# SYNERGISTIC EFFICACY OF BOTANICAL INSECTICIDE, *AZADIRACHTIN* AND A SYNTHETIC INSECTICIDE, *CYPERMETHRIN* AGAINST STORED PRODUCT PEST, *TRIBOLIUM CASTANEUM*

**D.Bhavani[1] and M.S. Nalina Sundari[2].**
Research scholar[1], Assistant professor[2]
Department of Zoology, Queen Mary's College, Mylapore, Chennai, TamilNadu.

**ABSTRACT**

Insect pests are the big enemies of farmers as they destroy crops, stored grains; act as a vector of diseases of livestock etc. *Tribolium castaneum* is a serious pest of stored products. It attacks stored grain products such as flour, cereals, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens. The infested products gives off a displeasing odour, and becomes unfit for human consumption. Though the chemical insecticides are used to protect stored grains, they have adverse effect on human beings and environment. However, increased concern by the consumers over insecticides residues call for new approaches to control stored product insects using botanical pesticides. Therefore the present investigation were aimed at studying the effect of botanical pesticide, Azadirachtin and chemical insecticide cypermethrin on *T. castaneum.*

keyword: *Tribolium castaneum,* Azadirachtin and Cypermethrin.

## 1. INTRODUCTION

Stored-grain insects often cause much loss after harvest. Post- harvest deterioration causes economic losses due to obvious decay and adverse changes in the odour, taste, appearance and nutrition value (Phillips and Burkholder, 1984; Mondal and Port, 1994). *Tribolium castaneum*, commonly called "red flour beetle" is a serious pest of stored products. It is of Indo-Austrian origin (Smith and Whitman, 1992) and is found in temperate areas, and also in the places, where there is central heat (Tripathi *et al.*, 2001). It affects many stored products including grains, seeds, rava, powders, biscuits, pasta, beans, flours, vegetables, dry fruits, oil cakes, museum specimens (Weston and Rattlingourd, 2000). They are attracted to grain with high moisture content and can cause a grey tint to the grain they are infested and a displeasing odour. Due to the significant increase in the human population and the consequent increase in the amounts of food and grains produced, many small scale farmers adopted the use of pesticides as a means of pest control. Synthetic insecticide "Cypermethrin", found in many household ant and cockroach killers, including raid (insecticide) and ant chalk. It is a pyrithroid, and is used by the farmers for dusting grains. However, increased use of insecticides for the control of stored product pests in general leads to environmental contamination and development of resistance. Botanical insecticide "Azadirachtin", a liminoid compound produced by the neem tree for protecting itself from damaging insects (Thomson, 1992). It is structurally similar to insect hormones called " Ecdysis", which control the process of metamorphosis as the insects pass from larva to pupa to adult (Martineau Jess, 1994). Owing to the above, a comparative study was done using azadirachtin and cypermethrin against stored product pest *T. castaneum* to evaluate the effect of botanical insecticide.

## 2. MATERIALS AND METHODS

INSECT CULTURE

The live specimen culture of *T. castaneum* was obtained from rice storage and accurately identified based upon distinct characteristics. The test insect *T. castaneum* were mass cultured in the Research laboratory, Department of Zoology, Queen Mary's College, Chennai. A cohort of 100 *T. castaneum* adults were introduced in 200g wheat rava in a plastic container measuring 17.5 x 10.5 x 9.5. Then the plastic container was covered with muslin cloth and placed in an ambient environment of 28 ± 2ºc and 60 - 95% Rh and allowed to breed for about 1 month. This was maintained as the stock culture.

BOTANICAL INSECTICIDE- AZADIRACHTIN

Azadirachtin 1% was used in this study. Azadirachtin 1% 1.5% and 3% (Comercially available) is a high concentration neem based botanical insecticide with an Azadirachtin content of 10000 ppm. It is very effective in eliminating the pest and will replace widely used chemical pesticides (U.S. Environmental protection agency, 1993). It control the process of metamorphosis as the insect pass from larva to pupa to adult (Marteneau Jess, 1994). This formulation of neem extract contains more than 60 pesticide elements. Several research has shown that neem extracts have an effect on nearly 200 species of insects.

SYNTHETIC INSECTICIDE- CYPERMETHRIN

Cypermethrin is a synthetic pyrethroid used as an insecticide in large scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast acting neurotoxin in insects. It is easily degradable in soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerates its decomposition. It is found in many household ant and cockroach killers, including raid and ant chalk. Cypermethrin is one of the most commonly used insecticide dusts among farmers.

BIOASSAY

Newly moulted 20 larvae of *T. castaneum* were isolated from the stock culture and place in a container containing rava mixed with different concentrations of Azadirachtin and Cypermethrin prepared with distilled water in 1:4 ratio (Insecticide: water). Three different concentrations were chosen and 20 larvae (in triplicate) were maintained in each concentration. Insects were also placed in containers containing rava without any treatment and were maintained as control.

Similar seperate experiments were carried out for the combination Azadirachtin and Cypermethrin to see the synergistic toxicity. For the present study Azadirachtin was mixed with Cypermethrin in 1:1 ratio and tested for its efficacy in mortality (%) of *T. castaneum*.

TOXICITY STUDIES

The assessment of toxicity was based on mortality (%) of *T. castaneum* against three different concentrations of Azadirachtin ,Cypermethrin and Synergistic mixture in three different durations 24hrs, 48hrs and 72hrs.

Sub lethal dose were fixed from the $LC_{50}$ value and were chosen for biochemical analysis. Freshly moulted third instar larvae were introduced into containers and were fed with treated rava and the control with untreated rava. Three replicates were maintained. Before pupation the larvae were sacrificed, oven dried and powdered. Powdered tissues were taken seperately for analysis of Protein and chitinase.

BIOCHEMICAL ANALYSIS

Estimation of Protein:

The Protein estimation was quantified by following the method of Lowry *et al*., (1951).

Estimation of chitinase:

The activity of chitinase was quantified ,estimated and the calculation was done as discribed by Monreal and Reese, (1969).

STATISTICAL ANALYSIS

Results obtained from the experiments were subjected to statistical analysis.

## RESULTS AND DISCUSSION

The assessment of toxicity was based on the mortality of the third instar larvae of *T. castaneum*. The toxicity of Azadirachtin and Cypermethrin was studied against *T. castaneum*. The $LC_{50}$ value of Azadirachtin on *T. castaneum* was found to be 0.15% (Table. 1). Whereas the $LC_{50}$ value of cypermethrin on *T. castaneum* was found to be 0.14% (Table. 2).

Synergistic effect of Azadirachtin along with cypermethrin is also studied against *T. castaneum.* When we mix Azadirachtin and Cypermethrin the $LC_{50}$ value of synergistic mixture on *T. castaneum* was found to be 0.10% (Table. 3). These results showed that there is synergism when Cypermethrin mixed with Azadirachtin. The $LC_{50}$ value of larvae of *T. castaneum* was reduced from 0.14% (treated with Cypermethrin alone) to 0.10% ( treated with Synergistic mixture) also the value reduced from 0.15% (treated with Azadirachtin alone) to 0.10% (treated with synergistic mixture).

BIOCHEMICAL CONSTITUENTS

The total Protein quantity in the treated insects were found to be decreased when compared with control. However, an increased activity of chitinase was observed when insects fed with Azadirachtin and cypermethrin mixture. This increased in activity of chitinase may further accentuate the inhibition of chitin build up. However, this raise in activity of chitinase, is found to decrease as the day's prolonged.

Jilani and Saxena (1990) found that neem oil and Margosan-O, a neem based insecticide, was a feeding deterrent for *R. dominica* adults. However, Xie *et al.,(1995)*, used azadirachtin and neem extracts, they noted that the adults mortality of *S. oryzae*, *Tribolium castaneum* (Herbst), and *Cryptolestes ferrugineus* (Stephens) is likely to be consequence of antifeedant effect, rather than a toxic action. Mishra *et al*., (2012) carried out studies on repellent efficacy of essential oils of Eucalyptus globulus (Myrtaceae) and Ocimum basilicum (Lamiaceae) leaves against adults of *T. castaneum* and *S. oryzae*. They reported that the repellent effects of *E. globulus* and *O. basilicum* were significantly higher even at very low concentration against *T. castaneum*. Novo *et al*., (1997) also recorded promising repellent activity of several crude extracts against *T. castaneum* while working on four native plants of Argentina.

The above findings of several workers who had demonstrated the toxic and highly repellent action of different neem preparations against a wide range of storage pests. Similarly our experiments shows that Azadirachtin preparations could be expected to reduce infestation by *T. castaneum* in very effective manner than the synthetic pesticides. Neem products have been reported to cause growth inhibition, malformation and mortality, especially when applied to the larval stages, as well as repellency. The effects of insect growth regulators (IGR) include slow growth, delayed moulting, moult abnormalities, inability to complete moulting, insects remaining as ‘‘over-aged’’ larvae for a greatly extended period of time and, finally, mortality.

The results from the current study revealed that the Azadirachtin have good potential for the practical use as grain protectant for stored product pest control. On the basis of results, it can be concluded that Azadirachtin ensured good control of *T. castaneum* at 0.15% concentration in terms of percent morality and restricted progeny development.

TABLE 1: Impact of Azadirachtin on the mortality of third instar larvae of ***T. castaneum*.**

| S.NO | CONCENTRATION (%) | MORTALITY (%) | | |
|---|---|---|---|---|
| | | 24HRS | 48HRS | 72HRS |
| 1. | CONTROL | 0 | 0 | 0 |
| 2. | 0.05 | 10 | 15 | 30 |
| 3. | 0.10 | 15 | 20 | 30 |
| 4. | 0.15 | 25 | 40 | 55 |

TABLE 2: Impact of Cypermethrin on the mortality of third instar larvae of ***T. castaneum*.**

| S.NO | CONCENTRATION (%) | MORTALITY (%) | | |
|---|---|---|---|---|
| | | 24HRS | 48HRS | 72HRS |
| 1. | CONTROL | 0 | 0 | 0 |
| 2. | 0.05 | 15 | 25 | 35 |
| 3. | 0.10 | 20 | 30 | 40 |
| 4. | 0.15 | 25 | 40 | 50 |

TABLE 3: Synergistic effect of Azadirachtin with Cypermethrin on the mortality of third instar larvae of ***T. castaneum*.**

| S.NO | CONCENTRATION (%) | MORTALITY (%) | | |
|---|---|---|---|---|
| | | 24HRS | 48HRS | 72HRS |
| 1. | CONTROL | 0 | 0 | 0 |
| 2. | 0.05 | 20 | 30 | 40 |
| 3. | 0.10 | 30 | 40 | 50 |
| 4. | 0.15 | 35 | 45 | 60 |

TABLE 4: Impact of Azadirachtin on the chitinase activity of ***T. castaneum*.**

| S.NO | Concentration of Azadirachtin % | Protein (mg/g. wet tissue) Mean±S.D | Chitinase (mg N-Acetyl glucosamine released/mg protein) Mean±S.D |
|---|---|---|---|
| 1 | Control | 2.66±0.98 | 18.11±1.02 |
| 2 | Treated insects (0.15) | 1.63±0.54 | 7.56±0.44 |

## CONCLUSION

The results revealed that Azadirachtin is highly effective even in low concentration against *T. castaneum*. However, the botanical insecticide-Azadirachtin competes favourably with synthetic insecticides. Finally, it should be emphasized that products based on plants used herein are considered as safe both for the environment and health and they can be recommended for use in plant protection. Thus a persual of the work reported here indicates that a number of plant derived pesticides can be developed to manage the insect pests. The present study has thrown light for future planning of this type of work in the field of insect pest management.

**REFERENCE:**


Golob P., Dales M., Fidgen A., Evans J. and Gudrus I., (1999). The use of spices and medicinals as bioactive protectants for grains. Rome, Food and Agriculture organisation of the united nations.

Gwinner J., Harnisch R. and Mück O., (1996). Manuel of the Prevention of Post-harvest Grain Losses. Post-harvest Project. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Eschborn, Germany. 1996.

Jilani G. and Saxena R. C., (1990). Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). ***J. Econ. Entomol. 83***: 629- 634.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. ***J. Biol. Chem***. **193**: 265–275.

Martineau Jess. (1994). AgriDyne Technologies, Inc. January 26, 1994, MSDS for Azatin-EC Biological Insecticide.

Mishra B.B., Tripathi S.P. and Tripathi C.P.M., (2012). Repellent effect of leaves essential oils from Eucalyptus globulus (Myrtaceae) and Ocimum basilicum (Lamiaceae) against two major stored grain insect pests of Coleopterons. ***Journal of Nature & Science 10***: 50–54.

Mondal K. and Port G.R., (1994). Pheromones of Tribolium species and their potential in pest management. ***Agricul Zool Rev 6***:121-48.

Monreal J. and Reese E.T., (1969). The chitinase of Serratia marcescens. ***Canadian Journal of Microbiology 15***: 689-696.

Novo R. J., Viglianco A. and Nassetta M., (1997). Actividad repelente de diferentes extractos. Retrieved from nutshellnutrition.wordpress.com.

Perls, (2009). Prussian blue original formula and uses. 8th edition.

Phillips J .K. and Burkholder W. E., (1984). Health hazards of insects and mites in food. In: Braur F J (ed) Insect Management for Food Storage and Processing. Pp 280-92.The American Association of Cereal Chemists, St. Paul M. N.

Rai R.S., Lal P. and Srivastava P.K.,(1987). Impregnation of jute bags with insecticide for protecting stored food grains. Comparative efficacy of impregnation method vis-à-vis existing method of prophylactic chemical treatment against cross infestation of different stored grain insect pests. ***Pesticides., 21( 8)*****:** 39-42.

Smith E.H. and Whitman R.C., (1992). Field Guide to Structural Pests. National Pest Management Association, Dunn Loring, VA.

Thomson W.T., (1992). Agricultural Chemicals. Book I: Insecticides. Thomson Publications, Fresno, CA.

Tripathi A.K., Prajapati V., Aggarwal K.K. and Kumar S., (2001). Toxicity, feeding deterrence, and effect of activity of 1,8,-Cineole from Artemisia annua on progeny production of Tribolium castaneum (Coleoptera: Tenebrionidae). ***Journal of Economic Entomology 94***:979–983.

U. S. Environmental Protection Agency. (1993). Azadirachtin: Toloerance Exemption. Federal Register. Vol. 58, No. 30. Rules and Regulations. Wednesday, February 17, 1993.

Weaver D. and Subramanyam B., (2000). Botanicals. In: B. Subramanyam and D. Hafstrum (Eds.), Alternatives to pesticides in stored- product IPM, Dordrecht, Kluwer Academics Publishers. pp303-320.

Weston P.A. and Rattlingourd P.L., (2000). Progeny production by Tribolium castaneum (Coleoptera: Tenebrionidae) and Oryzaephilus surinamensis (Coleoptera: Silvanidae) on maize previously infested by Sitotroga cerealla (Lepidoptera: Gelechiidae). ***Journal of Economic Entomology 93***:533–536.

Xie Y. S., Fields P. G. and Isman M. B., (1995). Repellency and toxicity of azadirachtin and neem concentrates to three stored-product beetles. ***J. Econ. Entomol. 88***: 1024-1031.