THE EFFICACY OF VEGETABLE OILS AS SURFACE PROTECTANTS ON SEEDS OF THE COMMON BEAN *PHASEOLUS VULGARIS* L., AGAINST THE BEAN BRUCHID *ACANTHOSCELIDES OBTECTUS* (SAY) (COLEOPTERA:BRUCHIDAE) INFESTATION

by

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1986

DECLARATION

I CATHERINE ONGECHA NGAAH declare that this thesis is my original work and has not been presented for a degree in any other University

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I DR. GEORGE KARUHIZE declare that this thesis has been submitted for examination with my approval as the Principal University Supervisor

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ABSTRACT

Six vegetable oils namely, castor oil, clove oil, maize germ oil, almond oil, olive oil and mustard oil, each at the dose of 8 ml/kg of bean seed Phaseolus vulgaris L., Rose-coco GLP-288 variety were applied on the seed surface to compare their insecticidal effectiveness against the bean bruchid Acanthoscelides obtectus (Say.).

All the vegetable oils caused high adult mortality. Clove oil gave the best protection. It attained standards of commercial kill, of 100% mortality after one day, similar to the standard recommended storage insecticide malathion. There were statistically significant differences in the levels of control, between the different vegetable oils. The residual toxicity of the vegetable oils on treated bean seeds decreased with time except for castor oil, which showed no significant difference (P=0.05) in toxicity to adult bruchids for a period of upto six months storage. Topical application of 1μl vegetable oil on adult bruchids caused high mortalities. Clove and mustard oils were the most outstanding, and killed all the test bruchids in one day; castor oil was again the least effective, and caused only 75% mortality after five days. The fecundity of the bean bruchids was seriously impaired by the vegetable oil treatments; the least effective oil was castor oil, the mean number of eggs laid in this treatment being 13% of the control. The emergence of progeny of the bean bruchid reared on oil treated beans, was completely prevented by all the

vegetable oils. This protection lasts for upto six months.

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1:10 GENERAL INTRODUCTION

Next to maize, grain legumes such as pigeon peas (Cajanus cajan), cowpeas (Vigna ungiculata), the common beans (Phaseolus vulgaris L.), soyabean (Glycine max), chickpeas (Cicer arietinum) etc., considered together constitute the most important food in Kenya. Amongst these, the common bean constitutes the largest portion of grain legume production in Kenya (Khamala, 1978).

The common beans are amongst the oldest established crop plants, their antiquity being equal to that of maize. Radio carbon dates show that Phaseolus vulgaris was domesticated in Central America, about 7,000 years ago (Kaplan, 1965). Kaplan also reported that the common bean reached Europe in the 16th Century, and was probably spread to the coastal parts of Africa by the Portuguese explorers during the colonial era.

Bean production in Kenya is difficult to estimate, the reason being that most beans (69%) grown in Kenya are inter-planted with other crops. However, the estimated area of bean cultivation per year is 162,000 hectares; and the bulk of the bean crop is produced in Central and Eastern provinces of Kenya (Table 1). The main production areas of these provinces are the drier zones with bimodal rainfall of 700-800 mm per annum.

Table 1

THE AREAS OF BEAN CULTIVATION PER PROVINCE 1969/70

(van Eijanatten 1975)

Province	X 1,000 hectares
Rift Valley	9.3
Central	113.7
Nyanza	40.0
Western	20.4
Coast	8.3
Eastern	130.9

For instance, in Machakos District, nearly half of the land is devoted to bean production (Khamala, 1978). Stanton (1966) and Rachie (1972) listed some of the inherent advantages of growing legumes as follows:-

(a) They have the ability to grow vigorously under a wide range of environments.

(b) They can withstand water stress or low moisture.

(c) They can grow in poor soils without supplemental nitrogen supplied by artificial fertilizers since they have the capacity to fix their own nitrogen through symbiotic bacteria found in nodules on their roots.

On the other hand, grain legumes have an added advantage in that the seed is very rich in protein (Table 2 FAO, 1970), sufficiently rich to be an inexpensive meat substitute. This potential would alleviate human malnutrition, due to inadequate protein, and calorie deficiency experienced in the predominantly starchy African diets (Stanton,1966; FAO, 1979; Rachie, 1972). The protein content of most dry legumes varies between 20-25 percent. This is about two to three times the protein value of most cereals (Stanton, 1966; Bressani, 1972).

Whilst in storage, grain legumes have been reported to suffer considerable losses due to insect pests. The F.A.O., estimate for storage losses is 30% for Africa; and losses in beans were estimated at 40% annually (Hall, 1970). Among the

TABLE 2. Protein content g/100g and essential amino composition (mg/100N) of some grain legumes, compared to that of whole fresh chicken egg (FAO 1970)

Protein and amino acids	Common bean	Cowpea	Groundnut	Field peas	Pigeon peas	Soya beans	Chicken Egg
Protein	22.1	23.4	25.6	22.5	20.9	30.8	12.4
Arginine	355	400	697	595	309	452	381
Histidine	177	204	148	143	232	158	1152
Isoleucine	262	239	221	267	194	284	393
Leucine	476	440	400	425	394	486	557
Methionine	66	73	72	57	32	79	210
Pheylalanine	326	323	311	287	517	309	358
Threonine	248	255	163	254	182	241	320
Tryptophan	63	68	65	56	35	80	93
Valine	287	282	261	294	225	300	429
Total essential amino acids	2710	2682	2559	2848	2596	2788	3322

insect pests that attack grain legumes including the common bean whilst in storage are the beetles found in the family bruchidae (Mookherjee et al, 1970). In this family, the bean bruchid Acanthoscelides obtectus (Say.), (Plate 1) is the principal pest of stored beans. It is rarely observed on other legume seeds (Schoonhoven, 1976; Southgate, 1978). A. obtectus is a principal pest of beans throughout Kenya and other East African countries as well (De Pury, 1968; De Lima, 1976). The bean bruchid probably originated from equatorial regions of Latin America and has followed the spreading of P. vulgaris crops to the American continent, Europe and Africa (Huignard, 1976).

More than 50 per cent of Kenyan farmers store grain legumes for a period of between two to six months (Schonherr and Mbogua, 1976). These authors reported severe insect damage to these seeds during storage. Their survey carried out in 1973/75 in the main bean growing areas, revealed that severe damage due to bean bruchids was experienced by 57 and 73 per cent of the farmers in Central and Eastern Provinces respectively, despite the use of various control measures.

Losses to stored food produce due to infestation and damage by storage insect pests occurs in two forms, viz; weight loss, nutritional and chemical changes. Infestation reduces part of the grain leading to loss of nutrients, increases fat

Plate 1 Adult <u>Acanthoscelides</u> <u>obtectus</u> (Say.)

acidity causes slight denaturation of protein, and consequently reduces the germinative capacity and palatability of the grain (Pingale et al, 1954; Venkat Rao et al 1959; Venkat Rao et al 1960; Caswell, 1970; Khare et al, 1974; Southgate, 1978).

Furthermore, consumption of foodstuffs infested with storage insects causes digestive disturbances and pulmonary disorders (Soysa et al, 1945; Venkat Rao, 1959; Southgate, 1978). If these losses could be prevented it would assist to alleviate the seasonal food shortages inherent in many tropical countries and diseases caused by nutritional deficiences observed especially in children manifested as kwashakor, marasmus and mental retardation (Nicholls, 1951; Mottram, 1979). It is therefore of vital importance to protect bean grains from infestation by storage pests.

Measures currently used to control storage insect pests include the use of chemical pesticides. These are not only a drain on the farmer's meagre financial resources, but are often used beyond recommended dose levels (Lepigre and Pointel, 1977). Likewise due to the heavy reliance on chemicals and the frequency by which these chemicals are applied in order to achieve satisfactory control, several problems have been created such as contamination of ecosystems, pesticide residues on treated commodities and residues in processed foodstuffs (Chee and Benvenue, 1969; Desmarchelier et al, 1971; Bullock, 1974; Henser, 1975; Rowlands, 1976; Siminatis, 1976; Venkat Rao et al, 1977; Hascoet, 1978; Anon, 1979; Wilken *et al*, 1981). Another important problem caused by excessive use of chemicals is the development of genetic resistance to insecticides by storage insect pests, which has arisen at an alarming rate (Parkin, 1965; Hoskins and Gordon, 1965; Spiers *et al*, 1967; Dyte and Blackman, 1970; Bhatia and Bansode, 1971; Zettler, 1972; Zettler *et al*, 1973; Zettler, 1974; Wang and Ku, 1982; Halisack and Beeman, 1983). Established contact insecticides are generally regarded as having little or no effect on seed viability. However, Srivastava, (1958), Potgeiter and de Beer (1972), and Lochner, (1962), reported that wheat lost its viability after four months storage when treated with lindane at the rate of 467 ppm. Moreover, Grogan *et al* (1959) found that lindane severely reduced germination of maize after three months of storage, and Quintanilla *et al* (1972) also reported that the viability of wheat was impaired twelve months after treatment with 10 ppm Phoxim, and upto 10 ppm Phenothoate.

In view of these disadvantages, the situation makes it imperative to search for alternative, safer, and effective control strategies, which are not entirely dependant on chemical control of the bean bruchid. The studies reported here were therefore conducted to evaluate the potential of some locally available vegetable oils, in protecting stored beans against *A. obtectus*. It was also hoped that the studies would provide information which could be useful in the development of bruchid pest management on beans, or other pests of grain legumes.

1:20 LITERATURE REVIEW

Treatment of stored grain with vegetable oils to prevent weevil attack is an old traditional practice in the rural areas of India where split pigeon peas and other grain legumes are usually mixed with edible oils before storage in the home (Mital, 1971). In recent years, because of the increasing awareness of environmental pollution and chemical residues in food, increasing attention has been given to other alternative methods to control storage insect pests including the use of vegetable oils.

Investigations have been conducted by various workers on the properties of vegetable oils as grain protectants against insect pests of stored grain. The effect of vegetable oils on adult survival was studied by Montes Deoca *et al*, (1978), Schoonhoven (1978), Ali *et al* (1983), and Ivbijaro (1984). They reported high mortalities of the rice weevil *Sitophilus oryzae* (L.), the Angoumois moth *Sitotroga cerealella* (Olivier), the mexican bean weevil *Zabrotes subfaciatus* (Boheman) and the pulse beetle *Callosobruchus chinensis* (L.), on oil treated grains within a very short time of exposure. The vegetable oils significantly reduced their survivorship. Contrary to these findings Tsen *et al* (1981) found *S. oryzae* fairly resistant, and very high oil concentrations were required to stop its infestation on rice, wheat and bran. On the other hand, Taylor and Evans (1980) quoted by Golob and Webely (1980) found vegetable oils ineffective against the maize weevil *Sitophilus zeamais* (L.) and the confused flour beetle *Tribolium confusum* (Jacquelin du

Val.), infestation on maize. Moreover Gunathilagaraj and Kumaraswamy (1978) demonstrated that vegetable oils when directly applied to storage insect pests in the absence of grain are toxic to the insects. They reported that clove oil was toxic when topically applied to *C. chinensis.*

The mechanisms involved in the protection of oil treated seeds is not clear. deLuca (1979) investigated the mode of action of vegetable oils on storage bruchids *Z. subfaciatus* by coating bean seeds with vegetable oils. He reported that the oils killed the insects by blocking their tracheae. However, Tikku and Saxena (1981) doubted this finding. This was because studies on insect survival in oxygen depleted environments, showed that oils were more lethal than the lack of oxygen (Gunther and Jeppson, 1960). Furthermore, Hill and Schoonhoven (1981a) examined the effectiveness of African palm oil, and found that the active ingredient of the oil in hindering *Z. subfaciatus* infestation is located in the triglyceride fraction of the oil. It increased adult mortality, and reduced reproduction; and of the fatty acids tested oleic acid had insecticidal effectiveness.

Studies on the influence of vegetable oils on the adult progeny emergence, and their residual effects have been conducted by various workers. Such investigations have shown that vegetable oils are effective in inhibitng the emergence of adult *Z. subfaciatus* and *C. chinensis* on beans and cowpeas respectively (Anon, 1979; Tikku et al 1981). Furthermore, Qi and Burkholder (1981) also achieved complete control of the granary weevil Sitophilus granarius (L.) using vegetable oils, and the residual toxicity of these oils was still effective after two months. Similarly, the residual effect of vegetable oils prevented progeny emergence of C. maculatus, S. cerealella, C. chinensis and the lesser grain borer Rhizopertha dominica (F.), for a period between six to ten months (Sangappa, 1977; Varma and Pandey, 1978; Singh et al, 1978, Gunathilagaraj and Kumaraswamy, 1978; Parkash, 1980; Pandey et al, 1981; Devi and Mohandas, 1982).

The effect of vegetable oils on the oviposition of storage insect pests was also studied by Varma and Pandey (1978), Ali et al (1983), and Ivbijaro (1984). They reported that oviposition of C. maculatus, C. chinensis and S. oryzae on green-gram and maize seeds, was completely inhibited. On the other hand, Schoonhoven (1978) and Pereira (1983) reported that vegetable oils did not inhibit the oviposition of Z. subfaciatus and C. maculatus, though there was reduced oviposition. Pereira (1983), also observed that cowpea seeds treated with vegetable oils, were effective in reducing the oviposition of female C. maculatus 30 days after treatment of the seeds, than on newly treated seeds. Furthermore, subsequent studies conducted on the influence of vegetable oils on the oviposition of Z. subfaciatus by Hill and Schoonhoven (1981b) revealed that it was the fatty acid linolenic, and arachidionic acid components of the oils that were responsible for the reduced oviposition.

Messina and Renwick (1983) worked on egg viability of C. maculatus laid on cowpea seeds coated with vegetable oils. They found that most eggs failed to hatch. However, this effect was short-lived as most eggs on stored seeds later hatched. This indicated that the residual persistence of ovicidal properties of the oils decreased with storage. Moreover, the ovicidal properties of vegetable oils vary on different produce. Pereira (1983) pointed out that the ovicidal properties of vegetable oils were higher on bambara groundnuts than on cowpeas. Similarly, Montes Deoca et al (1978) reported that when oil treated maize seeds were infested with eggs of S. oryzae, there was no progeny emergence. However, when oil treated sorghum and wheat seeds were infested with eggs of S. oryzae, there was progeny production, but it was significantly less than on the control. Moreover, Singh et al (1978) reported that a drop of 1µl groundnut oil had an ovicidal effect if placed at the posterior end of 1-5 day old C. maculatus eggs. The oil entered the eggs through the micropyle, and had a toxic effect on the protoplasm and on the partially or fully formed embryo. Furthermore, Shaaya and Ikan (1979) showed that C_9, C_{10}, C_{11} straight-chain fatty acids isolated from cottonseed oil, and mixed with chickpea seeds not only deterred C. chinensis from ovipositing, but also acted as ovicides by interferring with late stage embryogenesis. de Luca (1979) demonstrated that oil coated bean seeds acted as asphyxiants killing embryos of unhatched eggs of *Z. subfaciatus.*

It has been demonstrated that vegetable oils posses yet another important property, by being able to kill the larvae of storage pests. The larvicidal properties of vegetable oils on *C. maculatus* larvae have been reported by Messina and Renwick (1983). However, the oils had no effect on larvae that had successfully penetrated into the seeds. Schoonhoven (1978) also reported larvicidal properties of vegetable oils on *Z. subfaciatus* larvae, but, contrary to reports by Messina and Renwick (1983), oil treatments had some effect on larvae that were already inside the seeds.

Apart from studies reported by van Rheenen *et al* (1983) working at the Horticultural Research Station, Thika documentation on the pest management of the bean bruchids *A. obtectus,* using vegetable oils is virtually non-existant throughout the world. Studies on the effects of vegetable oils on other storage insect pests have been reported by several workers (Montes Deoca, 1978; Varma and Pandey, 1978; Qi and Burkholder, 1981; Pereira, 1983; Ivbijaro, 1984). These workers demonstrated the effects of vegetable oils which resulted in high adult mortality, reduced fecundity, reduced progeny emergence, and residual toxicity of the oils. van Rheenen *et al* (1983) demonstrated that maize germ oil, and sunflower oil were able to control *A. obtectus* infestation on bean seeds.

However, they did not study the potential of other locally available vegetable oils. The use of vegetable oils against stored product insect pests, is an attractive alternative because it would eliminate the problems that have arisen from the continued use of chemical pesticides including hazards from handling toxic compounds, development of insect resistant strains, residues in food and the pollution of the environment.

1:30 GENERAL MATERIALS AND METHODS

The adult bean bruchids (Acanthoscelides obtectus) used in these studies were obtained from a laboratory colony reared at the National Agricultural Laboratories, Crop Storage, Entomology section. The bruchids were reared and cultures maintained in wide mouth 1 litre kilner jars using Strong's method (Strong et al, 1968). Briefly, this method involves introducing 200 unsexed, one day old bruchids into 300 grams of sterilized and conditioned beans. Bruchids lay their eggs at a maximum rate during the first five days after emergence (Howe and Currie, 1964). After five days the adult bruchids were removed from the cultures by sieving them out using an Endecotts (filter) sieve, mesh number 6, aperture 0.2812 cms and 2812 micrometers. The eggs that passed through or adhered to the sieve were gently returned onto the bean seeds using a fine horsetail brush, so they could develop. All the experiments were conducted in an experimental room maintained at a temperature of 27 $\pm$ 1^{o}C, using an electric heater controlled by a thermostat, and at a relative humidity of 70 $\pm$ 1%, using a fan and a water bath. These are optimum conditions for A. obtectus development (Howe and Currie, 1964; Olubayo, 1980). The temperatures and relative humidity were monitored by a thermohygrograph.

Experimental seeds of a variety Rose coco GLP-288 which is susceptible to bean bruchid attack were purchased from Nyama Kima market in Nairobi. The seeds were disinfected prior to use by sterilizing them in an oven at 60°C for four hours to stop further bruchid development from field infestation. In order to ensure uniformity of the moisture content in the beans used in different experiments, and to obtain a moisture content favourable for _A. obtectus_ development all the bean seeds were conditioned in shallow trays for 2 weeks at 27 $\pm$ 1°C and 70 $\pm$ 1% relative humidity prior to all oil treatments.

The experimental results were statistically analysed by the ANOVAR test, and when comparisons were made they were based on Duncan's new multiple range test (Duncan 1955).

CHAPTER 2

A COMPARATIVE STUDY OF THE EFFECTS OF SIX VEGETABLE OIL TYPES, MALATHION INSECTICIDE, AND BEAN ASH ON THE LONGEVITY OF THE ADULT BEAN BRUCHID *ACANTHOSCELIDES OBTECTUS* (SAY.) REARED ON ROSE COCO CLP - 288 VARIETY.

2:10 INTRODUCTION

The longevity or lifespan of *A. obtectus* at optimal conditions of 27 ± 1°C and 70% ± 1% relative humidity, has been found to be on average 11 days. (Howe and Currie, 1964; Schoonhoven, 1976; Olubayo, 1980). It would be a good control measure if it could be demonstrated that vegetable oils, applied on bean seeds may limit the longevity of *A. obtectus*, and thus preventing them from reaching sexual maturity, mating and reproducing. Gunther and Jeppson (1960), demonstrated that the longevity of storage insects was shorter in oil treated, rather than in oxygen depleted environments. In these studies, the influence of six vegetable oils on the longevity of *A. obtectus* reared under optimal condition of temperature and relative humidity are compared. The effect of these oils were compared with those of a standard insecticide malathion and, the traditional practice of using bean ash (van Rheenen *et al* 1983).

2:11 MATERIALS AND METHODS

Preliminary tests carried out (Table 3), showed that when

Table 3 The toxicity of vegetable oils on bean bruchids after fresh treatment of beans with various vegetable oils; at different concentrations malathion and ash separately, measured as per cent mortality of *A. obtectus* five days after infestation of the bean seeds

Treatment	Concentration ml/kg	Cumulative per cent mortality* at indicated days after introduction of insects into treated seeds				
		Day 1	Day 2	Day 3	Day 4	Day 5
Castor oil	2.5	0.00	2.50	6.25	16.25	25.00
	8	1.25	53.75	80.00	93.75	96.25
	15	33.75	100.00	100.00	100.00	100.00
Clove oil	2.5	100.00	100.00	100.00	100.00	100.00
	8	100.00	100.00	100.00	100.00	100.00
	15	100.00	100.00	100.00	100.00	100.00
Maize germ oil	2.5	0.00	1.25	8.75	15.00	37.50
	8	63.75	98.75	100.00	100.00	100.00
	15	92.50	100.00	100.00	100.00	100.00
Almond oil	2.5	0.00	2.50	10.00	20.00	43.75
	8	61.25	97.50	98.75	100.00	100.00
	15	73.75	100.00	100.00	100.00	100.00
Olive oil	2.5	0.00	2.50	13.75	28.75	43.75
	8	42.50	92.50	100.00	100.00	100.00
	15	82.50	100.00	100.00	100.00	100.00
Mustard oil	2.5	0.00	1.25	5.00	17.50	46.26
	8	82.50	97.50	100.00	100.00	100.00
	15	98.75	98.75	100.00	100.00	100.00
Malathion	2.5g/kg	98.75	100.00	100.00	100.00	100.00
Ash	33g/kg	2.50	6.25	23.75	80.00	100.00
Control		0.00	0.00	0.00	0.00	0.00
Statistical Analysis						
F values		52.8***	246.7***	103.4***	58.5***	32.3***
Standard error of treatment mean		0.23	0.11	0.15	0.17	0.17
Coefficient of variability		16.8%	6.68%	8.49%	9.08%	8.63%

*** F values significant at 0.1% level

* Average of four replicates with 10g seed/replicate infested with 10 pairs of adults

vegetable oil is applied to beans at dosages of 2.5, 8 and 15ml/kg seed, the dose of 8 ml/kg seed had a significant effect on the survival of adult A. obtectus. For this reason, this dosage was selected for all the oil treatments. Aliquotes of 0.08 mls of oil calculated to give a dose of 8 ml/kg seed were pipetted into samples of 10 grams bean seeds in 10.3 x 2.5 cm glass vials. Another 10 grams of beans were treated with 0.025 grams of malathion 2% dust formulation , calculated to give a dosage of 2.5g /kg seed. A third sample of 10 grams bean seeds was treated with 0.33 g bean ash calculated to give a dosage of 33 g/kg seed. A control was set up which consisted of an untreated sample of 10 grams bean seeds. Four replicates were set up for each oil type, malathion, ash and control treatments. The malathion, oil and ash treatments were coated onto the bean seeds by vigorously shaking the vials for 5 minutes to thoroughly mix them with the bean seeds. The control was also subjected to the shaking action. Each replicate was infested with 10 pairs of 1-3 day old adult bruchids from laboratory maintained cultures. The insects were sieved out of the cultures at three day intervals to obtain uniformly aged adults. Insects were sexed using Halstead (1963) method. The pygidium in males is vertical and only partly seen from above, whereas in the females, it is oblique and in full view from above. Immediately after infestation the glass vials were covered with muslin cloth of 4 x 4 cm and bored corks and then placed in the

the experimental room. The treated grains were examined daily for five consecutive days, and the number of live and dead insects were determined. A duration of 5 days was taken because previous tests showed that beyond this duration, there was natural mortality in the controls. Therefore, insecticidal activity of any treatment could best be seen between day 1 and 5 after infestation. After examination live insects were returned to the vials, while the dead insects were discarded. After 5 days, all the remaining insects were removed from the glass vials, and the beans left to incubate. The toxicity of the oils was measured as the mean per cent adult mortality.

2.12 RESULTS

The surface protectant property of vegetable oils, malathion 2% dust and bean ash is shown in Table 4. The vegetable oils differed in their abilities to hinder insect infestation of the treated bean seeds. Analysis of variance of the data revealed that the differences in the adult mortalities in the oils, malathion, ash and control treatments in 5 days were statistically significant (P=0.1). All the oils were toxic to adult beetles, but clove oil appeared to be superior to all other oil treatments and caused total mortality of the pest after only one day of infestation. Malathion treatment was also as effective since only single survivors were observed.

Table 4 The toxicity of vegetable oils on bean bruchids, after fresh treatment of beans with various vegetable oils; at the concentration of 8ml/kg malathion and ash separately measured as per cent mortality of A. obtectus five days after infestation of the bean seeds

Treatment	Concentration ml/kg	Cumulative per cent mortality* at indicated days after introduction of insects into treated seeds				
		Day 1	Day 2	Day 3	Day 4	Day 5
Castor oil	8	1.25	53.75	80.00	93.75	96.75
Clove oil	8	100.00	100.00	100.00	100.00	100.00
Maize germ oil	8	63.75	98.75	100.00	100.00	100.00
Almond oil	8	61.25	97.50	98.75	100.00	100.00
Olive oil	8	42.50	92.50	100.00	100.00	100.00
Mustard oil	8	82.50	97.50	100.00	100.00	100.00
Malathion	2.5g/kg	98.75	100.00	100.00	100.00	100.00
Ash	33g/kg	2.50	6.25	23.75	80.00	100.00
Control		0.00	0.00	0.00	0.00	0.00
Statistical Analysis F values		45.60***	38.94***	59.80***	46.07***	166.30***
Standard error of treatment mean		0.24	0.25	0.18	0.18	0.08
Coefficient of variability		16.54%	14.18%	9.54%	9.24%	4.06%

*** F values significant at 0.1 level

* Average of four replicates with 10g seed per replicate, infested with 10 pairs of adults

No other oil treatment caused total mortality after only one day of infestation. Castor oil treatment proved to be the least effective after one day of infestation and caused very low mortality of 1.25% compared to ash and control treatments 2.5% and 0.00% respectively. Mustard oil caused high mortality of 82.50% and was almost as effective as clove and malathion treatments. After two days 100% kill was given by the malathion treatment. The treatments of maize germ, almond, olive and mustard oils were almost as effective, and gave very high mortalities greater than 90%. The latter oils with the exception of almond oil, caused complete mortality after three days, while it took four days for almond oil to cause the same mortality. Bean ash also exhibited insecticidal properties. The bruchid mortalities increased progressively with time, and after five days 100% mortality of the bruchids was achieved. All the oils except for castor oil, caused 100% mortalities by day five after infestation. Even though castor oil initially caused very low mortality one day after infestation, after five days it caused a very high mortality of 96.25%.

2.13 DISCUSSION

It is evident from the data collected during these studies, that the vegetable oils tested were highly effective in controlling *A. obtectus* infestation. The vegetable oils caused high, or total mortality of the adult bruchids after one day of exposure to the oil treated beans. This finding is quite important, and this is the first time such an observation on the toxicity of vegetable oils on *A. obtectus* has been reported. Similar findings have also been reported by Schoonhoven (1978), Ali et al (1983), and Ivbijaro (1984), working on Z. subfaciatus, C. chinensis and S. oryzae respectively. Observations during the current studies also showed that differences existed between the six vegetable oils, because of their differing properties in effectively reducing the survival of the adult bean bruchids. This, therefore suggests that the bruchid mortality was caused by toxic components of the cils, rather than by the physical property of the oil coating. It is known that vegetable oils are mixed triglycerides of fatty acids and differ from one another in composition and pattern of distribution; this perhaps is the reason for the differing properties of the oils in effectively limiting the survival of adult A. obtectus, as Hill and Schoonhoven (1981a) have reported that triglycerides of vegetable oils are the components that determine their insecticidal effectiveness.

CHAPTER 3

A COMPARATIVE STUDY OF THE RESIDUAL PROPERTIES OF SIX VEGETABLE OIL TYPES, MALATHION INSECTICIDE, AND BEAN ASH TO ADULT BEAN BRUCHIDS *ACANTHOSCELIDES OBTECTUS*

3:10 INTRODUCTION

More than 50% small scale Kenyan farmers store beans for a period of between two to six months; and severe insect damage to seeds during storage has been reported (Schonherr and Mbogua 1976). Measures currently used to control pests include the use of synthetic insecticides, which are not only a drain on the farmer's meagre resources, but also their excessive use has created several problems, such as the development of genetic resistance by storage insects, pesticide residues on treated commodities, and contamination of ecosystems (Desmarchelier, 1971; Zettler, 1972). There is a need for alternative control strategies which are safer and give prolonged protection from infestation. This investigation was therefore undertaken to determine whether vegetable oils remain effective against *A. obtectus* after treated bean seeds have been stored for upto six months.

3:20 MATERIALS AND METHODS

Residual properties of vegetable oils were assessed in an experimental set up similar to one described above in section 2:10. The residual toxicity at 2, 4 and 6 month intervals following oil application to the beans was determined. Samples of 10 grams bean seeds were treated with pipetted aliquotes of different oils namely; castor, clove, maize germ, almond, olive and mustard, calculated to give the dose of 8 ml/kg seed. Four replicates were maintained for each different oil type, malathion 2% dust (2.5 g/kg), bean ash (33 g/kg) and the control treatments. Two similar batches of the above treatments were prepared and kept in the experimental room. Ten pairs of 1-3 day old bruchids were infested into each replicate of different batches 2, 4 and 6 months after treatment. Insect mortalities at each of the 2 month intervals were determined 5 days after infestation. After 5 days, all the surviving and dead insects were removed and the beans left to incubate.

3:30 RESULTS

The residual properties of the different oils, was measured by their action on A. obtectus, as shown in Table 5. There was progressive decline in the insecticidal activity of the vegetable oils, on the adult bruchid with duration of storage. The results showed that the difference in bruchid mortalities

Table 5 Residual properties of 6 vegetable oils, malathion 2% dust, and bean ash, 2, 4 and 6 months after treatment of bean seeds, measured as per cent mortality of *A. obtectus* five days after infestation of the bean seeds

Treatment	Concentration ml/kg	Cumulative per cent mortality[a] at indicated time intervals after treatment of the bean seeds				STATISTICAL ANALYSIS		
		Freshly treated	2 months	4 months	6 months	Treatment F	Standard error of treatment mean	Coefficier of Variabilit
Castor oil	8	96.25b	73.75a	87.75a	88.75a	NS	0.31	14.64
Clove oil	8	100.00a	86.25a	41.25cd	20.00c	11.52***	0.35	20.68
Maize germ oil	8	100.00a	73.75a	67.50abc	17.50c	18.36***	0.27	15.18
Almond oil	8	100.00a	71.25a	57.50bc	50.75b	2.47*	0.35	18.32
Olive oil	8	100.00a	82.50ab	82.50ab	41.25b	4.13**	0.36	18.71
Mustard oil	8	100.00a	27.50b	27.50d	13.75cd	12.96***	0.34	24.98
Malathion	2.5g/kg	100.00a	100.00a	100.00a	100.00a	NS	1.56	6.98
Ash	33g/kg	100.00a	100.00a	87.50a	88.75ab	NS	0.19	9.09
Control		0.00c	0.00c	0.00e	0.00d	-	-	-
Statistical Analysis								
F values		1959.03***	15.14***	16.36***	13.11***			
Standard error of treatment mean		0.03	0.31	0.30	0.37			
Coefficient of Variability		1.40	17.81	17.89	26.20			

(a) Average of four replicated with 10g seed per replicate infested with 10 pairs of adults

*** F values significant at 0.1% level

** F values significant at 0.5% level

* F values significant at 0.05% level

NS F values not significant

Figures in the same column followed by the same letter are not significantly different at 5% level of significance analysed by analysis of variance followed by Duncan's multiple range test Duncan.

in the different treatments was highly significant (P = 0.1), when treated beans were infested at different time intervals after storage. On freshly treated beans, all the oil, malathion, and ash treatments caused very high mortalities, and were significantly different from the control. After storing treated beans for two moths, mustard oil treatment showed a marked loss in insecticidal activity. It caused very low bruchid mortality (27.50%), as opposed to all the other oils and ash treatments which caused mortalities greater than 70% and were not significantly different from malathion treatment which still exhibited 100.00% mortality. However, mustard oil treatment was significantly different from the control. A conspicous reduction in residual toxicity was observed in beans treated with clove and almond oils, stored for four months prior to infestation. These treatments were significantly different from malathion treatment, and the control. Oil treatments of castor, maize germ, olive, and ash treatment were not significantly different from malathion treatment. Treated bean seeds stored for six months, showed a decline in residual toxicity in maize germ oil and olive oil treatments. Castor oil and ash treatments are not significantly different from malathion 2% treatment. There was no significant difference between mustard oil treatment, and the control. Statistical analysis of each individual treatment revealed that throughout the six month storage period, there was no significant difference in toxicity of castor oil, ash and malathion treatment, while with the other vegetable oil treatments their insecticidal acitivity declined with time.

3:12 DISCUSSION

For practical purposes the protective quality of vegetable oils must persist in storage. Results of this investigation reported here show that all the vegetable oils significantly increased adult mortalities on beans freshly treated with vegetable oils. However, after storing the oil treated beans for upto six months, some of the oils were less effective in giving prolonged protection from infestation by adult A. obtectus. The loss in the residual activity was observed in all the oil treatments, and differences in the loss of effectiveness of the oil treatments was also noted. The reason for the loss of residual toxicity is not clear. Hill (1981) pointed out that, the amount of oil to which the insect is exposed to depends on a number of factors including, the degree of penetration of oil into the seed. Messina and Renwick (1983) suggested that changes in the surface layer of oil over time, reduces toxicity, and maybe the oil layer becomes absorbed into the seed. There is no evidence obtained in the present study, to support these claims. Even though, it is possible that the vegetable oils penetrated into the bean seeds, hence the amount of oil to which the bruchids were exposed to was reduced, causing the decline in toxicity of the oils. In conformity with the results of the present study Qi and Burkholder (1981) van Rheenen et al (1983), and Ivbijaro (1984) have reported a progressive decline in vegetable oil toxicity and weevil mortality with an increase of storage period of between 2 to 3 months. Singh et al (1978) also reported a decline in groundnut oil toxicity to C. maculatus. On the other hand, six months after treatment of cowpea seeds, the groundnut oil prevented the emergence of adult progeny of C. maculatus rather than affecting the mortality of adult weevils. From this investigation it can be concluded that castor oil may be recommended as the practical means of controlling adult bean bruchids, since there is a small reduction in its residual toxicity with duration of storage for upto six months.

CHAPTER 4

A COMPARATIVE STUDY OF THE EFFECTS OF SIX VEGETABLE OILS, AND MALATHION PESTICIDE ON THE FECUNDITY OF THE BEAN BRUCHID *ACANTHOSCELIDES OBTECTUS* (SAY.) REARED ON ROSE COCO GLP-288

4:10 INTRODUCTION

The reproduction of *A. obtectus* is adapted to storage conditions; this explains the development of numerous successive generations once the seeds in the store have been contaminated. The presence of bean seeds stimulate oogenesis and egg laying of bean bruchids (Huignard, 1978). The influence of beans on reproduction is important and one of the major approaches to insect pest control has therefore been to check insect reproductivity. Previous observations have indicated that oviposition of *C. chinensis* and *C. maculatus* could be prevented or reduced by treating greengrams and cow-peas with vegetable oils (Ali *et al*, 1983; Pereira, 1983). The female bean bruchid *A. obtectus* lays an average of 63.0 eggs. The eggs are laid at a maximum rate during the first five days after adult emergence (Howe and Currie, 1964). These investigations were therefore undertaken to determine whether some locally available vegetable oils affect the reproduction of the bean bruchid *A. obtectus*.

5:11 MATERIALS AND METHODS

Vegetable oils each at the dose of 8ml/kg were pipetted into samples of 10 grams bean seeds in glass vials. Another sample of 10 grams of beans, was treated with 2% malathion dust at a dosage of 2.5 g/kg seed. A control consisted of 10 grams of untreated bean seeds was also set up. Four replicates were maintained for each different oil, malathion dust and the untreated control. The oils and malathion were manually coated on the bean seeds, by vigorously shaking each glass vial for 5 minutes. Ten pairs of one day old adult bruchids were introduced into each replicate and the vials were then covered with muslin and bored corks and left undisturbed for a period of five days. Five days after infestation, the beans in each replicate were separately poured out onto a polythene paper 9.5 X 9 cm. The eggs adhering to the beans and those that remained in the vials were counted with the help of a hand lens and a tally counter. The number of eggs in each treatment was taken as a measure of the effect of different vegetable oils and malathion 2% dust on the reproduction of <u>A. obtectus.</u>

5:12 RESULTS

The number of eggs laid on beans treated with vegetable oils, by adult bean bruchids is shown in Table 6. The mean number of eggs laid on the oil and malathion treated beans was significantly less (P=0.05) than on the untreated control. It

Table 6. The influence of vegetable oils and malathion on the facundity of adult *A. obtecutus* on Rose coco GLP - 288 variety

Treatment	Concentration Ml/kg	Mean number of eggs laid
Castor oil	8	25.0b
Clove oil	8	0.0c
Maize germ oil	8	2.0c
Almond oil	8	2.0c
Olive oil	8	1.0c
Mustard oil	8	0.0c
Malathion	2.5g/kg	0.0c
Ash	33g/kg	-
Control		197.0a
Statistical Analysis		
F value		76.07***
Standard error of treatment mean		0.52
Coefficient of Variability		33.62%

*** Figures significant at 0.1% level

Figures in the same column followed by the same letter are not significantly different at 5% level of significance analysed by analysis of variance followed by Duncan's multiple range test.

is clear from Table 6 that oil treatment of clove and mustard, and malathion dust were the most effective and completely inhibited bruchids from laying eggs. In beans treated with maize germ, almond and olive oils, although egg laying was not completely deterred it was drastically reduced. Only one to two eggs were the mean number seen in the treatments. There was no significant difference (P=0.05) observed between the mean number o eggs laid on the beans treated with oils of clove, maize germ, almond, olive and mustard. However, there was a significant difference between the mean number of eggs laid on castor oil treatment, the other vegetable oils and malathion dust treatments. The highest mean number of eggs (25) in all the oil treatments and malathion treatment, were laid on beans treated with castor oil. Although this was highest mean number of eggs compared to the other oil and malathion treatments, it was signifantly less than the mean number of eggs laid in the control which was 197. Vegetable oils therefore, deterred or reduced fecundity of *A. obtectus*.

5:13 DISCUSSION

It was clear from the results obtained during this study that there was a distinct inhibition or reduction in the fecundity of *A. obtectus*, by vegetable oils. This observation confirms existing reports by various workers. In similar investigations, Schoonhoven (1978); Varma and Pandey (1978) and

Ivbijaro (1984), reported that fecundity of Z. subfaciatus, C. maculatus and S. oryzae was suppressed or completely inhibited by vegetable oils. It is logical to assume that bruchid fecundity is impeded due to the reduction in adult survival, which is affected by the toxicity of the vegetable oils, thus, suggesting that the adult bruchids die before they have laid eggs. However, Hill and Schoonhoven (1981a) have reported that the insecticidal fraction of vegetable oils triglyceride, reduces fecundity of Z. subfaciatus. Therefore, it could be inferred that the reproductive capacity of A. obtectus may have been influenced by the toxic constituents of the vegetable oils.

CHAPTER 5

THE INFLUENCE OF OIL TREATED BEAN SEEDS ON THE BREEDING OF *A. OBTECTUS*, AND THE DEGREE OF DAMAGE CAUSED

5:10 INTRODUCTION

Storage insects constitute a very high threat to farmer's produce in store. Losses of stored grain due to infestation by stored pests occur as the loss of weight, nutritive and aesthetic value of the produce, seed viability and the marketability of the produce, (Pingale *et al*, 1954; Pingale *et al*, 1956; Venkat Rao *et al*, 1960; Caswell, 1970; Khare *et al*, 1974). The damage caused to stored beans by bean bruchids *A. obtectus*, is a result of the larvae feeding and developing inside the seeds, extensively damaging the beans and eventually emerging as adults. It has been reported that use of vegetable oil on stored grain to prevent the breeding of stored produce insects, has been in practice in India for a long time (Cotton, 1963). The main objective of the study reported here was to determine whether vegetable oils had any effect on the breeding of *A. obtectus* reared on common beans, measured by adult progeny emergence, and consequently the damage caused.

5:11 MATERIALS AND METHODS

To determine if bean seeds freshly treated with vegetable oils affected progeny emergence, the beans used in the experiment described above in section 2:10, were left to incubate for 29 days from the day of infestation, this being the average time for the life cycle of A. obtectus (Howe and Currie, 1964; Olubayo, 1980). After incubating the bean seeds for 29 days, adults emerging from oil, malathion and ash treated beans as well as the control were sieved out and recorded every other day. The counting of adult emergence was terminated four weeks after the first emergence to avoid inclusion of F_2 generation. To measure the effect of the residual persistance of vegetable oils on the breeding of A. obtectus,the bean seeds infested 2, 4 and 6 months after oil application in the experiment described above 3:10, were allowed to incubate for 29 days, from the onset of each infestation, thereafter F_1 adult progeny were sieved out and recorded on every alternate day. Four weeks from the onset of adult progeny emergence the recording was terminated.

The amount of bean damage caused by bruchid infestation in the treated bean seeds in the experiments 2:10 and 3:10 described above, was determined by counting bean seeds with emergence holes in a sample of 10 grams. The percentage of damaged seed per treatment was taken as a measure of the efficacy of the oils.

5:12 RESULTS

Table 7 shows the emergence of F_1 adult progeny from beans treated with vegetable oils, malathion 2% dust and bean ash. The Table also shows the residual properties of various treatments in suppressing adult bruchid emergence for upto six months after treatment of the beans. It was shown in these experiments that, on freshly treated beans, no progeny emerged from the beans treated with vegetable oils and malathion, whereas 44.0 and 49.0 were the mean number of adult bruchids that emerged from ash treated beans and the untreated control respectively. It has previously been shown in Table 6 that clove oil, mustard oil and malathion treatments completely inhibited fecundity, and this could have probably been responsible for the lack of progeny emergence in these treatments. In bean seeds treated two months prior to infestation, again there was no adult progeny emergence in all the oil treatments, and malathion treatment, there was adult progeny emergence in the ash treatment and control. This activity of preventing adult progeny emergence, thus inhibiting breeding of _A. obtectus_, was retained even after storing beans treated with vegetable oils or malathion for six months. On the contrary, the ash treatment failed to suppress adult progeny emergence throughout the six month period.

Grain damage (Table 7) was completely prevented on bean seeds freshly treated with vegetable oils, malathion 2% dust and infested with adult bean bruchids. Very high damage was observed in the untreated control 61.0% and the ash treatment 52.0%. No damage was observed on beans infested two months after

Table 7 Adult <u>A. obtectus</u> progeny emergence and average percent of beans damaged when adult bruchids were freshly introduced into treated beans at stated intervals of months after admixture with various vegetable oils; malathion and ash separately

Treatment	Concentration ml/kg	Freshly treated		2 months after treatment		4 months after treatment		6 months treatment	
		Mean no adults emerged	Mean % damage	Mean no adults emerged	Mean % damage	Mean no adults emerged	Mean % damage	Mean no adults emerged	Mean % damage
Castor oil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Clove oil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maize germ oil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Almond oil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Olive oil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mustard oil	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Malathion	2.5g/kg	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ash	33g/kg	44.0	52.0	21.0	41.0	23.3	56.0	28.0	37.6
Control		49.3	61.0	28.25	73.0	59.3	70.0	40.75	58.2
Statistical Analysis									
F values		47.27***		4.25***		13.89***		23.04***	
Standard error of treatment mean		0.41		0.75		0.74		0.45	
Coefficient of Variability		37.51%		100.64%		70.86%		50.42%	

*** F values significant at 0.1% level

** F values significant at 1% level

Averages obtained from four replicates

oil application to the beans, or on beans treated with malathion 2% dust. All oil and malathion treatments also effectively prevented bean damage for upto six months after treatment of the beans, while very high damage was observed in the ash treatment and control during the same period. Consequent to the effectiveness of vegetable oils in preventing the breeding of A. obtectus, the oils effectively prevented damage of the treated bean seeds.

4:13 DISCUSSION

Observations of this investigation revealed that all oil treatments were highly effective in controlling the breeding and multiplication of bean bruchids. Results of this study tally with findings reported by Mummigatti and Ragunathan (1977), and Schoonhoven (1978). They noted that progeny production of C. chinensis and Z. subfaciatus respectively, was significantly affected by vegetable oils. Contrary to the present findings, Mujumder et al (1970) reported that clove oil reduced the population of stored grain insects, but did not inhibit the growth and multiplication completely. It was also demonstrated in the present studies that, oil treatments of beans prevented A. obtectus progeny emergence for upto six months in storage. This is indicative of the residual persistance of protective properties of the oils with duration of storage. Similarly, various oils have been found to be effective in preventing multiplication and progeny emergence on treated produce for more than six months (Cruz and Cardona, 1981; Gunathilagaraj and Kumaraswamy, 1978). Other studies have shown that mustard and castor oils effectively checked multiplication of C. chinensis for four and a half months only as opposed to six months according to findings of the present investigations (Sangappa, 1977).

Grain damage of all oil treated and malathion treated beans was prevented on freshly treated beans, and throughout the six month experimental period. These results contradict reports made by van Rheenen et al (1983) who observed A. obtectus damage on beans treated with vegetable oils three months prior to infestation. Furthermore, Varma and Pandey (1978), reported that the average percent damage caused by C. maculatus to green grams treated with mustard oil, increased progressively with time, contrary to the observation of the present study. Nevertheless, the results of the present investigations are in conformity with the findings of Singh et al (1978). They observed that castor oil prevented cowpea damage by C. chinensis for six months.

CHAPTER 6

A COMPARATIVE STUDY OF THE TOPICAL TOXICITY OF SIX VEGETABLE OILS AND MALATHION PESTICIDE APPLIED DIRECTLY ONTO THE BEAN BRUCHIDS *ACANTHOSCELIDES OBTECTUS* (SAY.)

6:10 INTRODUCTION

Previous observations have indicated that vegetable oils cause high adult mortality of stored insect pests (Singh *et al*, 1978, Schoonhoven, 1978, Hill, 1981). There has been a lot of controversy about how vegetable oils protect stored produce against insect pests and their mode of action has not been completely elucidated and remains obscure. de Luca (1979) postulated that vegetable oils kill the insect pests by interferring with their respiratory system and this was refuted by Gunther and Jeppson (1960) who demonstrated that vegetable oils cause death by some means other than by asphyxiation. These experiments were therefore conducted to determine the toxicity of vegetable oils, and malathion when applied directly to adult bean bruchids and the possible mode of action.

6:11 MATERIALS AND METHODS

Preliminary experiments showed that of the three volumes 0.25μl, 0.5μl and 1μl (Table 8) the volume of 1μl was the most effective. Adult bruchids aged 1-3 days old were each picked up individually using the

Table 8 — Toxicity of various vegetable oils and malathion at different dose levels topically applied to adult bruchids measured as per cent mortality of *A. obtectus*

Treatment	Dose ul/insect	Day 1	Day 2	Day 3	Day 4	Day 5
		Cumulative per cent mortality* on indicated days after treatment				
Castor oil	0.25	0.00	0.00	0.00	1.00	5.00
	0.5	8.00	9.00	13.00	18.00	26.00
	1	56.00	60.00	63.00	68.00	75.00
Clove oil	0.25	0.00	0.00	5.00	10.00	13.00
	0.5	100.00	100.00	100.00	100.00	100.00
	1	100.00	100.00	100.00	100.00	100.00
Maize germ oil	0.25	0.00	3.00	3.00	3.00	9.00
	0.5	38.00	45.00	54.00	58.00	75.00
	1	91.00	94.00	99.00	100.00	100.00
Almond oil	0.25	0.00	0.00	0.00	3.00	9.00
	0.5	38.00	49.00	61.00	80.00	90.00
	1	68.00	75.00	91.00	91.00	100.00
Olive oil	0.25	0.00	0.00	4.00	8.00	15.00
	0.5	66.00	70.00	81.00	90.00	98.00
	1	79.00	84.00	93.00	96.00	99.00
Mustard oil	0.25	0.00	0.00	0.00	5.00	5.00
	0.5	89.00	95.00	98.00	99.00	100.00
	1	100.00	100.00	100.00	100.00	100.00
Malathion	0.25	83.00	100.00	100.00	100.00	100.00
	0.5	100.00	100.00	100.00	100.00	100.00
	1	100.00	100.00	100.00	100.00	100.00
Acetone	o.25	3.00	3.00	3.00	3.00	9.00
	0.5	4.05	4.00	4.00	4.00	9.00
	1	4.00	10.00	13.00	15.00	21.00
Control		0.00	0.00	0.00	0.00	5.00
Statistical Analysis						
F values		106.2***	108.0***	122.2***	90.5***	543***
Standard error of treatment mean		0.16	0.16	0.15	0.17	0.20
Coefficient of variability		12.4%	12.0%	10.7%	11.5%	12.8%

*** F values significant at 0.1% level

thumb and forefinger. Each bruchid was topically applied with 1µl of each oil on the dorsal surface of the thorax using an electric Burkard microapplicator with a calibrated microsyringe (Plate 2,3). Each oil treatment was replicated four times with 10 pairs of insects per replicate. The treated bruchids were confined in 9cm petri-dishes with no beans. The same number of insects were treated with Malathion 2% emulsifiable concentrate as a standard insecticide for comparison; 10 pairs of insects were left untreated as the control. The malathion and control were also replicated four times. The petri-dishes were placed in the experimental room at 27 $\pm$ 1°C and 70 $\pm$ % r.h. Mortalities of bruchids in each treatment were recorded daily for 5 consecutive days after the topical treatments. Insects that did not move when touched gently with a small pointed object were considered dead.

6:12 RESULTS

There was a highly significant difference (P=0.1) in the toxicity of all the treatments throughout all the five days of observation (Table 9). In treatments of clove oil, mustard oil and malathion 2% e.c. acute adult toxicity of 100% mortality occurred one day after topical treatment of the bruchids. No other oil caused total mortality of the bruchids, one day after topical application of the oil. Three days after topical treatment maize germ oil was highly effective. Total mortality was achieved four days after topical treatment. Almond oil

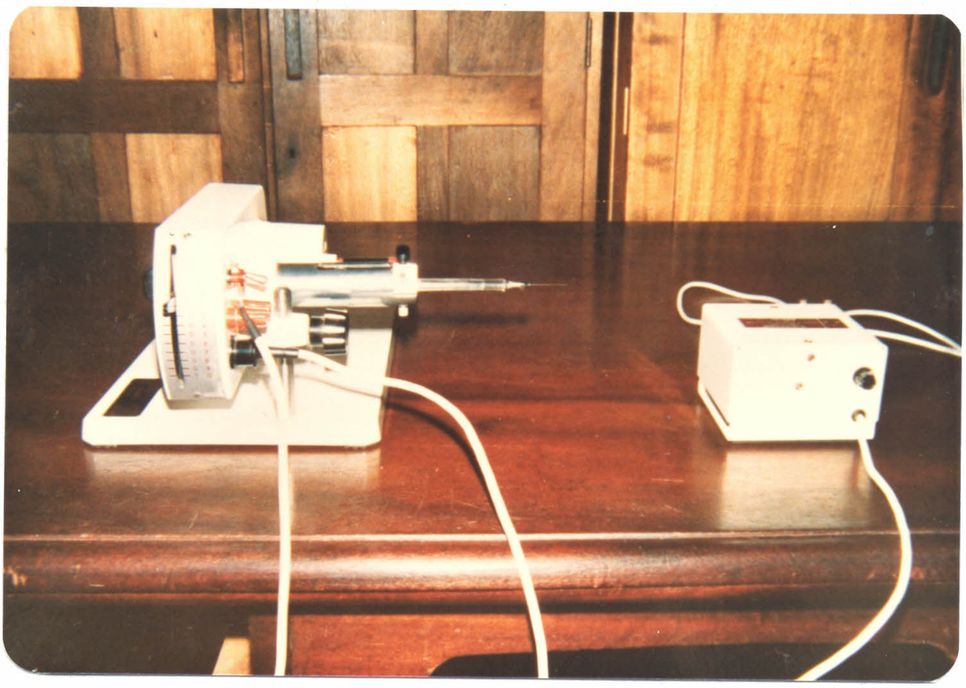

Plate 2 The Burkard Arnold Microapplicator

Plate 3 Topical application of vegetable oil on the adult bean bruchid

Table 9 Toxicity of various vegetable oils and malathion at the dose of 1μl/insect topically applied to adult bean bruchids measured as per cent mortality of A. obtectus

Treatment	Dose ul/insect	Cumulative per cent mortality* on indicated days after treatment				
		Day 1	Day 2	Day 3	Day 4	Day 5
Castor oil	1	56.00	60.00	63.00	68.00	75.00
Clove oil	1	100.00	100.00	100.00	100.00	100.00
Maize germ oil	1	91.00	94.00	99.00	100.00	100.00
Almond oil	1	68.00	75.00	91.00	95.00	100.00
Olive oil	1	79.00	84.00	93.00	96.00	99.00
Mustard oil	1	100.00	100.00	100.00	100.00	100.00
Malathion	1	100.00	100.00	100.00	100.00	100.00
Control		0.00	0.00	0.00	0.00	0.00
Statistical Analysis						
F values		80.77***	69.68***	72.53***	83.76***	39.52***
Standard error of treatment mean		0.17	0.17	0.17	0.16	0.21
Coefficient of variability		9.81%	9.81%	9.45%	8.55%	11.00%

*** F values significant at 0.1% level

* Average of four replicates with 10 pairs of adults per replicate

treatment caused total mortality five days after topical treatment of the adults. Olive oil elicited a very high mortality of 99% five days after topical treatment. Castor oil had the least effect in killing the insects, whereas all the other vegetable oils caused total mortalities or very high mortalities five days after topical treatment, castor oil caused the least mortality of 75% five days after topical treatment. The results of the present investigations clearly indicated that vegetable oils are highly toxic to bean bruchids when applied directly to the bruchids.

6:13 DISCUSSION

The vegetable oils when topically applied to adult bruchids were effective and caused high mortalities. Clove and mustard oils were as effective as malathion 2% treatment. A close comparison of the results of the present study with the work of the other authors is not possible due to lack of published data. It is however possible to make some comparisons with the work of Gunathilagaraj and Kumaraswamy (1978). They reported that clove oil was insecticidal when topically applied to C. chinensis adults. The results of the present investigations clearly reveal that vegetable oils were toxic and caused adult mortalities, and their effectiveness varied from oil to oil indicative of toxic components of oils (Schoonhoven, 1978). Previous considerations on the mode of action of vegetable oils on storage pests have been reported to be by interference with the insect's respiratory mechanism. The oils block the trachea and cause anoxia (Luca, 1979). However, Gunther and Jeppson (1960) in a previous investigation reported that comparisons of insect survival in anaerobic and oil treated environments,showed that oil was more lethal than asphyxiation. In the present investigations, the vegetable oils were applied onto the dorsal aspect of the insect's thorax, which does not bear any spiracles, and yet the vegetable oils caused high adult mortalities. This suggests as reported by Gunther and Jeppson (1960), that factors other than oxygen starvation play a role in the mode of action of the vegetable oils. The adult bruchid does not feed on the beans (Howe and Currie, 1964). Therefore, this rules out the possibility of the vegetable oils acting as stomach poisons. It is possible that the vapours of vegetable oils are toxic to stored product insect pests. However, there have been no reports on this phenomenon. It is possible that the vegetable oils are taken into the body and interfere with the nervous system.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

From the results of these investigations, it is evident that bean seeds treated with vegetable oils are toxic to *A. obtectus*, and as a result adult longevity was reduced, 100% mortality being achieved in 1-3 days in most of the oil treatments. From this observation, it is clear that vegetable oils can be used to protect beans against bruchids. Very little published information is available on the effect of vegetable oils on insect pests of stored beans, throughout the world. The only previous experiment on the use of vegetable oils to control *A. obtectus* was carried out by van Rheenen *et al* (1983) in Kenya using maize germ and sunflower oils. The vegetable oils in the present study exhibited different degrees of toxicity to the bean bruchids. This, clearly indicated different properties in the insecticidal effectiveness of the vegetable oils. Although the oils were not subject to biochemical analysis, it is possible that different toxic constituents in the oils caused the shortened lifespan of the adult bruchids. Similar observations have been made by Varma and Pandey (1982) who demonstrated that minor toxic constituents of vegetable oils could protect greengrams from infestation by *C. maculatus*. The mechanisms involved in the protection of oil treated seeds is not however clear.

Another observation from the current studies was that of progressive reduction in the residual toxicity of the vegetable oils to the adult bruchids with increase of storage period. It would therefore appear that the toxic constituents of the effectiveness became ineffective with time. Schoonhoven (1978), demonstrated that oil treatment of larval infested bean seeds significantly reduced progeny adult emergence of Z. subfaciatus. This indicated that the vegetable oils penetrated into the bean seeds. Pereira (1983) later reported that neem oil penetrated into the testa of oil treated cowpea seeds that had been stored for three months. Although there is no conclusive evidence, it is possible that the vegetable oils were increasingly absorbed into the testa of the bean seeds with length of storage. Thus the amount of oil to which the insects were exposed to progressively decreased, reducing the effectiveness of the oils as suggested by Hill (1981). However, during this investigation no evidence was obtained to show that the residual properties of the oils were lost by this mechanisim. Further investigations are needed to draw definite conclusions.

Despite the different properties of the vegetable oils, they were equally effective in preventing bruchid breeding and damage on the oil treated bean seeds. The vegetable oils completely prevented the emergence of A. obtectus progeny for a period of upto six months. Although the toxicity of oil treated beans to the adult bruchids decreased with length of storage, the vegetable oils were still effective in inhibiting bruchid multiplication. The reason for this has not been elucidated. Pereira (1983), reported that there was no C. maculatus progeny emergence, from the adults exposed on cowpea seeds treated with neem oil and stored for three months, whereas when the cowpea testa were peeled off the cowpea seeds three months after treatment there was adult progeny emergence. This demonstrated that the toxic activity of the neem oil was retained in the cowpea testa. Similarly it is possible that the toxic activity of the vegetable oils could have been retained in the testa of the oil treated bean seeds, thus preventing the bruchids from breeding for a period of upto six months. This may also provide a possible explanation as to why with length of storage, the oil treated bean seeds were more effective in preventing the bean bruchids from breeding than affecting the survival of the adult bruchids.

The vegetable oils all significantly reduced the fecundity of bean bruchids exposed to oil treated beans. Fecundity studies showed that some oils did not completely inhibit fecundity. On the other hand, other observations in this study revealed that adult progeny emergence from the oil treated beans was completely inhibited. Therefore, it could be concluded that in conjunction with reducing oviposition, the oils caused mortality of the immature stages of the bean bruchid. This is confirmed by results of various previous workers who found that vegetable oils exhibited significant ovicidal properties (Sangappa, 1977; Singh et al, 1978; Pandey and Varma 1982; Messina and Renswick, 1983). Singh et al (1978) and

Pandey and Varma (1982) using C. maculatus eggs demonstrated that vegetable oils entered the eggs through the micropyle and killed the embryo. Larvicidal properties of vegetable oils have also been reported by Schoonhoven (1978) and Messina and Renwsick (1983). This phenomenon of the ovicidal and larvicidal action could provide possible explanations for the lack of progeny emergence of the bean bruchids in the oil treated beans despite some oviposition by some adult bruchids.

The vegetable oils topically applied to adult bruchids were toxic and reduced their longevity. It is clear that vegetable oils contain certain insecticidal properties, which vary from oil to oil. The mode of action of vegetable oils on stored product insects is however not completely elucidated. Tikku et al (1981) pointed out that a previous consideration that, the insectiicial effect of vegetable oils on storage insect pests is due to the interference with the respiratory mechainism was not valid. Bhatnagar - Thamal and Pal (1984) have observed that garlic oil induces similar symptoms of poisoning in larvae and adults of the housefly Musca domestica nebulo Fabr and the khapra beetle Trogoderma granarium Everts, as those induced by organic insecticides, known to express their lethality by affecting various physiological and biochemical processes including disruption of nerve impulses (Narahashi 1971). It is likely that vegetable oils act as contact insecticides, and possibly interfere with the functioning of the nervous system. Nevertheless this needs to be accertained.

In conclusion, many chemical insecticides have been found to be effective in protecting stored produce from insect infestation, but for a small scale farmer such inputs are expensive and often not easily available in rural areas. It is essential that alternate sources of insecticides be found that are not only safe, effective, economical and locally available in rural area, but also that may be applied without the need of complex application equipment. It is proposed that vegetable oil treatment may provide another inexpensive and convenient method for preventing insect infestation of stored produce on small scale farms and in households. Vegetable oil is a product found in every household and can be easily applied to seed by manual mixing.

Finally, from these studies, it is clear that the protective action of castor, clove, maize germ, almond, olive and mustard oils applied to bean seeds, is by a combination of toxicity to adult bruchids, reduced adult progeny emergence, reduced fecundity, as well as by residual persistence of vegetable oil toxicity. These studies also showed that the vegetable oils were insecticidal when applied directly to adult bruchids. Further research could be directed towards the analysis of toxic factors, for example triglycerides in these oils. Studies on the exact mode of action of these oils would also help to confirm the insecticidal properties of vegetable oils against _A. obtectus_.

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