase للشغالات، بينما **في حالة الجنود** كانت درجة الحرارة المثلى و درجة الحموضة °70 درجة مئوية، 6 درجة الحموضة لـ endo-β-1,4-glucanase ، و °40 درجة مئوية، 6 درجة الحموضة بالنسبة للسيلوبيز (β-glucosidase) و°60 درجة مئوية ، 7 درجة الحموضة لـ amylase.

ويعتبر هذا العمل هو الأول في مصر لدراسة النشاط الأنزيمى للجهاز الهضمي للنمل الأبيض وربما يعتبر خطوة أولية للمساهمة فى مكافحة هذا النوع من النمل الأبيض بتطوير مبيدات جديدة للنمل الأبيض