IJCST

International Journal of Crop Science and Technology

Volume 2, Issue 2, 2016, Page: 60-68 ISSN: 2458-7540

The Effects of Biopesticide Azadirachtin on the Fifth Instar *Galleria mellonella* L. (Lepidoptera: Pyralidae) Larval Integument

Sadettin Ünsal[1]*, Emine Güner[2]

[1,2]*Department of Biology, Science Faculty, Selçuk University, Konya, TURKEY*

*Corresponding author e-mail: unsal@selcuk.edu.tr

ABSTRACT

The chitin synthesis inhibitory (CSI) effects of azadirachtin on the fifth instar *Galleria mellonella* L. larval integument were investigated. Using probit analysis, the LC95 value for *G. mellonella* larvae was detected as 3991 ppm. In this study, 3991 ppm and the reduced 1995, 997, and 498 ppm concentrations were used. It was observed that azadirachtin had different effects on the integument when the larvae were fed semi-synthetic feed containing 498, 997, 1995, or 3991 ppm azadirachtin. Morphologically failed molting, disruption of cuticle structure, hemolymph loss, and darkening were observed in azadirachtin-administered larvae. In the experimental groups, depending on the dose/time relationship, high doses of azadirachtin blocked cuticle secretion in higher levels. In larvae fed a diet containing azadirachtin, cuticle secretion was reduced by 21%–24% compared with the control group.

Keywords*:* *Galleria mellonella*, greater wax moth, azadirachtin (AZ), chitin synthesis inhibitor (CSI), integument, cuticle.

INTRODUCTION

Pesticides have both acute and chronic effects on humans and all other warm-blooded living things. The results of acute poisoning are observed within a short period of time. However, millions of people are not aware that they are exposed to poisons every day. In pesticide applications, lower than 0.1% of the amount used reaches the target organisms, whereas the rest mixes into the ecosystem and leads to the deterioration of the ongoing natural balance (Yıldız et al., 2005). Today, due to the important problems caused by pesticides, alternatives that can be used instead of these chemical compounds are sought in naturally occurring sources. Extracts, including meliantriol, sallanin and azadirachtin found in the seeds of *Azadirachta indica* A.Juss. (Meliaceae) (Adhikary, 1984), comprise perfect alternatives to synthetic insecticides for insect pest control. In recent years, among these substances, azadirachtin (AZ) has been the subject of most of the studies. The tetranontriterpenoid AZ and other neem derivatives have highly selective effects: adverse effects are not observed on beneficial organisms. They are generally safe as insect growth regulators (IGRs) against non-target organisms (Mordue (Luntz) et al., 1993). As a result of the studies on the effects of AZ on insects, it has been determined that it inhibits feeding (Garcia et al., 1984; Arnason et al., 1985; Koul et al., 1991), growth, and development (Ascher, 1993; Riba et al., 2003, Grisakova et al.,2006) by negatively affecting reproductive behaviors and egg maturation (Webb et al., 1983; Mordue (Luntz), 2004), and that high doses have toxic effects (Arnason et al., 1985; Schmutterer, 1988). These effects have been observed particularly in many species in the Orthoptera, Homoptera, Heteroptera, Lepidoptera, Coleoptera, Diptera, and Hymenoptera orders (Mordue (Luntz) et al., 2000). Usually designated as third-generation insecticides, IGRs are categorized into three

groups based on their mode of action: (I) juvenile hormone and its analogs; (II) ecdysone agonists; and (III) chitin synthesis inhibitors (CSI) (Mondal et al., 2000). IGRs are more effective in the insect's larval stage rather than during the adult stage, and one of their effective targets is the larval integument. The majority of insect integument consists of procuticle, which itself consists of 25%–60% chitin and is synthesized by epidermal cells. Although there are many previous studies on AZ's effects on the neuroendocrine system as an IGR (Dorn et al., 1986; Cassier et al., 1988; Mordue (Luntz) et al., 1993; Manal & Sehnal, 2000), there are very few studies on the effects of AZ as a chitin synthesis inhibitor on the larval integument. The aim of this study was to investigate the chitin synthesis inhibitor effects of lethal and sublethal doses of AZ, which is commonly used in many countries on the fifth instar *Galleria mellonella* L. (greater wax moth) larval integument and molting properties.

MATERIALS AND METHOD

Larvae

G.mellonella larvae used in the study were reared using stock culture at 78% relative humidity in a completely dark medium in glass jars (150×250mm) in a 28 ± 2®C incubator and fed with semi synthetic feed (600g honey + 492g glycerol + 20g honeycomb + 1200g bran) (Hegazy et al., 1980).

Azadirachtin

Neem Azal TS (AZA) (10g/1000ml) as the source of AZ was obtained from Trifolio M Gmbh (Germany). Neem Azal TS was dissolved in water for the application and shaken vigorously for 15 minutes. Using probit analysis (Finney,1971), the LC_{95} values for *G. mellonella* larvae was detected as 3991 ppm. In the trials, LC_{95} and its sub-lethal concentrations 1995, 997, and 498 ppm Neem Azal TS were used.

Insecticidal Procedure

In the trials, newly molted fifth instar *G.mellonella* larvae were used. To maintain equal feeding before AZ, larvae were starved for four hours (Clarke et al., 1990). The LC_{95} dose of 3991 ppm AZ and its reduced concentrations 1995, 997, and 498 ppm were mixed into the 100-g semi-synthetic feed used in the study. In the control group, that feed was mixed with water and placed in glass jars. Fifty larvae were placed in each glass jar containing the control group feed and feed containing AZ. Four larvae samples were taken from each control group, and AZ group larvae that were especially affected by the AZ (color darkening) at the 12th, 24th, 48th, 60th, 72nd, 84th, 96th, 108th, and 120th hours. Dead larvae were discarded to avoid histopathological changes that may have occurred due to death.

Histology

Samples taken from experimental and control groups were anesthetized with ether and cut from the head and abdomen, then fixed in Bouin solution for 48 hours. The samples underwent thorough washing, dehydration, clearing, soft paraffin and hard paraffin treatments. Sample blocks were prepared, and 6-µm thick sections were taken from the prepared blocks. Sections were dyed with Harris's hematoxylin-eosin, Harris's hematoxylin-eosin/aniline blue, and Luxol fast blue/aniline blue combinations. An Olympus (BX51/BX2-FBL3-000) light microscope was used to measure cuticle thickness at ×400 magnification and Leica (DM 1000) light microscopy was used for photography at ×400 and ×1000 magnifications.

Statistical Analysis

Cuticle thickness values were measured using at least 10 samples prepared with the larvae samples taken at the predetermined times. The measurements were conducted in four repetitions. Arithmetic means and standard errors of these values were calculated. Analysis of variance was utilized

for the comparison between the average cuticle thickness of the larvae in the experimental and the control groups. The Duncan test was applied for the groups in which the differences between the average values were significantly different ($P<0.05$).

RESULTS AND DISCUSSION

Integument Structure of the Control Group

In the histological sections, it was observed that in *G. mellonella*, the integument comprised epidermis consisting of a single line of cells, and an epicuticle and procuticle that were formed by the secretion of these cells. Exocuticle/endocuticle differentiation in the procuticle. and clarification of the endocuticle lamella occurred toward the end of the larval instar (Figure 1 A). The differentiation of procuticle could not be observed at other times (Figure 1 B). Newly synthesized cuticular and epicuticular tubercles were clearly observed in the epidermal cells in the control group at the beginning of the instar (Figure 1 C).

The Effect of Azadirachtin on Integument

Azadirachtin administration at 498, 997, 1995,and 3991 ppm doses caused excessive darkening and blackening in the integuments of *G.mellonella* larvae. Growth inhibition, immobility, hemolymph loss, and failed molting toward the end of the instar were observed in 997, 1995, and 3991 ppm dose experimental groups as compared with those in the control group. Larvae with those effects were particularly selected for histological sections. Another effect of AZ on *G. mellonella* larvae integument was the deterioration of the molting period. In control group larvae, it was observed that apolysis occurred following the formation of interzone cuticle (Figure 1 D), which is the beginning of molting. This was followed by the formation of epicuticle, exocuticle, and endocuticle in turn, and, with the addition of endocuticle lamella as a result of chitin synthesis by epidermal cells, molting occurred following the newly synthesized cuticle attaining maximum size. It was observed that molting occurred in the healthy larvae in a short time—such as a few hours. It was observed that cuticle thicknesses in larvae at the beginning of the instar in the control group increased continuously from the 12th hour and reached maximum at the 84th hour. Molting occurred at the 96th hour, and the larvae passed onto the next instar (Table 1) (Figure 2). Although molting in larvae in the 498 ppm experimental group occurred at the same time as that observed in the control group (84th hour), those larvae could not continue their development in the next instar and died. Larvae in the 997, 1995, and 3991 ppm experimental groups had previously died related to the increased dose (Table 1).

The Effect of Azadirachtin on Cuticle Thickness

The average cuticle thicknesses in 498, 997, 1995, and 3991 ppm AZ-dose experimental groups were obtained in histological sections (Table 1). In the experimental groups, cuticle synthesis rates in 498 ppm dose larvae in 12th–8th hours were close to those determined in the control group. Additionally, it was observed that the larvae in this group passed onto the next instar during ecdysis before they reached the cuticle thickness values observed in the control group; thus, they were unable to continue their development and died. Epidermal cells of the larvae in the 997 and 1995 ppm dose groups continued to synthesize new cuticle from the beginning of the instar; however, they synthesized less cuticle compared with those synthesized in 498 ppm dose group and died at the 72nd hour without molting. In the 2991 ppm lethal dose group, it was interesting that the larval cuticle was reduced below the thickness of previously formed cuticles before death. Reductions in cuticle thicknesses in 498, 997, 1995, and 3991 ppm AZ dose groups was compared with the cuticle thickness in the control group (Table 2). Although cuticle synthesis in the 498 ppm dose experimental group larvae decreased by 21%, larvae in this

group passed onto an upper instar (sixth larval instar). In other dose groups, cuticle synthesis decreased 23% at the 72nd hour in 997 ppm dose, 24% at the 72nd hour in 1995 ppm dose, 22% at the 24th hour in 3991 ppm dose; deaths occurred without passing onto the next instar.

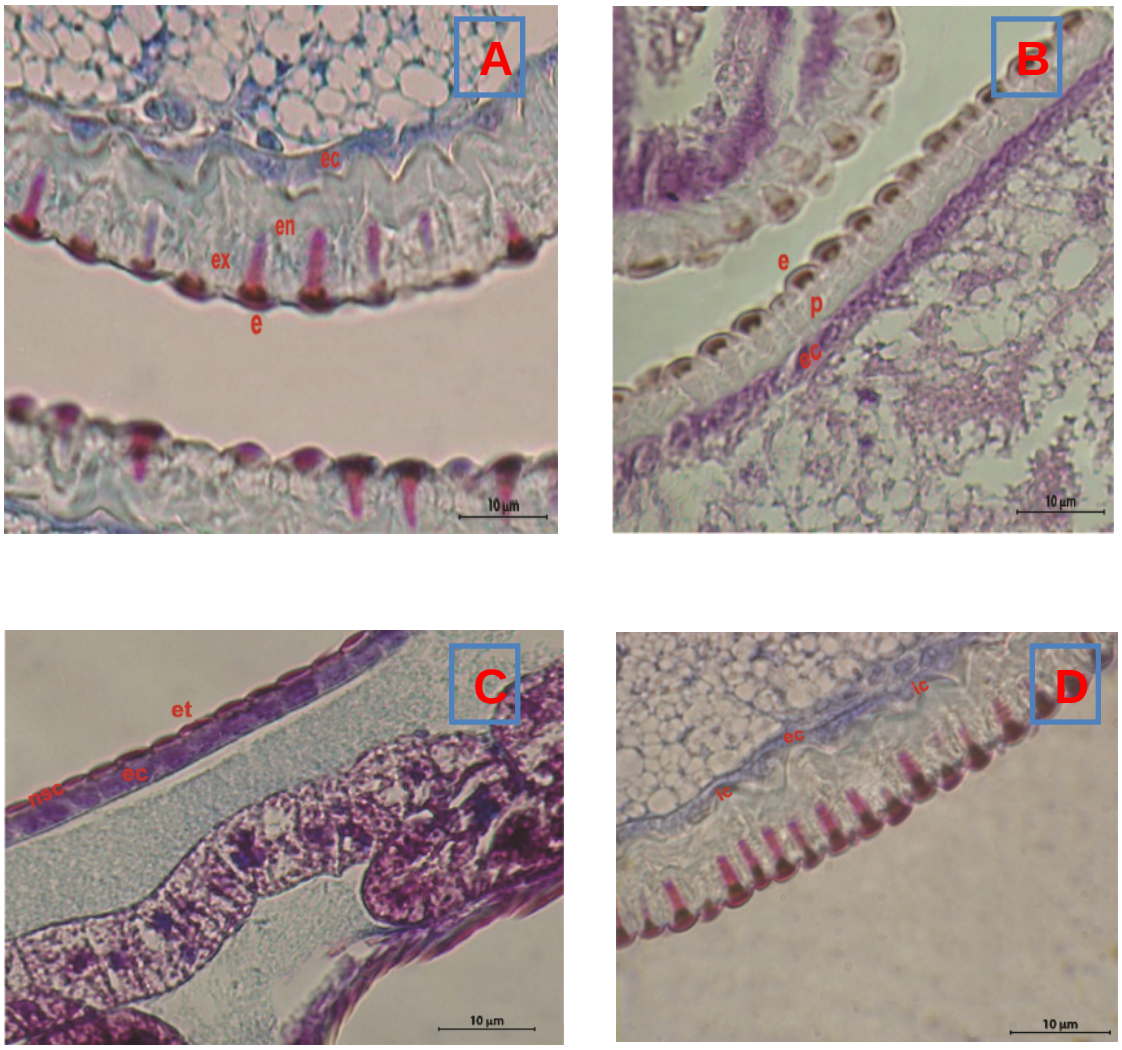

Figure 1. (A) Clarification of the cuticular layer in the fifth instar control group *G.mellonella* larvae, Luxol fast blue/aniline blue, X400. (B) The structure of the fifth instar control group *G.mellonella* larvae procuticle at the beginning and the middle of the instar ,Harris's hematoxylin-eosin, X1000. (C) Newly synthesized cuticular and epicuticular tubercles in fifth instar control group *G.mellonella* larvae, hematoxylin-eosin, X1000. (D) Interzone cuticle formed toward the end of the instar in fifth instar control group *G.mellonella*, Harris's hematoxylin-eosin, X400. (e: epicuticle, ec: epidermal cell, en: endocuticle, et: epicuticular tubercle, ex: exocuticle, ic: interzone cuticle, nsc: newly synthesized cuticle, p: procuticle)

Table 1.The average cuticle thicknesses (µm) in fifth instar *G.mellonella* L. larvae in the control group and azadirachtin-administered groups at different doses. (Mean value ± SD, four repetitions, ten measurements).

Time/dose (hours/ppm)	Control	500 (ppm)	1000 (ppm)	2000 (ppm)	4000 (ppm)
12	7.25±0.45 [a]	7.07±0.38 [a]	6.75±0.38 [ab]	6.46±0.35 [bc]	6.14±0.33 [c]
24	8.16±0.33 [a]	7.54±0.43 [b]	7.29±0.47 [bc]	6.73±0.29 [c]	5.54±0.39 [d]
36	8.91±0.38 [a]	8.44±0.49 [ab]	7.75±0.64 [bc]	7.36±0.53 [c]	-
48	9.81±0.55 [a]	9.24±0.46 [ab]	8.29±0.66 [bc]	7.84±0.86 [c]	-
60	10.90±0.32 [a]	9.98±0.41 [a]	8,875±075 [b]	8.05±0.98 [b]	-
72	11.99±0.40 [a]	10.19±0.30 [b]	9.16±0.93 [bc]	8.83±0.47 [c]	-
84	13.10±0.44 [a]	10.41±0.27 [b]	-	-	-

*(a-d) Changes in different doses on the same line at the same time period differ from the control group. The comparison of different averages was evaluated using the Duncan Test (P<0.05).

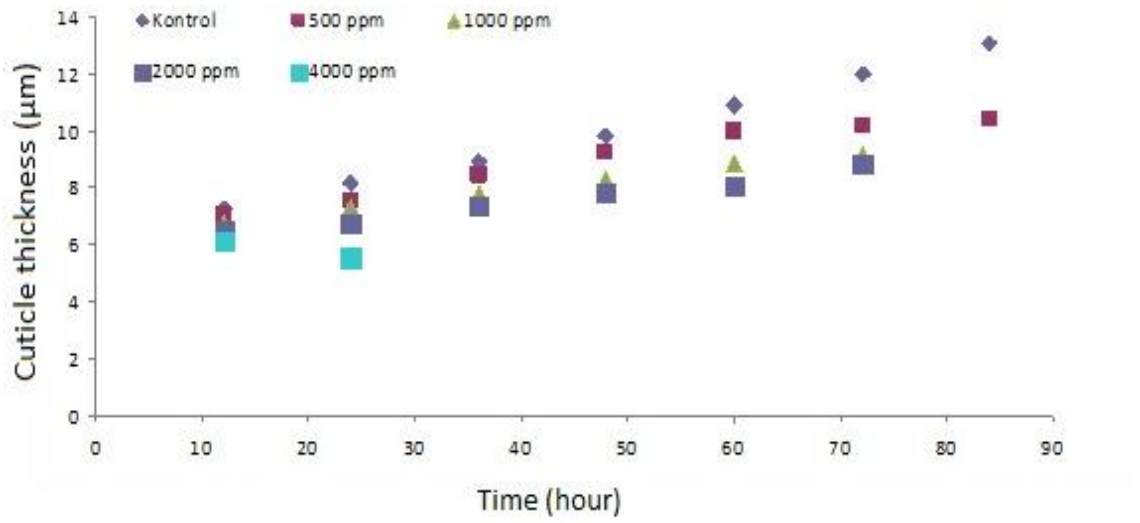

Figure 2.The increase in cuticle thickness in fifth instar *G.mellonella* L. larvae in the control and azadirachtin-administered groups, and the dose–response relationship.

Table 2. Reductions (%) in fifth instar *G. mellonella* L. larvae cuticle caused by 498, 997, 1995, and 3991 ppm azadirachtin doses.

Time/dose (hours/ppm)	Control	498 (ppm)	997 (ppm)	1995 (ppm)	3991 (ppm)
12	7.25±0.45	3	7	11	13
24	8.16±0.33	8	11	18	22
36	8.91±0.38	5	13	17	-
48	9.81±0.55	6	13	20	-
60	10.90±0.32	8	20	16	-
72	11.99±0.40	15	23	24	-
84	13.10±0.44	21	-	-	-

Changes in different doses on the same line at the same time period differing from the control group were shown in terms of percentage (%).

According to Hegazy et al. (1980), the cuticle structure of *G. mellonella* larvae is composed of an epicuticle and a procuticle. The procuticle is composed of chitin fibrils with pore channels in the lamellar structure parallel to each other. Chitin is generally the main component of exo- and endocuticle. Although there is no chitin in the epicuticle, the majority of the non-chitin material in the procuticle is protein. Lee et al. (1990) and Yin-Chang et al. (1990) have reported that endocuticular lamellae joined the procuticle in the intermolt period in lepidopteran larvae. Similarly, in the control group *G. mellonella* larvae, thickening of the cuticle occurred with the new lamella addition to the endocuticle. Currently commonly used in pest control globally, AZ is a chitin-synthesis inhibitor in addition to being an ecdysone antagonist (Schmutterer, 1988). Therefore, the chitin synthesis inhibitory (CSI) effects of AZ were compared with those of other CSI conventional insecticides. In this study, 3991 ppm and the reduced 1995, 997, and 498 ppm concentrations of AZ were used for examination of *G. mellonella* larvae. Cuticle in thickness in the control group fifth instar *G. mellonella* larva continuously increased (7.25–13.10µm), and the larvae passed onto the sixth instar following molting at the 84th hour. The cuticle synthesis rate decreased in the experimental groups dependent on the dose/time relationship. In low doses (498 ppm), the chitin synthesis rate decreased; however, the amount of synthesized cuticle was higher than in those synthesized in the medium and high dose (997, 1995, 3991 ppm) groups. In the 498 ppm dose group, although not so much as in the control group, cuticle synthesis continued between the 12th and the 84th hours (7.07–10.41µm), and the larvae in this group passed onto an upper instar at the 84th hour. However, they could not continue their development in this instar and died. In 997 and 1995 ppm medium doses of AZ, depending on the increased dose, cuticle synthesis rate decreased between the 12th and 72nd hours (6.75–9.16/6.46–8.83µm), and larvae that were exposed to these doses died at the 72nd hour. In the LC_{95} 3991 ppm dose group, cuticle thickness of the larvae was lower than the

previously synthesized cuticle thickness (6.14–5.54µm), and the larvae in this group died at the 72nd hour.

The effects of AZ observed in our study were similar to those reported by Hegazy et al. (1980) on *G. mellonella* larvae and Mitsui et al. (1980) on *Manduca sexta* larvae. Four different concentrations used in the present study caused significant reductions in the fifth instar cuticle thickness. These effects were similar to those obtained using other chitin synthesis inhibitors (Soltani, 1984; Vinuela, 1994; Rahimin, 1999). The cuticle synthesis rate was affected by AZ depending on the dose/time relationship. This can be explained as the effect of the blocked cuticle secretion on chitin synthesis in epidermal cells. In sixth-seventh instar *G. mellonella* electron microscopy cuticle studies, it has been observed that DFB had no effect on the protein epicuticular structure following the injection, and the formation of chitin lamellae in procuticle was prevented (Hegazy, 1980). In another study, it was reported that lamellar structure in endocuticle was partially disrupted due to the incomplete or defectively formed chitin microfibrils following the DFB LC_{50} in *Leptinotarsa decemlineata* (Say) larvae (Hegazy et al., 1989). Lee et al. (1990) has reported that chitin microfibril accumulation was halted, lamella formation was prevented and an amorphous cuticle structure was observed following the topical DFB and flufenoxuron applications to the cuticle of *Spodoptera littoralis* (Boisd) larvae. Hegazy et al. (1992), in their study on Musca *domestica* L. fed with a diet containing DFB (50 ppm), reported that the structure of epicuticle was not affected; however, the lamellar structure in the procuticle disappeared. Also, spherical structures and extra layers were observed between the endocuticle and epidermis cells in *Tenebrio molitor* and *Mythimna separata* larvae treated with DFB (Ren et al., 1988). Chitin synthesis rates in *G. mellonella* experimental groups were reduced by 21% in the 498 ppm dose, 23% in the 997 ppm dose, 24% in the 1995 ppm dose, and 22% in the 3991 ppm dose. Similar results were observed in the study by Unsal et al (2004), in which chitin synthesis rate decreased by 50% in *G. mellonella* L. larvae that were fed a diet containing an LD_{95} 1000 ppm dose DFB and the reduced 500 and 250 ppm doses; also, the chitin synthesis rate decreased by 20% in *S. littoralis* larvae (Osman, 1985), and by 50% in *L. decemlineata* (Say) larvae (Hagazy et al., 1989). The new cuticle was not sufficiently hardened, as the epidermal cells could not perform chitin synthesis; fifth instar *G. mellonella* larvae that had not undergone molting died at the 108th hour in the 498 ppm dose, at the 72nd hour in 997 and 1996 ppm doses, and at the 24th hour in the 3991 ppm dose. Reynolds (1987) reported that the molting cycle was initiated in larvae fed with DFB as usual; however, the weakened insect was not able to remove the old cuticle when the molting phase was initiated, as the hardness and firmness of the new cuticle was seriously disrupted. Ker (1978) reported that an insect fed with DFB could normally initiate ecdysis; however, it would die slowly, as it would not be able to manage molting. That researcher has associated this with the serious weakening in the cuticle resulting from lack of chitin.

CONCLUSION

In pest control, natural herbal pesticides, of which very low amounts can be effective on the pests, should be used, and these pests should have no acute or chronic poisonous effects on humans and other warm-blooded animals. The advantages of AZ and derivatives, which are natural components, can be summarized as being decomposable through natural processes, have remarkable selectivity, and are therefore not harmful to beneficial and non-target organisms. In consideration of these properties, AZ and its derivatives are potentially suitable for IPM (Integrated Pest Management) programs.

ACKNOWLEDGEMENTS

This study was supported by Selcuk University Scientific Research Projects Coordination Unit (BAP).

REFERENCES

Adhikary, S., 1984. Results of field trials to control common insect. pests okra, *Hibuscus esculentus* L., in Togo by application of crude methanolic extracts of leaves and seed kernels of the neem tree, *Azadirachta indica*. A. Juss. *Zeitsclirift für angewandte Entomologie*, 98(4), 327-331.

Arnason, J.T., Philogene, B.J.R., Donskov, N., Hudon, Mc Dougall, M.C.G., Fortier, P., Morand, D., Gardner and Lambert, J., 1985. Antifeedant and insecticidal properties of azadirachtin to the European corn borer, *Ostrinia nubilatis. Entomologia Experimentalis et Applicata*, 38(1), 29-34.

Ascher, K.R.S., 1993. Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica. Archives of Insect Biochemistry and physiology*, 22:433-449.

Cassier, P., Bagdassarian, D., De Besse, N., Papillion, M., Baldalla, L., Porcheron, P., 1988. Ecdysteroids and activation of epidermal cells in the Locust *Locusta migratoria. Journal of Insect Physiology*, 34(7), 669-677.

Clarke, B. S., Jewess, P.J., 1990**.** The The inhibition of chitin synthesis in *Spodeptera littoralis* larvae by flufenoxuron, teflubenzuron and diflubenzuron. *Pesticide Science*, *28:*377-388

Dorn, A., Redemacher, J.M. and Sehn, E., 1986. Effects of azadirachtin on the moulting cycle, endocrine system, and ovaries in last-instar larvae of the milkweed bug, *Oncopeltus fasciatus*. *Journal of Insect Physiology,* 32(3), 231-238.

Finney, D.J., 1971. *Probit analysis* (3 rd. Edn.) London: Cambridge University press.

Garcia, E.S., Azamburja, P., Forster, H. and Rembold, H., 1984**.** Feeding and molt inhibition by azadirachtins A, B and 7-acethyl-azadirachtin A in *Rhodnius prolixus* nymphs. *Zeitschrift für Naturforschung*, 39:155-1158.

Hegazy, G., Van De Veire, M. and Degheele, D., 1980. Effect of the chitin synthesis inhibitor diflubenzuron on the sixth and seventh instar of Gallaria mellonella L.: Importance of application time. *Meded Fac Londbouvw. Rijksuniv. Gent*, 45:453-463.

Hegazy, G., De Cock, A., Auda, M. and Degheele, D., 1989. Diflubenzuron toxicity effect on the cuticle ultrastucture and chitin and protein content of Colarado potato beetle *Leptinotursa decemlineata* (Say) (Coleoptera:Chrysomelidae). *Meded Fac Landbouw Rijks univ.Gent*, 54:89-101.

Hegazy, G. and Degheele, D., 1992. The effect of DFB on the ultrastructure of the Integument during moult events of *Musca domestica. Parasitica*, 48:3-14.

Ker, R.F., 1978. The effects of diflubenzuron on the growth of insect cuticle. *Pesticide Science*, 9:259-265.

Koul, O. and Isman M.O., 1991. Effect of azadirachtin on the dietary utilization and development of the varriegated cutworm *Peridroma saucia. Journal of Insect Physiology,* 37:591-598.

Lee, S.A., Clarke, B.S., Jenner, D.W. and Williamson, F.A., 1990. Cytochemical demonstration of the effects of the acylureas flufenoxuron and diflubenzuron on the incorporation of chitin into insect cuticle. *Pesticide Science*, *28:*367-375.

Manal, M.. Adel, F.S., 2000. Azadirachtin poten tiates the action of ecdysteroid agonist RH- 2485 in *Spodeptera littoralis. Journal of insect physiology*,46:267-274.

Grisakova, M., Metspalu, L., Jogar, K., Hiiesaar, K., Kuusik, A. and Poldma, P., 2006. Effect of biopesticide Neem EC on the large White Butterfly, *Pieris brassicae* L.(Lepidoptera, Pieridae) *Agronomy Research* 4 (Special issue):181-186.

Mitsui, T., Nobusava, C., Fukami, J., Colins, J. and Riddiford, L.M., 1980. Inhibition of chitin synthesis by diflubenzuron in Manduca larvae. *Journal of Pesticide Science,* 5:335- 341.

Mondal, K.A.M.S.H. and Parveen, S., 2000. Insect growth regulators and potential in the menagement of stored- product insect pests. *Integrated pest management*, 5:255-295.

Mordue (Luntz), A. J. and Blackwell, A., 1993**.** Azadirachtin: An update. *Journal of insect physiology*, 39:903-924.

Mordue (Luntz), A.J., Nısbet, A.J., 2000. Azadirachtin from neem tree *Azadirachta indica*: Its action against insects. *Anais da Sociedade Entomologica do Brasil*, 29(4), 615-632.

Mordue (Luntz), A.J., 2004. Present *consepts of the mode of action of azadirachtin from neem* In Koul, O. S. (eds): *Neem: today and in the new millennium.* Kluser Academic publishers, London. 229-249.

Osman, S.E., 1985. Effect of antimoulting agent "Dimilin" on the blood picture and cuticle formation in *Spodoptera littoralis (*Boisd) Larvae. *Bulltein of the Entomological Society of Egypt*, 14:37-46.

Rahimin, N. and Soltani, N., 1999. Laboratory evaluation of Alsystin, a chitin synthesis inhibitor, against *Culex pipiens pipiens* L. (Dip, Culicidae): effect on development and cuticle secretion. *Journal of Applied Entomology*, 123:437-441.

Ren, J.C., Ma, Y. and Chang, J.T., 1988. Microscopic observation on the histopatological changes of cuticle induced by DFB in two insect larvae. *Acta Entomologica Sinica*, 31:366-370 (Chinese, with Engilish abstract).

Reynolds, S.E., 1987. The cuticle, growth and moulting in insects: the essential background to the action acylurea insecticides. *Pesticide Science*, 20:131-146.

Riba, M.J.M. and Sans, A., 2003. Influence of Azadirachtin on development of reproduction of *Nezara viridula* L. (Het., Pentomidae). *Journal of Applied Entomology*, 127:37-41.

Schmutterer, H., 1988. Potential of azadirachtin–containing pesticides for integrated pest control in developing and industralized countries. *Journal of insect physiology*, 34(7), 713-719.

Soltani, N., Besson M. T. and Delchambre, J., 1984. Effect of diflubenzuron on the pupal adult development of *Tenebrio molitor* L. (Coleoptera,Tenebrionidae): Growth and development, cuticle secretion, epidermal cell density and DNA synthesis. *Pesticide Biochemistry and Physiology*, 21:256-264.

Unsal, S., Ozparlak, H. and Aktumsek, A., 2004. Effect of Diflubenzuron on the Integument of Fifth Instar *Galleria mellonella* L. larvae. *Phytoparasitica*, 32 (1), 43-51.

Vinuela, E. and Budia, F., 1994. Ultrastructure *of Ceratitis capitata* Wiedemann larval integument and changes induced by the IGI cyromazine. *Pesticide Biochemistry and Physiology*, 48:191-201.

Webb, R.E.M.A., Hinebaugh, R., Lindguist, K. and Jacobson, M., 1983. Evaluation of aquareous solution of neem seed extract against *Liriomyza sativae* and L. *trifolii* (Diptera, Agromyzidae*). Journal Icon Entomology*, 76(2), 357-362

Yıldız, M., Gürkan, M.O., Turgut, C., Kaya, V., Ünal, G., 2005. *Tarımsal savaşımda kullanılan pestisitlerin yol açtığı çevre sorunları*. VI. Türkiye Ziraat Mühendisliği Teknik Kongresi 3-7 Ocak, 2005. Ankara.

Yin- Chang, W. Rong-Sheng, C., Chang-Kun, C. and Zi- Ping, Y., 1990. Studies on cuticular structures in two species of cutworms *Acta Entomologica Sinica,* 33:309-313 (Chinese, with English abstract).