DEVELOPMENT AND ASSESSMENT OF NEWER BIOASSAY TECHNIQUES FOR THE MANAGEMENT OF STORED-PRODUCT INSECT PESTS

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Thesis submitted in part fulfilment of the requirements for the award of the degree of ***Master of Science (Agriculture) in Agricultural Entomology*** *to the Tamil Nadu Agricultural University, Coimbatore – 641 003.*

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CERTIFICATE

This is to certify that the thesis entitled **" DEVELOPMENT AND ASSESSMENT OF NEWER BIOASSAY TECHNIQUES FOR THE MANAGEMENT OF STORED-PRODUCT INSECT PESTS"** submitted in part fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in **Agricultural Entomology** to the **Tamil Nadu Agricultural University**, Coimbatore, is a record of bonafide research work carried out by **S.Brabeen Anusha** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

DEVELOPMENT AND ASSESSMENT OF NEWER BIOASSAY TECHNIQUES FOR THE MANAGEMENT OF STORED-PRODUCT INSECT PESTS

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Studies were carried out on the development and testing of newer bioassay techniques for the management of stored-product insect pests.

An improved petriplate bioassay has been developed to determine the efficacy of natural products to rice weevil (*Sitophilus oryzae* L.), lesser grain borer (*Rhyzopertha dominica* Feb.), saw-thoothed grain beetle (*Oryzaephilus surinamensis* L.) and red flour beetle (*Tribolium castaneum* Herbst.).

This technique determines the response of insects to the potential natural products by measuring their rate of movement in paddy grain treated with neem (*Azadirachta indica* A. Juss.), sweet flag or vasambu (*Acorus calamus* L.), notchi (*Vitex negundo* L.) and pungam (*Pongamia glabra* Vent.), spread as a mono layer in a petriplate. Among the

natural products, sweet flag or vasambu and neem arrested the insect movement effectively *vis-à-vis* to notchi and pungam.

Another simple, novel, and multipurpose Three-in-One bioassay technique has been developed to assess that the products are repellent or attractant to stored-product insect pests. It mimics storage condition. This technique determines the response of insects to potential repellents and attractants by measuring their movement by using trapping technique.

Several known natural products were tested for repellency *viz.* neem, sweet flag, notchi and pungam and for attractants *viz.* rice bran, ground nut powder and wheat flour by using the Three-in-One bioassay technique. The practical utility of this method was compared with standard probe trap method and its efficacy validated.

Three-in-One bioassay technique was also used for the detection of timely emergence of field carry-over stored-product insect pests. This detection will help the farmers for taking timely control measures. As the name indicates, it can be used for three purposes namely,

1. To assess the efficacy of natural products for their repellent property
2. To assess the efficacy of natural products for their attractant property
3. To detect the timely emergence of field carry-over stored-product insect pests.

In all the cases, the new Three-in-One bioassay technique proved effective. Hence the newer techniques will form an important tool in Integrated Pest Management of stored-product insect pests.

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CHAPTER I

INTRODUCTION

In India, agriculture is the primary source of livelihood for almost two thirds of the workforce, which has always been the most important economic sector. Nevertheless, agricultural productivity depends on the vagaries of monsoon. In order to feed the huge population of India, it is imperative to store the food grains as buffer. Almost, all stored-products and seeds are liable to insect attack (Saxena, 1995). These fortuitous survivors are able to multiply in a short period (Rajendran, 1992) and start their damage from the field itself (Mohan and Subba Rao, 2000).

Annual post-harvest losses due to insect damage, microbial deterioration and other factors are estimated to be 10-25 per cent of agricultural production worldwide (Matthews, 1993). In India, overall post-harvest losses of food grains account for ten per cent of total food production, of which losses due to insects alone are estimated to be two per cent (Singh and Sharma, 1981).

Rice is the most important food crop of antiquity in Asia and nearly three-fourth of Indian population subsists on it. About twenty species of insects damage paddy and rice during storage (Pathak and Jha, 2003). Out of which, six are major pests that includes rice weevil (*Sitophilus oryzae* L.), angoumois grain moth / paddy moth (*Sitotroga cerealella* Olivier), lesser grain borer (*Rhyzopertha dominica* Feb.), rice moth (*Corcyra* cephalonica Stainton), saw-thoothed grain beetle (*Oryzaephilus surinamensis* L.) and red flour beetle (*Tribolium castaneum* Herbst.) (Prakash and Rao, 2002).

Food grains are commonly protected by insecticides or fumigants and such practices pose health risks. Most of the stored-product insect pests have become resistant to most insecticides and fumigants (Champ, 1985; Srivastava *et al*., 2001; Huang *et al*., 2004) and hence, require increased dose of application. This would pollute the storage environment and lead to build-up of toxic residues in food grains. Alternatives for stored product protection to replace synthetic chemical insecticides are highly desirable.

Natural products such as fumes of gazelle dung, spices, waste water from olive pressings and neem, have been used traditionally to control stored-product insect pests since the dawn of agriculture (Levinson and Levinson, 1998). In addition, many natural products are also considered as promising repellents or attractants against stored-product insect pests (Malik and Naqvi, 1984).

Botanicals are considered as natural insecticides against stored-product pests and relatively safer than synthetic pesticides (Schumutterer, 1990). Many plant based products / compounds have been reported to have deleterious effects against insect pests (Koul and Dhaliwal, 2001; Weaver and Subramanyam, 2000). Among the natural products neem (Xie *et al.*, 1995), sweet flag (Chander *et al*., 1998), pungam (Anuradha *et al*., 2002), notchi (Prakash and Rao, 2002) etc., are reported as promising repellents against stored-product insects. On the other hand, attractants are also used to manage stored-product pests (Nansen and Phillips, 2004; Mohan, 1993).

Several laboratory bioassays have been conducted to test the efficacy of many attractants and repellents against stored-product insect pests (Burkholder, 1990; Dowdy *et al*., 1993). However, these techniques do not mimic field conditions (White *et al*., 1991; Morgan *et al*., 1998) or required large amounts (0.6-1 kg/test) of grain to be treated (Loschiavo, 1952) or special gadgets like perforated cups (Mohan and Fields, 2002).

Choosing the right tools and technology for storage management is a challenge. To meet this challenge, there is a need to develop simple and rapid techniques to time the control measures. This can be done by assessing the natural products that either repel or attract stored-product insect pests.

In light of the above facts, the present study was undertaken with the following objectives

1. To develop and validate an improved petriplate bioassay technique for assessing the efficacy of natural products that arrest stored-product insect pests movement.
2. To design, develop and test a simple, novel and rapid newer technique for assessing the efficacy of natural products that repel or attract stored-product insect pests.
3. To detect the timely emergence of field carry-over infestation of stored-product insect pests by newer technique.

CHAPTER II
REVIEW OF LITERATURE

Stored-product insects play a major role in the deterioration of food grains causing both quantitative and qualitative losses (Sinha and Watters, 1985). There are many ways of managing the dreaded insect pests in storage ecosystem. Synthetic pesticides are currently the method of choice for protection against stored product pests. However, their widespread use has led to the development of resistant strains to insecticides (Zettler & Cuperus, 1990). Problems in the use of chemical pesticides have resulted in increased use of botanicals. Weaver and Subramanyam (2000) demonstrated the insecticidal activity of many plant derivatives. Botanicals are preferred over chemical insecticides as they are eco-friendly, low cost coupled with promising bioefficacy in reducing the population and damage of the stored-pest species.

2.1. Effect of natural products against stored-product insect pests

Natural products have been used to control the stored-product insect pests since the dawn of agriculture (Levinson and Levinson, 1998). Several wild plants and even weeds act as a potential insecticidal source. It has been estimated that there are about 5,00,000 described plant species available in the world today. Out of these, only 10 per cent has been examined chemically, indicating that there is enormous scope for further research in this aspect (Benner, 1993).

Sreenivasamurthy and Krishnamurthy (1959) and Jilani (1985) found that turmeric, *Curcuma longa* (L.), was a good repellent against stored-product insects.

Su (1977) reported the insecticidal activity of black pepper, *Piper nigrum* L. and red pepper, *Capsicum frutescens* L. against the rice weevil, *Sitophilus oryzae* L. Malik and Naqvi (1984) showed the effectiveness of two per cent *Piper longum* L. against *Tribolium castaneum* Herbst.

Yutani and Burkholder (1981) reported the use of different vegetable oils such as cotton seed, soybean and groundnut to check the damage of granary weevil, *Sitophilus granarius* (L.) in stored wheat. The topical application of chenopodium, *Chenopodium ambrosioides* L. oil to wheat were found to reduce the infestation of *S. oryzae* at 2000 ppm (Su, 1991).

Yadhava and Bhatnagar (1987) studied the protection of stored cowpea against *Callosobruchus* spp. by six indigenous plant products. Of these neem leaf powder and *Datura* leaf powder commanded equal status in seed protection. There was no impairment of seed germination due to either of these treatments.

Arnason (1989) stated that plants are a rich source of compounds that have insecticidal activity. It also contains secondary metabolites, which exhibit insecticidal properties. Some of the secondary metabolites are alkaloids, limonoids, terpenoids, polyacetylene, lactone and saffrole (Metcalf and Metcalf, 1992).

Natural products of plants with good insecticidal activity were more likely to be used as leads for the synthesis of new insecticides (Benner, 1993). Regnault Roger and Hamraoui (1993) inferred that botanical insecticides were found to be highly useful to farmers for the better post-harvest storage of the harvested food commodities.

Curry leaf, *Murraya koenigii* L. and turmeric, *C. longa* rhizomes at one per cent level as bag treatments effectively controlled red flour beetle, *T. castaneum* and rice weevil, *S. oryzae* (Ahuja *et al*., 2000). They also reported that the two extracts exhibited good repellency against those insects even after three months of ageing under laboratory conditions, thereby substantiating the results obtained under warehouse conditions.

Hou and Fields (2003) recorded that 0.1 per cent protein-enriched pea flour treated barley had reduced adult numbers of *S. oryzae* by 93 per cent, *T. castaneum* by 66 per cent and *Cryptolestes ferrugineus* (Stephens) by 58 per cent, respectively. Pretheepkumar *et al*. (2004) also reported that one per cent concentration of pea flour extract admixed with milled rice repel rice weevil, *S. oryzae*.

Ogendo *et al.* (2004) revealed that *Lantana camera* L. and *Tephrosia vogelii* Hook possess toxic and repellent effects against insect pests of stored grains.

2.2. Repellents for stored-product insect pests management

Repellents have been defined *sensu strictu* as chemicals that cause insects to make oriented movements away from their source (Dethier, 1963).

Among the natural products which have repellent properties neem, notchi, vasambu and pungam were noticed as good repellents and are widely used for the management of stored-product insect pests.

2.2.1. Neem (*Azadirachta indica* A. Juss.)

Mixing of dried neem leaves with stored grains to control stored-product insects has been an age-old practice in rural India (Saxena *et al*., 1988; Mordue (Luntz) and Blackwell, 1933).

Jotwani and Sircar (1965) reported that the mixture of 1.2 per cent neem seed powder with wheat kernels provided 9-12 months protection against *Trogoderma granarium* Everts.

Jotwani and Sircar (1967) studied the repellency effect of crushed neem seed against bruchid infesting seeds of mung, bengal gram, cowpea and pea. The increased percentage damage at different intervals in various treatments revealed the degree of loss in the repellent property of neem seed. The results obtained showed that seeds of mung, bengal gram, cowpea and peas can be effectively protected from the damage by *Callosobruchus maculates* (L.) for 8, 11, 9 and 9 months, respectively. There was no impairment of seed germination due to either of these treatments.

Water and ethanol extracts of leaves and seeds of neem repelled the red flour beetle, *T. castaneum*; the khapra beetle, *Trogoderma granarium* Everts, and the lesser grain borer, *Rhyzopertha dominica* (Feb.) (Jilani and Malik, 1973).

Paper strips dipped in 30 per cent neem seed extract solutions were highly repellent to the larvae of the Mediterranean flour moth, *Anagastra kuhniella* (Zeller) (Roomi and Atiquiddin, 1977).

Jilani *et al.* (1988) reported that Margosan-O not only repel the insects but they also interfere with its normal reproduction and development. Saxena (1989) opined that the main active constituent of neem is the limonoid, azadirachtin, which is well known for its repellent, antifeedant, toxic and growth regulating effects on insects.

Xie *et al*. (1995) reported that the neem treatments containing >50 ppm azadirachtin provided good control of *C. ferrugineus*.

Zahoor *et al*. (2002) reported that dried neem kernel powder at 10 per cent protected wheat against lesser grain borer, *R. dominica* for up to one year.

Di-*n*-propyl disulfide and diallyl disulfide from neem were shown toxic and deterrent to *S. oryzae* and *T. castaneum* (Koul, 2004).

2.2.2. Notchi / Lagundi (*Vitex negundo* L.)

Bhuiyah (1988) evaluated the protectant effect of whole and powdered leaves of notchi, *V. negundo* on stored maize against maize weevil, *Sitophilus zeamaize* Motsch. The protectant effect of five per cent notchi leaf powder and one per cent whole leaf hold good for a period of one month storage.

Prakash and Rao (1989) studied the efficiency of dried leaves of begunia, *V. negundo* against pulse beetle, *C. chinensis* in stored grains of a local cultivar of blackgram under controlled and natural conditions of insect infestation. Under laboratory condition, oviposition and adult emergence of this beetle was significantly reduced when *Vitex* leaves were admixed with blackgram seeds and there was 100 to 94.18 per cent seed protection for a storage period of 3 to 9 months.

Branches of *V. negundo* with green leaves when kept below and around the gunny bags containing rice grains, repelled the storage pests (Roy and Mondal, 1998). Anuradha *et al.* (2002) showed that more than 87 per cent mortality of the stored pests was recorded in seven days in the presence of *V. negundo* treated green gram, black gram and bengal gram.

Dakshinamoorthy and Selvanarayanan (2002) studied the efficacy of certain natural products against *C. maculatus* (Fab.) infesting stored greengram seeds. The observations revealed that the 100 per cent mortality of beetle, was observed seven days after treating the seeds with castor oil followed by neem leaf powder (91.66% mortality), while the notchi and thulasi leaves treatment recorded 80 and 73.33 per cent adult mortality, respectively.

2.2.3. Vasambu / Sweet flag (*Acorus calamus* L.)

Sweet flag extract had chemosterilant effect on *T. granarium* (Kalpana *et al*., 1978). The dried rhizomes of sweet flag, *A. calamus* comprise 1.5-3.5% of an aromatic essential oil, whose main active component is cis-β-asarone (1,2,5-trimethoxy-4-propenylbenzene) (Baxter *et al*., 1960; Schmidt, 1986).

Paul *et al*., (1965) reported that application of the rhizomes extract of sweet flag, *A. calamus* were found toxic to the rice weevil, *S. oryzae*, long-headed flour beetle, *Letheticus oryzae* Waterhouse and *T. castaneum*.

Jilani and Haq (1984) reported that sweet flag oil completely checked the development of *R. dominica*, *S. oryzae* and the angoumois grain moth, *S. cereallela* in stored wheat.

Rhizomes of sweet flag applied as two per cent dust formulation offered best protection against the pulse beetle, *C. chinensis* in green gram (Reddy and Reddy, 1987).

The antifeedant effect of rhizome extract of sweet flag was also confirmed by the studies conducted against several insects species by Yadav (1971) and Jacob and Sheela, (1994). They also found that more than 60 per cent of the adults were killed within 24 days after treatment.

Levinson and Levinson (1998) reported the use of *A. calamus* as a fumigant. Chander et al. (1998) showed that sweet flag at one per cent caused more than twenty five per cent mortality of *T. castaneum*. In 2001, they also confirmed the phagodeterrent property of rhizome extract of sweet flag against *T. castaneum*.

2.2.4. Pungam (*Pongamia glabra* Vent.)

P. glabra have been tested for insecticidal properties against *C. macalatus* on cow pea (*Vigna anguiculata*), chickpea (*Cicer arietinum*) and green gram (*Vigna radiata*) and reported that more than 87 per cent of the pests in 7 days (Anuradha *et al*., 2002).

Lohra *et al*. (2002) reported that pungam or karanj, *P. pinnata* at 1.0 ml / 100 mg seeds effectively reduced the survival percentage of *T. castaneum*.

2.3. Attractants for stored-product insect pests management

Attractants are those chemicals, which cause the insects to make oriented movements towards its source (Dethier *et al.*, 1960). An insight into the use of food attractant might provide cues for monitoring, sampling and detection of stored-product pests as documented by Mohan (1993).

Strong (1970) made a first record of stored-product insect food baits for the survey of *Trogoderma* sp. in California. Later, Loschiavo and Okumura (1979) reported that the food baited bags acts as an effective tool for survey and monitoring of stored-product insect pests in Hawaii. Later, this document was supported by Hodges *et al.* (1985) in Indonesia and Wright *et al*. (1988) in Kansas, USA grain stores.

Bains *et al*. (1976) used food baits for *Trogoderma* spp. survey in Punjab. A modified insect bait technique using jute bags with rolled barley, whole wheat and corn for attracting *Trogoderma* spp.

Chambers *et al*. (1986) reported that the acidic volatiles present in the carob attract *Oryzaephilus surinamensis* by using electro antennogram technique (EAG).

Pinniger (1990) demonstrated the importance of cereal oil in place of wheat and carob distillate in place of carob for attracting stored product insect. Mohan (1993) revealed that *T. castaneum* Herbst. was attracted by rice bran.

Attractants serve as a suppression tool for the management of stored-product insects as stated by Nansen and Phillips (2004).

2.4. Bioassays

In order to find out whether the natural products act as a repellent or attractant, there is a need for suitable bioassay studies for screening.

Loschiavo (1965) reported that, thin disks of elder pith / soft white substance found in tree trunk has been used for studying chemicals affecting the aggregation and feeding behaviour of red flour beetle (McDonald *et al*., 1970). He further combined this method with photometric evaluation of disks, envisages the useful qualitative estimates of feeding, but this requires special equipment resulted in the unsuitability for routine screening of a large number of samples.

Jilani *et al*. (1988) used paper strip method to test the repellency of natural products and concluded that more than 50 per cent of *T. castaneum* adults were repelled by filter paper strips treated with turmeric oil. Jilani and Saxena (1990) and Talukder and Hocose (1994) also used this method widely for evaluating plant extracts as toxicants, repellents or attractants against stored product insects, in which a non food material was used and provided data only as repellency and toxicity.

Alonso – Amelot *et al*. (1994) introduced flour wafer method, in which a disk of artificial diet bioassay have been used. They designed a new bioassay to test plant extract activity against stored – product pests. Also, Jacob and Sheela (1994) conducted one bioassay technique for antifeedant tests, in that the botanical extract was applied to wafer discs, filter paper or to paper packing materials. The number of holes made by boring insects are then counted per unit time, usually for a seven-day exposure period.

Xie *et al.* (1995) investigated the response of stored – product insects by using flour disk bioassay and reported that *T. castaneum* was more sensitive to the repellent action of neem.

Studies on flour disk bioassay were conducted by Pratheepkumar (2001) and revealed that pea protein at one per cent concentration significantly reduced the growth of food consumption and utilization of flour disks by *T. castaneum*.

Mohan and Fields (2002) developed cup bioassay technique to determine the products for their repellent or attractant action against stored-product insects. Per cent insects left from the grains collected by the cup have been taken into consideration and it requires special gadgets.

2.5. Field carry-over pests

Many stored-product insects start their infestation from the field itself (Cotton *et al*., 1960). *S. cereallela*, *R. dominica*, *S. oryzae* and bruchids infest standing crop at ripening stage. The damage is also seen on seeds in fields (Prakash *et al*., 1981; Dobie, 1984).

The incidence of *S. cereallela* in maize field was increased from eight per cent in the roasting ear stage to 62 per cent as maize dried and was ready for harvest (Koone, 1952).

Cotton (1963) stated that the rice moth, *S. cerealella* infestation on stored rice results in reduction of viability of grains rendering them unsuitable for seeding and human consumption.

Raina (1971) reported the field carry-over infestation by bruchids, *C. chinensis* and *C. maculataus*. Experiments were carried out by them in the laboratory which revealed that females laid eggs on whole pods of cowpea, greengram, blackgram and pigeonpea.

Khanvilkar and Dalvi (1984) observed the subsequent build up of *C. maculatus* population from field to storage.

Ragumoorthy and Gunathilagaraj (1988) surveyed the field incidence of *S. cereallella* Olivier in Tamil Nadu, and reported that *S. cereallella* emerged from 124 of 213 samples at Madurai and from 59 of 151 samples at Aduthurai.

Edward and Gunathilagaraj (1994) reported that greengram cv. VGG-2 and black gram cv. Vamban-1 offered resistance to *C. maculatus* in the field.

The first record of *C. maculatus* infesting blackgram and greengram under field conditions in Tamil Nadu was made by Mohan and Subba Rao (2000). They also inferred that there was carry-over infestation from field to store house.

2.6. Techniques for the timely detection of field carry-over insect pests

The field carry-over infestation is the root cause for multiplication of stored-product insect pests during storage. There are several techniques to assess the field carry-over pest infestation viz., indicator device, pitfall trap and insect removal devices (Mohan *et al*., 2004). However, all these methods require large amount of grains and more time to detect the infestation. Hence there is a need to develop a simple and rapid technique to detect the timely emergence of stored-product insects.

Longstaff and Pocknall (1983) developed a new technique for the removal of adult weevils present in grain.

Probe traps were considered as an important tool for catching of insects in bulk and less grain storage (Loschiavo and Atkinson, 1967; Mohan, 1993). Futher, Mohan, (1993) substantiated the effectiveness of probe traps for capturing insects like, *S. oryzae, T. castaneum* and *R. dominica*.

Tamil Nadu Agricultural University (TNAU) automatic insect removal bin was invented by Mohan (1994), which exploits the wandering behaviour of insects. Mohan (1995) documented that wandering nature of stored-product pests ensured them towards the aerated region, so that they enter through the perforation getting slipped off and fell down in the collection device by a pitfall mechanism.

Mohan (1996) reported that the insect removal bin could be used to remove more than 90 per cent of insects namely *R. dominica*, *S. oryzae*, *O. surinamensis* and *T. castaneum* within a period of 20 days.

About 94 per cent of the adults of *S. oryzae* were removed within a period of 10 days when an artificially infested milled rice was stored in the automatic insect removal bin (Mohan (1997).

Studies conducted by Mohan (1998) revealed that the TNAU storage container model removed 38 to 41 per cent of bruchid grubs from the artificially infested *Tamarindus indica* L. seeds.

Insect removal devices play a vital role in the rapid removal of grain eating insects during storage. The shorter life cycle and high fecundity of this insect resulted upto 80 per cent loss within six months of storage (Kumar, 2001). Mohan and Subba Rao (2000) stated that the proper monitoring of stored product insects immediately after harvest reduce the storage loss.

CHAPTER III
MATERIALS AND METHODS

Studies were carried out on the development of newer bioassay techniques to assess the efficiency of certain natural products for their repellency and attractant properties against stored-product insect pests. The efficiency of the newer technique (Three-in-One bioassay) for the detection of timely emergence of field carry-over stored-product insect pests was also tested and its validity was examined *vis-à-vis* with standard trapping method.

3.1. Development of newer bioassay techniques

a. Improved petriplate bioassay technique
b. Three-in-One bioassay technique

3.2. Improved petriplate bioassay

The bioassay consists of a glass petriplate (100 X 15 mm) with glass lid. This method exploits the wandering behaviour of stored-product insects and their reaction to natural products with respect to movement. Preliminary work on petri dish assay was done by Liu *et al*. (personal communication). This petri dish assay was found to have the following *lacunae*

a. The plastic petri dish was used for the study. This along with the repellent may produce static charge.
b. The test insects were simply captured and released resulting in agitated movement in the released insects which may interfere with the experimental results as natural wandering behaviour of insects was exploited in this method.

However, in the present investigation, improvement has been made by incorporating the following aspects:

a. In lieu of using plastic petri dish, glass petriplates were used thus nullifying static charge effect over the repellents in the newer improved petriplate bioassay technique.
b. The capture and release cause the insects to be agitated. To circumvent this problem, insects were allowed to wander an hour before starting the experiment.

3.2.1. Mass culturing of test insects

Adults of *Sitophilus oryzae* L., *Rhyzopertha dominica* (Feb.), *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Herbst. were collected from Paddy Breeding Station and Millet Breeding Station, Tamil Nadu Agricultural University (TNAU), Coimbatore for building up a laboratory culture. The culture was further multiplied in rice, sorghum, ground nut and wheat flour, respectively.

Five pairs of adult beetles in each species were introduced in a plastic container having respective food materials. After five days, the adults were removed and the culture was kept for further multiplication. The mouth of the container was covered with muslin cloth, held tight with rubber bands (Plate 1) and maintained at ambient laboratory temperature of 30 ± 1°C and 75 ± 5% Relative Humidity (RH). Unsexed cohort of insects was used for different experiments.

3.2.2. Test products

To assess the effectiveness of the petriplate bioassay, the insects were tested against some botanicals, which are known to possess biological activity against insects namely, neem (*Azadirachta indica* A. Juss.) seeds, pungam (*Pongamia glabra* Vent.)

seeds, sweet flag (*Acorus calamus* L.) rhizomes and notchi (*Vitex negundo* L.) leaves. These were purchased from wholesale market, Coimbatore, Tamil Nadu, India. All the products were dried, ground to fine powder (30 mesh) (Plate 2a) and mixed with the grains at different concentrations such as 0, 0.05, 0.1, 0.5, and 1.0 per cent w/w basis. To assess the repellent and attractant properties of natural products, paddy, *Oryza sativa* L. var. ADT 43 (moisture content (m.c.) 12 ± 1%) grains were used.

3.2.3. Test insects

Unsexed adults of *S. oryzae*, *R. dominica*, *O. surinamensis* and *T. castaneum* were tested at the rate of one per replication.

3.2.4. Test procedure

Paddy grains of 50g per petriplate were spread as a monolayer in the petriplate. The grains were treated with the repellents at test doses. An unsexed insect per replication was released on to the middle of the petriplate, and kept for an hour to prevent agitation. After an hour, with the help of a permanent marker, the moving path of the released insect was carefully traced over the petriplate without disturbance for a period of two minutes. In all the trials, the movement of insects in grains without treatment was also traced parallel as control. The rate of movement, that is, the distance travelled per second was calculated. Five replications were maintained for each treatment. All the replications run simultaneously. This experiment was conducted at 28 ± 2°C and 75 ± 5% RH.

3.2.5. Record of observations

The repellency of the natural products was measured in terms of response movement shown by the insects. The movement was traced and marked carefully without much disturbance for a period of two minutes using a permanent marker (Plate 3a). The distance was then measured and the rate of movement was expressed as mm per second. For *T. castaneum* and *S. oryzae*, the path was marked over the lid. For *O. surinamensis* and *R. dominica* due to their small size, the petriplate have been placed in a tripod stand (Plate 3b) and the path was traced from the bottom of the petriplate. Natural light was the only source for illumination throughout the observation period. This method has been proposed based on the fact that if a test material was repellent, at some concentration, insect shows non-locomotory reactions (Dethier, 1960).

3.3. Three-in-One bioassay

Laboratory bioassays have been used for testing attractants and repellents (Dowdy *et al*., 1993). All these techniques have *lacunae* over the other; some do not mimic field conditions (White *et al*., 1991; Morgan *et al*., 1998), required large amounts (0.6-1 kg/test) of grain to be treated (Loschiavo, 1952) or special gadgets like perforated cups (Mohan and Fields, 2002). Hence, a simple and rapid bioassay technique (Three-in-One bioassay) has been developed exploiting the oriented movement of insects away from or towards the natural products using grain as a source of medium instead of filter paper treated with repellents or attractants. This technique consists of simple apparatus.

The basic components of the Three-in-One bioassay apparatus are as follows

a. Tray (to place grain)
b. Trapping unit
c. Tripod stand

The tray is made up of stainless steel. In the center of the tray, there is a cylindrical trap unit (TNAU trap) (Mohan, 2004) with perforation (2 mm size per perforation). The trap is placed in the center of the plate in such a way that two layers of perforation are over the plate. The perforated part of the trapping unit can be closed with the help of a plastic container. This is done to prevent insect trapping, which occurs immediately after introduction due to the agitated behaviour of insects. The tray was covered by muslin cloth and fastened tightly with rubber bands, to mimic storage condition, thereby ensured good supply of air and light. The entire apparatus rests on a tripod stand (Plate 4 and Figure 1).

3.3.1. Test grains

To assess the repellency and attractiveness of natural products, paddy, *O. sativa* var. ADT 43 grains were used. All grains used in these tests had 12±1% m.c. (Plate 5).

3.3.2. Test insects

Unsexed adults of *S. oryzae*, *R. dominica*, *O. surinamensis* and *T. castaneum* were tested at the rate of ten per replication.

3.3.3. Test products

a. Repellents

To assess the effectiveness of the Three-in-One bioassay model, the insects were tested against the following promising botanicals, which are known to possess biological activity against insects namely neem seeds, pungam seeds, sweet flag rhizomes and notchi leaves. These were purchased from wholesale market, Coimbatore, Tamil Nadu, India. All the products were dried, ground to a fine powder (30 mesh) for experimentation. The per cent repellency was measured in terms of number of insects collected in the collection unit of the apparatus at different periods *viz.* 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h after the experiment was started.

b. Attractants

The various attractants like rice bran, groundnut powder, wheat flour (Mohan, 1994) (Plate 2b) were used to assess the effectiveness of three-in-One bioassay technique against the test insects. They were kept in the collection unit of the apparatus at the rate of 1 gram per replication. The per cent attraction was measured in terms of number of insects collected in the collection unit of the apparatus 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h after the start of the experiment.

3.3.4. Studies to assess the efficacy of natural products by Three-in-One bioassay technique against stored-product insects from the grains

a. Repellent tests

Paddy grains of 100g per replication were spread on the plate. The grains were treated with the repellents at test doses such as 0, 0.05, 0.1, 0.5, and 1.0 per cent w/w basis. For each treatment five replications were maintained. Before releasing insects, the cylindrical single trap unit was closed with the plastic container (Plate 6) to prevent trapping due to agitation by insects. A cohort of ten unsexed insects of similar age were released onto the grains, which gave equi-spatial spreading and the apparatus was covered with muslin cloth held tightly with rubber bands to prevent the escape of insects. This mimicked storage condition and ensures aeration and light. After an hour, the plastic container was removed and the unit was covered with muslin cloth as described above (Plate 7). Five replications were maintained per treatment.

b. Attractant tests

Paddy grains of 100g per replication were spread on the plate. The attractants at the rate of one gram per replication were kept in the removable collection or trapping unit of the apparatus (Mohan, 1993). The rest of the test procedure followed was as same as repellent experiment (Plate 8).

3.3.5. Record of observations

Observations were made on 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h after the start of the experiment. The rate of insects leaving the grain was determined by counting the insects that were collected in the cone. There were five replicates per treatment. All the replications were run simultaneously. All the experiments were conducted at 28 ± 2°C and 75 ± 5% RH.

3.4. Comparison of the newer technique with standard trapping method

Standard method of using probe trap to study the repellency (Hou and Fields, 2003; Mohan, 1993; Pretheepkumar *et al*., 2004) of natural products against stored-product insects was also run parallel to compare the newer technique (Three-in-One bioassay) with best test concentration (one per cent) for all the test products namely neem, vasambu, notchi and pungam. In order to find out the efficiency of the newer technique, it was expected to compare with the standard method. Hence, the repellent study alone was taken into consideration, as majority of the natural products are known to possess repellent action against stored-product insect pests. Repellency of the product was measured in terms of number of insects trapped as a result of oriented movement away from the source. Paddy grains (m.c.12 ± 1%) var.ADT 43, mixed with the repellants at the 1 per cent concentration, were filled in cylindrical containers (diameter 50mm x 100mm height), which can hold 1 kg of paddy. A probe trap (Mohan *et al*., 2004), of 15 cm height (trapping area) and having 667 holes at 2mm equi-spatial perforations, was inserted vertically into the container, which holds the grain with it's cap alone outside the grain surface. Ten unsexed laboratory reared test insects were sprinkled gently over the surface of the grain, preferably using a long-stemmed funnel to have even distribution (Loschiavo, 1974). The trap was examined after 30 minutes, 1 h, 3 h, 6 h, 12 h and 24 h after releasing the test insects (Plate 9). All experiments were conducted at 28 ± 2°C and 75 ± 5% RH with five replications.

3.5. Investigations on the impact of newer technique on field carry over pests.

To have a multipurpose use of the newer Three-in-One bioassay, the same unit was evaluated to detect the timely emergence of field carry-over stored product insect pests during storage of grains.

Paddy samples (var. MDU 5) were collected from Paddy Breeding Station, TNAU, Coimbatore, India and green gram (var.CO 4) from New area, TNAU, Coimbatore, India, immediately after harvest and dried below ten per cent moisture level. Five samples, each of 100 g were placed on the new Three-in-One bioassay device and covered with muslin cloth, fastened tightly with rubber bands (Plate 10). Observations were made on the emergence of field carry-over insect pests up to a period of 40 days, and the number of adults emerging from the collected samples was recorded daily. This experiment was conducted during December 2005 to January 2006.

3.6. Statistical analysis

The data obtained on the bioassay laboratory experiments were analysed in Completely Randomized Design (CRD). All the data were transformed into the arcsin of the square root values and subjected to one-way ANOVA. Means to determine the effective dose of attractants and repellents were separated by Dunnet’s test. However, the effects of different treatments were compared at each duration employing Student-

Newman-Keuls method. All the analyses were performed using SPSS 7.5.1. Package. All the mean per cent data were converted into whole numbers (Mohan and Fields, 2002).

CHAPTER IV

RESULTS

The results of the studies carried out to find the efficacy of newer bioassay techniques namely improved petriplate bioassay technique and Three-in-One bioassay technique for the management of stored-product insect pests are presented in this chapter.

4.1 Development of newer bioassay techniques

An improved petriplate bioassay technique was developed to test the efficacy of botanicals against stored-product insect pests. A simple, novel and rapid Three-in-One bioassay technique was also developed to assess the efficacy of natural products that repel or attract stored-product insect pests. The Three-in-One bioassay device was also tested for the detection of the timely emergence of field carry-over insect pests.

4.2. Improved petriplate bioassay

This improved petriplate bioassay technique method exploited the wandering behaviour of stored-product insects and their reaction to natural products with respect to movement (mm per second). The results of the movement responses of stored-product insect pests to the botanicals treated paddy grains were presented below.

a. *Sitophilus oryzae* L.

The improved petriplate bioassay technique proved effective for assessing the efficacy of all botanicals tested against *S. oryzae*. Vasambu or sweet flag at 1 per cent concentration was found more effective than other natural products in arresting the movement of *S. oryzae*. The mean insect movement was 0.32 mm per second compared 1.15 mm per second in untreated control (Table 1).

The arrestant effect of natural products in *S. oryzae* was in the order of:

Vasambu > Neem > Notchi > Pungam

b. *Oryzaephilus surinamensis* L.

The movement response of *O. surinamensis*, at one per cent concentration was significantly different from other concentrations for all test products. Vasambu at one percent concentration (0.14 mm per second) had better effect when compared with control (1.17 mm per second) (Table 2). The effect of natural products against *O. surinamensis* was in the following order:

Vasambu > Pungam > Neem = Notchi

c. *Rhyzopertha dominica* (Feb.)

In *R. dominica*, the movement (mm per second) was significantly arrested in vasambu at one per cent concentration (0.02) compared to untreated control (0.34) (Table 3). The movement response of *R. dominica* to different botanicals at 1 per cent concentration was in the order of:

Vasambu > Pungam > Notchi > Neem

d. *Tribolium castaneum* Herbst.

Vasambu at one percent concentration significantly reduced the movement of *T. castaneum* (0.25 mm per second) compared to untreated control (1.31 mm per second) (Table 4). The effectiveness of *T. castaneum* to different natural products at one per cent concentration was in the following order:

Vasambu > Pungam > Notchi > Neem

4.3. Three-in-One bioassay

The Three-in-One bioassay technique designed and tested, to find out the efficacy of natural products that repel or attract stored-product insect pests was found effective. It was also used to monitor the field carry-over insect pests successfully. The per cent repellency or attraction was measured in terms of number of insects collected in the collection unit of the apparatus. The results obtained from this technique were presented below.

4.3.1. Effect of test insects to neem, pungam, vasambu and notchi

The Three-in-One bioassay technique tested for the efficacy of natural products *viz.*, neem, vasambu, notchi and pungam for their repellency against *S. oryzae*, *R. dominica*, *O. surinamensis* and *T. castaneum* proved effective. The results were presented below.

a. *S. oryzae*

The responses of *S. oryzae* to paddy grains treated with different natural products at different concentrations such as 0, 0.05, 0.1, and 0.5 per cent were as follows.

Neem at 0.5 per cent concentration repelled 50 per cent of insects in 24 h. While at the same level it was 16 per cent in untreated control (Table 5).

In pungam, at one per cent concentration in 24 h duration, 52 per cent emigration was noticed when compared with the control was 10 per cent (Table 6).

In the same period of 24 h at one per cent concentration, 70 per cent repellency of the insect was noticed in vasambu treated paddy grains as compared to 16 per cent in control (Table 7)

b. *O. surinamensis*

The neem caused significantly increased emigration of *O.surinamensis* from the treated paddy grains at the highest (1 per cent) dosage level (16 per cent) in 24 h duration (Table 8).

At 24 h, 16 per cent of the insects left the pungam treated paddy grains (1 per cent level) compared to 6.00 per cent in control (Table 9).

Vasambu at 1 per cent concentration repelled 14 percent of insects at 24 h duration whereas in control only 6 per cent of insects were trapped (Table 10).

In case of notchi, 0.5% per cent concentration noticed significantly higher trapping (22 per cent) (Table 9), compared to control (6 per cent) in 24 h (Table 11).

c. *R. dominica*

The results obtained from the experiment for assessing the efficacy of natural products against *R. dominica* were presented below.

At 24 h, one per cent concentration was found effective i.e., 44 per cent of insects were removed from the grain, when compared to control (12 per cent) (Table 12).

Twenty eight per cent insects left the pungam treated grains (1 per cent) in 24 h duration, compared to 2 per cent in control (Table 13).

In the same period of 24 h at one per cent concentration, 54 percent repellency was noticed as compared to 18 per cent in control, in case of vasambu (Table 14).

In notchi, at one per cent concentration, 20 per cent emigration was noticed in 24 h duration. But in the control the emigration was only 4 per cent (Table 15).

d. *T. castaneum*

One hundred per cent repellency was noticed in neem treated paddy grain (1 per cent concentration) in 12 h and compared to 28 per cent in control. But in 6 h, 98 per cent of the insects left the grain (Table 16).

In pungam, at one per cent concentration in 12 h duration there was 62 per cent emigration compared to 30 per cent in control (Table 17).

Vasumbu at 0.5 per cent concentration in 12 h repelled 82 per cent of the insects compared to 30 per cent in control (Table 18).

In notchi, at the same contentration (0.5 per cent), 64 per cent insects left the grain compared to only 30 per cent in control within 24h (Table 19).

4.3.2. Comparative response of test insects to individual natural products

In order to find out the overall effect of natural products by the new Three-in-One bioassay technique to test insects, data collected from the repellent studies at 1 per cent level alone was pooled and compared.

a. Neem

In neem, 100 per cent emigration of *T. castaneam* adults was noticed at 12 h duration and 98 per cent in 6 h duration. The per cent repellency to various test insects noticed was in the order of:

T. castaneum > *R. dominica* > *O. surinamenis* > *S. oryzae* (Table 20).

b. Pungam

In pungam, *R. dominca* responded significantly well when compared to other test insects. The percent repellency was 52 per cent. The response of insects to pungam was in the following order:

R. dominica >*T. castaneum* > *O. surinamenis* > *S. oryzae* (Table 21).

c. Vasambu

T. castaneam and *R. dominica* was found to give good and significant response to vasambu in 24 h. The per cent repellency of both the insects were 70 per cent. The effectiveness of vasambu to test insects was in the order of:

T. castaneum = *R. dominica* > *O. surinamenis* > *S. oryzae* (Table 22).

d. Notchi

The per cent emigration of *T. castaneam* was 56 per cent and found effective compared to other insects. The order of effectiveness of notchi to different test insects was:

T. castaneum > *O. surinamenis* > *R. dominica* > *S. oryzae* (Table 23).

4.3.3. Attractiveness of test insects to ground nut powder, rice bran and wheat flour

The Three-in-One bioassay technique was also tested for the efficacy of attractants *viz.*, rice bran, groundnut powder and wheat flour against *S. oryzae*, *R. dominica*, *O. surinamensis* and *T. castaneum* and proved effective. The results were presented below.

a. *S. oryzae*

Ground nut powder, was found effective against *S. oryzae*. One hundred per cent trapping in the collection unit was noticed, in 24 h. In wheat flour and groundnut, 78 per cent and 68 percent of insects respectively was noticed (Table 24).

b. *O. surinamensis*

For *O. surinamensis*, 98 per cent of the insects were attracted towards rice bran in 24 h compared to other products (Table 25)

c. *R. dominica*

The per cent insects attracted for rice bran as 82 percent in 24 h and it was found significant compared to other insects (Table 26).

d. *T. castaneum*

In *T. castaneum*, 100 per cent of insects attracted towards rice bran and found significantly superior compared to other products (Table 27).

4.3.4. Comparative response of insects to different attractants

In order to find out the overall effect of attractants by the new Three-in-One bioassay technique to test insects, data collected from the attractant studies were pooled and compared.

a. Groundnut powder

Groundnut powder was preferred as a food attractant by *T. castaneum* compared to other test insects. This was evident from 100 per cent trapping at 1 h duration. Movement response shown by the insects in relation to groundnut powder was in the order of:

T. castaneum > *O. surinamensis* > *S. oryzae* > *R. dominica* (Table 28).

b. Rice bran

For rice bran, *S. oryzae* responded well when compared to other insects. The percent insect attracted was 100 per cent within 24 h. The attraction of insects towards rice bran was in the following order:

S. oryzae > *O. surinamensis*> *T. castaneum* > *R. dominica* (Table 29).

c. Wheat flour

The per cent attraction of *T. castaneam* was 90 per cent for wheat flour. This was significantly more compared to other test insects. The order of attraction to wheat flour by test insects was:

T. castaneum > *S. oryzae* > *R. dominica* > *O. surinamensis* (Table 30).

4.4. Three-in-One bioassay vis-à-vis standard trap method

The new Three-in-One bioassay was compared with the standard probe trap method at the best dose tested (1 per cent concentration) for repellency test. In general, the new (Three-in-One) bioassay was found to be on par with the standard probe trap method, thus proving that the new Three-in-One bioassay can be a reliable tool to assess the natural products for their repellent property for the management of stored-product insect pests (Table 31).

4.5. Investigations on the efficiency of Three-in-one bioassay for timely detection on the emergence of stored-product insect pests

Studies were conducted to find out the efficiency of Three-in-one bioassay for timely detection of the emergence of stored-product insect pests in paddy and pulse grains. Adults of *Sitotroga cerealella* Olivier in paddy samples and *Callosobruchus Chinensis* (L.) in pulse samples emergence started from seven days after storage and continued upto 35 days after storage with peak emergence during 31^{st} day in paddy and 16^{th} day in green gram (Table 32). Thus, this Three-in-one bioassay apparatus can also be used as indicator device for timely detection of the emergence of field carry-over stored-product insect pests.

CHAPTER V

DISCUSSION

The prime objective of the present study is to develop and validate newer bioassay techniques for detection and management of stored-product insect pests. Also the developed Three-in-One bioassay was tested for timely detection of the emergence of field carried over insect pests. The results obtained on the above studies are discussed in this chapter.

5.1. Development of newer bioassay techniques

Bioassay techniques are useful for assessing the efficacy of natural products for the management of stored-product insect pests. Chemical insecticides and fumigants prompt health hazards to human being. To overcome this problem, now we are focussing on alternative to the dreadful chemicals. Many botanicals were found so far to control stored- product insect pests. Of that, all are not effective to control the pests. Nevertheless, there is a need for suitable bioassays for assessing the efficiency of natural products. Hence, the simple, novel and rapid bioassay techniques were developed for assessing the natural products for their efficacy.

5.2. Improved petriplate bioassay technique

The improved petriplate bioassay technique developed and tested proved more effective for assessing the efficacy of natural products.

In this method, the movement of insects was measured in terms of mm per second. The movement decreased with increase in concentration of natural products. All the test insects displayed an obvious response to different doses tested in this study, particularly at 1 per cent concentration as shown in figures 2, 3, 4 and 5.

The results obtained in this study clearly revealed that vasambu at 1 per cent concentration arrested the locomotion of the test insects. Usually, stored-product insect pests have the natural tendency to wander around the grain and show affinity towards the aerated regions whereas in the new improved petriplate bioassay method, the insect has no way to orient itself away from the treated grains. Hence, there is an arrested movement. Several workers reported that vasambu has active biochemical constituent cis-β-asarone (Baxler *et al.*, 1960; Schmidt, 1986) having insecticidal property against stored-product insect pests (Tiku *et al.*, and Jilani and Haq, 1984). The reduced movement may be due to its physiological behaviour (Salin *et al.*, 1999) (Figure 6, 7, 8 and 9).

Next to vasambu, neem was observed to arrest the locomotion of insects. Neem at 0.5 per cent dosage was highly effective against *O. surinamensis* (Figure 7) as it contains a wide assay of active compounds (Mutarak and Kulatilleke, 1990). All the natural products tested 1.0 per cent arrested the locomotion *vis-à-vis* to control (Dethier, 1960).

The goal of this study was to develop a more simple and rapid technique using grain as a medium instead of using filter paper and glass arenas (Burkholder, 1990, Dowdy *et al.*, 1993) for preliminary screening of large number of plant materials or natural products under laboratory condition. The results of the improved petriplate bioassay method showed that it seems to be a simple technique for preliminary screening of the efficacy of natural products with the assumption that a potential natural product reduces or arrest the speed of movement (Dethier, 1960) of the insects significantly with the increased dosage of the product. This method does not require voluminous amount of grains and also require less time. This seems to be an advantage over the previous bioassay methods (Loschiavo, 1952; Mohan and Fields, 2002).

Hence, this newer improved petriplate bioassay method can be a reliable bioassay technique in assessing the natural products for their efficacy against stored-product insect pests.

5.3. Three-in-One bioassay technique

The Three-in-One bioassay technique developed, tested proved more efficient, both in terms of assessing the efficacy of natural products as well as detecting the timely emergence of field carry-over stored-product insect pests.

Only a few bioassay techniques are available for determining if substances repel or attract stored product insect pests. Of that, one technique needs approximately 0.6 - 1 kg of grain to apply method of Loschiavo (1952), *vis-à-vis* 100 g required by this new method. Yet another method using filter paper or glass test arenas (Burkholder, 1990; Dowdy *et al*., 1993) does not allow testing of products on the grain. The recent cup bioassay technique developed by Mohan and Fields (2002) required large amount of perforated cups. However, the proposed new Three-in-One bioassay technique requires only a simple apparatus, less amount of grain and less time. This new method can also detect the timely emergence of field carry-over stored-product insect pests. Unlike other bioassays, this method allows three-dimensional movement of insects in grain, and also exploit the natural behaviour of insect (Hagstrum *et al*., 1990).

5.3.1. Studies on the repellency of natural products against stored product insect pests

Studies conducted for determining the efficacy of natural products for their repellency using the Three-in-One bioassay method proved effective. All the test products namely neem, vasambu, notchi and pungam, which are known to possess repellent property against stored-product insect pests showed repellency by Three-in-One bioassay technique also. This clearly indicates the Three-in-One bioassay technique can be a reliable tool to assess the repellent property against stored-product insect pests. These natural products have differential response to different stored-product insect pests (Jillani and Haq, 1984). In the present study also, this differential response was noticed. Neem at 1 per cent concentration effectively check *T. castaneum* and *O. surinamensis* as shown in figure 10 and 11. One hundred per cent repellency of *T. castaneum* was noticed. Similar effects of neem on *T. castaneum* was reported by Koul (2003), Jilani *et al*. (1988) and Jilani and Malik (1973). Figure 12 and 13 shows vasambu at one per cent concentration offered a good repellency for *S. oryzae* and *R. dominica* within 24 hours. Paul *et al*., 1965 and Jillani and Haq, 1984 reported that vasambu repelled the population of *S. oryzae* and *R. dominica*.

5.3.2. Studies on the attractiveness of natural products against stored product insect pests

Studies conducted for assessing the efficacy of various food attractants against stored-product insect pests using Three-in-One bioassay technique was proved effective. All the test products namely rice bran, groundnut powder and wheat flour, which are known to possess attractant property against stored-product insect pests showed insect attraction by Three-in-One bioassay technique also. This clearly indicates the Three-in-One bioassay technique can be a reliable tool to assess the attractant property of natural products against stored-product insect pests. The result indicated that *S. oryzae, O. surinamensis* and *R. dominica* were attracted to rice bran as shown in figure 14, 15 and 16. Mohan (1993) reported the attraction of stored product insects to rice bran. *T. castaneum* had a preferential response towards groundnut powder within 30 minutes compared to other insects (Figure 17). Mc Gregor (1964) reported preferential movement of *T. castaneum* to areas in the grain system that contained higher concentration of finer materials.

5.4. Three-in-one bioassay *vis-à-vis* probe trap

The studies conducted to compare the newer three-in-one bioassay technique with standard probe trap method proved effective. The new Three-in-One bioassay was compared with the standard probe trap method at the best dose tested (1 per cent concentration) for repellency test. In general, the new (Three-in-One) bioassay was found to be on par with the standard probe trap method. The results indicated that the three-in-one bioassay was efficient for testing the natural products for their efficacy i.e., the newer method requires a simple apparatus, less time and less amount of grain for a single test, but the probe trap requires large amount of grain samples (Loschiavo, 1952, 1974 and Mohan, 2004), thus proving that the new Three-in-One bioassay can be a reliable tool to assess the natural products for their repellent property for the management of stored-product insect pests (Figure 18).

5.5. Investigations on the efficiency of Three-in-one bioassay for timely detection on the emergence of stored-product insect pests

Investigations were conducted to find the efficacy of three-in-one bioassay for timely detection of the emergence of field carry-over insect pests. Results revealed that the emergence of *S. cerealella* adults in paddy samples and *C. chinensis* adults in pulse samples started seven days after harvest.

The peak emergence of *S. cerealella* adults was noticed on 31th day after harvest (Figure 19). This observation is in accordance with reports by Karan Singh and Majumder (1987). But in *C. chinensis*, the peak emergence was noticed on 16th day after harvest (Table 20). Similar observations was also reported by Mohan and Subba Rao (2000) and Kumar (2001).

Nevertheless, to observe the emergence of field carry over beetles from stored lot is time consuming and laborious. Hence, the three-in-one bioassay is an ideal device for use by farmers for detection and management of stored product insect pests. They can store random samples from the harvested produce in the newer model and place it in a frequently visited place by them. They can easily observe the emergence of field carry-over pests and take timely control measures.

Thus the newer bioassays can effectively be used as a reliable tool in the Integrated Pest Management (IPM) of stored-product insect pests.

CHAPTER VI

SUMMARY

Studies were carried out to find the efficacy of newer bioassay techniques namely improved petriplate bioassay technique and Three-in-One bioassay technique for assessing the efficacy of natural products for their repellent or attractant properties. The Three-in-One bioassay technique was also tested for the timely emergence of field carry-over stored-product insect pests. The results of these studies are summarized below.

Improved petriplate bioassay technique

1. An improved petriplate bioassay was developed and tested for assessing the efficacy of natural products against *S. oryzae*, *T. castaneum*, *O. surinamensis*, and *R. dominica* and proved effective.
2. Among the test products, vasambu was found to be a good locomotor arrestant than others.
3. Regarding insect movement, the movement response was in the order of:
   T. castaneum > *S. oryzae* > *O. surinamensis* > *R. dominica.*
4. Hence, the petriplate bioassay is a simple method to screen the natural products under laboratory condition.

Three-in-one bioassay

5. A newer technique was designed, developed and tested for determining the natural products that repel or attract stored-product insect pests. This was also tested for the detection of timely emergence of field carried over insect pests.
6. Neem and vasambu at 1 per cent concentration was found to be highly effective in their repellent action to all test insects than other tested products.
7. Among the four insect species tested, *T. castaneum* repelled quickly than other test insects.

8. Among the food attractants tested, rice bran was found to the more effective for *S. oryzae,O. surinamensis* and *R. dominica*. But, *T. castaneum* preferred groundnut powder compared to other test insects.
9. Studies made to compare the Three-in-One bioassay technique with standard method (trap method) revealed that the new method was reliable and more efficient.
10. Three-in-one bioassay technique was also found to be an ideal tool for detection of timely emergence of field carry-over stored-product insect pests
11. Thus, the Three-in-One bioassay is a simple, rapid and pragmatic method not only to assess the natural products for their efficacy but also for monitoring stored-product insect pests.

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***** Original not seen

Table 1. Movement of *Sitophilus oryzae* adults in response to different botanicals at various concentrations

Test Products	Distance travelled (mm / sec)					ANOVA	
	Concentrations						
	0%	0.05%	0.1%	0.5%	1%	F	P
Neem	1.05	0.82	0.97	0.52*	0.38*	5.53	0.004
Notchi	1.07	0.87	0.83	0.78	0.44*	2.07	0.124
Pungam	1.20	0.93	0.98	0.85	0.45*	1.93	0.145
Vasambu	1.15	0.77	0.62	0.38*	0.32*	4.58	0.009

Mean of five observations

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 2. Movement of *Oryzaephilus surinamensis* adults in response to different botanicals at various concentrations

Test Products	Distance travelled (mm / sec) Concentrations					ANOVA	
	0%	**0.05%**	**0.1%**	**0.5%**	**1%**	**F**	**P**
Neem	0.85	0.82	0.63	0.34*	0.23*	4.66	0.008
Notchi	1.01	0.67	0.50*	0.56*	0.23*	7.51	0.001
Pungam	1.06	0.88	0.62*	0.42*	0.19*	10.29	0.000
Vasambu	1.17	0.64	0.41*	0.33*	0.14*	13.73	0.000

Mean of five observations

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 3. Movement of *Rhyzopertha dominica* adults in response to different botanicals at various concentrations

Test Products	Distance travelled (mm / sec)					ANOVA	
	Concentrations						
	0%	**0.05%**	**0.1%**	**0.5%**	**1%**	**F**	**P**
Neem	0.27	0.16	0.14	0.15	0.19	0.91	0.476
Notchi	0.30	0.29	0.16*	0.15*	0.05*	11.12	0.000
Pungam	0.39	0.31	0.15*	0.17	0.05*	4.48	0.010
Vasambu	0.34	0.19	0.13*	0.11*	0.02*	7.64	0.001

Mean of five observations

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test (P < 0.05)

Table 4. Movement of *Tribolium castaneum* adults in response to different botanicals at various concentrations

Test Products	Distance travelled (mm / sec)					ANOVA	
	Concentrations						
	0%	**0.05%**	**0.1%**	**0.5%**	**1%**	**F**	**P**
Neem	1.36	1.10	1.09	0.94	0.69*	2.74	0.058
Notchi	1.44	1.19	0.77*	0.61*	0.49*	7.94	0.001
Pungam	1.12	0.99	1.04	0.80	0.38	2.06	0.124
Vasambu	1.31	0.95	0.74	0.68*	0.25*	6.24	0.002

Mean of five observations

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 5. Repellency of neem against *Sitophilus oryzae* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	4 (12.66)	2 (6.33)	4 (12.66)	2 (6.33)	10* (24.28)	0.88	0.492
1 h	6 (18.99)	4 (12.66)	10 (27.96)	2 (6.33)	18 (41.65)	4.04	0.015
3 h	12 (26.34)	12 (34.29)	24 (48.42)	8 (21.63)	20 (43.71)	2.36	0.088
6 h	12 (22.08)	12 (34.29)	34* (58.78)	28* (52.85)	46* (69.01)	8.24	<0.001
12 h	14 (28.41)	18 (42.23)	36* (60.42)	40* (64.00)	50* (72.29)	8.53	<0.001
24 h	16 (34.74)	18 (42.23)	36* (60.42)	50* (72.22)	50* (72.29)	10.51	<0.001

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 6. Repellency of pungam against *Sitophilus oryzae* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	4 (12.66)	8 (21.63)	8 (21.63)	18* (41.65)	18* (41.65)	3.21	0.034
1 h	6 (18.99)	2 (6.33)	10 (24.28)	14 (32.67)	10 (24.28)	1.19	0.344
3 h	10 (27.96)	4 (12.66)	16 (35.32)	18 (41.65)	12 (30.61)	1.99	0.136
6 h	10 (27.96)	8 (25.32)	22 (42.09)	24 (49.00)	24 (48.15)	2.54	0.072
12 h	12 (30.61)	10 (27.96)	26 (45.95)	26 (51.07)	36* (60.57)	3.54	0.024
24 h	10 (24.28)	12 (30.61)	30* (54.07)	34* (58.78)	52* (73.86)	9.56	<0.001

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 7. Repellency of vasambu against *Sitophilus oryzae* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations						
	0	0.05	0.10	0.50	1.0	F	P
30 min	2 (6.33)	2 (6.33)	4 (12.66)	8 (25.32)	10* (31.65)	3.67	0.021
1 h	2 (6.33)	6 (15.30)	4 (12.66)	10 (27.96)	14* (36.94)	2.97	0.045
3 h	2 (6.33)	6 (15.30)	10 (24.28)	14 (33.25)	14* (36.94)	2.47	0.078
6 h	6 (18.99)	12 (30.61)	16 (39.00)	34* (58.36)	38* (61.79)	8.30	<0.001
12 h	12 (34.29)	14 (33.25)	22 (45.50)	48* (70.50)	46* (68.86)	10.39	<0.001
24 h	16 (39.58)	22 (46.359)	32 (56.29)	46* (68.15)	70* (88.55)	12.46	<0.001

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 8. Repellency of neem against *Oryzaephilus surinamensis* at different time intervals

Duration	Per cent repellency of insects: Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	-
1 h	0 (0.00)	0 (0.00)	4 (12.66)	4 (12.66)	4 (12.66)	1.33	0.292
3 h	0 (0.00)	0 (0.00)	4 (12.66)	8* (25.32)	6 (18.99)	6.40	0.002
6 h	0 (0.00)	2 (6.33)	4 (12.66)	8 (25.32)	6 (18.99)	2.50	0.075
12 h	0 (0.00)	2 (6.33)	4 (12.66)	8* (25.32)	10* (31.65)	6.14	0.002
24 h	0 (0.00)	2 (6.330)	6 (15.30)	8* (25.32)	16* (39.58)	6.73	0.001

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test (P < 0.05)

Table 9. Repellency of pungam against *Oryzaephilus surinamensis* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	-
1 h	0 (0.00)	4 (12.66)	4 (12.66)	4 (12.66)	6 (18.99)	1.00	0.431
3 h	0 (0.00)	4 (12.66)	6 (18.99)	6 (18.99)	8 (25.32)	2.09	0.120
6 h	0 (0.00)	4 (12.66)	10* (27.96)	12* (30.61)	8* (25.32)	3.68	0.021
12 h	2 (6.33)	10 (24.28)	10 (27.96)	10 (24.28)	8 (21.63)	0.92	0.471
24 h	6 (15.30)	12 (26.92)	16 (35.32)	12 (30.61)	6 ((15.30)	0.89	0.490

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test (P < 0.05)

Table 10. Repellency of vasambu against *Oryzaephilus surinamensis* at different time intervals

Duration	Per cent repellency of insects Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1	F	P
30 min	0 (0.00)	2 (6.33)	10 (27.96)	4 (12.66)	2 (6.33)	-	-
1 h	0 (0.00)	4 (8.98)	16* (39.00)	10* (27.96)	4 (12.66)	2.90	0.048
3 h	0 (0.00)	14* (33.25)	20* (43.71)	14* (32.67)	4 (12.66)	5.56	0.004
6 h	2 (6.33)	14 (33.25)	22* (46.36)	12 (30.61)	6 (18.99)	6.27	0.002
12 h	4 (12.66)	12 (26.92)	22* (46.08)	18* (41.65)	12 (34.29)	4.38	0.010
24 h	6 (18.99)	16 (35.32)	28* (52.58)	24* (49.00)	14 (36.94)	3.70	0.021

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 11. Repellency of notchi against *Oryzaephilus surinamensis* at different time intervals

Duration	Per cent repellency of insects – Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	-
1 h	0 (0.00)	0 (0.00)	0 (0.00)	6 (15.30)	2 (6.33)	1.71	0.188
3 h	0 (0.00)	2 (6.33)	4 (12.66)	8 (21.63)	2 (6.33)	1.49	0.242
6 h	2 (6.33)	4 (12.66)	4 (12.66)	14 (33.25)	4 (12.66)	1.79	0.171
12 h	2 (6.33)	8 (17.95)	4 (12.66)	16 (35.32)	2 (6.33)	2.04	0.128
24 h	6 (18.99)	24 (48.15)	10 (24.28)	22* (45.50)	12 (34.29)	3.36	0.029

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test (P < 0.05)

Table 12. Repellency of neem against *Rhyzopertha dominica* at different time intervals

Duration	Per cent repellency of insects – Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	4 (8.98)	0 (0.00)	2 (6.33)	0.76	0.561
1 h	6 (18.99)	4 (8.98)	6 (15.30)	4 (12.66)	6 (18.99)	0.26	0.899
3 h	8 (21.63)	10 (23.70)	10 (27.96)	16 (30.20)	18 (36.11)	0.29	0.884
6 h	8 (21.63)	16 (39.00)	14 (32.67)	28* (52.86)	32* (56.29)	4.36	0.011
12 h	8 (21.63)	18 (41.65)	20* (43.71)	30* (54.65)	34* (58.36)	5.99	0.002
24 h	12 (26.34)	20 (44.29)	26* (51.07)	32* (56.71)	44* (67.22)	6.44	0.002

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 13. Repellency of pungam against *Rhyzopertha dominica* at different time intervals

Duration	Per cent repellency of insects – Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	0 (0.00)	4 (12.66)	0 (0.00)	2.67	0.062
1 h	0 (0.00)	0 (0.00)	0 (0.00)	4 (12.66)	2 (6.33)	1.60	0.213
3 h	2 (6.33)	0 (0.00)	6 (15.30)	10 (27.96)	8 (25.32)	3.16	0.037
6 h	2 (6.33)	2* (6.33)	6 (15.30)	12 (30.61)	16* (39.00)	4.13	0.013
12 h	2 (6.33)	2 (6.33)	16 (35.32)	14 (28.41)	18 (37.38)	2.74	0.058
24 h	2 (6.330)	4 (8.975)	24* (48.42)	20 (34.63)	28* (52.28)	6.051	0.002

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 14. Repellency of vasambu against *Rhyzopertha dominica* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	2 (6.33)	4 (12.66)	2 (6.33)	1.00	0.431
1 h	0 (0.00)	0 (0.00)	4 (12.66)	6 (18.99)	2 (6.33)	2.13	0.115
3 h	8 (21.63)	4 (12.66)	8 (25.32)	12 (34.29)	22* (46.36)	3.93	0.016
6 h	8 (21.63)	6 (15.30)	12 (34.29)	12 (34.29)	26* (50.49)	4.33	0.011
12 h	6 (15.30)	8 (21.63)	14 (36.36)	22* (45.78)	40* (63.70)	7.432	0.001
24 h	18 (42.23)	20 (43.71)	30 (53.65)	40 (62.28)	54* (75.29)	4.331	0.011

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett’s Post hoc test ($P < 0.05$)

Table 15. Repellency of notchi against *Rhyzopertha dominica* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-	-
1 h	0 (0.00)	0 (0.00)	0 (0.00)	2 (6.33)	4 (12.66)	1.60	0.213
3 h	0 (0.00)	0 (0.00)	2 (6.33)	4 (12.66)	6 (18.99)	2.13	0.115
6 h	4 (12.66)	6 (15.30)	10 (27.96)	6 (18.99)	10 (27.96)	0.78	0.550
12 h	4 (12.66)	6 (15.30)	12 (30.61)	10 (27.96)	12 (30.61)	1.12	0.377
24 h	4 (12.66)	8 (17.37)	16 (39.00)	16 (35.32)	20 (43.71)	2.88	0.050

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett’s Post hoc test ($P < 0.05$)

Table 16. Repellency of neem against *Tribolium castaneum* at different time intervals

Duration	Per cent repellency of insects – Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1.0	F	P
30 min	0 (0.00)	8 (25.32)	14* (28.99)	24* (47.73)	54* (75.21)	15.10	<0.001
1 h	12 (30.61)	16 (39.58)	24 (48.42)	48* (69.99)	64* (83.36)	11.19	<0.001
3 h	16 (39.58)	20 (44.29)	34 (58.78)	58* (78.65)	88* (105.91)	33.32	<0.001
6 h	18 (41.65)	28 (52.86)	42* (65.79)	64* (83.77)	98* (121.43)	44.35	<0.001
12 h	28 (52.86)	46 (68.78)	58* (78.72)	74* (92.10)	100* (125.33)	33.58	<0.001
24 h	34 (58.36)	52 (73.86)	62* (81.86)	78* (97.91)	100* (125.33)	19.16	<0.001

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 17. Repellency of pungam against *Tribolium castaneum* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	2 (6.33)	8 (21.63)	8 (21.63)	18* (41.65)	34* (58.78)	8.94	<0.001
1 h	10 24.28)	8 (21.63)	18 (37.96)	24 (48.15)	38* (61.79)	4.13	0.013
3 h	12 (30.61)	14 (36.94)	24 (43.88)	24 (48.15)	40* (63.85)	3.16	0.036
6 h	14 (33.25)	22 (46.36)	32 (55.86)	26 (49.21)	44* (67.43)	3.96	0.016
12 h	30 (54.50)	28 (52.86)	38 (60.49)	44 (66.99)	62* (81.86)	3.81	0.018
24 h	34 (58.63)	30 (54.92)	44 (65.65)	54 (75.21)	58 (78.43)	2.65	0.063

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 18. Repellency of vasambu against *Tribolium castaneum* at different time intervals

Duration	Per cent repellency of insects Different concentrations (%)					ANOVA	
	0	0.05	0.10	0.50	1.0	F	P
30 min	2 (6.33)	14 (28.99)	6 (18.99)	46* (68.36)	14* (36.94)	9.17	<0.001
1 h	4 (12.66)	22* (46.36)	12 (30.61)	54* (74.79)	22* (46.36)	10.85	<0.001
3 h	10 (24.28)	28* (53.13)	28* (52.58)	62* (81.15)	32* (55.86)	7.77	0.001
6 h	10 (24.28)	34* ((58.78)	36* (59.72)	64* (82.80)	36* (59.14)	7.183	0.001
12 h	28 (52.44)	42 (65.43)	52* (73.86)	82* (98.46)	66* (85.67)	10.761	<0.001
24 h	30 ((54.50)	48 (70.65)	56* (77.00)	74* (92.03)	70* (88.88)	6.856	0.001

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett's Post hoc test (P < 0.05)

Table 19. Repellency of notchi against *Tribolium castaneum* at different time intervals

Duration	Per cent repellency of insects					ANOVA	
	Different concentrations (%)						
	0	0.05	0.10	0.50	1.0	F	P
30 min	6 (18.99)	8 (21.63)	6 (18.99)	16 (35.32)	2 (6.33)	1.58	0.217
1 h	6 (15.30)	10 (23.70)	10 (31.65)	22 (41.40)	2 (6.33)	2.46	0.078
3 h	10 (19.16)	10 (23.70)	14 (36.94)	28 (51.36)	6 (18.99)	2.37	0.087
6 h	10 (19.16)	18 (40.79)	24* (49.00)	34* (57.28)	14 (36.94)	3.62	0.022
12 h	24 (48.15)	36 (59.51)	40 (64.00)	38 (61.72)	26 (50.79)	1.75	0.178
24 h	30 (54.92)	40 (63.36)	44* (67.29)	64* (83.43)	56 (77.34)	4.69	0.008

Mean of five observations

Figures in parentheses are arcsine transformed values

Concentrations that are significantly different from the untreated control (0 per cent) are marked with an * by Dunnett’s Post hoc test (P < 0.05)

Table 20. Comparative movement responses of test insects to neem seed powder (1 per cent dose) treated paddy grains

Duration	Insect leaving grains (%)				ANOVA	
	Sitophilus oryzae	*Oryzaephilus surinamesis*	*Rhyzopertha dominica*	*Tribolium castaneum*		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	0 ±0.00b	2 ±0.00a	10 ±4.47ab	54 ±6.78c	26.51	<0.001
1 h	4 ±2.45c	6 ±2.45a	18 ±3.74b	64 ±6.78c	24.07	<0.001
3 h	0 ±0.00b	18 ±8.60a	20 ±4.47b	88 ±3.74c	40.19	<0.001
6 h	6 ±2.45b	32 ±5.83a	46 ±4.00b	98 ±2.00c	62.54	<0.001
12 h	10 ±0.00c	34 ±5.10a	50 ±3.16b	100 ±0.00d	22.62	<0.001
24 h	16 ±2.45b	44 ±±5.10a	50 ±3.16b	100 ±0.00c	147.17	<0.001

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 21. Comparative movement responses of test insects to pungam seed powder (1 per cent dose) treated paddy grains

Duration	Insect leaving grains (%)				ANOVA	
	Sitophilus oryzae	*Oryzaephilus surinamesis*	*Rhyzopertha dominica*	*Tribolium castaneum*		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	0 ±0.00c	0 ±0.00a	18 ±3.74a	8 ±3.74b	15.40	<0.001
1 h	6 ±2.45a	2 ±2.00a	10 ±4.47a	18 ±4.90a	2.30	0.116
3 h	8 ±2.00a	8 ±2.00a	12 ±3.74a	24 ±6.79a	1.11	0.375
6 h	8 ±2.00b	16 ±4.00a	24 ±5.10ab	32 ±5.83b	5.20	0.011
12 h	8 ±3.74b	18 ±5.83a	36 ±2.45ab	38 ±10.20b	5.10	0.012
24 h	6 ±4.00b	28 ±4.90a	52 ±3.74b	44 ±10.30b	11.94	<0.001

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 22. Comparative movement responses of test insects to vasambu rhizome powder (1 per cent dose) treated paddy grains

Duration	Insect leaving grains (%)				ANOVA	
	Sitophilus oryzae	***Oryzaephilus surinamesis***	***Rhyzopertha dominica***	***Tribolium castaneum***		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	2 ±2.00b	2 ±2.00a	10 ±0.00a	14 ±2.45b	11.71	<0.001
1 h	4 ±2.45b	2 ±2.00a	14 ±2.45a	22 ±3.74b	11.31	<0.001
3 h	4 ±2.45b	22 ±3.74a	14 ±2.45b	32 ±5.83b	10.66	<0.001
6 h	6 ±2.45b	26 ±4.00a	38 ±5.83b	36 ±7.48b	9.25	0.001
12 h	12 ±2.00b	40 ±5.50a	46 ±5.10b	66 ±10.30c	15.24	<0.001
24 h	14 ±2.45b	54 ±8.12a	70 ±7.07b	70 ±8.94b	16.50	<0.001

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 23. Comparative movement responses of test insects to notchi leaf powder (1 per cent dose) treated paddy grains

Duration	Insect leaving grains (%)				ANOVA	
	Sitophilus oryzae	***Oryzaephilus surinamesis***	***Rhyzopertha dominica***	***Tribolium castaneum***		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	0 ±0.00a	0 ±0.00a	4 ±2.45a	2 ±2.00a	1.47	0.261
1 h	2 ±2.00a	4 ±2.45a	4 ±2.45a	2 ±2.00a	0.27	0.848
3 h	2 ±2.00a	6 ±2.45a	4 ±2.45a	6 ±2.50a	0.67	0.585
6 h	4 ±2.45a	10 ±3.16a	8 ±2.00a	14 ±2.45a	2.42	0.104
12 h	2 ±2.00ab	12 ±3.74a	10 ±3.16ab	26 ±4.00b	7.44	0.002
24 h	12 ±2.00a	20 ±4.47a	16 ±4.00a	56 ±9.27b	13.03	0.000

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 28. Comparative movement responses of test insects to groundnut powder

Duration	Per cent attraction				ANOVA	
	Sitophilus oryzae	***Oryzaephilus surinamesis***	***Rhyzopertha dominica***	***Tribolium castaneum***		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	26 ±6.00a	14 ±5.10a	14 ±2.45a	98 ±2.00b	45.35	<0.001
1 h	40 ±7.07b	40 ±7.07b	16 ±2.45a	100 ±0.00c	61.29	<0.001
3 h	46 ±5.99b	56 ±6.00b	20 ±3.16a	100 ±0.00c	84.23	<0.001
6 h	52 ±5.83b	70 ±7.07c	22 ±3.74a	100 ±0.00d	57.77	<0.001
12 h	58 ±6.63b	82 ±5.83c	32 ±4.90a	100 ±0.00d	36.05	<0.001
24 h	68 ±8.00b	92. ±3.74c	36 ±4.00a	100 ±0.00c	37.64	<0.001

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 29. Comparative movement responses of test insects to rice bran

Duration	Per cent attraction				ANOVA	
	Sitophilus oryzae	***Oryzaephilus surinamesis***	***Rhyzopertha dominica***	***Tribolium castaneum***		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	50 ±4.47b	40 ±7.071b	12 ±2.00a	32 ±5.83b	11.97	<0.001
1 h	56 ±6.78b	50 ±7.071b	14 ±2.45a	40 ±7.07b	11.37	<0.001
3 h	80 ±5.48b	92 ±3.74b	28 ±3.74a	44 ±6.78a	28.79	<0.001
6 h	92 ±3.74b	94 ±2.45b	42 ±3.74a	56 ±9.27a	19.14	<0.001
12 h	98 ±2.00b	96 ±2.45b	60 ±7.07a	66 ±9.27a	14.22	<0.001
24 h	100 ±0.00b	98 ±2.00b	82 ±3.74a	92 ±3.74b	9.49	<0.001

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 30. Comparative movement responses of test insects to wheat flour

Duration	Per cent attraction				ANOVA	
	Sitophilus oryzae	***Oryzaephilus surinamesis***	***Rhyzopertha dominica***	***Tribolium castaneum***		
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	16 ±4.00a	14 ±2.45a	18 ±3.74a	26 ±5.10a	1.61	0.226
1 h	20 ±3.16a	14 ±2.45a	18 ±3.74a	36 ±4.00b	7.34	0.003
3 h	26 ±2.45a	22 ±3.74a	20 ±3.16a	42 ±3.74b	7.50	0.002
6 h	42 ±5.83ab	26 ±5.10a	26 ±5.10a	62 ±8.60b	6.78	0.004
12 h	58 ±3.74ab	40 ±4.47a	50 ±7.07a	76 ±9.80b	5.24	0.010
24 h	78 ±5.83ab	62 ±5.83a	70 ±7.07a	90 ±5.48b	4.47	0.018

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, $P<0.05$)

Table 24. Attraction of different food attractants to *S. oryzae*

Duration	Per cent attraction			ANOVA	
	Groundnut	Rice Bran	Wheat Flour		
	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	26 ±6.00a	50 ±4.47b	16 ±4.00a	11.41	0.002
1 h	40 ±7.07b	56 ±6.78b	20 ±3.16a	9.89	0.003
3 h	46 ±5.10b	80 ±5.48c	26 ±2.45a	34.49	0.000
6 h	52 ±5.83a	92 ±3.74b	42 ±5.83a	22.99	0.000
12 h	58 ±6.63a	98 ±2.00b	58 ±3.74a	35.09	0.000
24 h	68 ±8.00a	100 ±0.00b	78 ±5.83a	18.63	0.000

Mean of five observations

Products that are significantly different have different letters (Student-Newman-Keuls, $P<0.05$)

Table 25. Attraction of different food attractants to *O. surinamensis*

Duration	Per cent attraction			ANOVA	
	Groundnut	Rice Bran	Wheat Flour		
	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	14 ±5.10a	40 ±7.07b	14 ±2.45a	6.12	0.015
1 h	40 ±7.07b	50 ±7.07b	14 ±2.45a	12.11	0.001
3 h	56 ±6.00b	92 ±3.74c	22 ±3.74a	42.55	0.000
6 h	70 ±7.07b	94 ±2.45c	26 ±5.10a	34.69	0.000
12 h	82 ±5.83b	96 ±2.45c	40 ±4.47c	26.24	0.000
24 h	92 ±3.74b	98 ±2.00b	62 ±5.83a	18.06	0.000

Mean of five observations

Products that are significantly different have different letters (Student-Newman-Keuls, $P<0.05$)

Table 26. Attraction of different food attractants to *R. dominica*

Duration	Per cent attraction			ANOVA	
	Groundnut	Rice Bran	Wheat Flour		
	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	14 ±2.45a	12 ±2.00a	18 ±3.74a	1.10	0.363
1 h	16 ±2.45a	14 ±2.45a	18 ±3.74a	0.41	0.675
3 h	20 ±3.16a	28 ±3.74a	20 ±3.16a	1.78	0.211
6 h	22 ±3.74a	42 ±3.74b	26 ±5.10a	5.22	0.023
12 h	32 ±4.90a	60 ±7.07b	50 ±7.07ab	5.09	0.025
24 h	36 ±4.00a	82 ±3.74b	70 ±7.07b	20.24	0.000

Mean of five observations

Products that are significantly different have different letters (Student-Newman-Keuls, P<0.05)

Table 27. Attraction of different food attractants to *T. castaneum*

Duration	Per cent attraction			ANOVA	
	Groundnut	Rice Bran	Wheat Flour		
	Mean ±SE	Mean ±SE	Mean ±SE	F	P
30 min	98 ±2.00b	32 ±5.83a	26 ±5.10a	62.20	0.000
1 h	100 ±0.00b	40 ±7.07a	36 ±4.00a	79.63	0.000
3 h	100 ±0.00b	44 ±6.78a	42 ±3.74a	82.96	0.000
6 h	100 ±0.00b	56 ±9.27a	62 ±8.60a	19.65	0.000
12 h	100 ±0.00b	66 ±9.27a	76 ±9.80a	10.60	0.002
24 h	100 ±0.00a	92 ±3.74a	90 ±5.48a	2.53	0.121

Mean of five observations

Test insects that are significantly different have different letters (Student-Newman-Keuls, $P<0.05$)

able 31. Comparison of Three-in-One bioassay technique with standard Probe trap method

Product	Time	Method	Mean	Std. Error Mean	t value	p value
Neem	30 min	Three-in-One bioassay	16.50	5.39	1.02	0.32
		Standard Trapping Method	8.00	2.96		
	1 h	Three-in-One bioassay	23.00	5.90	2.33	0.03
		Standard Trapping Method	9.00	3.15		
	3 h	Three-in-One bioassay	31.50	8.06	1.223	0.23
		Standard Trapping Method	16.50	3.35		
	6 h	Three-in-One bioassay	45.50	7.90	2.24	0.03*
		Standard Trapping Method	23.00	3.56		
	12 h	Three-in-One bioassay	48.50	7.69	2.24	0.03*
		Standard Trapping Method	29.50	4.84		
	24 h	Three-in-One bioassay	52.50	7.10	1.77	0.09
		Standard Trapping Method	38.50	6.21		
Notchi	30 min	Three-in-One bioassay	1.50	0.82	-0.41	0.69
		Standard Trapping Method	2.00	0.92		
	1 h	Three-in-One bioassay	3.00	1.05	-0.14	0.89
		Standard Trapping Method	3.50	1.31		
	3 h	Three-in-One bioassay	4.50	1.14	-0.27	0.79
		Standard Trapping Method	6.50	2.09		
	6 h	Three-in-One bioassay	9.00	1.43	0.09	0.93
		Standard Trapping Method	11.00	2.50		
	12 h	Three-in-One bioassay	12.50	2.50	-0.99	0.33
		Standard Trapping Method	17.00	3.17		
	24 h	Three-in-One bioassay	26.00	4.78	0.31	0.76
		Standard Trapping Method	25.50	4.32		

contd..

Pungam	30 min	Three-in-One bioassay	6.50	2.09	-0.17	0.87
		Standard Trapping Method	6.50	1.82		
	1 h	Three-in-One bioassay	9.00	2.16	-0.24	0.81
		Standard Trapping Method	9.50	2.11		
	3 h	Three-in-One bioassay	13.00	2.42	-0.26	0.80
		Standard Trapping Method	14.00	2.22		
	6 h	Three-in-One bioassay	20.00	2.90	1.41	0.17
		Standard Trapping Method	15.00	2.35		
	12 h	Three-in-One bioassay	25.00	4.07	0.47	0.64
		Standard Trapping Method	21.50	3.50		
	24 h	Three-in-One bioassay	32.50	4.97	0.27	0.79
		Standard Trapping Method	27.50	3.96		

Vasambu	30 min	Three-in-One bioassay	7.00	1.47	0.34	0.74
		Standard Trapping Method	7.00	1.93		
	1 h	Three-in-One bioassay	10.50	2.23	0.58	0.57
		Standard Trapping Method	9.00	2.28		
	3 h	Three-in-One bioassay	18.00	2.96	0.29	0.78
		Standard Trapping Method	16.00	2.22		
	6 h	Three-in-One bioassay	26.50	3.79	0.56	0.58
		Standard Trapping Method	21.50	2.64		
	12 h	Three-in-One bioassay	41.00	5.33	1.04	0.31
		Standard Trapping Method	33.50	3.65		
	24 h	Three-in-One bioassay	52.00	6.18	0.43	0.67
		Standard Trapping Method	48.00	5.16		

Mean of five observations

Concentrations that are significantly different are marked with an * by Dunnett's Post hoc test ($P < 0.05$)

Table 32. Daily totals of field carry-over insect pests collected in Three-in-One bioassay during December 2005 to January 2006 in paddy and pulse samples

Days	Mean number of adults of *S. cerealella*			Mean number of adults of *C. Chinensis*		
	Mean	±	S.E.	Mean	±	S.E.
1	0.00	±	0.00	0.00	±	0.00
2	0.00	±	0.00	0.00	±	0.00
3	0.00	±	0.00	0.00	±	0.00
4	0.00	±	0.00	0.00	±	0.00
5	0.00	±	0.00	0.00	±	0.00
6	0.00	±	0.00	0.00	±	0.00
7	0.33	±	0.58	0.67	±	0.58
8	1.00	±	0.00	0.67	±	0.58
9	1.67	±	0.58	2.00	±	0.00
10	3.33	±	2.31	3.00	±	0.00
11	4.67	±	1.53	3.33	±	0.58
12	5.33	±	1.53	4.33	±	0.58
13	5.66	±	1.15	5.67	±	0.58
14	6.33	±	0.58	6.67	±	0.58
15	7.33	±	0.58	7.67	±	0.58
16	8.00	±	0.00	8.00	±	0.00
17	7.00	±	2.00	7.67	±	1.15
18	9.33	±	0.58	7.33	±	0.58
19	10.67	±	1.15	6.67	±	0.58
20	11.33	±	0.58	6.33	±	0.58
21	12.00	±	1.00	5.67	±	0.58
22	13.00	±	0.00	5.67	±	0.58
23	14.67	±	0.58	4.67	±	0.58
24	15.67	±	0.58	3.67	±	0.58
25	17.00	±	0.00	3.00	±	0.00
26	17.33	±	0.58	2.33	±	0.58
27	16.00	±	2.00	2.00	±	0.00
28	16.67	±	3.21	2.00	±	0.00
29	20.33	±	0.58	1.33	±	0.58
30	24.67	±	1.15	0.67	±	0.58
31	26.67	±	0.58	0.67	±	0.58
32	23.67	±	1.53	0.33	±	0.58
33	21.33	±	1.53	0.00	±	0.00
34	17.00	±	1.73	0.00	±	0.00
35	14.67	±	3.21	0.00	±	0.00
36	12.67	±	3.21	0.00	±	0.00
37	7.00	±	2.65	0.00	±	0.00
38	2.00	±	1.00	0.00	±	0.00
39	0.33	±	0.58	0.00	±	0.00
40	0.00	±	0.00	0.00	±	0.00

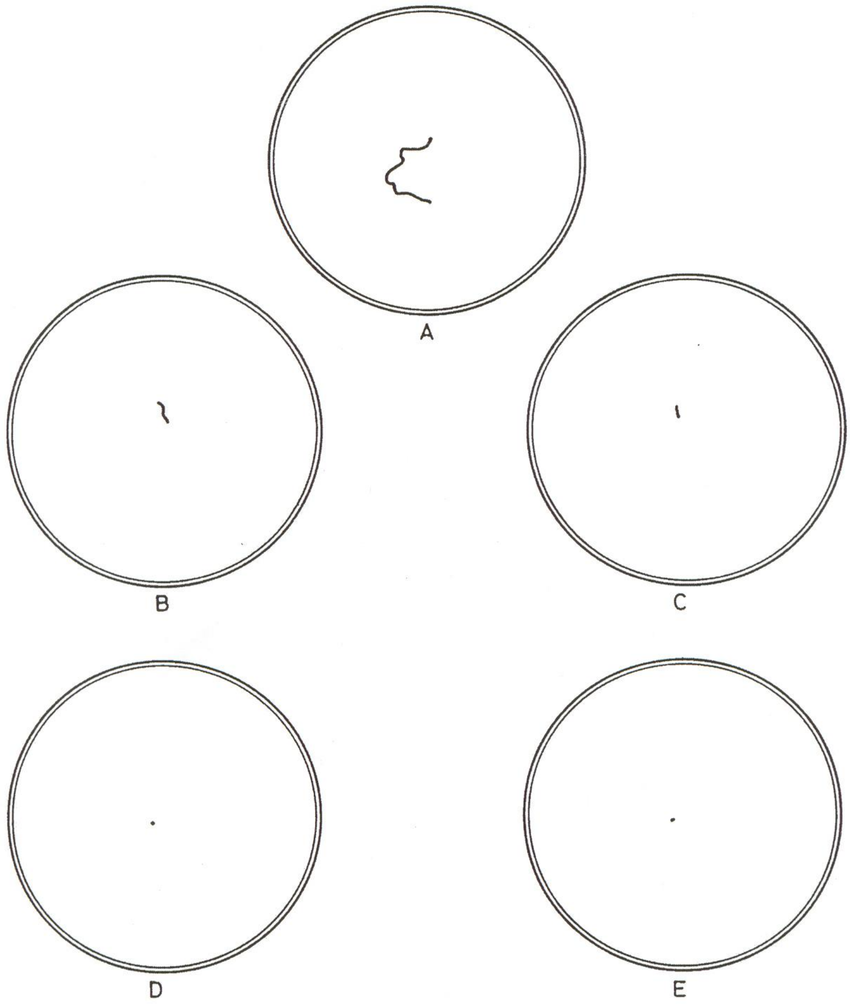


Fig 5. Typical path tracings of *R. dominica* at different dose of vasambu in petriplate bioassay method (Figure shown the moment of insect which was measured as cm for 2 min) A- 0%, B- 0.05%, C- 0.1%, D – 0.5%

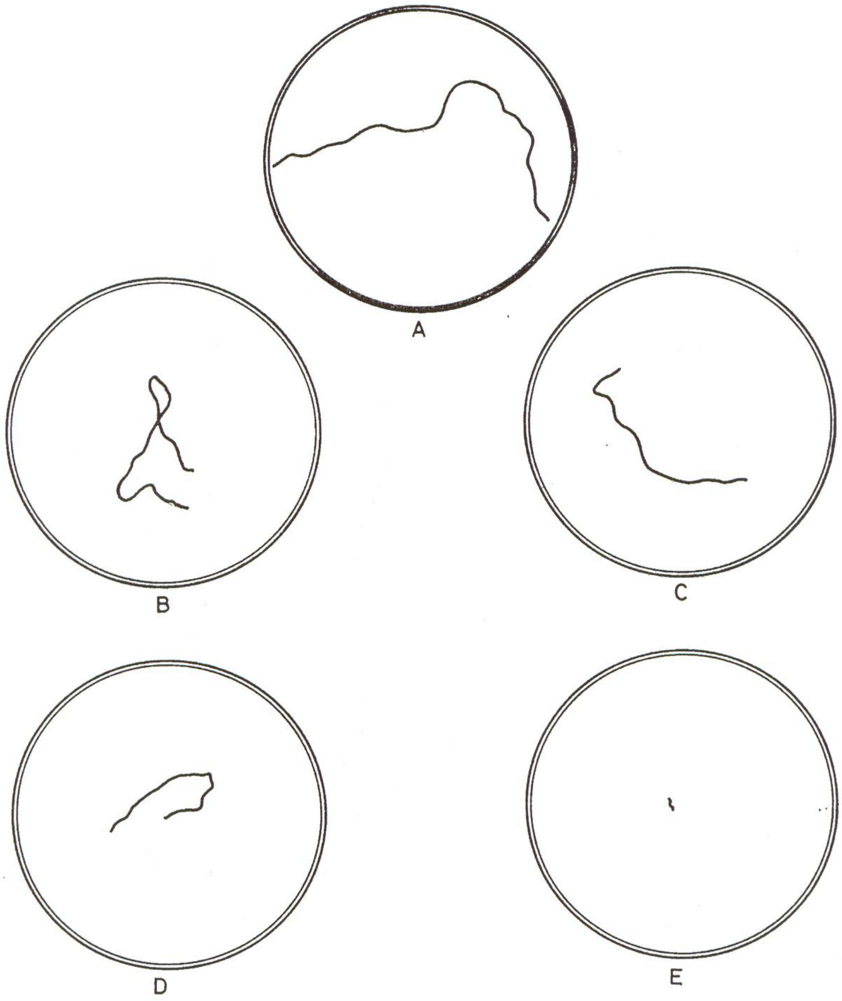


Fig 4. Typical path tracings of *O. surinamensis* at different dose of vasambu in petriplate bioassay method (Figure shown the moment of insect which was measured as cm for 2 min) A- 0%, B- 0.05%, C- 0.1%, D – 0.5%

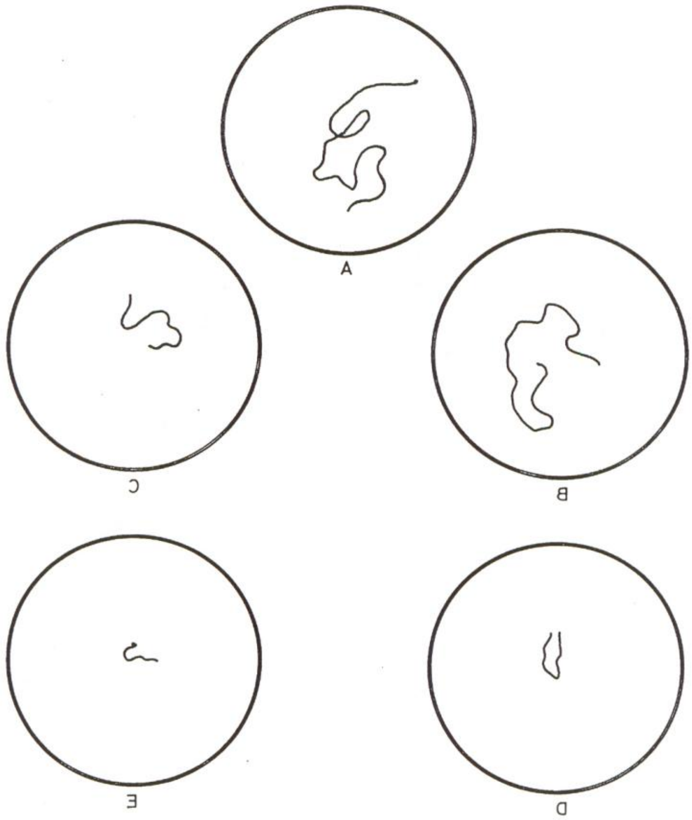


Fig 3 Typical path tracings of *S. oryzae* at different dose of vasambu in petriplate bioassay method (Figure shown the moment of insect which was measured as cm for 2 min) A- 0%, B- 0.05%, C- 0.1%, D – 0.5%

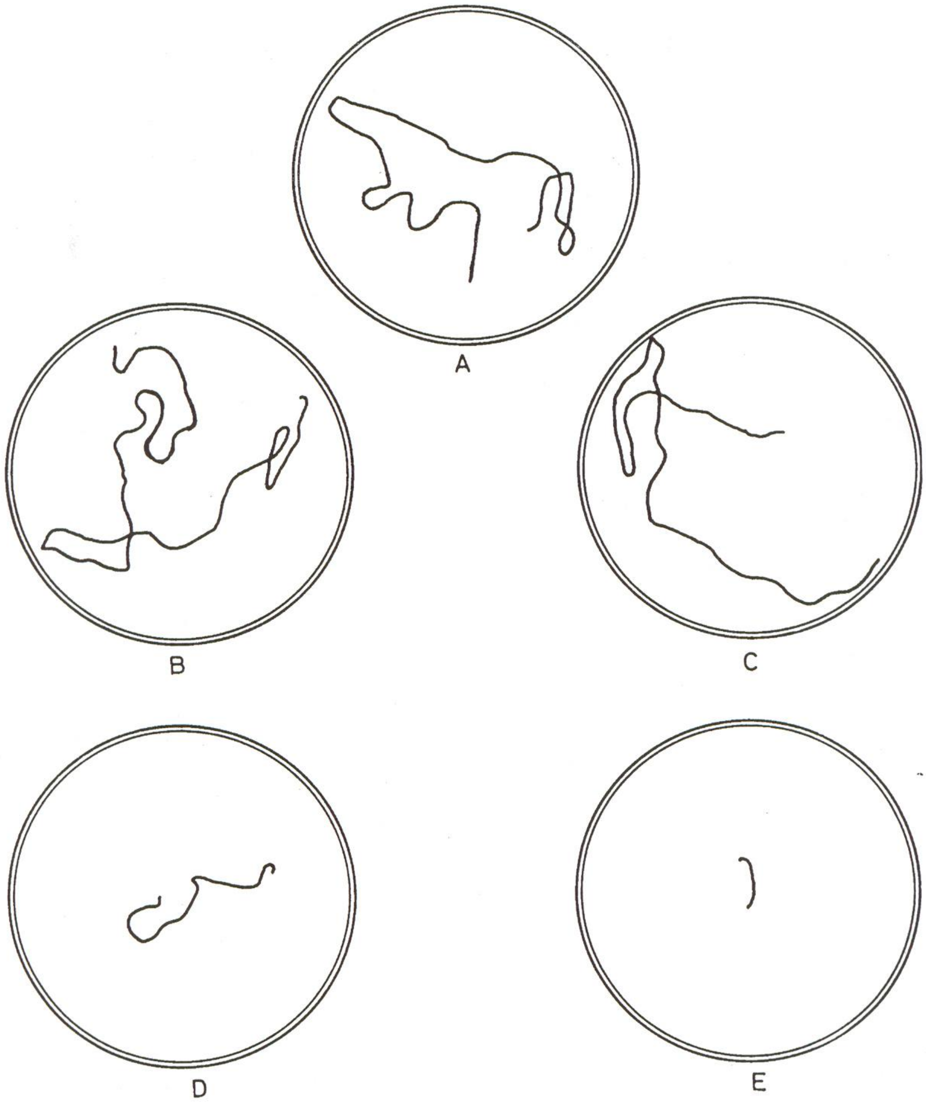


Fig. 2. Typical path tracings of *T. castaneum* at different dose of vasambu in petriplate bioassay method (Figure shown the moment of insect which was measured as cm for 2 min) A- 0%, B- 0.05%, C- 0.1%, D – 0.5%

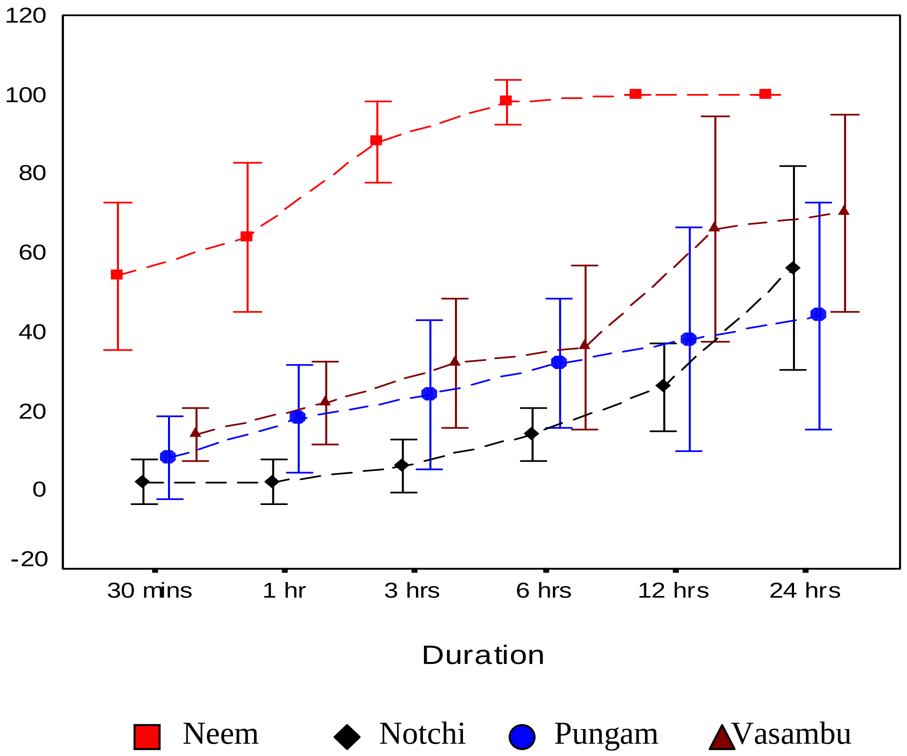


Figure 10. Repellency of natural products at 1 per cent concentration against *T. castaneum* at different time intervals

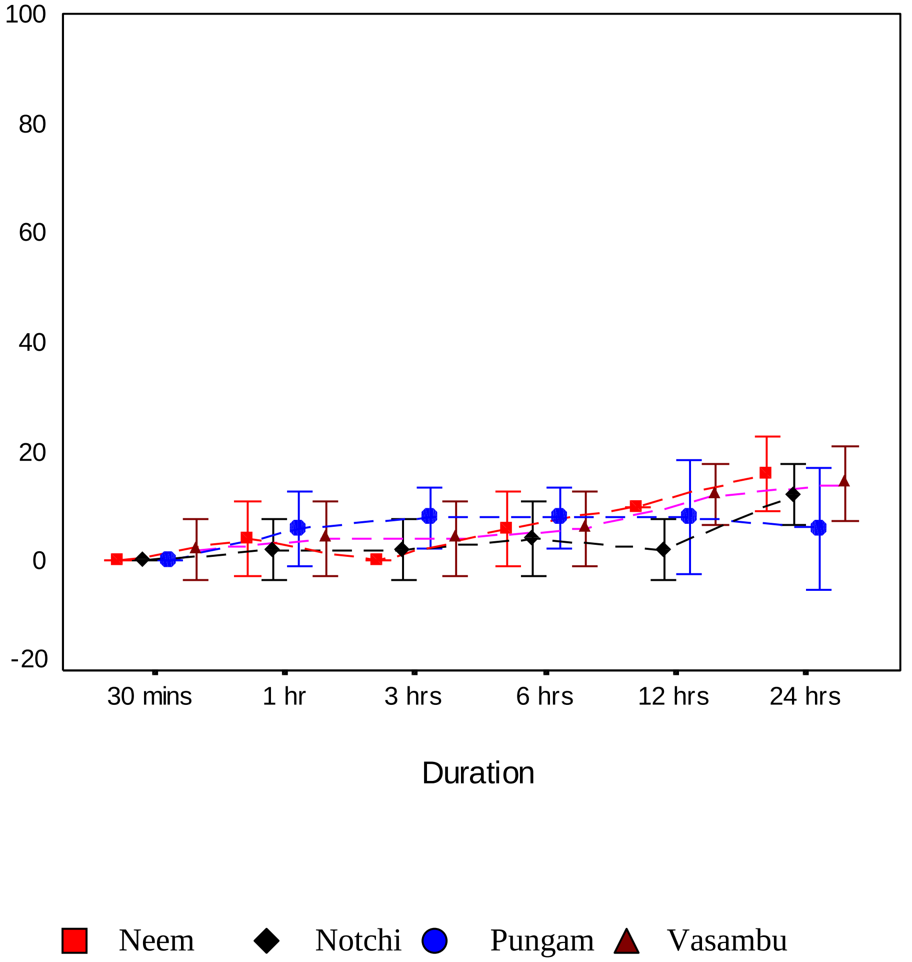


Figure 11. Repellency of natural products at 1 per cent concentration against *O. surinamensis* at different time intervals

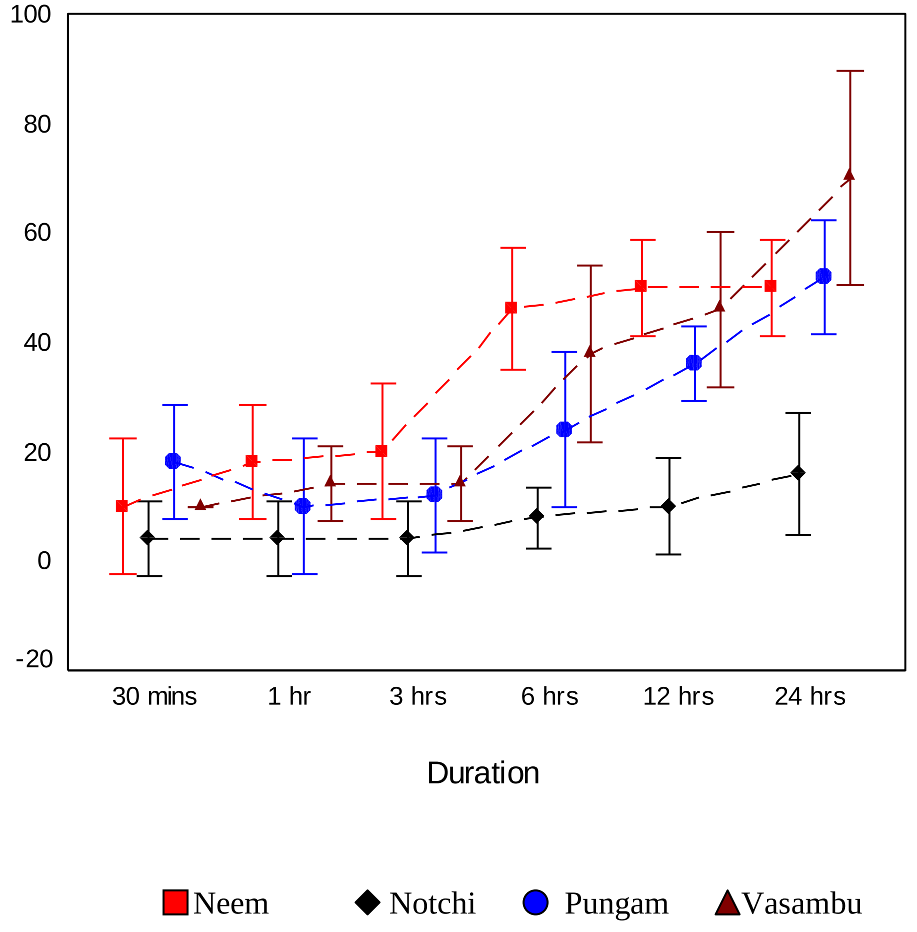


Figure 12. Repellency of natural products at 1 per cent concentration against *S. oryzae* at different time intervals

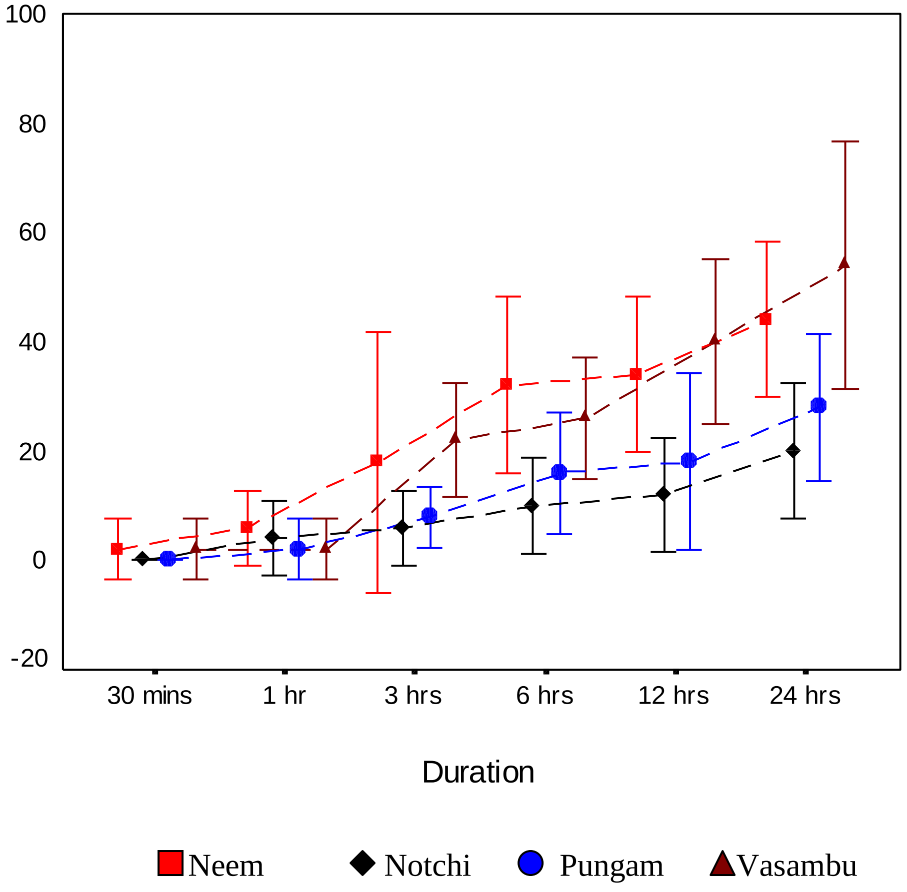


Figure 13. Repellency of natural products at 1 per cent concentration against *R. dominica* at different time intervals

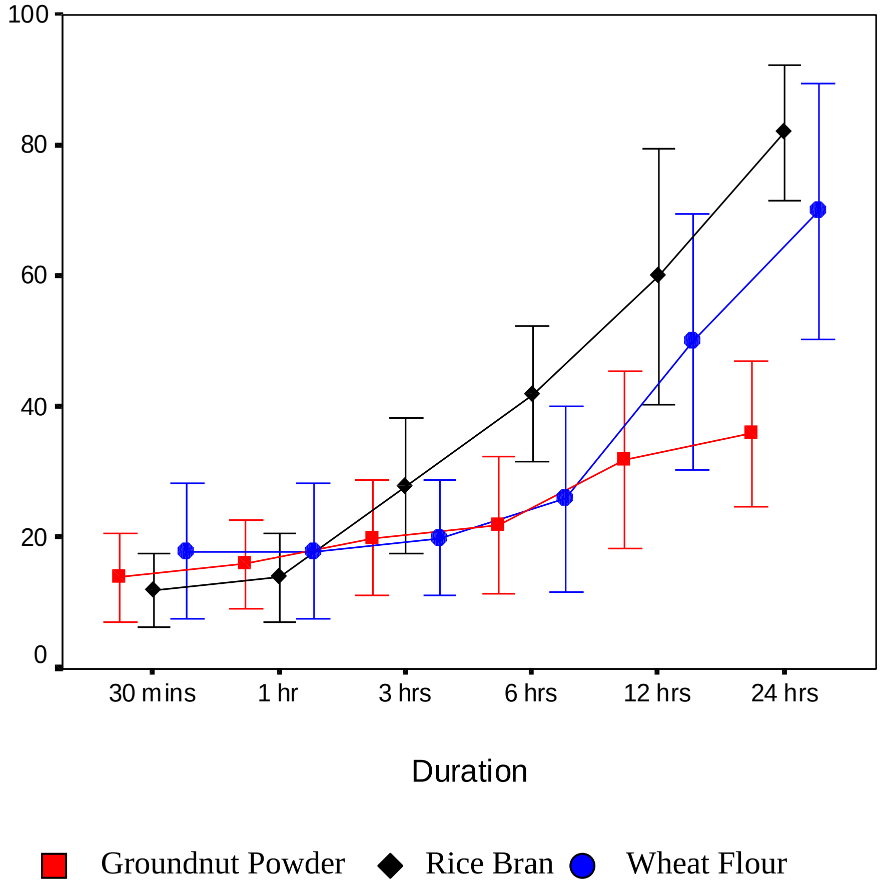


Figure 16. Efficacy of different attractants at 1 per cent concentration against *R. dominica* at various time intervals

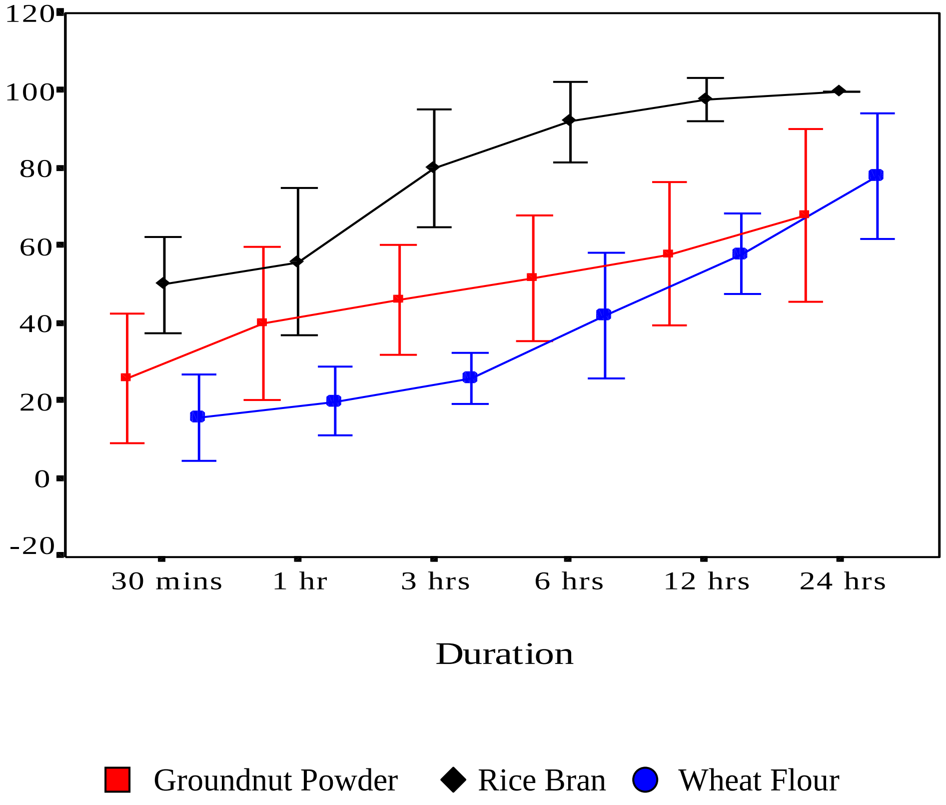


Figure 14. Efficacy of different attractants at 1 per cent concentration against *S. oryzae* at various time intervals

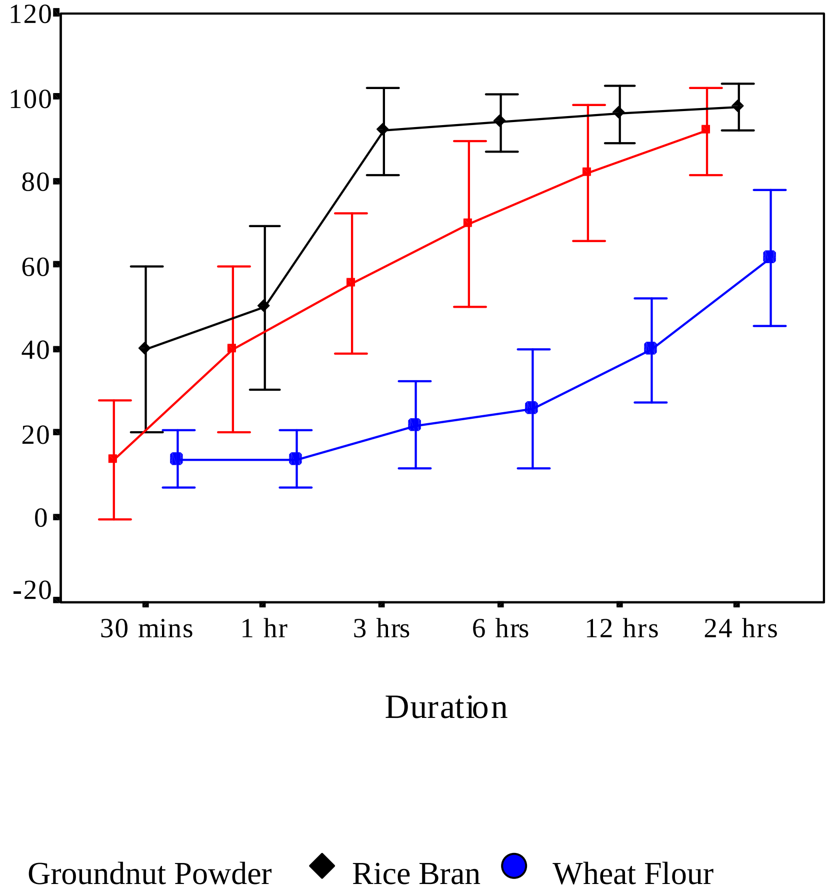


Figure 15. Efficacy of different attractants at 1 per cent concentration against *O. surinamensis* at various time intervals

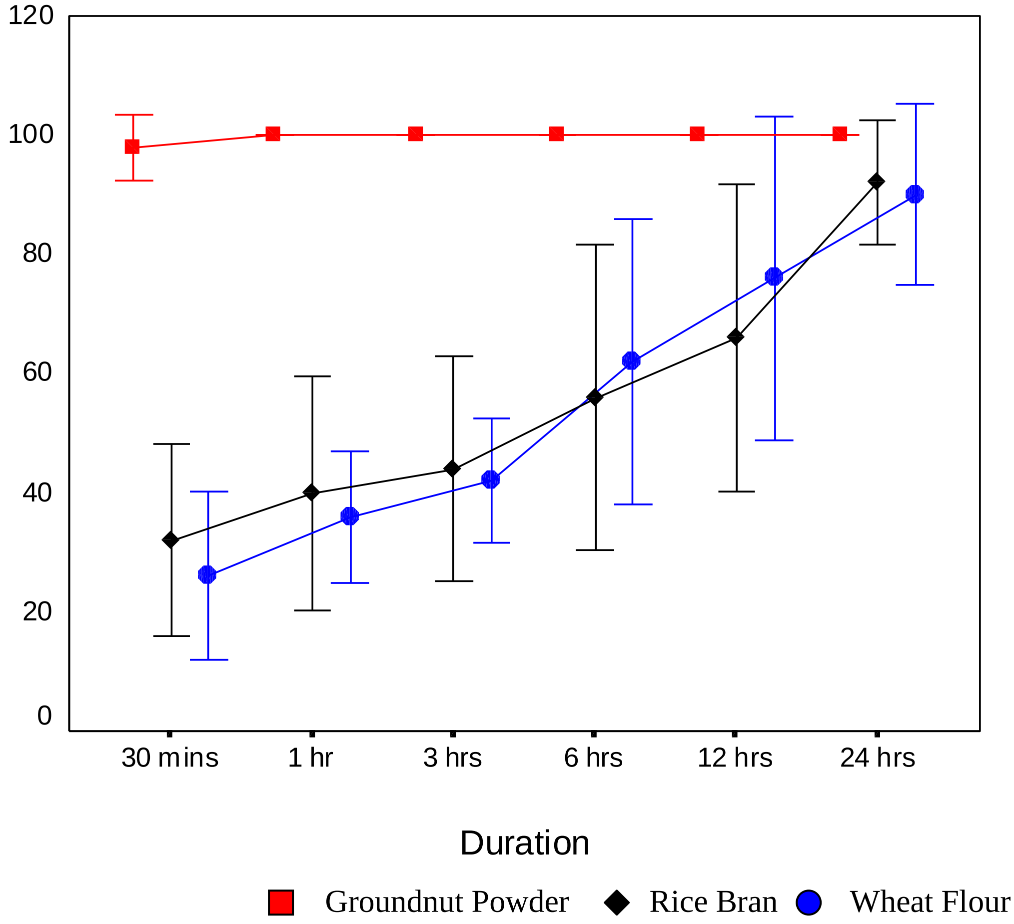


Figure 17. Efficacy of different attractants at 1 per cent concentration against *T. castaneum* at various time intervals

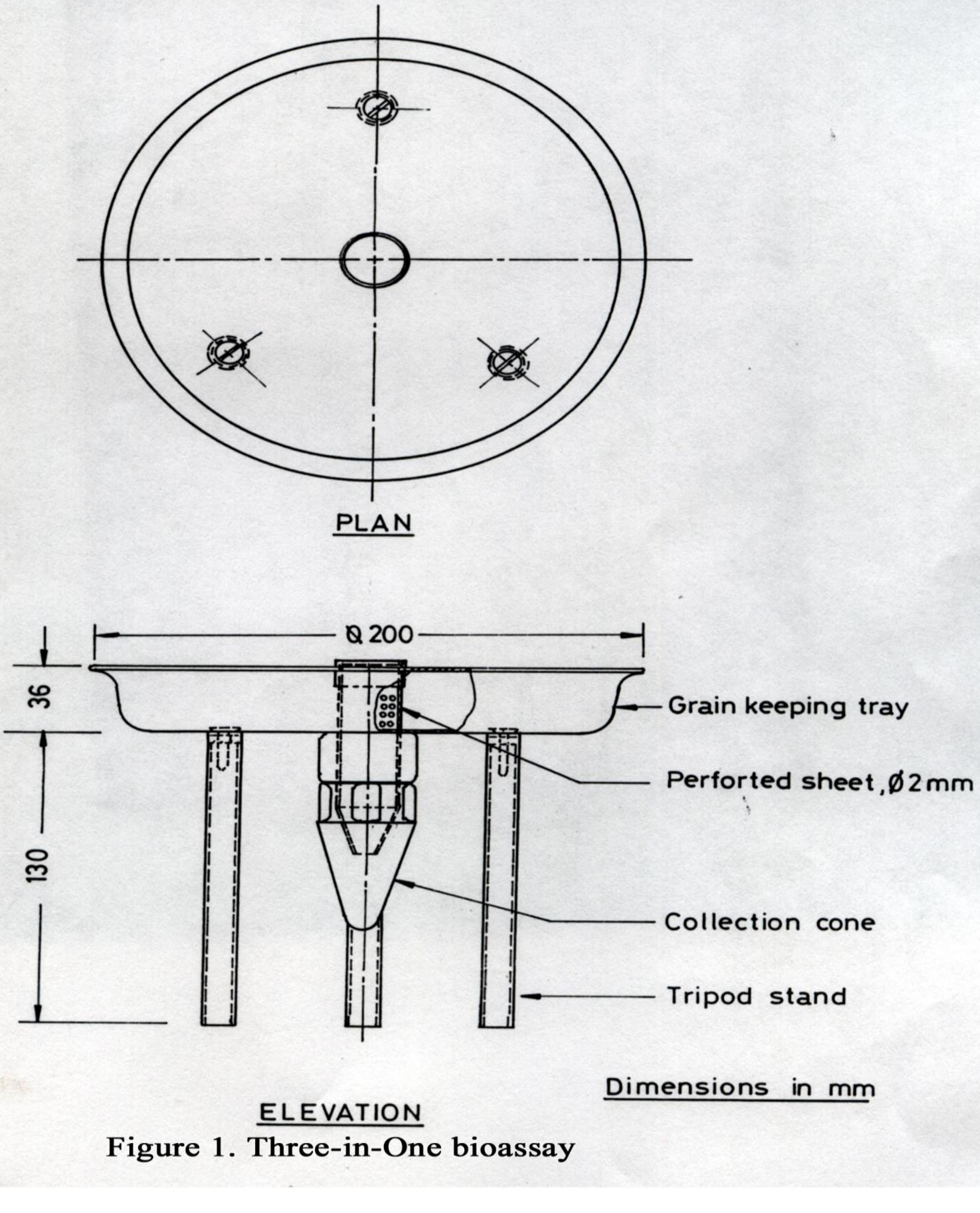
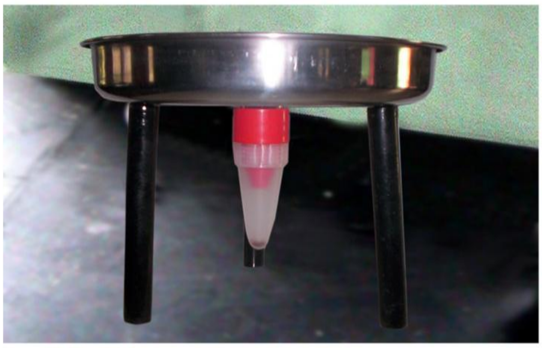


Figure 1. Three-in-One bioassay

a. Overall view



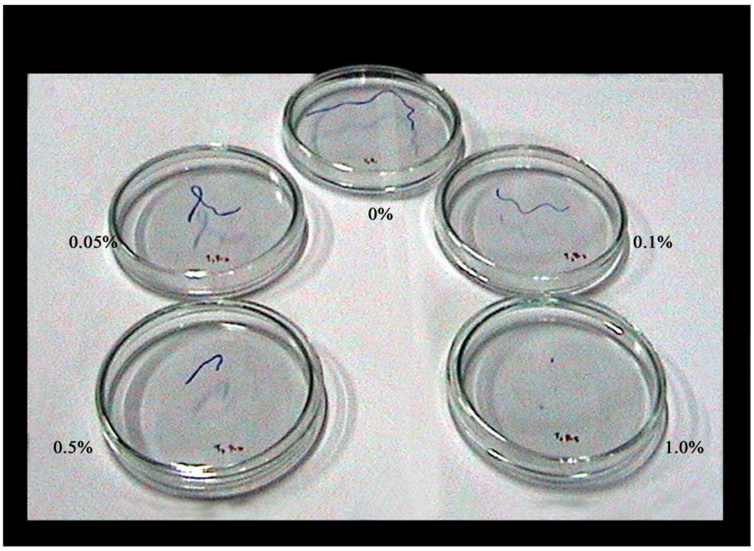
b. Outer view

c. Inner view

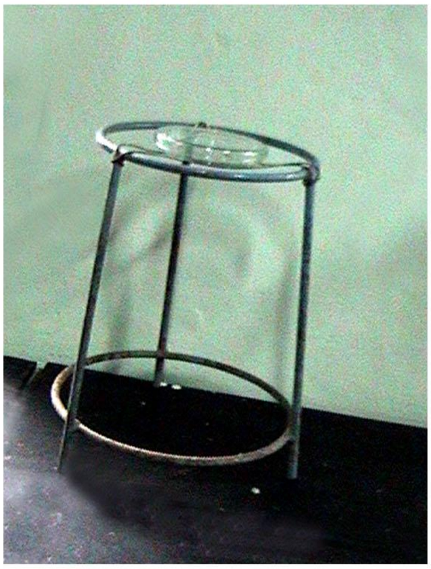
Plate 4. Three-in-One bioassay unit

Plate 9. Laboratory set up of probe trap studies

Plate 10. Laboratory set up of field carry-over insect pests studies



a. Moving path tracings above the petriplate



b. Moving path tracings below the petriplate

Plate 3. Typical insect moving path tracings of test insect at different doses of vasambu from petriplate bioassay method

Plate 1. Mass culturing of insects

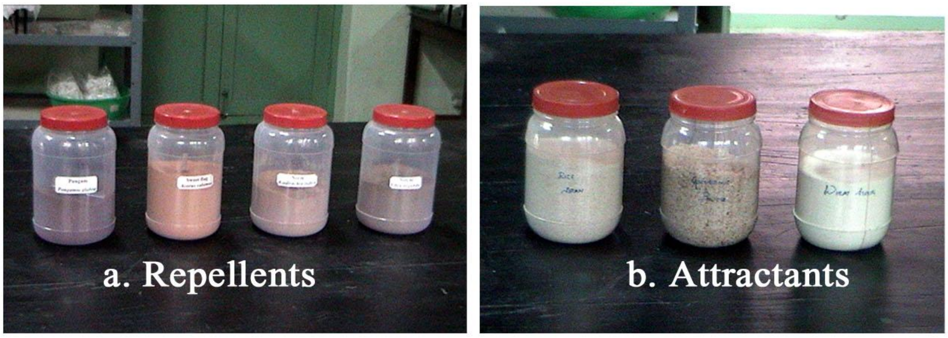


Plate 2. Test products

Plate 5. Three-in-One bioassay unit with test grains

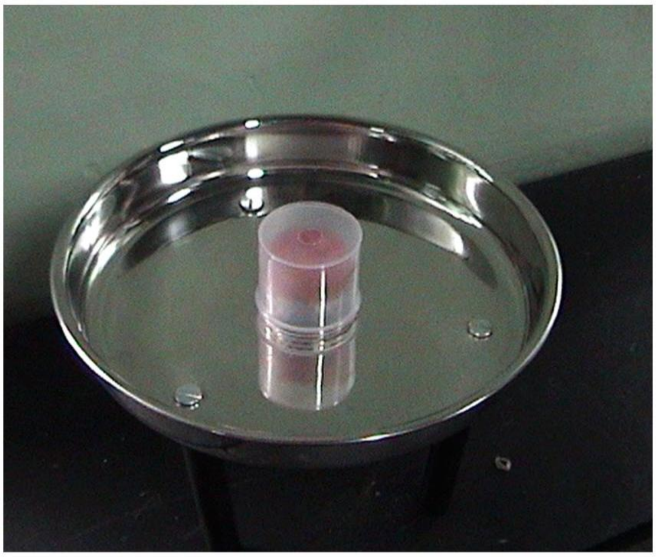


Plate 6. Perforated trapping unit with plastic container

Plate 7. Laboratory set up of repellent studies

Plate 8. Laboratory set up of attractant studies