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Original research

Effects of force-fed boric acid on the life history traits of greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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Abstract: Pest biological fitness and normal development are important factors for pest population sustainability. Sub-lethal, lethal and sub-acute effects of boric acid on the life history traits of the greater wax moth *Galleria mellonella* were investigated. Boric acid concentrations (LC_{30}, LC_{50} and, LC_{70}) were administered to 5th instar *G. mellonella* larvae via force-feeding method. The individuals in both experimental and control groups were observed daily and the larval and pupal developmental times, adult longevity as well as pupal and adult weights were recorded. Results showed that treating *G. mellonella* with boric acid significantly decreased pupal and adult weights in a concentration dependent manner when compared to the control. Additionally, while larval developmental time increased post boric acid treatment, it was observed that pupal developmental time and adult longevity significantly shortened when compared to the control. Moreover, at the two highest boric acid concentrations, pupal developmental time and adult longevity decreased by almost 50% when compared to the control. With these findings, we infer that boric acid significantly affects the normal development of pest insect *G. mellonella* and thus affects it biological fitness and survivability.

Keywords: Boric acid, force-feeding, *Galleria mellonella*, life-history traits, model insect

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Introduction

Today in agriculture, various chemicals are used to improve product quality, obtain more product from a limited arable area and to ensure product resistance to pests and diseases. The most important of these chemicals is undoubtedly pesticides. However, the uncontrolled use of pesticides provides cancerous, teratogenic and mutagenic effects (Senthil-Nathan, 2013) and resistance developed by pests (Klatt et al., 2016; Brevik et al., 2018). For this reason, current research about pest control is focused on the implementation of methods and use of chemicals with a wide spectrum and the least or no harm to the environment and human health (Jeschke et al., 2010; Casida and Durkin, 2013).

Among such chemicals is boric acid (BA), a slow acting (Cornwel, 1976) insecticide commonly used to control mites and cockroaches (Miles, 1994; Cochran, 1995). Besides having a suitable safety record and very low absorption via unbroken skin (Ebeling, 1995; Fail et al., 1998), BA also has a relatively low toxicity on honeybees (Atkins, 1987; Harper et al., 2012), aquatic invertebrates, birds and fish, (Weir and fisher, 1972; USDA forest service report, 2006). BA is classified as a Category III toxic agent (U.S EPA, 1996) and Group E carcinogen (U.S. EPA 1993, p. 27) since it has a low toxicity and, also poses no risk of carcinogenicity. On the other hand, BA is used in various industrial activities like the manufacture of paints, fungicides (Harper et al., 2012), and pharmaceuticals (Avino-Martinez et al., 2008) and thus, many living organisms both terrestrial and aquatic are inevitably exposed to it in their ecosystems.

Reports in literature have showed that BA possess insecticidal effects (Xue and Barnard, 2003; Cruz et al., 2010; Büyükgüzel et al., 2013; Gwokyalya and Altuntaş, 2019), destructs the intestinal lining and ultrastructure of the midgut (Cochran, 1995; Habes et al., 2006; Büyükgüzel et al., 2013), reduce fecundity (Kilani-Morakchi et al., 2009), affects the cuticle profiles of insects (Kilani-Morakchi et al., 2005), alters protein activity and biological fitness (Hyršl et al., 2007) and, induces oxidative stress (Hyršl et al., 2008; Büyükgüzel et al., 2013) in insects. Moreover, recently, Gwokyalya and Altuntaş (2019) showed that BA suppresses cellular mediated immunity of *G. mellonella* larvae and that it also possesses genotoxic effects. *Galleria mellonella* (Lepidoptera: Pyralidae), the greater wax moth, is a major pest in apiculture since its larval stage feeds on apiary products. *G. mellonella* larvae are commonly used as model organisms in laboratories for immunological and toxicological studies (Ramarao et al., 2012; Wojda, 2017) since they show immune responses similar to mammals when treated with various chemicals (Kavanagh and Reeves, 2007).

In pest control campaigns, the main goal is to reduce pest populations to the minimum possible and this can be achieved by altering the biological fitness and normal development of the pest in question (Gupta and Dikshit, 2010) i.e. altering the developmental times, supressing fecundity, reducing weights and/or preventing pests from developing from one growth stage to another. There are several reports in literature showing how different xenobiotics affect the survivability and biological fitness of storage pest *G. mellonella* (Çalık et al., 2016; Sugeçti et al., 2016; Er et al., 2017; Zorlu et al., 2018; Çelik et al., 2019). Since BA has been reported to be insecticidal to various insects, this study investigated the effects of force-fed BA on the life history traits of storage pest and model insect *G. mellonella.*

Materials and Methods

***Galleria mellonella* stock culture**

The cultivation of *G. mellonella* was carried out in an insectarium (insect room, D51-41) in the animal physiology laboratory, department of Biology, Faculty of Science, Eskisehir Technical University, Eskişehir. Photoperiodical conditions in the insectarium were maintained at 28 ± 2 °C temperature, 60 ± 5% relative humidity in continuous darkness to ensure stock and progressive culture continuity. The larvae were fed on a semi-artificial diet for *G. mellonella* mixed according Altuntaş et al., (2012).

In order to establish stock cultures, 5 females and 5 males *G. mellonella* adult individuals were put into 1 liter glass jars containing semi-artificial diet (100g) given in Table 1. The jars were covered with gauze and perforated covers (to avoid escaping of the larvae). Adult individuals were removed from the culture medium following mating of the female and male individuals. Routine culture maintenance was done three times a week to check the nutrient needs of the larvae and to remove any wastes. Depending on the density of the larvae, feeds were added to the jars from in sufficient quantities to feed the hatching larvae. If crowded, the larvae were optimally distributed to different jars. During weekly maintenance, the larvae approaching the final larval stage were transferred into 0.5 liter jars containing folded papers to facilitate pupation. Pupa jars were checked 7-10 days later and followed up daily till adult individuals emerged. Mature individuals were then mated to ensure the continuity of the stock cultures.

Experimental assays/Lethal concentrations of BA

Boric acid (H_3BO_3 CARLO ERBA) used in this study was purchased as a pure powder. Recently, Gwokyalya and Altuntaş, (2019) determined the lethal concentrations of force-fed BA for *G. mellonella* larvae. It was therefore decided that LC_{30}, LC_{50} and LC_{70} concentrations determined in this previous study be used to investigate the effects of BA on the life history trait of *G. mellonella* in the current study. From the stock culture, larvae weighing 0.16 ± 0.01 gr were randomly selected and each to each one of them 5µl of the different BA concentrations was fed via force-feeding method (Dere et al., 2015; Gwokyalya and Altuntaş, 2019).

Prior force-feeding, the larvae were starved for 3 hours after which, they were kept on ice for 2 minutes to be anesthetized and then 5µl of BA were administered orally to each larva through the oesophagus using a 10µl Hamilton injector (26-gauge sharp tip). For the control group, 5µl of distilled water were fed to each larva. Each of the treated larvae was transferred to a 15 × 60mm petri dish containing 2 g of the synthetic diet and kept in the insectarium. Both control and experimental groups were observed daily to observe the developmental times and changes. Following the developmental process of the experimental and control groups (LC_{30}, LC_{50} and LC_{70}), larval, pupal and adult life developmental times (day) and the pupal and adult weights of *G. mellonella* were determined. For both experimental and control groups 15 larvae were used, and all analyses were done in 3 replicates.

Statistical analysis

All data were expressed as mean ± standard errors determined using the data obtained from the experiments. The SPSS data analysis software program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used for all statistical analyses. All data from the experimental analyses were tested for normal distribution using Lavene's test. Results did not follow normal distribution thus, to compare means, Kruskal Wallis and Mann Whitney U tests were performed to determine the statistical differences. All results obtained in the experiments were evaluated as statistically significant at a 95% confidence interval ($p < 0.05$).

Results

Effects of BA on the biological parameters of Galleria mellonella

Results obtained in this study showed that, treating *G. mellonella* larvae with different BA concentrations significantly increased the larval developmental time at all BA concentrations when compared to the control ($\chi^2(3) = 21.35$; $p = 0.000$). However, there was no significant difference observed in the larval developmental time among the (BA treated) experimental groups. Additionally, BA significantly reduced the pupal developmental time ($\chi^2(3) = 85.046$; $p = 0.000$) and adult longevity of *G. mellonella* ($\chi^2(3) = 42.36$; $p = 0.000$) at all BA concentrations in a concentration-dependent manner when compared to the control (Fig. 1). For the experimental groups however, a significant difference in adult longevity was only observed at LC_{30} when compared to the 2 highest concentrations (LC_{50} and LC_{70}). Furthermore, the number of days recorded for both larval development and adult longevity reduced by more than a half at the two highest BA concentrations (LC_{50} and LC_{70}).

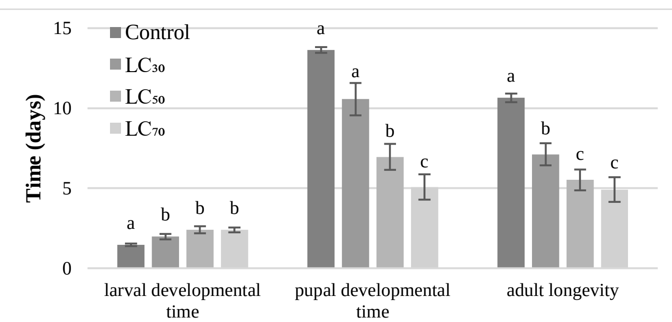

Figure 1. Boric acid induced changes in the adult longevity and larval and pupal developmental time (days) of *Galleria mellonella*. (**Each bar represents the mean ± S.E. Significant differences are denoted by different letters (a - d) (Mann Whitney U test, P < 0.05)*)

Additionally, BA treatment led to significant reductions in pupal ($\chi^2(3) = 119.987$; $p = 0.000$) and adult ($\chi^2(3) = 116.807$; $p = 0.000$) weights in all BA treated groups when compared to the control. (Fig. 2). Moreover, at the 2 highest BA concentrations (LC_{50} and LC_{70}), both pupal and adult weights decreased by approximately 50% and 60% respectively when compared to the control.

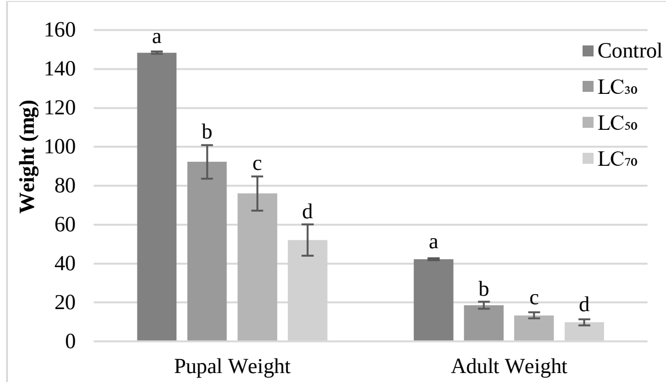

Figure 2. Effects of BA on the pupal and adult weights of *Galleria mellonella*. (**Each bar represents the mean ± S.E. Significant differences are denoted by different letters (a - d) (Mann Whitney U test, P < 0.05)*)

Discussion

BA a widely used inorganic insecticide has proved to be effective against various pests. Recently, insecticidal properties of BA on *G. mellonella* (Hyršl et al., 2007; Büyükgüzel et al., 2013), cockroaches (Cochran, 1995; Habes et al., 2006) and termites (Farid et al., 2015) have been reported. Studies also reported that BA induced oxidative stress, altered protein profiles (Hyršl et al., 2007; Büyükgüzel et al., 2013), prolonged larval and pupal development (Hyrsl et al., 2007) and caused cuticular abrasion and destruction of the digestive tract wall (Cochran, 1995; Ebeling, 1995; Habes, 2005). Kilani-Morakchi et al., (2009) also reported that BA affects the reproductive potential of *Blattella germanica.* In this study therefore, we present the sublethal and subacute effects of force-fed BA on the larval, pupal and adult developmental periods/longevity and the weights of the pupae and adults.

Results obtained from the current study showed that administering BA to *G. mellonella* larvae significantly affects the developmental time of the species' larval, pupal and adult stages and, also reduces the weight of both the pupal and adult stages. We observed that as BA concentrations increased, larval developmental time was prolonged while pupal developmental time and adult longevity decreased significantly among all experimental groups when compared to the control. Similar to our findings, Hyršl et al., (2007) reported that BA added into first instar larvae diets at concentrations of 156, 620, 1,250, or 2,500 ppm led to prolonged larval developmental time and shortened adult longevity in *G. mellonella*. The same study also showed that dietary BA prolonged pupal developmental time at the highest concentration a result which is contrary to the findings recorded in our study. We suggest that the shortened lifespan of *G. mellonella* pupae and adults post BA treatment could be related to BA induced lipid peroxidation (Hyršl et al., 2007; Büyükgüzel et al., 2013), immunotoxicity and genotoxicity (Gwokyalya and Altuntaş, 2019) in the larval stage. Furthermore, Hyrsl et al., (2008) reported that dietary BA altered the protein profiles and Büyükgüzel et al., (2013) showed that BA induces lipid peroxidation of *G. mellonella* larvae. It is known that proteins and lipids are one of the major feed nutrients that aid physiological functioning of insects (Van der Horst et al., 1997) since they are the primary components of most hormones and enzymes which regulate/control growth and development and other physiological processes (Deber and Benham, 1984; Kim et al., 2012). Therefore, any alteration in the composition, amount or functions of proteins and lipids directly affects endocrine system functioning and subsequently interferes with the normal development of the pupae and adult wax moths, whence, the observed changes in the growth and development of *G. mellonella* pupae and adults following BA treatment. A report by Kilani-Morakchi et al., (2009) showing that BA affects the life traits by supressing productivity and ovarian constituents in cockroaches support our findings that BA indeed affects the survivability and the life history traits of insects. The shortened adult longevity post BA treatment may be a very helpful in the control of *G. mellonella* as the adults lay significantly fewer eggs during their shortened active reproductive stage leading to fewer populations in the future. The prolonged larval developmental time may be related to inadequate feeds and insufficient nutrients and their related stress due to damaged intestinal lining and poor digestion (Habes et al., 2006; Cochran, 1995); these may slow down the normal physiological functions of the larvae post BA treatment thus halting normal larval development which leads to prolonged larval developmental periods.

Similar to previous studies which reported reduced weights in rat (Price et al. 1996; Harrouk et al., 2005; U.S EPA, 2006) and mice offsprings (Heindel et al., 1992) following BA administration, it was determined in the current study that treating *G. mellonella* with BA led to significant decrements in both pupal and adult weights in the BA treated groups when compared to the control. It is known that lepidopteran species do not feed during their pupal and adult stages and thus, larval nutrition is very important for aiding and sustaining their growth from the 1st instars to the adults (Schopf, 1991). Mohamed et al. (2014) presented evidence showing that the quality and quantity of diet ingested by the larvae play a very huge role in the growth, development and survivability of *G. mellonella*. Additionally, studies reported detrimental intestinal wall damaging in insects following that BA treatment leading to starvation (Cochran, 1995; Ebeling, 1995, Habes et al., 2006, Büyükgüzel et al., 2013). Due to the damaged alimentary canal, it is possible that the BA treated larvae could not efficiently feed or digest and utilize the consumed feeds leading to starvation and subsequent weight loss in both the pupae and the adults. Also, weight loss among various animal species as they evolve from one development stage to another is a common phenomenon (Davidowitz et al., 2003); the weight loss may be due to the loss of some water and the cuticle, molting and, a brief period of fasting. Development from one stage to another for instance from larvae to pupa and pupa to adult, necessitates a high demand for energy and nutrients. However, since the pupal and adult stages of *G. mellonella* do not feed (Jorjão et al., 2018), there is no external/supplementary source of such nutrient and energy requirements. Consequently, the insects rely on the energy reserves like the fat body for metabolism maintenance which leads to weight loss from one development stage to another. Furthermore, BA induced lipid peroxidation (Büyükgüzel et al., 2013) and its related stress may be one other reason for the observed alterations in the life traits of *G. mellonella* post BA treatment. Lipid peroxidation leads to depletion of glycogen reserves and other carbohydrates, fats and proteins (McCarthy 1994) in the fat body and the midgut. This inevitably leads to weight loss and, also shortens the normal developmental times of the affected organisms.

In conclusion, treating *G. mellonella* with boric acid even at very low concentrations significantly affects the life history traits and normal development of the model insect thus affecting its biological fitness and survivability.

References

Altuntaş H., Kılıç A.Y., Uçkan F., and Ergin E. 2012. Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Environmental Entomology, 41: 688-96.

Atkins E.L. 1987. Laboratory Bee Adult Toxicity Tests (BATDT) for boric acid, powdered, 100% technical. MRID 40269201.

Avino-Martinez J.A., España-Gregori E., Peris-Martinez C.P., Blanes M., 2008. Successful boric acid treatment of *Aspergillus niger* infection in an exenterated orbit. Ophthalmic Plastic and Reconstructive Surgery, 24: 79–81.

Brevik K., Lindström L., Mckay S. D. Chen Y. H. 2018. Transgenerational effects of insecticides-implications for rapid pest evolution in agroecosystems, Current Opinion in Insect Science.

Büyükgüzel E. Büyükgüzel K., Snela M., Erdem M., Radtke K., Ziemnicki K., and Adamski Z. 2013. Effect of boric acid on antioxidant enzyme activity, lipid peroxidation, and ultrastructure of midgut and fat body of *Galleria mellonella*. Cell Biology and Toxicology, 29: 117–129.

Casida J. E., Durkin K. A. 2013. Neuroactive insecticides: Targets, selectivity, resistance, and secondary effects. Annual Review of Entomology, 58: 99-117.

Cochran D.G. 1995. Toxic effects of boric acid on the German cockroach. Cellular and Molecular Life Sciences, 51: 561-563. https://doi:10.1007/BF02128743.

Cornwell P. B. 1976. The Cockroach, vol. II. St. Martins Press, New York.

Cruz D. A., Zacarin E.C.M., Bueno O.C., Malaspina O. 2010. Morphological alterations induced by boric acid and fipronil in the midgut of worker honeybee (*Apis mellifera* L.) larvae. Cell Biology and Toxicology, 26 (2): 165-176.

Çalık G., Büyükgüzel K., Büyükgüzel E. 2016. Reduced fitness in adults from Larval, *Galleria mellonella* (Lepidoptera: Pyralidae) reared on media amended with the antihelmintic, mebendazole. Journal of Economic Entomology, 109(1): 182-187.

Çelik C., Büyükgüzel K., Büyükgüzel E. 2019. The effects of oxyclozanide on survival, development and total protein of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Journal of the Entomological Research Society, 21(1): 95-108.

Davidowitz G., D'Amico L.J., Nijhout H.F. 2003. Critical weight in the development of insect body size. Evolution & development, 5: 2, 188-197.

Deber C.M., Behnam B.A. 1984. Role of membrane lipids in peptide hormone function: binding of enkephalins to micelles. Proceedings of the National Academy of Sciences of the United States of America, 81 (1): 61-65.

Dere B., Altuntaş H., Nurullahoğlu, Z. U. 2015. Insecticidal and oxidative effects of azadirachtin on the model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae). Archives of Insect Biochemistry and Physiology, 89: 138-152. https://doi:10.1002/arch.21231.

Dere B., Nurullahoğlu Z.U., Altuntaş H. 2019. Effects of azadirachtin on development of model insect *Galleria mellonella* l. (Lepidoptera: pyralidae). Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology, 8(1): 85-91.

Ebeling W. 1995. Inorganic insecticides and dusts, pp. 193-230. In M. K. Rust, J. M. Owens, and D. A. Reierson [eds.], Understanding and controlling the German cockroach. Oxford University Press. New York.

Er A., Taşkıran D. Sak O. 2017. Azadirachtin-induced effects on various life history traits and cellular immune reactions of *Galleria mellonella* (Lepidoptera: Pyralidae). Archives of Biological Sciences, 69(2): 335-344.

Fail P. A., Chapin R. E., Price C. J., Heindel J. J. 1998. General, reproductive, developmental, and endocrine toxicity of boronated compounds. Reproductive Toxicology, 12: 1-18.

Farid A., Zaman M., Saeed M., Khan M., Bad shah T. 2015. Evaluation of boric acid as a slow-acting toxicant against subterranean termites (Heterotermes and Odontotermes). Journal of Entomology and Zoology Studies, 3: 213-216.

Gupta. S., Dikshit. A. K. 2010. Biopesticides: An ecofriendly approach for pest control. Journal of Biopesticides, 3(1): 186-188.

Gwokyalya R., Altuntaş H. 2019. Boric acid-induced immunotoxicity and genotoxicity in model insect *Galleria mellonella* L. (Lepidoptera: Pyralidae). Archives of insect biochemistry and physiology. doi: 10.1002/arch.21588.

Habes D., Morakchi S., Aribi N., Farine J.P., Soltani N. 2006. Boric acid toxicity to the German cockroach, *Blattella germanica*: alterations in midgut structure, and acethylcholinesterase and glutathione Stransferase activity. Pesticide Biochemistry and Physiology, 84: 17-24. https://doi:10.1016/j.pestbp.2005.05.002,

Harper B., Gervais J.A., Buhl K., Stone D. 2012. Boric Acid Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services.

Harrouk W.A., Wheeler K.E., Kimmel G.L., Hogan K.A., Kimmel C.A., 2005. Effects of Hyperthermia and Boric Acid on Skeletal Development in Rat Embryos. Birth Defects Research. Part B, Developmental and Reproductive Toxicology, 74(3): 268-76.

Heindel J.J., Price C.J., Schwetz B.A. 1994. The developmental toxicity of boric acid in mice, rats, and rabbits. Environmental Health Perspectives, 102(7): 107-112. doi: 10.1289/ehp.94102s7107.

Hyršl P., Büyükgüzel E., Büyükgüzel K. 2007. The effects of boric acid-induced oxidative stress on antioxidant enzymes and survivorship in *Galleria mellonella*. Archives of Insect Biochemistry and Physiology, 66: 23-31.

Hyršl P., Büyükgüzel E., Büyükgüzel K. 2008. Boric acid-induced effects on protein profiles of *Galleria mellonella* hemolymph and fat body. Acta Biologica Hungarica, 59: 281-288.

Jeschke P., Nauen R., Schindler M., Elbert A. 2010. Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry, 59(7): 2897-2908.

Jorjão A.L., Oliveiraa L.D., Scorzonia L., Figueiredo-Godoia L.M.A., Pratab M.C.A., Jorgea A.O.C., Junqueiraa, J.C. 2018. From moths to caterpillars: Ideal conditions for *Galleria mellonella* rearing for in vivo microbiological studies. Virulence, 9(1): 383-389. https://doi.org/10.1080/21505594.2017.1397871.

Kavanagh K., Reeves P. E. 2007. Insects and mammalian innate immune responses are much alike. Microbe, 2: 596–599.

Kilani-Morakchi S., Aribi N., Farine J.P., Everaerts, C., Soltani, N. 2005. Effets de l'acide borique sur les profiles d'hydrocarbures cuticulaires chez un insecte à intérêt médical, *Blattella germanica* (Dictyoptera: Blattellidae). Journal de la Société Algérienne de Chimie, 15: 225-231.

Kilani-Morakchi S., Aribi N., Soltani N. 2009. Activity of boric acid on German cockroaches: Analysis of residues and effects on reproduction. African Journal of Biotechnology, 8 (4): 703-708.

Kim K.D., Li S.G., Kang, Y.J., Kim K.W., Son M.H. 2012. Effects of Dietary Protein and Lipid Levels on Growth and Body Composition of Juvenile Far Eastern Catfish *Silurus asotus*. Asian-Australas Journal of Animal Sciences, 25(3): 369-374.

Klatt B. K., Rundlöf M., Smith H. G. 2016. Maintaining the restriction on neonicotinoids in the European Union–benefits and risks to bees and pollination services. Frontiers in Ecology and Evolution, 4: 4.

McCarthy M.F. 1994. Promotion of hepatic lipid oxidation and glucogenesis as a strategy for appetite control. Medical Hypotheses, 42(4): 215-225.

Miles T. G. 1994. Use of disodium octaborate tetrahydrate to protect aspen waferboard from termites. Forest Products Journal, 44(9): 33-36.

Mohamed A.A., Ansari M.J., AL-Ghamdi A., Mohamed M.O., Kaur M. 2014. Effect of larval nutrition on the development and mortality of *Galleria mellonella* (Lepidoptera: Pyralidae). Revista Colombiana de Entomología, 40 (1): 49-54.

Price C.J., Strong P.L., Marr C.B., Myers C.B., Murray F.J. 1996. Developmental toxicity NOAEL and postnatal recovery in rats fed boric acid during gestation. Fundamental and Applied Toxicology, 32: 179-193.

Ramarao N., Nielsen-Leroux C., Lereclus D. 2012. The insect *Galleria mellonella* as a powerful infection model to investigate bacterial pathogenesis. Journal of Visualised Experiments, 11:e4392. https://doi:10.3791/4392.

Schopf A. 1991. The Effect of Host Age of *Lymantria dispar* Larvae (Lep: Lymantriidae) on the Development of *Glyptapanteles liparidis* (Hym: Braconidae). Entomophaga, 36: 4, 593-604.

Senthil-Nathan S. 2013. Physiological and biochemical effect of neem and other meliaceae plants secondary metabolites against lepidopteran insects. Frontiers in Physiology, 4: 1-17.

Sugeçti S., Büyükgüzel K., Büyükgüzel E. 2016. Laboratory assays of the effects of oxfendazole on biological parameters of *Galleria mellonella* (Lepidoptera: Pyralidae). Journal of Entomological Science, 51(2): 129-137.

U.S. EPA (U.S. Environmental Protection Agency). 1993. RED on boric acid and sodium Salts. Available at: http://cfpub.epa.gov/oppref/rereg/status.cfm?Show=rereg.

U.S. EPA. 1996). EPA exposure factors Handbook. Available at http://www.epa.gov/ordntrnt/ORD/WebPubs/exposure/.

USDA report. 2006. Human Health and Ecological Risk Assessment for Borax (Sporax)®. Final report. Forest Health Protection USDA Forest Service.

Van der Horst D.J., Vroemen S.F., Van Marrewijk W.J.A. 1997. Metabolism of stored reserves in insect fat body: hormonal signal transduction implicated in glycogen mobilization and biosynthesis of the lipophorin System. Comparative Biochemistry and Physiology, 117(4): 463-474.

Weir RJ., Fisher RS. 1972. Toxicologic studies on borax and boric acid. Toxicology and Applied Pharmacology, 23: 351-364. https://doi:10.1016/0041-008X(72)90037-3.

Wojda I. 2017. Immunity of the greater wax moth *Galleria mellonella*. Insect Science, 24: 342–357. https://doi:10.1111/1744-7917.12325.

Xue R.D., Bernard D.R. 2003. Boric acid bait kills adult mosquitoes (Diptera: Culicidae) Journal of Economic Entomology, 96: 1559-1562.

Zorlu T., Nurullahoğlu Z.U., Altuntaş H. 2018. Influence of dietary titanium dioxide nanoparticles on the biology and antioxidant system of model insect, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Journal of the Entomological Research Society, 20(3): 89-103.