Insecticidal Activities and Chemical Investigation of Common Cocklebur and Bloodleaf

A Thesis

By

Abul Hayat Md. Shahjalal
Student No. : 0705008
Session : 2007-2008
Semester : January-June

Master of Science (M.S.)
In
Agricultural Chemistry

Department of Agricultural Chemistry
Hajee Mohammed Danesh Science & Technology University, Dinajpur

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Dedicated To

My Beloved Mother,
Supervisor and Co-Supervisor

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The Author

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ABSTRACT

Aqueos extracts of common cocklebur, (*Xanthium strumarium*) leaf and fruit and leaves of bloodleaf plant (*Iresine lindenii*), plant were used to test the repellency, direct toxicity, adult emergence, seed damage, fecundity and oviposition inhibition by pulse beetle, (*Callosobruchus chinensis*) with emphasis on chemical investigation of extract. Mortality percentage at 2 days after treatment indicated that 4% (w/v) solution of common cocklebur leaf showed the highest mortality rate (36%) and 1% (w/v) solution of bloodleaf plant showed the lowest mortality rate (12%). Common cocklebur leaf extract 4% (w/v) showed the lowest fecundity (65), adult emergence rate (48%), seed damage (26%), and 1% (w/v) bloodleaf plant extract showed the highest fecundity (202), the highest adult emergence rate (88%) and the highest seed damage rate (65.3%). Bloodleaf plant extract at 1% (w/v) was found to be the lowest oviposition inhibition (3.6%), whereas 4% common cocklebur leaf extract showed the highest oviposition inhibition (47.5%). Common cocklebur leaf extract 4% (w/v) showed the highest repellency (60%) at 5 hours after treatment and bloodleaf plant extract showed the lowest repellency (20 %) at 2 hours after treatment. The thin layer chromatography (TLC) examination of ethanol extract of common cocklebur leaves showed two distinct compounds in Hexane:Ethylacetate (7:1 ,v/v). After purification of the crude compound and spectral studies (^{1}H- NMR and IR) it was indicated that S_1 is an aromatic ester like n-hexyle salicylate or o-hydroxy-n-hexyl-benzoate and S_2 may consider as long chain ketone.

CHAPTER I

INTRODUCTION

Botanicals are promising sources of pest control compounds. The pool of plants possessing insecticidal substances is enormous. These are generated extra- ordinary interest in recent years as potential sources of natural insect control agents. In the middle of the 17th century, pyrethrum, nicotine, and rotenone were recognized as effective insect-control agents (Silva-Aguayo, 2004). Roy *et al.*, (2005) revealed that the leaf extracts of shiyalmutra, (*Blumea lacera*) as a botanical insecticides against lesser grain borer and rice weevil. Azadirachtin, a limonoids from seeds of the neem tree (*Azadirachta indica*, Meliaceae) possesses strong antifeedant and growth inhibitory effects against various insect pests (Isman, 1997).

More than 200 major species of insects and mites infest important crops and stored products in storage (Das, 1998). Infestation of pulse beetle causes both qualitative and quantitative losses in legume seeds. Gujar and Yadav (1978) recorded 55-60% losses in seed weight and 45.5-66.3% losses in protein content. According to Choudhury (1961) the extent of damage by *Callosobruchus chinensis* might be upto 100% in mungbean seed during a period of one year in storage. Rahman (1971) reported 12.0% loss due to pulse beetle infestation in pulses stored in warehouses which leads to reduction of the commercial value and germination percentage. *C. chinensis* caused great losses of blackgram as 56.26%, mungbean 46.70%, chick pea 44.08% and pea 5.00-9.00% in storage (Rustammani *et al.*, 1985). The damage in store is more important than field (Hill, 1987; Yamamoto, 1990).

There are several methods for controlling pulse beetle in the field and in the storage. These are mechanical, cultural, physical, biological, chemical, use

of botanical insecticides etc. But in storage, this pest is controlled by synthetic insecticides, which have got many limitations and undesirable side effects. Chemical pesticides have been used for a long time with serious drawbacks. Indiscriminate use of insecticides to protect pulse beetle in storage may cause serious health hazard and their residual effects remain in the stored grain and also in the environment. In addition, the development of resistance in pest population and subsequent resurgence as well as destruction of beneficial insects ultimately create serious imbalance in environment (Kavadia *et al.*, 1984; Desmarchelier, 1985; Fishwich, 1988; Singh, 1989).

In this condition, alternative methods of insect control utilizing botanical products are being used in many countries. Botanical insecticides are biodegradable, relatively specific in the mode of action and easy to use (Das, 1986). Plant products are environmentally safe, less hazardous, less expensive and readily available (Ahmed et al.,1993).

Thymol, a monoterpenoid phenol, is a major constituent of garden thyme, *Thymus vulgaris*, Lamiaceae and *Origanum vulgare*, Lamiaceae and trans-anethole, a phenylpropanoid from the anise plant, *Pimpinella anisum*, are toxic to *Spodoptera litura* (Hummelbrunner and Isman, 2001). The toxicity of trans- anethole has also been demonostrated against a number of species, including various beetles, weevils, mosquitoes, and moths (Sarac and Tunc, 1995a.b; Ho *et al.*, 1997; Kelm *et al.*, 1997).

The undesirable side effects and high price of synthetic insecticides on stored grain encouraged to conduct the present study with the evaluation of plant products as a seed protectant against pulse beetle, *Callosobruchus chinensis*. The objectives of the present study are given below:

- ❖ To investigate the insecticidal activities (repellency, residual toxicity, fecundity, adult emergence and assessment of seed damage) of common cocklebur and bloodleaf on pulse beetle.
- ❖ To isolate the insecticidal active compounds from effective aqueous extracts.
- ❖ To determine the structure of the isolated compounds.

CHAPTER II

REVIEW OF LITERATURE

Stored grains suffer seriously from attack of a number of insect pests. The management of insect pests by the botanicals is an age-old practice and till today it plays a vital role. Now a days, the botanical products have been recognized as potential pest control measures all over the world. In development countries, numerous research have been conducted due mainly to hazards free, ecosafe and effective insecticides both in the field and in the storage. Several species of insect pests have been controlled by the application of botanical products such as powder, extract and oil as potential source of antifeedant, repellent and growth inhibitor. Some review of literatue are cited here to reveal some information about the research works done in different countries all over the world including Bangladesh on the use of botanical insecticides for the management of the stored product insect pests under the following sub-headings.

2.1. Effect on Direct toxiciry and Repellency

Chaubey *et al.*, (2009) reported essential oils were extracted from dried fruits of *Myristica fragrans* and *Illicium verum* by hydrodistillation method and its toxic and developmental inhibitory activities were determined against wheat flour beetle *Tribolium castaneum*. These essential oils caused toxicity against larvae and adults of *T. castaneum* when fumigated. Median lethal concentrations (LC_{50}) against the larvae were 12.67 µl and 18.43 µl and against adults were 14.23 µl and 19.87 µl for *Myristica fragrans* and *Illicium verum* oils respectively.These two oils reduced oviposition potential of the *T. castaneum*. Transformation of larvae into pupae and pupae into adults was inhibited by the essential oil vapours. Median effective

concentrations (EC_{50}) that reduced the transformation of larval population to pupa to half were 6.08 µl and 11.97 µl for *M. fragrans* and *I. verum* oils respectively. The developmental period of the insect was increased significantly when treated by these essential oils. All the responses were found dose-dependent.

Shimizu and Hori (2009) compared the repellency and toxicity against adzuki bean beetles among six troponoid compounds, and examined the relationship between their structure and activity. γ-Thujaplicin showed the highest repellency against the beetles among the compounds tested, while the repellency of tropiliden was quite low. The results suggested that the keto and hydroxyl groups are important in the repellent properties of troponoid compounds. Although an isopropyl group was also important in repellency, the effect varied according to its position on the seven-member ring; the farther the isopropyl group was from the keto and hydroxyl groups, the higher the repellency became. As with its repellency effect, the toxicity of tropiliden was quite low. Tropone showed the highest toxicity among the compounds tested while its repellency was relatively low. Similar to its repellency, γ-thujaplicin showed the highest toxicity among the thujaplicins. However, the toxicities of α-thujaplicin and β-thujaplicin (hinokitiol) were similar, unlike the repellency. In summary, it appears that the toxicity of troponoid compounds does not always coincide with their repellency. Furthermore, the repellency and toxicity of a mixture of γ-thujaplicin and β-thujaplicin, whose production cost is cheaper than that of hinokitiol alone, were investigated. The mixture strongly repelled the adzuki bean beetles. We conclude that the mixture of γ-thujaplicin and β-thujaplicin is a promising repellent.

Arabi *et al.*, (2008) observed *Perovskia abrotanoides*, Karel is a wild growing plant in Iran and has been used in traditional Iranian herbal medicine. The present study was conducted to investigate chemical composition and fumigant toxicity of the essential oil from *P. abrotanoides* against *Sitophilus oryzae* and *Tribolium castaneum*. Dry flowering aerial parts of the plant were subjected to hydrodistillation using a modified clevenger-type apparatus. The composition of the essential oil was analysed by gas chromatography (GC) and GC mass spectrophotometry. Twenty-four compounds representing 98.8% of total oil were identified. The predominant components in the oil were camphor (28.38%) and 1, 8-cineole (23.18%). Fumigant toxicity was tested against 1- to 7-day-old adults of *S. oryzae* and *T. castaneum* with five replications at 25 ± 1 °C and 65 ± 5% relative humidity in dark conditions. The mortality was increased with concentrations of 32, 161, 322, 483 and 645 µl/l air and with exposure time from 2 to 15 h. The lowest concentration (32 µl/l air) of the oil induced 100% mortality of *S. oryzae* and *T. castaneum* after 15 and 8 h exposure, respectively. The oil at 322 µl/l air caused 100% mortality for *S. oryzae* and *T. castaneum* within 13 and 7 h exposure, respectively. At 645 µl/l air, the LT_{50} values (lethal time for 50% mortality) were 8 and 2.84 h for *S. oryzae* and *T. castaneum*, respectively. In the probit analysis, LC_{50} values (lethal concentration for 50% mortality) showed that *T. castaneum* (LC_{50} = 11.39 µl/l) was more susceptible than *S. oryzae* (LC_{50} = 18.75 µl/l). The essential oil of *P. abrotanoides* can play an important role in stored grain protection and reduce the need for the same, and also the risks associated with the use of synthetic insecticides.

Shah *et al.*, (2008) stated leaves of six indigenous plants viz., *Typhonium trilobatum*, *Cleome viscosa*, *Cassia occidentalis*, *Pongamia pinnata*, *Mesua ferrea*, and *Trewia nudiflora* were extracted using acetone, ethanol and

water solvents were used for botanical pesticides. These extracts were evaluated for their repellent effect against *Oryzaephilus surinamensis* at 2.5, 5.0, 7.5, and 10.0% concentrations. Extracts of water solvent showed higher repellent effect than that of others except ethanol extract of *M. ferrea*. Considering mean repellency rate, extracts of three solvents of all six plants were in the same repellency class i.e. class II except water extract of *P. pinnata* (class III). It was found that the rate of repellency increased with the increase of dose level. At 10.0% dose level all plant extracts showed the highest repellency rate and were in repellency class III. The repellency rate of acetone, ethanol and water solvents extract of six plants showed insignificant at different hours after treatment. But numerically the repellency rate of all the extracts was higher at one hour after treatment than two or three hours after treatment except few.

Rozman *et al.*, (2007) stated the compounds 1, 8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate and linalyl acetate occur naturally in the essential oils of the aromatic plants *Lavandula angustifolia*, *Rosmarinus officinalis*, *Thymus vulgaris* and *Laurus nobilis*. These compounds were evaluated for fumigant activity against adults of *Sitophilus oryzae*, *Rhyzopertha dominica and Tribolium castaneum*. The insecticidal activities varied with insect species, compound and the exposure time. The most sensitive species was *S. oryzae*, followed by *R. dominica*, *T. castaneum* was highly tolerant of the tested compounds. 1, 8-cineole, borneol and thymol were highly effective against *S. oryzae* when applied for 24 h at the lowest dose (0.1 µl/720 ml volume). For *R. dominica* camphor and linalool were highly effective and produced 100% mortality in the same conditions. Against *T. castaneum* no oil compounds achieved more than 20% mortality after exposure for 24 h, even with the highest dose (100 µl/720 ml volume). However, after 7 days exposure 1, 8-cineole

produced 92.5% mortality, followed by camphor (77.5%) and linalool (70.0%). These compounds may be suitable as fumigants because of their high volatility, effectiveness and their safety.

Rahman *et al.*, (2006) found ethanolic extract of Melgota is used for repellency, insecticidal activity against rice weevil, *Sitophilus oryzae* with emphasis on chemical investgation. The observed mortality percentage increased with increase in time intervals after treatment. Mortality percentage at 0.25, 050, 0.75, 1.00, and 1.50 hours after treatment (HAT) indicated that 4% solution showed the highest mortality (34.0%) in *S. oryzae* at 1.50 HAT compared to *Pediculus humanus*. Mortality percentage showed parallel response to the level of concentration at different time intervals after treatment. 1% fruit extract of Melgota, *M. postulata* showed the lowest repellency 9.84% in case rice weevil. On the other side, 2% showed 12.76% and 4% showed 22.43% respectively. TLC of crude ethanol extract of Melgota, *M. postulata* showed six distinct compounds at uv-visible light.

Rotimi *et al.*, (2006) investigated the efficacy of crude stem extracts of forest anchomanes, *Anchomanes difformis* a plant occurring in West African forests, against the pulse beetle *Callosobruchus maculatus*. Crude stem extracts at 3% concentration showed high contact toxicity to adult beetles within 24 h after application, while it was moderately toxic to the beetles at the lowest (1%) concentration. At the highest application rate, the plant extract provided good protection to grains stored for 90 days. Grain viability and water absorption capacity were not affected by treatments with ethanol extracts of *A. difformis*. The significance of these findings is discussed in relation to biopesticide-means of controlling cowpea bruchids.

Regnault-Roger *et al.*, (2005) reported the bioactivity of 22 essential oils from aromatic and medicinal plants was tested upon *Acanthoscelides obtectus*, Coleoptera; Bruchidae, a pest of kidney bean, *Phaseolus vulgaris*. The insecticidal effect was evaluated by determination of 24- and 48-hr LC_{50} and LC_{50} (from 1.50 mg/ dm^3 to more than 1000 mg/dm^3). Isoprenoids and phenylpropanoids were identified by gas chromatography. The most efficient essential oils were extracted from plants belonging to Labiatae; *Origanum marjorana* and *Thymus serpyllum* essential oils were the most toxic.

Roy *et al.*, (2005) reported that leaves *Blumea lacera*, shiyalmutra or Kukurshunga for studying their repellency and toxicity against the lesser grain borer, (*Rhyzopertha dominica*) and rice weevil, (*Sitophilus oryzae*), with the attempt for chemical investigation of each extract. In the first experiment petroleum ether extract of dried leaves (1, 2 and 3% by volume) were used on the adult beetle of lesser grain borer and rice weevil to evaluate their repellency for mortality/direct toxicity effects. Results for the two experiments indicated that 1, 2 and 3% petroleum ether extract of leave *of Blumea lacera* species had repellency as well as direct toxicity, while 3% showed strong repellency (55.71%, 55.34%) and toxicity (57.41%, 56.71%) effects among the other extracts on both lesser grain and rice weevil.

Dwivedi and Shekhawat (2004) observed six aboriginal plant species were screened to observe possible repellent action against khapra beetle. Repellent property has been confirmed in all the plant species using olfactometer. Acetone extract of *Emblica officinalis* exhibited maximum repellency (88.66%) whereas minimum repellency was recorded (66.22%) in *Ziziphus jujube* ether extract.

Konar *et al.*, (2005) stated that in stored seeds of red gram, *Cajanas cajan* application of malathion 50EC (@ 1 ml/litre of water) as surface treatment was found most effective in achieving 100% mortality of pulse beetle, *Callosobruchus chinensis* followed by *Ipomoea* leaf powder (@ 100 g/litre of methanol) and azadirachtin 5000 ppm (@ 6 ml/litre of water), respectively at 1, 3 and 6 hours after treatment. *Ipomoea* also reduced egg laying and adult emergence of pulse beetle.

Ogendo *et al.*, (2003) observed that the insecticidal and repellent properties of *Lantana camara* and *Tephrosia vogelii* were evaluated against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in stored maize grain. Five treatment rates (1.0, 2.5, 5.0, 7.5 and 10.0% w/w) of each powdered plant material, an untreated control and a synthetic insecticide (Actellic Super™ 2% dust) were used to investigate treatment efficacy on mortality of the adult insect (five to eight days old), F_1 progeny emergence and repellency against *S. zeamais* adults. After 21 days, *L. camara* and *T. vogelii* caused 82.7-90.0% and 85.0-93.7% insect mortality, respectively. The mean lethal exposure times (LT_{50}) to achieve 50% mortality varied from five to six days (7.5-10.0% w/w) to seven to eight days (2.5-5.0% w/w) for both plants. Probit regression analysis showed a significant relationship between plant powder concentration and insect mortality. The plant powders and synthetic insecticide reduced adult F_1 insects by more than 75% compared to the untreated control. *Tephrosia vogelii* was most repellent to *S. zeamais* at 7.5-10.0% (w/ w), repelling 87.5% of the insects, followed by *T. vogelii* at 2.5% w/w and *L. camara* at 10% w/w which repelled 65.0 and 62.5% of insects respectively. The implications of these results are discussed in the context of smallscale farmer usage of these plants for stored product protection.

Rahman *et al.*, (2001) conducted a bioassay to evaluate the seed oils of castor *(Ricinus communis)*, neem *(Azadirachta indica)*, pithraj *(Aphanamixis polystachya)*, safflower and sesame against *Alphitobius diaperinus* (Panjab), adult insects were fed on wheat grains with the oils at concentrations of 1, 2, 4, and 5%. Insect mortality was recorded at 24, 48, 72, and 96 hours after treatment (HAT). All seed oils exhibited significant repellent property against *Alphitobius diaperinus*, with repellant increasing with rate and exposure time. The highest mean repellency was recorded in 5% pithraj oil (86.69%).

Shahjahan and Amin (2000) reported that water extracts and powdered material of akanda, *Asclepias calotropis*, biskatali, *Polygonum hydropiper* and neem, *Azadirachta indica* were evaluated for their repellency, feeding deterrency and direct toxicity effects on rice weevil and their potentiality as grain protectant. Result indicated that 2, 3 and 4% water extracts of all three plants had repellent and insect mortality activities; while extrcts at 3% showed strong feeding deterrent effects. The ground leaves 2, 3 and 4% by weight provided good protection of grains by reducing emergence and grain infestation rates. The maximum dose of the plant extracts gave maximum effects. Neem and biskatali were more effective than akanda.

2. 2. Effect on Fecundity and Adult Emergence

Boateng and Kusi (2008) observed the susceptibility of *C. maculatus* and *D. basalis* to *Jatropha* seed oil was evaluated under laboratory conditions. The adults of *C. maculatus* and *D. basalis* had the same susceptibility to *Jatropha* seed oil but the parasitoid was relatively more susceptible than its host at all treatment levels. The oil was also repellent to *C. maculatus* but its persistency declined from 15 to 60 days in storage. The eggs of *C. maculatus* were comparatively more susceptible to the *Jatropha* seed oil

than those of the parasitoid due to the protection afforded by the grain. However, the larvae and pupae of *C. maculatus* showed a relatively lower susceptibility to the oil. It is possible to incorporate the oil in a well designed pest management programme taking advantage of the short persistency of the oil on grains and its relatively ineffectiveness against the *C. maculatus* pupae developing inside the grain.

Chaubey (2008) observed the essential oil from seven common spices, *Anethum graveolens*, *Cuminum cyminum*, *Illicium verum*, *Myristica fragrans*, *Nigella sativa*, *Piper nigrum* and *Trachyspermum ammi* was isolated and its insecticidal, oviposition, egg hatching and developmental inhibitory activities were determined against pulse beetle, *Callosobruchus chinensis*. Essential oils were isolated by hydrodistillation method using clevenger apparatus. These essential oils caused death of adults and larvae of *Callosobruchus chinensis* when fumigated. The 24-h LC_{50} values against the adults of the insect were 8.9 µl, 10.8 µl, 11.0 µl, 12.5 µl, 13.6 µl, 14.8 µl and 15.6 µl for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils respectively. On the other hand, against larval stage these values were 6.4 µl, 7.9 µl, 8.9 µl, 11.1 µl, 11.7 µl, 12.2 µl and 13.5 µl for *N. sativa*, *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* respectively. These essential oils reduced the oviposition potential, egg hatching rate, pupal formation and emergence of adults of F_1 progeny of the insect when fumigated with sublethal concentrations. These essential oils also caused chronic toxicity as the fumigated insects caused less damage to the stored grains. The essential oil of *N. sativa* was found most effective against all the different stages of the *Callosobruchus chinensis* followed by *A. graveolens*, *C. cyminum*, *I. verum*, *P. nigrum*, *M. fragrans* and *T. ammi* oils. All the responses were found concentration-dependent. The toxic and developmental inhibitory effects

may be due to suffocation and inhibition of various biosynthetic processes of the insects at different developmental stages.

Sathyaseelan *et al.*, (2008) studied effect of indigenous pesticidal plants viz., *Prosophis sp.*, *Nerium sp.*, *Ocimum sp.*, *Acalypha sp.*, *Catheranthus sp.*, and *Vitex sp.*were tested against pulse beetle *Callosobruchus chinensis* in green gram. Leaf extracts of all plants caused significant ovipositional deterrent effect against pulse beetle. Five percent leaf extract *Vitex sp.* was the most effective in inhibiting the ovipositiion (26.6 eggs/ female) as that of 79.4 eggs/ female in untreated control. At 5% level, leaf extract of *Vitex sp.* caused maximum reduction in egg viability (61.7%) followed by *Catheranthus sp.* leaf extract (56.7%). The egg viability gradually decreased maximum reduction in adult emergence (85.0%) followed by *Catheranthus sp.* (83.7%), *Acalypha sp.* (73.3%), *Nerium sp.* (70.0%), *Ocimum sp.* (68.7%) and minimum reduction was recorded in case of *Prosophis sp.* (68%). No adverse effect was observed on the germination of green gram up to 90 days after treatment.

Raja and William (2008) observed the essential oils of plants namely Citronella, *Cymbopogan winterianus*, Citrodora, *Eculyptus citrodora*, Lemon grass, *Cymbopogan flexuosus*, Vetiver, *Vetiveria zizanioides*, and Palmorosa, *Cymbopogan martini* were tested for their insecticidal and ovicidal activities against adults and eggs of *Callosobruchus maculatus* at 5% concentration at 96 hours of exposure. The results revealed that the highest mortality and ovicidal activity was recorded in Citrodora oil (96%, 88.43%) followed by lemon grass oil (92%, 45.25%) at 96 h of exposure.

Moreira *et al.*, (2007) assessed the insecticide activity of hexane and ethanol extracts from basil benth, *O. selloi*, rue, *R. graveolens*, lion's ear, *L. nepetifolia*, jimson weed, *D. stramonium*, baleeira herb, *C. verbenacea*,

mint, *M. piperita*, wild balsam apple, *M. charantia*, and billygoat weed or mentrasto, *A. conyzoides* on *R. dominica* was evaluated against *R. dominica*, *S. zeamais* and *O. surinamensis*. Among them,only hexane extract of *A. conyzoides* showed insecticide activity and 5,6,7,8,3',4',5' heptamethoxy flavone; 5,6,7,8,3'-pentamethoxy-4',5'-methilenedioxyflavone and coumarin were identified from *A. conyzoides* crude. However, only coumarin showed insecticide activity against three insect pests (LD_{50} from 2.72 to 39.71 mg g^{-1} a.i.).

Srivastava and Gupta (2007) found the pulse beetle *Callosobruchus chinensis*, Coleoptera: Bruchidae is one of the major pests infesting stored pulses and is distributed worldwide. Plants and plant products possessing insecticidal properties have been used as an alternative to control the infestation caused by this pest. The present study was undertaken to study the effect of different formulations viz., aqueous suspension, aqueous extract and ether extracts of 10, 5, 2.5 and 1% concentrations of various parts (root, stem, leaf, fruit) of plant *Solanum surratense*, Solanaceae on egg laying by the pulse beetle *C. chinensis*. A significant reduction in the oviposition (eggs laid per pair) of insects was observed in various experimental sets. It went down to 2 - 5 eggs /pair in sets treated with 10% aqueous extract and aqueous suspension of fruits. It can therefore be suggested that the plant under study is potent enough against *C. chinensis* and can be at least partially.

Sarkar (2006) observed the pulse beetle (*C. chinensis*) laid the lowest number of egg (19.33) on the grain treated with 10% concentration of turmeric followed by 7.5% turmeric (37.33), 10% black pepper (45.00), 5% turmeric (60.33), 7.5% 10% black pepper (70.00) and 10% eucalyptus (72.00). The lowest number of adult (13.67) was found to emerge in the

grains treated with 10% turmeric and the highest number (112.67) was emerged from the grain treated with 2.5% garlic.

Upadhyay *et al.*, (2006) reported extracts of *Capparis decidua* stems and flowers showed insecticidal and oviposition inhibitory activities against *Bruchus chinensis*. The LC_{50} values of these extracts were found to increase with the increase in the polarity of the extract at different exposure periods. For instance, after 96 h, the LC_{50} values were found to be 3.619, 7.319, and 10.151 microg for CD_1, CD_2, and CD_3, respectively. Extract CD_7 was effective only at higher doses. The toxicity was found to be dose-and time-dependent. The females laid lesser number of eggs, when exposed to sublethal doses of different extracts and pure compounds, as compared to control. The maximum oviposition deterrence index was found for extract CD_1 followed in decreasing order by CD_2, CD_3, and CD_7. From extract CD_1, two compounds were isolated and characterized as triacontanol (C_1) and 2-carboxy-1, 1-dimethylpyrrolidine (C_2). When the females were exposed to sublethal doses of these compounds, they laid lesser number of eggs as compared to the control. C_2 was found to have a slightly greater oviposition inhibition effect than C_1. From fraction CD_7, one novel compound labeled as CDF_1 has been isolated and identified as 6-(1-hydroxy-non-3-enyl) tetrahydropyran-2-one. CDF_1 has also shown insecticidal and oviposition inhibitory activities against *B. chinensis* at low concentrations.

Hussein *et al.*, (2005) found saponin extract from alfalfa roots, azadirachtin from the neem seed oil, synthetic ecdysteroid agonist RH-2485, and the juvenoid hydroprene disturb the development and reproduction of *Tropinota squalida*. Feeding beetles on diets containing 750 p.p.m. saponins, 7.5 p.p.m. RH-2485, and 1.13 p.p.m. azadirachtin reduces their progeny from 51 second instar larvae per female to 24, 15, and 15 larvae, respectively. When the larvae of untreated adults are fed for 1 week on dung

with 75 p.p.m. saponins, 50 p.p.m. RH-2485, and 0.45 p.p.m. azadirachtin, the rate of adult emergence drops from 80% (controls) to 20, 0 and 13%, respectively. No adults emerge when the treatment is continued through the second and third larval instars. Two topical treatments of larvae with 0.2 μg hydroprene decrease the rate of adult emergence from 90 to 11%, and treatments with 2 μg prevent adult development in all insects. The observed effects warrant testing of azadirachtin, RH-2485, and hydroprene in the field. Several types of their application for the control of *T. squalida* are suggested.

Salunke *et al.*, (2005) reported the effects of partially purified flavonoids obtained from *Calotropis procera* (Ait.) R. Br. and six standard flavonoids on the adults and eggs of *Callosobruchus chinensis* (L.), reared on mung beans (*Vigna radiata* L.), were studied. All flavonoids were toxic to adults and eggs depending on dose and exposure period. Flavonoids obtained from *C. procera* showed the highest contact toxicity followed by standard quercetin, rutin and quercitrin at 10 mg/ml doses in filter paper diffusion assay. Significant reduction in oviposition was found for all flavonoids at the doses of 5 and 10 mg/ml on grains in plastic jars. Flavonoids also showed an ovicidal effect on bruchid eggs as well as affecting the number and weight of the emerging adults as a function of concentration.

Yadav (2004) investigatd the effect of vegetable oils on the orientation and oviposition of pulse beetle (*Callosobruchus maculatus*) on green gram during storage, seasame, coconut, karanja, groundnut and soybean or non-edible oils (mahua, castor, karanja and neem) were mixed with seeds at 10ml/ kg seeds. Seeds were exposed to insect at 1, 10, 30, and 75 days after seed treatment. Vegetable oil reduce beetle incidence on seeds to 5.91-7.50 beetles, compared to control (16 beetles). Among vegetable oils, mahua oil has the most effective. Oviposition was recorded by vegetable oil treatment.

Neem oil reduced the number of oviposited eggs to 3.58eggs, compared to untreated control (91.25 eggs).

Nandi *et al.*, (2004) observed the bioefficacy of nimbicidine against pulse beetle, *Callosobruchus maculatus* in the laboratory. Adult beetles were exposed to gram seeds treatd with 1.00, 0.50, 0.25, 0.12 and 0.06% concentration of nimbicidine. They found that nimbicidine strongly caused a significant reduce in oviposition, adult progeny development and severity of seed damage. Seeds treated with 1.00 % concentration of nimbicidine were less preferred and performed lawest pest fecundity (16.33).

Ba- Angood and Al-Sunadi (2003) conducted experiments to compare the effect of neem oil, *Azadirachta indica* powder of seeds of yellow oleander (*Thevetia neriifolia*), castor (*Ricinus* communis) and *Lantana camara* on the oviposition of *C. chinensis* and hatchibility of its eggs on stored cowpea (*Vigna chinensis*) seeds. However, the least effective treatment was found in *L. camara* seeds, where the average number of eggs laid was approximately 47 eggs, compared with 58 eggs on the untreated control. In terms of egg hatchability, yellow oleander treatment gave the best result, recording no hatched eggs. On the other hand, treatment with *R. communis* seed powder was the least effective, recording a mean percentage of 90.08 % eggs hatched compared to 93.0% in the untreatd control.

Kemabonta *et al.*, (2002) ovserved *Chenopodium ambroisoides*, Chenopodiaceae was investigated for its insecticidal and ovipositional activity against *C. maculatus*, Coleoptera. Ethanol extract of the plant was applied to one day-old eggs and topically, on adult *C. maculatus*. Adults that emerged from treated eggs and treated F_1 adults decreased significantly in number when compared with the control. Application of *C. ambroisoides* (5.0% extract) caused 54% mortality of *C. maculatus* adults after 5 days,

reduced oviposition by 72.5% as compared to the control and thereafter, reduced emergence of F_1 adults to 55% as compared to 81% in the control.

Anil *et al.*, (2000) reported neem, sesame, groundnut, soybean and mustard oils at 10 ml per kg seed acted effectively as ovicidal agent against *Callosobruchus maculatus* on cow pea seeds. The number of laid eggs (8.9) was lowest in neem oil treated seeds. There was a reduction in efficacy with the delay in treatment time.

2.3. Effect on Grain Infestation and F_1 Adult Inhibition

Sahayaraj *et al.*, (2008) stated the impact of ethanol extract of *Pedalium murex* (Linn.) (Family: Pedaliaceae) root (0.1, 0.2, 0.4 and 0.8%) were screened for its antifeedant and insecticidal activities against third, fourth and fifth instar larvae of *Spodoptera litura* (Fab.) by leaf-dip method. The larval mortality more than 50 percent at higher concentration (0.8%) was observed in the ethanol root extract. Stage dependant LC_{50} value was observed for *S. litura* (0.100, 0.118 and 0.258% for third, fourth and fifth nymphal instars). *P. murex* reduced the food consumption index, growth rate, approximate digestability, eficiency of conversion of ingested food, efficiency of conversion of digested food of *S. litura* indicating the antifeedant activity of this plant. Qualitative analysis of *P. murex* root extract revealed that it contains phytochemical such as, steroids, terpenoids, phenolics, saponines, tannins and flavanoids. Phenol, 2-(5,6-dimethyl pyrazinyl) methyl (molecular weight 214); O-Terphenyl-13C (molecular weight 230) and 3, 3A, 4, 9B-Tetrahydro- 2H-Furo (3, 2-C) (1) Benzopyran (molecular weight 206) were identified from the ethanol root extract of *P. murex* by using GC-MS. *P. murex* impact was more than the neembased biopesticide neemgold. Hence this plant can be explored as biopesticidal plant in the near future.

Ngamo Tinkeu *et al.*, (2007) ovserved essential oils of aromatic plants are more considered as good control alternative tools. The amount of active volatile of essential oils present in granaries is almost as infra lethal doses. The present work aimed to analyse the chronic toxicity of low doses of essential oils of *Annona senegalensis*, *Hyptis spicigera* and *Lippia rugosa*. These plants are toxic to the pest at high doses. At the dose 2.5 x 10-2 ml/ml, they all reduced the oviposition of *S. zeamais*. Moreover, *L. rugosa* and *H. spicigera* were the most active of the biological potential of *S. zeamais* reducing significantly its amount of grains attacked (F = 8.63**) and that of the rejected flour (F = 41.04***). This chronic toxicity therefore prevents grains from destruction.

Koona *et al.*, (2007) observed powdered dried leaves of *Tephrosia vogelii* were extracted using hexane, acetone, and ethanol. The extracts were tested for their ability to protect stored maize from damage by *S. zeamais*, the major maize weevil in Africa. The acetone and ethanol extracts were ineffective, but the hexane extract had a relatively high efficacy, producing within 7 days a slow reduction in adult survival, reduced numbers of eggs laid and reduced numbers of F_1 progeny, resulting in seed damage averaging 8.8% compared with 98.6% in the untreated control after one generation. This hexane extract provided control of *S. zeamais* at a higher level compared with neem (the botanical control) but a lower level compared with pirimiphos-methyl (the synthetic control).

Shukla *et al.*, (2007) found among the plant powders tested, *Murraya koenigii* and *Eupatorium cannabinum* were found to be the most effective in reducing the orientation, oviposition and causing the mortality of bruchids at dose of 2% (w/w). The F_1 emergence from the infested chick pea was significantly reduced in treatments to which powders of *M. koenigii* (90.62%) and *E. cannabinum* (86.46%) had been added.

Mishra *et al.*, (2006) observed the solvent extracted vegetable seed oils of Cucurbitaceae family viz. Bitter gourd, *Momordica charentia*, Small bitter gourd, *Momordica dioica*, Bottle gourd, *Lagenaria siceraria* and Ridge gourd, *Luffa acutangula* were evaluated as grain protectant against *Callosobruchus chinensis* on the stored legume-pulse grains. All the vegetable seed oils were found effective as legume-pulse grain protectant, which provided negligible weight loss at the oil-application rate of 6-8 ml/kg in legume-pulse grain after 60 days storage at laboratory conditions. The use of solvent extracted Small bitter gourd seed oil at the level of 6-8 ml/kg of legume-pulse grain sample resulted in the improved apparent degree of dehusking from 40.0 to 72.59, 59.88 to 92.44, 63.39 to 87.50 and 57.0 to 79.43 for pigeonpea, chickpea, urdbean and mungbean respectively.

Singh (2004) studied the effect of neem oil as surface protectant at 0, 0.5, 1.0, 1.5 and 2.0% (w/w) 100g of lentil seeds against the pulse beetle, *Callosobruchus chinensis*. Observations of percentage of seed damage and insect adult mortality were performed periodically during summer, rainy and winter seasons. Neem cause significant reduction in seed damage and insect population in treated grains. During summer and winter seasons, the beetle populations were low having high mortality, where as during rainy season the population was high and low mortality. Average percentage damage caused by the pest progressively increased in the control with increase in time, and the damage in treated seeds was inversely proportional to the neem oil. All the concentrations were effective. The highest toxicity was recorded with 2.0 ml neem oil/100g seeds.

Umarao and Verma (2002) assessed the efficacy to various plant products; leaf powder of dharek, *Melia azadarach* and sadabahar, *Ipomoea carnea* at 10g/kg grain and oils of coconut, mustard, and ground nut and neem products such as achook, nimbicidine and neem gold at 1 ml/kg against

pulse beetle *Callosobruchus chinensis* based on the percentage of grains damage and weight loss. Nimbicidine and achook appeared to be the most effective in minimizing the damage by the pests in grains, 1.97 and 2.36%, respectively, followed by the neem gold treatment (2.61%) over that the control (70.50%). The loss in weight was as high as 45.20% in the untreated grains, which considerably decreased to a level of 0.52, 0.93 and 1.07% by the application of achook, nimbicidine and neem gold repectively.

Singh *et al.*, (2001) tested leaf powder of lantana, sadabahar neem, madar and tulsi at a dose rate 10 g/kg grain and oils of castor, neem and mahua 2ml/kg grain against pulse beetle. The result revealed that neem oil and neem leaf powder appeared to be most effective for minimizing the damage.

Tripathy *et al.*, (2001) examined the efficacy of 8 different vegetable oils (castor, neem, pongamia, coconut, Indian mustard, sesame, soybean, and sunflower) at 2 and 4 ml/kg. 8 plants *(Annona squamosa, Eucalyptus glandosum, Lantana camara, Strychons nux-vomica, Tridax procumbance, Datura fistula, Spoeranthus indica* and *Azadirachta indica)* powders at 20 40 ml/kg, and 3 plants *(Lantana camara, Ageratum cnyzoides* and *Vitex negundo)* extracts at 2.5, 5.0, 7.5, and 10.0 ml/kg against pulse beetle *(Callosobruchus chinensis)* infesting on black gram (cv.T_9). All the oil treatments were superior in protecting the seeds from the pulse beetle attack compare to the malathion treatment (60 ppm) or control. The oils of neem, castor and coconut at both doses proved the most effective in protecting seeds about 9 month after treatment. Among the plant powders *L. camara* and *Tridax procumbance* at both concentrations and *L. camara* extracts at all concentrations were effective in protecting the seeds.

Amin *et al.*, (2000) observed four laboratory experiment is conducted with the leaves of three plant species viz, akanda, *Asclepios calotropis*, biskatali,

Polygonum hydropiper and neem, *Azadirachta indica* for studing their relative efficacy against the lesser grain borer, *Rhyzopertha dominica*. Results from the first three experiments indicated that 2, 3 and 4% water extracts of all three plant species had repellency as well as direct toxicity while the 3% showed strong feeding deterrency effect. In the last experiment, powdered leaves of 2, 3 and 4% dust provided adequate protection of wheat grains by reducing both the F_1 progeny emergence and grain infestation rates. The higest doses of all the powdered formulations gave the highest effects.

Rahman (1998) treated the wheat grains with 5% powder of pithraj, castor and neem seed against *Sitophilus oryzae*. By releasing insect on 20 days after treatment the inhibition rate of F_1 progeny of the insect was found to be 24.89, 32.19 and 40.06% for pithraj, castor and neem respectively.

Patro *et al.*,(1997) observed the developmental behaviour of *Callosobruchus chinenns* reared on green gram (Var. K-851) treated with aqueous extracts of seeds of *Azadirachta indica* and *Annona squamosa* and rhizomes of *Acorus calamus* and *Curcuma longa* each at 0.05, 0.08, 0.1 and 0.2 % concentration. *A. squamosa*, *A. calamus* and *C. longa* expressed 17.23 to 69.17, 12.76 to 57.19, 11.84 to 62.91 and 11.64 to 63.42% growth inhibition at their respective test concentration ranges.

Raguraman *et al.*, (1997) studied Oils obtained from neem seed kernel, the himalayan cedar wood and their combination (1:1) each at 3, 2 and 1% concentrations were tested against adults of pulse beetle. Cedar wood oil exhibited highest fumigant potential at 3, 2 and 1% concentrations showing corrected inhibition (knock-down) of 100, 100 and 96%, respectively. While neem oil + cedar wood oil (1:1) at 3, 2 and 1% concentrations inhibited 96, 84 and 51.66%.

CHAPTER III

MATERIALS AND METHODS

Experiments on the insecticidal activities and chemical investigation of common cocklebur and bloodleaf against the pulse beetle, *Callosobruchus chinensis* (L.) were carried out in the laboratory of the Department of Agricultural chemistry , Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, during the period of August, 2007 to August, 2008.

3.1. The Test Plants

The experiments were conducted with two plants namely common cocklebur, (*Xanthium strumarium*) and bloodleaf, (*Iresine lindenii*). Common cocklebur leaf, common cocklebur fruit and bloodleaf leaf were collected from HSTU campus, Dinajpur. A brief account of the plants is given below:

Plant No. 1: Common cocklebur

Local name: Shakto Ghagra

English name: Common cocklebur

Scientific name: Xanthium strumarium

Family: Asteraceae

Morphology of Common Cocklebur

A summer annual that produces a conspicuous prickly 'cocklebur' and ranges from 0.50 to 6.50 feet in height. Common cocklebur is found

throughout the United States and is primarily a weed of agronomic, horticultural crops, nurseries and occasionally pastures.

Seedlings: The stem below the cotyledons (hypocotyls) is purple at the base and often green in the upper portion. Cotyledons are linear to oblong in outline, waxy, smooth, fleshy, thick, approximately 0.75 to 1.75 inches long and usually no more than 0.5 inch wide. The first true leaves are opposite, while all subsequent leaves are alternate.

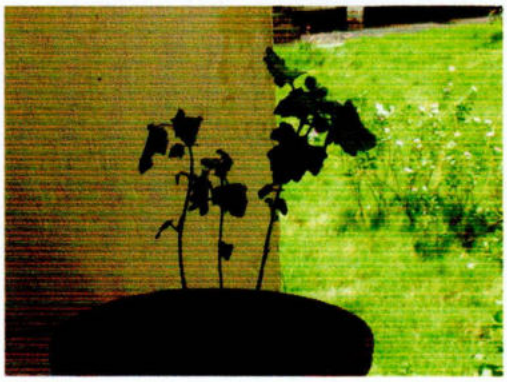


Fig.1: Common cocklebur plant in a pot

Fig.2: Common cocklebur plant in the field

Leaves: The first true leaves are opposite, all subsequent leaves are alternate. Leaves are triangular to ovate in outline, have stiff hairs, and are approximately 2 to 6 inches long. Leave are irregularly lobed with leaf margins that have relatively inconspicuous teeth. Leaves occur on long petioles and also have three prominent veins on the upper surface of the leaf that arise from the same point.

Stems: Erect, branched, stout and covered with a dense cover of short stiff ascending hairs and 'bumps'. Stems are ridged longitudinally and green with maroon to black spots. Stems may reach 6.5 feet in height.

Roots: A taproot is generally observed.

Flowers: Inconspicuous, greenish in color, arising from the area between the leaf petioles and the stems (axillary flowers) and at the ends of the erect stems (terminal flowers).

Fruit: An elliptic to egg-shaped two-chambered bur, 0.5 to 1.5 inches long and covered with hooked prickles. Each bur contains two seeds, one that grows during the first year and one that grows a year later. Two prickles that are longer and wider than the remaining prickles project from the tip of the bur.

Distribution

Common cocklebur is native to America but has spread throughout the dryer warmer regions of the world including Bangladesh.

Uses

It is used as fuel and fence in rural area. Now, it may be used as botanical insecticide.
(http://www.fs.fed.us/database/feis/plants/forb/xanstr/all.html#DISTRIBUTION%20AND%20OCCURRENCE)

Plant No. 2: Blood leaf

Local name: Bish korobi

English name: Bloodleaf

Scientific name: *Iresine lindenii*

Family: Amaranthaceae

Morphology of Bloodleaf

Iresine is a group of tender perenials grown mainly for their colorful foliage. They are frost tender, requiring minimum temperatures of 50-59 degrees F. They can also be grown as an indoor plant or treated as an annual. Prefer full sun, loamy well drained soil. Pinch the tips to promote bushy growth during the growing season.

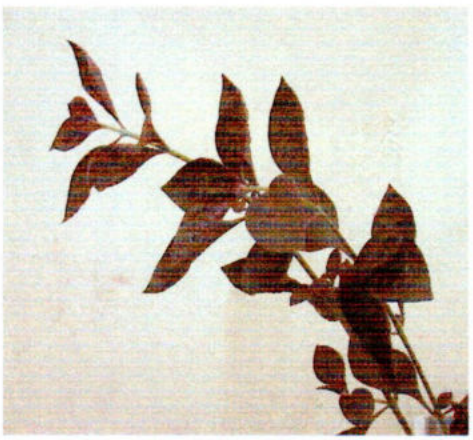


Fig.3: A Twig of bloodleaf

Fig.4: Bushy appearence of bloodleaf

Plant height of bloodleaf (*I. lindenii*) is 3ft. to 3ft. and width is 3 ft. to 3 ft. and is bushy, upright, compact grower with blood red stems, pointed egg-shaped leaves 2-4 inches long with apparent deep or light red veins, foliage color is red to burgundy, leaf size medium.

Distribution

Bloodleaf is native to the Northern Peruvian Andis but it has spread throughout the dryer warmer regions of the world including Bangladesh.

Uses

It is used as mainly medicinal plant but now, it may be used as botanical insecticide. (http://www.backyardgardener.com/plantname/pda_e69b.html)

3.2. The Test Insect

The pulse beetle, *Callosobruchus chinensis* was used as the test insect in the present studies, figure 5. It is a major economic pest, originated in Asia, but is now cosmopolitan in the tropics and subtropics (Alam. 1971; Schmutterer, 1977; Begum *et al.*, 1982; Mensah, 1986). It is a notorious pest of pulses.

Systemic position

Phyllum-Arthropoda

Sub-phyllum- Mandibulata

Class- Insecta

Sub-class- Pterygota

Division- Endopterygota

Super-order- Coleopteriodae

Order- Coleoptera

Sub-order- Polyphaga

Family- Bruchidae

Genus- *Callosobruchus*

Species- *Callosobruchus chinensis* L.

Distribution

C. chinensis has been reported from the USA, Mauritius, Formosa, Africa. China, the Philippines, Japan, Srilanka, Myanmar, Bangladesh and India (Atwal 1976) that is, throughout the world. In Bangladesh, it is commonly called as the pulse beetle. But in America and Japan, it is known as the cowpea weevil or adzuki bean-seed beetle.

Morphology

Adults small, brownish in colour, 2-3 mm long and rather square in body shape. Antennae pectinate in the male and slightly serrate in the female, the hind femora have a pair of parallel ridges on the ventral edge, each with an apical spine (tooth). The markings on the elytra vary somewhat, but the dark patches can be quite conspicuous. The eyes characteristically emerginate, the elytra do not quite cover the tip of the abdomen. Adults can fly quite well (usually up to one kilometer), but they do not feed on stored products and thus short-lived up to 12 days usually (Hill, 1990). The following are identifying characteristics of the male and female pulse beetle (Halstead, 1963).

Characters	Male	Female
Antenna	Pectinate, curved towards each other. Apical segment of antenna is elongate and oblong in shape. Pectination becomes prominent from the 4^{th} to the apical segment.	Serrate, straight. Apical segment is somewhat round to ovate. Serration becomes prominent from the 5^{th} to the apical segment.

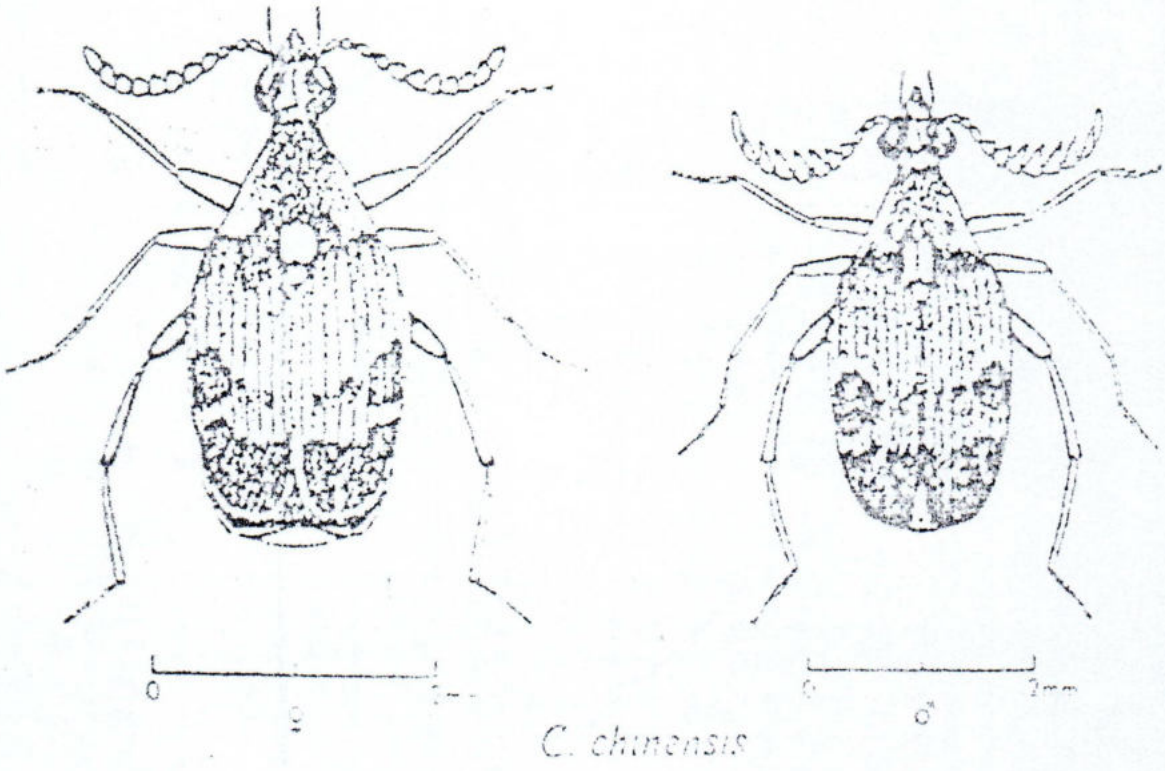


Fig.5: Morphological feature of pulse beetle *C. chinensis*

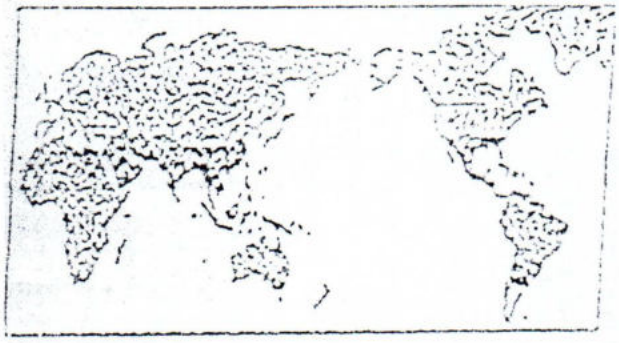


Fig.6: Distribution of pulse beetle *C. chinensis*

Biology

Eggs are laid on to the developing pod in the field, or on to the surface of seeds in pods, or on to seeds in store. 100 eggs are laid per female, glued firmly to the seed surface and incubation takes 5-6 days. The larvae after hatching bite through the base of the eggs, directly through the testa and into the cotyledons. The larva is scarabaeiform and the final instars develop in about 20 days, the whole time being spent within one seed. Pupation takes place inside the seed in a chamber covered by a thin window of testa materials, and requires about 7 days to come out as adult. It takes 36 days for the completion of life cycle and 6-7 generations per year are usual.

Ecology

Optimum conditions for development are about 32°C and 90% relative humidity. The life cycle can be completed in 21-23 days in this condition.

Nature of Damage

Larvae bore into the cotyledons and eventually hollow out the seed within the testa, typically 1-3 larvae bore per infestations start in the field and eggs are laid on the surface of maturing pods, later eggs are laid on the seed surface. They attack all pulses in topical regions like chickpea, *Cicer arietinum*; lentil, *Lens culinaris* ; mungbean, *Phaseolus vulgaris* ; green gram, *Vigna radiata*; adzuki bean, *V. angularis* and cowpea, *V. unguiculate* as well as cotton seed, sorghum and maize (Atwal, 1976). Due to the infestation of pulse beetle, grains become unsuitable for human consumption and loose their viability to germinate, and thus become unfit for sowing in the field.

3.3. Mass Rearing:

A stock culture of the insects was maintained in the laboratory, Department of agricultural chemistry and biochemistry, Hajee Mohammad Danesh Science &Technology University, Dinajpur, at 28-32°C temperature and 75-85% relative humidity. Fifty pairs of adult pulse beetles (about 1-3 days old) were placed in glass jar containing the rearing material (black gram). The jar was then sealed and was allowed for free mating and oviposition for a maximum period of 7 days. The parent stocks were removed and the grain or pulses containing eggs were transferred to preconditioned food material (black gram) in the breeding jar. The jars were covered with pieces of cloth, fastened with rubber bands to prevent the contamination and escape of insects. Rearing of these insects was being continued for experimental purpose.

Fig.7: Mass rearing of the test insect *C. chinensis*

3.4. Preparation of Plant Dust & Extracts

3.4.1. Collection of plant materials

Fresh leaves, seeds common cocklebur and fresh leaves of bloodleaf turmeric were collected from the pond side of the HSTU and from the front side of Domitory-1 of HSTU respectively. After collection of the plant materials, then they were weighed by electric balance. The weight of the fresh leaf, fresh fruit of common cocklebur and fresh leaf of bloodleaf were 2.7kg and 1.6kg and 2.9kg respectively. After weighing, they were washed in running tap water in the laboratory. The common cocklebur fruits were cut into small pieces by secature for suitable drying and grinding.

3.4.2. Preparation of Plant Dusts and Extracts

Fresh plant materials were dried in sunlight and then they were dried in the oven at 50-60°C for 24 hours to gain constant weight. The dried materials were ground with the help of a grinder. The weight of the ground powder of common cocklebur leaf, fruit and bloodleaf leaf were 404g, 243g and 204g repectively. The powder of 100g of each plant materials was taken in 2.5 litre reagent bottle and 1600 ml ditilled water was added in each reagent bottle.That was kept for 72 hours with a interval of shaking. After 72 hours it was then filtered. The filtrates were being used as insect bioassay, which were stored in a refrigerator before use.

3.4.3. Preparation of Stock Solution

Extracts were considered as 6.25% solution. From which 1%, 2% and 4% solution were prepared.

3.5. Insect Bioassay

Insect bioassays were done in the laboratory under ambient conditions. The present study was categorized into following bioassays.

3.5.1. Direct Toxicity Test

Direct toxicity test with pulse beetle was done following the method of Talukdar and Howse (1993). Insects were chilled for a period of 10 minutes. The unmobilized insects were individually picked up and one milliliter solutions of different concentrations (0.0, 1.0, 2.0 and 4.0 % W/V) were applied to the dorsal surface of the thorax of each insect by using micro capillary tube. Ten insects per replication were treated.

Fig.8: Direct toxicity test of different plant extracts on pulse beetle, *C. chinensis*

The insects were then transferred into a 9 cm diameter petridishes containing food. Insect mortality rate was recorded after 1, 2, 3and 4 day after treatment (DAT). All the experiments were conducted completely ramdomized design with five replications and turned to statistical analysis. Finally, the mean values were compared using DMRT (Duncan, 1957).

$$\text{Mortality (\%)} = \frac{\text{Total number of mortality of pulse bettles in each Petridish}}{\text{Total number of pulse bettles released in each Petridish}}$$

3.5.2. Repellency Test

The repellency test was conducted according to the method of Talukder and Howse (1993). For repellency test (figure 9) plant extracts were diluted with respective solvents to prepare (1, 2, & 4%) solutions. Petridishes were divided into two parts, treated and fresh grain portion (untreated). With the help of a pipette, 1.0 ml solution of each plant extract was applied to one half of the petridish.

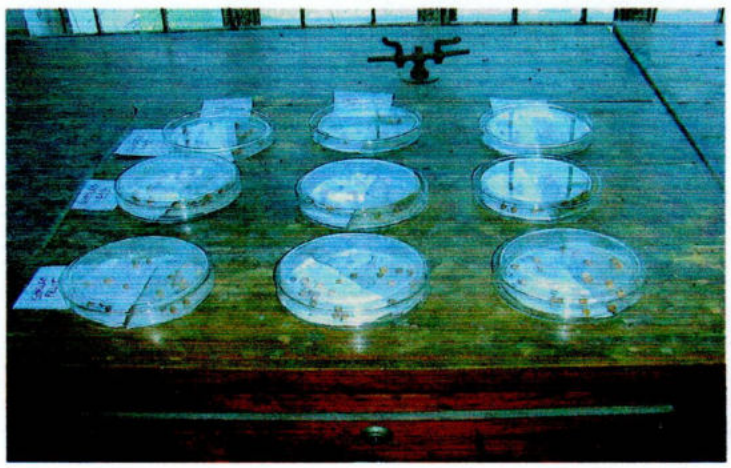


Fig.9: Repellency test of different plant extracts on pulse beetle, *C. chinensis*

The treated half was then air-dried. Ten insects (5 male and 5 female) were released at the centre of each Petridish and a cover was placed on the Petridish. There were three replications for each plant extract and each dose. Then the insects present on each portion were counted at hourly intervals up to fifth hour. The data were expressed as percentage repulsion (PR %) by the following formula:

$$PR\ (\%) = (Nc-50) \times 2$$

Where,

Nc = The percentage of insects present in the control half.

Positive (+) values expressed repressed repellency and negative (-) values attractency. Data (PR %) was analysed using analysis of variance (ANOVA) after transforming them into arcsine percentages values. The average values were then categorized according to the following classes (McDonald *et al.* 1970).

Class	Repellency rate (%)
0.	> 0.01 to 0.1
i.	0.1 to 20.0 0
ii.	20.1 to 40.0
iii.	40.1 to 60 .0
iv.	60.1 to 80.0
v.	80.1 to 100.0

3.5.3. Fecundity Test

Five pair of newly emerged beetles was released in the Petridishes containing black gram seeds treated with different concentrations of each plant extracts for recording oviposition and fecundity. Male and female insects were always maintained as 1: 1 ratio. Control treatments were done side by side. There were three replications for each treatment. The oviposition and fecundity rate was recorded after 7 days of the release of beetles.

Fig.10: Fecundity test of different plant extracts on pulse beetle, *C. chinensis*

The eggs laid on black-grarn seeds of each treatment in the Petridish were counted individually by using hand lens.

3.5.4. Adult Emergence Test

The pulse beetle started to emerge after 30 days of egg laying. The emerged beetles were counted and removed every day from the container.

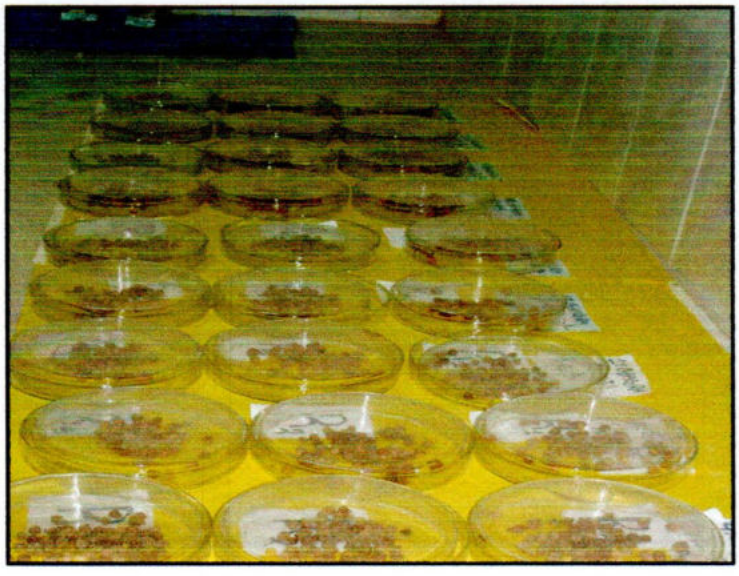


Fig.11: Adult emergence test of different plant extracts on pulse beetle, *C. chinensis*

The numbers of adult beetles were counted daily from the date of first emergence to at least 10 days. The adult emergence rate was calculated and the inhibition rates (IR %) were calculated by using the following formula:

$$IR\ (\%) = \frac{Cn - Tn}{Cn} \times 100$$

Where,

C_n = Number of insects in control Petri-dish.

T_n = Number of insects in treated Petri-dish.

3.5.5. Seed Damage Test

Each and every seed was taken out from the Petridishes after the completion of counting the adult beetles to determine the number of hole(s) on each seed after feeding inside. Seeds containing, hole(s) were considered as damaged seeds. The number of damaged black gram seeds were counted and recorded for each replication.

Fig.12: Seed damage test of different plant extracts on pulse beetle, C. chinensis

3.6. Meteorological Data

During the entire period of research, temperature was recorded daily at 12.00 noon from the dry and wet bulb thermometer placed in the laboratory, Department of agricultural chemistry and biochemistryof HSTU.

The relative humidity was calculated from the dry and wet bulb reading of the thermometer (ZEAL Model) by using the following formula: $L = t_1 - G(t_1 - t_2)$ and $R = f/F \times 100$

L = Dew point, t_1 = Dry bulb temperature, t_2 = Wet bulb temperature, G = Glacial coefficient of t_1°c – temperature, R – Relative humidity, f – Atmospheric pressure at dew point (t°c), F = Atmospheric pressure at air temperature (t_1°c).

3.7. Statistical Analysis

The experimental data were statisticaly analysed by Completely Randomized Design (factorial CRD) using MSTAT statistical software in a microcomputer. The mean values were adjusted by LSD Test.

3.8. Chemical Investigation on Effective Plant Extract

3.8.1. Isolation of Crude Compounds from Effective Plant Extract Using Chloroform

100 g of common cocklebur leaf powder was taken in a 2.5 liter reagent bottle and 250 ml chloroform was to add it. It was then kept 72 hour with regular interval of shaking. After 72 hours it was filtered by using Whatman filter paper No.1. The extract was collected in 500 ml reagent bottle and 200 ml of chloroform was added to the residue again, the reagent bottle was again kept for next 72 hours with also regular interval of shaking. After 72

hours it was then filtered. The extracting processes were repeated for at least three times. The chloroform extracts were combined together. The solvent was evaporated by using Thin Film Rotary Evaporator under reduced pressure. 9.3g of crude compound was obtained, which was stored in refrigerator at 0°C for further investigation.

3.8.2. Isolation of Crude Compounds from Effective Plant Extract Using Ethylalcohol:

Similarly, 100 g of common cocklebur leaf powder was taken in a 2.5 liter reagent bottle and 200 ml of ethyl-alcohol was added to it. It was kept for 72 hours with several interval of shaking. After 72 hours it was filtered by using Whatmann filter paper No.1. The extract was collected in 500 ml reagent bottle and 200 ml of ethyl-alcohol was added to the residue again, the reagent bottle was again kept for next 72 hours with also regular interval of shaking. After 72 hours it was then filtered. The process was also repeated for three times. The ethylalcohol extracts of individual plant were combined together. The solvent was evaporated by using thin film rotary evaporator under reduced pressure. 17.00g of crude compound was also stored in refrigerator at 0°C for further investigation.

3.8.3. Examination of Crude compounds by Thin Layer Chromatography (TLC):

Thin Layer Chromatography (TLC) is one of the most important techniques, by which we are able to detect or identify the presence the number of compounds or number of components present in a crude extract or crude compound in which R_f value of each component was calculated by using this formulae:

$$R_f = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent front}}$$

Thin Layer Chromatography (TLC) was carried on glass plates (slides) coated with silica gel G type 60 (BDH, England).

3.8.4. Procedure for Preparation of TLC Plates:

Slurry was prepared by the slow addition with shaking 30 gm of absorbent (silica gel) to 100 ml of chloroform in a wide-racked capped bottle. A pair of microscopic slides was held together and dipped into the slurry, slowly with drawn and allowed to drain momentarily while held over the bottle. The slides were parted carefully and placed horizontally in a rack; it was then dried in sunlight or in oven at 30- 40° C for10-15 minutes (Furniss *et al.*, 1989)

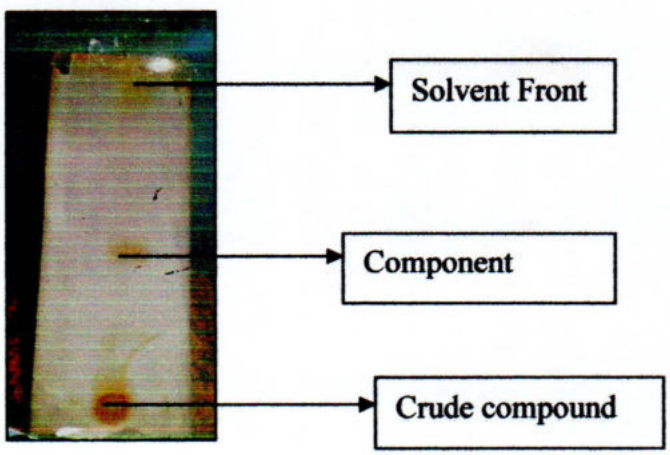


Fig.13: A TLC (Thin Layer Chromatography) plate

3.8.5. Purification of Crude Product by Column Chromatography:

The crude product containing a mixture of compounds was separated individually by using column chromatography after preliminary TLC examination, where silica gel was used as stationary phase and solvent or mixture of solvent (eluent) was treated as mobile phase.

3.8.6. Preparation of Column Chromatography:

The column was prepared by slurry method, silica gel (70- 230 mesh, BDH England) being the stationary phase; the column was first washed well with washing mixture and then with distilled water and then dried with a drier. It was then rinsed with the solvent used in the preparation of silica gel slurry and again the column was dried and then fitted with a cotton plug at the bottom. The column was half filled with the appropriate solvent/eluent (non polar solvent, hexane) and the slurry was then poured into the column so that the packing was compact and uniform. Air bubble was avoided by making the column as quickly as possible. The crude extracts (40 times of crude product) were carefully placed on the surface of the column with glass dropper so that the surface of the column was not disturbed and then crystal silica gel was added. The little amount of cotton was placed again at the top of surface of the column. The elution was continued until the desired fractions were eluted out. It was carefully marked that the slurry was always under the required solvent or solvent ratio.

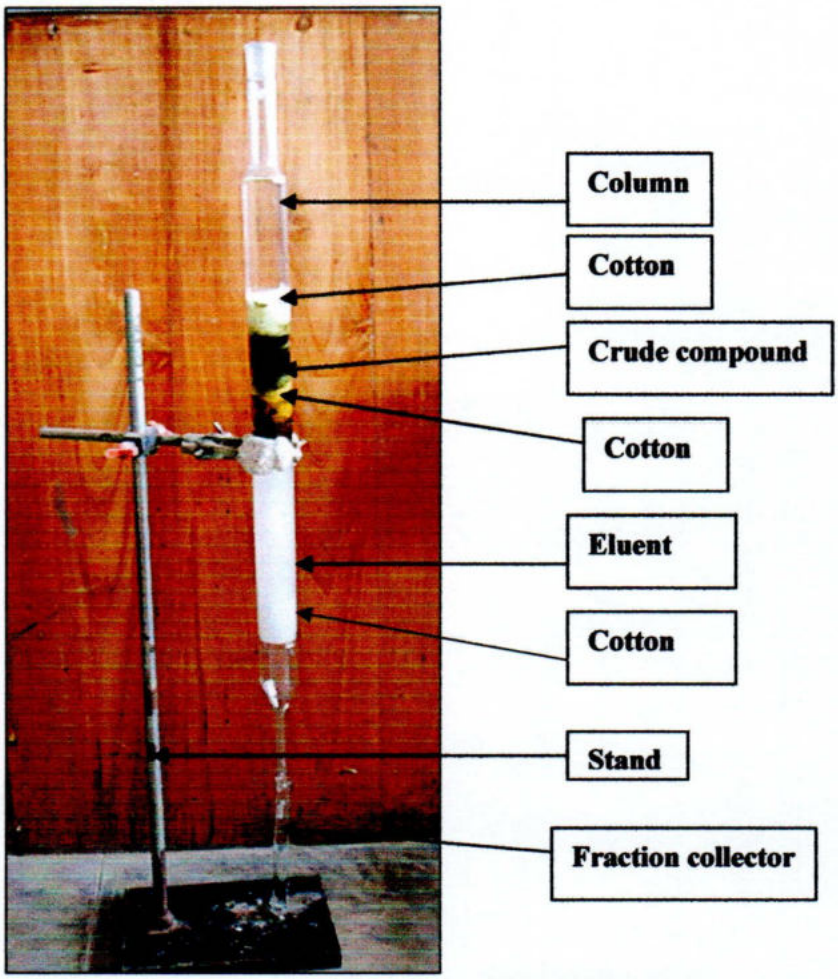


Fig.14: Preparation of a manual column chromatography

3.9. Structure Determination of Purified Compounds:

Structures of purified compounds were determined by the following spectroscopic methods.

3.9.1. Infrared (IR) Spectroscopy Study:

IR (Infrared Spectroscopy) was measured with SCHIMAZU IR spectrometer from BCSIR (Bangladesh Council of Scientific and Industrial Research) Dhaka, Bangladesh.

3.9.2. ^{1}H-NMR (Nuclear Magnetic Resonance) Study:

^{1}H-NMR spectra were measured with BRUKER 400 MHz NMR spectrometer from BCSIR (Bangladesh Council of Scientific and Industrial Research) Dhaka, Bangladesh.

CHAPTER IV

RESULTS AND DISCUSSION

Part 1: Effects of Bloodleaf Plant Extract on *C. Chinensis*

4.1.1. Effect on Direct Toxicity

The efficacy of bloodleaf plant extract as protectant for black gram grains has been evaluated by direct toxicity by direct exposing the target species at different DAT and the results are shown in figure 15. When the adults were exposing at different concentrations of bloodleaf plant extract, the highest mortality (18%) was observed in 4% concentration at 2 DAT and the lowest mortality (2%) was observed in control. Toxicity effects of bloodleaf plant extract at 1 DAT, 3 DAT and 4 DAT were statistically identical.

Fig.15: Effect of bloodleaf plant extract on the mortality rate of *C. chinensis*

Amin *et al.*, (2000) studied the toxicity effect of biskatali, neem and akanda plant extract on lesser grain borer and reported that 4% biskatali extract showed strong toxicity effect (80.11%). Plant materials possessed some chemicals viz. azadirachtin, cymarin, digitoxin, toosendanin, xanthotoxin, etc are toxic chemicals that showed mortality effect on insect. The present

study plants may have some toxic chemicals that heve showed toxicity on pulse beetle. This finding showed in agreement with Kemabonta *et al.*, (2002) who reported that application of *C. ambroisoides* (5.0% extract) caused 54% mortality of *C. maculatus* adults after 5 days. Allelochemicals (cymarin, digitoxin, toosendanin, xanthotoxin, trans-anethole) of *C. ambroisoides* were responsible for mortality effect of pulse beetle. Aggarwal *et al.*, (2003) who also observed that 1,8-cineole, one of the components of the essential oil of *Artemisia annwa* was evaluated for repellency and toxicity against three stored product coleopterans;*C. maculatus*, *R. dominica* and *S. oryzae*. The compound was more effective as a fumigant and gave 93-100% mortality against all three pest species at the dose of 1.0 µl/l air under empty jar conditions as compared to treatment of jars filled with grain (11-26% mortality).

4.1.2. Effect as Repellent

The repellency effects of bloodleaf plant extract at different HAT have represented in figure 16. The highest repellency rate (46.7%) was found after 3HAT when 4% bloodleaf plant extract was applied as repellent. The lowest repellency rate (20.0%) was observed after 2HAT when 1% concentration of bloodleaf plant extract was applied as repellent. It is also stated that repellent effects of bloodleaf plant extract at 1HAT to 5 HAT were statistically indifferent.

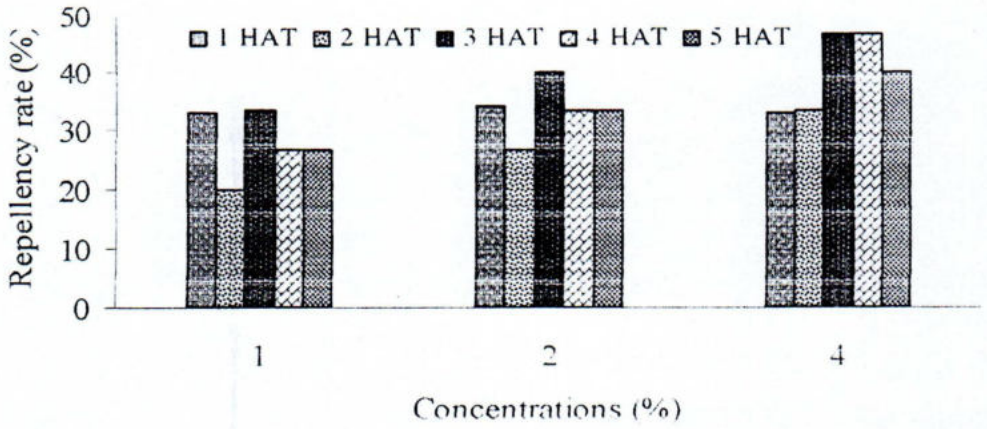


Fig.16: Effect of bloodleaf plant extract on the repellency rate of *C. chinensis*

Shahjahan *et al.*, (2000) studied the repellency effect of biskatali, neem and akanda plant extract on rice weevil and reported that the highest repellency effect (73.6%) was found in case of 4% neem followed by biskatali (68.0%) and akanda (58%). Plant materials possessed some chemicals viz. azadhirachtin, nicotin, cymarin, digitoxin etc biochemicals were toxic chemicals that showed repellency effect on insect. The present study plants may have some toxic chemicals that have showed repellency on pulse beetle. This experiment was also in agreement with Aggarwal *et al.*, (2003) who observed that 1, 8-cineole, one of the components of the essential oil of *Artemisia annwa* showed repellency against three stored product coleopterans; *C. maculatus*, *R. dominica* and *S. oryzae*. Rahman *et al.*, (2006) assessed 1% fruit extract of Melgota, *Macaranga postulata* the lowest repellency (9.84%) on the rice weevil. On the other hand 2% showed 12.76% and 4% showed 22.43% repellency. They also observed that TLC of crude ethanol extract of Melgota, *M. postulata* showed six distinct compounds which were responsible for repellency of *S. oryzae*.

4.1.3. Effect on Fecundity

Figure 17 showed the effect of different concentration of bloodleaf plant extract on the number of eggs laid by the female of *C. chinensis*. Results showed that the fecundity rates of the beetles were lowest (111.7) at 4% concentration followed by 2% (151.3), 1% (202) and control (213.3) and there were significantly different.

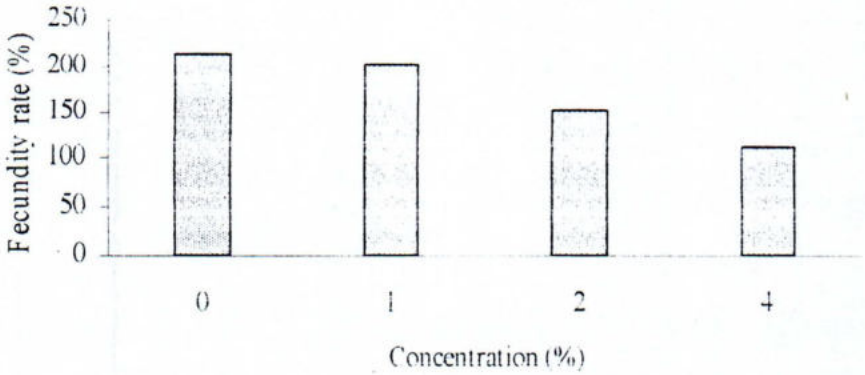


Fig.17: Effect of bloodleaf plant extract on the fecundity rate of *C. chinensis*

Nandi *et al.*, (2004) had noted the similar fecundity effect against pulse beetle and also observed the bioefficacy of nimbicidine against pulse beetle, *C. maculatus* in the laboratory. Adult beetles were exposed to gram seeds treatd with 1.00, 0.50, 0.25, 0.12 and 0.06% concentration of nimbicidine and seeds treated with 1.00 % concentration of nimbicidine were less preferred and performed lawest pest fecundity (16.33). This study shows in agreement with Salunke *et al.*, (2005) who possessed the partially purified flavonoids obtained from *C. procera* and observed the highest contact toxicity against *C. chinensis* followed by standard quercetin, rutin and quercitrin at 10 mg ml^{-1} doses in filter paper diffusion assay. Significant reduction in oviposition was found for all partially purified flavonoids at the doses of 5 and 10 mg ml^{-1} on grains in plastic jars, respectively. Upadhyay

et al., (2006) reported that extracts of *Capparis decidua* stems and flowers showed insecticidal and oviposition inhibitory activities against *C. chinensis*. For instance, after 96 h, the LC_{50} values were found to be 3.619, 7.319, and 10.151 microg for CD_1, CD_2, and CD_3, respectively. The maximum oviposition deterrence index was found for extract CD_1 followed in decreasing order by CD_2, CD_3, and CD_7. From extract CD_1, two compounds were isolated and characterized as triacontanol (C_1) and 2-carboxy-1, 1-dimethylpyrrolidine (C_2). When the females were exposed to sublethal doses of these compounds, they laid lower number of eggs as compared to the control. C_2 was found to have a slightly greater oviposition inhibition effect than C_1. Raja and William (2008) also reported the essential oils of plants namely Citronella, *Cymbopogan winterianus*, Citrodora, *Eculyptus citrodora*, Lemon grass, *Cymbopogan flexuosus*, Vetiver, *Vetiveria zizanioides*, and Palmorosa, *Cymbopogan martini* were tested for their insecticidal or ovicidal activities against adults and eggs of *C. maculatus* at 5% concentration and beside this, the results revealed that the highest ovicidal activity was recorded in Citrodora oil (88.43%) followed by lemon grass oil (45.25%) at 96 h of exposure due to presence of terpenoids, alkanoids, flavonoids etc in lemon grass oil.

4.1.4. Effect on Adult Emergence

The adult emergence effect of bloodleaf plant extract was statistically different at 1% level of significance. The lowest number of adult emergence (65.3%) was found in the grains treated with 4% bloodleaf plant extract and the highest number (91.3%) was found in control (figure 18).

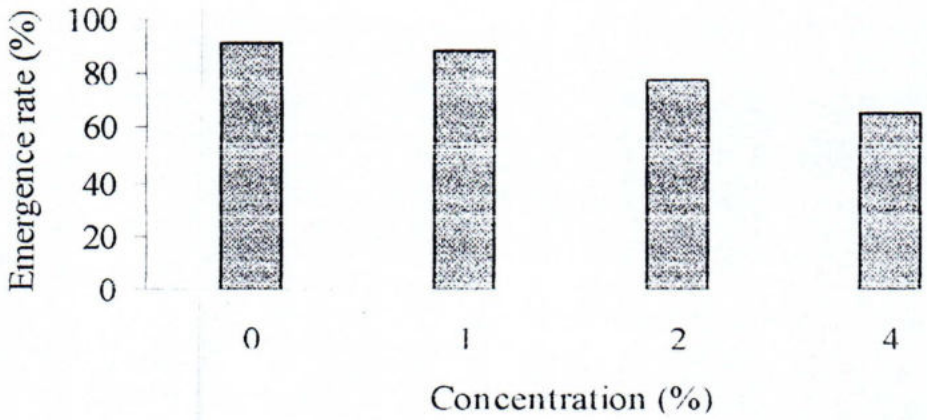


Fig.18: Effect of bloodleaf plant extract on the adult emergence rate of *C. chinensis*

Shahjahan *et al.*, (2000) studied the F_1 adult emergence effects of biskatali, neem and akanda dusts on rice weevil *S. oryzae* and reported that the lowest number of F_1, rice weevil *S. oryzae* adult (28.80%) emerged from the rice grains treated with 2% neem dust followed by biskatali (29.40%) and akanda (31.40%). Similar trend was also observed for 3% and 4% (w/w) mixtures. Plant materials possessed some chemicals viz. kulactone, limocinin, azdirol, salanin, nimocinolide etc biochemicals were toxic chemicals that showed reducing of F_1 adult emergence effect on insect. The present study plants may have some toxic chemicals that have showed toxicity on pulse beetle. Hussein *et al.*, (2005) applied saponin extract from alfalfa roots, azadirachtin from the neem seed oil, synthetic ecdysteroid agonist RH-2485, and juvenoid hydroprene on *Tropinota squalida* and observed that the reproduction of insects were disturbed. They also possessed that when the larvae of untreated adults were fed for 1 week on dung with 75 p.p.m. saponins, 50 p.p.m. RH-2485, and 0.45 p.p.m. azadirachtin, the rate of adult emergence droped from 80% (controls) to 20, 0 and 13%, respectively. No adults emerged when the treatments were continued through the second and third larval instars. Sarkar (2006) studied the adult emergence effect of turmeric, black pepper and eucalyptus plant

dust on *C. chinensis* and reported that the lowest number of adult (13.67) was found to emerge in the grains treated with 10% turmeric and the highest number (112.67) was emerged from the grain treated with 2.5% garlic. Plant materials possessed some chemicals viz. steroids, terpenoids, phenolics, saponines, tannins and flavanoids etc are toxic chemicals that showed reducing of adult emergence effect on insect.

4.1.5. Effect on Seed Damage

Figure 19 represented the minimum seed damage rate (43.7%) was found at 4% concentration of bloobleaf plant extract and the maximum rate (92.3 %) was found in control. All the concentrations revealed significant difference with control ($p \leq 0.05$).

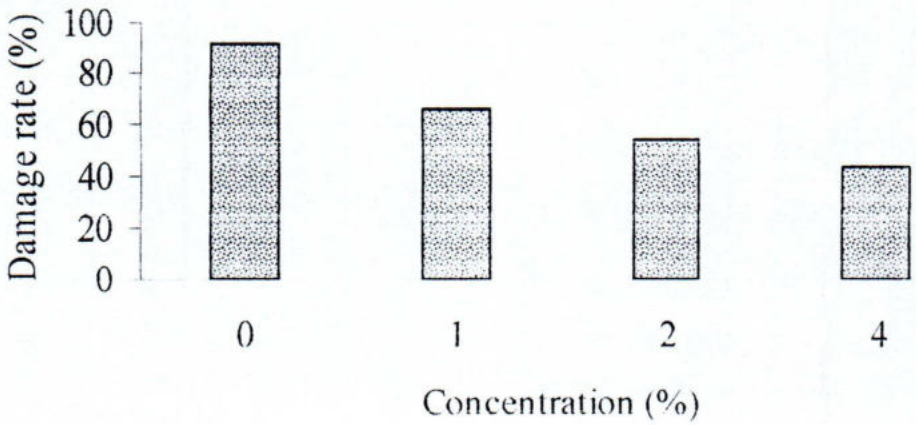


Fig.19: Effect of bloodleaf plant extract on the seed damage rate of *C. chinensis*

Umarao and Verma (2002) assessed the efficacy to various plant products; leaf powder of dharek, *Melia azadarach* and sadabahar, *Ipomoea carnea* at 10 g/kg grain and oils of coconut, mustard, and ground nut and neem products such as achook, nimbicidine and neem gold at 1ml/kg pulse beetle *C. chinensis* based on the percentage of grains damage. Nimbicidine and achook appeared to be the most effective in minimizing the damage by the pests. Mishra *et al.*, (2006) had cited the similar grain damage effect of

vegetable seed oils of cucurbitaceae family against *C. chinensis* and also observed the use of solvent extracted small bitter gourd seed oil at the level of 6-8 ml/kg of legume-pulse grain sample resulted in the improved apparent degree of dehusking from 40.0 to 72.59, 59.88 to 92.44, 63.39 to 87.50 and 57.0 to 79.43 for pigeonpea, chickpea, urdbean and mungbean respectively.

4.1.6. Effect on Oviposition Inhibition

Figure 20 showed the oviposition inhibition rates of pulse beetle at different concentrations of bloodleaf plant extract. The oviposition rates ranged from 3.65 to 28.3%. The maximum and minimum inhibition rates were found at 4 and 1% concentrations, respectively. Oviposition inhibition rates were statistically different ($p \leq 0.05$) in all the concentrations.

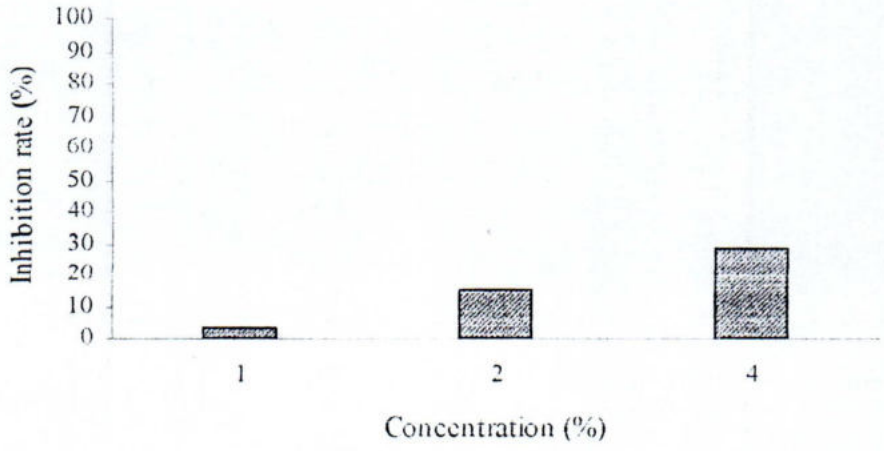


Fig.20: Effect of bloodleaf plant extract on the oviposition inhibition rate of *C. chinensis*

Rahman (1998) treated the wheat grains with 5% powder of pithraj, castor and neem seed against *Sitophilus oryzae*. By releasing insect on 20 days after treatment, the inhibition rate of F_1 progeny of the insect was found to be 24.89, 32.19and 40.06% for pithraj, castor and neem, respectively. As a

result, the inhibition of F_1 *S. oryzae* progeny by neem seed dust was showed better performance for the reason of containing limocinin, azdirol, salanin, nimocinolide and other bioactive compounds. Shahjahan *et al.*, (2000) examined the rice cereals treated with 4% leaf dusts and found that the highest F_1 adult inhibition of rice weevil, *S. oryzae* (43.63%) was observed in case of biskatali and the lowest in case of akanda (39.81%). Amin *et al.*, (2000) reported that the maximum inhibition of F_1 progeny of lesser grain borer (36.83%) was recorded in wheat grains treated with 2% neem dust and the minimum (32.38%) was with akanda. Similar trend of F_1 adult inhibition was also observed in case of 3% and 4% dusts.

Part 2: Effects of Common Cocklebur Leaf Extract on *C. Chinensis*

4.2.1. Direct Toxicity Effect

When the adults were exposing at different concentrations of common cocklebur leaf extract, the highest mortality rate (36 %) was observed in 4% concentration at 2 DAT and the lowest mortality (2%) was observed in control (figure 21). Common cocklebur leaf extract at 1, 2 and 4% concentrations showed significant effect on the mortality of *C. chinensis* at 2 DAT.

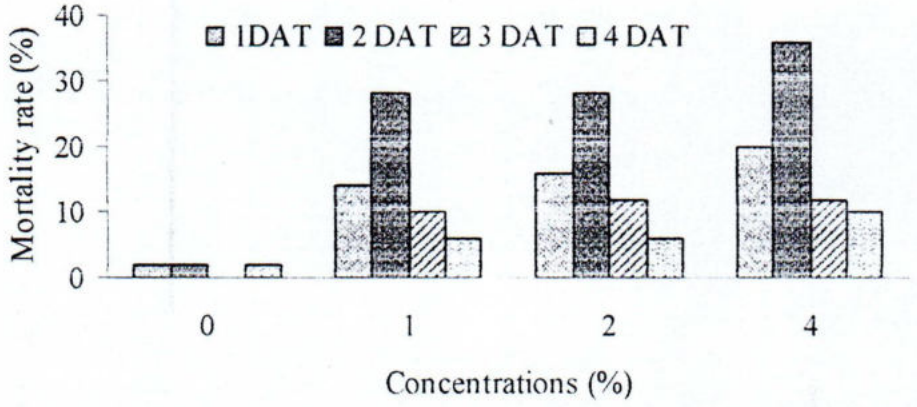


Fig.21: Effect of common cocklebur leaf extract on the mortality rate of *C. chinensis*

Aggarwal *et al.*, (2003) who also observed that 1,8-cineole, one of the components of the essential oil of *Artemisia annwa* showed for toxicity against three stored product coleopterans ; *C. maculatus*, *R. dominica* and *S. oryzae*. The compound was more effective as a fumigant and gave 93-100% mortality at the dose of 1.0 µl/l air under empty jar conditions as compared to treatment of jars filled with grain (11-26% mortality). Rahman *et al.*, (2006) had noted similar toxicity effect of Melgota, *Macaranga postulata* against rice weevil, *Sitophilus oryzae*. They explained that the observed

mortality percentage increased with increase in time. Mortality percentage at 0.25, 050, 0.75, 1.00, and 1.50 hours after treatment (HAT) indicated that 4% solution showed the highest mortality (34.0%) in *S. oryzae* at 1.50 HAT. They also informed that six distinct compounds were found in ethanolic crude of Melgota, *M. postulata* from TLC examination and these six compounds were the toxoic chemicals which caused the mortality of insect.

Raja and William (2008) revealed that the highest mortality activity was recorded in citrodora oil (96%) followed by lemon grass oil (92%) at 96 h of exposure. As a result, toxicity of biochemicals (xanthotoxin, trans-anethole, kulactone, limocinin etc.) of citrodora oil was more effective than lemon grass oil.

4.2.2. Effect as Repellent

In figure 22, it is represented that the repellency effect of 4% common cocklebur leaf extract was higher (60%) than 2% extract (53.3%) and 1% extract (46.7%) at 5HAT but repellent effects of common cocklebur leaf extract at different hours after treatment are statistically indifferent.

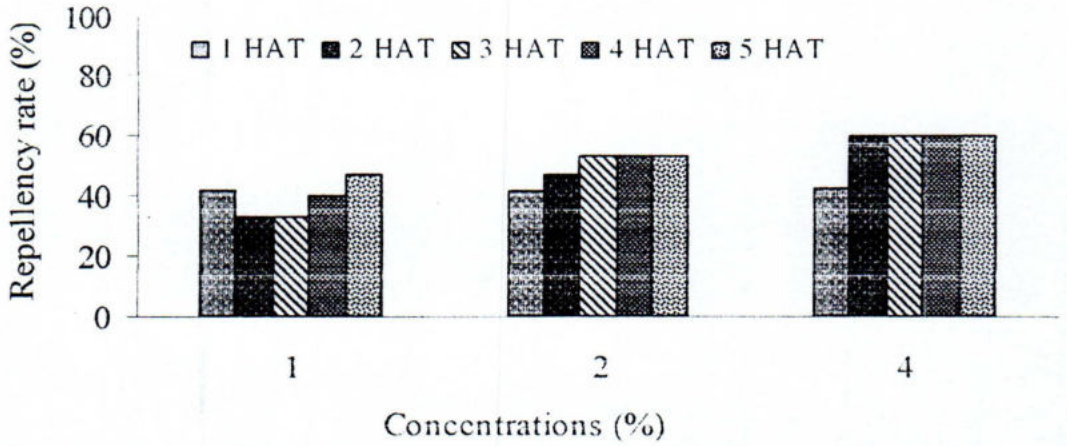


Fig.22: Effect of common cocklebur leaf extract on the repellency rate of *C. chinensis*

Above results were in agreement with Dwivedi and Shekhawat (2004) who observed that Six aboriginal plant species were screened to observe possible repellent action against khapra beetle. Repellent property has been confirmed in all the plant species using olfactometer. Acetone extract of *Emblica officinalis* exhibited maximum repellency (88.66%), whereas minimum repellency was recorded (66.22%) in *Ziziphus jujube* ether extract due to presence of various types of terpenoids, alkanoids, limonoides etc.

Shimizu and Hori (2009) examined six troponoid comoound to compare the repellency effect against adzuki bean beetles. Among the six troponoid compounds, γ-Thujaplicin showed the highest repellency and tropiliden showed the lowest against the beetles. The results suggested that the keto and hydroxyl groups are important in the repellent properties of troponoid compounds.

4.2.3. Effect on Fecundity

Figure 23 shows that the beetles laid the minimum number of eggs (65) on the grain treated with 4% concentration of common cocklebur leaf extract followed by 2% (80.7), 1% (95.7) and control (205). The fecundity of the beetle was statistically different at different concentrations.

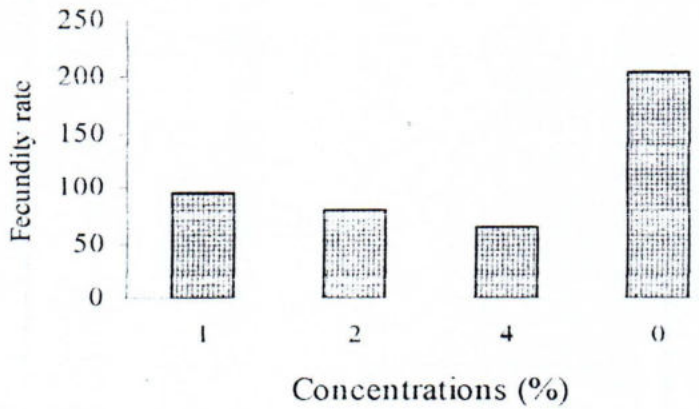


Fig.23: Effect of common cocklebur leaf extract on the fecundity rate of *C. chinensis*

This finding was in agreement with Yadav (2004) who investigatd the effect of vegetable oils on the orientation and oviposition of pulse beetle, *C. maculatus* on green gram. During storage, seasame, coconut, karanja, groundnut and soybean or non-edible oils (mahua, castor, karanja and neem) were mixed with seeds at 10ml/ kg seeds. Seeds were exposed to insect at 1, 10, 30, and 75 days after seed treatment. Neem oil reduced the number of oviposited eggs to 3.58 eggs, compared to untreated (91.25 eggs). Sarkar (2006) observed the pulse beetle *C. chinensis* laid the lowest number of egg (19.33) on the grain treated with 10% concentration of turmeric followed by 7.5% turmeric (37.33), 10% black pepper (45.00), 5% turmeric (60.33), 7.5% black pepper (70.00) and 10% eucalyptus (72.00). As a result, turmeric dust (10%, w/w) showed the best performanec against the fecundity of *C. chinensis* in presencence of cymarin, digitoxin, toosendanin, xanthotoxin, trans-anethole etc.

4.2.4. Adult Emergence Effect

The lowest number of adult emergence (48 %) was found in the grains treated with 4% common cocklebur leaf extract and the highest number of adult emergence (91.7%) was found in control (figure 24). The adult emergence rates were statistically different ($p \leq 0.05$) at all the concentrations.

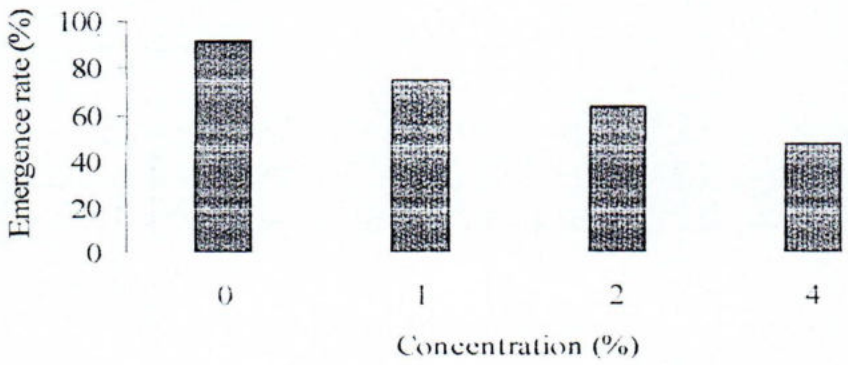


Fig.24: Effect of c. cocklebur leaf extract on the adult emergence rate of *C. chinensis*

Amin *et al.*, (2000) studied the F_1 adult emergence of lesser grain borer, *R. dominica* and reported that the lowest number of F_1 lesser grain borer (25.40%) emerged from wheat grains treated with 2% biskatali and neem leaf dusts. Almost similar trend of F_1 adult emergence was observed in the cases of 3% and 4% dust applications. Plant materials possessed some chemicals viz. azadhirachtin, nicotin, cymarin, digitoxin etc biochemicals were toxic chemicals that showed reducing of F_1 adult emergence on insect. The present study plants may have some toxic chemicals that have showed toxicity on pulse beetle. Kemabonta *et al.*, (2002) observed the similar F_1 adult emergence effect of *Chenopodium ambroisoides* against *C. maculatus*. They also reported application of *C. ambroisoides* (5.0% extract) reduced emergence of F_1 adults to (55%) as compared to the control (81%). As a result, *Chenopodium ambroisoides* must be contained some allelochemicals such as terpenoids, limonoids, flavonoids, alkaloids etc. Hussein *et al.*, (2005) applied Saponin extract from alfalfa roots, azadirachtin from the neem seed oil, synthetic ecdysteroid agonist RH-2485, and the juvenoid hydroprene disturbed the development and reproduction of *Tropinota squalida*. They also possessed that the larvae of untreated adults were fed for 1 week on dung with 75 p.p.m. saponins, 50 p.p.m. RH-2485, and 0.45 p.p.m. azadirachtin, the rate of adult emergence drops from 80% (controls) to 20, 0 and 13%, respectively. No adults emerge when the treatment is continued through the second and third larval instars. Two topical treatments of larvae with 0.2 μg hydroprene decrease the rate of adult emergence from 90 to 11%, and treatments with 2 μg prevent adult development in all insects.

4.2.5. Seed Damage Effect

Figure 25 showed that the minimum seed damage rate (26%) was found at 4% concentration of common cocklebur leaf extract and the maximum (87.3 %) was found in control, and seed damage rates at all concentrations were statistically different.

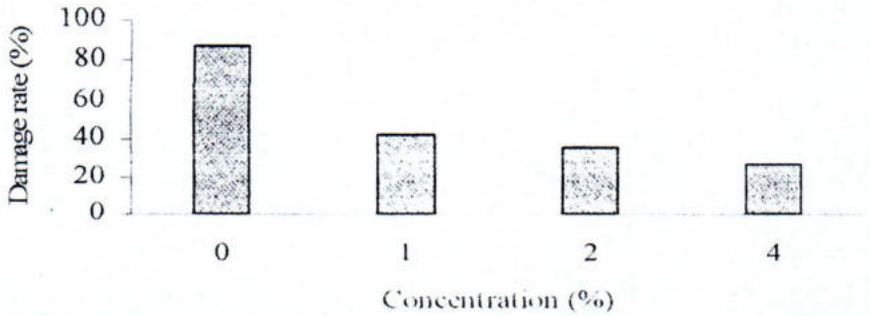


Fig.25: Effect of c. cocklebur leaf extract on seed damage rate of *C. chinensis*

Shahjahan *et al.*, (2000) observed the minimum grain damage rate of rice weevil, *S. oryzae* (16.00%) was rice cereals were treated with 2% neem dust followed by biskatali (19.40%) and akanda (19.80%). Almost similar trend was observed when treated the cereals with 3% and 4% dusts. Seed damage rate of neem leaf dust showed better performance for the reason of containing limociin, azdirol, salanin, nimocinolide and other bioactive compounds. Amin *et al.*, (2000) reported in presence of bioinsecticidal substances such as nimbinin, azadiractin, kulactone, limocinin etc, the lowest grain damage rate of lesser grain borer, *R. dominica* (16.40%) was recorded from the wheat sample treated with 2% neem dust followed by biskatali (18.18%) and akanda (20.40%). Umarao and Verma (2002) assessed the efficacy of various plant products; leaf powder of dharek, *Melia azadarach* and sadabahar, *Ipomoea carnea* at 10 g/kg grain and oils of coconut, mustard, and ground nut and neem products such as achook, nimbicidine and neem gold at 1ml/kg pulse beetle *Callosobruchus chinensis* based on the percentage of grains damage. Nimbicidine and achook appeared to be the most effective in minimizing the damage.

4.2.6. Oviposition Inhibition Effect

The highest oviposition inhibition rate (47.5%) was found in 4% common cocklebur leaf extract and the lowest (18.1%) was found in 1% extract (figure 26).Oviposition inhibition rates were statistically different ($p \leq 0.05$) at all the concentrations.

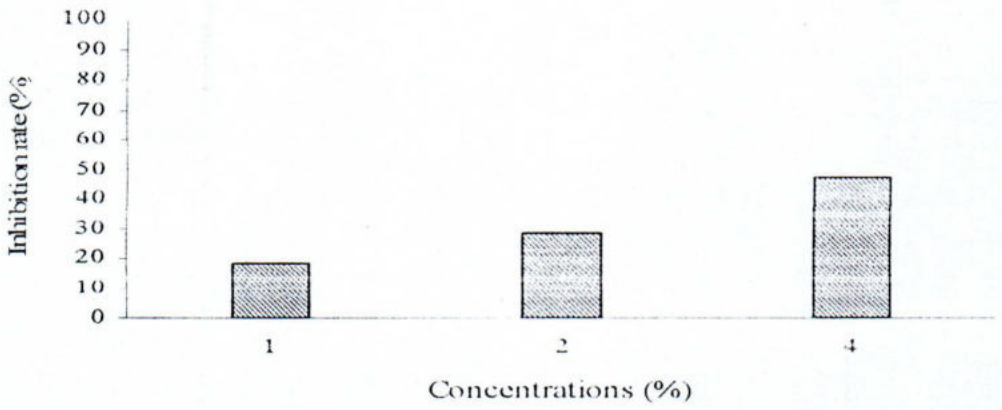


Fig.26: Effect of c. cocklebur leaf extract on the inhibition rate of *C. chinensis*

Raguraman *et al.*, (1997) ovserved the effects of oils obtained from neem (*Azadirachta indica*) seed kernel, the himalayan cedar wood, *Cedrus deodara* and their combination (1:1), at 3, 2 and 1% concentrations against adults of pulse beetle *Callosobruchus chinensis*. Cedar wood oil exhibited highest fumigant potential and at 3, 2 and 1% concentrations showed corrected inhibition (knock-down) of 100, 100 and 96%, respectively. While neem oil + cedar wood oil (1:1) at 3, 2 and 1% concentrations inhibited 96, 84 and 51.66%, and bioinsecticidal chemicals (tannins, flavanoids, benzopyran, steroids, terpenoids etc) were increased the inhibition rate of cedar wood oil.

Shahjahan *et al.*, (2000) treated rice cereals with 4% leaf dusts and reported that the highest F_1 adult IR (%) of rice weevil, *S. oryzae* (43.63%) was observed in case of biskatali and the lowest in case of akanda (39.81%).

Part 3: Effects of Common Cocklebur Fruit Extract on *C. Chinensis*

4.3.1. Effect on Direct Toxicity

Figure 27 showed the efficacy of common cocklebur fruit extract on black gram as protectant by direct toxicity by direct exposing the target species at different concentrations at different DAT. When the adults were exposing at different concentrations of common cocklebur fruit extract, the highest mortality rate (26 %) was observed in 4% concentration at 2 DAT and the lowest rate (2%) was observed in control. Toxicity effects at different concentrations of common cocklebur fruit extract at 1 DAT, 3 DAT and 4 DAT were statistically indifferent.

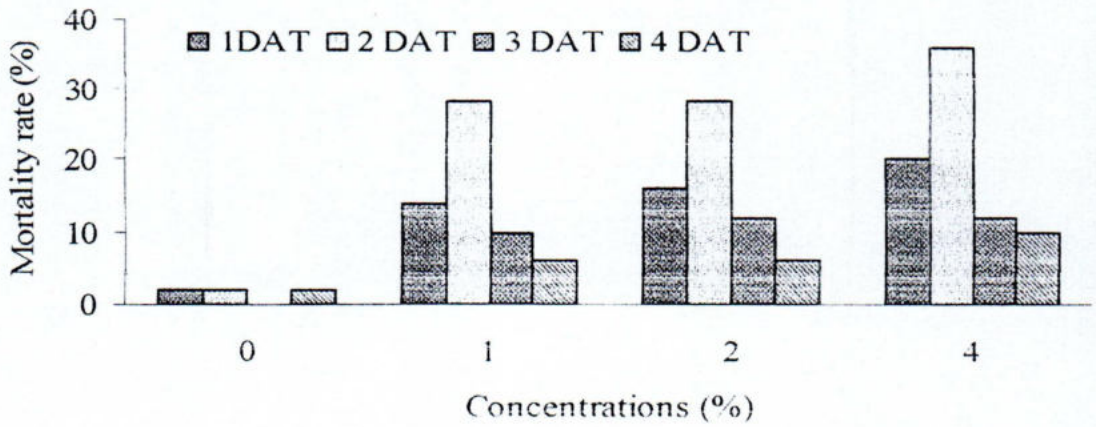


Fig.27: Effect of common cocklebur fruit extract on the mortality rate of *C. chinensis*

Shahjahan *et al.*, (2000) studied the toxicity effect of biskatali, neem and akanda plant extract on rice weevil and reported that 4% biskatali extract have strong toxicity effect (62.02%). Plant materials possessed some chemicals viz. azadirachtin, cymarin, digitoxin, toosendanin, xanthotoxin, etc are toxic chemicals that showed mortality effect on insect. The present study plants may have some toxic chemicals that have showed toxicity on

pulse beetle. Arabi *et al.*, (2008) observed the fumigant toxicity of *P. abrotanoides* oil when tested against 1- to 7-day-old adults of *S. oryzae* and *T. castaneum*. The lowest concentration (32 µl/l air) of the oil induced 100% mortality of *S. oryzae* and *T. castaneum* after 15 and 8 h exposure. At the higest dose (645 µl/l air), the LT_{50} values (lethal time for 50% mortality) were 8 and 2.84 h for *S. oryzae* and *T. castaneum*, respectively. Sahayaraj *et al.*, (2008) reported that impact of ethanol extract of *Pedalium murex* root (0.1, 0.2, 0.4 and 0.8%) were screened for its antifeedant and insecticidal activities against third, fourth and fifth instar larvae of *Spodoptera litura* by leaf-dip method. The larval mortality was more than 50 percent at higher concentration (0.8%) in the ethanol root extract. Qualitative analysis of *P. murex* root extract revealed that it contains phytochemical such as, steroids, terpenoids, phenolics, saponines, tannins and flavanoids. Phenol, 2-(5,6-dimethyl pyrazinyl) methyl (molecular weight 214); O-Terphenyl-13C (molecular weight 230) and 3, 3A, 4, 9B - Tetrahydro- 2H-Furo (3, 2-C) (1) Benzopyran (molecular weight 206).

4.3.2. Effect as Repellent

The repellency effects at different concentrations of common cocklebur fruit extract at different HAT have presented in figure 28. The highest repellency rate (53.3 %) was found after 3HAT when 4% common cocklebur fruit extract was applied as repellent. The lowest repellency rate (26.7%) was observed after 2HAT when 1% concentration of common cocklebur fruit extract was applied as repellent. It is also stated that repellency effects at different concentrations of common cocklebur fruit extract at 1HAT to 5 HAT were statistically indifferent.

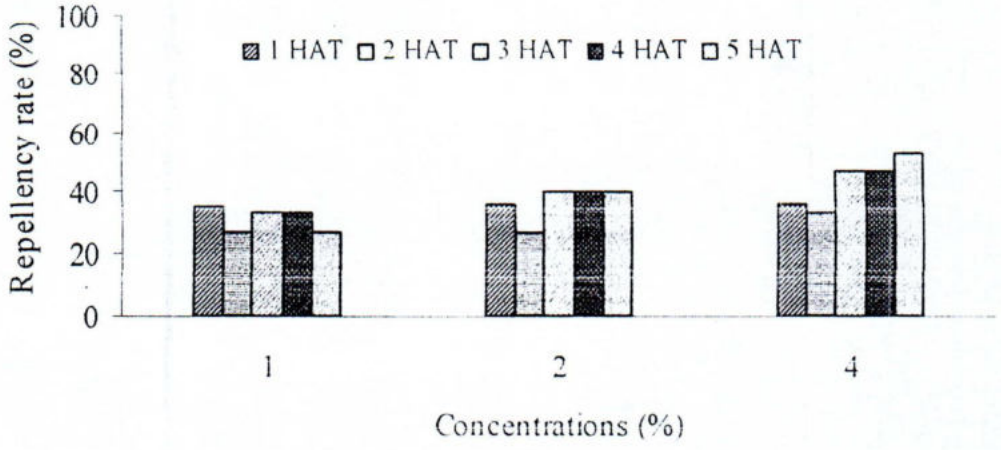


Fig.28: Effect of common cocklebur fruit extract on the repellency rate of *C. chinensis*

Amin *et al.*, (2000) studied the toxicity effect of biskatali, neem and akanda plant extract on lesser grain borer and reported that among the extracts, 2% akanda, *Asclepios calotropis* showed the lowest repellency (37.6%) while higest repellency (77.6%) was found with 4% biskatali and neem. Plant materials possessed some chemicals viz. azadhirachtin, nicotin, cymarin, digitoxin etc biochemicals were toxic chemicals that showed mortality effect on insect. The present study plants may have some toxic chemicals that have showed toxicity on pulse beetle. Rahman *et al.*, (2006) assessed that 1% fruit extract of Melgota, *M. postulata* showed the lowest repellency (9.84%) of rice weevil. On the other hand, 2% showed 12.76% and 4% showed 22.43%, respectively. They also obsertved that TLC of crude ethanol extract of Melgota, *Macaranga postulata* showed six distinct compounds which were responsible for repellency of *S. oryzae*. Shimizu and Hori (2009) compared the repellency and toxicity of six troponoid compounds against adzuki bean beetles and examined the relationship between their structure and activity. They also found that γ-Thujaplicin showed the highest repellency against the beetles among the compounds tested. The results suggested that the keto and hydroxyl groups are important in the repellent properties of troponoid compounds.

4.3.3. Effect on Fecundity

Figure 29 shows the effect of different concentration of common cocklebur fruit extract on the number of eggs laid by the female of *C. chinensis*. Results showed that the fecundity rates of the beetles were lowest (113.7) at 4% concentration followed by 2% (140), 1% (158.7) and control (185) and there were significant difference.

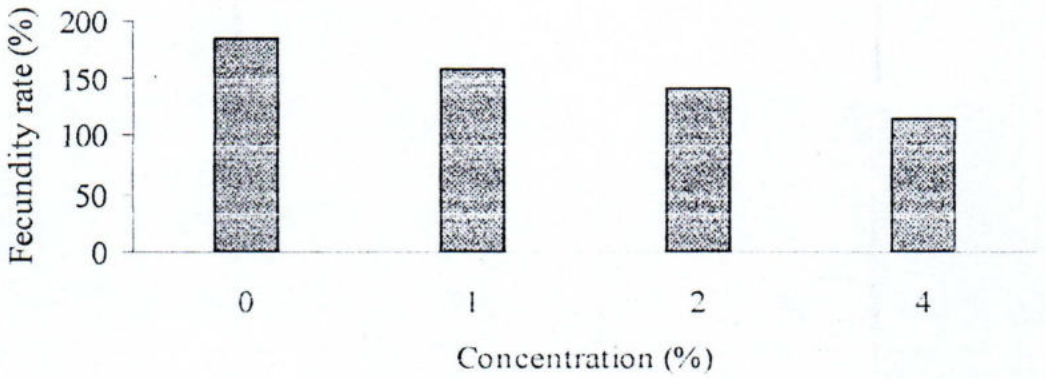


Fig.29: Effect of common cocklebur fruit extract on the fecundity rate of *C. chinensis*

This finding showed agreement with Salunke *et al.*, (2005) who possessed the partially purified flavonoids from *C. procera* and showed that the highest toxicity against *C. chinensis* followed by standard quercetin, rutin and quercitrin at 10 mg ml^{-1} doses in filter paper diffusion assay. Significant reduction in oviposition was found for all partially purified flavonoids at the doses of 5 and 10 mg ml^{-1} on grains in plastic jars respectively.

4.3.4. Effect on Adult Emergence

In figure 30, the lowest number of adult emergence (56.7%) was found in the grains treated with 4% common cocklebur fruit extract and the highest number of adult emergence (87%) was found in control. The adult emergence rates were statistically different at different concentrations.

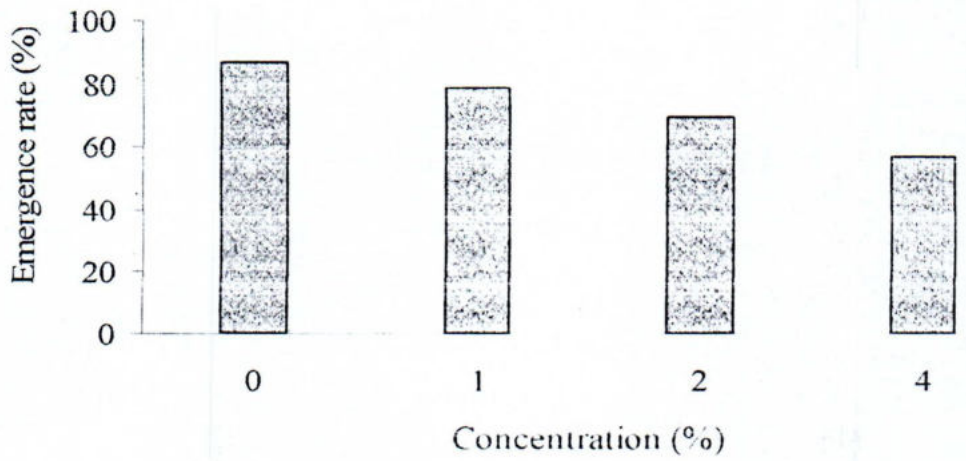


Fig.30: Effect of c. cocklebur fruit extract on the adult emergence rate of *C. chinensis*

Above results also conducted with Hussein *et al.*, (2005) who stated that saponin extract from alfalfa roots, azadirachtin from the neem seed oil, synthetic ecdysteroid agonist RH-2485 and juvenoid hydroprene disturbed the development and reproduction of *Tropinota squalida*. They also possessed that when the larvae of untreated adults were fed for 1 week on dung with 75 p.p.m. saponins, 50 p.p.m. RH-2485, and 0.45 p.p.m. azadirachtin, the rate of adult emergence droped from 80% (controls) to 20, 0 and 13%, respectively. No adults emerged when the treatments were continued through the second and third larval instars. Sathyaseelan *et al.*, (2008) had reported the similar F_1 adult emergence effect of indigenous pesticidal plants viz., *Prosophis sp.*, *Nerium sp.*, *Ocimum sp.*, *Acalypha sp.*, *Catheranthus sp.*, and *Vitex sp.* against pulse beetle *C. chinensis* in green gram. At 5% level, leaf extract of *Vitex sp.* caused maximum reduction in adult emergence (85.0%) followed by *Catheranthus sp.* (83.7%), *Acalypha sp.*(73.3%), *Nerium sp.* (70.0%), *Ocimum sp.*(68.7%) and minimum reduction was recorded in case of *Prosophis sp.*(68%). So, the effecacy of biochemicals such as cymarin, digitoxin, toosendanin, xanthotoxin, trans-anethole etc of *Vitex sp.* leaf extract was more than others.

4.3.5. Effect on Seed Damage

From figure 31, it is confirmed that the maximum seed damage rate (91 %) was found in control and the minimum seed damage (42.3%) was found in the grains treated with 4% concentration of common cocklebur fruit extract. All the concentrations revealed significant difference with control ($p \leq 0.05$)

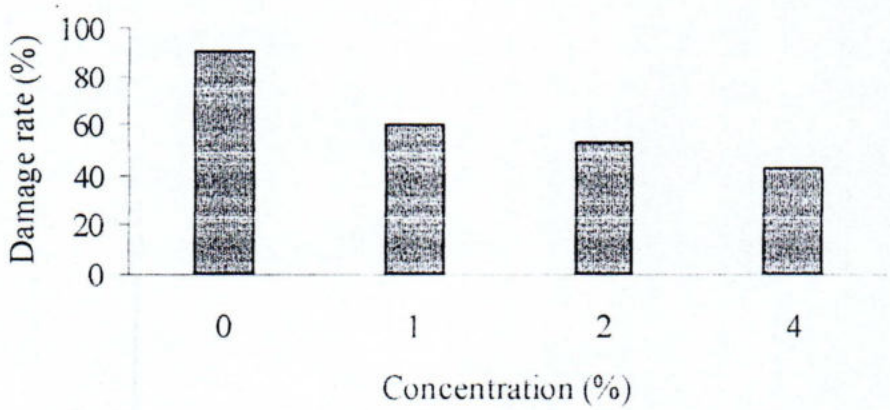


Fig.31: Effect of common cocklebur fruit extract on seed damage rate of *C. chinensis*

Mishra *et al.*, (2006) cited the grain damage effect of vegetable seed oils of Cucurbitaceae family against *C. chinensis* and observed that the use of solvent extracted small bitter gourd seed oil at the level of 6-8 ml/kg of legume-pulse grain sample resulted in the improved apparent degree of dehusking from 40.0 to 72.59, 59.88 to 92.44, 63.39 to 87.50 and 57.0 to 79.43 for pigeonpea, chickpea, urdbean and mungbean, respectively. As a rersult, small bitter gourd seed oil must be contained some bioinsecticidal chemicals such as *trans*-phytol, linalool, *trans*-2-methylcyclopentanol, *β*-caryophyllene, *m*-Cymene, nonanal, l-*α*-terpineol, *β*-cyclocitral, nerol, *trans*-geraniol, carvacrol, *β*-ionone, *transformations*, nerolidol etc. Koona *et al.*, (2007) observed powdered dried leaf of *Tephrosia vogelii* for their

ability to protect stored maize from damage by *S. zeamais*. The hexane extract had a relatively high efficacy to protect seed damage averaging 8.8% compared with 98.6% in the untreated control after one generation. This insecticidal activity ensured that terpenoids, alkaloids, limonoids and flavonoids and other natural substances were remained in hexane extract of *T. vogelii* leaf.

4.3.6. Effect on Oviposition Inhibition

Figure 32 showed the highest oviposition inhibition rate (37%) in 4% common cocklebur fruit extract and the lowest inhibition rate (11%) was in 1% extract. Oviposition inhibition rates were statistically different ($p \leq 0.05$) at all the concentrations.

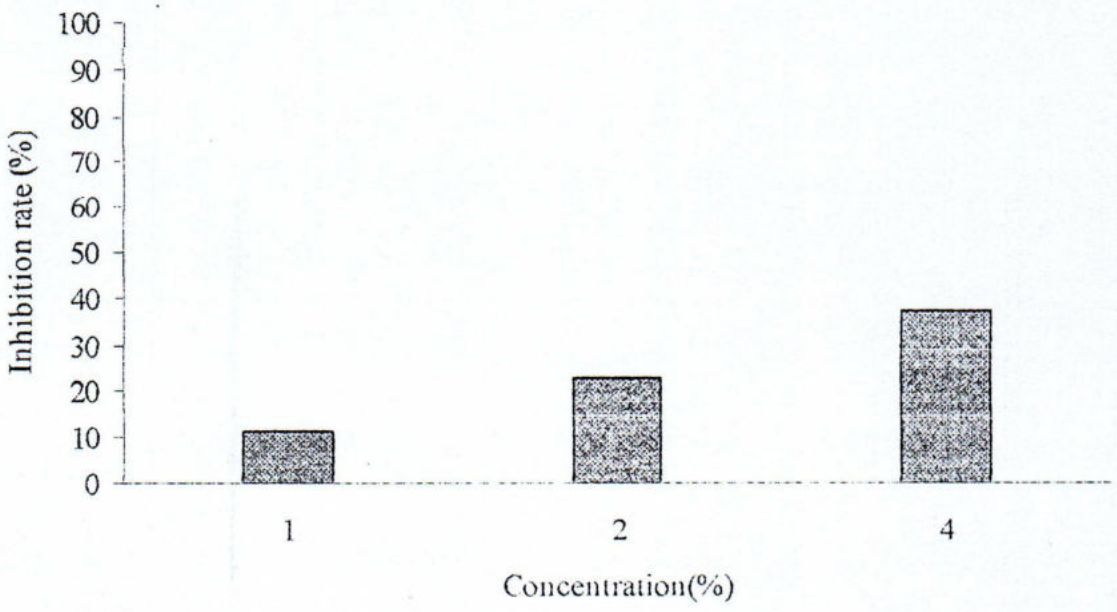


Fig.32: Effect of c.cocklebur fruit extract on the inhibition rate of *C. chinensis*

Rahman (1998) treated the wheat grains with 5% powder of pithraj, castor and neem seed against *Sitophilus oryzae*. By releasing insect on 20 days after treatment the inhibition rate of F_1 progeny of the insect was found to be 24.89, 32.19and 40.06% for pithraj, castor and neem respectively. As a

result, the inhibition of F_1 *S. oryzae* progeny by neem seed dust was showed better performance for the reason of containing limocinin, azdirol, salanin, nimocinolide and other bioactive compounds.

Amin *et al.*, (2000) observed the inhibition of F_1 progeny of lesser grain borer, *R. dominica* when feeding wheat grain treated with plant extract. They also reported that the maximum IR (%) (36.83%) was recorded in wheat grains treated with 2% neem dust and the minimum IR (%) (32.38%) was with akanda. Similar trend of F_1 adult inhibition was also observed in case of 3% and 4% dusts. The biochemicals (nimbinin, azadiractin, salanin, nimocinolide, kulactone, limocinin, azdirol etc) of neem leaf dust were affacted the F_1 adult emergence of *R. dominica*.

4.4. Chemical Investigation on Aqueous Extract of Common Cocklebur Leaf:

The results in this experiment indicate that the aqueous extract of common cocklebur leaf showed insecticidal activities on pulse beetle. The above interesting results encourage us to take why and how this type of plant is responsible for insecticidal activities. For this reason, the crude compounds were extracted from the powder of respective plant species with non-polar and polar solvents like chloroform and ethanol, respectively. The crude compound was then procceded for TLC examination.

4.5. TLC (Thin Layer Chromatography) of Ethanol Crude Extract of Common Cocklebur Leaf (Xanthium strumarium):

The TLC (Thin Layer Chromatography) of ethanol extract of common cocklebur leaf (*Xanthium strumarium*) was showed distinctly two compounds at Hexane: Ethylacetate (7:1 v/v, fig.33), this result suggested that it contained two distinct compounds, designated as S1 and S_2,

respectively. These compounds were detected in iodine tank and the following R_f values were calculated by using the formula (Furniss *et al.*, 1989). R_f value of crude extract at different solvent system were mentioned in table -1.

Name of plant species	Ratio of Hexane and Ethylacetate	Detected component	R_f value
Common Cocklebur Leaf (Xanthium strumarium)	7:1	S_1	0.397
		S_2	0.161
	5:1	S_1	0.66
		S_2	0.56

Table 1. R_f values of detected components of c. cocklebur leaf extract

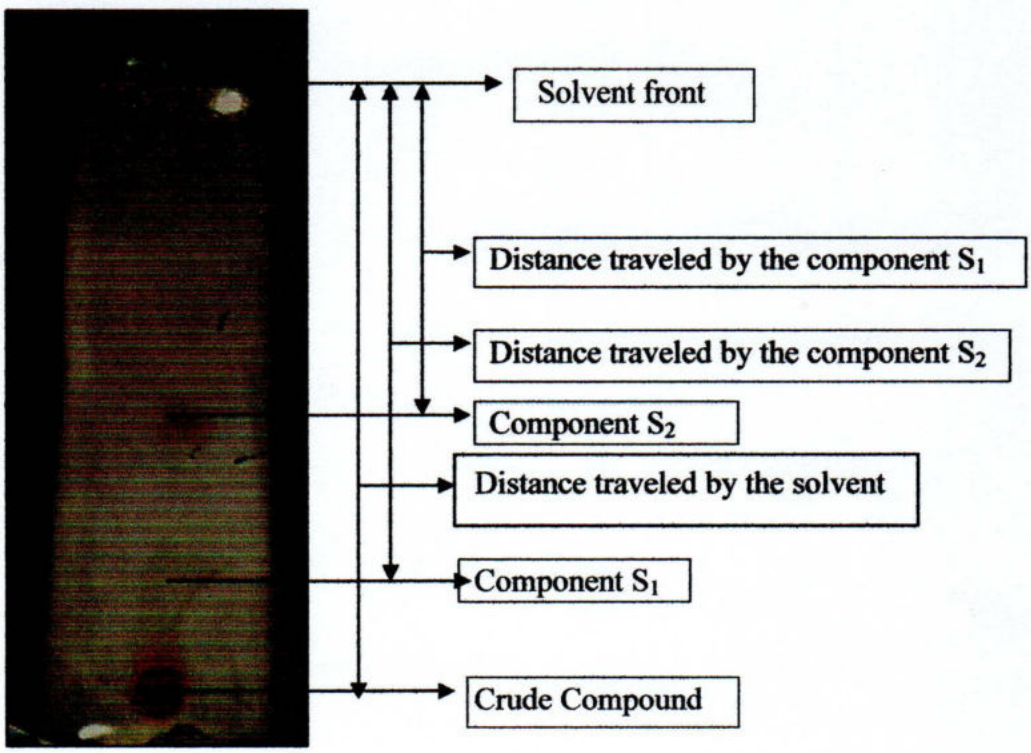


Fig.33: Determination of R_f value

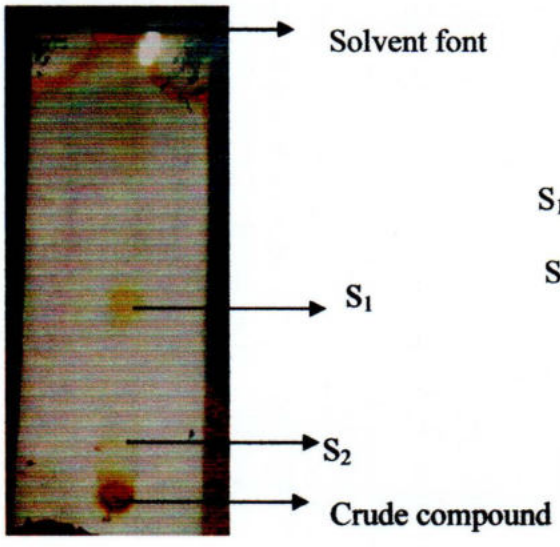


Fig.34: TLC of ethanol crude extract
Plant Species: (*Xanthium strumarium*)
Bangla Name: Shakto Ghagra
English Name: Common Cocklebur
Solvent Ratio: Hexane: Ethylacetate (7:1)

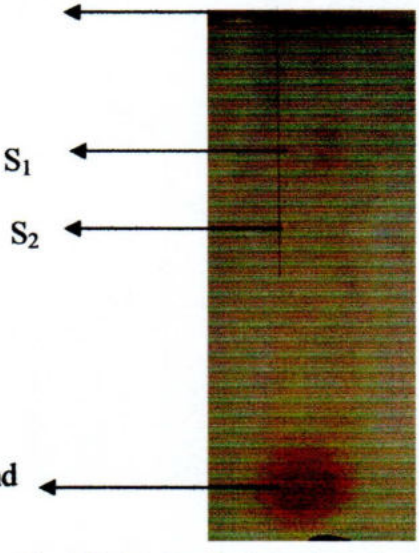


Fig.35: TLC of ethanol crude extract
Plant Species: (*Xanthium strumarium*)
Bangla Name: Shakto Ghagra
English Name: Common Cocklebur
Solvent Ratio: Hexane: Ethylacetate (5:1)

4.6. Column Chromatography of Chloroform Crude Extract of Common Cocklebur Leaf (Xanthium strumarium):

The crude extract of common cocklebur leaf was undertaken for column chromatography eluting with hexane: ethylacetate (35:1, 25:1, 10:1, 5:1 and 1:1) respectively. The fractions S_1 and S_2 were collected respecto found in right way eluting with hexane: ethylacetate (1:1, v/v). Comparative TLC examination (fig.36) of the above fractions indicated that clear single spot. The all fractions of S_1 and S_2 were combined together and were collected in different round bottom flasks. The solvent of S_1 and S_2 were evaporated under reduced pressure using a Thin Layer Rotary Film Evaporator,which were then stored in refrigerator for further study.

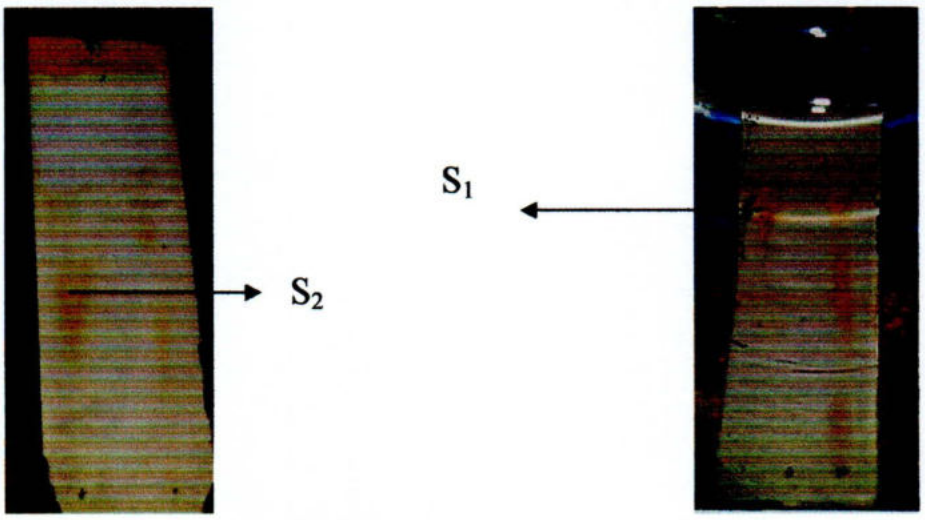


Fig.36: Comparative TLC of crude compound and purified compound after Column Chromatography
Bangla Name: Shakto Ghagra
English Name: Common Cocklebur
Solvent Ratio: Hexane: Ethylacetate (2:1)

4.7. Spectral Study for Determination of Structure of Purified Compounds:

4.7.1. Infrared Spectroscopy (IR) Study:

IR of S_1 ($CDCl_3$): υmax = 3224 cm^{-1}(br), 2958(s), 2343(s), 1728(s), 1273(br), 1122.5(s), 1072.3(s), 1039(s), 960.5(br).

IR of S_2 ($CDCl_3$): υmax = 3385 cm^{-1}(br), 2362(br), 1728.1(s), 1458(br), 1343(s), 1273(br), 1122(s), 1072(s), 1039(s), 959(br).

4.7.2. ^{1}H-NMR Spectroscopy Study:

^{1}H-NMR of S_1 (400 MHz, $CDCl_3$): δ = 7.69(m), 7.52(m), 4.21(m), 2.1-2.3 (m), 1.67(m), 1.31(m), 0.929(t).

^{1}H-NMR of S_2 (400 MHz, $CDCl_3$): δ = 2.15(m), 1.91- 1.75(m), 1.24 (m), 0.93- 0.89(m).

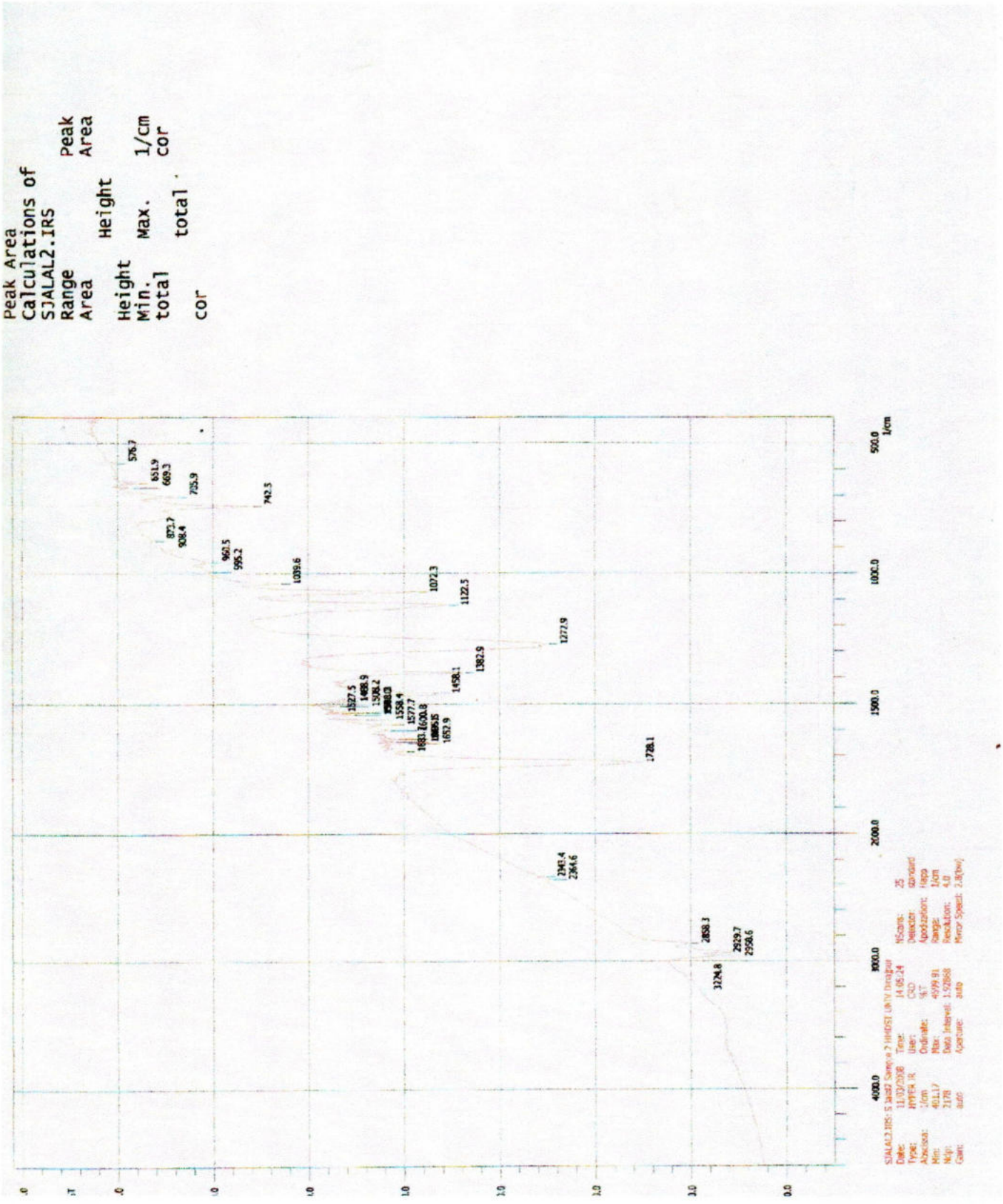
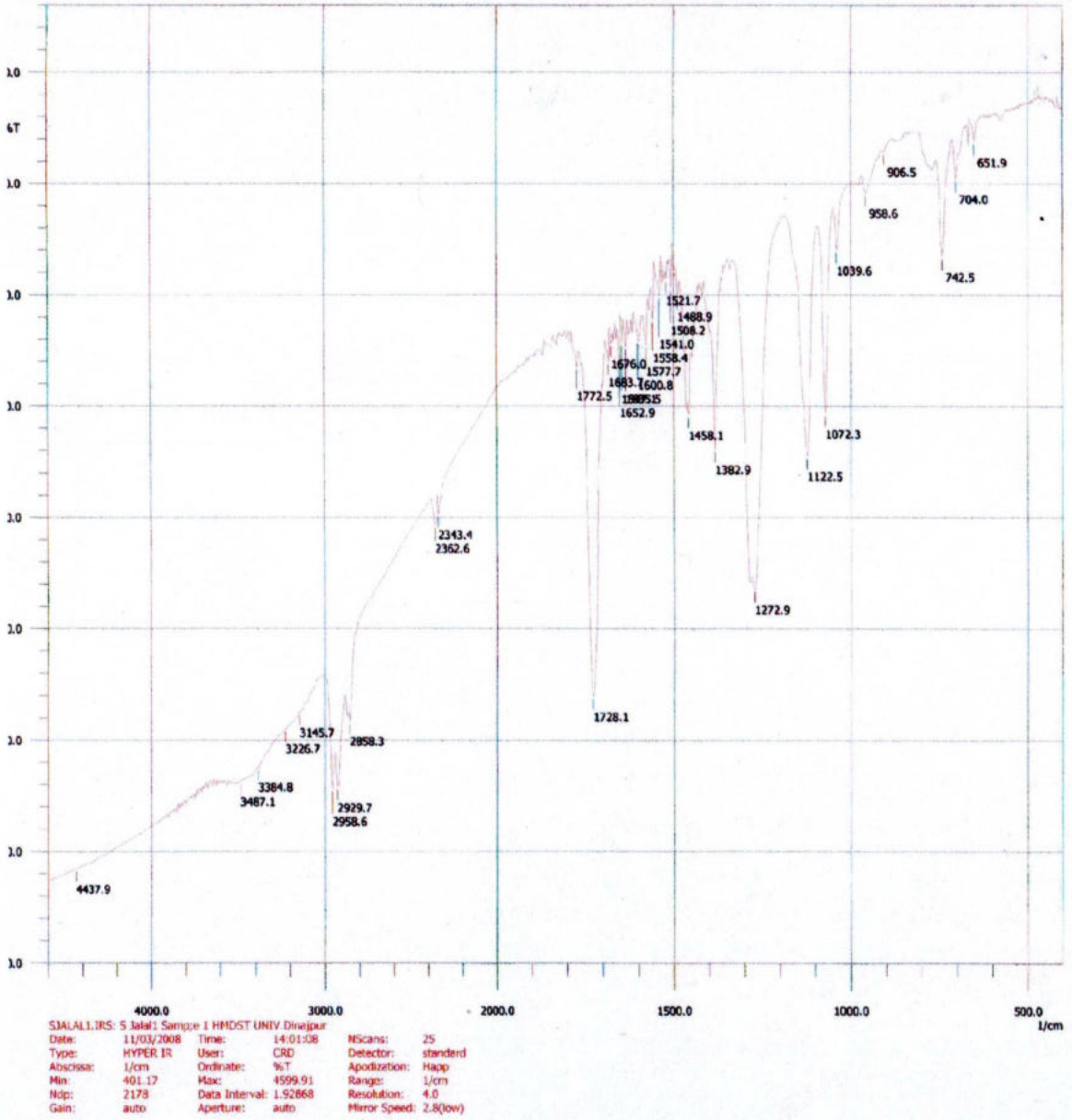


Fig.38: IR spectrum of S_2



Peak Area Calculations of SJALAL1.IRS

Range Area		Peak Area Height		Height
Min.	Max.	1/cm	total	cor total	cor

Fig.37: IR spectrum of S_1

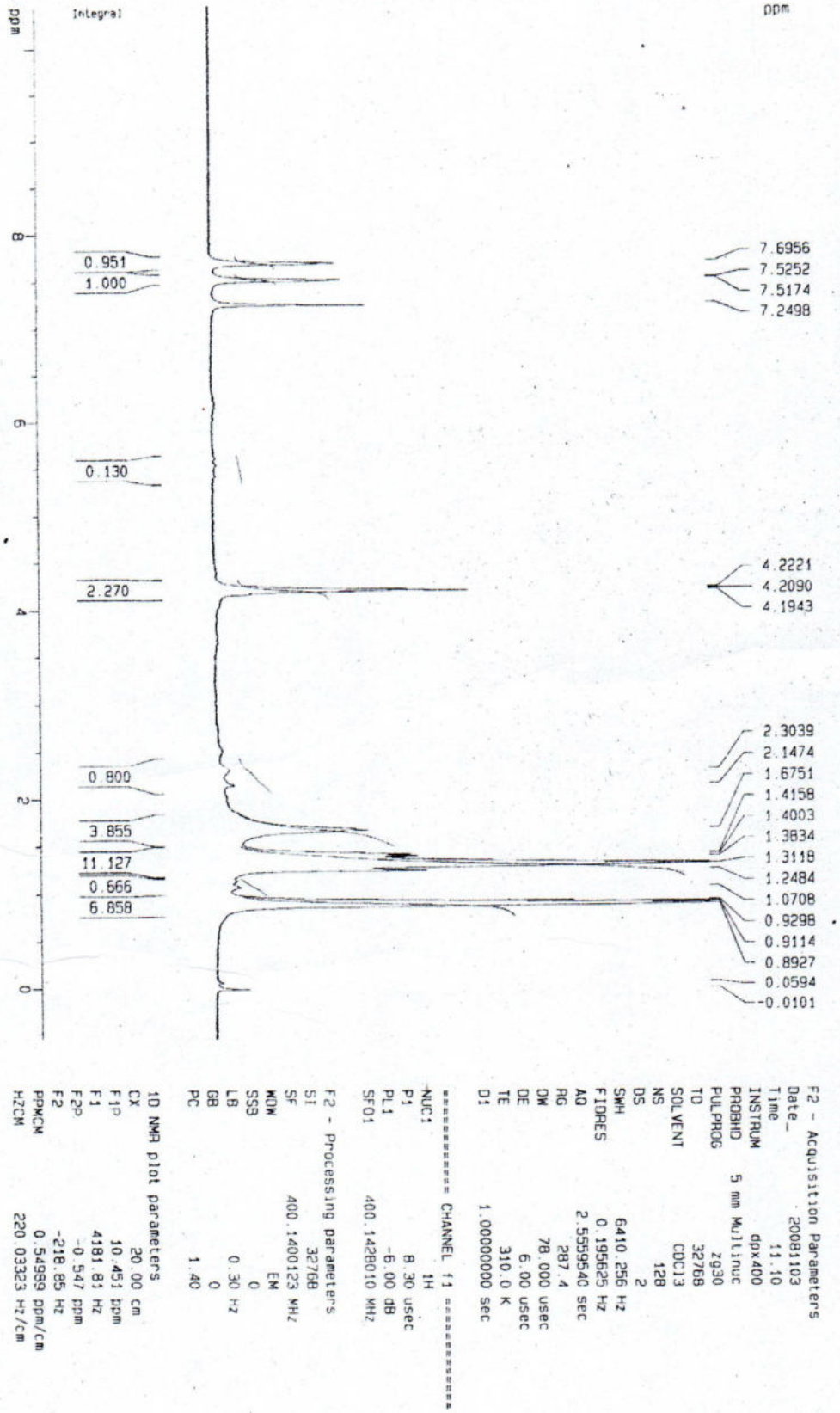


Fig. 39: ^{1}H-NMR of S_1 (a)

Fig. 40: ^{1}H-NMR of S_1 (b)

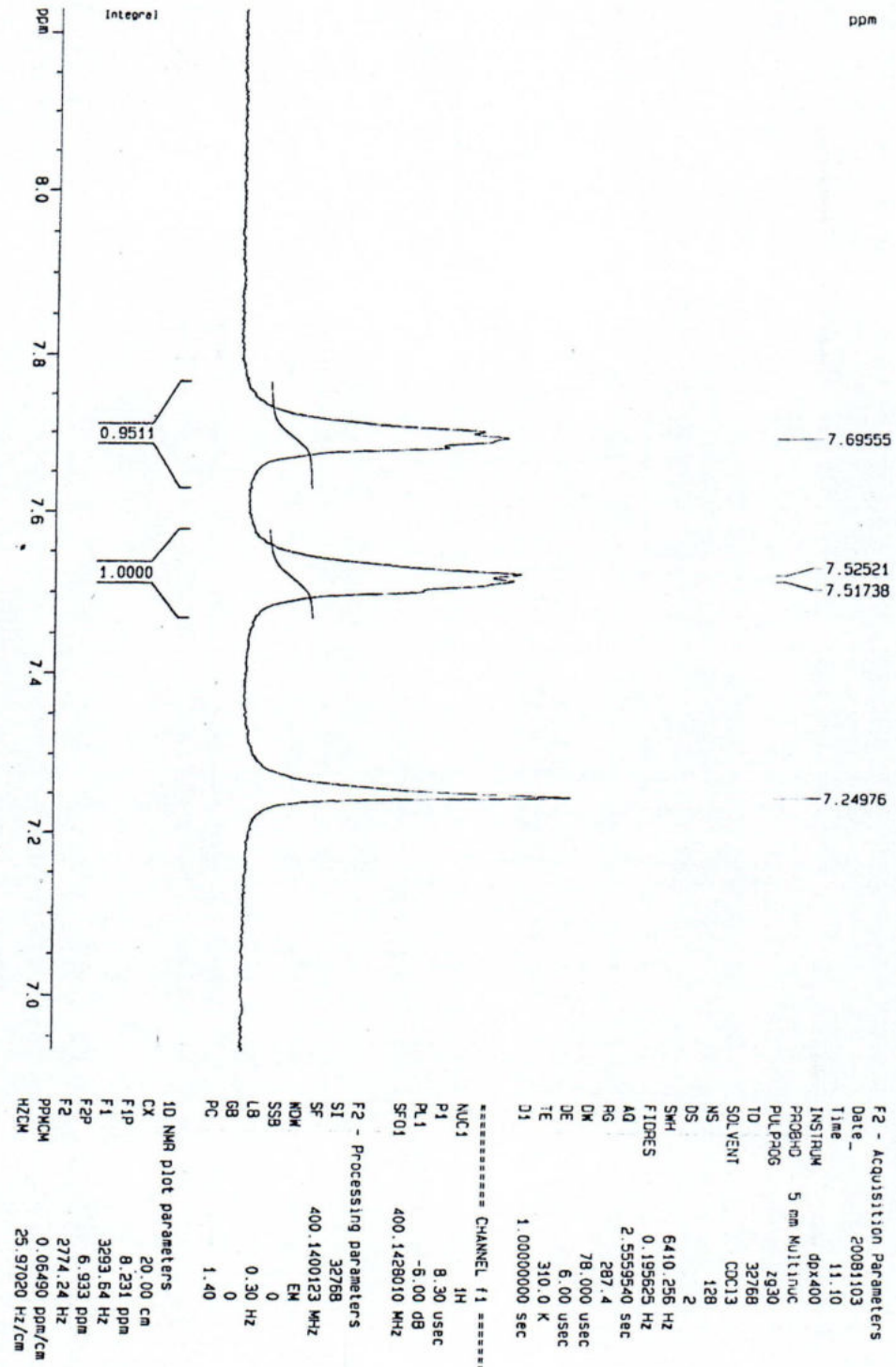


Fig.41: ^{1}H-NMR of S_1 (c)

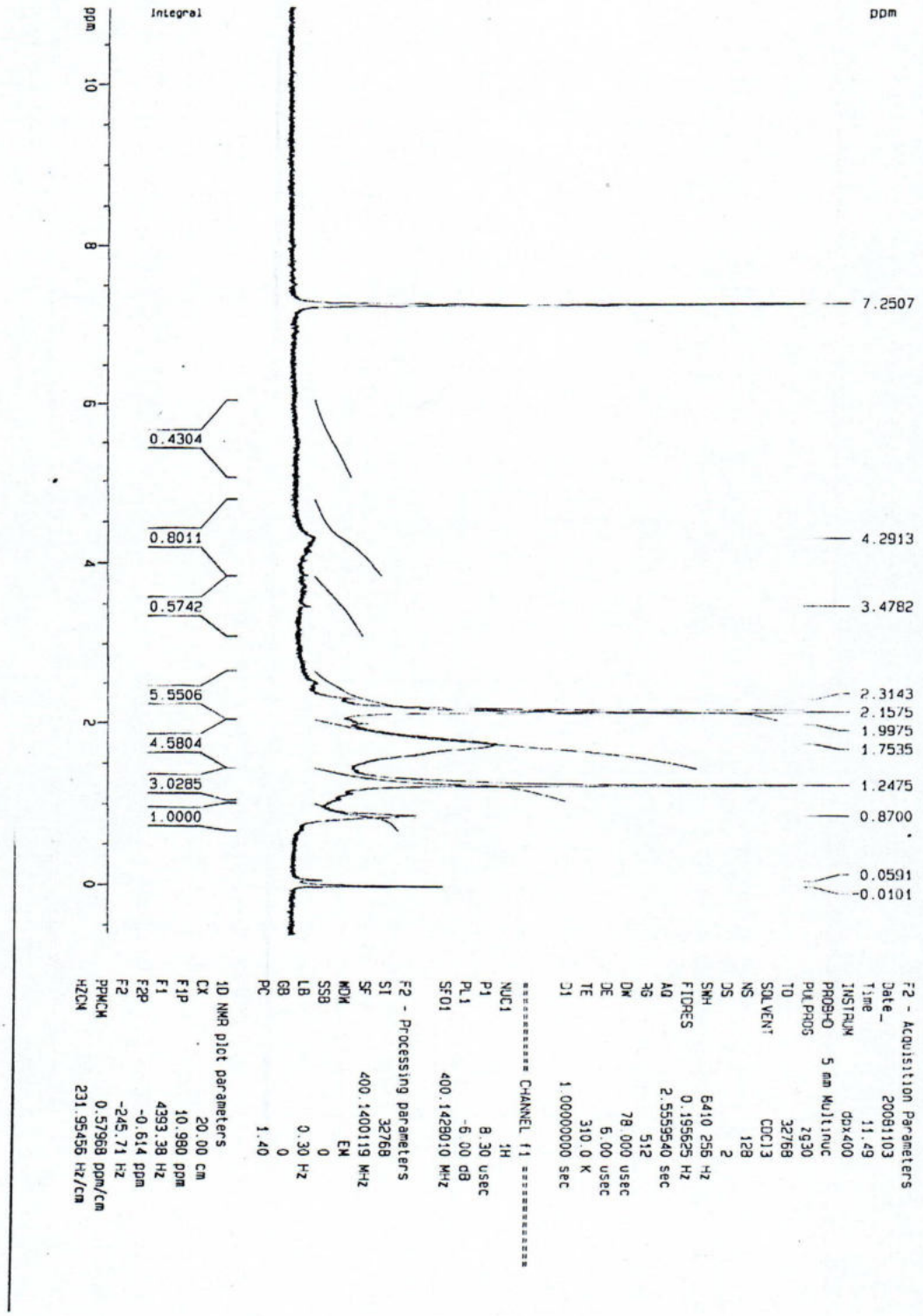


Fig.42: ^{1}H-NMR of S_2 (a)

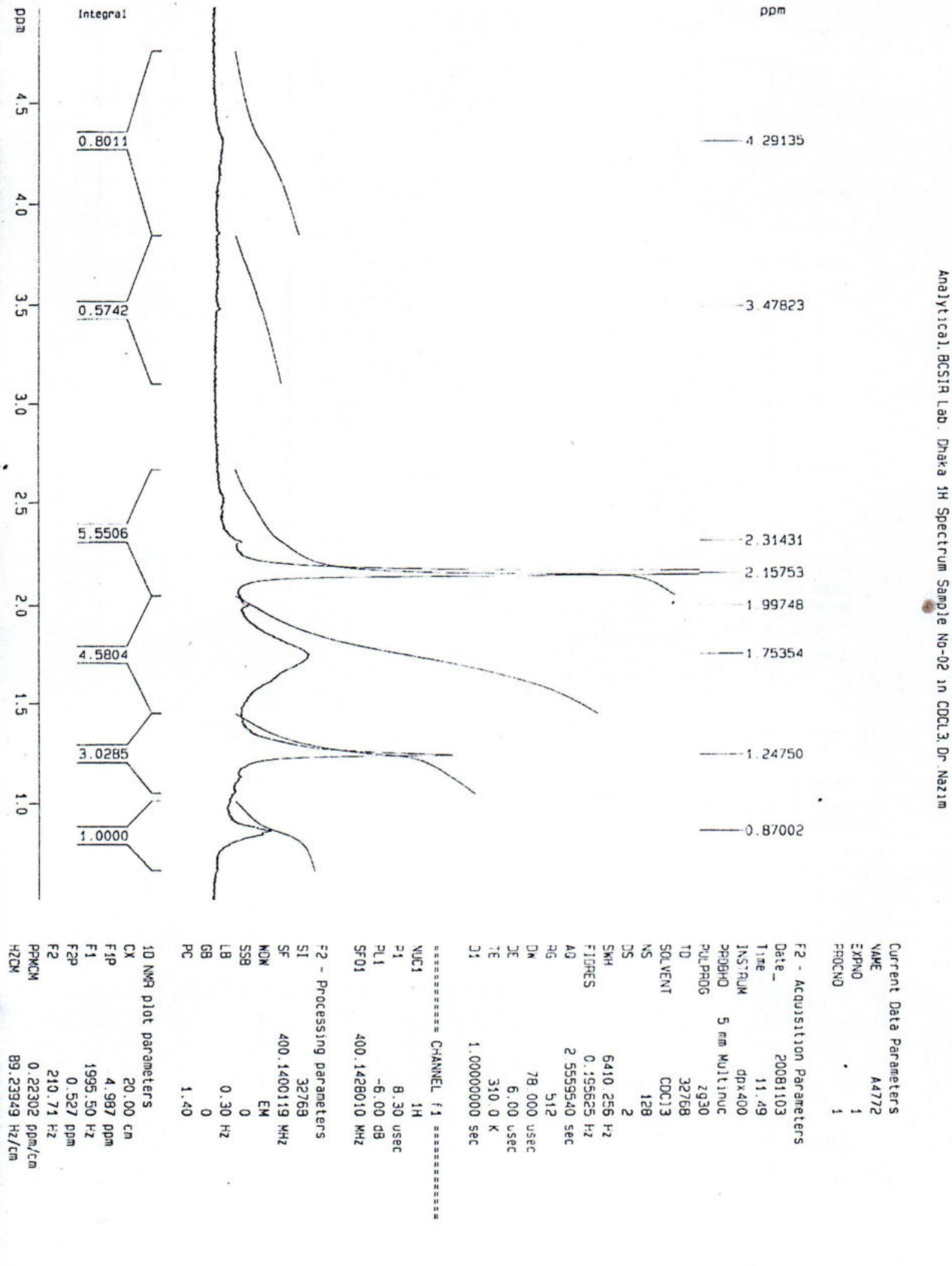
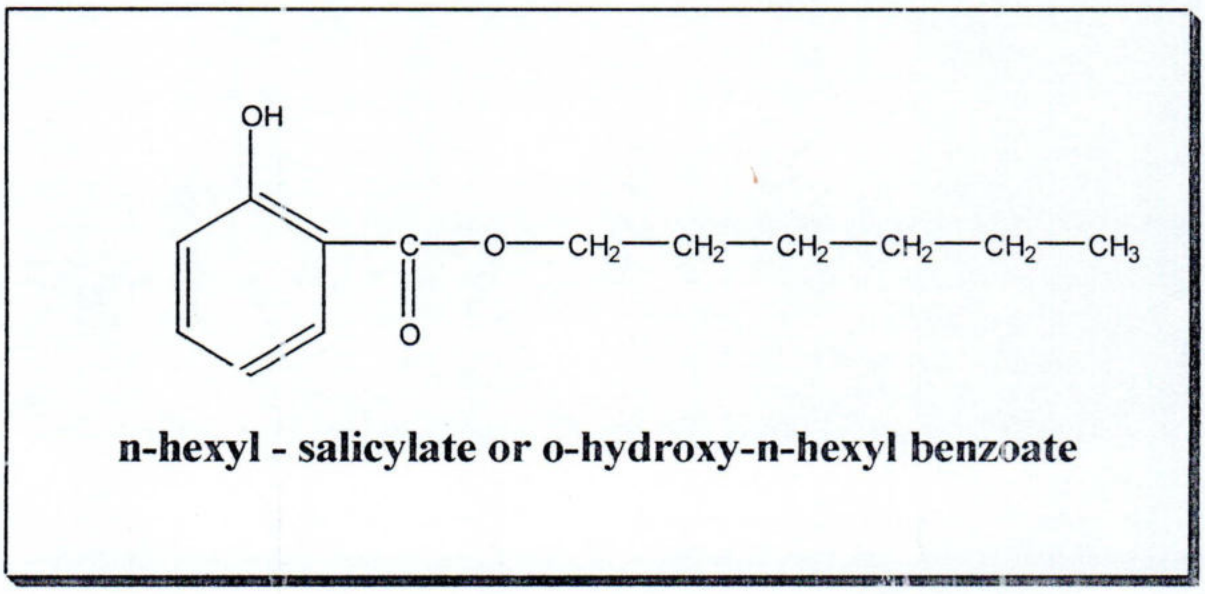


Fig.43: ^{1}H-NMR of S_2 (b)

4.8. Determination of Structure of Purified Compounds:

From IR and ^{1}H-NMR studies it was observed S_1 showed strong absorption at 1728 cm^{-1} indicating the presence of carbonyl group of ester, and broad absorption peak at 3224 cm^{-1} indicating the presence of –OH group and in ^{1}H-NMR proton observed at δ = 7.69 (multiplet) and 7.51(multiplet) indicate the presence of aromatic ring. At δ 4.20 (m) for (6) -O-CH_2 (methylene proton) and δ 1.67(m) for -O-CH_2-CH_2 proton (i.e. proton of next –O- CH_2 group), δ 1.31(multiplet) for the three methylene group of –O-CH_2-CH_2-$(CH_2)_3$ and finally peak observed at δ 0.929 (Triplet) for –CH_3 group of –n-hexyl like –O-CH_2-CH_2-$(CH_2)_3$. So, from above spectral information; the possible structure for compound S_1 may be construct as,



n-hexyl - salicylate or o-hydroxy-n-hexyl benzoate

Fig.45: Structure of n-hexyl- salicylate or o-hydroxy-n-hexyl benzoate

Similarly, from IR and ^{1}H-NMR studies, it was observed that S_2 showed strong absorption at 1728 cm^{-1} indicating the presence of a carbonyl group and in ^{1}H-NMR δ 2.15 (multiplet) for methylene group of -CH_2- $\underset{\underset{O}{\|}}{C}$ - CH_2-

δ 1.75-1.24 (multiplet) for unknown number of $(CH_2)_x$ and ~ 0.87(multiplet) for $-CH_3$ group of both sides. So, from above information, the possible stucture for compound S_2 may be designed as,

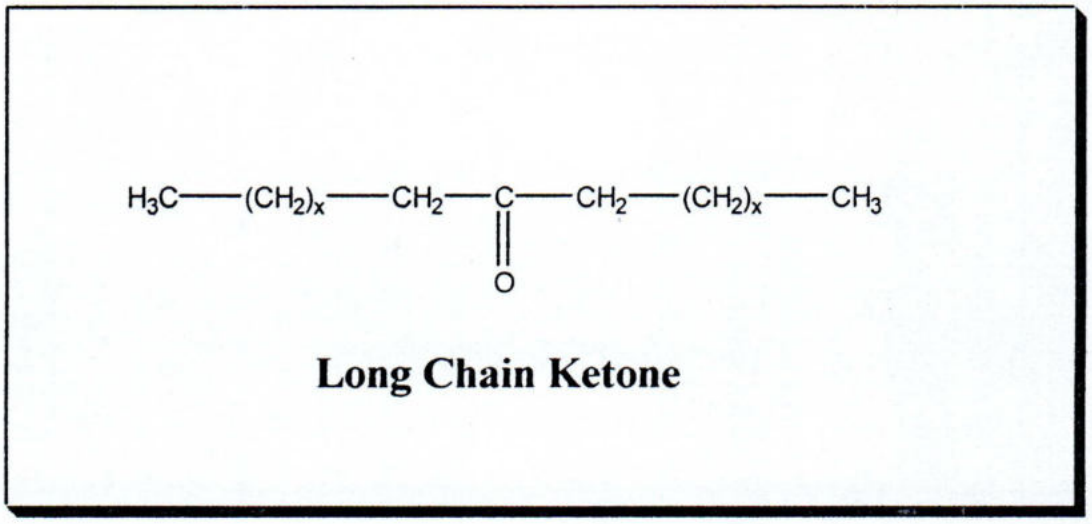


Fig.46: Structure of long chain ketone

CHAPTER V

SUMMARY

Laboratory experiments were carried out to determine the direct toxicity actions, surface protection effects and repellent effects of common cocklebur leaf, common cocklebur fruit and bloodleaf plant extracts against the pulse beetle. The present study revealed that plant materials were effective against the pulse beetle .

It was observed from the results that the plant extracts had significant repellent and direct toxicity effects, surface protectant effects and reduced the number of eggs laid, thus affecting the adult emergence of pulse beetle. Mortality percentage was found to vary among different concentrations of plant extracts. The highest mortality (36%) was observed in grains treated with highest concentrations (4%) of common cocklebur leaf extract followed by that of common cocklebur fruit (26%) and bloodleaf plant (18%) extracts at 2 DAT. Direct toxicity effect of different plant extracts had lost gradually after 2 (DAT).

All the plant extracts were found effective in protecting black gram seeds from pest infestation. Common cocklebur leaf extract possessed toxic effect on fecundity of pulse beetle. 4% common cocklebur leaf extract treated seeds showed lowest number of eggs (65) followed by extracts of common cocklebur fruit (113.67) and bloodleaf plant (111.67) eggs laid by pulse beetle respectively.

The lowest adult emergence (48%) was found in 4% common cocklebur leaf extract treated seeds like that of common cocklebur fruit extract treated seeds (56.67%) and bloodleaf plant extract treated seeds (65.33%).

It was evidient that the number of damage seeds recorded in control treatment was significantly higher than that of all other treatments. The highest seed damage (65.33 %) was found in 1% bloodleaf plant extract treated seeds and the lowest (26%) was in 4% common cocklebur leaf extract treated seeds.

The results of oviposition performance of *Callosobruchus chinensis* showed that common cocklebur leaf extract significantly inhibited oviposition on the gram seeds, common cocklebur leaf extract at 4% concentration caused the highest reduction (47.5%) of oviposition of *Callosobruchus chinensis* than 4% extracts of common cocklebur fruit (37.0%) and bloodleaf plant (28.34%).

The 4% extract of common cocklebur leaf, common cocklebur fruit and bloodleaf plant have good repellent action. The highest repellency was observed with 4% common cocklebur leaf extract (60%) at 5 HAT. The results also indicated that repellent effect increased proportionally with the increase of concentrations of plant extracts.

From the results of present studies, toxicity of plant extracts were found in the order common cocklebur leaf > common cocklebur fruit > bloodleaf.

Chemical investigation of ethanol extract of common cocklebur leaf had showed two distinguishing compounds such as S_1 and S_2. After spectral studies (IR and ^{1}H-NMR) S_1 was constructed as n-hexyl salicylate or o-hydroxy-n-hexyl benzoate and S_2 was a long chain ketone. After structure determination, it is clear that S_1 and S_2 were different type compounds. S_1 may consider aromatic ester i.e. ester of benzoic acid or salicyclic acid, which may toxic effect to insect. So, to ensure it the purification of S_1 and S_2 in large scale may in progress by our reseach group. After that individual

component will be checked for further insecticidal activity to find out which compound is responsible for this type of work, which will be reported in due course. The strong insecticidal effects such as direct toxicity repellency, seed damage, adult emergence, oviposition inhibition and fecundity of pulse beetle were observed in the aqueous extract of common cocklebur leaf probably due to containing above mentioned and other bioactive compound.

Use of indigenous plants as botanical insecticides will benefit in our agricultural sector, as these substances are not only cheaper but also environmentally friendly and do not leave any hazardous on the environment and food. Therefore, the application of plant products in our country will be highly effective against stored product insects. Finally, these initial efficacy tests of this present experiment will be helpful to identify the potential of botanical pesticides for controlling stored grain pesto. These plant products will reduce our dependency on dangerous synthetic insecticides and will act as one of the effective tools for integrated post management.

CHAPTER VI

CONCLUSION

The aqueous extract of common cocklebur leaf showed strong insecticidal activities such as direct toxicity, repellency, seed damage, adult emergence, oviposition inhibition and fecundity of pulse beetle on pulse seed.

From present chemical investigation, it is concluded that the ethenol extract of common cocklebur leaf may contain n- hexyl salicylate or o-hydroxy-n-hexyl-benzoate and another compound as long chain ketone.

This research can suggested the farmer's to use aqueous or ethanol extract of common cocklebur leaf as botanical insecticide to save the pulse seed from pulse beetle after further large scale study.

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APPENDICES

Appendix-I

Table 2. Mortality rates of pulse beetle by different plant extracts at different concentration at different DAT

Plant Extract	Concentration (%)	Insect Mortality (%)			
		1 DAT	2DAT	3 DAT	4 DAT
Blood leaf	0	2 f	2 g	2 c	2 e
	1	10 e	12 f	10 b	4 d
	2	10 c	14 c	10 b	4 d
	4	16 b	18 d	10 b	8 b
Common cocklebur fruit	0	2 f	2 g	2 c	0 f
	1	12 d	14 c	10 b	6 c
	2	14 c	18 d	10 b	6 c
	4	16 b	26 c	10 b	6 c
Common cocklebur leaf	0	2 f	2 g	0 d	2 e
	1	14 c	28 b	10 b	6 c
	2	16 b	28 b	12 a	6 c
	4	20 a	36 a	12 a	10 a
Sx-		0.57	0.62	0.33	0.61
lsd value		1.6	1.77	0.95	1.74
Probability level		NS	0.01	NS	NS
CV (%)		11.21	14.98	13.62	14.87

Appendix-II

Table 3. Repellency effects of different plant extracts on pulse beetle at different concentrations at different HAT

Plant extract	Concentration (%)	Repellency rate (%)					Mean repellency	Repellency class
		1 HAT	2 HAT	3 HAT	4 HAT	5 HAT		
Blood leaf	1	33 b	20 d	33.3 d	26.7e	26.7 e	27.93 g	ii
	2	34 b	26.7cd	40 cd	33.3de	33.3de	33.46 e	ii
	4	33 b	33.3c	46.7bc	46.7c	40cd	39.93 cd	ii
Common cocklebur fruit	1	35.3b	26.7cd	33.3 d	33.3de	26.7 e	31.06 ef	ii
	2	35.7b	26.7cd	40 cd	40 cd	40 cd	36.46 d	ii
	4	35.7b	33.3c	46.7bc	46.7c	53.3ab	43.13 c	iii
Common cocklebur leaf	1	42 a	33.3c	33.3 d	40 cd	46.7bc	43.06 c	iii
	2	42 a	46.7b	53.3ab	53.3b	53.3ab	49.73 b	iii
	4	42.7a	60 a	60 a	60 a	60 a	56.53 a	iii
Sx-		1.91	2.34	2.56	2.22	2.56	2.32	-
lsd value		5.68	6.96	7.62	6.60	7.62	6.90	-
Probability level		NS	NS	NS	NS	NS	-	-
CV (%)		13.84	14.23	14.51	13.35	14.58	-	-

Appendix-III

Table 4. Effect of different plant extracts on fecundity of pulse beetle at different concentrations

Plant Extract	Concentration (%)	No. of Eggs Laid
Blood leaf	1	202 b
	2	151.33 e
	4	111.67 g
	Control	213.33 a
Common Cocklebur Fruit	1	158.67 d
	2	140 f
	4	113.67 g
	Control	185 c
Common Cocklebur Leaf	1	95.67 h
	2	80.67 i
	4	65 j
	Control	205 b
Sx-		1.95
lsd value		5.693
Probability level		0.01
CV (%)		8.15

Appendix-IV

Table 5. Effect of different plant extracts on adult emergence of pulse beetle at different concentrations

Plant Extract	Concentration (%)	Adult emergence (%)
Blood leaf	1	88 c
	2	77.33 e
	4	65.33 h
	Control	91.33 ab
Common Cocklebur Fruit	1	80.00 d
	2	69.33 g
	4	56.67 i
	Control	90 b
Common Cocklebur Leaf	1	75 f
	2	65.33 h
	4	48 j
	Control	91.67 a
Sx-		0.5234
lsd value		1.528
Probability level		0.01

Appendix-V

Table 6. Effects of different plant extracts on seed damage percentage of blackgram seeds caused by pulse beetle

Plant Extract	Concentrations (%)	(%) of Damage seed
Blood leaf	1	65.33 c
	2	54.33 e
	4	43.67 f
	0	92.33 a
Common cocklebur fruit	1	60.33 d
	2	53 e
	4	42.33 f
	0	91 a
Common cocklebur leaf	1	42.33 f
	2	36 g
	4	26 h
	0	87.33 b
Sx-		0.7
lsd value		2.04
Probability level		0.01
CV (%)		7.28

Appendix-VI

Table 7. Effect of different plant extracts on inhibition rates IR (%) of pulse beetle at different concentrations

Plant Extract	Concentration (%)	Inhibition Rate(%)
Bloodleaf	1	3.64 h
	2	15.28 f
	4	28.34 c
	Control	0 i
Common Cocklebur Fruit	1	10.98 g
	2	22.88 d
	4	37.01 b
	Control	0 i
Common Cocklebur Leaf	1	18.11 e
	2	28.78 c
	4	47.5 a
	Control	0 i
Sx		0.64
lsd value		1.87
Probability level		1.00%
CV (%)		12.71

1 DAT	Concentration	3	1911.667	637.222	33.2464	0.0000
	Plant Extract	2	123.333	61.667	3.2174	0.0488 *
	Plant Extract & Conc.	6	63.333	10.556	0.5507	-
	Error	48	920.000	19.167		
	Total	59	3018.333			
2 DAT	Concentration	3	4920.000	1640.000	70.2857	0.0000 **
	Plant Extract	2	1523.333	761.667	32.6429	0.0000 **
	Plant Extract & Conc.	6	570.000	95.000	4.0714	0.0022 **
	Error	48	1120.000	23.333		
	Total	59	8133.333			
3 DAT	Concentration	3	938.333	312.778	46.9167	0.0000 **
	Plant Extract	2	3.333	1.667	0.2500	-
	Plant Extract & Conc.	6	36.667	6.111	0.9167	-
	Error	48	320.000	6.667		
	Total	59	1298.333			
4 DAT	Concentration	3	340.000	113.333	5.0370	0.0041**
	Plant Extract	2	30.000	15.000	0.6667	-
	Plant Extract & Conc.	6	50.000	8.333	0.3704	-
	Error	48	1080.000	22.500		
	Total	59	1500.000			

** Indicated 1% Level of Significant
* Indicated 5% Level of Significant

Appendix – VIII

Analysis of variance (ANOVA) of Repellent effects of *Callosobruchus chinensis* after treatment with common cocklebur leaf, common cocklebur fruit and bloodleaf plant extracts at different HAT

Duration of Time	Sources of Variations	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability Level
1 HAT	Concentration	2	2.296	1.148	0.0116	-
	Plant Extract	2	385.185	192.593	1.9490	0.1713
	Plant Extract & Conc.	4	0.815	0.204	0.0021	-.
	Error	18	1778.667	98.815		
	Total	26	2166.963			
2 HAT	Concentration	2	1866.667	933.333	6.3000	0.0084 **
	Plant Extract	2	1866.667	933.333	6.3000	0.0084 **
	Plant Extract & Conc.	4	266.667	66.667	0.4500	-
	Error	18	2666.667	148.148		
	Total	26	6666.667			
3 HAT	Concentration	2	562.963	281.481	1.5833	0.2326
	Plant Extract	2	1451.852	725.926	4.0833	0.0345 *
	Plant Extract & Conc.	4	59.259	14.815	0.0833	-
	Error	18	3200.000	177.778		
	Total	26	5274.074			
4 HAT	Concentration	2	1422.222	711.111	5.3333	0.0152 *
	Plant Extract	2	1155.556	577.778	4.3333	0.0291 *
	Plant Extract & Conc.	4	88.889	22.222	0.1667	-
	Error	18	2400.000	133.333		
	Total	26	5066.667			
5 HAT	Concentration	2	1422.222	711.111	4.0000	0.0365 *
	Plant Extract	2	1866.667	933.333	5.2500	0.0160 **
	Plant Extract & Conc.	4	177.778	44.444	0.2500	-
	Error	18	3200.000	177.778		
	Total	26	6666.667			

** Indicated 1% Level of Significant

* Indicated 5% Level of Significant

Analysis of variance (ANOVA) of number of eggs laid per 100 seeds of gram due to oviposition of *Callosobruchus chinensis* after treatment with common cocklebur leaf, common cocklebur fruit and bloodleaf plant extracts

Sources of Variations	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability Level
Concentration	3	20796.500	10398.250	75.9304	0.0000 **
Plant Extract	2	53607.667	17869.222	130.4852	0.0000 **
Plant Extract & Conc.	6	10822.167	1803.694	13.1710	0.0000 **
Error	24	3286.667	136.944		
Total	35	88513.000			

** Indicated 1% Level of Significant
* Indicated 5% Level of Significant

Appendix - X

Analysis of variance (ANOVA) of number of adult emereged per 100 seeds of gram due to oviposition of *Callosobruchus chinensis* after treatment with common cocklebur leaf, common cocklebur fruit and bloodleaf plant extracts.

Sources of Variations	Degrees of freedom	Sum of Squares	Mean Square	F Value	Probability Level
Concentration	3	674.000	337.000	34.174	0.0000 **
Plant Extract	2	5821.000	1940.333	196.7662	0.0000 **
Plant Extract & Conc.	6	263.333	43.889	4.4507	0.0037 **
Error	24	236.667	9.861		
Total	35	6995.000			

** Indicated 1% Level of Significant
* Indicated 5% Level of Significant

Appendix - XI

Analysis of variance (ANOVA) of Seed damage effects of *Callosobruchus chinensis* after treatment with common cocklebur leaf, common cocklebur fruit and bloodleaf plant extracts.

Sources of Variations	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability Level
Concentration	3	1518.167	759.083	42.8323	0.0000 **
Plant Extract	2	14163.889	4721.296	266.4054	0.0000 **
Plant Extract & Conc.	6	607.611	101.269	5.7142	0.0008 **
Error	24	425.333	17.722		
Total	35	16715.000			

** Indicated 1% Level of Significant
* Indicated 5% Level of Significant

Appendix - XII

Analysis of variance (ANOVA) of Inhibition Rate IR (%) of *Callosobruchus chinensis* on gram seeds after treatment with common cocklebur leaf, common cocklebur fruit and bloodleaf plant extracts

Sources of Variations	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability Level
Concentration	3	6996.588	2332.196	157.6768	0.0000 **
Plant Extract	2	832.765	416.382	28.1511	0.0000 **
Plant Extract & Conc.	6	308.186	51.364	3.4727	0.0130 **
Error	24	354.984	14.791		
Total	35	8492.522			

** Indicated 1% Level of Significant
* Indicated 5% Level of Significant

Appendix XIII.

Data for temperature and relative humidity

2007, Date	Day of week	Temperature (^{0}C)			Relative Humidity (%)		
		8.00 am	12 am	6.00 pm	8.00 am	12 am	6.00 pm
16/08	Fri	28.8	29.8	30.6	86	87	88
17/08	Sater	29.0	29.8	29.7	87	87	88
18/08	Sun	28.8	27.7	29.6	84	86	88
19/08	Mon	29.0	30.6	30.8	91	84	83
20/08	Tues	30.0	31.4	30.1	80	81	85
21/08	Wednes	28.5	30.4	30.0	86	79	85
22/08	Thrus	29.0	30.2	30.5	90	85	84
23/08	Fri	30.6	31.7	30.4	86	80	85
24/08	Sater	30.0	30.9	30.1	87	80	83
25/08	Sun	29.5	29.4	29.7	90	89	89
26/08	Mon	29.2	29.9	29.5	90	87	89
27/08	Tues	28.4	28.8	28.8	89	86	88
28/08	Wednes	28.2	28.5	28.4	91	87	90
29/08	Thrus	28.1	28.3	28.5	91	88	88
30/08	Fri	29.5	29.7	29.7	92	86	89