ISSN : 0974 - 7508 Volume 7 Issue 5

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 7(5), 2011 [279-286]

Insecticidal potential of *Calotropis procera* and *Annona squamosa* ethanol extracts against *Musca domestica*

Nighat Begum[1], Bechan Sharma[2], Ravi S. Pandey[1*]

Departments of Zoology[1] and Biochemistry[2], University of Allahabad, Allahabad-211002, (INDIA)

E-mail : rspandey2004@yahoo.com

Received: 13th July, 2011 ; Accepted: 13th August, 2011

ABSTRACT

Present study has been undertaken for the assessment of the larvicidal activities of crude ethanol extracts of *Calotropis procera* and *Annona squamosa* seeds against *Musca domestica*. The third instar larvae of housefly were treated with the different concentrations of both the extracts by dipping method for 48 h. The larvae were exposed to 5 and 10% concentrations of the LC_{50} value of each extract along with their control sets to evaluate their effect on metamorphosis, nucleic acid and protein content in different developmental stages. Extract treatment resulted in several pupal deformities as well as inhibition of adult emergence. The data indicate that the seed extracts of these plants may be utilized as the probable candidates for the development of bioinsecticides to control the population of *Musca domestica* as safer and economic alternatives to the synthetic insecticides.

KEYWORDS

Musca domestica;
Calotropis procera;
Annona squamosa;
Bioinsecticide;
LC_{50}.

INTRODUCTION

The indiscriminate use of synthetic insecticides has caused environmental contamination and toxicity to living organisms[1,2], indicating the need for the development of products that are not hazardous to the environment. Realizing the adverse effects of chemical insecticides, attention has now been focussed in favour of non-chemical methods of pest management. Insecticidal activity of many plants against several insects has been demonstratedb[3,4]. Seed as well as foliar extracts of several plants have been reported to have toxic and potent growth reducing activity to insects[5]. The deleterious effect of plant extracts or pure natural/ synthetic compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility.

Exposure of toxic agents to animals can cause changes even at the molecular level. Nucleic acids (DNA and RNA) and protein contents are regarded as important biomarkers of the metabolic potential of cells, as they play the main role in regulating the different activities of cells. Changes in the amount of nucleic acid can be used to detect whether toxic agents affect cellular proliferation and cell death[6.7].

The common housefly *Musca domestica* (Diptera:Muscidae) is an important mechanical vector of several bacterial and pathogenic organisms of human and animals[8]. Recently some reports have indi-

Full Paper

cated that on prolonged exposure to chemical insecticides worldwide, houseflies have developed resistance to them e.g. spinasod[9], diflubenzuron[10] and synthetic insecticides[11].

Calotropis procera is a member of the plant family, Asclepiadaceae, a shrub widely distributed in West Africa, Asia and other parts of the tropics. The plant is erect, tall, large, branched and perennial with milky latex throughout. A large quantity of latex can be easily collected from its green parts[12]. Local people use it successfully to combat some cutaneous fungal infections. The abundance of latex (containing alkaloids) in the green parts of the plant reinforces the idea that it produced and accumulated latex as a defence strategy against organisms such as virus, fungi and insects[13]. The presence of plant defence related proteins such as hevein, an alpha-amylase inhibitor has been described to occur in the latex secretion of other plants[14]. However there are no reports to indicate that *Calotropis* seed extracts exhibit insecticidal properties against *Musca domestica*.

The Annonaceae (Custard- apple family) is a large family of almost exclusively tropical trees and shrubs comprising about 130 genera and 2300 species. Some plants of this family have been used traditionally as insecticides[15]. For example, the powdered seeds and leaf juices of *Annona* spp. are used to kill head and body lice, and bark extract of *Goniothalamus macrophyllus* is used as mosquito repellents. Annonaceous acetogenins extracted from the tree leaves, bark and seeds have pesticidal and/or insect antifeedant properties[16]. However there is no report to indicate that *Annona squamosa* seed extract possess insecticidal activity against housefly. Keeping these facts in view, the present study was undertaken to investigate the larvicidal activities of ethanolic extracts of *C. procera* and *A. squamosa* seeds under laboratory conditions as well to assess the chemical nature of the active components present in the extracts along with their effect on nucleic acids and protein content in different developmental stages of housefly.

EXPERIMENTAL

Rearing Technique: Adult house flies were collected from local areas using a sweep net and reared in the laboratory at 26±2°C, 60±10% RH, photoperiod 12:12 (L:D). The rearing method described by Kristensen and Jespersen[17] was adopted in the present study. In brief, the adults of *M. domestica* were fed milk and dry sugar. Mixture of wheat flour and milk was prepared at a weight ratio of 1:3, and 35g of this mixture was placed on a Petri dish with wet cotton as an oviposition site.

Collection and Processing of Plants Sample: Seeds of *C. procera* and *A. squamosa* were collected from the Botanical Garden of University of Allahabad. Seeds were properly cleaned and shade dried for 10-13 days at 32-35°C and relative humidity 50-60%. The dried leaves were powdered mechanically using commercial electrical stainless steel blender (Remi Anupam Mixie Ltd., India). The samples were stored in air tight container at room temperature in dark for further analysis.

Extraction of Plant Extracts: The seeds leaves of *C. procera* and *A. squamosa* were extracted with 1 litre of 90% ethanol in a Soxhlet apparatus (Borosil, India) using the method described by Mishra *et al*.[18]. The extracts were concentrated at 50°C and the residue obtained was stored at 4°C.

Method of Treatment: Twenty number of late 3rd instar larvae of *M. domestica* identified by shortening and thickening of size and shape, respectively, darker in color and presence of three spiracles at the posterior end of the body[19] were selected for each set of treatment. Seven numbers of glass beakers of 250 ml capacity were taken and labeled for different concentrations in addition to one for check and one for control. In case of control, water and for check, ethanol was added in place of extract. Larvae were dipped into the solution for two minutes and then transferred back in the rearing medium (composition mentioned above).

The experiment was repeated three times on subsequent days. Same method of treatment was applied for both the extracts. The LC_{50} was calculated by Karbers method[20]. The LC_{50} of the seed extracts of *C. procera* and *A. squamosa* was calculated to be 870.50 and 345 mg/l respectively (unpublished data). Morphological appearance of the resultant pupae and the subsequent emergence of the adults from it were also observed. To evaluate the insecticidal effect of the seed extracts of *C. procera* and *A. squamosa*, the 3rd instar larvae of *M. domestica* were treated with 5% and 10% (equivalent to 1/20 and 1/10 of LC_{50} value)

Full Paper

for 24 and 48h. The control group was exposed only with the equal volume of ethanol, the solvent in which the extract was prepared.

BIOCHEMICAL ANALYSIS

Homogenate preparation

After 48 h of exposure larvae were homogenized (10%, w/v) in 50 mM Tris–HCl buffer (pH 7.5) on ice using Potter-Elvehjam homogenizer fitted with a Teflon-coated pestle (Remi, India). The homogenates were centrifuged at 4°C for 10 min at 15,000 g in a refrigerated centrifuge (Sigma, model-3K30, Germany). The corresponding supernatants were either used fresh or kept frozen at -20°C until further use for determining the concentration of different biomolecules.

Total protein content estimation

Protein levels were estimated following the method of Lowry *et al*.,[21] using bovine serum albumin as standard. In brief, the protein samples (tissue extracts) were mixed with the protein reagent (alkaline copper sulphate solution), incubated for 10 min at room temperature followed by addition of Folin-ciocalteau reagent. The reaction mixture was further incubated at room temperature for 30 min. The absorbance of blue color was monitored at 660 nm using spectrophotometer (Systronics Visiscan 167, India). Simultaneously, a blank was also processed containing all the reagents except the protein. Bovine serum albumin (BSA) was used as a standard.

Extraction and estimation of nucleic acids

Nucleic acids (DNA and RNA) were extracted using the method described by Schneider[22]. Calf thymus DNA was used as a standard. Total RNA was estimated by the orcinol[22] using yeast RNA as a standard.

STATISTICAL ANALYSIS

All values in the table are given as mean ± standard error of mean (SEM) of three independent experiments. The mean, standard deviation, standard error of mean for each set of data were calculated by using student's 't' test. Graph pad version 3 software was used to analyze the data and obtain level of significance. *and **represent the values significant at P<0.05(significant) and P<0.01(very significant).

RESULTS

Pupal deformities followed by *C. procera* treatment included dorsoventral compression, reduction in size, collapsed appendages and their failure to metamorphose into adults. Other morphological appearances of the pupae were not much different from that of control excepting that of reduction in (Figure 1).

The morphological changes like reduced size, darkening of the puparium, condensed appendages and failure to metamorphose were recorded due to the treatment of *A. squamosa* seed extract. Pupae with certain

a b

Figure 1: *Musca domestica* pupae showing the morphological changes due to the treatment with the seed extract of *Calotropis procera* under experimental conditions as mentioned in the Materials and Methods, bar represents 5mm.
a –Treated with water (Control) or ethanol (check) for 2 min daily for two days
b –Treated with *C. procera* seed extract daily for two days

a b

Figure 2: *Musca domestica* pupae showing the morphological changes due to the treatment with *Annona squamosa* seed extract under experimental conditions as mentioned in the Materials and Methods, bar represents 5mm.
a –Treated with water (Control) or ethanol (check) for 2 min daily for two days
b –Treated with *Annona squamosa* seed extract daily for two days

larval characters were also observed (Figure 2). The results clearly indicated that the mortality of the larvae in both the cases was dose dependent. It was observed

Full Paper

that during the course of metamorphosis, the tendency for the development of test larvae to pupae decreased with a rise in the concentration of the ethanol extracts (TABLE 1). The larvae treated with the extract of *C. procera* seeds at 1400 mg/l could not develop to pupae completely. At lower concentration, some larvae could continuously develop. Similarly, no pupa formation was recorded in case of 500 mg/l concentration of ethanol extract of *A. squamosa* as it caused 100% mortality of the larvae during the exposure period. In the untreated condition, most of these larvae developed to pupae within 5 days but the pupation period got delayed up to 6-7 days due to treatment with these extracts.

TABLE 1 : Effect of *Calotropis procera* and *Annona squamosa* seed ethanol extracts on the metamorphosis of *Musca domestica*

Ethanol extract from plant seed	Concentration (mg / l)	Percentage of larvae developed into pupae*	Percentage of pupae emerged into adult **
Calotropis procera	0	100%(20/20)	100%(20/20)
	200	100%(20/20)	95% (19/20)
	400	90% (18/20)	77.77%14/18
	600	70% (14/20)	71.42%(10/14)
	800	60% (12/20)	66.66% (8/12
	1000	40% (8/20)	62.50(5/8)
	1200	25% (5/20)	60%(3/5)
	1400	0% (0/20)	0% (0/0)
Annona squamosa	0	100%(20/20)	100%(20/20)
	100	95% (19/20)	89.47%(17/19)
	200	75% (15/20)	80%(12/15)
	300	60% (12/20)	58.33% (7/12)
	400	45% (9/20)	44.44% (4/9)
	500	0% (0/20)	0% (0/0)

*** Each value in parenthesis is the number of total pupae developed from the larvae/ total number of larvae**

**** Each value in parenthesis is the number of pupae developed to adults/the number of total pupae**

The rate of emergence of adults was also significantly inhibited due to the treatment of both of these extracts and the impact was concentration dependent. The results suggest inhibition of adult emergence by 60% due to the treatment of *C. procera* extract (1200 mg/l), whereas 44.44% emergence was recorded at 400mg/l concentration of *A. squamosa* extract. However, at highest concentrations (1400 and 500 mg/l, respectively) of *C. procera* and *A. squamosa* extracts, no emergence was observed (TABLE 1). In the control set of experiments, the adults emerged from pupae within eight days but in case of treated groups the pupation period got delayed resulting in emergence of the adults on 10-11th day.

Total nucleic acid as well as protein levels were reduced significantly ($p < 0.05$) followed by plant extract exposures. At higher dose *C. procera* extract i.e. 87.05ppm the reduction was more significant (TABLE 2). Total protein content was reduced by 19% as compared to the control in the larval stage. The reduction in the level of protein was found to be 40% and 11% in adult and pupa respectively as compared to their con-

TABLE 2 : Effect of ethanol extract of *Calotropis. procera* seed on the level of nucleic acids and protein after 48 h exposure of *Musca domestica*

Stage	DNA mg/g wet wt. of tissue			RNA mg/g wet wt. of tissue			Protein mg/g wet wt. of tissue		
	C	5%	10%	C	5%	10%	C	5%	10%
Larva	0.382±0.0007	0.236±0.001 (-28.05)	0.175*± 0.0016 (-46.64)	0.241±0.002	0.239 ±0.0021 (-0.83)	0.229 ±0.0024 (-4.98)	415.22±0.00	355.04 ±0.0017 (-14.49)	337.12* ±0.0015 (-18.77)
Pupa	1.446±0.002	0.976±0.002 (32.50)	0.653* ±0.001 (-54.84)	0.168 ±0.00	0.167 ±0.001 (-0.60)	0.152 ±0.002 (-9.52)	309.63±0.0064	292.11 ±0.0052 (-5.66)	274.94* ±0.0041 (-11.20)
Adult	1.719±0.0015	1.513 ±0.003 (-11.98)	1.478* ±0.001 (-14.02)	0.159±0.0015	0.145 ±0.0023 (-8.80)	0.125* ±0.0032 (-21.38)	299.12±0.0065	192.10 ±0.0052 (-35.77)	179.92* ±0.0063 (-39.85)

Data were analyzed by Graphpad software,, values given as mean ± SEM Values in parentheses are percent change over control. Significance(*) of data is shown in superscripts, ns P>0.05(non significant), *P<0.05(significant), **P<0.01(very significant), C-Control (0%), 5% & 10% are 1/20 and 1/10 of LC_{50}.

Full Paper

TABLE 3 : Effect of ethanol extract of *Annona squamosa* seed on the level of nucleic acids and protein after 48 h exposure of *Musca domestica*

Stage	DNA mg/g wet wt. of tissue			RNA mg/g wet wt. of tissue			Protein mg/g wet wt. of tissue		
	C	5%	10%	C	5%	10%	C	5%	10%
Larva	0.382±0.0007	0.201 * ±0.0018 (-38.72)	0.0792 ** ±0.0001 (-75.85)	0.241±0.002	0.233 * ±0.0018 (-3.32)	0.198** ±0.0022 (-17.84)	415.22±0.00	327.80* ±0.002 (-21.05)	305.25** ±0,0025 (-26.48)
Pupa	1.446±0.002	0.904 * ±0.0016 (-37.48)	0.592** ±0.0018 (-59.06)	0.168 ±0.00	0.160* ±0.0025 (-4.76)	0.120** ±.003 (-28.57)	309.63±0.0064	272.07 ±0.0046 (-12.13)	251.45* ±0.0031 (-18.79)
Adult	1.719±0.0015	1.432 ±0.002 (-16.70)	1.405** ±0.004 (-18.26)	0.159±0.0015	0.121 ±0.0039 (-23.90)	0.109 * ±0.0040 (-31.45)	299.12±0.0065	165.34* ±0.0050 (-45.39)	152.89** ±0.0062 (-48.89)

sData were analyzed by Graphpad software,, values given as mean ± SEM Values in parentheses are percent change over control. Significance(*) of data is shown in superscripts, ns P>0.05(non significant), *P<0.05(significant), **P<0.01(very significant), C-Control (0%), 5% & 10% are 1/20 and 1/10 of LC_{50}.

trol ones, after treatment with 87.05ppm for 48 h. *A. squamosa* extract higher dose treatment caused reduction of 49% in adult stage protein level (TABLE 2).

After *C. procera* extract higher concentration exposure, RNA level was reduced by 5% of the control in the larvae while in pupal and adult stage it was 10% and 21% as compared to their controls respectively. A reduction of 32% in the RNA level of adult stage was recorded in the *A. squamosa* extract treated set as compared to control ones.

Similar trend of reduction of effect from larval to adult stage was recorded in case of DNA.In *C. procera* extract treated set; it was decreased by to 47% as compared to the control level during the larval stage at higher concentration. In pupal and adult stages the level declined by 55% and 14% respectively. However higher dose of *A. squamosa* extract proved to be more effective causing a depletion of 76, 59 and 18% in larval, pupal and adult stages respectively.

DISCUSSION

In the present study, the ethanol extracts of the seeds of the plants, *C. procera* and *A. squamosa,* were quite effective against the housefly larvae. These extracts drastically affected the pupation and emergence of the adults from pupae in dose dependent manner. The lethal effect of both of these extracts recorded in this study may be due to the crude nature of the ethanol extracts used in the present study. The crude plant extracts might be containing many active compounds in addition to those that are inactive. Some of these may act synergistically to enhance a specific bioactivity whereas some may act antagonistically to mask certain activities.

Several morphological changes (Figure 2) were recorded in the present study following *A. squamosa* extract treatment. Pupal deformities included dorsoventral compression, reduction in size, collapsed appendages and their failure to metamorphose into adults. Prolonged larval duration leading to late pupation was also observed. The morphological changes in the case of *C. procera* comprised of reduced size of the pupa and darkening of the puparium as compared to control. These changes were observed only at the higher doses of extract.

Toxicity potential of *C. procera* and *A. squamosa* leaf extracts against M. domestica and their effect on metamorphosis has already been reported[23].

At higher concentrations i.e. 1200 and 1400ppm of *C. procera* and 400 and 500ppm of *A. squamosa*, mortality was accompanied with feeding inhibition. This suggests that insect death was due to a combination of starvation and contact toxicity of the extract. It was also reported that ascimin, an acetogenin from the bark of paw-paw tree, *Asimina triloba* (Annonaceae) has both toxic and antifeedant properties[24,25], as reported with Azadirachtin from the neem tree, *Azadirachta indica* (Meliaceae)[26,27].

Previous investigations on annonaceous acetogenin, the bioactive principle of the plant family Annonaceae, have shown that it may have pesticidal or antifeedant properties[24,28]. Seed oil of *A. squamosa* has been re-

ported to reduce survival of leaf hopper, *Nephotettix virescens* (Hemiptera: Cicadellidae) and transmission of rice tungro virus[29,30].

Though reports on nematicidal[31], antimicrobial and antihelminthic[32] activities of *C. procera* extract and its use in the treatment of toothache, cough and subcutaneous diseases[33] exist, there is no report at all regarding the insecticidal property the alcoholic extract of *C. procera* seed against *M. domestica*. The laboratory study on larvicidal properties of leaf extract of *C. procera* against mosquito larvae is known[34]. The toxicity of crude Neem extract against 2nd instar larvae of *M. domestica* has been established earlier[35]. The results from the present study indicate the inhibitory property of the *Calotropis* and *Annona* extracts which may be due to the different compounds present in the extract possessing different bioactivities.

Deranged or halted program of pupation in the present study may be due to the lack of adequate concentration of ecdysteroids needed for achieving larval pupal transformation normally[36]. In coherence to our finding, the production of malformed pupa following azadirachtin treatment has also been reported[37]. It is well known that juvenile hormone and ecdysone play important role in metamorphosis. Delay in the development of larvae may be due to the presence of high level of juvenile hormone in the larvae or else due to the ascribed chemical compounds in the plant extract preventing normal pupation and adult emergence. Earlier studies reported that azadirachtin acts as an antiecdysone and/or inhibits the neuroendocrine control of ecdysteroid and extends its developmental period[38]. Death of the larvae exposed to the ethanol extracts of these two plants in present study may be due to the inability of the moulting bodies to take up sufficient volume of air to split the old cuticle and expand the new one during ecdysis or metamorphosis. The inhibitory effects of plant extracts are possibly due to the perturbations in the hormonal regulation in the insect[39].

As shown in TABLE 1, the alterations in the rate of pupation and adult emergence due to treatment of the plant extracts were dose dependent. This may be due to the effect of some active ingredients present in the extracts which exhibit potential to cause interference into the normal metabolism of the insects[40].

Sub acute doses of the *C. procera* and *A. squamosa* seed extract for 48 h (2 min. daily), caused highly significant alterations in total protein and nucleic acids (DNA and RNA) levels in different developmental stages (larvae, pupa and adult) of *Musca domestica* (TABLES 2 and 3). Though the exposure was given to only larval stage but its effect lasted in the pupal and adult stages, indicating towards the residual effect of the extract.

In case of *C. procera* extract exposure the level of DNA was reduced to 53% of the control during the larval stage. In pupal and adult stages the level was 44% and 86% of their controls respectively after the treatment with higher dose. The maximum reduction of 76% in the DNA level was observed in larval stage at higher dose of exposure of *A. squamosa* leaves extract.

Similarly, when 6th instar larvae of *Tribolium castaneum* were treated with higher doses of fenpropathrin, RNA and DNA contents were reduced by 21% and 20%, respectively[41].

Nucleic acids and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the different activities of cells. Since insects have very little carbohydrate, protein is used to meet the increased energy demand. Proteins are mainly involved in the architecture of the cell which is the chief source of nitrogenous metabolism. Decrease in its level during exposure to plant extract may have been due to their degradation and possible utilization for metabolic purposes. In the present study highest reduction in the protein content was 40% and 49% in case of *C. procera* and *A. squamosa* extract high dose treatment respectively.

The decreases in total protein level in the different developing stages suggest the high protein hydrolytic activity due to elevation of protease activity. Inhibition of DNA synthesis, thus, might affect both protein as well as protein synthesis machinery. The results of this study suggest that the extracted compound (s) is a potent inhibitor of DNA synthesis, which in turn results in the reduction of RNA level. In the present finding highest decline of 21% and 31% was observed in the RNA level in adult in case of *C. procera* and *A. squamosa* extract respectively. Thus, it is possible that extracted compounds might have inhibited the enzymes necessary for DNA synthesis. Carbohydrates are the primary and immediate sources of energy. In stress condi-

tion, carbohydrate reserve is depleted to meet energy demand. An inhibition of 30.23% and 19.30% of RNA and DNA by neem compounds and 17.70% and15.63% by solfac respectively, was reported in *Musca domestica*[42].

CONCLUSION

The data obtained from the present study clearly indicate that *C. procera* and *A. squamosa* seed extracts were quite effective as larvicides for providing a better and excellent alternate for the control of *M. domestica*. Among the two extracts tested *A. squamosa* seed extract proved to be more effective. Further validation of these extracts through multidimensional biochemical and molecular approaches and their field trials may be useful in evaluating its suitability as safer, economic and ecofriendly insecticide.

ACKNOWLEDGEMENTS

The authors express their gratitude to Prof. P. Gaur, Ex-Head, Department of Zoology, University of Allahabad for providing necessary facilities. Financial assistance to one of the authors (NB) from the University of Allahabad (UGC) and UGC-SAP (RFSMS) to the Department of Zoology is gratefully acknowledged.

REFERENCES

[1] R.B.Raizada, M.K.Srivastava, R.A.Kaushal, R.P.Singh; Food.Chem.Toxicol., **39**, 477-483 **(2001)**.
[2] M.Abdollahi, A.Ranjbar, S.Shadnia, S.Nikfar, A.Rezaie; Med.Sci.Monit., **10**, 141-147 **(2004)**.
[3] D.Singh, D.C.Jain; Ind.J.Exp.Biol., **25**, 560-570 **(1987)**.
[4] C.R.Carlini, M.F.Grossi-de Sa; Toxicon, **40**, 1515-1539 **(2002)**.
[5] D.E.Champagne, M.B.Ismam, K.R.Downum, G.H.N.Towers; Chemology, **4**, 165-173 **(1993)**.
[6] T.G.Wilson; Integr.Comp.Biol., **45**, 127-136 **(2005)**.
[7] A.Kumar, B.Sharma, R.S.Pandey; J. Environ.Sci.Health., Part B., **44**, 564-570 **(2009)**.
[8] D.C.Chavasse, R.P.Shler, O.A.Murphy, S.R.A.Hutlly, S.N.Lousens, T.Akhtar; Lancet, **353**, 22-25 **(1999)**.
[9] T.Shono, J.G.Scott; Pest.Biochem.Physiol., **75**, 1-7 **(2003a)**.
[10] T.Shono, J.G.Scott; J.Econ.Entomol., **96**, 1300-1306 **(2003b)**.
[11] T.Y.Su, M.Zaim, M.S.Mulla; J.Amer.Mosquito Control Asso., **19**, 408-418 **(2003)**.
[12] F.R.Irvine; Woody plants of Ghana Oxford University Press, London 48-50 **(1961)**.
[13] M.Larhsini, M.Bousad, H.B.Lazrek, M.Jana, H.Amarouch; Fitoterapia, **68**, 371-373 **(1997)**.
[14] D.Wititsuwannakul, C.Sakulborirug, R.Wititsuwannakul; Phytochem., **47**, 183-187 **(1998)**.
[15] L.E.Castillo-Sánchez, J.J.Jiménez-Osornio, M.A.Delgado-Herrera; Trop.Subtrop.Agroecosys., **12**, 445-462 **(2010)**.
[16] J.K.Rupprecht, Y.H.Hui, J.L.McLaughlin; Review.J.Nat.Prod., **53**, 237-276 **(1990)**.
[17] M.Kristensen, J.B.Jespersen; J.Econ.Entomol., **96**, 1300-1306 **(2003)**.
[18] A.K.Mishra, A.Mishra, H.K.Kehri, B.Sharma, A.K.Pandey; Antimicrobiol., **8**, 9-12 **(2009)**.
[19] D.D.Bowman, J.R.Georgi; In: Georgis' Parasitology for Veterinarians – 9th Edition., Sauders Elsevier, St. Louis, Missouri, USA, Chapter **2**, 15-16 **(2008)**.
[20] G.Karber; Arch. für Experimentelle Pathologie und Pharmakologie **162**, 480–483 **(1931)**.
[21] O.H.Lowry, N.J.Rosenbrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193**, 265-275 **(1951)**.
[22] W.C.Schneider; Determination of nucleic acids in tissues by pentose analysis, In: S.P.Colowick and N.O.Kaplan, (eds) Methods in enzymology, 3, Academic Press, New York, 680-684 **(1957)**.
[23] N.Begum, B.Sharma, R.S.Pandey; Evaluation of Insecticidal efficacy of Calotropis procera and Annona squamosa ethanol extracts against Musca domestica.J.Biofertil.Biopestici., 1:1,.doi.org/ 10.4172/2155-6202, 1000101 **(2010)**.
[24] A.Alkofahi, J.K.Rupprecht, J.E.Anderson, J.L.McLaughlin, K.L.Mikolajczak, B.Scott; A Search for new pesticides from higher plants, Insecticides of plant origin, ACS symposium series 387, Washington, D.C: 24-25 **(1987)**.
[25] K.L.Mikolajczak, Mc.J.L.Laughlin, J.K.Rupprecht; Control of pests with annonaceous acetogenins. U.S. Patent No.4721727 **(1988)**.
[26] L.M.Schoonhoven; Entomol.Exp.Appl., **31**, 57-69 **(1982)**.
[27] H.Schmutterer; Ann.Rev.Entomol., **35**, 271-297 **(1990)**.

Full Paper

[28] H.F.Moeshler, W.Pfuger, D.Wendlisch; Pure annonin and a process for the preparation thereof, U.S. Patent No.4689323 **(1987)**.

[29] V.Mariapan, R.C.Saxena; J.Econ.Entomol., **76**, 573-576 **(1983)**.

[30] V.Mariapan, S.Jayaraj, R.C.Saxena; J.Econ.Entomol., **81**, 1369-1373 **(1988)**.

[31] P.Khirstova, M.Tissot; Biores.Tech., **53**, 670-672 **(1995)**.

[32] Z.Iqbal, M.Lateef, A.Jabbar, G.Muhamma, M.N.Khan; J.Ethnopharmacol., **102**, 256-261 **(2005)**.

[33] F.Kew; The useful plants of West Tropical Africa, Families A-D (2nd edition) (ed Burkill,H M). Royal Botanical Gardens, **1**, 219-222 **(1985)**.

[34] R.K.Singh, P.K.Mittal, R.C.Dhiman; J.Commun.Dis., **37**, 109-113 **(2005)**.

[35] M.W.Khan, S.N.H.Naqvi, I.Ahmad, R.Tabassum, F.A.Mohammad; Pak.J.Pharmacol.Sci., **4**, 77-81 **(1991)**.

[36] V.Jagannath, V.S.K.Nair; Physiol.Entomol., **17**, 56-61 **(1992)**.

[37] S.L.Smith, M.J.Mitchell; Biochem.Biophys.Res. Commun., **154**, 559-563 **(1988)**.

[38] C.P.W.Zebitz; Aus.J.Entomol., **39**, 208-211 **(1984)**.

[39] Z.Al-Sharook, K.Balan, Y.Jiang, H.Rembold; J.App.Entomol., **111**, 425-530 **(1991)**.

[40] G.Sripongpun; Songklanakarin J.Sci.Technol., **30**, 667-672 **(2008)**.

[41] A.R.Shakoori, M.Fayyaz, M.A.Saleem; J.Stored Prod.Res., **24**, 215-220 **(1996)**.

[42] S.N.H.Naqvi, R.Tabassum; Pak.J.Entomol., **7**, 9-16 **(1992)**.