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Assessment of Oviposition Deterrent and Insecticidal Activities of *Clitoria ternatea L.* Plant Extracts Against Stored Grain Pest Pulse Beetle, *Callosobruchus maculatus*(Fabr.) (Coleoptera: Bruchidae)

KEYWORDS: Clitoria ternatea, oviposition deterrent, Insecticidal, Callosobruchus maculatus

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ABSTRACT *Methanolic extracts of Clitoria ternatea L. plants (family: Fabaceae) were evaluated under laboratory conditions for their insecticidal and oviposition deterrent activity against Callosobruchus maculatus on green gram. All the methanolic extracts of plant parts viz. leaf, flower and seed showed significant mortality and reduction of egg laying when compared to the control. However, among the plant parts evaluated, seed extract of C.ternatea was found to be most effective causing 75% adult mortality and 98.4% oviposition deterrent activity. This was followed by leaf extract causing 63.33% adult mortality and 89.51% oviposition deterrent activity and Flower extract causing 40% adult mortality and 82.51% oviposition deterrent activity. Susceptibility of seed and leaf extract was found to be more in case of female adult when it was compared with the control sample. Extract of flower of C.ternatea did not show much difference between female and male mortality . Mortality of adult andoviposition deterrent activity of the plant as observed in the present investigation suggests that the extracts from this plant may be one of the most effective botanicals that could be used as green gram protectants in controlling infestation of C. maculatus.*

Damages and losses due to infestation of insect under stored condition are the most serious threat in developing countries because of improper storage and unhygienic facilities which encourages insect attack (Talukder ,2005; Talukder et al,2004)

Pulse bruchids *Callosobruchus* species are among the most destructive storage grain pests and known to occur throughout the world on different types of pulses. These are considered as notorious pest of green gram, chick pea, black gram, peas, cowpea, lentil and pigeon pea (Aslam et.al. 2002). Perhaps no other storage pest is as difficult to control as these bruchids due to their concealing behavior. Their attack on pulses under storage not only reduces the quantity but it also affects the quality of the pulses and due to this their market value is reduced and pulse growing enterprise is rendered unprofitable.

Bruchid lay eggs on the surface of seed coat and after hatching they enter and feed on endosperm. Once the insect larvae enters into inside the pulse grain, control is almost impossible thereafter. The hidden larvae feed inside and the adults only comeout after emergence. Anumber of insecticides and fumigants have been recommended for the control of this storage pest but fumigants are most effective solution to reduce their population.

Fumigants are used for the protection of pulses and other stored products to prevent economic and quality losses due to insect pest attack. Methyl bromide (MB) and phosphine are mainly two important fumigants which are used to control store grain insect pest. MB is a commercially desirable fumigants due to its rapid action and remarkable penetration activity. But owning to adverse effects of these fumigants, regulatory agencies have restricted the fumigant registration. Presently very few fumigants are allowed to use. Besides residues, MB has also adverse impact on stratospheric ozone layer depletion and thus it has already been phased out in developed countries since 2005 and developing countries will also follow the suit and will phase it out by 2015.

Thus there is an urgent need for safe and biodegradable fumigant for the control of stored grain pests. Plants are rich in substances which may provide the potential alternatives to the chemical insecticides and fumigants.

Botanicals contain an array of compounds which are virtually an untapped reservoir of pesticides which can be utilized directly or as templates for currently used synthetic pesticides(Singh and Sehgal, 2001). Potential plant preparations like; Plant powders, Extracts, , Essential oil from diversity of plant sources are currently being investigated for their pesticidal activities.

As of now investigation regarding oviposition activity of plants are at preliminary stage of studies except neem. Thus the present studies have focused on the insecticidal and oviposition deterrent activity of solvent extract of *Clitorea ternatea* against *Callossobruchus maculatus* under laboratory condition to protect green gram pulse under storage condition.

Materials and Methods:

Different parts of the plant like ; leaves, flowers and Seeds were collected from the state of Rajasthan and brought to the laboratory.

- **Preparation of powders:**

The collected roots, leaves, flowers and seeds of *C. ternatea* were properly cleaned, washed and dried in the shade and pulverized into fine powder using an electric grinder.

- **Preparation of extracts:**

Fifty gram powder of each of the plant parts except seeds was extracted in 250 ml of methanol with soxhlet apparatus for 8-12 hrs. The collected extracts were concentrated with the help of rotary evaporator under low pressure, below 60^0C and residues were stored at 4^0C until the use.

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- **Preparation of seed extracts:**

Seeds of *C. ternatea* were surface sterilized and soaked overnight in water. Further the absorbed seeds was macerated in water with the help of mortar and pestle and filtered through several layers of cheese cloth. The Filtrate was centrifuged and the supernatant was collected to evaluate theirbiocidal activity against the insects.

- **Collection and maintenance of Insects:**

The pulse beetle *C. maculatus* was obtained from naturally infested pulses like green gram or cowpea seeds from a local market, in Delhi. These beetles were separated on the basis of their physical morphology and was reared on clean and un-infested green gram (*Vigna radiata* L.). Around 50 adults was released in 250 gm of green gram and red gram seeds in a jar covered with muslin cloth to ensure ventilation. The culture of these beetles was maintained under controlled temperature (28+2^{0}C) and relative humidity (70± 5%) in BOD. After 48 h the adults were removed and the jars were kept for around 25 days to obtain adult beetles for the current studies.

- **Evaluation of Insecticidal activity of extracts and powders:**

Methanolicextracts of leaves, flowers and seeds were evaluated at different concentrations for insecticidal activity. The required amount of powder and extracts was mixed thoroughly with 20 gm. green gram seeds. Ten pairs of 24-48 h of adults was released into each container which were covered with muslin cloth. The no. of dead beetles were recorded after 48 hrs. of treatment. The per cent mortality of beetles was calculated using the corrected formula of Abbott (1925). The total no of eggs laid on the surface of seed was recorded after 7 day after the treatment and per cent deterrence of oviposition was calculated according to Elhag (2000) .

- **Oviposition deterrence:**

Fifty gram seed of green in small container were mixed with 5 ml of methanolic extract of *C.ternatea*. For control grains were treated with solvent only. Minimum three replicates of each sample were considered for each treatment. After application of extract grains were air dried before releasing the insects. Ten pairs of 0-12 hrs. old adults were introduced into each replicate and observations were made accordingly.

- **Seed Viability test:**

To test the viability of green gram seeds, seeds were treated with extracts of *Clitoria*. Fifty seeds were cleaned and mixed with extracts and then kept on moist filter paper in a petri dish. Water was added to the filter paper whenever required. The number of seeds germinated were recorded up to 7 days and compared to control.

Result and Discussion:

Callosobruchus species larvae feed inside the grain of pulses and utilized all the nutrients and during its feeding stage. Potential of *C. ternatea* has been reported by Kelumu *et al* (2004) against bean beetles. In the present study the effectiveness of the different plant parts of C.ternatea was evaluated to control one of the most destructive stored grain pest *Callsobruchus maculatus*. All the extracts of the plant parts viz. leaf, flower and seed caused significant mortality andoviposition deterrent activity against the adult of the insect.

The highest mortality was observed in the higher concentration (10000 ppm) of the seed extract where adult mortality was 75 percent after 7 days. After 96hrs. the mortality was 51.6 percent (Table 1). Among all of the plant parts seed extracts was found to be most effective followed by the extract of leaves.In case of leaves extract mortality was 63.3 percent after 7 days and 3.3percent after 96 hrs. The lowest mortality among the evaluated plants was recorded in the flower extracts where after 7 days the mortality was only 40 percent. Females were found to be more susceptible in comparison to adult males (Fig.1)where mortality was highest in seed treatment i.e. 45% in female and 30% in male. In case of flower and leaves treatment gender sensitivity was less affected as in leaves mortality was 30% in females and 25 % in males. The present

Table1. Toxicological response of *Callosobruchus maculatus* to extracts of *Clitoria ternatea* plant parts.

Name of sample	Number of Adults released	Stage of the Adult	Concentration	Avg mortality after 4 days (Mean± S.E.)	Avg. mortality after 7 days (Mean± S.E.)	Percent mortality of adults	
						After 4 days	After 7 days
Control	30 Pair	0-12 hrs. old	1 %	1 ±0.82	2.66 ±0.47	5	13.33
Clitoria Leaves	30 Pair	0-12 hrs. old	1 %	6.66 ±1.24	12.66 ±1.70	33.33	63.33
Clitoria Flower	30 Pair	0-12 hrs. old	1 %	5.66 ±0.47	8.0 ±0.82	28.33	40
Clitoria Seed	30 Pair	0-12 hrs. old	1 %	10.33 ±0.94	15 ±0.82	51.66	75

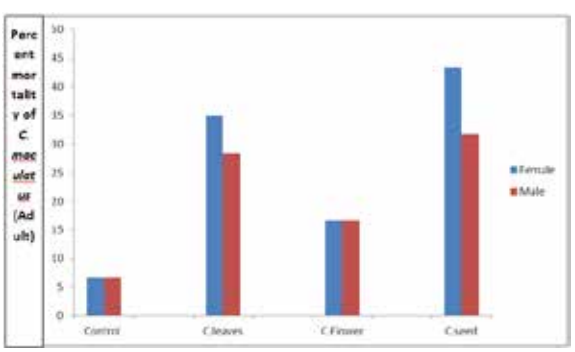

Fig.1.Mortality response of female and male adults after the treatment of *C.ternatea*

investigation revealed that maximum deterrent activity was found in the seed extracts of the plant , where complete cessation of egg laying was observed after 96 hrs. of the treatment (Table.2). During the studies it has also been observed that in the early stage of treatment insects tried to avoid the treated pulse grains and as time passed out either the adults were unable to lay eggs or fewer eggs were laid by them. The hatching of eggs did not take place in the higher concentration of the treatment.

The survival and egglaying of *C.maculatus* was significantly affected by the treatment of the extracts which suggest

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the presence of insecticidal and ovipositional active compound in the extracts which delayed and completely inhibited the oviposition of the insect. The insecticidal activity of the seed extract supports the findings of kelemu*et al*, 2004 where they have reported that the presence of finotin (a protein) in the seed extract of *C.ternatea* caused mortality in *Zabrortus subfaciatus* and *Acanthoscelides obtectus* have potential to control the insect pest. They have reported that seeds of *C. ternatea* have broad range of activity against insects as well as bacterial and fungal pathogens. The present investigations have revealed the potentiality of *C. ternatea* for the protection of green gram. The insecticidal activity of the protein of the plant have been also reported by Kelumu *et al,* 2004, where they have used artificial seeds technique for

Table 2. Oviposition deterrent activity of extracts Clitoria ternatea against *Callosobruchus maculatus*

Name of sample	Stage of the Adult	Concentration		Number of eggs laid		Oviposition deterrence (Percent)	
				After 96 hrs.	After 7days		
			Number of Adults released	Avg	Avg	After 96hrs.	After 7 days.
Control	0-12 hrs. old	1 %	30 Pair	56.33	143	-	-
Clitoria Leaves	0-12 hrs. old	1 %	30 Pair	7	15	87.07	89.51
Clitoria Flower	0-12 hrs. old	1 %	30 Pair	12	25	78.82	82.51
Clitoria Seed	0-12 hrs. old	1 %	30Pair	Nil	1.6	100	98.88

insecticidal activity against *Zabrortus subfaciatus* and *Acanthoscelides obectus* and found that finotin protein from seeds of *C.ternatea* is effective for the protection of common bean variety against the infestation of these bruchid species.Importance of plant as seed protectant by solvent extracts, plant powders, essential oils have been reported by various workers ((Ngamo et al., 2007; Zahra Sahaf and Moharramipour, 2008; Othira et al., 2009). vanmathi et al (2010) have reported that higher concentation of plant extracts (5%) significantly reduced the activity of egg laying. The oviposition activity was reduced more than 50% in pulse beetle *Callosobruchus maculatus* even at the lower doses (1% & 3%). According to them deterrence activity of the insect might be attributed to the change in the behavior and physiology of the insect after the treatment of the extracts. Previously Olafa and Erhum(1998) have shown that piper guineense considerably reduced the egg laying. The oviposition of *C.maculatus* was also significantly reduced by the treatment of Eucalyptus leaf extract, turmeric powder, black pepper powder and grarlic clove powder also(Gahlot and Singhvi,2006; Ravinder, 2011).

Conclusion:

From the present investigation we conclude that the extracts of *C.ternatea* could be proved to be an alternative of synthetic pesticides used to manage *C. maculatus* on green gram under storage condition. The timely application of the extract could provide protection against the insect by its oviposition deterrent as well its insecticidal activity. More studies may be carried out to isolate and characterize the active compounds in the *C. ternatea* extracts.

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