*Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)*
*An Online International Journal Available at http://www.cibtech.org/jls.htm*
*2011 Vol. 1 (2) April – June, pp. 32-36/Arora et al.*
***Research Article***

# Larvicidal Property of Aqueous Extracts of *Withania somnifera* on *Tribolium castenum*

***Mona Arora[1], Jyotshana Sharma[2], Ambica Singh[2], RS Negi[2]**
[1]Department of Zoology, Mahatma Gandhi Institute of Applied Sciences, JECRC Foundation, Jaipur
[2]Department of Biotechnology, Mahatma Gandhi Institute of Applied Sciences, JECRC Foundation, Jaipur
****Author for Correspondence:*** E-mail: monaaroravmn@gmail.com

**ABSTRACT**
The use of insecticide to control food pest is causing serious health problems in society. *Withania* a medicinal plant was evaluated for its larvicidal property against mature larvae of *Tribolium castenum* species. *Tribolium* is most common food pest infesting most of the tropical food stores and thus causing a great commercial loss. The extracts of different parts of *Withania* were prepared and the late instar larvae of *Tribolium castenum* (Herbst) were treated with them. Morphological abnormalities and significant mortality was observed in treated larval forms at higher dose levels of root extracts.

***Key Words:*** Larvicidal Activity, Medicinal Plants, *Withania somnifera*, *Tribolium*

## INTRODUCTION

Stored food pests are economically important and are responsible for commercial loss every year. Many insect species are active under suitable conditions. Safe storage of grains and food products against insect damage is of serious concern (Haq *et al*., 2005). Food products that are left undisturbed on the shelves for long periods are particularly susceptible to infestation. However, foods of any age can become infested (Chittenden, 1987). It has been estimated that about 9% of the world's grain production is lost to post harvest insect and mite's infestation (Padin *et al*., 2002; Tooba *et al*., 2005; Rahman *et al*., 2009).

Iteroparous beetles of genus *Tribolium* have been associated with stored food for more than 4000 years and are therefore considered pest of primary economic importance for food industry (Sokoloff *et al*, 1984; Levinson and Levinson, 1985). *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* (DuVal), the confused flour beetle, are major pests of stored products, and when insecticides were tested against these species, the order of susceptibility was found dependent upon the specific insecticide and its formulation (Arthur 1998a, b, 2000).

The widespread use of synthetic chemicals to control insect pest has caused numerous environmental problems. Pesticides formulated with herbal extracts are in practice as a safer alternative and has become part of leading research all over the world (Silva *et al*., 2002; Clemente *et al.*, 2003).

Plants constitute a rich source of bioactive compound which might act deadly on the insect physiological system and kill them (Daoubi *et al.*, 2005; Kim *et al*., 2005). Recent studies have demonstrated the insecticidal properties of chemical derived from plants that are active against specific target species, are biodegradable and potentially suitable for use in integrated management program (Markouk *et al.*, 2000; Tare *et al*., 2004). Many plant extracts are considered important products for pest management in Ancient China, Egypt, Greece and India (Isman, 2006; Long *et al.*, 2006).

To the present, more than 2000 plant species are known to have insecticidal properties, where the Euphorbiaceae, Asteraceae, Labiatae, Fabaceae, Meliaceae and Solanaceae families stand out (García *et al.*, 2004). 60% to 70% of the species of Solanaceae family produce alkaloids which play an important role against pathogens and herbivores. They have toxic and feed deterrent effect on insects (Eich 2008). The extracts of the Solanaceae family have been tested on various groups of insects of agricultural importance (Braga *et al.*, 2004; Bouchelta *et al.*, 2005; Bastos *et al.*, 2009).

*Withania somnifera*, one of the member of solanaceae family is a widely used medicinal plant in Indian traditional medical practice and its extracts are variously used and consumed by human beings. This Indian ginseng has been tested by many workers for identifying its various properties. It is prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), (Anbalagan and Sadique 1981; 1984) and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy (Chatterjee and Pakrashi 1995; Bone,

1996). Many studies have been conducted to investigate its use as an antistress (Dhuley, 1998; Archana and Namasivayan 1999) and antitumor properties (Singh *et al*., 1986; Devi *et al*., 1992; Devi 1996)

*Withania somnifera* having medicinal property was thus investigated for its insecticidal property. The study was aimed to investigate the larvicidal property of extracts obtained from different parts of *Withania somnifera* collected from local vicinity of Jaipur.

## MATERIALS AND METHODS

### *Plant Collection*

*Withania somnifera* was collected from the local areas of Jaipur city. Plant parts (roots, leaves, stems) were washed and were shade dried in a well ventilated room for 7 days at room temperature. They were separately grinded into a fine powder with the help of electric blender and were kept in airtight packed jar prior to use. The powdered part was used to prepare aqueous extracts for the purpose of investigation.

### *Plant Extraction*

25gm of the powdered material was weighed and was mixed with 125 ml of double distilled water (1:5) in the round bottom flask and extraction process was carried out. The solvent was concentrated under reduced pressure and temperature. The output extract was removed to glass vials and preserved in a refrigerator at 4˚C temperatures with proper labeling. The drug was then prepared in different concentration levels and mixed with the diet of larva to treat them.

### *Test Organism*

Initial stock of *Tribolium castenum* was obtained from infested wheat grain bought from the local market in Jaipur, Rajasthan and was reared in whole wheat grain in the laboratory, Department of Zoology, Mahatma Gandhi Institute of Applied Sciences, Jaipur. The grains were sterilized at 60˚C for 24 hours in an oven. A standard mixture of whole wheat grain with 5% powdered dry yeast was used as food medium throughout the experimental period (Parker and Frank 1984; Zyromska-Rudzka, 1996). The *Tribolium* were reared in a glass jar covered with muslin cloth to ensure ventilation. The cultures were maintained in dark in incubator at 30±5˚C and relative humidity of 65-70% throughout the experimental period. Mature late instar larvae were selected for the preset study.

### *Morphological Abnormalities in Larvae*

Morphological abnormalities of the treated live larvae were recorded on the termination of the experiment in each group. Abnormal individuals were separated and the deformed characters were studied under magnification.

***Larvicidal Test:*** Healthy mature instar larvae were chosen for the present study. The plant extracts were prepared in different dose concentration (2%, 4%, 6%, 8% and 10%). Six groups having 25 larvae each were made with five replicas of each group; 5 treated and 1 control. Larvae of each group were released in muslin cloth covered beaker containing treated food with different dose concentrations. Larvae of control group were provided with untreated and sterile food. After every three days the food material was replaced by a fresh one, treated with the same dose and compound to avoid conditioning by the larvae (Mondal, 1984). The observation for mortality was made on 5th day of the study. The mortality percentage was corrected using Abbot's formula (Abbot 1925) as given below:

$$\%\text{age of mortality} = \frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \text{ X100}$$

$$\text{Corrected \%age of mortality} = \frac{\text{n in T after treatment}}{\text{n in C after treatment}} \text{ X100}$$

Where *n= number of larvae, T= treatment* and *C= control*

## RESULT AND DISCUSSION

### *Morphological Abnormalities in Larvae*

Morphological abnormalities were observed in the treated live larvae. It was observed that larvae treated with high dose levels were reduced in body size and showed incomplete metamorphosis. Stiffness in the cuticle was also observed in few cases.

### *Larvicidal Test*

Effect of different extracts of *Withania somnifera* are summarized in Table 1.

No mortality occurred in larvae fed with control diet. Treatments with extracts from roots, stems and leaves of *Withania* showed significant effect on mortality of *Tribolium* larvae. The larvae mortality was observed to have a direct relationship with concentration of dosages irrespective to the source of extracts, been maximum when treated with extracts of 10% concentration and minimum when treated with 2% aqueous extract concentration. As we can see root extracts is more effective in killing the larvae and stem extracts being least effective comparatively. Maximum percentage mortality of 46.20±2.06% by the root extracts (at 10% dose) and minimum percentage mortality of 35.86±2.07% by the stem extract (at 10% dose) were observed. Synthetic chemical pesticides are causing serious health problems and even seriously affecting our

**Table 1. Mortality rate of *Tribolium* larva treated with different extracts of *Withania somnifera***

| Plant Parts | Mortality % in different aqueous extracts concentration | | | | |
|---|---|---|---|---|---|
| | 2% (Mean± SE) | 4% (Mean±SE) | 6% (Mean± SE) | 8% (Mean± SE) | 10% (Mean± SE) |
| **Root** | 15.17±2.07 | 29.65±2.07 | 42.06±2.53 | 44.13±2.53 | 46.20±2.06 |
| **Leaf** | 8.96±2.07 | 29.65±2.07 | 33.74±2.53 | 35.86±2.07 | 39.99±2.06 |
| **Stem** | 8.07± 2.07 | 19.30±2.06 | 21.37±2.53 | 31.72± 2.53 | 35.86±2.07 |
| **Control** | | Nil | | | |

environment. Research is going on to replace synthetic chemicals by better and safe natural products to control storage pests (Bekele *et al*., 1997; Prates *et al*., 1998; Isman, 2000; Shaaya and Kostyukovysky, 2006).

Larvicidal studies with extracts of medicinal plants have been an interest of many scientists. Rachid, *et al*., (2006) studied the larvicidal activity of four medicinal plant extracts *Peganum harmala* (Zygophyllaceae), *Ajuga iva* (Labiateae), *Aristolochia baetica* (Aristolochiaceae) and *Raphanus raphanistrum* (Brassicaceae) against *Triboloium castaneum*. All treatments provoked highly significant effects on mortality. Extracts of *P. harmala* caused 58% mortality and at the same time, mortality rates for the extracts from *Aristolochia baetica*, *Ajuga iva* and *R. raphanistrum* reached 34, 31 and 26% respectively. Bell (1978) showed that pupae may exhibit a higher tolerance to chemical agent than active stages.

In another study Sadek (2003), observed that the time of pupation of *Spodoptera littoralis* (boisdual) of larvae increased by the extracts of *Adhatoda vasica*. Jeyabalan *et al*., (2003) reported that extracts of *Pelargonium citrosa*, prolonged the duration of larvae instars and the total development time of *Anopheles stephensi*.

Madhumathy *et al*., (2007), observed the effect of capsaicin on larval system and showed that larvae became slowly inactive within 18 h and began to fall towards the bottom of the beaker. Microscopic examination of dead larvae revealed that the extract has penetrated into larval digestive system. The treated larvae showed curling up, agitation, vigorous body movements which are the characteristic of neurotoxicity. The results suggested a possible utilization of the cheap and readily available chilly fruits for possible control of mosquitoes as a part of the integrated vector management programme. Nascimento *et al*., (2004) used some *Aristolochia* and *Tubercula* species to test their insecticidal activity. They recommended the use of acetone and ethanol extracts of the *Tubercula* and several compounds isolated from *Aristolochia pubescens* as potential botanical insecticide agents against *Anticarsia gemmatalis* larvae. The compound inhibited larvae growth and induce malformed adults.

The observation made in the present study shows some similarity with the result of other scientists. Extracts obtained from root were found more effective on larvae in comparison to extracts obtained from other parts of the plant. The differences observed in mortality due to extracts from different parts of the plant might be because of the variation in the concentration of the secondary metabolite. The result from the present study shows an initial step towards controlling the larval stage of *Tribolium* using extracts obtained from *Withania*, further investigation is under process.

## ACKNOWLEDGEMENT

We are grateful to Department of Science and Technology, Rajasthan, for providing financial assistant to complete this project work. Authors express their sincere thanks to Prof. C.K. Ojha, Director Academics, MGIAS, JECRC foundation for providing necessary facilities.

## REFERENCES

**Abbott WS (1925).** A Method of Computing the effectiveness of an Insecticide. *Journal of Economic Entomology* **18** 265-267.

**Anbalagan K and Sadique J (1981).** Influence of an Indian medicine (Ashwagandha) on acutephase reactants in inflammation. *Indian Journal of Experimental Biology* **19** 245-249.

**Anbalagan K and Sadique J (1984).** Role of prostaglandins in acute phase proteins in inflammation. *Biochemistry Medical* **31** 236-245.

**Anbalagan K and Sadique J (1984).** Role of prostaglandins in acute phase proteins in inflammation. *Biochemistry Medical* **31** 236-245.

**Archana R and Namasivayan A (1999).** Antistressor effect of *Withania somnifera*. *Journal of Ethnopharmacology* **64** 91-93.

**Arthur FH (1998a).** Residual studies with cyfluthrin wettable powder: toxicity towards red flour beetles (Coleoptera: *Tenebrionidae*) exposed for short time intervals on treated concrete. *Journal of Economic Entomology* **91** 309–319.

**Arthur FH (1998b).** Residual toxicity of cyfluthrin wettable powder against *Tribolium confusum* exposed for short time intervals on concrete. *Journal of Stored Products Research* **34** 19–25.

**Arthur FH (2000).** Toxicity of diatomaceous earth to red flour beetles and confused flour beetles: effects of temperature and relative humidity. *Journal of Economic Entomology* **93** 526–532.

**Bastos S, Egewarth R, Sausen C, Soares Vy and Prado L (2009).** Action of plants extracts on oviposition and on mortality of diamondback moth. *Ciencia Rural* **39** 551-554.

**Bekele J, Obeng-Ofori D and A Hassanali (1997)** Evaluation of *Ocimum kenyense* (Ayobangira) as a source of repellents, toxicants and protectants in storage against three stored product insect pests. *Journal of Applied Entomology* **121** 169-173.

**Bell CH (1978).** Limiting concentrations for fumigant efficiency in the control of insect pests. In: Proceedings of the Second International Working Conference on Stored-Product Entomology, Ibadan, Negeria, 182-192.

**Bone K (1996).** Clinical Applications of Ayurvedic and Chinese Herbs. Monographs for the Western Herbal Practitioner. (*Australia: Phytotherapy Press*) 137-141.

**Bouchelta A, Boughdad Ay and Blenzar A (2005).** Effets biocides des alcaloides, des saponines et des flavonoides extraits de *Capsicum frutescens* L. (Solanaceae) sur *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Biotechnologie, Agronomie, Société et Environnement.* **9** 259-269.

**Braga P, Goetze My and Hermes, G (2004).** Efeito de extratos de plantas silvestres da família *Solanaceae* sobre o controle de *Brevicoryne brassicae* em couve (*Brassica oleracea* var. *acephala*). *Ciencia Rural* **34** 971-978.

**Chatterjee A and Pakrashi SC (1995).** The Treatise on Indian Medicinal Plants **4** 208-212.

**Chittenden, RH (1987)**. Some insects injurious to stored grains and their habits and control. (MC Graw Hill Book, Company, New York.) 925.

**Clemente S, Mareggiani G, Broussalis A, Martino Vy and Ferraro, G (2003)**. Insecticidal effects of Lamiaceae species against stored products insects. *Boletín de Sanidad Vegetal. Plagas* **29** 1-8.

**Daoubi M, Deligeorgopoulou A, Macias-Sanchez A J, Hermamdez-Galan R, Hitchcock PB, Hanson JR and Collado IG (2005).** Antifungal activity and biotrans formation of diisophorone by Botrytiscinerea. *Journal of Agricultural and Food Chemistry* **53** 6035-6039.

**Devi PU, Sharada AC, Solomon FE and Kamath MS (1992).** *In vivo* growth inhibitory effect of Withania somnifera (Ashwagandha) on a transplantable mouse tumor, Sarcoma 180. *Indian Journal of Experimental Biology* **30** 169-172.

**Devi PU (1996)** *Withania somnifera* dunal (ashwagandha): potential plant source of a promising drug for cancer chemotherapy and radiosensitization. *Indian Journal of Experimental Biology* **34** 927-932.

**Dhuley JN (1998)** Effect of ashwagandha on lipid peroxidation in stress-induced animals. *Journal of Ethnopharmacology* **60** 173-178.

**Eich, E (2008).** Solanaceae and Convolvulaceae. Secondary metabolites: biosynthesis, chemotaxonomy, biological and economics significance (Springer Alemania).

**García-Mateos R, Pérez PR, Rodríguez HCy and Soto HM (2004)**. Toxicidad de alcaloides de *Eythrina americana* en larvas de mosquito *Culex quinquefasciatus*. *Fitotecnia Mexicana* **27** 297-303.

**Haq T, Usmani NF and Abbas, T** (**2005**). Screening of plant leaves as grain protectants against *Tribolium castaneum* during storage. *Pakistan Journal of Botany* **37** 149–15.

**Isman MB (2000).** Plant essential oils for pest and disease management. *Crop Protection* **19** 603-608.

**Isman MB (2006).** Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* **51** 45-66.

**Jeyabalan D, Arul N and Thangamathi P (2003).** Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, Anophelesstephensi Liston. *Bioresource. Technology* **89** 185-189.

**Kim HG, Jeon JH, Kim MK and Lee HS (2005).** Pharmacological ectsofasaron aldehyde

isolated from Acorusgram in eusrhizome. *Food Science Biotechnology* **14** 685-688.

**Levinson, HZ and Levinson, A (1985)**. Storage and insect species of stored grain and tombs in ancient Egyptian. *Zeitschrift fuir angewandte entomologie* **100** 321-339.

**Long Z, Hock Sy and Hung S (2006).** Screening of Chinese medicinal herbs for bioactivity against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *Journal of Stored Products Research* **43** 290-296.

**Madhumathy A, Aivazi AY and Vijayan, V (2007).** Larvicidal efficacy of *Capsicum annum* against *Anopheles stephensi* and *Culex quinquefasciatus*. *Journal of Vector Borne Diseases* **44** 223-226.

**Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek HB and Jana M (2000).** Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. *Journal of Ethanopharmacology* **73** 293-297.

**Mondal, K** (**1984**). Repellent effect of pirimiphos methyl to larvae *Tribolium castaneum* (Herbst). *International Pest Control* **26** 98-99.

**Nascimento IR, Murata AT, Bortoli SA and Lopes LM (2004).** Insecticidal activity of chemical constituents from Aristolochia pubescens against Aticarsia gemmatalis larvae. *Pest Management Science* **60** 413-6.

**Padin, S, Dal Bello, G and fabrbrizo, M** (**2002**). Grain loss caused by *Tribolium castenum*, *Sitophilus oryzae* and *Acanthoscelides obectus* in stored drum wheat and beans treated with *Beauveria bassiana. Journal of stored product research* **3** 69-74.

**Parker, T and Frank, MB** (**1984**) The fecundity and development of the (2.5×10-5g) flour beetles, *Tribolium castaneum* and *Tribolium confusum* at three constant temperatures. *Ecology* **29** 368-375.

**Prates HT, Santos JP, Waquil JM, Fabris JD, Oliveira AB and JE Forster (1998)** Insecticidal activity of monoterpenes against *Rhyzoperhta domonica* (F.) and *Tribolium castaneum* (Herbst). *Journal of Stored Product Research* **34** 243-249.

**Rachid J, Abdeslam E and Fouad S (2006).** Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *African Journal of Biotechnology*. **5** 936-940.

**Rahman MM, Islam W and Ahmed KN** (**2009**). Functional response of the predator *Xylocoris flavipes* to three stored product insect pests. *International Journal of Agriculture and Biology*. **11** 316–320.

**Sadek, MM (2003).** Antifeedant and toxic activity of Adhatoda vasica leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). *Journal of Applied Entomology* **127** 396-404.

**Shaaya E and Kostyukovysky M (2006)** Essential oils: potency against stored product insects and mode of action. *Stewart Post harvest Review* **4** 5.

**Silva G, Lagunes A, Rodríguez Jy and Rodríguez D (2002).** Insecticidas vegetales: una vieja y nueva alternativa para el manejo de plagas. *Manejo Integrado de Plagas y Agroecología* **66** 4-12.

**Singh N, Singh SP, Nath R, *et al*. (1986).** Prevention of urethane-induced lung adenomas by *Withania somnifera* (L.) Dunal in albino mice. *International Journal of Crude Drug Research* **24** 90-100.

**Sokoloff A, Albers JC, Cavataio P, Mankau SK, McKibben S, Mills S, Perkins R, Roberts LD and Sandri, JJ (1984).** Observations on populations of *Tribolium brevicornis* (Le Conte) (Coleoptera, Tenebrionidae). *Research on Population Ecology* **22** 1-12.

**Tare V, Deshpande S and Sharma RN (2004)**. Susceptibility of two different strains of Aedes aegypti (Diptera:Culicidae) to plant oils. *Journal of Economic Entomology* **97** 1734-1736.

**Tooba, S Usmani, NF and Abbas, T (2005)**. Screening of plant leaves as grain protectant against *Tribolium castaneum* during storage. *Pakistan Journal of Botany* **37** 149–153.

**Zyromska–Rudzka, H (1966).** Abundance and emigration of *Tribolium* in laboratory model. Ekologia Polska- Seria A **14** 491-518.