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Research Article

Insecticidal Activity of Methanolic Extract of *Calotropis Procera* against *Callosobruchus Maculatus* using Moong Seeds (*Vigna Radiata*)

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ABSTRACT

Food quality and safety are directly depending on the worth of raw material used. Screening of plant extracts for deleterious effect on insects is one of the approaches in the search of novel biological insecticides for longer shelf life and quality of the seeds. Application of several medicinal plant products has drawn much attention as effective alternatives to the synthetic pesticides. *Calotropis Procera* exhibited several medicinal properties hence selected for the control of *Callosobruchus maculatus using moong* seeds (*Vigna radiata*). Different concentrations (1.0, 1.5 and 5.0%) of methanolic crude extract of *Calotropis procera* were evaluated for insecticidal activity against *C.maculatus using moong* seeds. Germination study was carried out before treatment (24.66%). Ovipostion and adult emergence was evaluated at three different concentrations. Maximum oviposition deterrent activity was observed in control sample (86.33%) followed by 1.0% (78%), 2.5% (40.33%) and 5.0% (30.66%). Reduction in adult emergence was also high after using the different extracts concentration i.e.1.0% (65.36%), 2.5% (21.76%) and 5.0% (8.10%). At 5.0 % concentration weight loss was found to be significant 5.99%. Germination rate was also improved as the concentration of extract increased which again shows the augmentation in germination of seeds. Significant results in all evaluated parameter at 5.0 % concentration specify insecticidal property of methanolic crude extract which after identification and characterization of inhibitory molecules may commercialize and further applied in food as well as other agricultural sector.

Key words: *Calotropis Procera*, *Vigna radiata*, *Callosobruchus maculatus*, oviposition deterrent activity, adult emergence

1. Introduction

Vigna radiata (green gram or mung bean) is the third most widely cultivated pulse grain in India (Swaminathan et al., 2012). India has 14.8 Million tonnes of pulse production whereas loss due to insect pest is 2.6 million tonnes (Dhaliwal et al., 2010). Loss due to *C. maculatus* in terms of weight against *Vigna radiata* is 19.2% (Ramzan et al., 1990) and if left untreated it can cause 100 percent damage to the crop. *C. maculatus* is a broad-based insect pest of cowpea. The larvae of cowpea beetle bore into the pulse grain (oviposition site) (Credland et al., 2006) and make the grain unsuitable for human consumption and affect the germination capacity of the plant (Rahman and Talukder, 2006). Suitable growth conditions are 30 °C and 70% relative humidity (Credland et al., 2006). The populations of insect increases rapidly and results in total destruction within a short duration of 3-4months (Rahman and Talukder, 2006). It multiplies very rapidly in storage (Ouedraogo et al, 1996) and reported 8.5% loss in pulses during post harvest handling and storage in India. The pest can contaminate stored products during transportation of grain or in warehouses and retail stores (Allahvaisi et al., 2010). Various botanical powders have been used as protectants against the insect while storage (Shukla et al., 2007). Insect feed on the protein content of the grain and damage caused ranges from 12-30 per cent in developing countries (Tsedeke, 1985 and FAO, 1994). Leaves of *Calotropis procera* is selected for the insecticidal activity. *Calotropis procera* (Akra) is a species of flowering plant belonging to the milkweed family has been used in many medicinal practices throughout the world (Mossa et al., 1991) and also known for several properties such as antimicrobial activity (Jain et al., 1996), anti-inflammatory,

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analgesic properties (Basu and Chaudhuri, 1991) anti-diabetic, abortifacient, spasmogenic and carminative properties, antidysentric, antisyphilitic, antirheumatic, antifungal, treatment of chronic illnesses such as leprosy, bronchial asthma and skin affliction (Sharma, 2001). Used for diarrhoea, stomatic, sinus fistula, and skin disease (Rasik et al., 1999) and the leaf part is used to treat jaundice (Murti et al., 2010). Giridhar et al., 1984 first demonstrated the insecticidal and larvicidal effects in the latex of *Calotropis procera* paving a path for more research.

Annie Bright (2001) and Raja et al.(2001) reported that botanicals inhibited adult emergence in *C. maculatus* in cowpea. They further stated that, when the eggs were laid on treated seeds, the toxic substance present in the extract may enter in to the egg through chorion and suppressed their embryonic development. It is in agreement with the present study that adult emergence was greatly reduced in treated seeds than control seeds. Raja et al.(2001), Keita et al. (2001) and Sathyaseelan et al. (2008) reported that various plant products were effective in reducing oviposition and adult emergence of *C. maculatus* only, but the seed quality and germination were not affected. These results are in accordance with our findings. The present investigation has brought out the efficacy of methanolic extracts of *calotropis procera* against *C. maculatus* using moong seeds (Vigna radiata)

2. METHODS AND MATERIALS

2.1. Insect culture and rearing

C. maculatus culture was procured from Division of Entomology, IARI Pusa, New Delhi. Insects were reared on mung bean seeds in a glass jar (15cm x 10.5 cm) and tightened with muslin cloth at 27±1.5O C and 70 % relative humidity. The growth chambers were sealed and the beetles were allowed for mating and ovipositions. The parental insects were removed and mung bean seeds containing eggs were transferred individually into fresh vials to avoid mating after their emergence. Individually emerged unmated insects were further used for experiments (Ranjan et al., 2016)

2.2. Collection of Plant Material

After on site identification of plant nearby fields of Amity University, Noida, aerial part of the herb was randomly collected. Sample was brought to the laboratory in sealed poly bag. Samples leaves were washed thrice and then allowed to dry at room temperature under a shaded (28±2^{O}C) ground and sieved to get fine powder and stored in tightly sealed polyethylene bags.

2.3. Preparation of Plant Extracts

5.0 % of extracts was prepared by using powder of *Calotropis procera.* 5g of powder were weighed and taken into 250 ml capacity conical flask and initially 50 ml of methanol was added to it and manually mix. 50 ml methanol was again added and shaken for 4 h in a mechanical shaker. Extracts was then taken in a centrifuge tube and mixed well by vortexing and shaking with hands for about 30 minutes (Kaushik and Chauhan 2007; Chauhan et al., 2010). This was centrifuged at 4000 rpm for 20 minutes at 25^{O}C. Supernatant was collected in conical flask. This 5 % extract (stock solution) was further diluted to make 2.5 and 1.0 % concentration. All three concentrations were used for efficacy (Fig. 1)

2.4. Collection of moong seeds (*Vigna radiata*)

Moong seeds were collected from local market and taken in plastic tray. Seeds were rinsed thoroughly with tap water followed by distilled water to remove oil and other coating on the surface of seeds. Seeds then allowed to air dry.

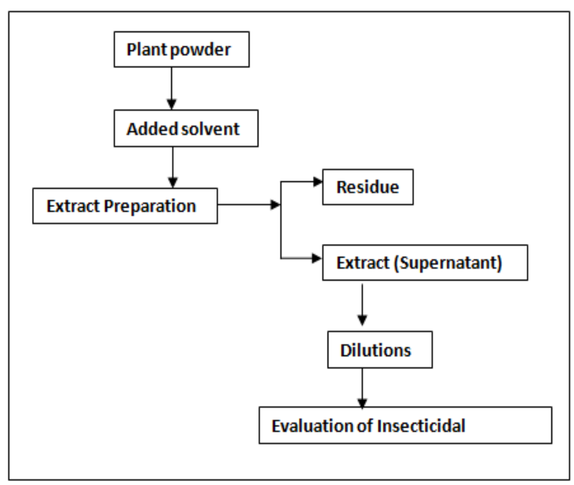

Figure 1: Flow diagram representing extraction of plant powder

2.5. Assessment of adult emergence and weight loss

10 gm of healthy Mung bean seeds (*Vigna radiata*) weighed in four replications for each

concentration and two controls in clean plastic vials. One control was taken as seeds without any treatment and another were seeds treated with emulsified water. Seeds were treated with respected concentration in a petri dish and allowed to air dry for 24 hours. Two pairs (male & female) of newly hatched and unmated pulse beetles *C. maculatus* were released in each vial and stored at 27±2 O C and 70% humidity. A week later after releasing insects, number of ovipositions was counted for each set of experiments. Adults started emerging after 18 days onwards of incubation period. Number of emergence of *C. maculatus* was recorded. Assessment of seed loss and weight loss were also carried out.

2.6. Germinations Study: Before beginning of experiments, mung beans seeds were checked with percentage germination. Paper beds were made by cutting germination papers exactly the size and shape of petri dishes. Paper beds were allowed to be wet by pouring distilled water. 100 mung beans seeds in five replications were tested for germination. Germination was also tested on seeds which were left over after finishing experiments. Observations were recorded for a week.

3. RESULTS

The results of several parameters studied are summarized in Table.1. Germination of seeds before treatment was 24.66 % which was increased to 28.66, 61.00 and 72.33 at 1.0, 2.5 and 5.0 % respectively. Highest germination rate was observed at 5.0 % concentration of methanolic extract. A week after setting up experiment and releasing insects in to experimental vials, number of oviposition was recorded. Maximum oviposition was in control which is 86.33±1.45and minimum ovipositions were at 5.0% which is 30.66±0.88. Relative ovipositions with respect to control were 78±1.73, 40.33±2.96, and 30.66±0.88 at 1.0 %, 2.50 %, and 5.0 % respectively. After 18th day of incubation period, adult started emerging and it was found to be 8.10±0.46 at 5.0%, 21.76±1.54 at 2.50% and 65.36±1.77at 1.0% concentrations. Maximum emergence was observed in control at 76.33±0.63%.

Table: 1 Insecticidal activity of crude extract of *C. procera*

Parameter Evaluated (in %)	**Without Treatment**	**Treatment (% Concentration of Methanolic extract of *C. procera)***		
	Control	**1.00**	**2.50**	**5.00**
Oviposition	86.33±1.45	78±1.73	40.33±2.96	30.66±0.88
Emergence	76.33±0.63	65.36±1.77	21.76±1.54	8.10±0.46
Weight loss	15.53±0.33	9.50±0.20	7.89±0.29	5.99±0.10
Germination	24.66±1.20	28.66±1.20	61.0±2.08	72.33±2.33

Figure 2: *Calotropis procera*: Identification, Collection and Extraction

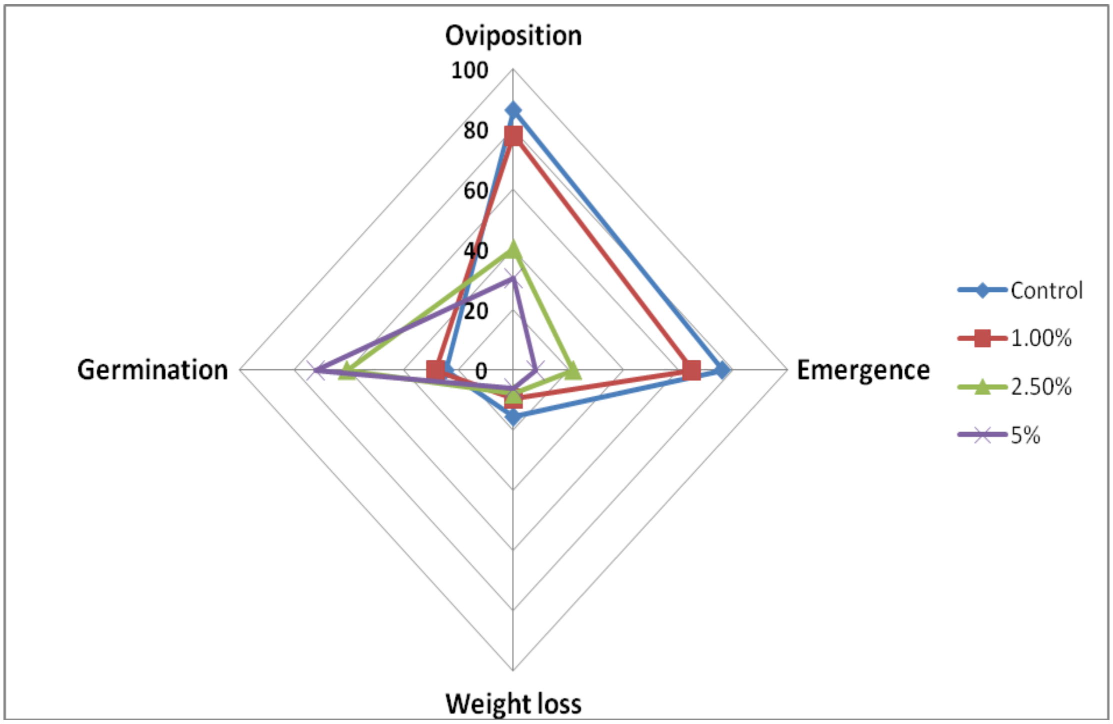

Figure 3: Comparison of several parameters study for insecticidal activity of methanolic extract of *Calotropis procera*

Adults of *C. maculatus* hatch out from the seeds by making exit holes. This causes major loss to crop. Treatment with 5.0% dose have shown lowest weight loss of 5.99±0.10 % and maximum of 15.53±0.33% which was control. Percent weight loss at 1.0%, 2.5.0%, and 5.0.0% were 9.50±0.20, 7.89±0.29 and 72.33±2.33 respectively.

4. DISCUSSION:

5.0% dosage of methanolic extract of *calotropis procera* helps in reducing ovipositions and emergence there by controlling seeds and weight loss of the crop. This dose also helps in protecting germplasm of the seeds which can be further used for cultivation. Data recommends 5.0% dose strongly signifies for reduced oviposition, weight loss, seed loss and germinations. Study was also supported by Nooshin (2012) who tested essential oil from *L. camara* and found have very strong repellent activity. Saxena (1992) studied petroleum ether and methanolic extracts (1-5%) of *L.camara* against *Callosobruchus chinensis* and observed 10-43 % mortality, complete feeding deterrence at 5% and anti ovipositions values was 40mg/100 gm with methanol extract of *L. camara*. Loganathan (1996) studied with 5, 10 and 20 % of acetone extract of *L. camara* reduces ovipositions by 22%, 37% and 62 % respectively. Gujar and Yadav (1978) recorded 55 to 60 % loss in seed weight and 45.50 to 66.30 % loss in protein content due to the damage by pulse weevil.

Earlier literature indicates the importance of plant extract in protecting seeds by way of direct mixing of the dried leaves, plant powders, solvent extracts, vegetable/ essential oils on seeds during post harvest storage (Rajapakse, 1996; Ngamoet al., 2007; Othiraetal., 2009). The reduction in oviposition was increased with the increase in dosage of each treatment. Earlier, Olaifa and Erhun (1998) found that higher concentration of the powder of *Piper guineense* significantly reduced the oviposition. These earlier findings are in conformation with the present study. Higher concentrations of plant extracts (5%) were found to be effective as compound to lower ones (1% and3%) in bringing down the egg laying by the pest insect. Present study revealed that, maximum oviposition deterrent activity was observed in *O. tenuiflorum* and *C. dactyl* on followed by *P. amarus*. It is noteworthy that all these plant extracts showed more than 50% of deterrent activity even at lower concentration. It appears that these plant extracts might possess repellent and/or oviposition deterrent principles. Oviposition detergency may be due to the changes induced in physiology and behavior in the adult of

C. maculatus as reflected by their egg laying capacity. The data shown in Table 2 revealed the effect of leaf extracts on adult emergence of pulse beetle. A significant reduction in adult emergence was among the treatments. It is added that efficacy of these selected plant extracts was much stronger against Fl than egg laying. Jayakumar et al. (2003) reported that plant extracts have obvious effects on postembryonic survival of the insect and resulting reduction in adult emergence in all the concentrations of different plants. In the present study, maximum reduction in the adult emergence was observed in the seeds treated with *Azadirachta indica*. Adult emergence was reduced to 5.66±0.66 at 1% 2.66±0.33 at 3% and 1.66±0.33 at 5% concentration of *Azadirachta indica*. It was followed by *T.purpurae* (82.11%) at 1% (88.62%) at 3% and (94.31%) at 5% concentrations. However, *O. tenuiflorum* was found to be effective in reducing the adult emergence at a higher concentration.

5. CONCLUSION:

Plant extract can be used as one of the component in Integrated Pest Management especially small go-downs or shop retailer for short term storage of food and farm commodities. Present study infers 5.0% dose of *Calotropis procera* methanol extract can efficiently protect mung bean seeds from infestation from *C. maculatus* by controlling oviposition and emergence thereby protecting from seed loss and weight loss. Since same dose was also able to protect germplasm of the seeds after laboratory validation and identification of inhibitory compounds can further commercialize and use for controlling insect for better longer storage life. After a perusal of the data it could be concluded that extract of *Calotropis procera* could be used as protectants of moong seeds against *C. maculatus.*

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