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Bioassays for Stored Grain Pests

Increasing awareness of the hazards to human health and environment has created a demand to develop either safe, effective and cheaper insecticide or alternatives as stored product protectants. Previous studies on different botanical insecticides have proved the potential use of plant materials and/or plant extracts from different plant parts to be used against storage insect pests in grain. Insecticidal properties of these natural products can be evaluated using several bioassay techniques. Toxicological properties and repellent properties of the natural products are tested using the bioassay techniques given below. However the Electroantennogram technique is used to identify bioactive compounds present in volatile samples such as essential oils.

Storage Pest Assays

- a. Toxicity bioassays
 - i. Fumigant toxicity assay
 - ii. Contact toxicity assay
- b. Repellent bioassays
 - i. Olfactometer bioassay
 - ii. Choice chamber bioassay
 - iii. Electroantennogram (EAG)

Fumigant Toxicity Assay

Callosobruchus maculatus will be used as the test insects in these assays. A Whatman no. 1 filter paper strip (1.0-cm diameter) is impregnated with appropriate amounts of test sample dissolved in a suitable solvent to obtain the required concentrations and the solvent is allowed to evaporate for 10 minutes. Subsequently the filter paper is placed on the undersurface of the screw cap of a glass vial (6.5 ml), and five pairs of 5-10 hour old *C. maculatus* are introduced into the vial (Figure 1). The neck of the vial is blocked with a metal mesh (25 mesh/cm²) to avoid the contact between the insects and the plant volatiles. Then the cap of each vial is screwed tightly and the insects are placed in the bioassay laboratory at the prevailing conditions and 12 h D: L period for 24 h. Thereafter, the insects

are transferred into clean vials (60 ml) each containing 50 cowpea seeds and the mouth of the vial was covered with a piece of polythene and kept

under same conditions for 10 days. Parallel experiment with standard insecticide, pirimiphos methyl (Actellic®) and the solvent are also conducted for comparison purposes. Experiments without treated cowpea seeds are carried out as controls. Experiment is a CRD with six replications.

Mortality of the bruchids and the number of eggs laid on seeds are recorded daily during the first 10 days. Whenever the first generation of adults (F_1) emerged, the numbers of F_1 adults are also recorded.

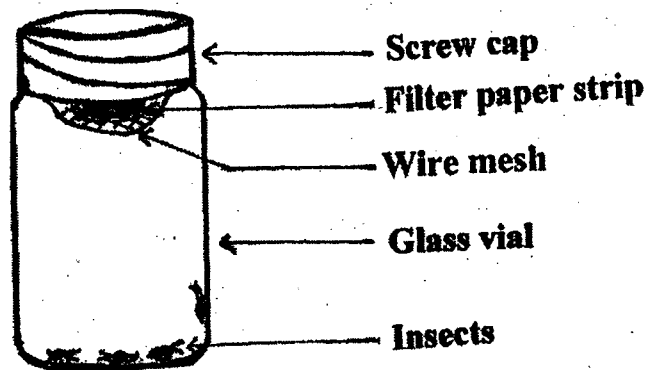


Figure 1: Setup for the fumigant toxicity bioassay

Contact Toxicity of Essential Oils

Contact toxicity bioassay is performed using the method described by Huang *et al.* (1999). A series of test samples treatments dissolved in a suitable solvent are applied evenly as possible on the inner surface of the glass vials (6.5 ml) and the screw caps separately. The solvent is allowed to evaporate for 10 minutes. Five pairs of 5-10 hour old *C. maculatus* are introduced into each vial and the cap is screwed tightly on to the vial (Figure 2). The vials are placed in the bioassay laboratory at the prevailing environmental conditions and 12 h D: L period for 24 h. After 24-hour exposure period, the *C. maculatus* are transferred to clean vials (60 ml) with 50 cowpea seeds and the mouth of the vial is covered with a piece of

polythene. The vials are kept under observation for 10 days. Parallel experiments with Actellic® and the solvent are carried out and samples without any treatment are used as the control. The experimental design is CRD with six-replicates per treatment. Same observations are made during the contact toxicity bioassay as fumigant toxicity.

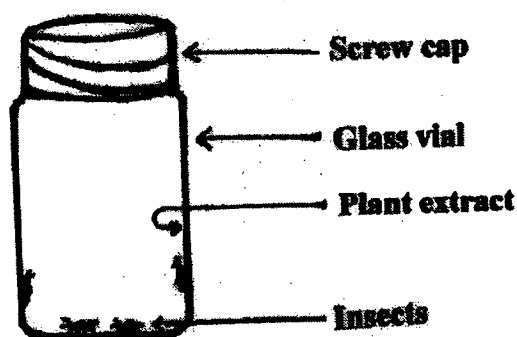


Figure 2: Setup for the contact toxicity bioassay.

Olfactometer Bioassay

A "Y" shaped olfactometer is made by connecting 3 glass tubes (10 cm long and 1 cm diameter) and there is an opening on the intersection of the 3 arms (Figure 3). This opening is used to circulate the airflow through the olfactometer. Two arms of the Y tube are connected to two perforated, wide mouthed, transparent containers (300 ml) through their lids and the other arm is used to introduce the bruchids. A Whaman no. 1 filter paper strip (2.5 cm x 3.0 cm) treated with known amount of essential oils dissolved in ethanol is attached to the middle of one container using a metal wire (baited arm). Another filter paper strip treated with the same amount of ethanol is attached to the other container (non-baited arm) of the olfactometer. The setup is placed upright position as the treated containers pointing towards the natural light. A vacuum pump is connected to the forth arm of the olfactometer and regulated the airflow. Then a round-bottomed flask (100 ml) with 50 unsexed adult bruchids is connected to the third arm of the olfactometer.

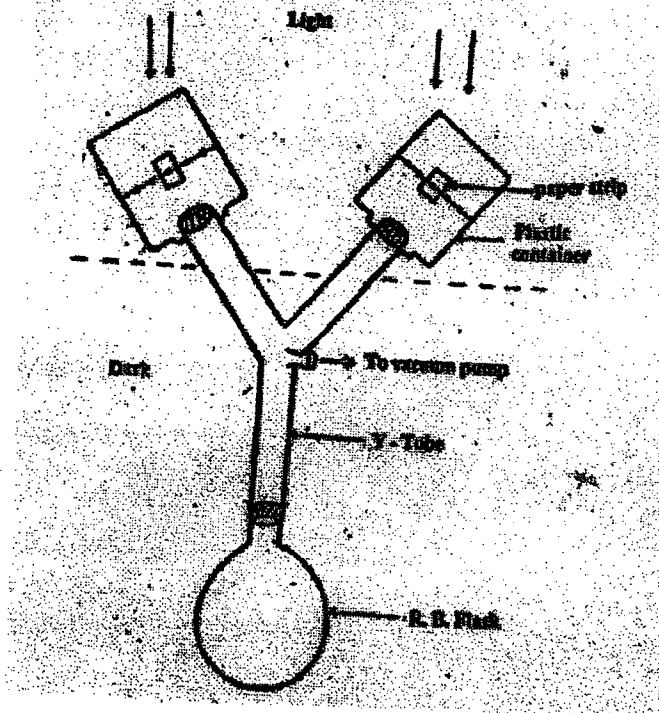


Figure 3. Setup for olfactometer bioassay

After 30 minutes, the number of test insects moved to the baited arm and non-baited arm are counted. Five different doses (10, 20, 40, 80 and 160 mg) of each volatile are tested separately with thoroughly cleaned setup with six replicates. All the bioassays are conducted between 7 a.m. - 11 a.m. The baited and the non-baited arms are interchanged in subsequent replicates.

Choice Chamber Bioassay

The choice chamber consisted of eight transparent wide mouthed plastic containers (300 ml), placed on a platform at equidistant to each other and also the containers are connected to a large transparent container (1 Liter) placed in the center of the platform through glass tubes (1cm diameter and 8 cm long). The experimental apparatus is placed in a plastic basin having a diameter of 42 cm and the height of 18 cm and the experimental apparatus is covered with black paper (Figure 4).

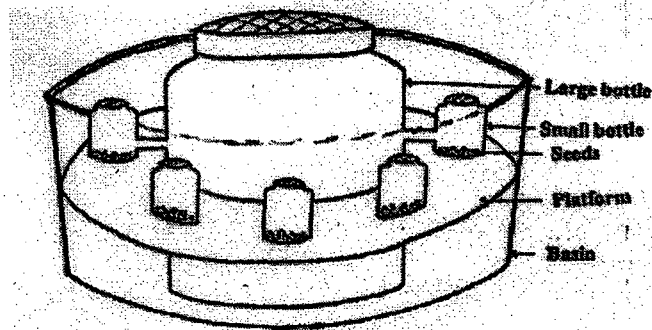


Figure 4

Five different doses of each volatile sample is placed on filter paper strips (2.5 cm x 3.0 cm) separately, and the solvent is allowed to evaporate. Each strip is placed in an appropriate container containing 50 cowpea seeds. Two containers containing 50 cowpea seeds without any treatment as controls and another one, with the solvent treated filter paper strip are used in the choice tests. Two hundred and fifty adult bruchids (unsexed, 1-3 d old) are introduced to the central large bottle, and the setup is placed in darkness. After 24 h, the numbers of bruchids moved in to each small container are recorded. The bioassay is replicated five times with thoroughly cleaned setup.

Electroantennogram (EAG) Studies of *C. citratus* Oil

Effect of test samples on antennal responses of test insects is studied using EAG. The antenna is excised out with the base and isolated in insect Ringer solution (NaCl 7.5 g, CaCl₂ 0.21 g, KCl 0.35 g & NaHCO₃ 0.2 g in 1 l water). The antenna is fixed on two gold electrodes which are placed in glass capillary columns filled with insect Ringer solution. Distal end of the antenna is damaged and inserted into the recording electrode and the basal end is fixed to the reference electrode. Total time of mounting did not exceed 5 minutes and the whole procedure is carried out under a stage microscope. The test compounds are delivered as 100 µl aliquots on a filter paper, which is placed inside a pasture pipette. The pasture pipette is connected to a syringe, which contained 1 ml of air. A vacuum outlet is placed 60 cm behind the setup in order to remove essential oil containing air from the surrounding. At least 0.5 minutes elapsed between stimulations for adequate receptor recovery. The responses of the antenna

to the volatiles are recorded as peaks. One millivolt external standard is used to convert peak heights to millivolts. The procedure is carried out for both male and female antenna separately. Firstly this experiment is carried out with a known sample to determine the best dose to be used as the standard stimulant. This procedure is carried out for the different doses of test volatile sample (figure 5).

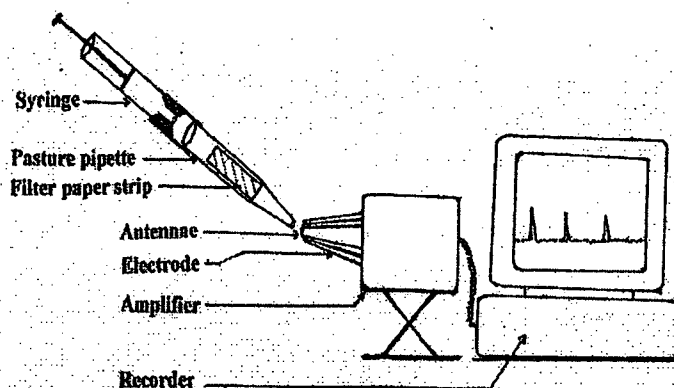


Figure 5

References

1. Adhikari, A.A.C.K., Paranagama, P.A., Abeywickrama, K.P. and Bandara, K.A.N.P. (2002): Behavioral studies of Cowpea bruchid, *Callosobruchus maculatus* (F.) against volatile extracts from leaves of Lemongrass (*Cymbopogon citratus*), Neem (*Azadirachta indica*) and curry leaf (*Murraya koenigii*). *Tropical Agricultural Research*, 14, 138-147.
2. Paranagama, P.A., Adhikari, A.A.C.K., Abeywickrama, K.P. and Bandara, K.A.N.P. (2003): Evaluation of volatile constituents of neem, *Azadirachta indica* A. Juss. (Neem) leaf extract against *Callosobruchus maculatus* (F.). *Journal of the National Science Foundation*, 31(3&4), 445-458.