ISJ 13: 153-163, 2016 ISSN 1824-307X

RESEARCH REPORT

# Effect of andrographolide on phosphatases activity and cytotoxicity against *Spodoptera litura*

**E Edwin[1], P Vasantha-Srinivasan[1], S Senthil-Nathan[1], A Thanigaivel[1], A Ponsankar[1], S Selin-Rani[1], K Kalaivani[2], WB Hunter[3], V Duraipandiyan[4], NA Al-Dhabi[4]**

*[1]Division of Biopesticides and Environmental Toxicology, Sri Paramakalyani Centre for Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi - 627 412, Tirunelveli, Tamil Nadu, India*
*[2]Post Graduate and Research Department of Zoology, Sri Parasakthi College for Women,Courtrallam-627 802, Tirunelveli, Tamil Nadu, India*
*[3]United States Department of Agriculture, ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA*
*[4]Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University, P.O.Box.2455, Riyadh 11451, Kingdom of Saudi Arabia*

*Accepted May 12, 2016*

**Abstract**

Andrographolide was isolated from ethanol extraction of *Andrographis paniculata* by column chromatography. Evaluation of larvicidal efficacy, enzymatic changes and cytotoxic activities against *Spodoptera litura* were conducted across a range of concentrations. The compound showed significant larvicidal activity between 5 - 25 ppm, post ingestion. Morphological deformities observed in larval-pupal stages. Enzymatic profiles were altered by reduction in acid phosphatase, ACP activity by 69.18 %, alkaline phosphatase, ALP activity 75.3 % and 74.9 % reduction in ATPase. Binding affinity to midgut epithelium cells suggests disintegration of cellular organelles observed was directly associated with ingestion of the compound. The results suggest that andrographolide has potential for development as a significant inhibitor of development against the pest *Spodoptera litura*.

**Key Words**: biorational insecticides; HPLC; GC-Mass; toxicity; Noctuidae; histology

## Introduction

Chemical pesticides provide important protection of crops, animals, and humans from pests and pathogens, improper use in developing countries has resulted in insecticide resistance development, and various environmental pollution disputes (Matsumura, 1975). Chemicals including synthetic pesticides can sometimes form by-products which can be linked to unwanted side effects, in beneficial insects (Abudulai *et al*., 2001). Increasing development of resistance to synthetic insecticides, promotes investigations into the potential of biorational chemistries isolated from plants (Abudulai *et al*., 2001). Plants are potential producer of novel chemical compounds which cannot yet be synthesized. Estimations suggest that over 2000 plant species have the potential to identify and develop new chemistries to reduce bacteria, fungal, and insect/arthropod pests (Klocke, 1989). The complexity in chemical compounds in biorational products can also make development of resistance by insect pests is more difficult (Regnault-Roger *et al*., 2002). These environmental issues have driven agricultural researchers to search for better ecofriendly based pesticides (Wood and Granados, 1991). Andrographolide is a labdane diterpene lactone from *Andrographis paniculata,* an annual herbaceous plant in the family Acanthaceae, is native to India and Sri Lanka, which has been used for its therapeutic activity. The therapeutic value is for its impact on enzyme induction (Meenatchi-Sundaram *et al*., 2009). Unfortunately the limitation to wider direct use as a therapeutic agent is due to poor solubility in water (Xiaoa *et al.,* 2013).

Acid phosphatase (ACP, E.C.3.1.3.2) and Alkaline phosphatase (ALP, E.C.3.1.3.1) are

*Corresponding author:*
S. Senthil-Nathan
Division of Biopesticides and Environmental Toxicology
Sri Paramakalyani Centre for Excellence in Environmental Sciences Manonmaniam Sundaranar University
Alwarkurichi - 627 412, Tirunelveli, Tamil Nadu, India
E-mail: senthil@msuniv.ac.in; senthilkalaidr@hotmail.com

enzymes, which supports to hydrolyse phosphomonoesters in acid or alkaline environments. Epithelim of intestine in animals is a common place for ALP which helps to provide phosphate ions for metabolic processes mainly in transphosphorylation reaction (Sakharov *et al*., 1989). During larval moulting stage the ACP and ALP activity decrease and increase after moulting (Miao, 2002; Ajamhassani *et al.,* 2012). Adenosine triphosphatases (ATPases) helps to transport glucose, amino acids, and other organic molecules. ATPases are present in the guts and nerve cells of the lepidopteron insects (Horie, 1958). Thus if a reduction in ATPase efficacy could be caused, there would be a defect in the gut physiology of the insect.

*Spodoptera litura* (Lepidoptera: Noctuidae) is an economically important polyphagous pest in India, China and Japan (Wan *et al*., 2014), causing major loss to many vegetables and field crops. The pest is active throughout the year on a wide variety of crops. The larvae are especially serious pests during the seedling stage (Chandrasekaran *et al*., 2012). Synthetic pesticides or chemical insecticides used for controlling *S. litura* have started to fail as populations of *S. litura* start to develop chemical resistance (Sintim *et al.,* 2009; Senthil-Nathan *et al*., 2013).

*A. paniculata* extracts have been reported as an antifeedant and ovicidal activity against insects (Hermawan *et al.,* 1993), but activity of andrographolide on *S. litura* has yet to be evaluated. Hence, the objectives of the present study includes, isolation and characterization of Andrographolide from *Andrographis paniculata* and evaluation of Andrographolide for its larvicidal efficacy, phosphatases and cytotoxic activities against *Spodoptera litura*.

## Materials and Methods

Spodoptera litura *culture*

*S. litura* larvae were collected from *Ricinus communis* plant in Nagercoil, Tamil Nadu, India was cultured and maintained according to Senthil-Nathan and Kalaivani (2005) and Senthil-Nathan *et al.* (2008a). Larvae were cultured in the laboratory on *R. communis* plant leaves. The *R. communis* plant leaves were collected from approximately 45 days old plant. For leaf assays and mass culturing, in-between young and mature leaves were used. Pre-pupae were provided with vermiculture soil as pupation sites. Adult moths emerged from the pupation site were transferred to the cages and fed on a 10 % sucrose solution. The fine cloths containing eggs were removed every 24hrs and eggs present were surface sterilized *in situ* by dipping in 10 % formaldehyde solution for approximately 3 mts, then washing with distilled water. The eggs in the fine cloths were moistened and kept in aerated containers for hatching. The culture and experiments were carried out at 27 (±3°C), 65 % relative humidity, with a 14:10 light: dark cycle.

*Isolation of plant compound andrographolide*

The ethanolic extracted plant material was analysed using chromatograph column chromatography with chloroform and methanol. Solutes were eluted with different gradients of 90:10, 80:20, 70:30, 60:40, 50:50 and 30:60 Chloroform: Methanol. Active fraction analyses by GC-MS revealed andrographolide as a major compound along with a few trace elements. Further purification by column chromatography and analyses by HPLC was compared with a purchased standard and chromatogram of andrographolide for confirmation.

*Insect bioassay*

Bioassays were performed with second, third, fourth and fifth instar larvae of *S. litura* using different concentrations. Methanol treated leaves was used as control. Five replication was performend with twenty larvae per concentration (n = 100). The fresh *R. communis* leaves (75 - 125 $cm^2$) were sprayed with different concentrations of (5, 10, 15, 25 ppm) on both surfaces and allowed for dry. All the treatment instars larvae were starved for 4 h, then fed with treated leaves. The uneaten leaves were removed every day and provide fresh leaves. Replication was done five times (totally n = *50*). Mortality was recorded every 24 h for all treatments. The percentage mortality was calculated using the formula (1) and corrections for mortality when necessary were done using Abbott's (1925) formula (2):

(1)

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

(2)

$$\text{Corrected percentage of mortality} = \left(1 - \frac{n \text{ in T after}}{n \text{ in C after}}\right) \times 100$$

The mean lethal concentration ($LC_{50}$ and $LC_{90}$) were calculated by subjecting mortality data to Probit analysis (Finney, 1971). From these results the developmental studies were performed.

*Developmental studies*

*S. litura* 3rd instar larvae were used for developmental studies. Individual larvae were reared in containers on the *R. communis* leaves treated with the lethal concentrations of active fraction as 3, 6, 9 and 12 ppm. Control leaves were treated with methanol. The containers of the treated and control groups were maintained at 27 (± 2 °C) in a 14L:10D photoperiod at 85 % relative humidity. Records were made daily to document deformities in the pupae and abnormalities in emerged adults.

*Enzymatic profile*

*Preparation of enzyme extract*

Treated larvae of third, fourth and fifth instars were used for enzyme activities at different concentrations of 3, 6 and 9 ppm respectively. The

extraction procedure as in Applebaum (1964) and Applebaum *et al*. (1961). Anaesthetized larvae were dissected out and entire digestive tract was treated within ice-cold insect Ringer's solution. The gut contents, malphigian tubules and adhering tissues were carefully removed. The separated gut regions was weighed (in mg) and homogenized in volume 300µl of ice-cold citrate-phosphate buffer (pH 6.8) with tissue grinder for 3 min at 4 C. The homogenate was suspended in ice-cold buffer bringing the volume up to 1ml with citrate buffer. Samples were centrifuged at 500x*g*, (Eppendorf 5415C table top centrifuge) for one min and the resultant supernatants were collected as the enzyme source.

*Acid phosphatase (E.C.3.1.3.2) and alkaline phosphatase (E.C.3.1.3.1)*

Bessey *et al*. (1946) procedure was used to carried out the experiment The 100 uL substrates were incubated for about 30 min and 1 mL of alkali (1.0M pH 8.0) was added to stop the reaction. The p-nitrophenolate spectral absorbance was maximal at 310 nm. The molar absorbance of p-nitrophenolate at 400 nm is about double that of p-nitrophenyl phosphate at 310 nm. On converting the p-nitrophenolate into p-nitrophenol by acidification, the absorption maximum is shifted to about 320 nm with no detectable absorption at 400 nm.

*Estimation of adenosine triphosphatase*

Shiosaka *et al*. (1971) protocols was followed during the experiment. In the gut sodium and potassium dependent ATPase activities were assayed. Fiske and Subbarow (1925) methodology was adopted for quantitative assay of inorganic phosphorous liberated during experiment. In detail first the protein is precipitated with trichloroacetic acid, then the protein-free filtrate is treated with acid molybdate solution and the phosphoric acid formed is reduced by the addition of 1-amino-2-naphthol-4-sulfonic acid (ANSA) reagent to produce blue color. The intensity of the color is proportional to the amount of phosphorous present.

*Histology*

The effects of the active fraction ingestion was studied on larvae of *S. litura*. Treated and control larval gut tissue was fixed in Bouin's Solution overnight. The blocks were cooled about 27 °C for 3 h and cut into 1.5 µm slices with an ultra-cryo-microtome (Cryocut 1800, Leica, Germany). The slices were stained with Delafield's haematoxylin and counter-stained with eosin, and mounted after drying. The sections were observed and photographed under Optika, Flow series HBO light microscope (model: B-600 TiFl-Italy).

*Statistical analysis*

Each experiment was replicated five times and the data's obtained from mortality were expressed in mean of five replications and arcsine-square root transformation of percentages was used to normalise the data. The analysis of variance (ANOVA) was used to identify the difference in percentage of mortality and were fitted with linear regression using Minitab®17 for lethal concentration. Differences between the treatments were determined using the Tukey-Kramer HSD test ($p \leq 0.05$). The highest mean difference detected by statistical testing are marked with letter "a" the next text lower "b" etc. (Snedecor and Cochran, 1989; SAS Institute, 2001). For enzyme activity linear regression technique of Microcal software (OriginPro 8) was used to graphs. The lethal concentrations ($LC_{50}$ and $LC_{90}$) were calculated using Probit analysis (Finney, 1971).

## Results

*Analysis of purified plant compound andrographolide*

The isolated plant compound from column chromatography analysed by HPLC revealed the purity at 88 % (Fig.1) when compared with the standard of andrographolide (Fig. 2). The analysed compound eluted at the Rt 3.01, which was detected at 224 nm compared with the standard chromatogram. The andrographolide standard eluted

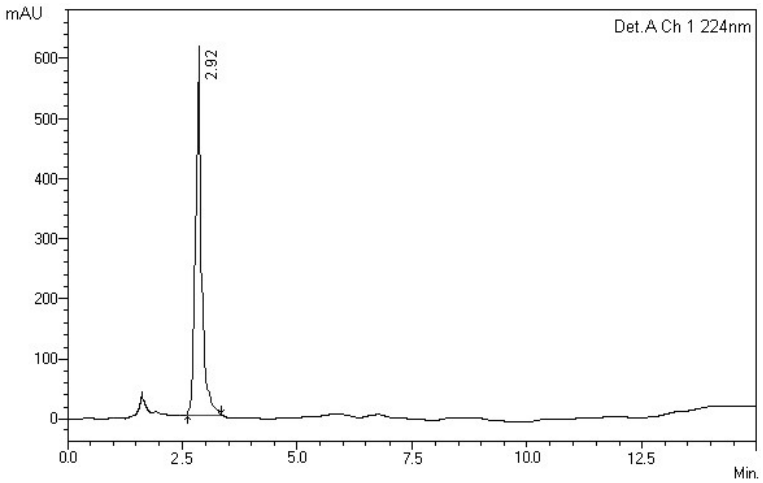


**Fig. 1** HPLC chromatogram of standard andrographolide

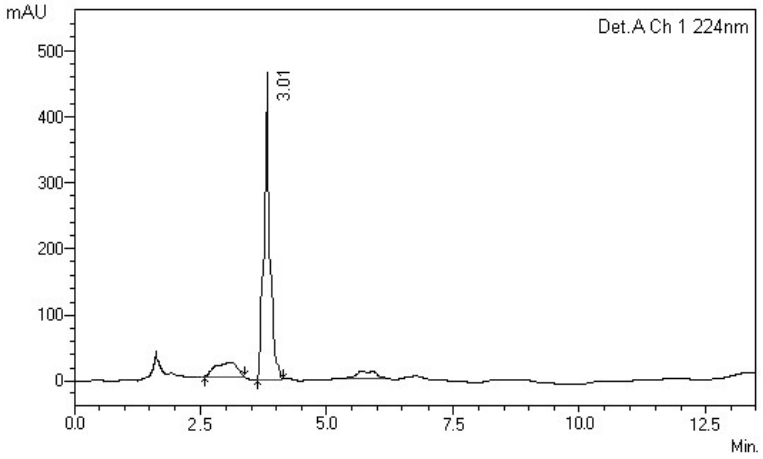


**Fig. 2** HPLC chromatogram of isolated compound andrographolide

at the Rt of 2.92. The comparison analyses confirms the compound isolated was andrographolide.

*Mortality of* S. litura *against andrographolide*

The active compound andrographolide had pronounced larvicidal activity against *S. litura*. Mortality rates of larvae increased gradually with increasing concentrations (5, 10, 15 and 25 ppm). Second instar larvae had the highest mortality. The mortality percentage of andrographolide against *S. litura* is shown in (Fig. 3). The leaf disc treated with andrographolide was permitted to feed and the larvae were observed at 24 h. Mortality rate showed significant difference between second instar ($F_{4,20}$ = 54.61, $p \leq 0.001$), third instar ($F_{4,20}$ = 56.62, $p \leq 0.001$), fourth instar ($F_{4,20}$ = 67.35, $P \leq 0.001$) and ($F_{4,20}$ = 29.02, $p \leq 0.001$) fifth instar larvae respectively. The greatest mortality rate was observed in all instar larvae at 25 ppm concentration. The results shown that the andrographolide had significant larvicidal activity. The lowest (5 ppm) concentration of andrographolide had the lowest mortality rate in all instars, but it caused several abnormalities in larval growth.

Low concentrations were ample enough to give prominent $LC_{50}$ and $LC_{90}$ values for all the instars. Lethal concentration ($LC_{50}$) for second instar larvae was observed at 8.31 and 28.86 ppm for $LC_{90}$. In third instar larvae $LC_{50}$ was observed at 9.59 and 30.78 ppm for $LC_{90}$. For fourth and fifth instar larvae the $LC_{50}$ and $LC_{90}$ was observed at 10.38 ppm, 34.53 ppm and 11.33 ppm, 36.88 ppm respectively.

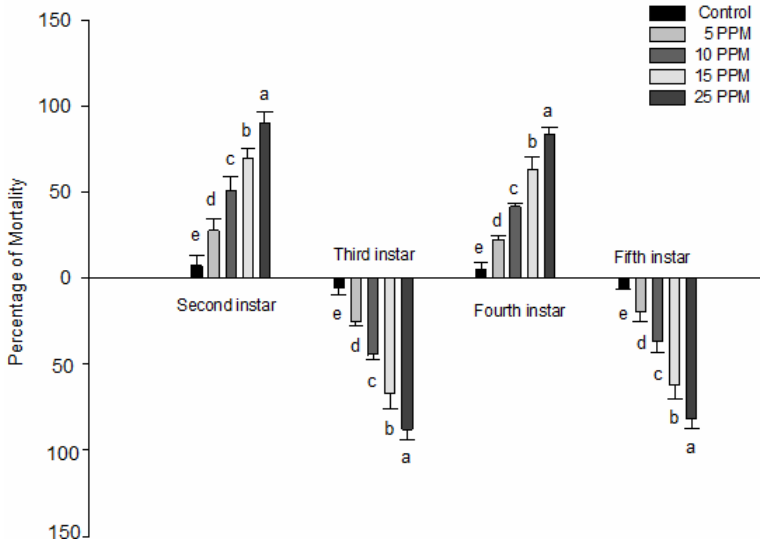


**Fig. 3** Percentage mortality of second, third, fourth and fifth instar of *S. litura* after treatment with andrographolide. Means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$) by using Probit analysis.

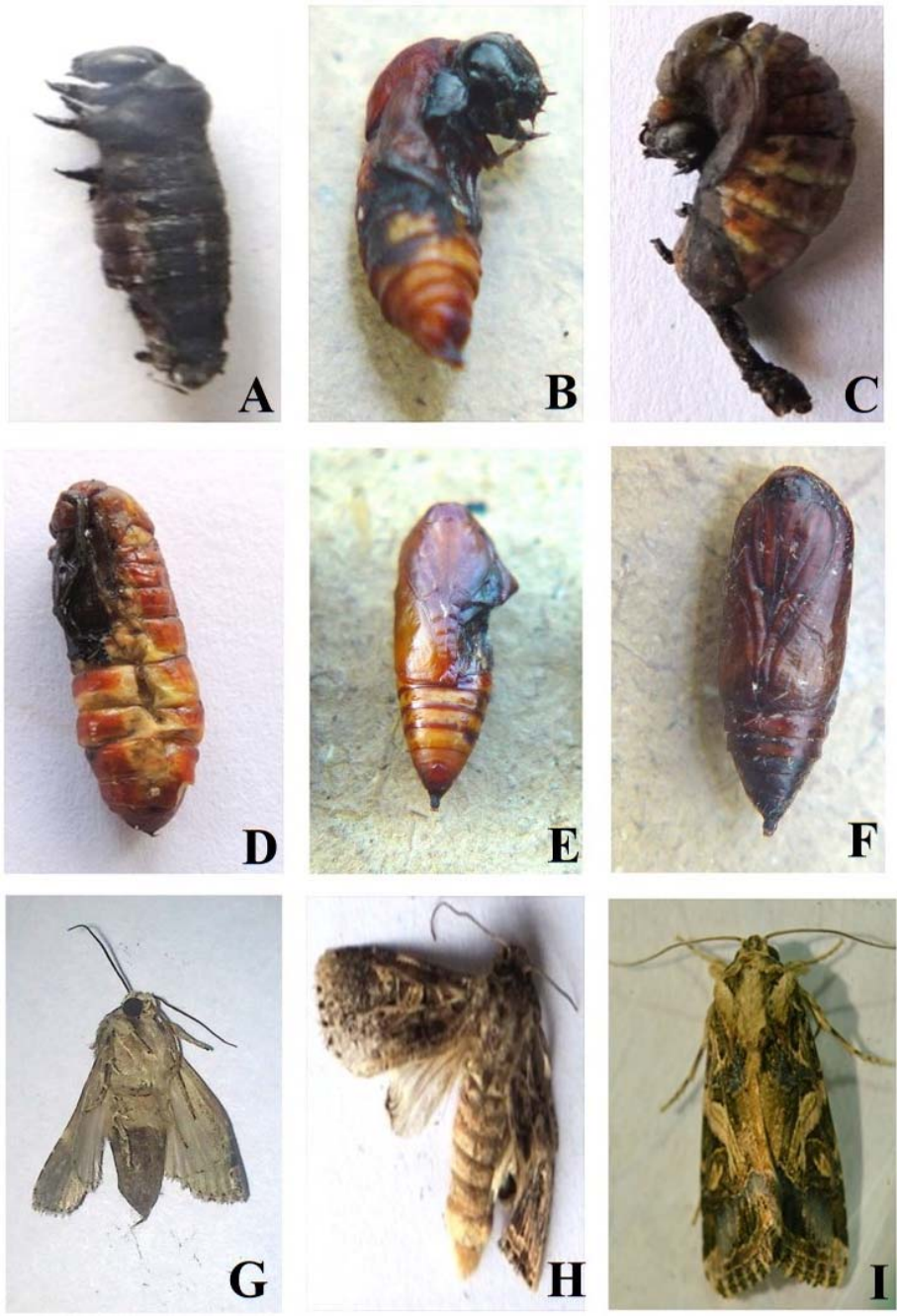


**Fig. 4** Morphological effects of andrographolide on *S. litura*. (A,B,C) larval pupal intermediate (D,E) pupal deformities (F) control pupa (G) treated and unhealthy adult (died) (H) adult emerged with wrinkled wings, molting disorder (shrinking of larval body) (I) healthy adult.

*Behavioural effects of* S. litura *against andrographolide*

Effective dosage of andrographolide produced a typical colour change, with fluid ooze out from the body of the *S. litura* larvae. Larval-pupal intermediates due to growth-regulatory effects of andrographolide and immature adults were noticed in all the treated concentrations but was more prominent at the highest concentrations (Fig. 4). In some cases pre-pupae were affected often causing death, or formation of abnormal pupae were noticed at higher concentrations of 9 and 12 ppm. In lower dosage (3 and 6 ppm) durations of the development life cycle became extended, larval growth was retarded and colour change occurred. At the concentration of 12 ppm larvae appeared unhealthy, with severely damaged cuticles. The adult emergence showed lower longevity, deformed wings and egg produced by the adults were nonviable, immature.

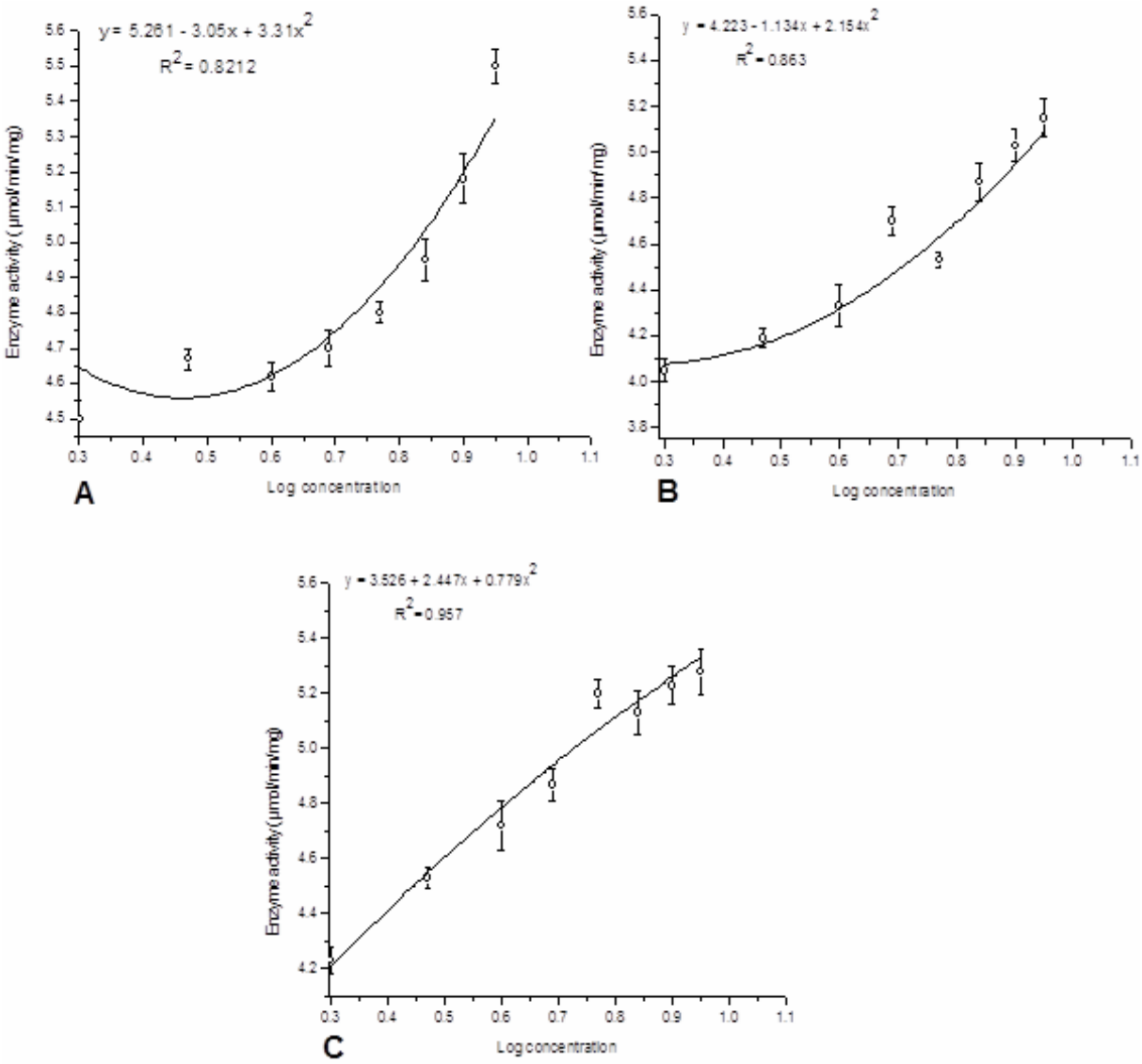


**Fig. 5** Enzyme activity (ACP) in (A) third, (B) fourth and (C) fifth instar larvae of *S. litura* after treatment with andrographolide.

*ACP activity*

Concentration used below the $LC_{50}$ value showed reduction in enzymatic activity. Acid phosphatase activity was reduced in larvae treated even at lower concentrations 3, 6 and 9 ppm. The ACP inhibition was significantly different for third ($F_{3,16}$ = 22.65; $p \leq 0.001$), fourth ($F_{3,16}$ = 42.01; $p \leq 0.001$) and fifth ($F_{3,16}$ = 49.99; $p \leq 0.001$) instar larvae (Fig. 5). When compared with control the estimated highest reduction of 69.18 % was observed in third instar larvae treated with 9 ppm concentration. The reduction in acid phosphatase level reduces metabolism by minimal release of phosphorus. The reduction observed suggests that the cause is predominantly due to the physiological activity of andrographolide.

*ALP activity*

Activity of andrographolide against *S. litura* showed ALP activity reduction across all concentrations. It showed significant reduction at 9 ppm. Inhibition rate was protuberant in third instar larvae compared with fourth and fifth instars of *S. litura* (Fig. 6). The greatest reduction of 75.3 % was observed in third instars treated with 9 ppm concentration of andrographolide.

*ATPase*

Adenosine triphosphate was reduced in treated larvae compared with control larvae. The greatest reduction of 74.9 % occurred with treatment of 9 ppm andrographolide (Fig. 7). The reduction rate of third, fourth and fifth instar larvae are significantly

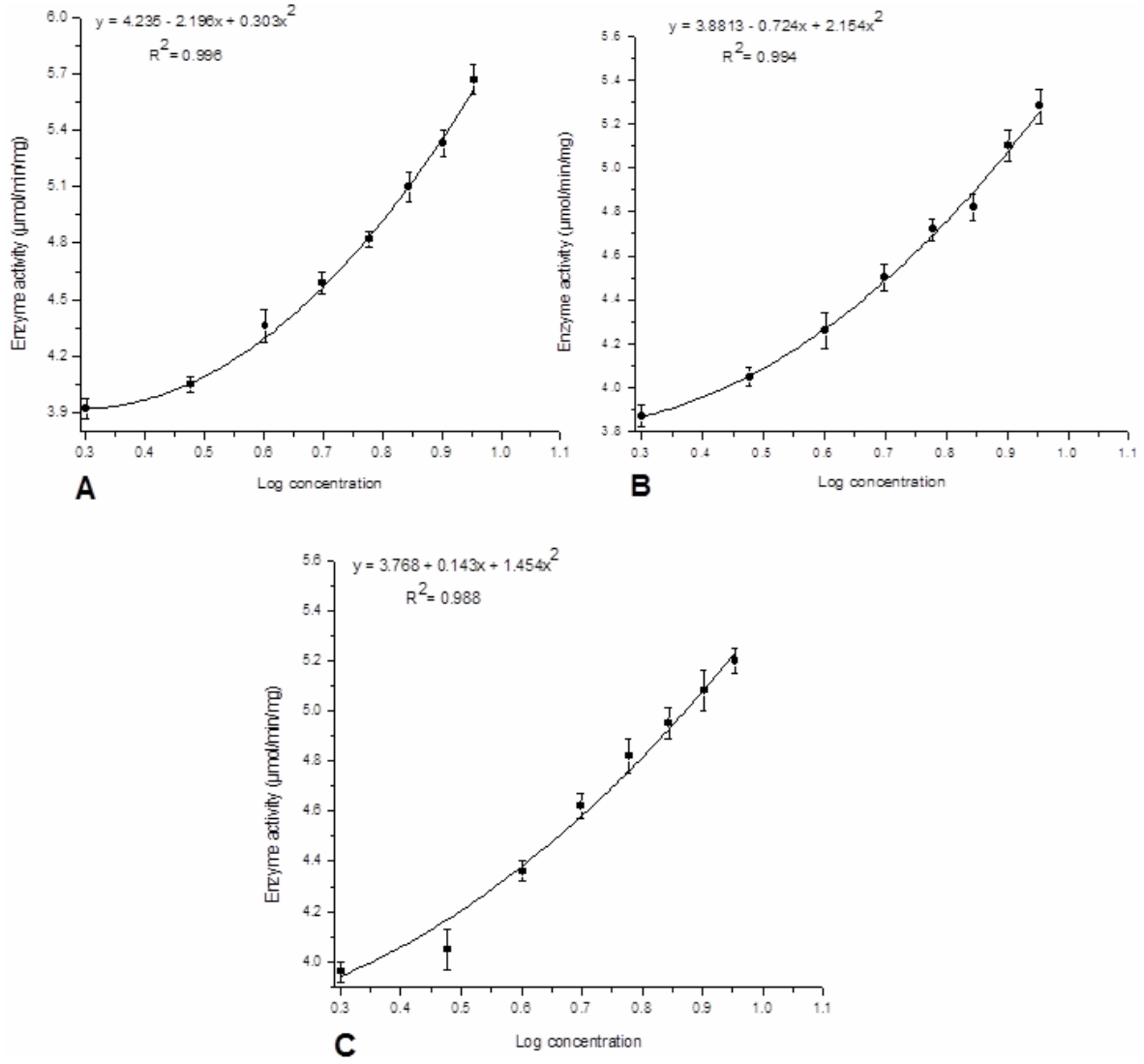


**Fig. 6** Enzyme activity (ALP) in (A) third, (B) fourth and (C) fifth instar larvae of *S. litura* after treatment with andrographolide.

different with each other ($p \leq 0.001$). This reduction affects the physiology of the gut by inhibiting the transphosphorylation activity. This activity clearly implies the compound can produce significant influence on enzymatic profile.

*Histological study of* S. litura

Differences in the midgut region was observed in 3, 6 and 9 ppm treated larvae of *S. litura.* The histology of treated larvae showed damaged cellular components to the epithelial and columnar cells. The untreated larvae had well distinguished epithelial and columnar cells, peritrophic membrane, brush border membrane and microvilli (Fig. 8A).

Larvae treated at 3 ppm showed some disturbed cells, epithelial cells were enlarged, getting to rupture and partially disintegrated gut lumen was observed (Fig. 8B). In 6 ppm treatment, larvae showed damaged gut lumen and cytoplasmic regions of oozing cell material into the alimentary tract that mixed within the food column. Columnar cells enlarged, brush boarder membrane was damaged with no clear identification of epithelial layer (Fig. 8C).

Larvae at 9 ppm concentration showed damage to cell organelles such as brush boarder membrane being reduced, columnar cells becoming thin, enlarged spacing of cells, mixing with other cell organelles and post disruption of membranes (Fig. 8D).

At higher concentration of 12 ppm, the epithelial cells were broken and fully collapsed. There was no distinct columnar cells, epithelial layer, cytoplasmic disappeared and other cellular components appeared to have disintegrated completely (Fig. 8E). The space between the cytoplasm appeared larger and very distinct. Thus at each increasing concentration the damage of cells became more significant and obvious.

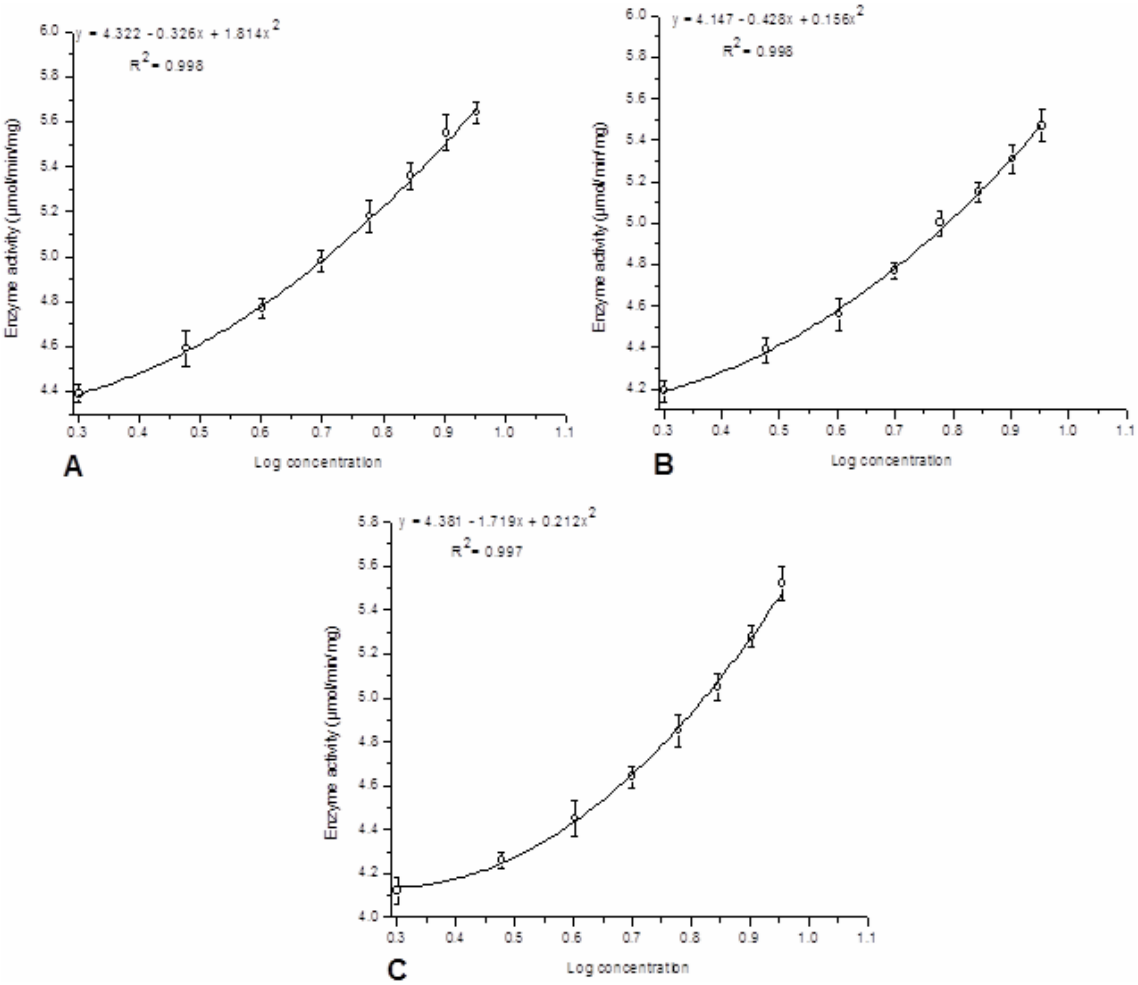


**Fig. 7** Percentage reductions of enzyme activity (ATP) in (A) third, (B) fourth and (C) fifth instar larvae of *S. litura* after treatment with andrographolide

## Discussion

Mortality rate was highest in second instar larvae compared with all other instars, possibly because of their earlier developmental stage. Hexane and chloroform extract of *A. paniculata* showed 100 % mortality at 1000 ppm treated mosquitoes, *Anopheles subpictus* (Elango *et al*., 2011). Young instars were more susceptible than older instars, as insects mature the mortality rate was reduced. This observation may be due to the increased physiological power of a maturing larvae which has greater capacity to excrete or breakdown ingested toxins. Earlier instars were observed to be more susceptible to andrographolide exposure, when compared with later instars.

Morphological changes in the lifecycle of the insect was due to inhibitory effects of andrographolide. Similar results were reported from ethanolic leaf extract of *Tribulus terrestris,* family (Zygophyllaceae), on *S. litura* (Gunasekaran and Chellaiah, 1985). The darkish black colour pupae was observed and unable to emerge into adults due to ingested andrographolide. The deformed adults showed, darker wings and unable to fly for a longer distance. Similar results were reported by Narendran *et al*. (1999), of deformities in head size, length of the body, remains of old cuticle, and darkened colouration on wings when treated with bark extracts of *Cassia fistula* and leaf extract of *Murraya koenigii,* L. family Rutaceae, at 1000 ppm. Martinez and van Emden. (2001) also showed that azadiractin induced a wide range of abnormalities such as larval, pupal and adult abnormalities and small sized adults of the lepidopteran, *Spodoptera littoralis.*

The enzyme activity was reduced due to ingestion of treated diet, which reduced gut enzymes such as ACP, ALP and ATPase. Reports suggest that these enzymes are the most sensitive to pesticide exposure (Wu and Lam, 1997; Diamantino *et al*., 2001; de Almeida *et al.,* 2014; Ottaviani, 2014). Decreased ACP levels from treatments suggests a decreased release of phosphorous needed metabolism (energy), which caused decline rate of transport of metabolites of that further reduced the overall rate of metabolism (Senthil-Nathan and Kalaivani, 2005, 2006; Senthil-Nathan *et al*. 2005a, b).

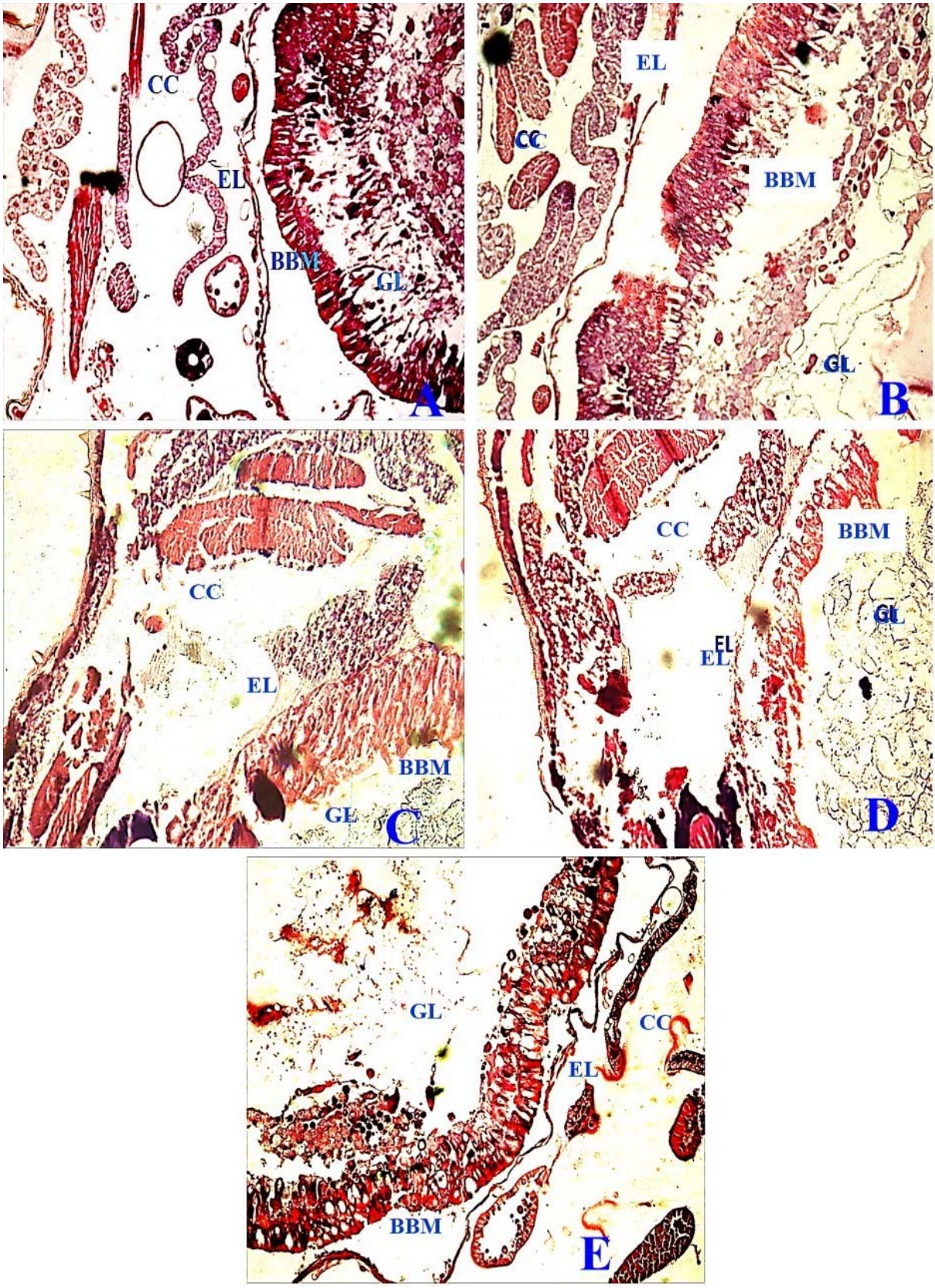


**Fig. 8** Histological section of the midgut region of fourth instar larvae of *S. litura* (A) control, (B) Treated concentrations of 3 ppm, (C) 6 ppm, (D) 9 ppm and (E) 12 ppm of andrographolide. CC- Columnar cells EL- Epithelial layer GL- Gut lumen BBM- Brush border membrane

Adenosine triphosphate (ATP) is considered the energy currency of cells, particularly in the epithelial cells of intestine which aid transport and metabolites reabsorption, nutrients and secondary transport of ions (Lechleitner and Phillips, 1988; Fogg *et al*., 1991; Zibaee *et al.,* 2010). As ingested food cannot be digested or oxidised, the metabolism stops, thus ATPase levels are reduced.

The digestion and absorption of nutrients occurs in midgut (Selin-Rani *et al*., 2016). Histological studies revealed that andrographolide treatment disrupted cell and organelle integrity in larval midguts of *S. litura.* Destruction of epithelial cells would reduce nutrition absorption of ingested food. The midgut cytoplasm were severely damaged at the highest concentration ingested by larvae. Corruption of the epithelial layer due to andrographolide ingestion led to oozing of cytoplasmic components which mixed with food in the digestive tract. Cell destruction leads to the reduction rate of digestion and food intake. Similar observations were reported by Senthil-Nathan *et al*. (2008a, b) on *S. exigua* which ingested styryl-pyrone in an artificial diet, and for *S. litura* treated with 100 ppm of *Melia* extract. *Melia* is a genus of flowering trees in the mahogany family, Meliaceae (Schmidt *et al*., 1997). The apoptotic histopathological changes support reports that andrographolide has strong effect on cell physiology, and midgut structure of *S. litura* larvae.

The results that the active compound bound to specific receptors on the midgut may have led to blockage of the digestive tract. Furthermore, the subsequently destructions of the transmembrane potential and the osmotic lysis of cells lining the midgut would have contributed to the death of the larvae (Aronson and Shai, 2001). The present study suggest that andrographolide derived from *A. paniculata* had acute toxicity to *S. litura* and also produced morphological changes in their lifecycles. The enzyme activity was also reduced due to ingestion of andrographolide through their diet. Similarly, andrographolide treatment strongly disrupted the midgut cells. Overall, our results gain evidence to develop novel and safer natural pesticides against the polyphagous pest *S. litura*. If compared to commercial pesticides, andrographolide is usually more biodegradable, eco-friendly and also decreases the chemical burden on the society.

**Acknowledgement**

The project was full financially supported by King Saud University, through Vice Deanship of Research Chairs.

**References**

Abbott W. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267, 1925.

Abudulai M, Shepard BM, Mitchell PL. Parasitism and predation on eggs of *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae) in cowpea: impact of endosulfan sprays. J. Agric. Urban Entomol. 18: 105-115, 2001.

Ajamhassani M, Sendi JJ, Farsi MJ, Zibaee A. Purification and characterization of phenoloxidase from the hemolymph of *Hyphantria cunea* (Lepidoptera: Arctiidae). Inv. Surv. J. 9: 64-71, 2012.

Applebaum SW, Jankovic M, Birk Y. Studies on the midgut amylase activity of *Tenebrio molitor* L. larvae. J. Insect. Physiol. 7: 100-108, 1961.

Applebaum SW. The action pattern and physiological role of *Tenebrio* larval amylase. J. Insect. Physiol. 10: 897-906, 1964.

Aronson AI, Shai Y. Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique feature of their mode of action-mini review. FEMS Microbiology Lett. 195: 1-8, 2001.

Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with the cubic millimeters of serum. J. Biol. Chem. 164: 321-329, 1946.

Chandrasekaran R, Revathi K, Nisha S, Kirubakaran SA, Sathish-Narayanan S, Senthil-Nathan S. Physiological effect of chitinase purified from *Bacillus subtilis* against the tobacco cutworm *Spodoptera litura* Fab. Pestic. Biochem. Phys. 104: 65-71, 2012.

de Almeida GD, Zanuncio JC, Senthil-Nathan S, Pratissoli D, Polanczyk RA, Azevedo DO, *et al*. Cytotoxicity in the midgut and fat body of *Anticarsia gemmatalis* (Lepidoptera: Geometridae) larvae exerted by neem seeds extract. Inv. Surv. J. 11: 79-86, 2014.

Diamantino TC, Amadeu E, Soaresa MVM, Guilherminoc L. Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna* straus. Chemosphere 45: 553-560, 2001.

Duraipandiyan V, Ignacimuthu S, Paulraj MG. Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. Saudi. J. Biol. Sci. 18: 129-133, 2011.

Elango G, Zahir AA, Bagavan A, Kamaraj C, Rajakumar G, Santhoshkumar T, Marimuthu S, Rahuman, AA. Efficacy of indigenous plant extracts on the malaria vector *Anopheles subpictus* Grassi (Diptera: Culicidae). Indian J. Med. Res. 134: 375-383, 2011.

Finney DJ. Probit Analysis, 3rd ed., Cambridge University Press, Cambridge, 1971.

Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400, 1925.

Fogg KH, Anstee JH, Hyde D. Studies on the subcellular distribution of (Na++ K+)-ATPase, K+-stimulated ATPase and $HCO_3$-stimulated ATPase activities in malpighian tubules of *Locusta migratoria* L. Insect Biochem. 21: 749-758, 1991.

Gunasekaran K, Chellaiah S. Antifeedant activity of *Andrographis paniculata* on *Spodoptera litura* F. Proceedings of the National Seminar on Behavioural Physiology and Appropriate Management of Crop Pests, TNAU, Coimbatore, pp 31-33, 1985.

Hermawan W, Tsukuda R, Fujisaki K, Kobayashi A, Nakasuji F. Influence of crude extracts from a tropical plant, *Andrographis paniculata* (Acanthaceae), on suppression of feeding by the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) and oviposition

by the azuki bean weevil, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Appl. Entomol. Zool. 28: 251-254, 1993.
Horie B. The alkaline phosphatase in the midgut of silkworm, *Bombyx mori* L. Bull. Seric. Exp. Stn. 15: 275-289, 1958.
Klocke JA. Plant compounds as source and models of insect control agents. In: Hosttettmann K (ed.), Economic and medicinal plant research. Academic, London, pp 103-144, 1989.
Lechleitner RA, Phillips JE. Anion stimulated ATPase in locust rectal epithelium. Can. J. Zool. 66: 431-438, 1988.
Martinez SS, Emden HFV. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by Azadirachtin. Neotrop. Entomol. 30: 113-125, 2001.
Matsumura F. Toxicology of Insecticides, Plenum, New York, USA, pp 453: 1975.
Meenatchisundaram S, Parameswari G, Subbraj T, Suganya T, Michael A. Medicinal and pharmacological activities of *Andrographis paniculata*-Review. Ethnobot. Leaflets 13: 55-58, 2009.
Miao YG. Studies on the activity of the alkaline phosphatase in the midgut of infected silkworm, *Bombyxmori* L. J. Appl. Entomol. 126: 138-142, 2002.
Narendran ST, Arivoli S, Ignacimuthu S. Evaluation of larvicidal and antifeedant activity of two plants, Proceedings of biopesticides in insect pest management. Phoenix Publishers, New Delhi, pp 152-155, 1999.
Ottaviani E. The importance of studying invertebrate immune-neuroendocrine functions. Inv. Surv. J. 11:1-3, 2014.
Regnault-Roger C, Philogene BJR, Vincent C. Biopesticides origine vegetale, Techiques and documents, Paris, 2002.
Sakharov IY, Makarova IE, Ermolin GA. Chemical modification and composition of tetrameric isozyme K of alkaline phosphatase from harp seal intestinal mucosa, Comp. Biochem. Physiol. 92B: 119-122, 1989.
SAS Institute. The SAS System for Windows, Release 8.1. Cary NC, 2001.
Schmidt GH, Ahmed AAI, Breuer M. Effect of *Melia azedarach* extract on larval development and reproduction parameters of *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) (Lep. Noctuidae). J. Pest. Sci. 70: 4-12, 1997.
Selin-Rani S, Senthil-Nathan S, Revathi K, Chandrasekaran R, Thanigaivel A, Vasantha-Srinivasan P, *et al.* Toxicity of *Alangium salvifolium* Wang chemical constituents against the tobacco cutworm *Spodoptera litura* Fab. Pestic. Biochem. Phys. 126: 92-101, 2016.
Senthil-Nathan S, Choi MY, Paik CH, Kalaivani K. The toxicity and physiological effect of goniothalamin, a styryl-pyrone, on the generalist herbivore, *Spodoptera exigua* Hubner. Chemosphere 72: 1393-1400, 2008a.
Senthil-Nathan S, Choia MY, Seoa HY, Paika CH, Kalaivani K, Kima JD. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown plant hopper *Nilaparvata lugens* (Stal). Ecotoxicol. Environ. Saf. 70: 244-250, 2008b
Senthil-Nathan S, Kalaivani K, Chung PG. The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Pestic. Biochem. Physiol. 83: 46-57, 2005a.
Senthil-Nathan S, Kalaivani K, Murugan K, Chung PG. The toxicity and physiological effect of neem limonoids on *Cnaphalocrocis medinalis* (Guenee) the rice leaffolder. Pestic. Biochem. Physiol. 81: 113-122, 2005b.
Senthil-Nathan S, Kalaivani K. Efficacy of nucleopolyhedrovirus and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Biol. Control. 34: 93-98, 2005.
Senthil-Nathan, S, Kalaivani K Combined effects of azadirachtin and nucleopolyhedrovirus (SpltNPV) on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) larvae. Biol. Control 36: 94-104, 2006.
Senthil-Nathan S. Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. Front. Physiol. 359: 1-17, 2013.
Shiosaka T, Okuda H, Fujii S. Mechanism of the phosphorylation of thymidine by the culture filtrate of *Clostridium perfringens* and rat liver extract. Biochim. Biophys. Acta 246: 171-183, 1971.
Sintim HO, Tashiro T, Motoyama N. Response of the cutworm *Spodoptera litura* to sesame leaves or crude extracts in diet. J. Insect. Sci. 9: 52, 2009.
Snedecor GW, Cochran WG. Statistical methods, eighth ed., Iowa State University Press, Ames, Iowa, 1989.
Wan H, Kang T, Zhan S, You H, Zhu F, Lee KS, Zhao H, Jin BR, Li J. Peroxiredoxin 5 from common cutworm (*Spodoptera litura*) acts as a potent antioxidant enzyme. Comp. Biochem. Physiol. 175B: 53-61, 2014.
Wood HA, Granados RR. Genetically engineered baculoviruses as agents for pest control. Annu. Rev. Microbiol. 45: 69-87, 1991.
Wu RSS, Lam PKS. Glucose-6-phosphate dehydrogenase and lactate dehydrogenase in the green-lipped mussel (*Perna viridis*). Possible biomarker for hypoxia in the marine environment. Water Res. 2797-2801, 1997.
Xiaoa X, Fua H, Luoa Y, Wei X. Potential anti-angiogenic sulfates of andrographolide. J. Asian Nat. Prod. Res. 15: 809-818, 2013.
Zibaee I, Bandani AR, Jalali-Sendi J, Talaei HR, Kouchaki B. Effects of *Bacillus thuringiensis* var. *kurstaki* and medicinal plants on *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). Inv. Surv. J. 7: 251-261, 2010.