JCBPS; Section B; August 2017 – October, 2017, Vol. 7, No. 4; 1331-1337, E- ISSN: 2249 –1929

[DOI: 10.24214/jcbps.B.7.4.133137.]

# Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

***Section B: Biological Sciences***

CODEN (USA): JCBPAT

Research Article

# Assessing the toxicity of NSO (Neem Seed Oil) and NSKP (Neem Seed Kernal Powder) to a bruchid

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**Received:** 19 September 2017**; Revised:** 08 October 2017; **Accepted:** 20 October 2017

**Abstract:** Bruchid pests are controlled in the field and in storage systems using chemical pesticides. Apart from chemical pesticides, botanical derivatives are also used in the management of bruchids. Neem oil and neem seed kernal powder prepared from fresh neem seeds are used in this study. These neem products are found to be very effective compared to commercial preparations. The protocol for the preparation of these neem products can be conveniently used by farmers in rural areas. The results of this study showed that *Callosobruchus maculatus* F. adults were more susceptible while young whereas the older adults were more resistant.

**Keywords:** NSO, NSKP, Bruchids, *Callosobruchus maculatus* F. mortality.

## INTRODUCTION

Bruchids are pests of cowpea, *V. ungulata* L., an economically important grain legume, grown successfully in extreme environments such as high temperature, low rainfall and poor soil with few inputs[1]. Subsistence farmers in semi-arid and sub humid regions of Africa are the major producers and consumers of cowpea. Cowpea seeds, a major source of dietary protein as well as vitamins and minerals in most developing countries[2,3] , can be consumed directly, made into flour, sprouts and used as weaning food for young children, thus ameliorating malnourishment, wasting and stunting [4]. Production of *V. ungulata* is restricted by abiotic and a biotic factors both in the field and in storage. Among, the biotic factors are insect pests [5]. Insect pests have been a threat to food products and seeds ever since man has started growing crops. Almost all stored foodstuffs and seed are liable to insect injury[6] . The damage in storage is more crucial than in the field. Losses caused by insects are not only by due to direct consumption of kernels, but also involve accumulations of fross, exuviae, webbing and insect cadavers. High levels of these insect detritus may result in grain that is unfit for human consumption. Insect induced changes in the storage environment may cause warm, moist 'hotspots' that are suitable for the development of storage fungi that cause further losses.

*Callosobruchus maculatus* F. an important cosmopolitan bruchid, widespread throughout tropical and subtropical regions of the world, mainly damage stored cowpeas and to a lesser extent, other legumes [7,8]. However, infection begins in the field and serious damage on the pods and/or seeds occurs during storage[9]. This problem is serious at small-scale farmer level, village traders and average households where storage conditions are inadequate[10] .It is necessary to protect stored grains from insect pests owing to their severe infestation and damage potential, in a short period. Management of this pest is primarily dependent upon continued application of pesticides. Cowpea production and storage is in the hands of the resource-poor farmers who could hardly afford efficient and effective storage systems or apply synthetic chemical insecticides to protect cowpea from bruchid infestation and damage[11].

An integrated pest-management strategy is ideal for the safe storage of post-harvest commodities. The use of effective hygienic measures and chemical protectants are integral parts of this strategy. However, the number of pesticides currently approved for the protection of stored commodities is very limited. Efficacy may also be affected by the development of pesticide resistance in populations. Thus, alternative non-chemical control measures are sought which can be incorporated into this pest management strategy [12]. Green plants act as a reservoirs of inexhaustible source of innocuous pesticides [13]. Plant derived products namely, azadirachtin from neem (*Azadirachta indica*) pyrethrin from pyrethrum (*Chrysanthemum cinerariaefolium*) and allyl isothiocynate from mustard (*Brassica nigra)* oil have received global attention due to their pesticidal properties and potential to protect several food commodities [14]. Essential oils produced by different plant genera have been reported to be biologically active and are endowed with insecticidal, antimicrobial and bio regulatory properties [14-16]. There may be a least chance of residual toxicity by treatment of food commodities with volatile substances of higher plants [17]. Botanical insecticides have broad spectrum insecticidal proerties with reduced persistence compared to organochlorines, organohosphates, carbamates and pyrethroids [18]. In this study Neem Seed Kernel Extract (NSKE) and Neem Seed Powder (NSP) are used for managing adult cowpea beetle, *C. maculatus.*

## MATERIALS AND METHODS

*C. maculatus* life stages were exposed to Neem Seed Oil (NSO) and Neem Seed Kernal Powder(NSKE). The two preparations were made in the laboratory.

**(i) Procurement of *C. maculatus:*** *C. maculatus* adults were trapped by placing *Vigna radiata* L. grains in a plastic container in a nearby grocery outlet. The adults that visited the grains oviposited on them. Grains with one or two eggs were segregated and placed in a wire-netted plastic container in the insect rearing area and the eggs were allowed to incubate. The hatched out larvae developed within the grains and the adults eclosing from the grains were used in the toxicity bioassay.

**(ii) Neem Seed Oil:** Mature neem seeds were collected from campuses where neem trees were abundant. Good and fully developed seeds were selected, dried well and pulverized in a mortar and pestle. The neem seed powder was extracted with petroleum ether and neem oil dissolved in petroleum ether was obtained. The solvent was evaporated from this mixture, in a vacuum desicator. Exactly 10 ml of the high quality neem oil obtained through the extraction was mixed with 90ml neutral paraffin oil of density 0.8g/ $cm^3$.This oil mixture (10% v/v) was used in all studies involving NSO

**(iii) Neem Seed Kernal Powder (NSKP):** Neem seeds, dried well under shade were pulverized in a blender. The powder obtained was oily and hence dried under shade for about a week. The dried powder was sifted using a standard plastic mesh. The powder was mixed with kaolin at 10 percent level (%w/w) and used for all studies involving NSKP.

**(iv)Toxicity bioassay:** The toxicity bioassay was designed to check the time taken for 100 percent mortality of adult *C.maculatus* exposed to fixed concentrations of NSO or NSKP. Treatment with oil or powder was given to adult *C. maculatus* belonging to different age groups. 0, 1, 2, 3, 4, 5 and 6 day old adults were exposed to the two neem-seed based products. The time taken for the natural mortality of the beetle was recorded on daily basis. The time taken for 100 per cent mortality of the treated *C. maculatus* was recorded alongside and compared with the normal mortality time.

**Mode of treatment**

**NSO treatment:** Exactly 100 n day old beetles were immersed in the oil for a period of 15sec and then placed on a strip of tissue paper, for the excess oil to drip off. Another set of 100 beetles of the same group were immersed in paraffin oil. Any mortality in this group was used as a correction factor.

**NSKP treatment:** Exactly 100 n day old *C. maculatus* adults were immersed in NSKP preparation (10% w/w) for 30sec. The beetles taken out were thoroughly brushed and allowed back in to large plastic containers. Another group of beetles was dipped in Kaolin powder and any mortality in this treatment was considered for making appropriate corrections.

Normally emerging 0, 1, 2 and 3 day old adult *C. maculatus* were sprayed with NSO preparation. The beetles were allowed to interact with the oil preparation for 3min. After treatment, the beetles were placed on paper tissue to remove excess oil and the hours for which each beetle survived was recorded. The 0, 1, 2 and 3 day old beetles were spread with NSKP formulation. The beetles were allowed to interact with NSP for 3min and then removed to fresh vials. The length of survival of each beetle was recorded.

## RESULTS AND DISCUSSION

Both (NSO) and (NSKP) are highly effective against *C. maculatus* adults. The adult beetles just eclosing from the pupae are less susceptible compared to the older beetles. ‘0’ day old adults treated with NSO recorded 100 percent mortality in 79h, whereas 6 day old adults in 54h. Similar trend was recorded for NSKP also **Table 1.**

The mortality of zero day to sixth day old adult *C. maculatus* from is time found for both NSO and NSKP. Tukey comparison shows that treatments are significantly different. Azadirachta, the key bioactive compound in *A. indica.,* is insecticidal in nature. Neem oil, neem seed oil cake and neem seed kernal powder are used for controlling insect pests [19]. Neem seeds are collected from places where the trees are abundant. A number of commercial preparations of neem oil and neem seed kernel are freely available in the market. Some of these preparations contain adjuvants and additives[20,21] .

Response of adult *C. maculatus* to neem seed oil (NSO) and neem seed kernel powder (NSKP) treatment

| Age of adult C. maculatus (days) | Mortality time (in hours) (100 percent mortality) | | |
|---|---|---|---|
| | Control | NSO | NSKP |
| 0 | 229.00±7.5[a] | 79.6± 4.6 [b] (-65.24) | 111.6±2.8 [c] (-51.26) |
| 1 | 207.33 ±7.9[a] | 74.2 ±8.1 [b] (-64.05) | 101.4 ±7.3 [c] (-51.09) |
| 2 | 180.83±8.0[a] | 70.6 ± 6.2 [b] (-60.95) | 98.4 ±6.1 [c] (-45.58) |
| 3 | 157.16 ±8.2[a] | 65.5 ±2.6 [b] (-58.32) | 90.1 ± 4.3 [c] (-42.66) |
| 4 | 137.16 ±6.9[a] | 61.6 ±3.4 [b] (-55.08) | 81.5 ± 4.3 [c] ( -42.66) |
| 5 | 110.57 ±8.1[a] | 55.4±6.5 [b] (-49.89) | 78.4 ± 2.6 [c] (-29.09) |
| 6 | 86.83 ± 8.6[a] | 54.1 ±2.2 [b](-37.69) | 73.8 ± 1.5 [c] (-14.43) |

Note: Tukey test:row wise comparision; different alphabets indicate significantly different mean values

This study was carried out to assess the toxicity of natural neem oil and neem seed kernel powder obtained from freshly picked neem seeds. Both the oil and the seed powder are found to be very effective in killing the adult cowpea beetle, In this study belonging to different age groups (0-6 days) were selected. The younger beetles were more susceptible compared to the adult ones. This was observed both for neem seed oil and kernel powder. The time taken for killing the entire population of *C. maculatus* exposed to the two plant preparations was recorded. The time calculation was on the basis of the mortality of the last member of the population. Each age group of beetles was exposed to only one standard concentration. For each age group the time taken for the natural mortality of the untreated population was considered as the reference index. The treatment may be carried out in grain store houses without damaging or bringing down the quality of the grains.

NSO and NSKP are commonly used for the management of insect pests. Vegetable oils cause the eggs and larvae to die before they can bore into the seed. If it has not been sufficiently coated with oil the larvae peneterate into the seed.Then the treatment produces no further effect and the larvae will develop normally. In some cases female are able to lay eggs, but the hatching of the larvae is prevented by the oil [17]. Oil may also kill the insect eggs when the egg is already present at the surface of the seed or inside the seed, the oil coating prevents gaseous exchanges. So the larvae inside the egg in the seed would die due to lack of oxygen. Singh *et al.*[22], used soybean oil as seed protectent against the infection on pigeonpea and studied the effectiveness of soybean oil as seed protectent against *Callosobruchus chinensis* Linn. The toxicity of three concentrations (5%, 10% and 20% w/v) and spraying schedules (2, 4 and 6 weekly applications) of an extract from black pepper (*Piper guineense*) for managing two major post-flowering pests: Maruca pod borers (MPBs) and pod sucking bugs (PSBs) of cowpea (*Vigna ungulata*) was investigated. The higher concentrations (10% and 20% w/v) and more frequent applications (4 and 6 week) significantly reduced the numbers of the two insect pests compared to the untreated control. Pod damage was significantly reduced and grain yields consequently increased in treated plots compared with the untreated control[23]. Traditional Indian tree neem oil is also a good insect-pest protectant. The toxicity of neem oil against the pulse beetle *Callosobruchus chinensis* Linn was studied in different pulse grains and persistent toxicity of neem oil was reported to be highest in the green peas followed by cowpea and lowest in the bengalgram. A mortality of 100percent was observed in treatment with neem oil upto 14 days of treatment in green gram and cowpea [24].


**J. Chem. Bio. Phy. Sci. Sec. B, August 2017 – October, 2017, Vol. 7, No. 4, 1331-1337.**
**[DOI:10.24214/jcbps.B.7.4.133137.]**

Oil and powder of spearmint *Mentha viridis* L. were effective against *C. maculatus* insect with respect to mortality of adults, significantly reducing the number of eggs laid and hatchability. Oil was significantly more effective than powder [25]. The insecticidal mode of action of the compounds in spearmint may be largely attributed to fumigant action, penetrating insect body through the respiratory system[26, 27]. The leaf oils of *Thymus vulgari* (L) and *Santolina chamaecyparissus* (L), the leaf oils of these plants that were extracted by hydro distillation and their effects on the biology of *C. chinensis* (fertility, longevity and sex ratio) was tested.While comparing these oils, thyme oil at a dose of 10µl exhibited great biocidal power 100% mortality only 72hours of exposure to treatment. Santolina produced 100% mortality at a dose of 20µl [28]. *Mahdian and Rahman*[29] investigated the insecticidal potency of some spices such as black pepper, *Piper nigrum* (L) cinnamon, *Cinnamom zeylanicum* (L) turmeric, *Curcuma longa* (L) and red chillies, *Capsicum frutescens* (L) against the pulse beetle, *C. maculatus* on stored black gram *Vigna mungo* (L). All the spices were effective as protectants of black gram seeds and black pepper was the most effective. Bioligical control methods of bruchids with the endemic larval parasitoid *Dinarmus basalis* Rondani was investigated in storage systems [30]. Introduction of *D. basalis* adults at the beginning of the storage period when the *C. maculatus* density is low, enabled an effective control of the bruchid population [31]. Parasitoids are often more susceptible than their hosts to synthetic insecticides [32-34] . The application of synthetic insecticides in wheat storage systems eliminated many species of natural enemies adversely affecting the biological control of pests. Chloroform extract of the root produced limited mortality of cowpea weevil (7.89 percent). Extraction with hexane and methanol showed a much higher insecticidal activity compared to the extraction with chloroform. Ho *et al.*[35], reported that the n-hexane extract of the dried fruit of star anise has higher contact toxicity to the stored product beetle, *Tribolium castaneum* Herbst than *Sitophilus zeamais* Motsch.

## CONCLUSION

NSO and NSKE are compounds of natural origin, which are non-toxic to human consumers. If used in low concentrations, they will not affect the table quality of the grains, protected using these neem products.

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**Online publication Date: 20.10.2017**