EVALUATION OF THE ACTIVE PRINCIPLES OF THE RHIZOME EXTRACTS OF *Acorus calamus* L. FOR THE MANAGEMENT OF MELON FLY, *Bactrocera cucurbitae* (Coq.) (TEPHRITIDAE : DIPTERA)

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

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Department of Agri. Entomology

COLLEGE OF HORTICULTURE

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DECLARATION

I hereby declare that this thesis entitled "Evaluation of the active principles of the rhizome extracts of *Acorus calamus* L. for the management of melon fly, *Bactrocera cucurbitae* (Coq.) (Tephritidae : Diptera)" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Place : Vellanikkara
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CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. SHAKUNTHALA NAIR a candidate for the Degree of Master of Science in Agriculture, agree that this thesis entitled "Evaluation of the active principles of the rhizome extracts of *Acorus calamus* L. for the management of melon fly, *Bactrocera cucurbitae* (Coq.) (Tephritidae : Diptera)" may be submitted by Ms. SHAKUNTHALA NAIR in partial fulfilment of the requirement for the Degree.

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SHAKUNTHALA NAIR

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INTRODUCTION

Cucurbits are important and popular vegetables, both in urban and rural areas as they can be easily grown even in small homesteads. They occupy a prominent position among vegetables cultivated in the summer season.

The melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (= *Dacus cucurbitae)* (Tephritidae:Diptera) is the most common and highly destructive pest species attacking cucurbitaceous vegetables such as cucumber, bittergourd, pumpkin, snakegourd, etc. Both wild and cultivated plants belonging to the Family Cucurbitaceae are attacked and often the damages caused are quite substantial. The pest is widespread in all tropical countries especially in the oriental region of South and South East Asia.

The adult is a reddish brown fly with lemon yellow coloured curved markings on the thorax and fuscous shadings on the outer margins of the wings. It measures about 8 mm across stretched wings. The eggs are laid in cavities made by the ovipositor, 2-4 mm deep, pierced on the fruit surface, and hatch in 1-2 days. The larvae bore into the fruits and feed on the internal contents. They are yellowish to dirty white in colour, broad at one end and tapering towards the other. They measure 9-10 mm when fully grown. The larvae fall to the ground after 7-8 days and pupate in the soil. The puparia are barrel shaped, reddish brown or yellowish brown in colour and 4-5 mm long. Adults emerge in about 7 days.

Plate I Life cycle of *Bactrocera cucurbitae*

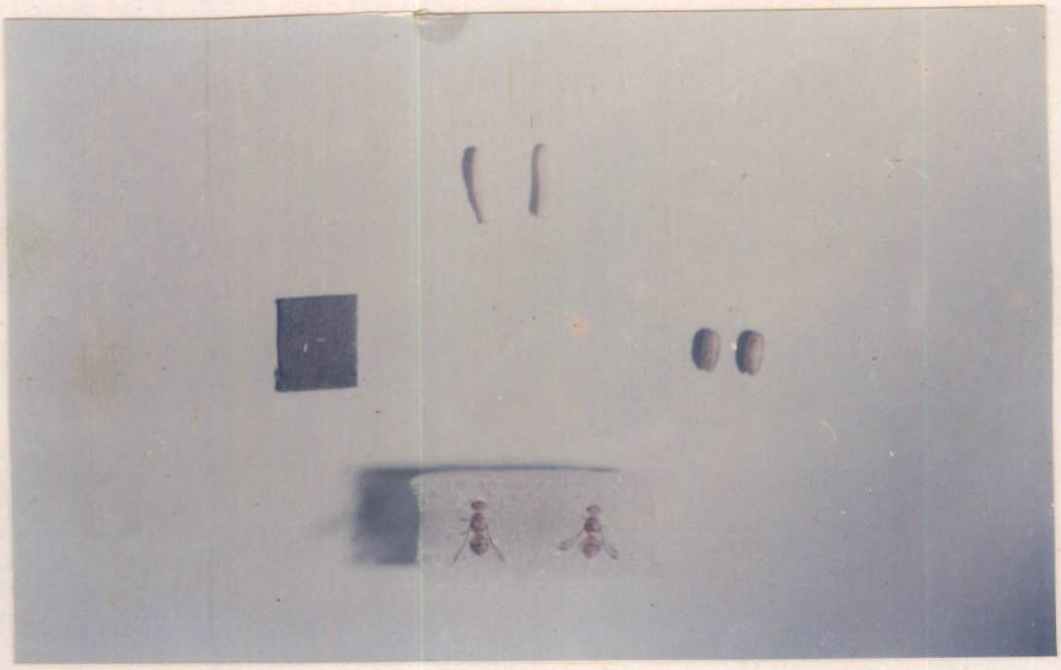


Plate II Damage caused by *Bactrocera cucurbitae*

The damage is caused by the maggots as a result of which the fruits rot and drop to the ground in large numbers. The life cycle takes about 14-20 days on an average.

Melon fly adults feed on honey or nectar and are fairly hardy insects. Some individuals may live long enough to keep up the pest population even during periods when host fruits suitable for oviposition are not available. As a result of its wide infestation, the fruits which are the marketable produce are severely damaged.

The control of fruit flies is very difficult due to several reasons. The adults visit the fruits to oviposit, but during the rest of the time they are seen to rest on the foliage of the host or other adjoining plants and are therefore difficult to locate for spot treatments. Besides they are highly active. The larvae (maggots) are seen inside the fruits and are hence quite inaccessible. The pupae are found in the soil, which again makes them less amenable to control treatments. The larvae nearing pupation have a habit of jumping and wriggling and they can move away from the surroundings of the host plant. As a result, the pupae are very much scattered in their distribution and escape from management practices. Once the pupae reach the soil, the life cycle goes on uninterrupted. The fecundity of the females is quite high and a single gravid female can lay about 100-400 eggs in its life time. Thus fruit flies have several factors favouring their survival and infestation potential.

The recommended management practices against the melon fly include raking up the soil under the plants to destroy the pupae, prompt disposal of the affected fruits, protection of the fruits with paper or polythene covers, spraying the foliage or poison baiting with 0.2% malathion or carbaryl with attractants (sugar or molasses) at fortnightly intervals (KAU, 1993). But these measures are not found to be effective in many cases, because of the various survival adaptations of the pest. Thus, there is a necessity to improve the existing methods as well as to devise more effective and safer methods to manage the fruit fly infestation.

Recently, the use of plant products in pest control is gaining importance. Due to the increasing importance of conserving the environment ecological considerations and the devastating effects of continued use of synthetic pesticides on the ecosystem, the trend has shifted towards natural products from plant and animal sources as safe pesticides. Several hundreds of species of plants have already been tested, evaluated and standardized for use against a variety of insect pests in agriculture, animal husbandry, household and in public health. These plant products are known to possess various principles with insecticidal, insectistatic, repellent, antifeedant or attractant, and sterilizing effects on various pest species.

Among these plants, *Acorus calamus* L.is attracting attention owing to its remarkable insecticidal and insectistatic effects. Rhizomes and rhizome extracts of this plant have been used against a variety of pest species, especially storage pests. *A. calamus,* known as sweet flag in English and 'Vayambu' in Malayalam

is a semi-aquatic, perennial herb, belonging to the Order Aroideae and Family Araceae. It is regarded as a native of Asia and introduced to other parts of the world because of its high medicinal value (Subramaniam, 1947).

The herb is found wild or cultivated throughout India (Mukerjea and Govind, 1959). It is also cultivated on a small scale in courtyards and other water logged places, mainly for its medicinally important rhizomes (Subramaniam, 1947).

The plant has a rich ethnobotanical history, dating back to the time of Moses in the Old Testament of the Bible. Sweet flag is thought to be indigenous to India, and spread along trade routes. Mention is made of this plant in ancient Ayurvedic literature.

A. calamus is cultivated and valued for its rhizomes which yield the important essential oil known as calamus oil. The oil has been used in Ayurvedic medicinal preparations and alcoholic beverages, as a fragrant essence in perfumes and cosmetic oils, etc. The extract and oil, apart from their medicinal value are also found to be having good insecticidal and vermicidal properties. Current research is investigating the value of sweet flag as an insecticidal, antibacterial and antifungal agent (Motley, 1994).

Renapurkar and Deshmukh (1984) have reported that extracts of *A. calamus* possess pulicidal activity against fleas, which are vectors of diseases like bubonic plague, marine typhus, etc. Fungitoxicity of cis-asarone, isolated from *A. calamus* against *Helminthosporium oryzae* was reported by Saxena *et al.* (1990).

The effect of *A. calamus* have been widely studied on various insect pests of both field and storage. However, the work on Diptera, especially *B. cucurbitae* is comparatively less.

In this background, the present study was carried out :

to evaluate the insecticidal and insectistatic principles in the rhizomes extracts of sweet flag, *Acorus calamus* L. against the melon fruit fly, *Bactrocera cucurbitae* (Coq.) by testing for the bio-efficiency in respect of the following attributes:

Repellent and feeding deterrent action

Oviposition deterrency

Ovicidal action

Topical contact toxicity

Chemosterilant effect

Review of Literature

REVIEW OF LITERATURE

Recently, the use of plant products in pest control is gaining importance from the IPM point of view. The rhizomes of *A. calamus* have been found to possess different insecticidal and insectistatic properties and have been tested and used against a wide variety of insects infesting stored grains, field crops, clothes and other household articles (Subramaniam, 1947 and Duke, 1985).

The extracts and oils of *A. calamus* have been reported to possess diverse biophysiological effects on various life stages of several crop pests. In many cases, the extracts were found to have insecticidal effect resulting in the mortality of both adults and larvae of many pest species.

2.1 Pests of stored products

Most of the work with *A. calamus* and its insecticidal effects has been done against storage pests and it has been used as an effective grain protectant since very early days (Subramaniam, 1942 and Mukerjea and Govind, 1959).

Callosobruchus spp. the pulse beetle was reported to be effectively controlled by *A. calamus* rhizomes in various forms (Chander and Ahmed, 1985; Khan and Borle, 1985; Chander and Ahmed, 1986a; Khan, 1986; El-Nahal *et al.*, 1989; Risha *et al.*, 1990; Su, 1991 and Rao *et al.*, 1993).

Other storage pests reported to be controlled by *A. calamus* are *Stitophilus oryzae* (Teotia and Pandey, 1979; Chander and Ahmed, 1985; El-Nahal *et al.*, 1989; Risha *et al.*, 1990; Schmidt and Risha, 1990; Schmidt *et al.*, 1991; Su, 1991; Paneru *et al.*, 1993 and Tiwari, 1993); *Sitophilus granarius* (El-Nahal *et al.*, 1989; Risha *et al.*, 1990; Schmidt and Risha, 1990 and Schmidt *et al.*, 1991); *Rhizopertha dominica* (Jilani and Saxena, 1990 and Tiwari, 1994); *Tribolium castaneum* (Prakash and Rao, 1986; Jilani *et al.*, 1988 and Joseph *et al.*, 1994); *Tribolium confusum* (Prakash and Rao, 1986 and Smet *et al.*, 1986); *Lasioderma serricorne* (Su, 1991); *Oryzaephilus surinamensis* (Prakash and Rao, 1986); *Prostephanus truncatus* (Pierce and Schmidt, 1993); *Corcyra cephalonica* (Chander and Ahmed, 1986b; Chauhan *et al.* 1989 and Ghatak and Bhusan, 1995a); *Ephestia kuehniella* (Smet *et al.*, 1986) and *Trogoderma granarium* (Chander and Ahmed, 1985).

2.2 Pests of field crops

Grainge *et al.*(1985) in their database have listed several pests which were affected by *A. calamus* including *Dacus cucurbitae, Dacus dorsalis* and *Ceratitis capitata.*

The toxic effects of *A. calamus* extracts were observed on many field crop pests like *Aphis* spp. (Pandey *et al.*, 1983; Bandara *et al.*, 1987; Bandara *et al.*, 1990 and Patil *et al.*, 1993); *Epilachna* spp. (Tewari and Moorthy, 1985 and Chandel *et al.*, 1988); *Dysdercus* spp. (Rajendran and Gopalan, 1979; Rameshbabu *et al.*, 1991 and Patil *et al.*, 1993); *Pyrilla perpusilla* (Pandey *et al.*, 1984); *Heteropsylla cubana*

(Sharma *et al.*, 1992); *Athalia proxima* (Pandey *et al.*, 1979 and Banerji *et al.*, 1982); *Bagrada cruciferarum* (Verma and Pandey, 1981); *Spodoptera litura* (Rajendran and Gopalan, 1979; Koul, 1987; Sharma *et al.*, 1990 and Prasad *et al.*, 1993); *Pericallia ricini* (Rajendran and Gopalan, 1979); *Plutella xylostella* and *Myzus persicae* (Rajavel and Veeraraghavathatham, 1989); *Spilosoma rhodophila* (Verma *et al.*, 1988); *Peridroma saucia* (Koul and Isman, 1990 and Koul, *et al.*, 1990) and *Atteva fabriciella* (Ahmed *et al.*, 1991).

2.3 Biophysiological effects

Calamus oil has been reported to possess neuropharmacological (Bhattacharya, 1968 and Dhalla and Bhattacharya, 1968), spasmolytic (Jerzy *et al.*, 1966) and antibacterial effects (Kar and Jain, 1971).

Mukerjea and Govind (1959) have stated that the root possesses principles with stimulant, toxic and antispasmodic properties.

Calamus oil was not found to be mutagenic, DNA-damaging or carcinogenic (Ramos - Ocampo and Hsia, 1988).

2.3.1 Antifeedant/repellent effect

The antifeedant and repellent effects of *Acorus calamus* have been tested against several pests.

Prakash and Rao (1986) have evaluated the antifeedant properties of *A. calamus* against rice storage pests *Tribolium castaneum* and *Oryzaephilus surinamensis*. They observed reduction in larval weight, increase in larval mortality and increase in pupation failures. Jilani and Saxena (1990) found that *Rhyzopertha dominica* adults made significantly fewer and smaller feeding punctures in the filter paper discs treated with plant oils like turmeric oil, sweet flag oil and neem oil. Among field crop pests, antifeedant effects of calamus have been tested against *Spodoptera litura* (Koul, 1987 and Sharma *et al.*, 1990); *Peridroma saucia* (Koul and Isman, 1990 and Koul *et al.*, 1990) and *Athalia proxima* (Banerji *et al.*, 1982).

Jilani and Saxena (1990) tested the repellent effects of several plant oils and found that sweet flag oil and turmeric oil were significantly more repellent to *R. dominica* than the other treatments for the first 2 weeks.

Jilani *et al.* (1988) reported that repellency of *T. castaneum* adults to sweet flag and other oils increased with increasing concentration of the oils.

2.3.2 Growth inhibitory effect

Several workers have found that the rhizomes of *A. calamus* possess growth inhibitory effects.

Koul and Isman (1990) conducted nutritional experiments with larvae of *Peridromia saucia* and confirmed that the oil of *A. calamus* induces both antifeedant

and growth inhibitory effects, the latter being possibly independent of feeding behaviour. Koul *et al.* (1990) identified the active constituents of *A. calamus* oil which inhibit growth and feeding in *P. saucia.* They found that cis-asarone significantly inhibited growth and feeding, while the trans- isomer had an antifeedant effect only.

Koul (1987) reported that calamus oil was effective in inducing a significant reduction in feeding and inhibition of growth in *Spodoptera litura.*

Jilani *et al.* (1988) working with *Tribolium castaneum* found that adults fed on wheat flour treated with calamus oil at 200 ppm produced fewer and under weight larvae, pupae and adults compared with untreated controls. Joseph *et al.*. (1994) reported that post-embryonic development and adult emergence of *T. castaneum* were adversely affected by *A. calamus* root extracts. There was a marked decline in the reproductive potential in terms of female fecundity and egg hatchability.

2.3.3 Oviposition deterrency/ ovicidal action

Chander and Ahmed (1986a) assayed oils from five medicinal plants for their ovicidal, repellent and other properties against *Callosobruchus chinensis* on green gram. They found that by treatment with *A. calamus* oils at 0.25 and 0.5%, oviposition on treated seeds was significantly reduced, mainly because of high insect mortality and repellency. Egg hatching was also significantly lower at the tested concentrations. Microscopic examinations of the unhatched eggs revealed that majority of eggs were killed at the very early stages of development.

Schmidt and Risha (1990)tested the effects of vapour from *A. calamus* oil to storage pests and found that the eggs and adults of *Callosobruchus chinensis* and *Sitophilus granarius* were the most susceptible. The period of exposure appeared to be the main factor affecting efficiency of vapours.

Mukerjea and Govind (1959) reported that the extract of *A. calamus* was ovicidal to the eggs of *Bombyx mori*. Higher concentrations of the extract were able to stop the development of the embryo almost immediately. Ovicidal activity of crude extracts of *A. calamus* was reported on *Corcyra cephalonica* (Ghatak and Bhusan, 1995a) and *Spilosoma obliqua* (Ghatak and Bhusan, 1995b).

2.3.4 Sterilant effect

The possibility that reduction in numbers of offspring of insects treated with *A. calamus* is due to the effect of the extracts on the reproductive system, has been considered by previous workers.

Smet *et al.* (1986)studied the effect of β-asarone vapours on mortality and reproduction of *Ephestia kuehniella* and *Tribolium confusum*. They found that treatment of adults over a period greatly reduced the numbers of first generation offspring.

Joseph *et al.* (1994) found a marked decline in the reproductive potential in terms of female fecundity and egg hatchability when extracts of *A. calamus* were applied topically or administered through diet to *T. castaneum*.

Calamus oil is reported to be having sterilizing effects on ants (Schmidt and Borchers, 1981).

Studies concerning non-toxic antigonadial agents causing sterility among insects are surprisingly few.

A. calamus has been found to be a sterilizing agent to male (Mathur and Saxena, 1975 and Saxena et al., 1977b), as well as female insects (Saxena and Rohdendorf, 1974; Saxena *et al.*, 1976; Koul *et al.*, 1977 and Tikku *et al.*, 1978).

Saxena *et al.* (1976) found that the vapours from oil of *A. calamus* reduced fecundity and caused regression in the terminal follicle of the vitellarium in treated females of *Tribolium castaneum, Sitophilus oryzae* L., *Callosobruchus chinensis, Trogoderma granarium* and *Anthrenus flavipes* Leconte (as *A. vorax* Waterhouse).

Saxena *et al.* (1977a) reported the identification of the component of *A. calamus* oil that inhibits interstitial cell activity. They also established the importance of substitute groups and a side chain by testing various allyl benzene analogues.

Koul *et al.*, (1977) and Tikku *et al.* (1978) have also reported follicular regression in females of *T. granarium.*

Tikku *et al.* (1978) have studied the effects of *A. calamus* oil on the histocytological picture of *T. granarium*. Their studies revealed disturbance in the differentiation of follicular epithelium and resorption of oocytes from the terminal end towards the germarium. This disturbance has been attributed to the imbalanced hormonal interplay. The effect of *A. calamus* vapours is immediate, compared to chemosterilants, which show inhibition in ovarian development after 48-72 hours.

Koul *et al.* (1977) studied the form and extent of regression in the developing ovaries of *T. granarium* treated with *A. calamus* oil vapours. They suggested that duration of exposure is an important factor in the treatment with calamus oil.

2.5 Forms of use

2.5.1 Solvent extracts

Extracts of *A. calamus* have been made using different solvents, by Soxhlet extraction as well as by cold extraction.

Rajavel and Veeraraghavathatham (1989) tested the efficacy of aqueous extracts of *A. calamus* rhizomes on cabbage pests like *Plutella xylostella* and *Myzus persicae*. They found that all plant extracts were more effective in controlling the pests than monocrotophos.

Several organic solvents have also been used to prepare *A. calamus* extracts and the solvents differ in their efficacy on different pests.

Alcohol extracts of *A. calamus* have been proved to be effective against *Spilosoma rhodophila* (Verma *et al.*, 1988), *Atteva fabriciella* (Ahmed *et al.*, 1991) and *Aphis gossypii* and *Dactynotus carthami* (Patil *et al.*, 1993).

Prasad *et al.* (1993) reported the efficacy of methanolic fractions of *A. calamus* on *Spodoptera litura.* The extracts also increased the efficacy of preparations of NPV against *S. litura.*

The dichloromethane extracts of *A. calamus* rhizomes were found to be highly toxic to *Aphis* spp. and the mortality caused by them was comparable with that of dimethoate 40 EC (Bandara *et al.*, 1987 and Bandara *et al.*, 1990).

The efficacy of petroleum ether extracts have been reported on *Corcyra cephalonica* (Chauhan *et al.*, 1989), *Sitophilus oryzae* (Teotia and Pandey, 1979), *Athalia lugens proxima* (Banerji *et al.*, 1982) and *Henosepilachna vigintioctopunctata* (Tewari and Moorthy, 1985).

Verma and Pandey (1981) found that acetone extracts of *A. calamus* resulted in 100% mortality of *Bagrada cruciferarum.* The efficacy of acetone extracts of *A. calamus* has also been proved on *Dysdercus cingulatus, S. litura* and *Pericallia ricini* (Rajendran and Gopalan, 1979).

Pandey *et al.* (1979) have reported the efficacy of other extracts of *A. calamus* on larvae of *Athalia lugens proxima*.

2.5.2 Rhizome powder

The use of *A. calamus* rhizome powder has been mainly for protection of stored grains. The efficacy of *A. calamus* powder has been tested on *Callosobruchus chinensis* (Chander and Ahmed, 1985; Khan and Borle, 1985; Khan, 1986 and Rao *et al.*, 1993); *Sitophilus oryzae* (Chander and Ahmed, 1985; Paneru *et al.* 1993 and Tiwari, 1993); *Corcyra cephalonica* (Chander and Ahmed, 1986b) and *Rhizopertha dominica* (Tiwari, 1994).

In field pests, *A. calamus* powder was used to protect apple trees against *Dyscerus fletchari* by treating the collar zone with the powder (Verma and Joshi, 1988).

Subramaniam (1947) tested the effectiveness of *A. calamus* powder against houseflies and arrived at promising results.

2.5.3 Oil

The essential oil of *A. calamus* has been tried effectively to protect stored products from insect infestation.

The effects of this oil have been reported on *Callosobruchus chinensis* (Chander and Ahmed, 1986a and Su, 1991); *Rhizopertha dominica* (Jilani and Saxena, 1990), *Tribolium castaneum* (Jilani *et al.*, 1988), and *Prostephanus truncatus* (Pierce and Schmidt, 1993).

Su (1991) evaluated the essential oil of *A. calamus* for its contact toxicity to adults of *C. chinensis, S. oryzae, Lasioderma serricorne* and *Tribolium confusum* and found that it was highly toxic to the pests, giving 100% protection from infestation.

Among field pests calamus oil has been found to exert antifeedant and growth inhibitory effects on caterpillars like *Spodoptera litura* (Koul, 1987 and Sharma *et al.*, 1990) and *Peridroma saucia* (Koul and Isman, 1990 and Koul *et al.*, 1990). Sharma *et al.* (1992) studied the toxic effects of *A. calamus* oil on *Heteropsylla cubana* and found that it caused high mortality at 0.05%.

2.5.4 Vapours

The vapours of *A. calamus* oil were found to protect stored grains against insect infestation (El-Nahal *et al.*, 1989; Risha *et al.*, 1990 and Schmidt and Risha, 1990).

Schmidt *et al.* (1991) reported reduction of progeny of some stored product Coleopteran pests by vapours of *A. calamus* oil.

2.6 Components/principles

Thirty four compounds have been identified in the oil and extracts of calamus, among which, the glucosidic bitter principle, acorin and the phenolic ether fraction, β asarone showed good biological activity (Duke, 1985).

The essential oil of *A. calamus* rhizomes from the foot hills of the Himalayas was obtained in a yield of 4.5% (w/w). GC-MS examination of the oil revealed the presence of eight known compounds, β asarone being major (92.68%) and four unidentified sesquiterpene alcohols. MS fragmentation data was recorded as being the criterion of their identity (Nigam *et al.*, 1990).

There is a lot of discrepancy and variability in the total yield of essential oil of *A. calamus* and its principles. The difference between Indian and other varieties of calamus oil is not due to the presence of any new constituents but due to the predominance of asarone in the Indian oil. The Indian oil contains 82% of asarone while usual commercial varieties have only 7%.

The percentage composition of the oil is approximately as follows:

α-pinene and camphene 0.2%, eugenol 0.3%, eugenol-methyl-ether 1.0%, asarone 82.0%, calamene 4.0%; calamenenol 5.0%, calameone 1.0% (Kelkar and Rao, 1933).

2.7 Effects of plant products on *Bactrocera* spp. (*Dacus* spp.)

Areekul *et al.* (1987) have tested the extracts of 165 plants for their toxicity against the Oriental fruit fly *Dacus dorsalis*. It was found that some of them were moderately to highly toxic, causing 61-85% mortality within 24 hours. These plants were *Alpinia officinarum, Zingiber officinale, Annona squamosa, Artemisia pallens, Euphorbia tirucalli, Croton tiglicum, Diospyros philippensis, Nicotiana tabacum, Pedilanthes tithymaloides, Piper nigrum* and *Tithonia diversifolia.*

The repellency of 130 plant extracts to *Dacus dorsalis* was assessed in the laboratory on the basis of rate of oviposition by 15 day old females. The extracts which showed high repellency were obtained from *Azadirachta indica* var. *siamensis* (neem); *Bixa orellana, Citrus hystrix, Cucumis melo* (melon), *Cymbopogon citratus, Hedychium coccineum* var. *angustifolium, Heliotropium indicum, Homalomena, Ocimum gratissimum, Ricinus communis, Ternstoemia japonica* [*T. gymnanthera*] and two unidentified species (Areekul *et al.*, 1988).

Extracts of the leaves of *Ocimum sanctum* were found to be a potent attractant for luring and trapping *D. ciliatus, D. zonatus* [*Bactrocera zonatus*], *D. dorsalis* [*B. dorsalis*] and *D. cucurbitae* [*B. cucurbitae*] (Roomi *et al.*, 1993).

The efficacy of extracts from the flower buds and leaves of *O. sanctum* in trapping *Dacus* spp. was also reported by Tan (1982).

The alcoholic extract of neem seed oil completely deterred oviposition by *D. cucurbitae* on bittergourds at 5% and was highly effective at 2.5%. The extract was also effective in inhibiting oviposition by the pest on guava although at 20% (Singh and Srivastava, 1985).

Hawaiian *Zanthoxylum* spp. (Rutaceae) was found to possess volatile insecticidal compounds. Marr and Tang (1992)bioassayed the extracts using eggs of *D. dorsalis* [*B. dorsalis*]. Several of the species prevented hatching.

Studies on the effect on oral feeding of petroleum ether extracts and alcohol extracts of *Pisum sativum* and *Melia azedarach* in the larval food medium on *D. cucurbitae* revealed that both the extracts affected egg viability and fecundity when males were treated; whereas treatment of females reduced fecundity (Bodhade and Borle, 1984).

Material and Methods

MATERIALS AND METHODS

The present investigation on the evaluation of bioactivity of the active principles in the rhizome extracts of sweet flag, *Acorus calamus* L. on the melon fruit fly, *Bactrocera cucurbitae* (Coquillett), was carried out at the College of Horticulture, Kerala Agricultural University, Vellanikkara during 1993-94.

3.1 Orientation responses

Orientation responses of the adult flies, were tested by assessing the repellency, feeding deterrency and oviposition deterrency of the flies to treated substrates.

3.1.1 Maintenance of laboratory culture of *B. cucurbitae*

Melon flies, *B. cucurbitae* (Coquillett) reared in the laboratory under controlled conditions were used for the tests.

Infested fruits of bitter gourd and snake gourd were collected from the field and brought to the laboratory and kept in large plastic basins with a layer of sand at the bottom. The basins were then closed securely with double layer of muslin cloth to prevent escape of the maggots which start crawling out of the fruits and jumping as the pupation stage approaches. The basins were examined regularly and the pupae were collected and transferred to net cages for adult emergence.

The adult flies were maintained in the net cages and were fed with sugar crystals or honey. Water was also provided in the cages. Pieces of the host fruit (pumpkin) were provided in the cages for the flies to oviposit and there were replaced daily. Pumpkin was selected for maintenance of the culture, since it retained its crispness and form longer.

The fruit pieces on which the flies had oviposited were collected daily and placed in rearing basins on a layer of moist sand to prevent desiccation.

The emerging maggots developed on the fruit pieces and pupated in the soil. The pupae were transferred to the net cages and the cycle was repeated. The age (date of emergence) of each batch of culture population was recorded.

3.1.2 Extraction of active principles from the rhizomes of *A. calamus* L.

Dried rhizomes of *A. calamus* procured from the local market were used to prepare the extracts. The rhizomes were macerated coarsely in a mortar and then finely powdered in a wearing blender.

3.1.2.1 Preparation of stock solution - Aqueous Extract

The stock solution was prepared by cold steeping method (Teotia and Pandey, 1979). Ten grams of finely powdered rhizomes were transferred to a 100 ml

volumetric flask and the volume was made up with distilled water. The flask was then shaken vigorously for 15 minutes and kept in a cool dark place for 24 hours. During this period, the flask was shaken periodically. At the end of the period, the contents were filtered and the extract was immediately used for bio efficacy studies. The stock solution was prepared afresh for each subsequent experiment in order to avoid fermentation.

3.1.2.2 Solvent extract

Five organic solvents, namely benzene, dichloromethane, solvent ether, petroleum ether and methanol were used to prepare the extracts. The procedure described in 3.1.2.1 was followed while using the solvents also.

3.1.2.3 Dilutions

The 10% stock solutions prepared in water were further diluted by adding the required quantities of distilled water. In the case of the solvent extract, the required quantity of stock solution was taken. The solvent was evaporated and the extract was redissolved in the required quantity of acetone. The dilutions of aqueous and solvent extracts are given in Table 1.

Table 1 Concentrations of *A. calamus* extracts used to test orientation responses

Sl.No.	Aqueous extract (%)	Solvent extract (%)
1	0.1	0.1
2	0.5	0.25
3	1.0	0.5
4	5.0	1.0
5	10.0	5.0

The treatments were as follows:

Design : CRD

Replications : 5

1. Repellency test

i) Aqueous extract-0.1% : RW_1

ii) " 0.5% : RW_2

iii) " 1 % : RW_3

iv) " 5 % : RW_4

v) " 10 % : RW_5

vi) Control : RW_6

vii) Solvent extract - 0.1% : RS_1

viii) " 0.25% : RS_2

ix) " 0.5% : RS_3

x) " 1 % : RS_4

xi) " 5 % : RS_5

xii) Control : RS_6

2. Feeding deterrency test

i)	Aqueous extract-0.1%	:	FW_1
ii)	" 0.5%	:	FW_2
iii)	" 1 %	:	FW_3
iv)	" 5 %	:	FW_4
v)	" 10 %	:	FW_5
vi)	Control	:	FW_6
vii)	Solvent extract - 0.1%	:	FS_1
viii)	" 0.25%	:	RS_2
ix)	" 0.5%	:	FS_3
x)	" 1 %	:	FS_4
xi)	" 5 %	:	FS_5
xii)	Control	:	FS_6

3. Oviposition deterrency test

A. Number of ovipunctures

i)	Aqueous extract-0.1%	:	OWA_1
ii)	" 0.5%	:	OWA_2
iii)	" 1 %	:	OWA_3
iv)	" 5 %	:	OWA_4
v)	" 10 %	:	OWA_5
vi)	Control	:	OWA_6

vii) Solvent extract - 0.1% : OSA_1

viii) " 0.25% : OSA_2

ix) " 0.5% : OSA_3

x) " 1 % : OSA_4

xi) Control : OSA_5

B. Fecundity realization

i) Aqueous extract-0.1% : OWB_1

ii) " 0.5% : OWB_2

iii) " 1 % : OWB_3

iv) " 5 % : OWB_4

v) " 10 % : OWB_5

vi) Control : OWB_6

vii) Solvent extract - 0.1% : OSB_1

viii) " 0.25% : OSB_2

ix) " 0.5% : OSB_3

x) " 1 % : OSB_4

xi) Control : OSB_5

Evaluation of orientation responses of *B. cucurbitae* towards extracts of *A. calamus*.

The adult flies were observed during different times of the day, before and after sunset. Based on these behavioral observations on the activity and feeding process of the flies, the best time to conduct repellency and feeding deterrency tests was found to be in the forenoon. Oviposition, however, was seen to occur throughout the day time.

The orientation responses of the flies were evaluated by determining the number of adult flies responding to the treated and untreated substrates, the substrates varying according to the response tested (Padmanabhan, 1989).

3.1.3 Repellency test

Repellency of the extracts was tested by determining the number of flies alighting on treated and untreated bitter gourd leaf substrates.

Two types of tests were conducted, namely, no-choice and multiple choice tests. The tests were conducted using aqueous and solvent extracts.

Test material

The treated surfaces, used for repellency test were bitter gourd leaves of uniform size (approximately the size shown in Fig. 1). The fourth leaf from the apex was chosen as the standard. The leaves were dipped separately in 1 ml each of the

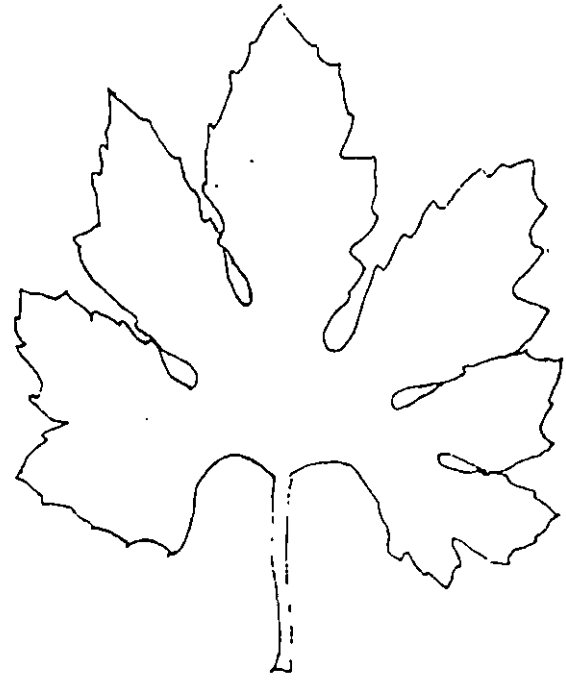
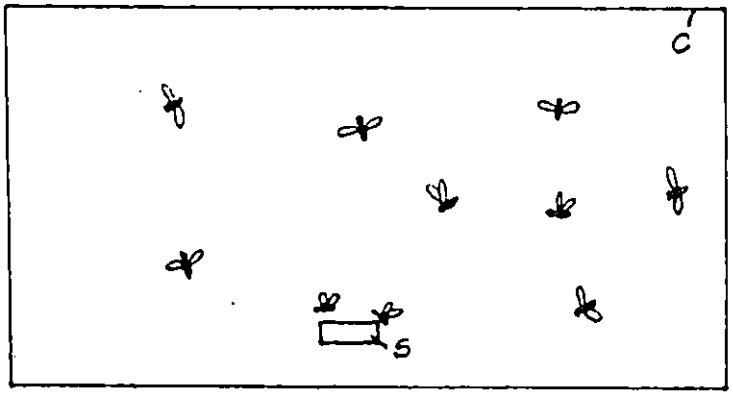
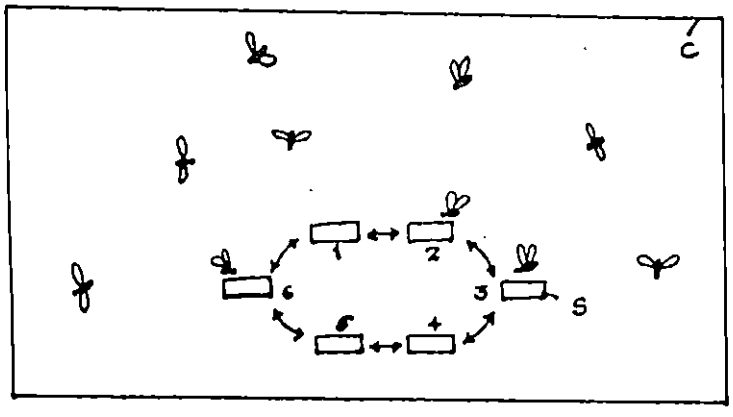


Fig.1 Bittergourd leaf of standard size used for repellency test

Fig.2 Scheme of experiments for testing orientation responses



a. No-choice test



b. Multiple-choice test

S - Substrate (Leaf/sugar/fruit piece)

C - Cage/chamber

extract taken in a Petri dish and dried under an electric fan. These leaves were then introduced into the cage with the flies. Ten flies of known age were used for the tests, and different sets of flies for each concentration.

3.1.3.1 No choice test

The test flies were exposed to a single dose of the extract at a time on the treated surface. The treated surface was introduced into a cage containing ten flies and the number of flies alighting on it in ten minutes was recorded. This constituted one count. Then the leaf was withdrawn and again introduced after an interval of ten minutes. This process was repeated to get five counts, which constituted one replication. The experiment was replicated five times and thereafter, the concentration of extract used was changed and the same procedure repeated. An untreated leaf, dipped in water/solvent and dried was used as control, to compare the difference in the degree of alightment by the flies.

3.1.3.2 Multiple choice test

The test flies were exposed to different doses of the *A. calamus* extract or different extracts at a time. For this, the test substrates, namely bitter gourd leaves of uniform size, treated with the different extracts along with an untreated control leaf, were introduced together into the cage containing ten flies. The number of flies alighting on each surface in ten minutes was recorded and this constituted one count. Then all substrates were withdrawn and again introduced together after an interval of

ten minutes. This process was repeated five times to get five sets of count data, which constituted one replication.

The positions of the test substrates were randomly changed after each count. The experiment was replicated five times.

Comparison of extracts in different solvents

The extracts in five different solvents, namely methanol, benzene, dichloromethane, petroleum ether and solvent ether, were compared by testing their repellency in a multiple choice test.

The solvent extracts were compared at 0.1% concentration.

Another multiple choice test comparing solvent extracts with aqueous extract was conducted. Here the concentration of all extracts was 1%.
As control, untreated substrates (leaves) were used.

3.1.4 Feeding deterrency test

Feeding deterrency was tested by determining the percentage alighting and sustained feeding of flies on the treated and untreated food material, namely, sugar crystals.

Two types of tests were conducted, namely, no choice and multiple choice tests. The tests were conducted using aqueous and solvent extracts.

Test material

For the feeding deterrency test, the test material used was the food given to the flies, namely, sugar crystals. One gram of sugar was taken in a small dish and mixed with one ml of the extract. This was introduced into the cage containing the flies. Ten flies of known age were used for the test.

3.1.4.1 No choice test

The food, namely, sugar crystals, mixed with a single dose of the test chemical extract was introduced into the cage containing ten flies. The number of flies alighting to feed in ten minutes was recorded. This constituted one count. Then the food was withdrawn and again introduced after an interval of ten minutes. This process was repeated to get five counts, which constituted one replication.

Then the concentration of the extract mixed with the food was changed and the same procedure repeated.

As control, pure sugar alone was introduced into the cage and the observation repeated.

3.1.4.2 Multiple choice test

Sugar crystals, mixed with different doses of the extract along with pure sugar as control were introduced together into the cage containing the test flies. The number of flies alighting to feed on each dish in ten minutes was recorded. This constituted

one count. Then all the dishes were withdrawn and again introduced together after an interval of ten minutes, after changing the positions of the dishes randomly. This process was repeated five times to get five counts which constituted one replication.

The experiment was replicated five times.

3.1.5 Oviposition deterrency test

Oviposition deterrency was tested by determining the number of ovipunctures and fecundity realization.

For this, two types of tests were conducted namely, no choice and multiple choice. The tests were conducted using aqueous and solvent extracts.

Test material

The test substrate used in the oviposition deterrency test was a piece of pumpkin fruit of size 2.5 x 2.5 x 2.5 cm^3. One ml of the extract was taken in a Petri dish and the fruit piece was dipped in it, such that the extract coated all sides of the piece uniformly. Then the piece was dried under an electric fan and provided to the flies for oviposition. As control, untreated fruit pieces were used. Pumpkin was selected for this test, as flies reared on pumpkin were used for the test and also because pumpkin retained its crispness and form longer.

Pairs of mating flies were secluded using a glass chimney and they were used for the tests. They were removed to a cage where they were provided with sugar to feed and pumpkin fruit pieces to oviposit on the next day.

On the following day, the fruit pieces kept for oviposition were taken out and the number of ovipunctures on each piece was recorded. This constituted the first part of the observation.

For the second part, the fruit pieces with the eggs were removed to small plastic dishes and kept overnight for hatching. The number of larvae emerging from each piece was recorded after 24 hours.

3.1.5.1 No choice test

A single fruit piece treated with one dose of the extract was provided to a pair of flies.

This experiment was replicated five times, along with untreated fruit pieces as control.

3.1.5.2 Multiple choice test

Several fruit pieces, each treated with different doses of the extracts, were provided simultaneously to a pair of flies. Observations were recorded from five sets of replications along with untreated fruit pieces as control.

3.2 Toxicity tests

Toxicity of *A. calamus* extracts to different life stages of *B. cucurbitae* was assessed by treating the various stages with the extracts and determining the mortality at required intervals of time. The concentration of extracts tested against various stages are presented in Table 2.

3.2.1 Ovicidal action

To test ovicidal action, first, the eggs were carefully extracted from the fruit pieces provided with the mated female for oviposition. For this, a cut was made along an ovipuncture site using a clean, sharp blade. This exposed the eggs in the ovipuncture. The eggs were then carefully taken out from the fruit flesh using a fine brush and collected in a glass dish containing one ml of the extract of required dose.

The eggs were kept dipped in the extract for one minute and then removed using a fine brush on to a slice of pumpkin fruit in a Petri dish.

The eggs thus treated with different concentrations of the extracts were collected in separate Petri dishes and kept to hatch overnight.

Six concentrations were tried. Each concentration was taken as a treatment and three replications were maintained for each treatment. Ten eggs were used for each replication. As untreated control, the eggs dipped in water/solvent were used along with the treatments.

Table 2 Concentrations of *A. calamus* extracts tested for toxicity against various stages of *B. cucurbitae*

Stage	Aqueous extract (%)	Solvent extract (%)
Eggs	0.1	0.08
	0.5	0.06
	1.0	0.04
	5.0	0.02
	10.0	0.01
Larvae	0.1	0.1
	0.5	0.5
	1.0	1.0
	5.0	5.0
	10.0	10.0
Pupae	0.1	0.1
	0.5	0.5
	1.0	1.0
	5.0	5.0
	10.0	10.0
Adults	0.1	0.1
	0.5	0.8
	1.0	0.06
	5.0	0.04
	10.0	0.02

On the following day, the number of eggs hatched was determined by counting the number of larvae emerged. The eggs which did not hatch after 24 hours were taken as dead.

From these mortality values, percentage mortality was calculated and the data were subjected to Probit Analysis according to Finney (1971).

The experiments were carried out with aqueous and solvent extracts.

3.2.3 Topical contact toxicity to larvae

Larvae of same age and uniform size were used for conducting the tests. The tests were conducted using aqueous and solvent extracts.

Ten larvae were selected and using a camel hair brush they were gently picked and placed in a glass dish containing one ml of the extract. The larvae were kept in the extract for one minute, after which they were released into Petri dishes with a piece of pumpkin fruit as feed.

Observations on mortality were recorded after 24 hours.

3.2.4 Topical contact toxicity to pupae

Uniform sized pupae of same age were used and the tests were conducted using aqueous and solvent extracts.

Ten pupae were collected and placed in a glass dish containing one ml of the extract. The pupae were kept in the extract for one minute, after which they were air dried and kept for adult emergence.

The pupae from which adults did not emerge were taken as dead.

3.2.4 Topical contact toxicity to adult flies

To determine the topical contact toxicity, bio-assay was carried out on the test flies using various doses of the extracts of *A. calamus* rhizomes.

Flies of same age reared under laboratory conditions were used for conducting the bio-assay.

Two methods of application were used, namely, topical application and residue film technique.

The tests were conducted using aqueous and solvent extracts. Concentrations of extracts giving mortality range of 20-80% were selected.

3.2.2.1 Topical application

Ten test flies of uniform age and size were collected in a glass tube and kept in the freezer compartment of a refrigerator for ten minutes, for temporary

immobilization of the flies. Application was done in this condition as otherwise the flies are very active.

One ml of the extract was taken in an atomizer and sprayed directly on the flies, after which they were released into glass chimneys provided with sugar as food. The narrow end of the chimneys was covered with netting and the wider end rested in Petri dishes.

The control set of flies were exposed to water spray alone.

Observations on mortality were taken at regular intervals. Percentage mortality was then calculated and the data were subjected to Probit Analysis according to Finney (1971).

3.2.2.2 Residue film technique

In this method, Petri dishes of 10 cm diameter were used.

One ml of the extract was poured into the upper and lower dish of each Petri dish. The dishes were then swirled to give a uniform coating of the extract over the inside of the dish. The dishes were then kept under an electric fan to dry.

Ten flies of uniform age and size were released in each Petri dish set, along with sugar as food. As control, Petri dishes treated with water/solvent alone and dried, were used.

Six concentrations were tried and each concentration was taken as a treatment. Each treatment had three replications.

Observations on the mortality of flies were taken at regular intervals. Then percentage mortality was calculated and the data on dosage mortality response was subjected to Probit Analysis according to Finney (1971).

3.3 Contact chemosterilant effect

To study the chemosterilant effect, the adult flies were treated with the methanol extracts of *A. calamus* rhizomes at doses of 0.1 to 0.01%.

The extracts were administered to the flies along with their food. One g of sugar mixed with 1 ml of the extract was provided to a set of ten flies (five males and five females) of uniform age. The treated food was replaced daily.

As control, ten flies (five males and five females) of uniform age were provided with pure sugar alone.

The flies were observed daily to note the occurrence of mating or any other changes in their behaviour.

After the pre-oviposition period of about 12-15 days, the flies were dissected.

In order to compare the normal and treated flies, the size of the reproductive organs - ovaries and testes - was studied.

The length and breadth of the ovaries and testes were measured using micrometry technique.

3.3.1 Micrometry

The size of the reproductive organs was measured using micrometry.

First, the calibrated value was determined. For this, the ocular and stage micrometers were brought into focus. Then the coinciding divisions of the stage and ocular micrometers were noted. Ten such sets of values were obtained. Then the calibrated value for each set of readings was calculated by the formula,

$$\text{Calibrated value} = \frac{\text{Stage reading}}{\text{Ocular reading}} \times 10$$

Then the average calibrated value was calculated.

After calibrating the ocular micrometer, the stage micrometer was removed and the specimens, arranged on a microscopic slide were measured.

The number of ocular divisions corresponding to the length and breadth of each specimen were recorded.

The length and breadth of each specimen were calculated by using the formula:

Actual measurement (μ) = Number of ocular divisions x Calibrated value

The dimensions of reproductive organs of treated and untreated flies at various stages of development were compared.

Results

RESULTS

Evaluation of the extracts from the rhizomes of *Acorus calamus* for their bioefficacy against the melon fly *Bactrocera cucurbitae* was carried out. Experiments were conducted to assess the repellency, feeding deterrency, oviposition deterrency, ovicidal action, topical contact toxicity and chemosterilant effects of the extracts. Observations recorded from these experiments on the above aspects were tabulated and the data statistically analysed. The results are presented in this chapter.

4.1 Orientation responses

The orientation responses of the flies to the extracts of *A. calamus* were evaluated by testing for repellency, feeding deterrency and oviposition deterrency.

4.1.1 Evaluation of rhizome extracts of *A. calamus* using different solvents

A comparison of extracts using different organic solvents at 0.1% was done by testing the repellency of these extracts to melon flies in a multiple choice test. The number of flies alighting on treated leaves of uniform size in 10 minutes was recorded. The data was subjected to analysis of variance and the ANOVA is presented in

Appendix I. The mean alightment of flies with different solvent extracts is presented in Table 3 and graphically depicted in Figure 3.

Table 3 Mean number of melon flies (*B. cucurbitae*) alighting on bittergourd leaves treated with extracts of *A. calamus* rhizomes in different organic solvents at 0.1% in 10 minutes

Treatment		Mean number of melon flies alighting per leaf*
Solvent ether	T_1	1.40 b
Petroleum ether	T_2	1.32 b
Benzene	T_3	1.36 b
Dichloromethane	T_4	0.52 bc
Methanol	T_5	0.36 c
Control	T_6	2.56 a
CD		0.93

Treatment means followed by common letters do not differ significantly at 5% level
*Bittergourd leaves of uniform size as shown in Fig.1

Among the extracts used, methanol extract offered the best repellency, and recorded a mean alightment of 0.36. The other extracts, solvent ether, benzene, petroleum ether and dichloromethane, were at par with respect to repellency. Dichloromethane extract was at par with the methanol extract. However all the extracts were significantly more repellent compared to the untreated control.

The organic solvent extracts were also compared with the aqueous extract of *A. calamus* rhizomes, at 1.0%, in another multiple choice repellency test. The mean alightment of flies in this test are presented in Table 4 and graphically presented in Figure 4.

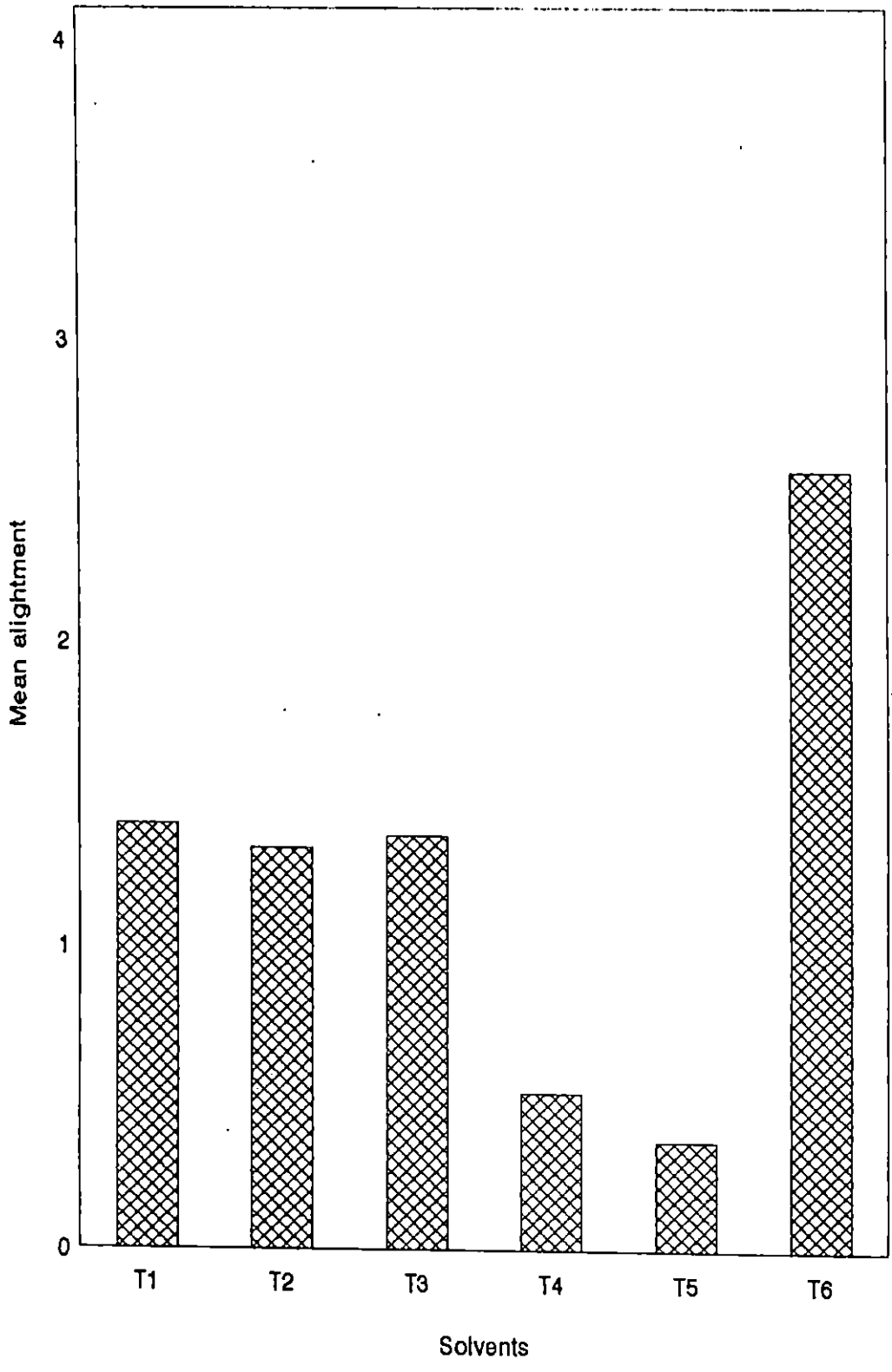


Fig.3 Comparison of repellency of *A. calamus extracts* in different organic solvents

Table 4 Mean number of melon flies (*B. cucurbitae*) alighting on bittergourd leaves treated with extracts of *A. calamus* in different organic solvents and water at 1% in 10 minutes

Treatment		Mean number of melon flies alighting per leaf*
Solvent ether	T_1	0.64 c
Petroleum ether	T_2	0.24 c
Benzene	T_3	0.20 c
Dichloromethane	T_4	0.32 c
Methanol	T_5	0.08 c
Water	T_6	1.40 b
Control	T_7	4.04 a
CD		0.67

Treatment means followed by common letters do not differ significantly at 5% level
*Bittergourd leaves of uniform size as shown in Fig.1

A perusal of the table shows that all organic solvents were significantly more repellent than the aqueous extract, which recorded a mean alightment of 1.4. All the other solvent extracts were at par in their repellency. Among them, methanol extract was seen to be most repellent, as it showed the lowest mean alightment of flies, 0.08.

All treatments were significantly different from the untreated control, where the mean alightment was 4.04.

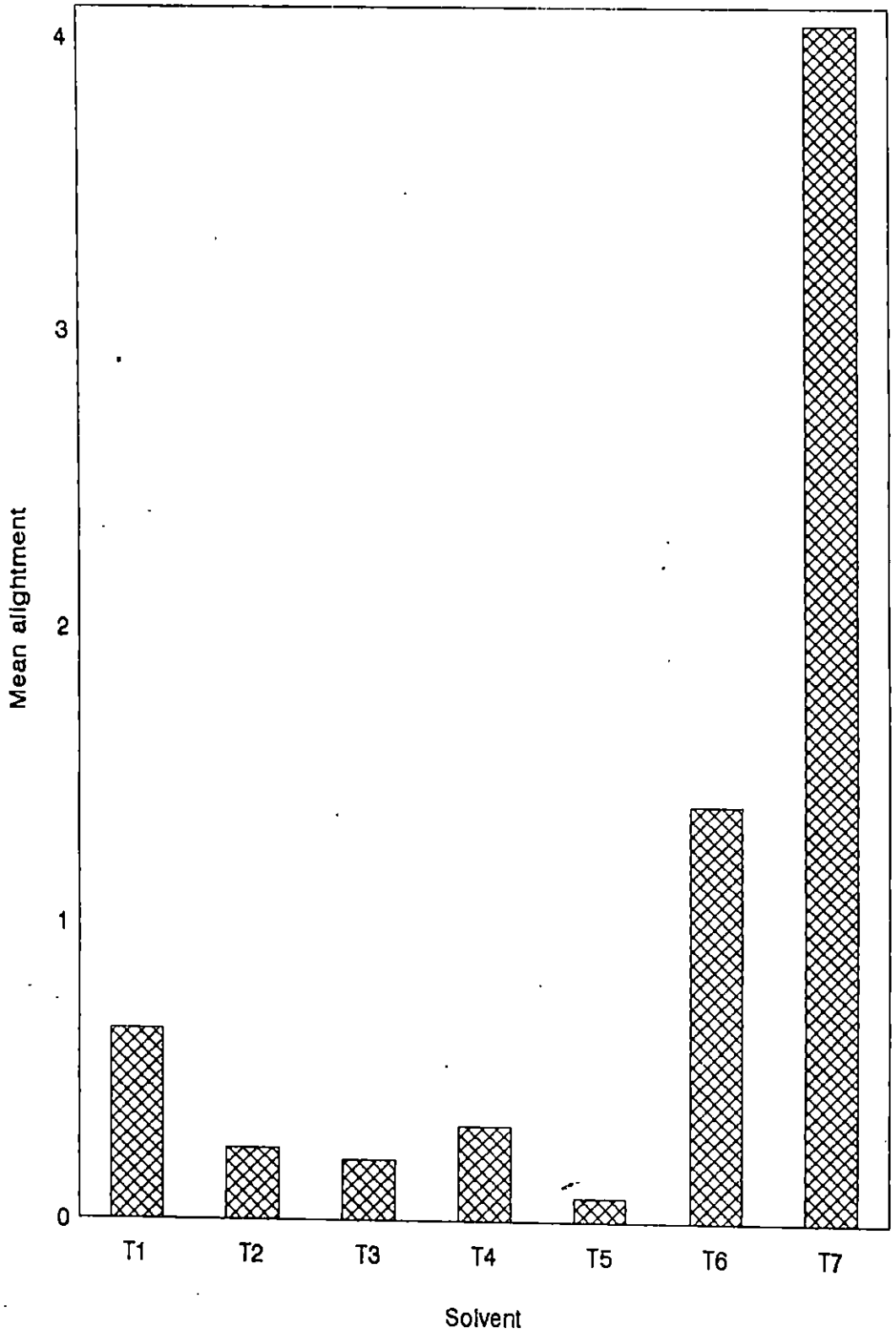


Fig.4 Comparison of repellency of *A. calamus* extracts in different organic solvents and water

4.1.2 Repellency

Repellency was assessed by two types of tests, namely, no choice and multiple choice tests and both tests were conducted using aqueous as well as solvent extracts.

The number of flies alighting on treated leaves of uniform size in 10 minutes was recorded and the data subjected to analysis. The ANOVA is presented in Appendix II. The mean alightment of flies with different extracts and concentrations is presented in Table 5 and graphically presented in Figure 5.

A perusal of the data revealed that the mean alightment values showed a decreasing trend with increase in concentration of the extracts used.

4.1.2.1 Aqueous extract

4.1.2.1a No choice test

In the no-choice test with aqueous extract, mean alightment values ranged from 8.48 to 2.08. The untreated control, RW_6 showed the highest mean alightment of 8.48.

Among the different concentrations of aqueous extracts used, RW_1 (0.1%) showed the lowest repellency with a mean alightment of 8.32 flies per leaf. RW_5 (10.0%) showed the highest repellency with a mean alightment of 2.08. The treatment RW_1 (0.1%) was on par with the untreated control, RW_6. All the other treatments were significantly different.

Table 5 Mean number of melon flies (*B. cucurbitae*) alighting on bitter gourd leaves treated with graded doses of *A. calamus* extracts

Treatment		Dose (%)	Mean number of melon flies alighting per leaf* (in 10 minutes)	
			No choice test	Multiple choice test
Aqueous extract	RW_1	0.10	8.32 a	3.44 b
"	RW_2	0.50	7.20 b	1.04 c
"	RW_3	1.00	5.52 c	0.36 cd
"	RW_4	5.00	4.08 d	0.48 cd
"	RW_5	10.00	2.08 c	0.20 d
"	RW_6	Control	8.48 a	4.44 a
CD			1.12	0.79
Methanol extract	RS_1	0.10	3.60 b	1.52 b
"	RS_2	0.25	2.28 c	0.20 c
"	RS_3	0.50	2.08 c	0.44 c
"	RS_4	1.00	1.32 d	0.16 c
"	RS_5	5.00	0.36 e	0.12 c
"	RS_6	Control	7.80 a	8.08 a
CD			0.52	0.55

Treatment means followed by common letters do not differ significantly at 5% level

*Bittergourd leaves of uniform size as shown in Fig.1

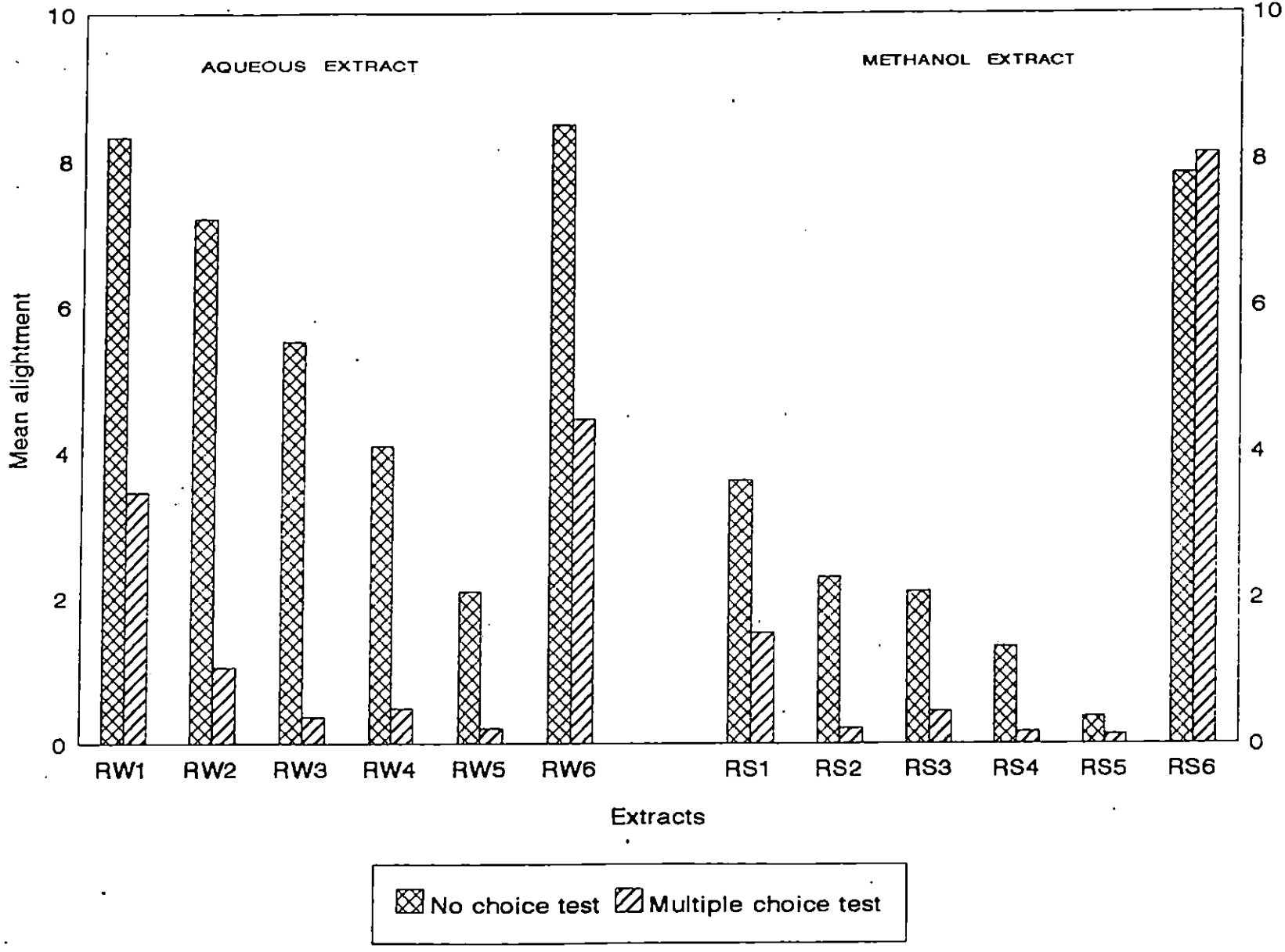


Fig.5 Repellent effect of A. calamus extracts

4.1.2.1b Multiple choice test

In the multiple choice test with aqueous extract, the highest mean alightment, 4.44 was recorded in RW_6, the untreated control. RW_1 (0.1%) showed the least repellency with a mean alightment of 3.44 flies per leaf. RW_2 (0.5%), RW_3 (1.0%) and RW_4 (5.0%) were on par with respect to their repellencies and differed significantly from RW_1 (0.1%). RW_3 (1.0%) and RW_4 (5.0%) were also on par with RW_5 (10.0%). RW_5 (10.0%) showed the highest repellency among the various concentrations, with a mean alightment of 0.2. All treatments were significantly different from the control.

4.1.2.2 Solvent extract (Methanol)

4.1.2.2a No choice test

Among the different concentrations, RS_5 (5.0% methanol extract) showed the highest repellency with mean alightment of 0.36, while the least repellent concentration was 0.1% (RS_1) with mean alightment of 3.6. The treatments RS_2 (0.25%) and RS_3 (0.5%) did not differ significantly, but all other treatments were significantly different from each other as well as from the untreated control (RS_6) where mean alightment was 7.8.

4.1.2.2b Multiple choice test

The mean alightment values ranged from 8.08 to 0.12. RS_5 (5.0%) showed the highest repellency among the extracts, giving a mean alightment of 0.12, which was significantly different from the control, RS_6, where the mean alightment was 8.08.

The control was also significantly different from all other treatments. The table shows that RS_5 (5.0%) was at par with RS_2 (0.25%), RS_3 (0.5%) and RS_4 (1.0%) in the repellency. However RS_1 (0.1%) was significantly different from all other treatments and recorded a mean alightment of 1.52 which shows that it was the least repellent.

4.1.3 Feeding deterrency

Feeding deterrent effect of the *A. calamus* (rhizome) extracts was determined by recording the number of flies alighting to feed on the food material, sugar crystals, treated with the extracts. No choice and multiple choice tests were conducted, each using aqueous and solvent extracts.

The number of flies alighting for sustained feeding on treated and untreated food material was recorded for 10 minutes. This data was analysed and the ANOVA is presented in Appendix III. The mean number of flies alighting for sustained feeding on food treated with different extracts and concentrations is presented in Table 6 and graphically depicted in Figure 6.

The alightment for sustained feeding was found to be considerably affected by treating the food with the extracts, thus showing the feeding deterrent activity of the extracts.

Table 6 Mean number of melon flies (*B. cucurbitae*) alighting to feed on sugar crystals treated with graded doses of *A. calamus* extracts .

Treatment		Dose (%)	Mean number of melon flies alighting to feed* (in 10 minutes)	
			No choice test	Multiple choice test
Aqueous extract	FW_1	0.10	8.08 b	1.84 b
"	FW_2	0.50	6.92 c	0.60 c
"	FW_3	1.00	6.32 c	0.20 cd
"	FW_4	5.00	4.12 d	0.20 cd
"	FW_5	10.00	2.04 e	0.16 d
"	FW_6	Control	8.96 a	6.08 a
CD			0.73	0.41
Methanol extract	FS_1	0.10	3.40 b	1.28 b
"	FS_2	0.25	2.28 c	0.44 c
"	FS_3	0.50	1.68 d	0.16 c
"	FS_4	1.00	0.64 e	0.08 c
"	FS_5	5.00	0.16 e	0.08 c
"	FS_6	Control	7.92 a	7.12 a
CD			0.55	0.52

Treatment means followed by common letters do not differ significantly at 5% level

*1 g of sugar treated with 1 ml of the extract

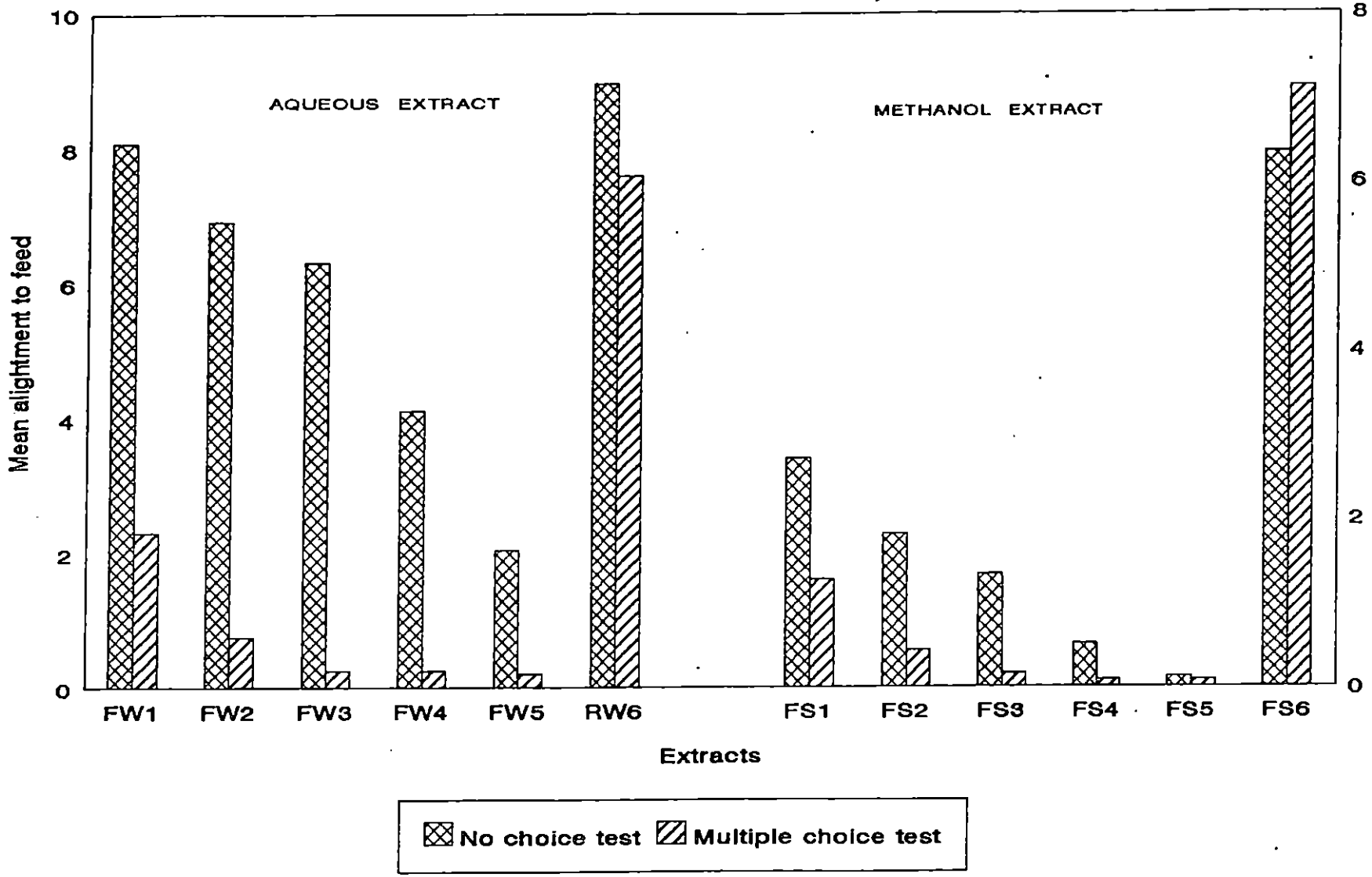


Fig.6 Feeding deterrent effect of A. calamus extracts

4.1.3.1 Aqueous extract

4.1.3.1a No choice test

The mean alightment values ranged from 8.96 to 2.04. Among the extracts, FW_1 (0.1%) gave the lowest feeding deterrency with a mean alightment of 8.08, which was significantly different from the untreated control FW_6, where the mean alightment was 8.96. FW_2 (0.5%) and FW_3 (1.0%) were at par with respect to feeding deterrency. FW_4 (5.0%) and FW_5 (10.0%) were significantly different from each other and from all other treatments also. The treatment FW_5 (10.0%) showed the highest feeding deterrency with a mean alightment value of 2.04.

4.1.3.1b Multiple choice test

FW_5 (10.0%) showed the maximum feeding deterrency, with a mean alightment of 0.16 and FW_1 (0.1%) recorded the highest mean alightment of 1.84 suggesting the lowest feeding deterrency.

FW_1 (0.1%) was significantly different from all other treatments, while the treatments FW_2 (0.5%), FW_3 (1.0%) and FW_4 (5.0%) were at par in their deterrency. FW_3 and FW_4 were also at par with FW_5 (10.0%).

However all treatments differed significantly from the untreated control FW_6 where the mean alightment recorded was 6.08.

4.1.3.2 Solvent extract (Methanol)

4.1.3.2a No choice test

The mean alightment values ranged from 0.16 to 7.92.

The lowest mean alightment value, 0.16, was recorded in FS_5 (5.0%) which showed the highest deterrency. FS_4 (1.0%) was on par with FS_5, with a mean alightment of 0.64. The other treatments, FS_1 (0.1%), FS_2 (0.25%) and FS_3 (0.5%) were significantly different from each other and also the other treatments.

The highest mean alightment among the treatments was shown by FS_1 (0.1%), which was 3.4.

All doses of the solvent (methanol) extract gave significantly lower values of mean alightment as compared to the untreated control, where the mean alightment was 7.92.

4.1.3.2b Multiple choice test

The treatments FS_5 (5.0%) and FS_4 (1.0%) showed the highest feeding deterrency, with a mean alightment of 0.08 each. These were at par with FS_2 (0.25%) and FS_3 (0.5%). FS_1 (0.1%) was significantly different from all other treatments and recorded a mean alightment of 1.28 which showed the lowest deterrency.

In the untreated control, the mean alightment was 7.12, and this was significantly higher than all the treatments.

4.1.4 Oviposition deterrency

Two sets of observations were made to assess the oviposition deterrency of *A. calamus* (rhizome) extracts to *B. cucurbitae*, namely the number of ovipunctures made in 24 hours on the fruit pieces exposed and the fecundity realization as indicated by the number of eggs hatching which is an index of the actual number of eggs laid.

No choice and multiple choice tests were conducted each with aqueous and solvent extracts. The number of ovipunctures made in 24 hours and fecundity realization were recorded and the data statistically analyzed. The ANOVA is presented in Appendix IV.

The mean number of ovipunctures and fecundity realization from substrates treated with different extracts and concentrations are presented in Tables 7 and 8 and graphically depicted in Figures 7 and 8 respectively.

4.1.4.1 Oviposition deterrency as indicated by the number of ovipunctures

4.1.4.1.1 Aqueous extract

4.1.4.1.1a No choice test

The mean number of ovipunctures ranged from 12.4 to 0.6. The treatment OWA_5 (10.0%) recorded the least number of ovipunctures, 0.6, showing that it was the most deterrent to oviposition by the flies. OWA_2 (0.5%), OWA_3 (1.0%) and OWA_3 (1.0%) and OWA_4 (5.0%) were at par with respect to oviposition deterrency, but differed significantly from the other treatments. The lowest oviposition deterrency

Table 7 Mean number of ovipunctures made by female melon flies (*B. cucurbitae*) on substrates* treated with graded doses of *A. calamus* extracts

Treatment		Dose (%)	Mean number of ovipunctures made in 24 hours	
			No choice test	Multiple choice test
Aqueous extract	OWA_1	0.10	10.2 b	3.6 b
"	OWA_2	0.50	7.8 c	2.6 bc
"	OWA_3	1.00	7.0 c	1.0 c
"	OWA_4	5.00	6.6 c	0.6 c
"	OWA_5	10.00	0.6 d	0.4 c
"	OWA_6	Control	12.4 a	9.8 a
CD			1.75	1.9
Methanol extract	OSA_1	0.10	4.8 b	2.4 b
"	OSA_2	0.25	3.2 c	2.0 b
"	OSA_3	0.50	2.6 c	1.2 bc
"	OSA_4	1.00	0.8 d	0.2 c
"	OSA_5	Control	12.6 a	11.6 a
CD			1.59	1.39

Treatment means followed by common letters do not differ significantly at 5% level

* 2.5 cm^3 piece of pumpkin treated with 1 ml of extract

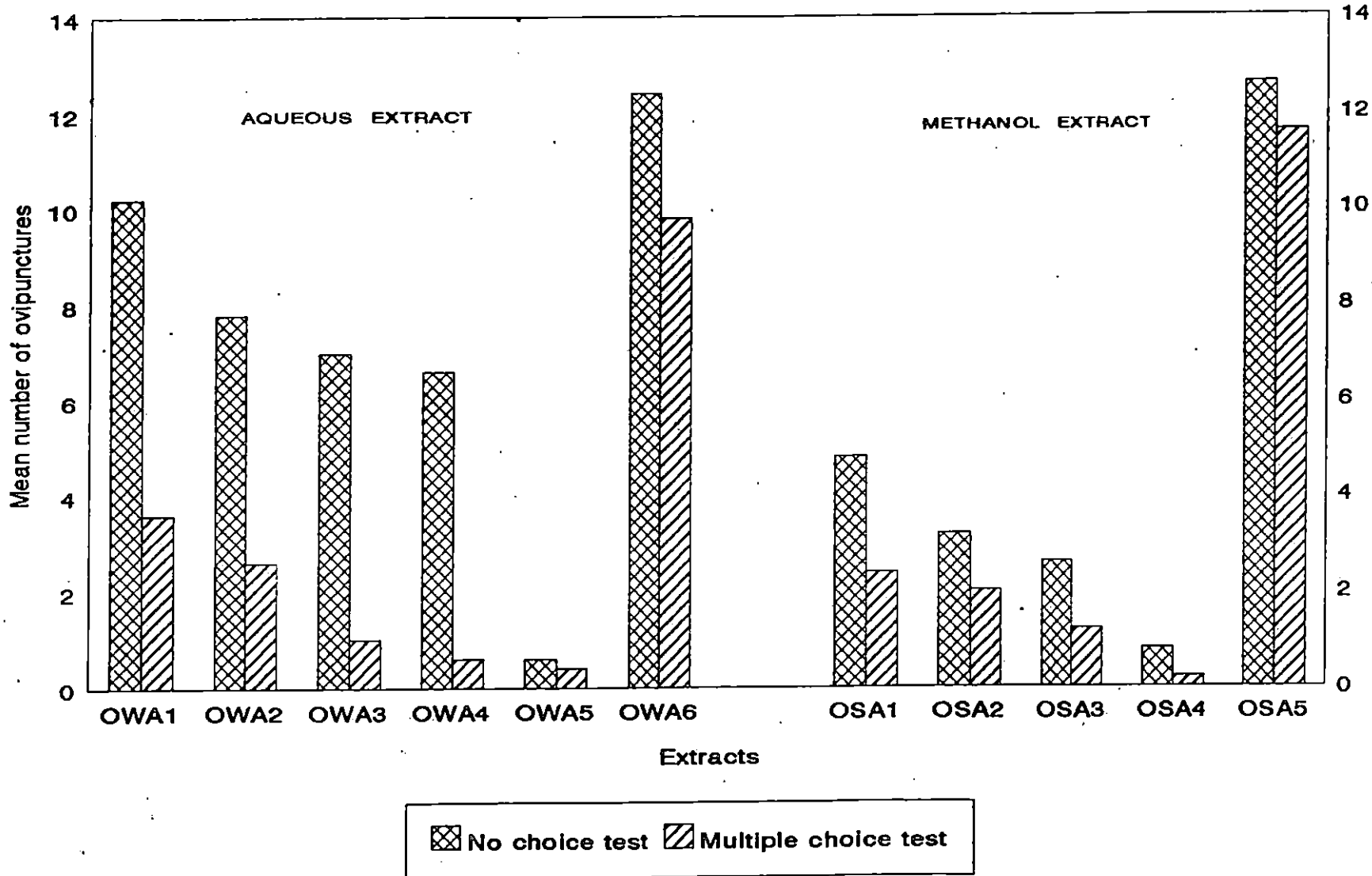


Fig.7 Oviposition deterrent effect of *A. calamus* extracts - Number of ovipunctures

Table 8 Mean fecundity realization of (*B. cucurbitae*) from substrates* treated with graded doses of *A. calamus* extracts

Treatment		Dose (%)	Mean number of larvae emerging after 24 hours	
			No choice test	Multiple choice test
Aqueous extract	OWB1	0.10	18.2 a	10.0 b
"	OWB_2	0.50	13.4 b	5.8 bc
"	OWB_3	1.00	13.0 b	4.4 c
"	OWB_4	5.00	10.4 b	2.2 c
"	OWB_5	10.00	1.2 c	1.4 c
"	OWB_6	Control	21.2 a	21.2 a
CD			3.4	4.5
Methanol extract	OSB_1	0.10	10.2 b	5.8 b
"	OSB_2	0.25	5.0 c	3.8 bc
"	OSB_3	0.50	3.2 cd	2.2 cd
"	OSB_4	1.00	1.4 d	0.4 d
"	OSB_5	Control	25.4 a	20.0 a
CD			2.42	2.74

Treatment means followed by common letters do not differ significantly at 5% level

* 2.5 cm^3 piece of pumpkin treated with 1 ml of extract

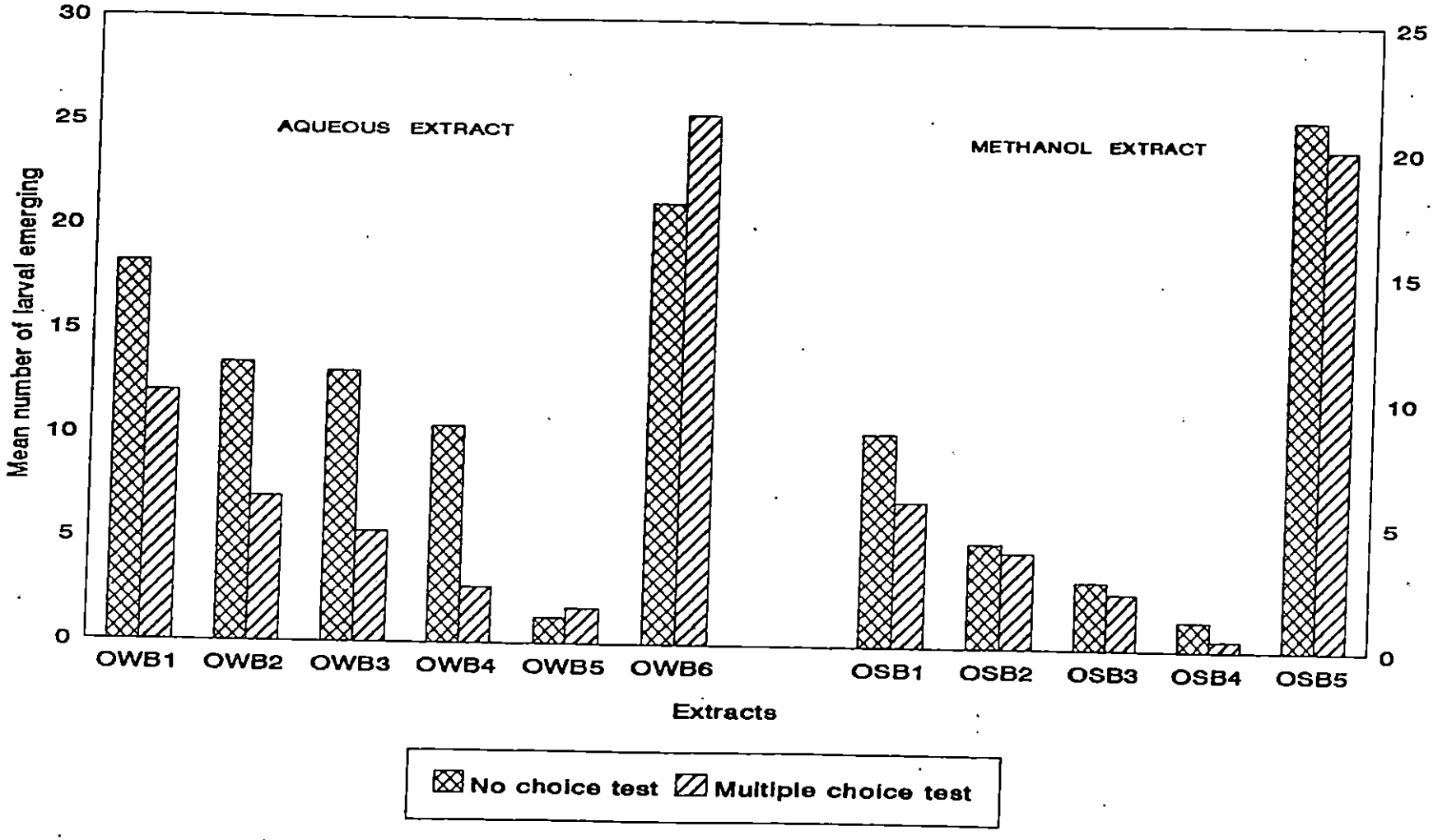


Fig.8 Oviposition deterrent effect of *A. calamus* extracts - Fecundity realization

was shown by OWA_1 (0.1%), with the mean number of ovipunctures being 10.2, which was also significantly different from all other treatments.

However all treatments differed significantly from the untreated control, OWA_6, where the mean number of ovipunctures was 12.4.

4.1.4.1.1b Multiple choice test

The treatment, OWA_1 (0.1%) showed the highest mean number of ovipunctures, 3.6, which indicated that it was the least deterrent to oviposition. This was on par with OWA_2 (0.5%). OWA_2 was also on par with OWA_3 (1.0%), OWA_4 (5.0%) and OWA_5 (10.0%) with respect to oviposition deterrency. The least number of ovipunctures, 0.4, was seen in OWA_5 (10.0%), indicating that it was had the most deterring action among the treatments. The untreated control, OWA_6, differed significantly from all the treatments.

4.1.4.1.2 Solvent extract

4.1.4.1.2a No choice test

4.8 was the highest mean number of ovipunctures, which was seen in OSA_1 (0.1% extract) and it was significantly different from all other treatments. OSA_2 (0.25%) and OSA_3 (0.5%) were at par in their oviposition deterrencies. OSA_4 (1.0%) showed the best oviposition deterrency among the treatments which is proved by the lowest mean number of ovipunctures recorded in this treatment, 0.8. This was also

significantly different from all other treatments. The untreated control OSA_5, recorded 12.6 ovipunctures which was also significantly different from all other treatments.

4.1.4.1.2b Multiple choice test

OSA_1 (0.1%), OSA_2 (0.25%) and OSA_3 (0.5%) were at par, among which OSA_1 (0.1%) was the least deterrent, as is evidenced by its highest number of ovipunctures, 2.4. OSA_3 (0.5%) was also at par with OSA_4 (1.0%). OSA_4 showed the best deterrency and the lowest number of ovipunctures, 0.2. OSA_5, the untreated control was however significantly different from all other treatments and recorded 11.6 ovipunctures.

4.1.4.2 Oviposition deterrency as indicated by the fecundity realization

4.1.4.2.1 Aqueous extract

4.1.4.2.1a No choice test

The fecundity realization ranged from 21.2 to 1.2 larvae. The highest number of larvae, 18.2 emerged in treatment OWB_1 (0.1%) showing the least oviposition deterrency. OWB_2 (0.5%), OWB_3 (1.0%) and OWB_4 (5.0%) were at par with respect to oviposition deterrency but differed significantly from the other treatments. OWB_5 (10.0%) was significantly more deterrent than all the other treatments, as only 1.2 larvae emerged. OWB_1 (0.1%) was at par with the untreated control, OWB_6, where the number of larvae emerged was 21.2.

4.1.4.2.1b Multiple choice test

The maximum number of larvae emerging, 10.0, was seen in OWB_1 (0.1%), which was at par with OWB_2 (0.5%). OWB_2 was also at par with OWB_3 (1.0%), OWB_4 (5.0%) and OWB_5 (10.0%). OWB_5 offered the highest deterrency as is shown by the lowest fecundity realization, 1.4, in this treatment. In the untreated control, the fecundity realization was 21.2, which was significantly different from all other treatments.

4.1.4.2.2 Solvent extract

4.1.4.2.2a No choice test

The treatment OSB_1 (0.1%) showed the least oviposition deterrency, where the fecundity realization was 10.2 larvae. The treatments OSB_2 (0.25%) and OSB_3 (0.5%) were at par with respect to their deterrency. OSB_3 was also at par with OSB_4 (1.0%) which offered the highest oviposition deterrency among the treatments, with a fecundity realization of 1.4. However the untreated control, OSB_5, showed the highest fecundity realization of 25.4, and it differed significantly from all the treatments.

4.1.4.2.2b Multiple choice test

The oviposition deterrency of the treatments OSB_1 (0.1%) and OSB_2 (0.25%) were at par; OSB_1 being the most deterrent, with fecundity realization 5.8. OSB_2 was also at par with OSB_3 (0.5%). OSB_3 was again at par with OSB_4 (1.0%) and offered the best deterrency, as fecundity realization was only 0.4.

In the untreated control, however, 20.0 larvae emerged, which was significantly higher than all other treatments.

4.2 Toxicity tests

4.2.1 Ovicidal action of *A. calamus* (rhizome) extracts

Ovicidal action was judged by exposing the eggs to *A. calamus* extracts and observing for the egg mortality as evidenced by the hatching percentage. The hatchability of treated eggs was observed and the egg mortality was recorded. From these, the percentage mortalities were calculated, which were then subjected to Probit Analysis (Finney, 1971).

The aqueous extract did not have ovicidal action. However, the solvent extracts showed ovicidal action. The concentrations which showed egg mortality in the range 70-30% were selected based on preliminary observations to calculate the LC_{50} to eggs. The LC_{50} of the methanol extract of *A. calamus* to eggs of *B. cucurbitae* was found to be 0.03% (Appendix V).

The concentrations tested and their corresponding percentage mortalities are presented in Table 9.

4.2.2 Toxicity to larvae and pupae

The toxicity of the extracts to larvae and pupae was judged by observing the mortality on treatment of the larvae and pupae with the extracts, by direct application.

Table 9 Mean percentage mortality of eggs of *B. cucurbitae* treated with graded doses of *A. calamus* extracts

Treatment	Dose (%)	Mean percentage mortality*
Aqueous extract	0.10	0
"	0.50	0
"	1.00	0
"	5.00	0
"	10.00	0
"	Control	0
Solvent extract	0.08	96.67
"	0.06	90.00
"	0.04	70.00
"	0.02	36.64
"	0.01	6.67
"	Control	0.00

* Mortality observed after 24 hours

The extracts were not toxic to the larvae and pupae upto 10% concentration (Table 10).

4.2.3 Topical contact toxicity to adult flies

Topical contact toxicity of *A. calamus* (rhizome) extracts to *B. cucurbitae* adults was judged by subjecting the flies to two methods of application, topical application and residue film technique.

4.2.3.1 Topical application

Immobilized adults were directly sprayed with the extracts. When aqueous extracts were used, no mortality was observed upto 10% concentration. In case of methanol extract, 100% mortality was observed in all cases, including the control, where the pure solvent (acetone) was sprayed. Hence the solvent was judged to be the cause of death.

In order to avoid the mortality caused by acetone, the stock solution of the solvent extract in methanol was diluted with water to the desired concentrations and sprayed on the flies. Here again there was no mortality at any level.

The concentrations of *A. calamus* extracts tested along with their corresponding percentage mortalities are presented in Table 11.

Table 10 Mean percentage mortality of larvae and pupae of *B. cucurbitae* treated with graded doses of *A. calamus* extracts

Treatment	Dose (%)	Mean percentage mortality	
		Larvae	Pupae
Aqueous extract	0.10	0	0
"	0.50	0	0
"	1.00	0	0
"	5.00	0	0
"	10.00	0	0
"	Control	0	0
Solvent extract	0.10	0	0
"	0.50	0	0
"	1.00	0	0
"	5.00	0	0
"	10.00	0	0
"	Control	0	0

Table 11 Mean percentage mortality of *B. cucurbitae* adults treated with graded doses of *A. calamus* extracts by topical application

Treatment	Dose (%)	Mean percentage mortality*
Aqueous extract	0.10	0
"	0.50	0
"	1.00	0
"	5.00	0
"	10.00	0
"	Control	0
Methanol extract (redissolved in acetone)	0.10	100
"	0.50	100
"	1.00	100
"	5.00	100
"	10.00	100
"	Control	100
Methanol extract (diluted with water)	0.10	0
"	0.50	0
"	1.00	0
"	5.00	0
"	10.00	0
"	Control	0

* Mortality observed after 24 hours

4.2.3.2 Residue film technique

Adult flies of uniform age and size were released in Petri dishes impregnated with the *A. calamus* extracts at different levels and the residual toxicity was assessed. The data were used to calculate percentage mortality, which were then subjected to Probit Analysis (Finney, 1971).

The aqueous extract was not toxic upto 10%, as observed in the topical spraying method also. However, the solvent extract showed residual contact toxicity with increasing concentrations of the extracts. The concentrations which gave mortalities in the range 70-30% were selected based on preliminary observations, to calculate the LC_{50} to adults. The LC_{50} of the methanol extract of *A. calamus* residue film technique, to the adults of *B. cucurbitae* was found to be 0.07% (Appendix VI).

The concentrations tested and their corresponding percentage mortalities are presented in Table 12.

LT_{50} values were also calculated for a range of concentrations, by observing the mortalities over different intervals of time. The LT_{50} values for different concentrations are presented in Table 13.

4.3 Chemosterilant effect

The chemosterilant effect was studied by administering the rhizome extracts of *A. calamus* at dosages from 0.1-0.01% mixed with the adult feed, sugar.

Table 12 Mean percentage mortality of *B. cucurbitae* adults treated with graded doses of *A. calamus* extracts by residue film technique

Treatment	Dose (%)	Mean percentage mortality*
Aqueous extract	0.10	0
"	0.50	0
"	1.00	0
"	5.00	0
"	10.00	0
"	Control	0
Methanol extract	0.10	100.0
"	0.08	73.33
"	0.06	23.33
"	0.04	6.67
"	0.02	0.00
"	Control	0

* Mortality observed after 24 hours

Table 13 LT_{50} values of various concentrations of *A. calamus* extracts on adults of *B. cucurbitae*

Concentration (%)	Heterogeneity (χ^2)	Regression equation	LT_{50} (hours)	Fiducial limits
10.0	0.039	Y = 4.54 + 11.54 x	1.10	1.16 - 0.96
5.0	-51.77	Y = 2.3 + 5.2 x	3.31	3.65 - 2.99
1.0	4.00	Y = -5.49 + 9.65 x	12.30	13.03 - 11.61
0.1	7.34	Y = 11.05 + 12.73 x	20.89	21.98 - 19.86
0.07	1.22	Y = 5.56 + 7.69 x	23.44	26.24 - 20.94
0.025	-9.34	Y = -18.36 + 13.05 x	61.66	102.32 - 37.15

Y = Probit kill x = Log LT_{50}

Table 14 Micrometry of reproductive organs of *B. cucurbitae* at magnification 40X - calculation of calibrated value of ocular micrometer

Stage reading	Ocular reading	C.V.[(S.R/O.R) x 10]
11	3	36.66
22	6	36.66
44	12	36.66
55	15	36.66
59	16	36.87
81	22	36.82
7	2	35.00
26	7	37.14
71	19	37.37
100	27	37.04
Average		36.85

By daily observation, it was noted that, by the 15th day, the control flies had mated and oviposition started. The treated flies, however, showed no signs of mating even on the 25th day. From the 27th day onwards, the treated flies began to die (Table 17).

In the replications of the above experiment, when the control flies started oviposition, the flies from all treatments were sampled and dissected to observe the morphological/anatomical changes in the internal organs of both sexes.

The dissections revealed changes in size of the reproductive organs of the treated flies. To compare the changes, the sizes of reproductive organs were measured using micrometry technique.

The data on calculation of calibrated value of the ocular micrometer is presented in Table 14. The comparisons of sizes of reproductive organs of treated and normal flies are presented in Tables 15 and 16.

4.3.1 Effect on female reproductive organs

The Table 15 compares the sizes of ovaries of normal mature female flies and treated flies treated with 0.01% methanol extract from the day of emergence, dissected on the 20th day after emergence. The sizes of normal immature ovaries, dissected 5 days after emergence is also presented for comparison.

Table 15 Comparison of size of female reproductive organs of normal and treated* *B. cucurbitae*

	Normal ovary (20th day)		Treated ovary (20th day)		Normal ovary (5th day)	
	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)
R_1	1658.25	1363.45	479.05	368.50	589.60	552.75
	1695.10	1326.60	442.20	368.50	626.45	663.30
R_2	1621.40	1289.75	405.35	405.35	552.75	552.75
	1658.25	1289.75	442.20	442.20	552.75	589.60
R_3	1695.10	1363.45	515.90	405.35	589.60	552.75
	1731.95	1289.75	479.05	442.20	626.45	552.75
R_4	1768.80	1289.75	405.35	368.50	663.30	575.90
	1768.80	1400.30	442.20	405.35	626.45	589.60
R_5	1768.80	1289.75	442.20	442.20	626.45	626.45
	1695.10	1363.45	515.90	368.50	626.45	663.30
Average	1706.15	1326.60	456.94	401.66	608.02	585.91

* 0.01% methanol extract of *A. calamus*

Plate III Ovary of mature, untreated *Bactrocera cucurbitae* female (20 days old) [Actual size: 1706.15 - 1326.60μ]

Plate IV Ovary of immature, untreated *Bactrocera cucurbitae* female (5 days old) [Actual size: 608.02 - 585.91μ]

Plate V Ovary of mature, *Bactrocera cucurbitae* female (20 days old) treated with *A. calamus* extracts [Actual size: 456.94 - 401.66μ]

The average size of a normal 20 days old ovary was 1706.15 μ - 1326.6μ, while that of the treated 20 day old ovary was found to be 456.94 μ - 406.66 μ. The size of a normal immature ovary was found to be 608.02 μ - 585.91 μ.

The treated ovary was seen to be considerably reduced in size. The differences can be clearly seen in Plates III, IV and V. The treated ovary was even reduced in size than a normal immature ovary.

4.3.2 Effect on male reproductive organs

Table 16 compares the sizes of the testes of normal mature male flies and treated flies (treated with 0.01% methanol extract from the day of emergence), dissected 20 days after emergence. The sizes of normal immature testes, dissected 5 days after emergence is also presented for comparison.

The average size of normal 20 day old testes as measured by micrometry was 1278.69 μ - 460.62 μ; and that of the treated 20 days old testes was found to be 843.86 μ - 361.13 μ. The immature 5 days old testes measured 604.34 μ - 453.25 μ.

The difference in size of normal treated testes is not conspicuous as marked as in the case of ovaries. The treated testes were seen to be larger than the normal immature testes. The dissected organs can be compared in Plates VI, VII and VIII.

Table 16 Comparison of size of male reproductive organs of normal and treated* *B. cucurbitae*

	Normal tests (20th day)		Treated tests (20th day)		Normal tests (5th day)	
	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)
R_1	1179.20	479.05	847.55	331.65	515.90	515.90
	1363.45	442.20	810.70	331.65	663.30	405.35
R_2	1252.90	475.05	958.10	368.50	552.75	442.20
	1289.75	405.35	994.95	368.50	589.60	479.05
R_3	1289.75	442.20	921.25	368.50	663.30	442.20
	1326.60	442.20	700.15	405.35	663.30	479.05
R_4	1289.75	479.05	773.85	294.80	626.45	552.75
	1363.45	479.05	810.70	331.65	589.60	368.50
R_5	1216.05	442.20	884.40	368.50	663.30	405.35
	1216.05	515.90	737.00	442.20	515.90	442.20
Average	1278.69	460.62	843.86	361.13	604.34	453.25

* 0.01% methanol extract of *A. calamus*

Plate VI Testes of mature untreated *Bactrocera cucurbitae* male (20 days old) [Actual size: 1278.69 - 460.62μ]

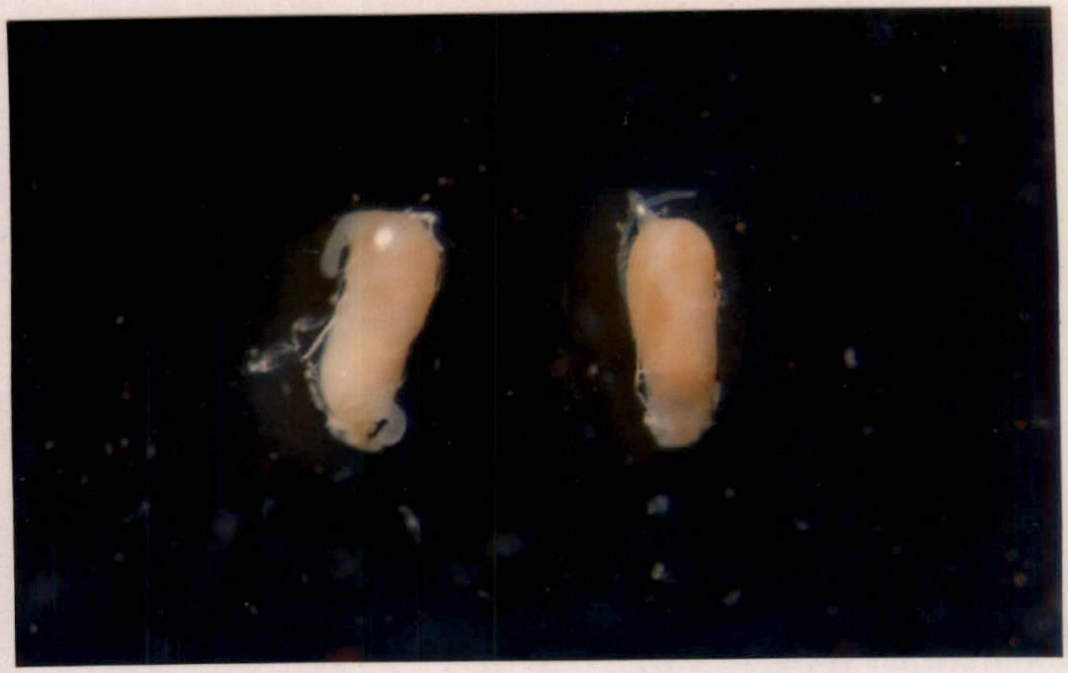


Plate VII Testes of immature untreated *Bactrocera cucurbitae* male (5 days old) [Actual size: 604.34 - 453.25μ]

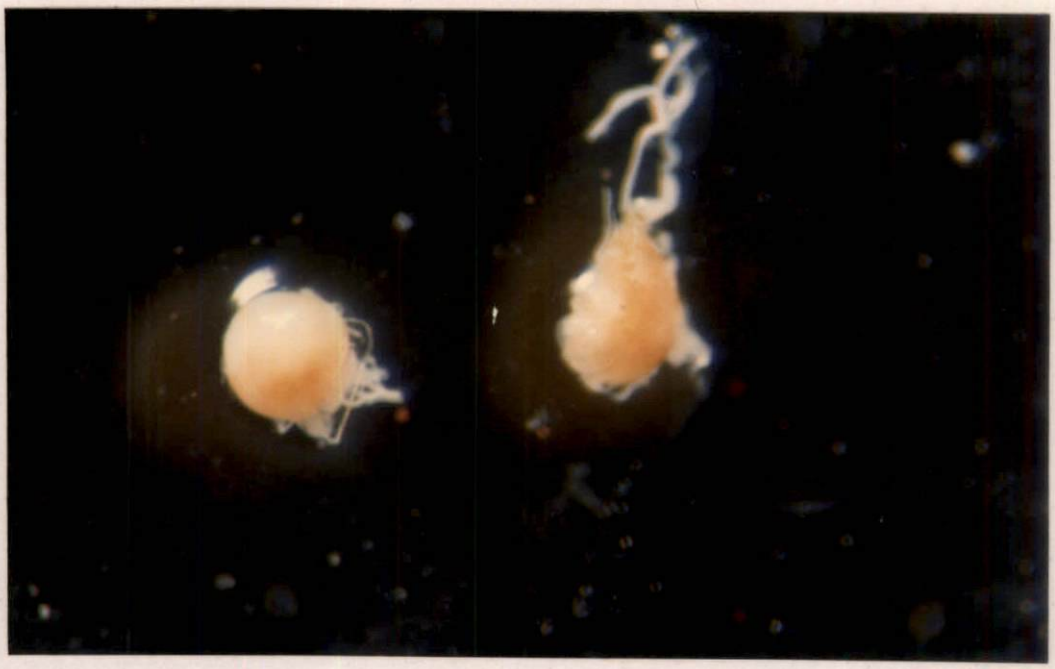
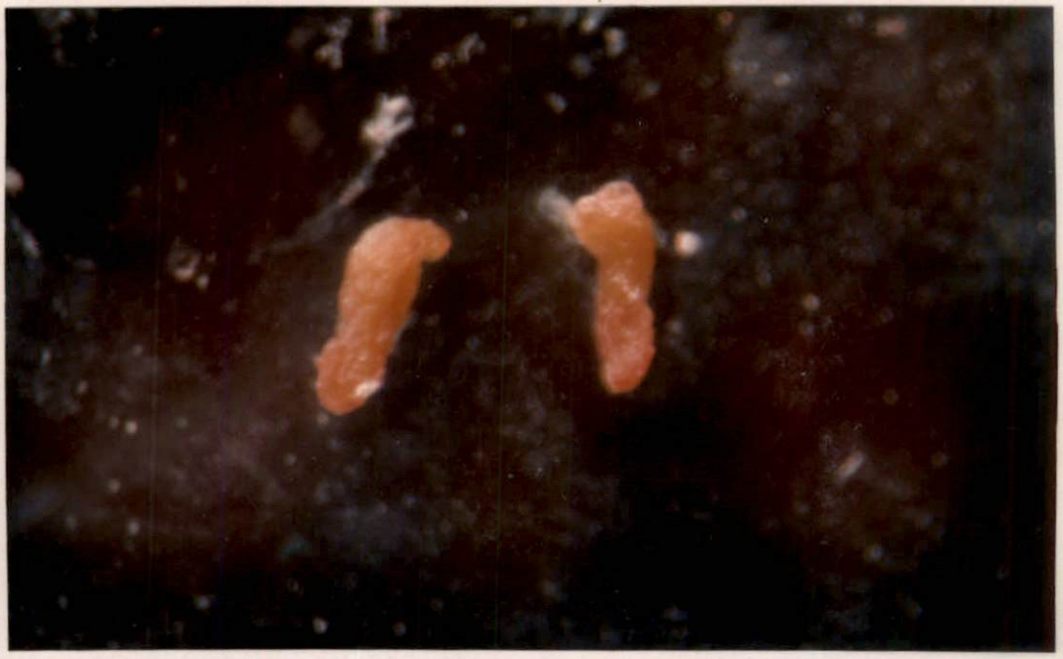


Plate VIII Testes of mature *Bactrocera cucurbitae* male (20 days old) treated with *A. calamus* extracts [Actual size: 843.86 - 361.13μ]



4.4 Other effects

The average survival period of adult flies was found to be considerably reduced on continuous exposure to lower doses of *A. calamus* rhizomes extracts. The average survival period of normal flies and flies treated with 0.1% solvent extract from the day of emergence is presented in Table 17.

Table 17 Average survival period of *B. cucurbitae* adults treated with 0.1% solvent extract from the day of emergence

Replication	Average survival period (days)	
	Treated flies	Normal flies
R_1	27	98
R_2	29	126
R_3	20	135
R_4	27	90
R_5	30	147
Mean	26.6	119.2

Discussion

DISCUSSION

The effects of *A. calamus* (rhizome) extracts on the melon fly *B. cucurbitae* were evaluated on the basis of repellency, feeding deterrency, oviposition deterrency, ovicidal action, topical contact toxicity and chemosterilant action.

Data obtained from observations on these aspects were analysed statistically and the results are presented in chapter 4. The results are discussed in this chapter.

5.1 Comparison between extracts in different solvents

The extracts were compared by testing their repellency to adult flies.

The results of the test indicate that among the various solvents used, methanol was the most effective, since it showed the least mean alightment of flies at both concentrations tested. All solvent extracts were more effective than the aqueous extract. This is naturally expected due to the fact that most of the bio-efficient ingredients are not soluble in aqueous medium.

Percentage recovery of active principle varies with different solvents. Solvent residue also has a key role in the variation of mortality. The efficacy of the methanol extract could be due to a cumulative effect of the active principles and solvent residue. Comparison of aqueous extract and solvent extracts were also supporting this because the active principles of *A. calamus* were effectively extracted in solvent and not in water.

Previous reports indicate the use of various solvents to prepare the extracts of *Acorus calamus*; and these have been proved to be effective against different pests.

Teotia and Pandey (1979); Banerji *et al.* (1982); Tiwari and Moorthy (1985) and Chauhan *et al.* (1989) have all reported the efficacy of petroleum other extracts of *A. calamus* against different storage pests. The acetone extract of *A. calamus* rhizomes resulted in 100% mortality of *Bagrada cruciferarum* (Verma and Pandey, 1981). Bandara *et al.* (1987) found that the dichloromethane extract of *A. calamus* was comparable to dimethoate 40EC, in its insecticidal activity against *Aphis fabae*. Ethanolic extracts of *A. calamus* caused 100% mortality of *Atteva fabriciella* and they were most effective as compared to acetone and ether extracts (Ahmed *et al.*, 1991).

5.2 Repellency/Antifeedancy

The repellent and antifeedant effect of the extracts was evidenced by the reduced alightment of flies on treated surfaces/food material. The aqueous extracts showed repellency and feeding deterrency from 5% onwards, while the methanol extract at 0.1% showed significant effect. The extracts may be exerting some action on the gustatory or chemoreceptors in the flies, which result in them being repelled.

A perusal of Tables 5 and 6 indicates that there is a definite reduction in mean alightment values of flies with increase in concentrations of the extracts, both in case of aqueous extract as well as solvent extracts. However, with aqueous extracts

higher concentrations were required to produce significant results which again suggests that the extractability of the active principles are more with organic solvents.

The mean alightment values in the multiple choice test are lower than those in the no choice tests. This can be explained by the fact that in multiple choice tests, the flies are exposed to several substrates treated with various doses of the extracts, at a time and hence the alightment is scattered, while in the no choice test, there is only one substrate at a time which makes them have a forced landing and feeding for survival.

Scattered alightment in the multiple choice tests is also indicative of the relatively weaker deterrent effect of the *A. calamus* extracts. In case of a high deterrent effect, the alightment on the control would have been 100% and there would be no alightment on the treated surfaces.

Among various biophysiological effects of *A. calamus* (rhizome) extracts, the repellent and antifeedant effects have been widely studied in several pests.

Grainge *et al.*,(1985)have listed a wide variety of pests species against which these extracts show repellent or antifeedant effects.

Jilani *et al.*,(1988) have reported the repellent effect of the oil at 100, 500 or 1000 ppm against *Tribolium castaneum.* The repellency increased with increasing

concentration of the oils. Jilani and Saxena (1990) have reported similar effects on *Rhizopertha dominica.*

Antifeedant activity of *A. calamus* rhizome extracts has been reported against several pests.

Jilani and Saxena (1990)studied the feeding deterrent effects of *A. calamus* oil on *R. dominica* and found that the beetles made fewer and smaller feeding punctures on filter paper discs treated with the test materials at 100, 500 or 1000 $\mu g/cm^2$. Prakash and Rao.(1986)evaluated the antifeedant properties of *A. calamus* against *T. castaneum* and *Oryzaephilus surinamensis.* They observed reduction in larval weight and increase in larval mortality and pupation failures, which were concluded to be caused due to reduced feeding on treated food.

Antifeedant effects of *A. calamus* have also been reported among field pests like *Athalia proxima, Epilachna* spp., *Spodoptera litura, Peridroma saucia* etc.

Banerji *et al.*(1982) found that 2.5, 1.0 and 0.5% petroleum ether extract sprays of *A. calamus* resulted in 100% protection of radish leaves against larvae of *A. proxima.*

Petroleum ether extract of *A. calamus* rhizomes at 0.1 and 0.5% gave 100% protection against *Henosepilachna vigintioctopunctata* (Tewari and Moorthy, 1985).

A. calamus oil at 2.5 and 5% gave 95 and 100% protection respectively against *S. litura* (Sharma *et al.*, 1990).

Koul (1987) studied the antifeedant effects of calamus oil on *S. litura* and found that it was effective at 0.5 and 1% in inducing a reduction in feeding. The reduction was attributed to the possible action of calamus oil on gustatory receptors.

The antifeedant effects of *A. calamus* on *P. saucia* were studied by Koul and Isman (1990). They found that calamus oil applied to cabbage leaf discs significantly inhibited feeding by 4th and 5th instar larvae. Koul *et al.* (1990) determined the active constituents of *A. calamus* oil which inhibited feeding in *P. saucia*. Cis-asarone was found to be toxic and inhibit growth, in addition to having strong antifeedant activity, while the trans-isomer had antifeedant effect only. The gross dietary utilization (efficiency of conversion of ingested food) was decreased when the diet was supplemented with cis-asarone or when it was topically applied. Inhibition of growth occurred, at even a moderate topical dose, primarily as a result of decreased efficiency of conversion of ingested food. Oral or topical treatment with trans-isomer also significantly inhibited carval growth, but here, the effects could be attributed to decreased consumption as dietary utilization was not affected.

Jacobson (1976) has reported that a distillate of the essential oil of *A. calamus* was highly attractive to fruit flies, *Ceratitis capitata, D. cucurbitae* and *D. dorsalis*. However he found that the commercial Indian calamus oil showed low attractancy

to the fruit flies which may be due to the presence of repellent compounds, which can be removed by distillation. Trans-asarone is reported to be highly repellent to certain sp. of insects (Dixit *et al.*, 1966 and Jacobson, 1975).

5.3 Oviposition deterrency

Oviposition deterrency was assessed based on two observations, namely number of ovipunctures and fecundity realization.

Tables 7 and 8 indicate the results of these observations. Perusal of the tables show that there is good correlation between the concentration of extracts used and the effects produced. The mean number of ovipunctures showed a decreasing trend with increase in concentrations used. The mean number of larvae emerging also showed a similar trend.

Chander and Ahmed (1986 a) found that *A. calamus* oil significantly reduced oviposition by pulse beetle on treated seeds of green gram, when applied at doses of 0.25 and 0.5 ml/kg seed. This was attributed mainly to the contact toxicity of the oil to the bruchid adults. The alcoholic extract of neem seed oil completely deterred oviposition by *D. cucurbitae* at 5% and it was highly effective at 2.5% (Singh and Srivastava, 1985).

The reduced oviposition in case of *B. cucurbitae* may be related to the repellent effect of the extracts to the gravid adult flies. The flies were found to probe

the substrate provided for oviposition thoroughly using chemoreceptors in their mouthparts and ovipositors, before oviposition. During such probing, they come into contact with the extract on the substrate and thus avoid oviposition on the treated surface.

In this test also, the solvent extracts were found to be more effective than the aqueous extracts in deterring oviposition.

5.4 Ovicidal action

The details of the extracts and doses tried for testing ovicidal action, with the corresponding percentage mortalities are presented in Table 9. The table reveals that aqueous extracts do not show ovicidal action and there is no mortality even with 10% aqueous extract.

However, solvent extracts did show ovicidal action. By subjecting the average percentage mortality values to Probit Analysis (Finney, 1971) the LC_{50} to eggs was calculated and found to be 0.03%.

Ovicidal action of *A. calamus* has also been reported by previous workers. Mukerjea and Govind (1959) reported the ovicidal action of *A. calamus* extract to eggs of *Bombyx mori*. Higher concentration (0.02 and 0.01%) were able to stop the development of the embryo almost immediately.

Schmidt and Risha (1990)found that the eggs of *Callosobruchus chinensis* and *Sitophilus granarius* were highly susceptible to the vapours of *A. calamus* oil. The period of exposure appeared to be the main factor affecting the efficacy of vapours. An exposure period of 72 hours was necessary to control newly laid eggs with a dose of 1 μl.

Chander and Ahmed (1986a)evaluated the ovicidal activity of *A. calamus* oils and found that the oil at 0.25 and 0.5% significantly reduced the expected adult emergence. Microscopic examination of the unhatched eggs revealed that majority of eggs were killed at the very early stages of development.

Ovicidal activity of crude extracts of *A. calamus* was reported on *Corcyra cephalonica* (Ghatak and Bhusan, 1995 a and *Spilosoma obliqua* (Ghatak and Bhusan, 1995 b).

5.5 Topical contact toxicity

The extracts were not found to be toxic to larvae and pupae (Table 10). However *A. calamus* extracts and oil have been found to be toxic to the larvae of many insects. Ahmed *et al.* (1991)reported that 5% ethanolic extract of *A. calamus* caused 100% mortality of *Atteva fabriciella*. *A. calamus* extracts also caused significant mortality of *Spodoptera litura* and *Pericallia ricini* larvae and *Dysdercus cingulatus* nymphs (Rajendran and Gopalan, 1979). The alcoholic extracts of

A. calamus rhizomes showed an LC_{50} of 1.698 to the 4th instar larvae of the arctiid, *Spilosoma rhodophila* (Verma *et al.*, 1988).

The larvae of *B. cucurbitae* were not affected by the *A. calamus* extracts, possibly because after treatment, they were released back into the fruit flesh. When the larvae tunnelled through the fruit flesh, the toxic material was lost since the larvae mostly remain inside the fruit, the chances of them picking up the toxic material on the treated dishes, were very few and thus they were not affected.

The pupae, with their tough pupal case were quite safe from the toxic effects of the extracts and hence they were also not affected. The pupal case protects the developing pupae from external influences.

The extracts were applied on the adult *(B. cucurbitae)* flies by topical application and by residue film technique.

Topical application by direct spraying was not found to be effective (Table 11).

In residue film technique, the aqueous extract did not show any toxicity even upto 10%. Hence it was concluded that the aqueous extract of *A. calamus* rhizomes were not lethal to *B. cucurbitae* adults due to absence or low concentration of lethal principle.

The solvent extracts showed changes in mortality with concentration. The concentrations which showed mortality percentage of flies in the range 30-70% were selected and by Probit Analysis, the LC_{50} of solvent extract to adult flies was found to be 0.07%.

The toxic effects of *A. calamus* extracts have been reported since early times. Mironov (1940) reported that adult *Anopheles maculipennis* and *Musca domestica* were killed within forty minutes when sprayed with the decoction made out of *A. calamus* rhizomes.

Several workers have used the rhizomes in various forms, such as extracts, oil, vapours, and powders, and have obtained varying toxic effects to various pests. However there are no reports of *A. calamus* rhizomes being toxic to *B. cucrbitae.*

5.6 Chemosterilant effect

Observations on the adult flies fed on the extracts of *A. calamus* with sugar showed that they inhibited mating at 0.1 - 0.01% concentrations.

These results point to the fact that the rhizome extracts of *A. calamus* acted as a mating inhibitory agent on adult *B. cucurbitae.*

The inhibition of the mating process could be due to hormonal imbalance consequent to oral feeding of the extracts. This hormonal imbalance maybe due to effects of the extracts on the reproductive system of the insects.

Another observation noted was the early death of treated insects. These insects showed a significantly lower average survival period than the control insects (Table 17). Thus due to a cumulative effect of mating inhibition and low survival, fecundity realization was not possible.

Dissections of the flies also revealed considerable changes in the sizes of reproductive organs of the treated flies (Tables 15 and 16, and Plates III to VIII).

It has been found that the vapours of *A. calamus* oil inhibit the reproductive system of insects through hormonal imbalance in the gonads. The activity of the isolated compound, β-asarone was established on a variety of insects like *Dysdercus koenigii, Sitophilus oryzae, Tribolium castaeneum, Trogoderma granarium, Musca domestica, Corcyra cephalonica, Callosobruchus chinensis* and *Anthrenus vorax* (RRL, Jammu, 1983).

Unlike other sterilizing agents that inhibit the germinal epithelium or the juvenile hormone analogues that suppress the ovarian maturation or the anti-JH compounds that suppress the JH production, the vapours of *A. calamus* posses a specific effect on egg resorption, the first target being the terminal oocyte of the ovarioles. This effect then shifts to the upper parts and ultimately makes the ovary a bunch of empty tubes without any follicular epithelium. This derailment has been found to be due to abnormal functioning of follicular cells making the gravid insects infecund. This is contrary to what has been reported for

classical chemosterilants or JH analogues where fecundity is not inhibited at this stage. In males, on the other hand, the sterility is because of the immobility, agglutination and mal-functioning of the sperms. This inhibition has been found to be due to the suppression in the secretory mechanism of the interstitial cells and the vas deferens. The said antigonadal activity was found to be neither neurosecretory cell mediated nor like the JH (gonadotropic hormone) or anti-JH compounds. It *de facto* effects the interstitial cells that have been proved to be responsible for the spermatogenesis and vitellogenesis in place of corpora allata secretion.

Bell and Bohm (1975)have reported that oosorption is a reproductive strategy which occurs while the egg is still in the ovariole, is characterized by the interruption of vitellogenesis, and eventually results in the death of the oocyte while still enveloped in the follicle. The remains of the oocyte are partially digested by lysosomes with some of the digested material being absorbed by follicle cells or other ovarian tissue. It is under specific hormonal control in that it can be induced by a lack of juvenile hormone and can be reversed by the addition of an external supply of the hormone. Oosorption is a transitory variable phenomenon in response to environmental behavioral or physiological factors and affects some or all follicles. It can be considered a special case of cell death, because the ovary can resume oogenesis when the factors that initiated oosorption are alleviated.

The reduced sizes of reproductive organs of treated flies show that they have been affected by the extracts of *A. calamus*. The reduction in size may be due to atrophy or under development of the organs or regression in the case of ovaries.

The sterilant effects of *A. calamus* have been previously reported.

Schmidt and Borchers (1981) reported the sterilizing effects of calamus oil on ants.

Saxena *et al.* (1976) found that the vapours of oil of *A. calamus* reduced fecundity and caused regression in the terminal follicles of the vitellarium in treated females of *T. castaneum*, *S. oryzae* (L.), *C. chinensis*, *Trogoderma granarium* and *Anthrenus flavipes* Leconte.

Koul *et al.* (1977) and Tikku *et al.* (1978) similarly reported follicular regression in *T. granarium*.

It is possible that if the treated flies survived longer, a lower fecundity realization could be noted provided mating occurred. It is also possible that, even if mating occurred, there could be no fecundity realization, indicating sterility among treated flies, in which case, it could be stated that the *A. calamus* extracts have chemosterilant effects of *B. cucurbitae*.

The significance of the study using *A. calamus* extracts also lies in the fact that the compound used may help as an autosterilizing compound that can induce sterility among the wild populations of menace creating insects, or course, without polluting the ecosystem and also reducing the larval population to a considerable level. Thus this compound also is quite useful as compared to known classical chemosterilants that possess toxic mutagenic and carcinogenic properties.

Summary

SUMMARY

Investigations were undertaken at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, during 1993-'94 to assess the efficacy of *Acorus calamus* rhizome extracts for the management of melon fruit fly, *Bactrocera cucurbitae*. Experiments were designed to evaluate the following effects of the *A. calamus* extracts.

* Repellency and feeding deterrency
* Oviposition deterrency and ovicidal action
* Topical contact toxicity
* Chemosterilant action

Extracts of *A. calamus* rhizomes prepared in water and methanol were tested on laboratory reared melon flies.

The results of the experiments are summarized below:

Among the various organic solvents tested, methanol was found to show the best repellency and hence it was selected for all further tests.

In the repellency and feeding deterrency tests, the effects were assessed based on number of flies alighting on treated substrates (leaves, in the case of repellency and

sugar crystals, in the case of feeding deterrency). The effects were evidenced by the reduced alightment of flies on treated substrates.

The aqueous extracts showed repellency and feeding deterrency from 5% onwards, while the methanol extract showed significant effect from 0.1%. Thus the methanol extract was found to be superior to the aqueous extract in effecting repellent or antifeedant effects. However, both extracts recorded a significantly lower alightment of flies compared to the untreated control, from which it could be concluded that the extracts do possess some repellent effect on the melon flies.

Oviposition deterrency was assessed based on the number of ovipunctures and fecundity realization recorded from treated substrates (pumpkin). Both observations showed a decreasing trend with the increase in concentration of extracts used. Here, again, the methanol extract was found to be superior to the aqueous extract in deterring oviposition, as it deterred oviposition effectively from 0.25% onwards while in case of the aqueous extract at least 5% concentration was required to produce a significant effect.

Toxicity of the extracts was tested separately on all stages. The aqueous extracts did not possess ovicidal action upto 10%. However, the methanol extracts showed 100% egg mortality at 0.1%. The mortalities at lower concentrations were observed and the LC_{50} calculated by Probit Analysis (Finney, 1971). The LC_{50} of methanol extracts to melon fly eggs was found to be 0.03%.

Both the extracts were not found to be toxic to the larvae and pupae upto 10%. In case of adult flies, the aqueous extract was seen to be ineffective, as there was no mortality observed upto 10% concentration, by topical application or residue film technique. The methanol extract was not effective when applied by topical application, as the solvent itself caused 100% mortality of the treated flies. By residual film technique, it was possible to obtain a gradation in the mortality. 0.1% methanol extract gave 100% mortality of adult flies. The mortality values were subjected to Probit Analysis (Finney, 1971) and the LC_{50} of the methanol extract to adult melon flies was found to be 0.07%.

To test for chemosterilant action, the adult flies were fed with the methanol extracts of *A. calamus* mixed with sugar, at doses of 0.1 to 0.01% from the day of emergence. After feeding on the treated sugar, mating was seen to be effectively inhibited. The treated flies did not mate even upto the 27th day after emergence, while the normal pre-oviposition period is 10-14 days. These flies showed a lower average survival period (26.6 days) than the normal flies (119.2 days). Thus fecundity realization was not possible due to early death of flies.

Dissections of the flies revealed remarkable changes in the sizes and appearance of the reproductive organs of treated and normal flies. This reduction in size could be due to atrophy or underdevelopment of the organs or regression of the follicles and oosorption in case of ovaries.

Thus it could be concluded that the methanol extracts of *A. calamus* acted as an effective mating inhibitory agent for *B. cucurbitae* adults, probably due to a hormonal imbalance created by affecting the reproductive system. If the flies survived long enough to mate and oviposit, a lower fecundity realization may have been noted, or there would have been no fecundity realization at all, because of the changes in the reproductive system. In this case, the chemosterilant effect could be confirmed.

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* Originals not seen

Appendices

APPENDIX I

Summary of analysis of variance tables of orientation responses of *B. cucurbitae* to extracts of *A. calamus* at different doses. Comparison between solvent extracts

Source	Comparison between organic solvents		Comparison between organic solvents and water	
	df	Mean square	df	Mean square
Between treatments	5	3.081	6	10.047
Within treatments	24	0.511	28	0.261

APPENDIX II

Summary of analysis of variance tables of orientation response of *B. cucurbitae* to extracts of *A. calamus* at different doses: Repellency

Source	df	Mean square			
		Water extract		Solvent extract	
		No choice test	Multiple choice test	No choice test	Multiple choice test
Between treatments	5	30.962	16.495	34.621	49.425
Within treatments	24	0.739	0.357	0.163	0.175

APPENDIX III

Summary of analysis of variance tables of orientation response of *B. cucurbitae* to extracts f *A. calamus* at different doses: Feeding Deterrency

Source	df	Mean square			
		Water extract		Solvent extract	
		No choice test	Multiple choice test	No choice test	Multiple choice test
Between treatments	5	33.221	27.077	39.648	38.581
Within treatments	24	0.301	0.091	0.182	0.157

APPENDIX IV(a)

Summary of analysis of variance tables of orientation response of *B. cucurbitae* to extracts of *A. calamus* at different doses: Oviposition deterrency - Number of ovipunctures

Source	df	Mean square			
		Water extract		Solvent extract	
		No choice test	Multiple choice test	No choice test	Multiple choice test
Between treatments	5	80.033	63.733	105.300	106.560
Within treatments	24	1.800	2.133	1.440	1.100

APPENDIX IV(b)

Summary of analysis of variance tables of orientation response of *B. cucurbitae* to extracts of *A. calamus* at different doses: Oviposition deterrency - Fecundity realization

Source	df	Mean square			
		Water extract		Solvent extract	
		No choice test	Multiple choice test	No choice test	Multiple choice test
Between treatments	5	240.380	271.740	472.240	307.140
Within treatments	24	6.867	12.200	3.400	4.280

APPENDIX V

Probit Analysis of Egg Mortality

Slope (b)	:	-540.48
Intercept (a)	:	154.94
Standard Error of mean	:	0.035
Chi-square (Heterogeneity)	:	-101.58(NS)
D.F.	:	3
Log LC_{50}	:	-1.58
LC_{50}	:	0.03
Regression equation	:	Y = 10.42 + 3.42x
Fiducial limits	:	0.03 - 44.66

APPENDIX VI

Probit Analysis of Adult Mortality

Slope (b)	:	-313.2
Intercept (a)	:	72.77
Standard Error of mean	:	0.014
Chi-square (Heterogeneity)	:	-6.67 (NS)
D.F.	:	3
Log LC_{50}	:	-1.17
LC_{50}	:	0.07
Regression equation	:	Y = 16.79 + 10.03x
Fiducial limits	:	0.07 - 15.74

EVALUATION OF THE ACTIVE PRINCIPLES OF THE RHIZOME EXTRACTS OF *Acorus calamus* L. FOR THE MANAGEMENT OF MELON FLY, *Bactrocera cucurbitae* (Coq.) (TEPHRITIDAE : DIPTERA)

By

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ABSTRACT OF A THESIS

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ABSTRACT

The melon fruit fly *Bactrocera cucurbitae* (coq.) (Tephritidae : Diptera) is one of the highly destructive pest species attacking cucurbits. The larvae hatching from eggs deposited within the fruits, tunnel and feed on the internal contents. As a result of this, the fruits are severely damaged, rot and fall to the ground, where pupation takes place.

As the recommended management practices against the melon fly are still proving inadequate, there is a need to devise newer and safer means to solve this problem. The latest trend in pest control is the use of natural products derived from plant and animal sources. Among the various plants tested, *Acorus calamus* L., is gaining importance owing to its insecticidal and insectistatic properties.

The present study was carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, during 1993-94, with the objective of evaluating the extracts of *A. calamus* for the management of the melon fly, *B. cucurbitae.* The experiments were conducted in order to assess effects like repellency, feeding deterrency, oviposition deterrency, ovicidal action, topical contact toxicity to larvae, pupae and adults and chemosterilant action.

Water and organic solvents were used to prepare the *A. calamus* extracts. Among the organic solvents tested, methanol was selected, owing its the better effect. Melon flies (*B. cucurbitae)* reared in the laboratory were used for all the tests, and the following results were obtained.

The repellent and feeding deterrent effects of the extracts was proved by reduced alightment of flies on the treated substrates. The aqueous extracts were effective at 5%, while the methanol extract at 0.1% was highly repellent., proving the superiority of the methanol extract.

In the oviposition deterrency test, the number of ovipunctures as well as the fecundity realization, showed a decreasing trend with the increase in concentration of the extracts. The methanol extract was found to be superior to the aqueous extract in deterring oviposition also, as it was effective at 0.25%, while in case of the aqueous extract a significant effect was produced only at a concentration of 5%.

Toxicity tests were conducted on all life stages of the melon fly. The eggs were not affected by the aqueous extract upto 10%, but there was 100% egg mortality (inhibition of hatching) when 0.1% methanol extract was used. The LC_{50} of methanol extracts to the eggs was found to be 0.03%. Both the aqueous extract and the methanol extract were found to be ineffective in causing mortality to larvae and pupae, upto 10%. In the case of adult flies, there was no mortality upto 10% of the aqueous extract by topical application or residue film technique. Topical application with methanol extract could not be followed, as the solvent itself caused 100% mortality. However by residual film application, it was possible to obtain a range of mortalities, and the LC_{50} of methanol extract to adult flies was found to be 0.07%.

The methanol extracts were found to inhibit mating completely, in adult flies fed with the extracts at 0.1 to 0.01% from the day of emergence. The average survival period of these flies was also significantly lower, because of which, fecundity realization was not seen.

The sizes of reproductive organs in the treated flies were found to be considerably reduced, probably due to regression or oosorption in the ovaries, or general atrophy of the organs, caused by the feeding of the extracts. This damage to the reproductive organs might have caused a hormonal imbalance, which resulted in the mating being inhibited.