



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2019; 8(3): 188-192

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www.thepharmajournal.com

Received: 20-01-2019

Accepted: 26-02-2019

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Evaluation of some seed protectants against pulse beetle, *Callosobruchus chinensis* (L.) in stored chickpea seeds under laboratory conditions

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Abstract

To determine the bioefficacy of commercial formulations of neem oil, deltamethrin, diflubenzuron and U.V. radiation as a preventive measure against pulse beetle, *Callosobruchus chinensis* (L.) on fresh chickpea seeds under laboratory conditions in the Department of Entomology and Agricultural Zoology, Banaras Hindu University, Varanasi, India, where constant doses of all seed protectants along with control in three replications per 25 g of fresh chickpea seeds was set and three pairs of *C. chinensis* were inoculated in each jar and kept at room temperature. The lowest mean seed damage was observed with the seeds treated with deltamethrin followed by diflubenzuron and neem oil whereas maximum seed damage was recorded on U.V. irradiated seeds when samples were drawn at various intervals of 24 h, 48 h, 72 h, 96 h and 120 hours after 30 days of storage. A similar trend was found even after 60 days, 90 days and 120 days of storage. All the treatments differed significantly among themselves and also differed significantly with control treatment. The losses were increased with increase in storage period.

Keywords: *C. chinensis*, preventive measure, seed damage, seed protectants and bioefficacy

Introduction

Pulse crop because of their high protein content plays an important role in the diet of common people of developing countries like India. In India, total pulse grown area is about 25.25 million hectares and producing 16.47 million tonnes (Anonymous, 2016) [3]. Among the pulses, chickpea [*Cicer arietinum* (L.)] play a vital role in the expansion of agricultural economics of developing countries. Chickpea is a major and cheap source of protein compared to animal protein. In India chickpea accounts for about 43.53% of total pulses produced in the country. Most of the cereals and pulses have to be stored by the producer in their home and by the traders and the Governmental agencies in go-downs for one year or more for future use. So, insect pests are the major problem for storing cereals and pulses. Chickpea is menaced by more than 200 species of insect pests under storage conditions in Indian context. Among these, the pulse beetles (*Callosobruchus chinensis* L.) are the major pests in stored pulse (Ahad, 2003 and Bhalla *et al.*, 2008) [1,7]. It has been reported that pulse beetle, *Callosobruchus chinensis* is a major economically importance pest of all pulses and causes 40-50 percent in losses of pulses under storage (Gosh and Durbey, 2003) [14]. Generally management of stored product pest is done through fumigation and also controlled by synthetic insecticides (Atwal and Dhaliwal, 2005) [4], which have many limitations and undesirable side effects. Insecticides 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 as well as destruction of beneficial insect and increasing costs of application (Singh *et al.*, 2001) [21]. Global warning has cautioned us and the adverse consequences of insecticide use are always alarming and also inducing pest out break because of pest resistance. 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) [8]; and are environmentally safe, less hazardous, less expensive and readily available (Ahmed *et al.*, 1993) [2]. Therefore, efforts are being made to minimize the use of harmful insecticides through different other alternative approaches such as irradiation, phytochemicals, bio-pesticides, insect hormones and natural enemies instead of traditional and synthetic chemical pesticides. Moreover as compared to conventional synthetic insecticides, synthetic pyrethroids have many advantages like low toxicity to mammals and high toxicity to insects

but still these approaches are destined to be non-hazardous to human health, eco-friendly, less expensive and more importantly, are more specific to the target pest population. Ionizing radiations like x-rays and gamma rays (Islam and Laz 2001, Follett 2006, Follett *et al.*, 2007 and Tandon *et al.*, 2009) [15, 11, 12, 23] and non-ionizing radiations like ultraviolet (U.V) rays (Islam *et al.*, 1992, Faruki *et al.*, 2005, 2007 and Begum *et al.*, 2007, 2009) [16, 10, 6, 5], and microwave radiation (Zhao *et al.*, 2007; and Gasemzadeh *et al.*, 2010) [24, 13] have been employed to limit reproduction and survival of a variety of insect pest species. Similarly, the chitin synthesis inhibitors (CSI) which regulate the insect population through the disruption of chitin synthesis have captured the interest of stored-product entomologists. Through greater selectivity of action, these compounds appear to fit the requirements for third generation pesticides that disrupt the normal development of several species of insects. These compounds are effective suppressors of development for the entire life cycle of insect pests. To replace the chemicals with alternatives, neem based bio-pesticide formulations have been scanned for their pesticidal properties with a view to reduce the problem associated with excessive use of synthetic insecticides.

Keeping these facts in mind, a study was undertaken in which the main objective was to assess the seed damage caused by *C. chinensis* in chickpea under laboratory conditions.

Materials and methods

A laboratory experiment was conducted on “estimation of seed damage in stored chickpea caused by pulse beetle, *Callosobruchus chinensis* L.” in the department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during year 2016-17.

Rearing and maintenance of culture

Collection of seeds

The chickpea seeds were collected from local market *i.e.* Susuwahi market near Banaras Hindu University, Varanasi.

Collection of test insect

The mother culture of the test insect was obtained from storage laboratory, Department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during 2016.

Rearing technique of test insect

During the investigation, *C. chinensis* was selected as test insect. The initial culture was maintained on disinfected seeds at 30±1 °C and relative humidity 70±5% in BOD incubator. Prior to release, the host grains were disinfested by fumigation with aluminium phosphide (celphos) @ three tablets 9 (gm) per tonne for 72 hours and then left in open for 24 hours to eliminate the hidden infestation and were conditioned. Fifteen pairs of one day old beetles from the initial culture were released in cylindrical jars measuring (25x15x10 cm) containing 500 gm seeds. The jars were covered with muslin cloth and tied with rubber band. The jars were kept at ambient condition in the laboratory in order to get a continuous supply of adults for further experimentation. The insects were handled carefully with the help of a pair of forceps having blunt ends, camel hair brush and aspirator were used invariably for transferring insects in glass jars.

Efficacy of seed protectants

Experiments were carried out for the study of bio-efficacy of commercial neem based bio-pesticide formulation, chemical insecticide (deltamethrin commercial formulation), U.V. radiation and diflubenzuron as preventive (fresh grains) on pulse beetle, *C. chinensis* L. in stored chick pea. The experiment was conducted at insect physiology and toxicology laboratory, Department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi in 2016-17. Neem oil, deltamethrin, U.V. radiation and diflubenzuron were tested as seed protectants against pulse beetle. At a constant dose for each treatment, one kg of fresh chickpea seeds in glass jars (18 cm x 20x10 cm) were treated through hand sprayer and exposed under U.V. radiation.

Table 1: Details of treatments used for management of *C. chinensis* are as follows

Sl. No.	Treatment	Trade name	Formulation	Working concentration (ppm)	Dose
T ₁	Neem oil	Nimbecidine (T. Stanes & Company Ltd.)	0.03% EC	6.00	10.00 ml/kg
T ₂	Diflubenzuron	Bi-Larv (Bayer crop science)	25% WP	1.00	0.002 mg/kg
T ₃	Deltamethrin	Decaguard (Gharda Chemicals Ltd.)	2.8 EC	1.40	0.025 ml/kg
T ₄	U.V. radiation	Philips lamp*	-	254 nm	5 minutes
T ₅	Untreated control	-	-	-	-

* U.V. lamp was fixed inside a locally made wooden desk type container.

Observations

A sample of 25 gm each from those bulk treated fresh chickpea seeds were drawn at 24 h, 48 h, 72 h, 96 h and 120 h after exposure to various treatments and three pairs of newly emerged male and female adults were released. Observations for different parameters were recorded at 30, 60, 90 and 120 days interval from the samples drawn at each hour of exposure. The chickpea seeds without treatment were served as control. After 10 days, the adults were removed and the jars containing the exposed grains were kept at room temperature for the adult emergence. Three replications were maintained for each treatment.

From the representative sample of each treatment, damaged and the total number of grains were counted and subjected to the formula given by Quitco and Quindoza (1986) [19]:

$$\text{Percent grains damaged} = \frac{\text{No. of damaged grains}}{\text{Total no. of grains}} \times 100$$

The experiment was carried out in laboratory, hence analysed through completely randomized design, replicated thrice with four treatments and a control to study the effect of these treatments on seed infestation. Observations were recorded at the mentioned intervals and statistical analysis was carried out thereafter.

Results and Discussion

Bioefficacy of commercial neem oil, deltamethrin, U.V. radiation and diflubenzuron as preventive measure (fresh seeds) on pulse beetle.

Percent seed infestation

To protect the seeds stored for next season, the requisite treatment is essential to protect the seed from the stored grain pest. Therefore, treatments with deltamethrin, diflubenzuron, neem oil and irradiation with U.V. radiation along with control (untreated) were tested under laboratory conditions as preventive measure. A sample of twenty five gram each from those bulk treated grains were drawn at 24 h, 48 h, 72 h, 96 h and 120 h after exposure to various treatments and three pairs of newly emerged adults were released. The data pertaining to the seed infestation in chickpea treated with various protectants and exposure under U.V. radiation at 30, 60, 90 and 120 days after storage are presented in Table 2.

30 days after storage

Under the bioefficacy trial deltamethrin 2.8 EC @ 1.40 ppm seed was found significantly superior giving complete protection till one month of storage as there was no development of bruchids due to its deleterious impact on the released adults. Diflubenzuron 25% WP @ 1.00 ppm seed was found second best effective treatment against pulse beetle in chickpea as minor infestation was found in the sample the one withdrawn 120 hours after treatment. Neem oil @ 6.00 ppm and U.V. radiation @ 254 nm intensity gave least protection against bruchid and seed damage were found in increasing pattern in the samples drawn from 24 to 120 hours after treatment and stored for one month (Table 2 & figure 1).

60, 90 and 120 days after storage

The damage observed after definitive intervals was similar as compared to the damage scenario observed after 30 days of storage of treated chickpea drawn at 24 h, 48 h, 72 h, 96 h and 120 hours of treatment. The commercial formulation treatments of deltamethrin 2.8 EC @ 1.40 ppm and diflubenzuron 25% WP @ 1.00 ppm seeds exhibited significantly less seed infestation compared to neem oil and U.V. radiation treatments. It is significant to note that zero percent seed infestation was observed with deltamethrin treated seeds even up to 120 days of storage. It is also clearly evident from the data (Table 2 & figure 1) that the treated

samples drawn after 120 hours of deltamethrin treatment and stored up to 120 days has recorded zero percent infestation, whereas the diflubenzuron treated seeds exhibited 5.13, 22.18, 30.03, 43.05 and 60.22 mean percent infestation of the stored chickpea grain after 120 days of storage from the samples drawn at 24 h, 48 h, 72 h, 96 h and 120 hours after the treatment, respectively. On the other hand, significant percent seed infestation as high as 98.10 was recorded from the seed irradiated with U.V. radiation @ 254 nm intensity after 120 days of storage and the sample drawn after 120 hours of treatment. Whereas, neem oil formulation @ 6.00 ppm seed recorded a mean percent infestation of 90.74 at this hour of observation.

All the treatments differed significantly among themselves and also differed significantly with control treatment. Cent percent mean seed infestation was recorded after 120 days of storage and no significant difference in mean percent seed infestation was observed when samples were drawn at various intervals of 24 h, 48 h, 72 h, 96 h and 120 hours of 30 days, 60 days, 90 days and 120 days of storage in control treatment. The mean percent seed damage was statistically at par between U.V. radiation and control treatment after 120 days of storage and the samples drawn after 120 hours after treatment recording 98.10 and 100.00 percent, respectively.

The present finding is in close accordance with the results of Srivastava and Jha (2007) [22], Ramzan (1994) [20], Jain and Yadav (1989) [17] and Lal and Dikshit (2001) [18] who reported full protection of pulses with deltamethrin against *C. chinensis*. From the present study, it can be concluded that deltamethrin 2.8 EC @ 1.40 ppm and diflubenzuron 25% WP @ 1.00 ppm seed can be used for successful protection of chickpea seeds up to four months of storage. These treatments are likely to be economical. U.V. radiation treatment as a preventive measure does not imply any significance as there was gradual increase in grain damage up to 120 days by the pulse beetle released on the exposed seed. As the beetles were not exposed to irradiation, the adult beetles continued to grow and multiply and caused damage to the grain to a time of 98.10% by end of 120 days of storage (Table 2 & figure 1).

Table 2: Efficacy of certain treatments as a preventive measure on percent seed infestation caused by *C. chinensis*

Treatments	Mean percent increase in seed infestation caused by <i>C. chinensis</i> over control at different hours of exposure after																			
	30 DAS					60 DAS					90 DAS					120 DAS				
	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
Deltamethrin	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Diflubenzuron	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.17 (18.47)	3.27 (10.37)	6.04 (14.22)	8.17 (16.49)	15.21 (22.86)	35.22 (36.38)	5.13 (13.05)	11.60 (19.85)	15.10 (22.85)	22.24 (28.12)	43.06 (41.00)	5.13 (13.05)	22.18 (28.06)	30.03 (33.21)	43.05 (41.00)	60.22 (50.90)
Neem oil	2.50 (8.94)	6.88 (15.08)	15.50 (23.18)	20.35 (26.76)	21.33 (27.50)	12.00 (20.23)	17.00 (24.22)	28.30 (32.13)	45.88 (42.64)	51.75 (46.00)	25.00 (29.98)	38.93 (38.58)	55.00 (47.88)	70.00 (56.83)	73.08 (58.76)	35.00 (36.25)	48.00 (43.85)	62.11 (52.02)	75.00 (60.07)	90.74 (72.31)
U.V. Radiation	3.50 (10.61)	10.71 (19.03)	19.58 (26.26)	24.77 (29.84)	25.57 (30.37)	16.00 (23.50)	25.00 (29.96)	33.00 (35.01)	49.16 (44.52)	54.81 (47.76)	30.00 (33.18)	48.00 (43.85)	62.00 (51.95)	73.80 (59.23)	77.51 (61.72)	42.00 (40.39)	58.00 (49.61)	75.29 (60.28)	82.00 (65.01)	98.10 (82.22)
Untreated	21.02 (27.26)	22.11 (28.01)	24.50 (29.66)	26.05 (30.68)	27.50 (31.62)	32.09 (34.50)	34.00 (35.65)	41.15 (39.89)	51.28 (45.73)	56.83 (48.93)	51.53 (45.87)	57.29 (49.20)	69.72 (56.62)	77.63 (61.78)	79.18 (62.86)	79.84 (63.33)	82.25 (65.09)	85.49 (67.81)	90.10 (71.77)	100.00 (90.00)
S.E (m)	0.946	1.070	0.396	0.761	0.856	0.968	1.228	1.279	1.009	0.844	0.869	1.086	0.889	0.980	0.934	0.889	0.933	1.565	1.489	0.635
C.D	3.021	3.417	1.264	2.428	2.734	3.089	3.920	4.081	3.219	2.694	2.775	3.467	2.839	3.128	2.982	2.839	2.978	4.994	4.751	2.026

*Figures in parenthesis are arc sin transformed values of percentage. DAS: Days after storage, HAT: Hours after treatment

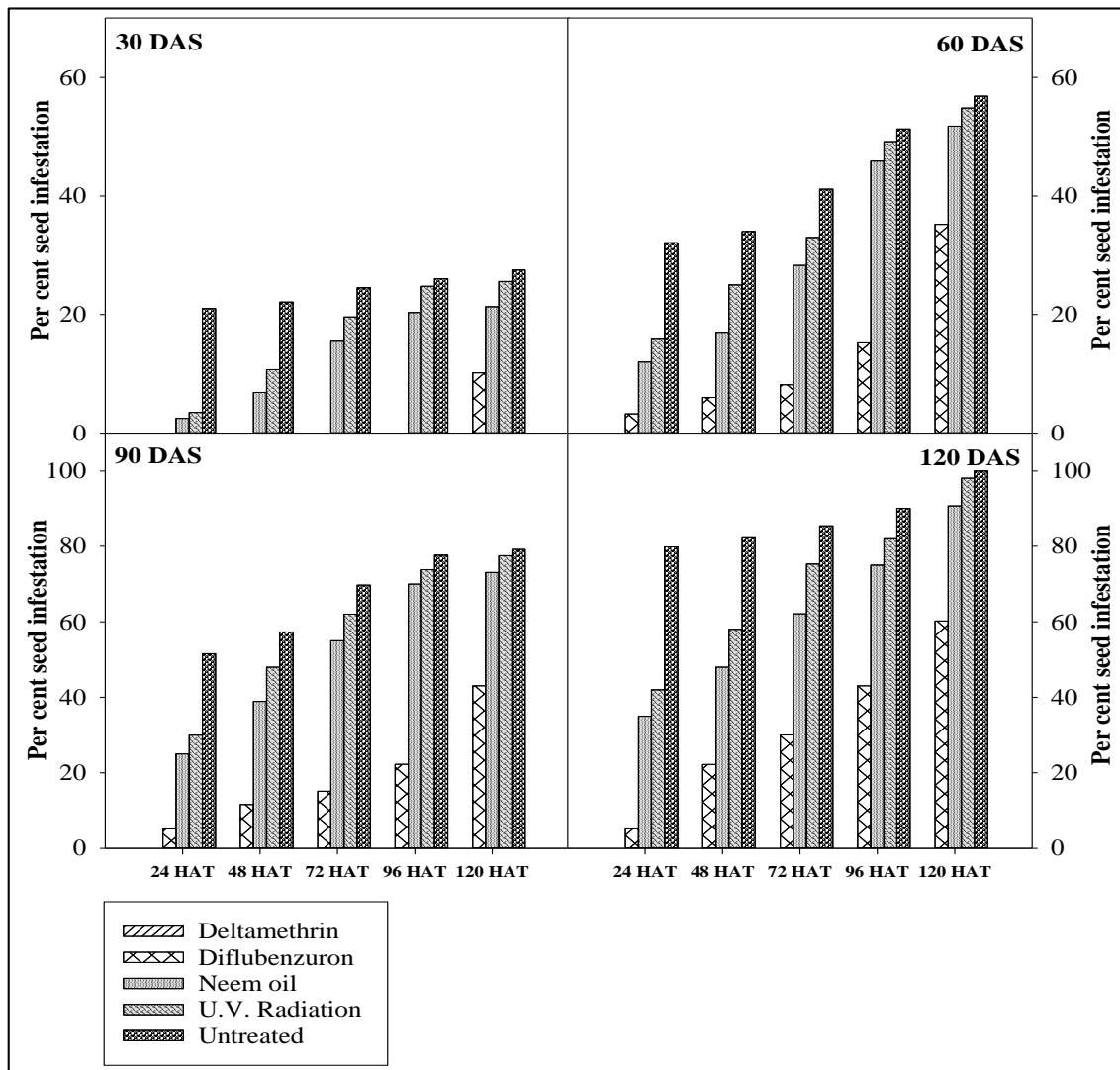


Fig 1: Efficacy of certain treatments as a preventive measure on percent seed infestation caused by *C. chinensis*

Conclusion

Based on present investigation, it is worthwhile to study extensively to control *C. chinensis* through deltamethrin because it has potential an alternative to chemical pesticides but still more explorations are needed to achieve the goals of long term and sustainable pest management strategies with minimal environmental impacts.

Acknowledgment

Author is highly thankful to Dr. Dinesh Kumar, professor, Department of Zoology, Banaras Hindu University, Varanasi for providing U.V. cabinet setup during the research work and also to university grant commission for funding to carry out this research.

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