Reduction of Trehalase Activity and Morphological Changes of Reproductive Organs in the Red Flour Beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), Induced by Latex from Paper Mulberry, *Broussonetia papyrifera*[1]

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J. Entomol. Sci. 60(4): 000–000 (Month 2024)
DOI: 10.118474/JES24-86

Abstract Plant latex contains various compounds related to plant defensive roles against herbivores. The paper mulberry, *Broussonetia papyrifera* L. (Moraceae), produces latex in every part of the tree. The composition and properties of *B. papyrifera* latex have been reported; however, its effects on insects have not been reported. This study was conducted to examine the effect of *B. papyrifera* latex on the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Trehalase activity was suppressed by latex extracts *in vitro* at various concentrations, and an examination of the fundamental properties of trehalase inhibitor revealed that it is a heat-stable and proteinaceous molecule larger than 10 kDa. The latex extract reduced insect body mass, egg production, and the number of larvae, pupae, and adults of *T. castaneum* in a concentration-dependent manner. The reproductive organs in both males and females were affected by latex treatment, resulting in a reduction in the size of mature oocytes and germarium in female beetles. Testicular lobes and accessory glands in male beetles also decreased in size. Trehalase activity in reproductive tissues was suppressed by latex, which led to the reduction of glucose content, but the amount of trehalose was not affected. These findings revealed that the latex of *B. papyrifera* contains components that suppress the trehalase activity, interfere with the growth and reproductive organs of the red flour beetle, and thus may contribute to the plant's defense strategy against this insect pest.

Key Words plant latex, trehalase, trehalose, red flour beetle, trehalase inhibitor

Plant latex contains many active compounds, such as alkaloids, proteins, starches, sugars, resins, and tannins. Latex is exuded from laticifers following physical damage to plants. Latex has been recognized for its importance in plant defense against herbivorous insects and plant pathogens (Mithöfer and Boland 2012). Latex from several plant families, such as Annonaceae, Solanaceae, Asteraceae, Cladophoraceae, Lamiaceae, Meliaceae, Rutaceae, and Moraceae, have deleterious effects on insects, with high toxicity, mortality, antifeedant activity, and inhibition of growth and reproductive activity (Konno 2011, Upadhyay 2011).

Trehalase enzyme (α-glucoside-1-glucohydrolase, EC 3.2.1.28) catalyzes the hydrolysis of trehalose to glucose molecules. Trehalose, the primary hemolymph

[1]Received 10 August 2024; accepted for publication 27 September 2024.
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sugar in insects, is maintained in the larval hemolymph, serving as the source of energy for adult development (Silva et al. 2004, Terra and Ferreira 1994). The latices from the mulberry, *Morus alba* L., the jackfruit, *Artocarpus heterophyllus* Lamarck, and the weeping fig, *Ficus benjamina* L., contain proteins that are responsible for defense mechanisms (Chen et al. 2020, Siritapetawee et al. 2012). The methanol extract of these three plant latices inhibited trehalase activity in the red flour beetle, *Tribolium castaneum* Herbst. It also decreased the expression of the soluble trehalase gene, causing an increase in trehalose concentration up to 140% and a decrease in glucose concentration, to approximately 72% of the control (Tatun et al. 2014). These plants belong to the Moraceae family, primarily distributed in tropical and subtropical regions. However, many species, including the paper mulberry, *Broussonetia papyrifera* L., are economically important and have been cultivated widely, but their latices have not been examined for the presence of trehalase inhibitors and for their biological effects on insects.

The paper mulberry is a deciduous tree distributed throughout Asia, including Thailand and has been cultivated for food, fiber, and medicine (Chen et al. 2020). In recent years, numerous effects, properties, and compositions of latex have been identified, and latex has been used in many applications, including medicinal and cosmetic (Verma et al. 2022). However, there are limited reports about the effect of paper mulberry latex on insects, especially insect pests. In a preliminary study of the inhibitory activity of trehalase against the red flour beetle, we used latex extracted from seven species belonging to the Moraceae family and found that latex from *B. papyrifera* had elevated levels of inhibitory activity compared with the other species tested. Thus, we undertook the present study to examine the effects of paper mulberry latex extract on the growth of *T. castaneum*. A biochemical study also was performed to examine the changes in trehalase activity and the concentrations of glucose and trehalose in *T. castaneum* treated with *B. papyrifera* latex extract. The morphological changes in the reproductive organs of the red flour beetle also were examined.

Materials and Methods

Insect cultures. The stock cultures of *T. castaneum* were maintained on sterilized wheat flour plus 5% yeast as a source of protein. The plastic container with perforated cap was kept at 30 $\pm$ 1°C and 70 $\pm$ 5% relative humidity. In this study, we used 1-wk-old adult beetles to assay trehalase inhibitory activity.

Plant latex preparation. The latex of *B. papyrifera* was collected by cutting the young branches and petioles of young leaves. Methanol (60 ml) was added to 15 ml of latex and kept at 4°C for 48 h for maximal extraction. After centrifugation at 14,000 $\times$ *g* for 15 min at 4°C, the resulting supernatant was dried in an evaporator at 40°C. The residue was dissolved in 15 ml of 20 mM sodium phosphate buffer (PB; NaH_2PO_4/Na_2HPO_4) (pH 6.5) and stored at $-$20°C until used.

Latex treatments. The methanol extract of *B. papyrifera* latex was then subjected to three distinct kinds of treatment: dialysis, heating, and trypsin digestion. The methanol extract was dialyzed in a dialysis bag with a 6–8-kDa cutoff (Spectrum Laboratories, Rancho Dominguez, CA) against 500 volumes of 20 mM PB (pH 6.5) for 24 h at 4°C; the dialysate was used directly for measurement of trehalase inhibition. The methanol extracts were heated at 100°C for 5 min to examine heat stability. The solution was

cooled at 4°C for 10 min and centrifuged at 10,000 × *g* for 5 min at 4°C to remove the denatured materials. The obtained supernatant was used to assay inhibitory activity against *T. castaneum* trehalase. For trypsin digestion, the methanol extracts were mixed with 10 μl of trypsin (10 mg/ml; BioBasic Inc., Markham, Ontario, Canada), and the solution was incubated for 5 h at 37°C. The protease inhibitor (10 μl) was added to stop the reaction (10 mg/ml; cocktail protease inhibitor, BioBasic). Then, the reaction mixture was centrifuged at 10,000 × *g* at 4°C for 10 min, and the supernatant was used for the trehalase inhibition assay.

Preparation of trehalase samples. For the *in vitro* study, 50 adult *T. castaneum* were homogenized in 50 ml of 20 mM PB (pH 6.0), filtered through a doubled layer of cheesecloth, and centrifuged at 10,000 × *g* at 4°C for 10 min. The resulting supernatant was subjected to acetone precipitation to eliminate other materials, including endogenous sugar from the insect extract. The supernatant was mixed with three volumes of ice-cold acetone and kept at −20°C for 3 h for protein precipitation. The pellet protein was collected by centrifugation at 10,000 × *g* at 4°C for 10 min, and residual acetone was removed by air drying. The precipitated protein was dissolved in 20 mM PB and used as the source of trehalase enzyme for inhibitory assay *in vitro*. For the *in vivo* assay, the insects treated with latex extract ($n = 10$) were homogenized in 500 μl of 20 mM PB (pH 6.0), and the sample was sonicated for 5 s, filtered through a doubled layer of cheesecloth, and centrifuged at 10,000 × *g* at 4°C for 10 min. The resulting supernatant was used for the trehalase inhibition assay.

Measurement of trehalase activity. Trehalase activity was determined according to the method of Hirayama et al. (2007). The reaction mixture consisted of 62.5 μl of 40 mM trehalose (Sigma, St. Louis, MO) in 20 mM PB, 10 μl of crude enzyme solution, and 177.5 μl of PB to adjust the final volume to 250 μl. The mixture was incubated at 37°C for 60 min, and the reaction was heated at 100°C for 5 min in a dry block heater to stop the reaction. The reaction tube was cooled on ice and then centrifuged at 10,000 × *g* for 10 min at 4°C to separate coagulated proteins, and the supernatant was used for a glucose assay with the hexokinase-glucose-6-phosphate dehydrogenase method. The reaction comprised 50 units of hexokinase, 100 units of glucose-6-phosphate dehydrogenase, 2 mM NADP, and 2.8 mM ATP (Roche Diagnostics GmbH, Mannheim, Germany). Trehalase activity was calculated with a glucose standard, and the activity was expressed in nanomoles of glucose per microgram of protein per hour. The quantity of protein in each insect sample solution was measured with the protein dye-binding method (Bio-Rad, Hercules, CA); bovine serum albumin was used as the standard.

Determination of inhibitory activity *in vitro*. The trehalase solution (10 μl) was mixed in microcentrifuge tubes with 140 μl of the three methanol extracts of plant latex: dialyzed, pepsin treated, and heated. The mixtures were incubated at 37°C for 30 min in a water bath, 60 μl of 40 mM trehalose and 60 μl of 20 mM PB were added to the mixture, and the mixture was incubated at 37°C for 60 min in a water bath. The mixture was then heated at 100°C for 5 min, cooled on ice, and centrifuged at 12,000 × *g* for 10 min at 4°C to remove the denatured protein. The supernatant was used to measure the amount of glucose as described above.

Determination of biological changes to *T. castaneum*. The wheat flour (5 g) for the diet of *T. castaneum* was supplemented with the methanolic extracts of *B. papyrifera* latex (5 ml), and suspensions were mixed vigorously. The flour mixture was then

dried at 40°C for 48 h. A control diet was prepared in the same way but without the added latex extract. Sixty adults (30 male, 30 female) were reared on a diet treated with latex extract at different concentrations for 7 d then removed from the diet. Body mass and mortality were measured after 7 d of continued treatment with the latex extract. The diet was then sieved to collect the eggs, and egg numbers were recorded. The eggs were reared on a controlled diet to observe the number of larvae, pupae, and adults. The experiment was conducted three times.

Morphological changes to reproductive organs. The adult beetles were dissected under a microscope in a glass Petri dish with a small drop of insect Ringer's solution. After wing removal, the dorsal side of the abdomen was opened with dissecting forceps. The reproductive organs were collected, and the attached tissue, including tracheoles and fat bodies, were separated. The morphology of the male and female reproductive organs were observed under a stereomicroscope (Olympus Corp., Tokyo, Japan), and photos were digitally captured with an microscope camera (4083 WiFi, Optika Srl, Ponteranica, Italy). The quality of the images was improved with Adobe Photoshop CS3 software. This software also was used to measure the width of the testicular lobes, the rod-shaped accessory gland, the tubular-shaped accessory gland in male beetles, and the mature oocytes and germarium in female beetles.

Measurement of trehalose and glucose. Samples of ovary and testis were collected from latex-ingesting adults ($n = 60$), weighed, and homogenized in 200 µl of distilled water, boiled for 10 min, chilled on ice, and centrifuged at 15,000 × *g* for 10 min. The supernatant was mixed with four volumes of cold methanol, incubated at 4°C for 2 h, and centrifuged at 15,000 × *g* for 10 min to remove precipitates. This supernatant was transferred to a new tube, and hexane was added at the same volume. The tube was shaken on a vortex mixer and centrifuged briefly to separate the hexane and aqueous methanol layers. The lower layer was transferred to a new tube, the methanol was evaporated, and the dried residue was dissolved in 20 mM PB. The resulting solution was divided into two equal parts to quantify the concentrations of trehalose and glucose. To determine the trehalose concentration in ovary and testis extracts, the sample solution was mixed with trehalase solution and incubated at 37°C for 24 h to digest the trehalose into glucose. The amount of glucose was determined with the hexokinase-glucose-6-phosphate dehydrogenase method described previously. The amount of glucose in the ovary and testis extract was determined with the same method; the glucose concentrations were expressed as nanomoles of glucose per milligram of tissue.

Statistical analysis. The data were analyzed with a one-way analysis of variance (SPSS Statistics 22, IBM, Armonk, NY) followed by a least significance difference multiple-range test. The significance level was set at $P < 0.05$.

Results

Inhibitory activity of latex of plants of the Moraceae family. The trehalase activity of *T. castaneum* was measured after mixing with the latices from seven species from the Moraceae family: *B. papyrifera, Ficus hispida* L., *Ficus curtipes* Corner, *Ficus religiosa* L., *Ficus virens* Aiton, *Streblus asper* Loureiro, and *Artocarpus altilis* (Parkinson) Fosberg. The inhibitory activity of latex in each species was calculated (Fig. 1A), and results revealed that *B. papyrifera, F. hispida, F. religiosa, F. virens,*

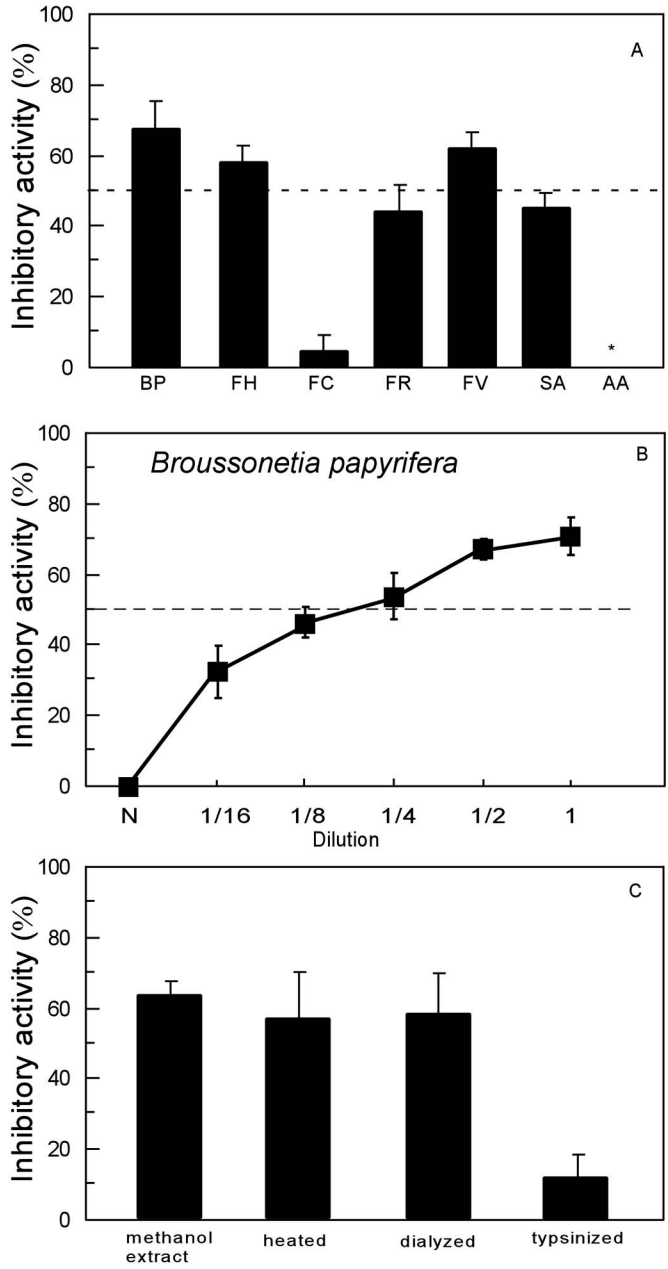

Fig. 1. ***In vitro*** **inhibition of** ***T. castaneum*** **trehalase activity by the latex of plant species of the Moraceae family. Error bars indicate the standard deviation from the mean. (A) Inhibitory activity of latex from seven species:** ***Broussonetia papyrifera*** **(BP),** ***Ficus hispida*** **(FH),** ***Ficus curtipes*** **(FC),**

and *S. asper* latices had evident inhibitory activity against trehalase. In contrast, low inhibitory activity was recorded for *F. curtipes* latex. No inhibitory activity was detected in the latex of *A. altilis*. The inhibitory activity *B. papyrifera* latex was higher than that of any other plant species tested. Further examination of the basic characteristics and effects of latex on *T. castaneum* in various aspects is required.

Characteristics of the inhibitor in *B. papyrifera* latex. The methanol extract of *B. papyrifera* latex was diluted and assessed for inhibitory activity against *T. castaneum*. Trehalase activity was reduced in a concentration-dependent manner with approximately 10-fold dilution needed for 50% inhibition, indicating that the latex contains methanol-extractable factors that reduce trehalase activity of *T. castaneum* (Fig. 1B). The methanol extract that was heated retained trehalase inhibitory activity at about 52% inhibition (Fig. 1C). After dialysis in a 10-kDa cutoff dialysis bag, the trehalase inhibitory activity was recorded at approximately 56% in the dialysate. The methanol extract latex that was digested by trypsin had decreased inhibitory activity, about 25% of that of the original latex extract, which had 65% inhibition.

Biological effects of *B. papyrifera* latex. We examined whether *B. papyrifera* latex was toxic to *T. castaneum*. Adults were fed with different concentrations of latex extract, and mortality rate and insect body mass were recorded after 7 d (Fig. 2). Insect body mass decreased in a concentration-dependent manner (Fig. 2A), and the cumulative mortality rate was higher when insects were fed on four-, two-, and onefold dilutions of latex extract (Fig. 2B). The number of eggs produced and the number of larvae, pupae, and adults were recorded after the adults were fed with latex extract of various dilutions (Fig. 3). The control group produced the highest number of eggs, but this number decreased significantly for insects reared in an eight-fold dilution of latex extract (Fig. 3A). The number of eggs produced dropped to the lowest level in the group fed the four-, two-, and onefold dilutions. Fewer larvae developed from the eggs produced by insects that were treated with two- and onefold dilutions of latex extract (Fig. 3B). Significantly fewer pupae developed when insects were fed on eight-, four-, and twofold dilutions of latex extract (Fig. 3C). No pupae were found in the onefold dilution group. Therefore, the number of adults in eight-, four-, and twofold dilution groups were greatly reduced. No adults were found in the group treated with a onefold dilution of latex extract because pupae did not develop (Fig. 3D).

Morphological changes in reproductive organs. The reproductive organs of the adult male and female beetles comprised all the essential parts following exposure to the latex; however, the sizes of these parts were reduced after latex treatment (Figs. 4, 5). Examination of the treated ovaries revealed that the mature oocytes and germarium were shorter than those in the control beetles (Fig. 4A);

←

***Ficus religiosa* (FR), *Ficus virens* (FV), *Streblus asper* (SA), and *Artocarpus altilis* (AA). Asterisk indicates no inhibitory activity detected. (B) Inhibitory activity of *B. papyrifera* latex serially diluted and incubated with the enzyme sample. N, a reaction mixture without the addition of latex extract. (C) Inhibitory activity of the latex extract treated by four methods.**

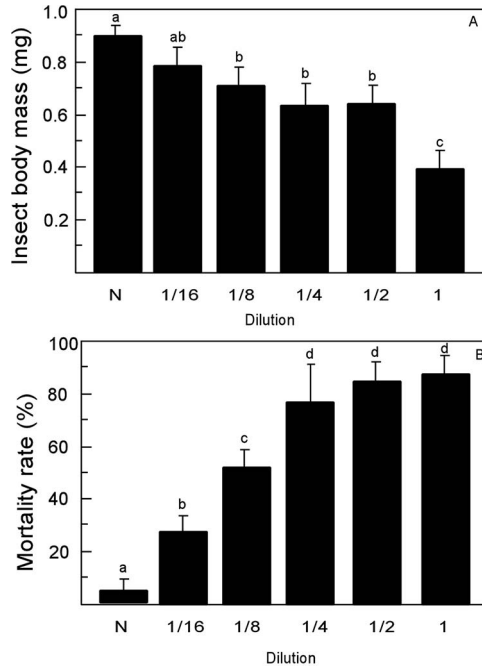

Fig. 2. Mean changes in insect body mass (A) and lethal effect (B) when *T. castaneum* adults were fed various dilutions of *B. papyrifera* latex extract mixed with food. N, insects were fed on a diet without latex extract. Error bars indicate the standard deviation from the mean. Values labeled with different letters are significantly different ($P < 0.05$).

the mature oocytes of beetles treated with the onefold dilution of latex were the smallest. The number of mature oocytes and germarium decreased markedly after latex treatment (Fig. 4B). The insects treated with the onefold dilution of latex could not develop mature oocytes.

Examination of reproductive organs in male adult beetles treated with latex revealed that the sizes of the testicular lobe, rod-shaped accessory gland, and tubular accessory gland were decreased markedly compared with the control (Fig. 5A). The testicular lobe of insects treated with high concentrations of latex were markedly different in size and appearance compared with the control. Each part of the testis in the treated beetles was opaque or milky white, whereas the testis in the control group had a relatively transparent surface (Fig. 5B). The tubular accessory gland was smaller in beetles treated with four-, two-, and onefold dilutions of the latex extract. In contrast, the size of the rod-shaped accessory gland was decreased significantly in the onefold dilution group.

Changes in trehalase activity and sugar content in reproductive organs. The development and function of reproductive organs are related to the metabolism of carbohydrates stored in the adult hemolymph, including trehalose and glucose. Trehalase activity plays a role in utilizing trehalose by hydrolysis into glucose molecules.

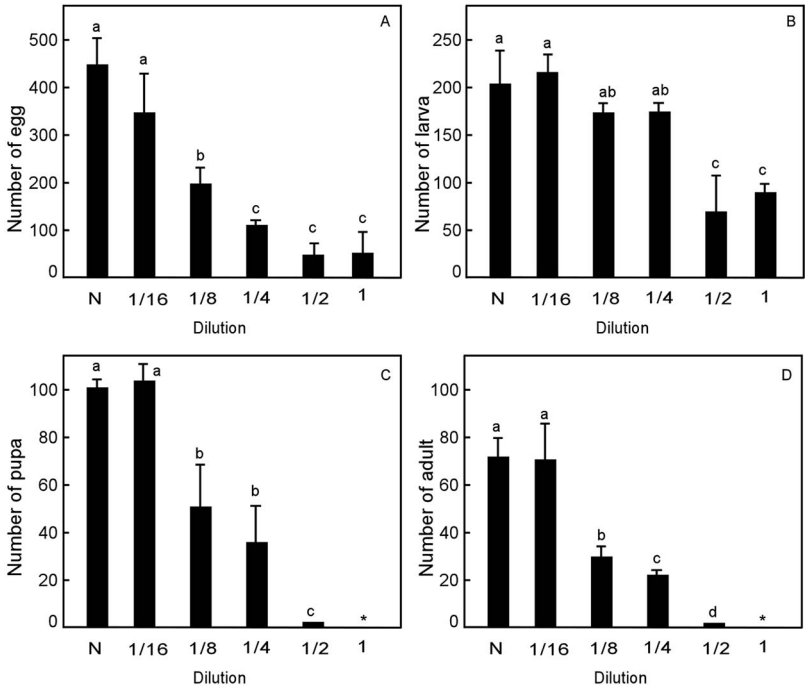

Fig. 3. Number of eggs (A), larvae (B), pupae (C), and adults (D) present after *T. castaneum* were fed with *B. papyrifera* latex extract at various dilutions. N, insect were fed on a diet without addition of latex extract. Asterisk (C, D) indicates that pupae and adults were not found. Error bars indicate the standard deviation from the mean. Values labeled with different letters are significantly different ($P < 0.05$).

Trehalose also can be transported into reproductive tissue via trehalose transporter. The trehalase activity and glucose concentration in the reproductive organs of the red flour beetle significantly decreased when insects were treated with latex extract at one- and twofold dilutions (Fig. 6A, B). In contrast, concentrations of trehalose did not differ between the control and treatment groups (Fig. 6C).

Discussion

This study revealed that the latex of six plant species within the Moraceae family, *B. papyrifera, F. hispida, F. curtipes, F. religiosa, F. virens,* and *S. asper,* contain inhibitors that suppressed trehalase enzymatic activity in the red flour beetle, *T. castaneum*. The inhibitors in the latex of *B. papyrifera* could be extracted with methanol and tolerated heat treatment. The latex lost inhibitory activity after being digested by proteolytic enzymes, suggesting that the inhibitor is a protein molecule with a molecular mass of 6–8 kDa or larger. Because plant latex contains phytochemicals, including proteins, alkaloids, sterols, and tannins, the inhibitor detected

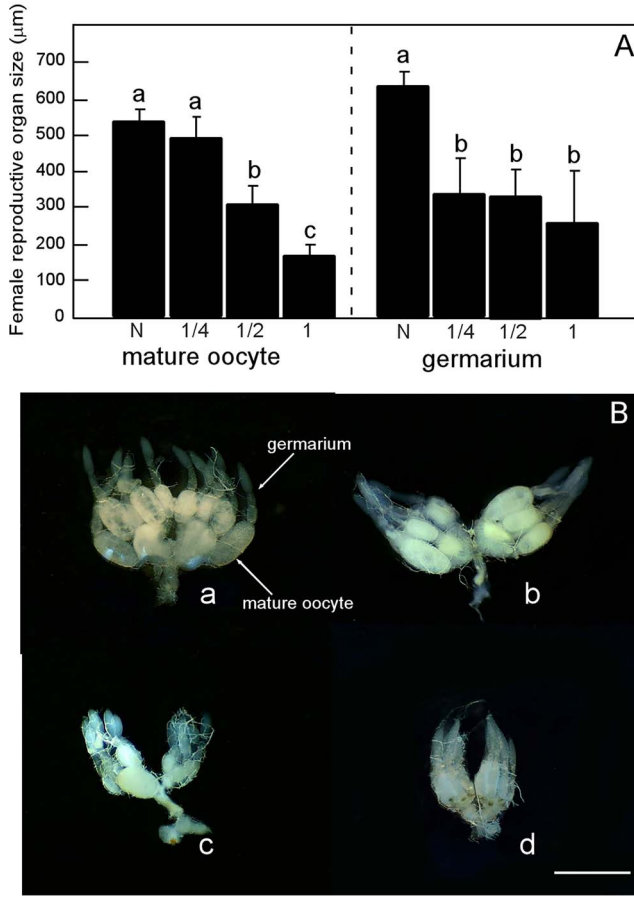

Fig. 4. (A) Size of mature oocytes and germarium of *T. castaneum* after beetles were fed with *B. papyrifera* latex extract at various dilutions. N, insects were fed on a diet without latex extract. (B) Morphological changes in female reproductive organs after ingesting latex extract: adult control (a) and adult fed with fourfold (b), twofold (c), and onefold (d) dilutions of latex extract. Error bars indicate the standard deviation from the mean. Values labeled with different letters are significantly different ($P < 0.05$). Scale bar = 1 mm.

in *B. papyrifera* latex may be a thermostable protein. Latex from plants in the family Moraceae has various components, including protein and nonprotein molecules. Mulberry, *M. alba,* produces latex containing several types of proteins that have insecticidal effects on *Drosophila melanogaster* Meingen, such as chitinase-like proteins molecular masses of 46–50 kDa that were purified from *M. alba* latex (Kitajima et al. 2010). In addition, the protein with 56 kDa molecular mass had growth-inhibiting activity in the larvae of the cabbage armyworm, *Mamestra brassicae* L., and the Eri silkworm, *Samia ricini* Donovan, but not the silkworm,

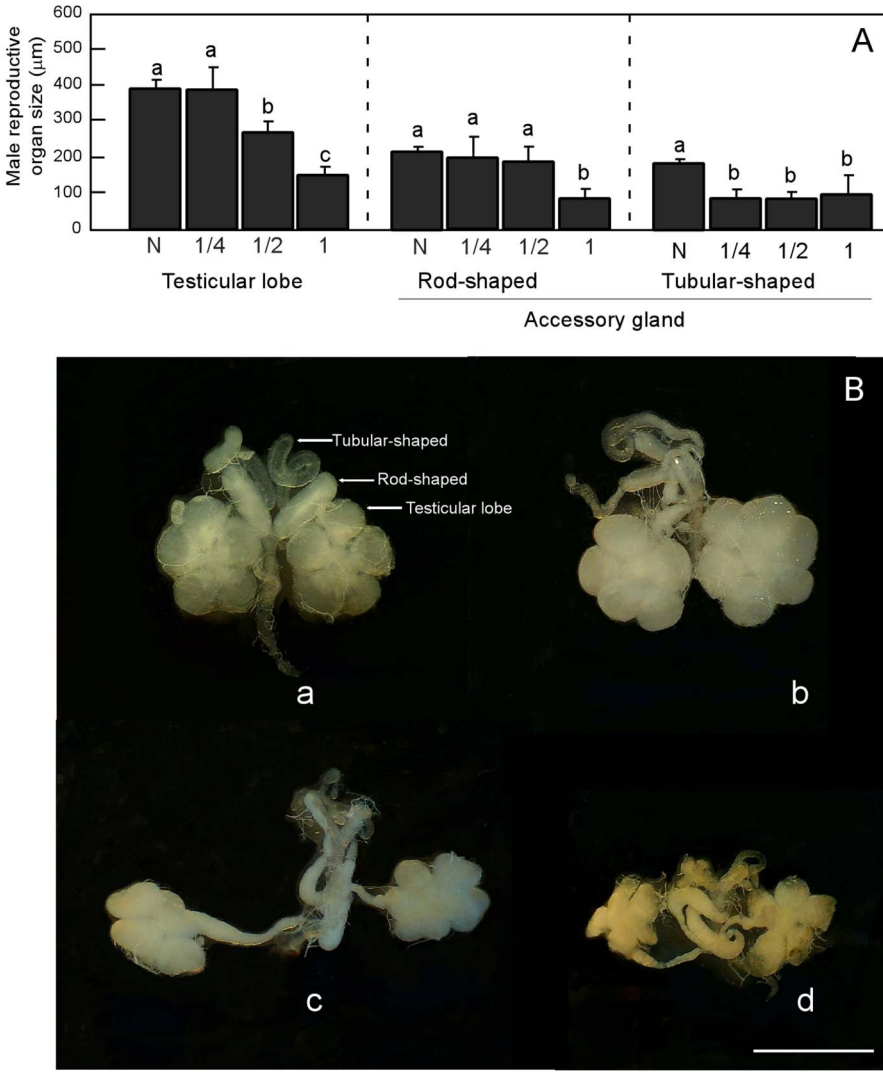

Fig. 5. (A) Size of the testicular lobe, rod-shaped accessory gland, and tubular accessory gland of *T. castaneum* after beetles were fed *B. papyrifera* latex extract at various dilutions. N, insects were fed on a diet without latex extract. (B) Morphological changes in male reproductive organs after ingesting latex extract: adult control (a) and adult fed with fourfold (b), twofold (c), and onefold (d) dilutions of latex extract. Error bars indicate the standard deviation from the mean. Values labeled with different letters are significantly different ($P < 0.05$). Scale bar = 1 mm.

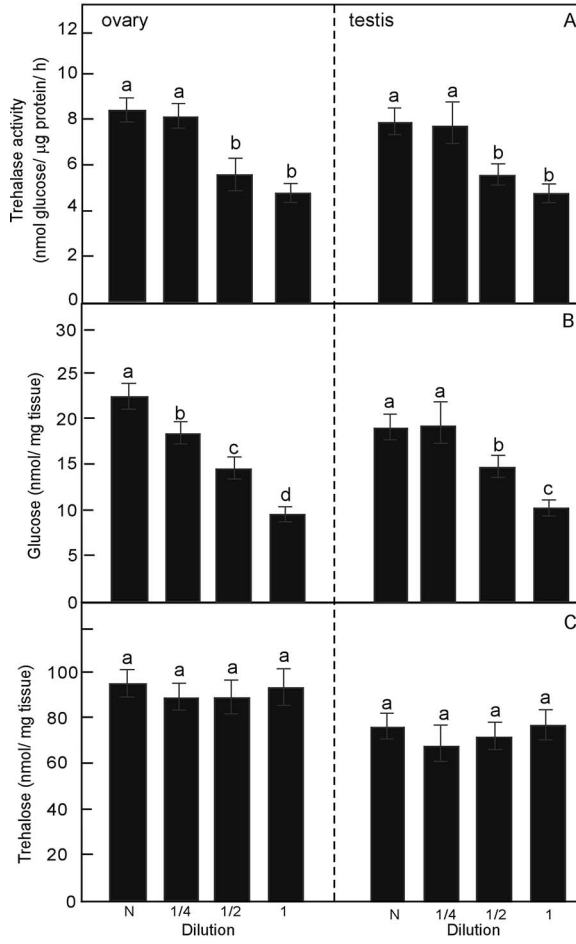

Fig. 6. The changes in trehalase activity (A), glucose content (B), and trehalose content (C) in the ovary and testis of *T. castaneum* beetles fed *B. papyrifera* latex extract at various dilutions. Trehalase activity was expressed as nanomoles of glucose per microgram of protein per hour. Error bars indicate the standard deviation from the mean. Values labeled with different letters are significantly different ($P < 0.05$).

B. mori, which is a mulberry specialist (Singh et al. 2023). The methanolic extract latex of *F. benjamina* was reported to have inhibitory activity against the trehalase enzyme of *T. castaneum* and was described as a heat-tolerant protein molecule larger than 8 kDa (Tatun et al. 2014). In contrast, plants from the same family, including *M. alba* and *A. heterophyllus,* produce latex that exhibits trehalase inhibitory activity. However, those molecules were reported to be nonproteins, indicating that plants belonging to the family Moraceae may use different defensive strategies to protect themselves from herbivorous insects.

Because the latex from *B. papyrifera* had the highest inhibitory activity against trehalase compared with the other plants in the present experiment, it was consequently selected to examine the biological effects on the red flour beetle. The latex extract affected the growth and development of *T. castaneum* by reducing the body mass of the treated beetles, the number of eggs produced, and the number of larvae, pupae, and adults. *T. castaneum* also had high mortality rates when beetles were fed with high concentrations of latex extract, indicating that the extract has insecticidal potential. The latex of wild fig, *Ficus virgata* Reinwardt ex Blume, is highly toxic to larvae of herbivorous insects (Singh et al. 2023). The latex of *Ficus glomerata* Roxburgh (Moraceae) was also toxic to *Spodoptera litura* F. larvae and reduced the larval body weight (Upadhyay 2013). When the latex extract of *F. glomerata* was fed to adults, it reduced oviposition rates and caused low levels of adult emergence of F1 progeny by interfering with their development. In the present study, latex extracts had significant lethal effects and caused reproductive or postreproductive inhibition of *T. castaneum*. Latex and its components could be considered potent natural insecticidal constituents for safe and ecofriendly control of insect pests (Ramos et al. 2019, Singh et al. 2023).

The beetles treated with *B. papyrifera* latex had fewer eggs and fewer developed larvae. The morphological abnormalities of reproductive organs in both male and female *T. castaneum* suggest that the *B. papyrifera* latex caused toxicity to the reproductive tissues, resulting in reduced egg production and fewer hatching larvae. The decrease in the number of developed larvae may be related to the abnormalities in both the ovary and testis in the parent beetles that ingested the plant latex. The extract of numerous plants can affect insect reproductive organs. Extract from *Annona squamosa* L. significantly reducing the size and weight of the testis and ovary in *Oryctes rhinoceros* L., with fewer and smaller spermatids (Sreeletha and Geetha 2012). The male reproductive organs of *Spodoptera littoralis* Boisduval treated with azadirachtin were shorter and narrower than those in the control, with poor spermatocyte and sperm development (Hatem et al. 2007). In contrast, female reproductive organs were not affected by azadirachtin treatment; the ovariole was longer than that of the control.

This study reveals that the carbohydrates and carbohydrate-metabolizing enzymes in *T. castaneum* were affected by *B. papyrifera* latex treatment. The enzyme trehalase is crucial for carbohydrate synthesis and mobilization from hemolymph into specific tissues, including reproductive organs (Kamei et al. 2011, Shukla et al. 2015, Su et al. 1994). Trehalase activity and gene expression have been recorded in various insects (Andrea et al. 2019, Takiguchi et al. 1992, Tang et al. 2016), including the ovary and testis of *T. castaneum,* indicating that trehalase has an essential physiological function in insect reproduction and not simply a digestive function when found in the gut. When *T. castaneum* was treated with *B. papyrifera* latex, trehalase activity decreased significantly, which was related to reduced glucose content in reproductive tissues, suggesting that the latex was absorbed into the ovary and testis tissues and consequently inhibited trehalase at the cell membrane or in the cytoplasm. Because the hydrolysis of trehalose by trehalase was inhibited by latex, the glucose content in the ovary and testis was lower than that in the control. However, the trehalose content in the reproductive tissues was not affected by *B. papyrifera* latex treatment. Trehalose can enter the cell via a trehalose transporter (Kanamori et al. 2010, Li et al. 2020, Tellis et al. 2023), indicating that trehalose detected in *T. castaneum* ovary and testis

tissues has been transported there from hemolymph. The treatment with *B. papyrifera* latex did not affect this transportation; however, further study is required to address this issue.

Changes in carbohydrate contents occurred after insects were exposed to insecticides and bioinsecticides, and changes in protein contents in insect reproductive tissues were noted (Ge et al. 2009). These results agree with those obtained after treatment of *Spodoptera mauritia* Boisduval with fenoxycarb, which reduces the amount of carbohydrates, proteins, and lipids in the gonads (Banu et al. 2022). The lack of nutrient transfer from hemolymph to the reproductive tissues is one of the reasons for reducing ovary and testis size in insects challenged with insecticides and plant extracts as bioinsecticides (Abdelsalam et al. 2016, Perveen and Miyata 2000). In the present study, the reduction of glucose in the ovary and testis in latex-treated *T. castaneum* may cause abnormal development of reproductive organs in these beetles. Further studies on this latex are recommended, including field trials to manage insect pests effectively.

Acknowledgments

This research was supported by the University of Phayao and the Thailand Science Research and Innovation Fund (Fundamental Fund 2022). This manuscript (no. 284280) was edited by Proofed Inc, Dover, DE.

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