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Effects of Turmeric oil, Sweetflag oil, and Margosan-O on the corn weevil *Sitophilus zeamais* Motsch.

CESAR G. DEMAYO[1], JOSE WIGAN P. DALUGDUG,[2] and ADELINA A. BARRION[2]

Abstract

The toxicity of three botanical extracts, namely turmeric oil, sweetflag oil, and Margosan-O on the corn weevil S. zeamais evaluated by determining percent mortality of the insects on each treatment. Amylase, acetylcholinesterase (ACTH), non-specific esterase activities, and total soluble proteins in treated insects were determined through spectrophotometry. The relative toxicity of the extracts are ranked as follows: turmeric oil > sweetflag oil > Margosan-O. Turmeric oil reduced amylase activity most, and Margosan-O the least. ACTH production generally decreased in all of the treatments of oil derivatives, with turmeric oil causing the highest while Margosan-O showed the reverse. Non-specific esterase activities, generally increased. Esterases using beta-napthyl esters as substrate showed greater activity than using alpha-napthyl esters. Margosan-O induced the highest esterase activity and turmeric oil the lowest. The total protein activity decreased as the concentration of the oil increased. Treatments with turmeric oil resulted to lowest protein content, followed by treatments with sweetflag oil and Margosan-O.

Key Words: *Azadirachta indica, Sitophilus zeamais*, turmeric, sweetflag, esterase

Introduction

Modern technology has accelerated grain production in Asian countries. Gains have been due to the use of high yielding varieties, improved irrigation, fertilizers, and crop protection measures involving the use of pesticides. However, such gains in production have been lessened considerably due to postharvest losses, caused mainly by infestation of storage pests.

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[1] Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology

[2] Genetics and Molecular Biology Division, Institute of Biological Science, College of Arts and Science, University of the Philippines Los Baños

Infestations by storage pests result to quantitative and qualitative losses. Losses in quality greatly decrease not only the market price but also the nutritive value of the grains.

In corn, the most common stored grain pest is the corn weevil *Sitophilus zeamais* Motsch. *S. zeamais* is a storage pest of corn. It belongs to family of Curculionidae or true weevils. Its prothorax is very densely set with round irregularly shaped punctures. The elytra has intervals usually distinctly narrower than striae of strial punctures; elytra has four reddish spots. Its hindwings are always present. The size vary from 4-5 mm. The larvae have no legs, and are stout and slightly curved. The larvae live entirely within the kernel, and produce white powdery excreta which, in large quantities, makes the grain unpalatable (Imms, 1951).

Morallo-Rejesus and Javier (1976) found that *S. zeamais* multiplies rapidly on sorghum than on corn and was more dominant in milled rice and corn over *S. oryzae*. This was due to the former's greater intrinsic rate of increase. In some instances, *S. zeamais* have been known to co-exist with *S. oryzae*, but the former maintains the competitive advantage.

Control of this pest is done by fumigation which makes use of synthetic insecticides and air-tight storage facilities. However, such a method is impractical if done in small scale and still liable to frequent reinfestation. Furthermore, majority of insecticides are expensive and hazardous to the environment and non-target organisms. Moreover, insecticides leave toxic residues in grains and bring about insecticide resistant strains of insect pests. Under such circumstances, search and development of an effective, inexpensive, pest specific, non-toxic to beneficial and non-target organisms, and less prone to pest resistance grain protectants was undertaken (Saxena, 1988).

Under these criteria, plant derivatives which have been traditionally used thru the ages by farmers showed a promising potential as an ideal source of grain protectants. Past testings have shown that they act as toxicants, oviposition deterrents, antifeedants, sterilants, repellents, and growth inhibitors among others. Oils of sweetflag (*Acorus calamus* [L.]), turmeric (*Curcuma longa* [L.]), and Margosan-O, a neem derivative have shown encouraging potentials as grain protectants. Isolated compounds from these plant oils were seen to be active against different species of insect pests.

Some naturally occurring compounds have been isolated from plants and shown to be active against different species of insect pests. One of which is *A. calamus* belonging to the family Araceae. Its root is believed to possess stimulant, toxic and antispasmodic properties (Mukerjea and Govind, 1959). It has attracted the attention of many workers (Mironov 1940, Subramaniam 1942, 1949, Israel and Vedamurthy 1953, Dixit et al. 1956, Mukerjea and Govind 1959). for its toxicity. Chopra et al. (1965) showed that rhizomes of *A. calamus* contained an essential oil composed of 82% of asarone which has powerful insecticidal activity.

C. longa, is a tropical herb of Zingiberaceae family indigenous to Southern Asia. Its aromatic yellow powder from its rhizome was used by Asian countries for many centuries as dye, condiment, medicine, and as an insect repellent in India (Sreenivasanurthy and Krishnamurthy, 1959, Watt and Breyer-Brandwijk, 1962). Turmeric contains an odoriferous oil which has turmerones as the chief component (Sesquiterpene ketones in the form of turmerone and ar-turmerone) (Mima, 1959, and Salzer, 1977). Su et al. (1982) have isolated these compounds and described them as a colorless oil (ar-turmerone) and a pale yellow oil with a faint sweet odor (turmerone).

Neem (*Azadirachtin indica* A. Juss) has been used in rural India as a natural insecticide.

This plant is characterized by its bitter taste to which its insecticidal properties is attributed. Moreover, its derivatives consist of limonoids, azadirachtin being the most active. Azadirachtin have been found to cause diverse behavioral and physiological effects on storage pests (Habacon, 1989). Neem derivatives were observed to be effective against 123 species of insect pests, including storage pests (Jacobson, 1986). One such derivative is Margosan-O, a neem-based commercial insecticide with insecticidal activity similar to that of the other neem-rich derivatives. It has been hailed as one of the best new pesticides capable of preventing toxic build up and increased resistance of the insects. It works as a repellent, an antifeedant, and as a growth regulator through hormonal disruption. Margosan-O contains 3000 ppm azadirachtin and is stable under ambient conditions for up to one year, and up to two if refrigerated.

It has shown protective activity in excess of 21 days after which it biodegrades in the soil. Its residual cake is said to have nematicidal activity and enriches the soil when plowed under (Neem newsl., 1988).

Majority of the studies done using plant extracts focused on the physiological and morphological effects on the insect pest. But very few have tackled on the biochemical effects of these extracts, specifically on the activity of the enzyme and total proteins. This study, therefore, investigates the effect of sweetflag, turmeric, and Margosan-O oils on the activity of amylase, acetylcholinesterase (ACTH), esterase enzyme, and total proteins in *S. zeamais*.

Materials and Methods

Collection of Insect Samples. *S. zeamais* were mass cultured in corn media at the Department of Entomology, International Rice Research Institute. The adult corn weevils were collected from their media through strainers of varying pore diameter. The strained corn weevils were collected individually and placed in covered containers. The insects were starved one day prior to treatment.

Treatment of Insects. Six kilograms of corn grains used as media were brought from the market. Fifty grams of the media were weighed using the Mettler electronic balance. Three replicates per treatment were prepared. Sterilized cans were used as containers of the media. The cans were properly labeled.

Margosan-O, turmeric oil and sweetflag oil were provided by the Department of Entomology at I.R.R.I. and the following dosages were prepared: 0, 200, 400, 800, 1600, 3200, 5000, 10000, 25000, 50000 ppm.

The weighed amount of each of the three oils were dissolved separately in 20ml acetone. The oil-acetone solutions were stirred until homogeneous solutions of each were attained. The oil-acetone solution of each of the three oils was added slowly to their respective media with constant stirring to facilitate a homogeneous distribution of the oil. The treated media of the three oils were placed uncovered in the fumehood overnight to ensure a thorough evaporation of acetone.

Fifty adult individuals of *S. zeamais* were placed in each replicate of the corn media in each oil. The cans were covered with fine-meshed cloth fastened tightly with rubber bands. The insects were than allowed to feed on the treated media in each oil for one week.

Collection of insects for mortality test and analysis was done by straining, using a wire strainer with decreasing pore diameter. The gathered insects for each replicate in each were then killed in the freezer with the temperature of -10 C.

The frozen insects were washed with cold distilled water. Foreign materials mixed with the insects were also removed. The insects were placed in a homogenizer with 0.04M phosphate buffer. The amount of phosphate buffer used per replicate was based on the number of insects found in each replicate (4 insects/ml.). They were manually grounded and poured into labelled test tubes. The test tubes were centrifuged. After the debris settled, the tubes were placed in the freezer. The same procedure was followed in all replicates in each oil variety.

Enzyme Assays

Amylase assay. One percent starch solution of 0.5 ml was pipetted into a series of labelled test tubes and a blank. Enzyme extracts of 500 ul was placed in each tube and 500 ul distilled water to the blank. The test tubes were then incubated for three minutes after which 1 ml of dinitrosalicyclic acid (DNSA) color reagent was added to each tube. The tubes were placed in a waterbath for 5 minutes and subsequently cooled in room temperature of 25oC. Three milliliters of distilled water was added to each tube. Absorbance was then read at 640 nm using a spectrophotometer. Micromoles of maltose were generated from the standard curve. The amount of maltose generated was computed per insect.

Esterase assay. Four milliliters of 0.04M phosphate buffer (ph 8) was pipetted into a series of labelled test tubes and a blank. Twenty five microliters of B-naphthyl acetate was added to each tube and to the blank. Incubation at 27 C for 30 minutes followed. After incubation, 1 ml of diazobluelauryl sulphate solution (DBLS) was added for color reaction. Absorbance was read at 555 nm using a spectrophotometer. Same procedure was followed in alpha esterase using 600 nm.

Acetylcholinesterase assay. A run typically uses: 40 ml pH 8 phosphate buffer, 300 ul sample, 100 ul dithiobisnitrobenzoic acid (DNTB) and acetylthiocholine iodide (Ach I). The blank consists of buffer, substrates Ach I and DNTB solutions. Transmittances were read after 20, 40, 60, 80 minutes after preparation of samples at 412nm. Conversion to absorbances were done using the formula: 2 - log(T). The rate of production of ACTH/min. was calculated using the formula: rate = absorbance/min. divided by 1.36 x 10. After obtaining the rate of production per minute of ACTH, the amount of ACTH produced per minute was calculated using the standard curve as reference.

General protein assay. Several dilutions of protein standard containing from 0.2 to about 1.4 mg/ml were prepared. Standards and appropriately diluted samples of 0.1 ml were placed in clean, dry test tubes. Sample buffer of 0.1 ml was placed in the "blank" test tube. Diluted dry reagent of 0.5 ml was added. Mixing several times by gentle inversion of the tubes were done. Excessive foaming in the tubes were avoided. After a period of 5 minutes to 1 hour, OD versus the reagent blank were measured. The OD versus the concentration of standards were plotted. And unknowns were read from the prepared standard curve.

Results and Discussion

The toxicity of the oil derivatives of sweetflag, turmeric and neem on *S. zeamais* is presented in Table 1. Analysis of variance revealed significant effects ($P \geq 0.05$) of the oil derivatives against the corn weevil.

Table 1. Toxicity of different concentration of sweetflag oil, turmeric oil, and Margosan-O on *S. zeamais*.

TREATMENT	MEANS (% MORTALITY)		
	Sweetflag oil	Margosan-O	Turmeric oil
200	3.40 g	12.03 g	42.13 ef
400	2.57 g	1.63 g	73.07 b-d
800	17.87 g	4.27 g	83.03 a-d
1600	43.77 e	12.37 g	95.83 ab
3200	71.90 cd	4.27 g	100.00 a
5000	79.90 a-d	5.03 g	100.00 a
10000	92.70 a-c	11.67 g	100.00 a
25000	70.00 d	22.80 fg	100.00 a
50000	100.00 a	70.20 d	100.00 a
Control	0.00 g	0.00 g	0.00 g

* In a column, unsorted means with a common letter are not significantly different at 5% level of DMRT.

Increasing pattern of mortality effects by the Sweetflag oil was observed. There were however, some variations observed in the pattern of toxicity on the different treatments. At concentrations lower than 1600 ppm, mortality was high although not significant with respect to the control. At higher concentrations, mortality was significantly high. Increasing mortality with increased concentration of the oils may be attributed to antifeedant effects of the oil.

The results observed with Margosan-O was similar to that of sweetflag oil. There was also an increasing trend in toxicity as the concentration of the oil was increased .

Analysis of variance showed significant effects of the treatments on mortality ($P \geq 0.05$). There are some small variations however as to the extent of toxic effects of the oil when compared to the control. This was generally attributed to the antifeedant properties of the oil. Compared to the results of an earlier study by Gayapa (1989), the results of this study confirms the less susceptibility of *S. zeamais* to neem oil. Higher dose (eg 50000 ppm) is needed to cause mortality of 50% unlike the other two oils which require very minimal amount to cause deaths.

Of the three oil derivatives, turmeric oil was observed to be the most toxic. Even at concentrations of 200 ppm, a substantial increase of almost 42.13% was already seen. All percent mortality values were highly significant when compared to the control. A definitive

trend can be clearly seen in turmeric oil. Increase in the concentration of the oil gave corresponding increase in mortality values. At 400 ppm alone for example, LD_{50} of turmeric oil was already reached and at 2300 ppm, 100% mortality was achieved1). With this we can argue that turmeric oil poses as a good grain protectant, since it requires only a minimal amount of the oil to cause death to the grain pest.

In general, the trend in the toxicity of the three botanical oil derivatives against *S. zeamais*, shows an increasing pattern as the concentration of the oil is increased. Turmeric oil was the most potent, followed by sweetflag oil and lastly, Margosan-O (Table 1).

Toxicity is the intensity of the virulence of a poison. Several studies have shown that indigenous plant materials traditionally used as grain protectants exhibit promising insecticidal properties. Sweetflag oil (*A. calamus*) was reported to be toxic to house insect pests in solvent extracts and in powdered form (Mironov, 1940, Subramaniam, 1942, Dixit et al., 1956). Mukerjea and Govind (1959) studied the toxic effect of *A. calamus* rhizome as compared to DDT. They reported that as a stomach poison, the ether extract was toxic to the larvae of *B. mori* (silk worm), and as a contact poison, the petroleum ether extract was 17 times less toxic than DDT against *M. nebulo*. Good control of *Callosobruchus chinensis*, *S. oryzae*, *Corcyra cephalonica*, and *Trogoderma granarium* were obtained when fed with treated seeds, cereals, and pulses of oil and crushed rhizomes of *A. calamus* (Yadava, 1971, Saxena and Srivastava, 1972). Further studies made by Toetia and Tewari (1977) showed that ether and petroleum ether extracts based on contact toxicity were lethal to the adults of *Sitotroga cerealeila* Oliv.. In a compiled report by Jilani (1984), *A. calamus* gave the highest insecticidal activity against *C. analis* and *S. oryzae*. Moreover, studies done on the *A. calamus* oil against four species of insects (*R. dominica*, *T. granarium*, *S. oryzae*, and *C. analis*), showed 100% mortality at different rates of application. Using filter papers, 100% mortality rate of *R. dominica* and *S. oryzae* were attained at 25ug/cm^2 and *T. granarium* at 50 ug/cm^2, relative to their respective control. At 100 ug/cm^2, 100% mortality of *C. analis* was achieved, relative to its control. In surface treatment of the grains, minimum applications at 50 ppm and 100 ppm attained 100% mortality against *S. oryzae*, *C. analis* and *R. dominica*, *T. granarium*, respectively. The dispenser technique, showed 100% mortality at 400 ppm in four insects, relative to their respective control. Residual toxicity was observed less in extracts of *A. calamus* (Mukerjea and Govind, 1959, Teotia and Pandey, 1979).

Using neem oil, Gayapa (1989) showed increasing % mortality in *S. zeamais* and *S. oryzae*, and slight toxicity in *T. castaneum*. The study elaborated *S. zeamais* being the most susceptible. Likewise, Habacon (1989) concluded *S. zeamais* was the most sensitive to azadirachtin in terms of toxicity.

The effects of sweetflag oil, turmeric oil and Margosan-O on the total proteins per individual *S. zeamais* were also investigated. The general pattern was decreasing in the amount of the protein as the concentrations of the oil were increased. The effect of each of the oils was however, only significant in sweetflag oil and Margosan-O (Tables 2a, 3a and 4a). Nevertheless, the different treatments have directly affected protein activity in the treated insects ($P>0.05$). Comparison of means showed differences in activities between insects treated with different concentrations of the oil (Table 2). Relative to the control, total soluble proteins per insect were observed to be minimal in all treatments when compared to the control. This observation was true to all the oil derivatives (Tables 2-4). The oils are believed to have affected the operations of metabolic pathways (Gomez et al. 1983 and

Amoranto 1989).

Table 2. Effect of different concentrations of sweetflag oil on total protein content (microgram/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	10.858	14.747	11.767	12.457 b
400	21.818	19.545	20.101	20.488 a
800	17.525	12.727	21.818	17.357 ab
1600	23.030	15.202	19.293	19.175 a
3200	19.798	22.424	17.525	19.916 a
5000	19.040	19.293	19.545	19.293 a
control	17.979	24.293	23.030	21.767 a

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 3. Effect of different concentrations of Margosan-O on total protein content (microgram/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	13.131	13.535	11.565	12.744 de
400	22.424	16.111	17.980	18.838 a-c
800	12.727	15.202	19.798	15.909 b-d
1600	11.565	11.212	10.858	11.212 e
3200	19.040	21.818	17.525	19.461 ab
5000	18.232	20.101	19.040	19.124 a-c
10000	13.131	12.323	18.485	14.646 c-e
25000	20.101	20.101	18.788	19.663 ab
50000	14.747	20.353	17.752	17.617 a-c
control	17.979	24.293	23.030	21.767 a

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 4. Effect of different concentrations of Margosan-O on total protein content (microgram/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	14.747	15.202	29.966	19.972 a
400	11.919	19.040	16.565	15.841 a
800	18.964	16.111	21.818	18.964 a
control	17.979	24.293	23.030	21.767 a

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

The effects Amylase activities were also investigated. Amylase is an enzyme that cleaves the glycosidic bond of starch, involving the uptake of one molecule of water in the

hydrolysis of one bond, to yield saccharides. Alpha amylase attacks randomly and degrades dextrin and then maltose and glucose. While, beta amylase hydrolyzes maltose only from one end. Branch linkages of the amylopectin are not attacked by beta amylases (Salisbury and Ross, 1969). Amylases are found in the endo-and ectoperitrophic spaces (Terra et al., 1979).

The amount of amylase activity is always correlated with the amount of starch present in the insect's body. The high amount of starch induces the synthesis of amylase to facilitate the hydrolysis of starch. High amylase activity for example, indicates high amount of starch present in the insect's body. Therefore, all factors affecting the intake of starch by the insect subsequently affect the amylase activity.

The amylase activities of *S. zeamais* treated with sweetflag oil, turmeric oil, and Margosan-O are shown in Tables 5,6,7. The F-test conducted on the three oil varieties were significant ($P>0.05$) except for Margosan-O . Comparison of means show the amylase activity in all treatments of the two oil derivatives were significantly lower compared to the control. The F-test in Margosan-O was not significant but qualitative inspection of the data show all treatments had relatively lower amylase activity when compared to the control.

Table 5. Effect of different concentrations of sweetflag oil on the activity of amylase (microgram/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	1.679	1.472	1.576	1.576 cd
400	2.012	1.804	2.467	2.094 b
800	1.804	1.472	2.012	1.763 bc
1600	2.012	1.680	1.783	1.825 bc
3200	2.363	1.804	2.115	2.094 b
5000	1.244	1.307	1.368	1.306 d
control	2.840	2.965	2.965	2.923 a

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 6. Effect of different concentrations of turmeric oil on the activity of amylase (microgram/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	1.244	2.592	1.057	1.631 b
400	0.560	1.681	1.472	1.238 b
800	1.244	0.954	0.954	1.051 b
control	2.840	2.965	2.965	2.923 a

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 7. Effect of different concentrations of Margosan-O on the activity of amylase (microgram/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	1.472	2.072	2.239	1.928 a
400	3.089	1.679	2.488	2.419 a
800	3.214	2.841	1.907	2.654 a
1600	1.368	1.472	3.338	2.059 a
3200	2.364	2.364	2.717	2.482 a
5000	2.115	0.207	2.012	1.445 a
10000	1.907	2.841	0.981	1.910 a
25000	2.717	2.841	2.115	2.558a
50000	2.841	2.488	1.679	2.339 a
control	2.840	2.964	2.965	2.923 a

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

The results shown in this study are consistent with the result that amylase activity will decline by an antifeedant effect such as the case of fentin acetate (Ascher and Ishaaya, 1973).

Repellency is characterized by the movement of an insect away from a chemical stimulus. A relative study done on turmeric oil, sweetflag oil, neem oil, and Margosan-O showed increasing repellency of the red flour beetle *Tribolium castaneum* with increasing concentrations of the test materials. Turmeric was the most repellent, followed by neem oil, sweetflag oil and Margosan-O (Jilani et al., 1988).

A similar study done on *R. dominica* demonstrated strong repellency more than 50% up to 8 weeks. At 400 ug/cm2 turmeric oil was repellent than Margosan-O and neem oil, but at par with sweetflag oil. It verified past results and the conclusion that repellency is dependent on the rate of application of the test plant materials (Jilani and Saxena, 1989).

In both studies, decrease in residual repellency of turmeric oil and sweetflag oil relative to neem oil and Margosan-O were observed due to higher volatilization rate of the active components of turmeric and sweetflag oils. These compounds have much lower molecular weights than azadirachtin, the active component of neem (Jilani et al., 1988). Other studies in the past, generally showed repellency against *T. castaneum* by *A. calamus*, *A. indica*, *V. afficinalis*, *X. armatum*, *A. maritima* (Jilani, 1984). Furthermore, petroleum ether extract of turmeric was found to be the most repellent against *T. castaneum* when repellency of petroleum ether, acetone, and ethanolic extracts of leaves of neem and rhizomes of turmeric were compared by Jilani and Su (1983).

Antifeedant chemicals act by retardation or disruption of the insect feeding habits by rendering plants unattractive and unpalatable. The resulting action made by these chemicals is termed as the antifeedant phenomenon. In a study by Jilani and Saxena (1989), the antifeedant effect was evaluated in terms of the number and size of the feeding punctures made by the insect in treated and control filter paper disks. *R. dominica* showed significantly less punctures in treated filter papers compared to that of the control. Margosan-O treated filter paper had less feeding punctures compared with the filter papers treated with turmeric oil, sweetflag oil, and neem oil, which have more or less equal antifeedant effect.

However, the neem oil treated paper which have more feeding punctures relative to Margosan-O treated filter paper, showed smaller size punctures indicating a feeding deterrent effect. Insect feeding was found to be dependent also on the application of the plant products.

The effects of the extracts on acetylcholinesterase (ACTH) and nonspecific esterases were also studied. Esterase is an enzyme that catalyzes the hydrolysis of as ester bonds without using any high-energy cofactor. They are found in organ tissues and body fluids. Esterase metabolizes polar molecules with an ester bond. Present classification grouped esterases into arylesterases, B-esterases (aliphatic, acetylesterases, lipase) and carboxylesterases (Augustinson 1959, 1961). Because of their importance in insecticide toxicity, specifically acetylcholinesterase of the B-esterase group, considerable attention has been paid to esterases in insects (Oosterbaan and Jansz, 1965, Dauterman, 1976, 1985, Ahmad and Forgash, 1976, Brattsten, 1979, Junge, 1984, Junge and Klees, 1984, and Heymann, 1980). Acetylcholinesterase is said to be inhibited by organophosphate and carbamate insecticides, resulting to tremors convulsions and physiological disturbances to insects. However, insensitivity to these compounds has been cited as the probable cause (Wilkinson, 1976). Consequently, reduced affinity of normal substrates contribute to the insensitivity of the insects (Smissaert, 1964). Enabling the insects to detoxify these insecticides as shown by the green leafhoppers (Hama. 1983), which could eventually lead to resistance development.

The effect of three oil derivatives on ACTH activity in *S. zeamais* are shown in Tables 8, 9, 10. The F-test done in all three oils showed significant effects on ACTH production. A general decreasing trend in ACTH production was observed as the concentration level is increased except for Margosan-O. In sweetflag oil, an initial increase in ACTH production was observed at 200 ppm to 500 ppm concentration but soon showed a significant and abrupt decrease starting at 10,000 ppm, relative to the control. Initial increase in ACTH production indicated only tolerance of AChE to sweetflag oil at these levels, as shown by the response. The inhibition of the enzyme would only be reached at 10,000 ppm and at higher concentration. The same trend was observed in turmeric oil. a more definitive decreasing pattern of ACTH production with increasing concentrations was observed (Table 9). However, compared with sweetflag oil, susceptibility of the AChe was reached at a lower concentration level. At only 400 ppm, a marked decrease in ACTH production was observed. The decrease was significantly different from the control.

In Margosan-O however, ACTH production showed a significantly increasing trend at 5000 ppm to 50,000 ppm. The activity would seem to have become enhanced. It is possible that the insect may have developed the capacity to tolerate or detoxify the insecticide (Hama, 1983).

The nonspecific esterase activity was determined using alpha and beta naphthyl acetate in 25 ul enzyme extracts. Analysis of variance on both of these extracts showed significant tests ($P>0.05$). An increase in esterase activity was generally observed as the concentrations of the oils increased in both extracts. Beta esterase however, was more prominent than alpha esterase activity.

Sweetflag oil has effected an increases in both alpha and beta esterase activities (Table 11). Beta esterase activity however, decreased in turmeric oil-treated *S. zeamais*. Treatments were noted to be significantly different relative to the control but insignificant with respect to each other (Table 12). In the case of Margosan-O, increasing trend in esterase activity was observed (Table 13).

Table 8. Effect of different concentrations of sweetflag oil on acetychlinesterase activity (unit/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	0.021	0.026	0.025	0.024 c
400	0.035	0.029	0.049	0.038 bc
800	0.049	0.044	0.045	0.046 b
1600	0.059	0.044	0.047	0.050 b
3200	0.089	0.115	0.065	0.090 a
5000	0.055	0.055	0.055	0.055 b
control	0.024	0.024	0.017	0.022 c

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 9. Effect of different concentrations of turmeric oil on acetychlinesterase activity (unit/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	0.055	0.045	0.036	0.045 a
400	0.052	0.044	0.047	0.048 a
800	0.044	0.045	0.044	0.044 a
control	0.024	0.024	0.017	0.022 b

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 10. Effect of different concentrations of Margosan-O on acetychlinesterase activity (unit/insect) of *S. zeamais*.

TREATMENT	REPLICATE			
	1	2	3	Mean
200	0.026	0.026	0.036	0.029 b-d
400	0.042	0.027	0.047	0.039 b-d
800	0.031	0.025	0.041	0.032 b-d
1600	0.026	0.039	0.034	0.033 b-d
3200	0.026	0.035	0.021	0.027 cd
5000	0.030	0.034	0.041	0.035 b-d
10000	0.047	0.045	0.035	0.042 bc
25000	0.037	0.054	0.052	0.048 b
50000	0.052	0.071	0.099	0.074 a
control	0.024	0.024	0.017	0.022 d

* In a column, means with a common letter are not significantly different at 5% level of DMRT.

Table 11. Effect of different concnetrations of sweetflag oil on non-specific esterase activity of *S. zeamais.*

TREATMENT	MEANS (ESTERASE ACTIVITY)	
	Beta-esterase	Alpha esterase
200	0.191 cd	0.105 ef
400	0.308 b	0.181 d
800	0.272 b	0.158 de
1600	0.293 b	0.159 de
3200	0.293 b	0.185 cd
5000	0.247 bc	0.163 de
control	0.387 a	0.072 f

* In a column, treatment means having a common letter are not significantly different at 5% level of DMRT.

Table 12. Effect of turmeric oil on non-specific esterase activity of *S. zeamais.*

TREATMENT	MEANS (ESTERASE ACTIVITY)	
	Beta-esterase	Alpha esterase
200	0.215 bc	0.170 cd
400	0.268 b	0.179 cd
800	0.141 d	0.150 d
control	0.387 a	0.072 e

* In a column, treatment means having a common letter are not significantly different at 5% level of DMRT.

Among the three, Margosan-O induced the greatest esterase activity and turmeric oil the least (Table 11, 12, 13). Increase in esterase activity was generally associated with the insects capability to detoxify the toxic compounds. Esterases are involved in detoxification process in the insect's body. While decreases reflect the strength of the toxins in inhibiting esterase activity.

Table 13. Effect of Margosan-O on npn-specific esterase activity of *S. zeamais.*

Treatment	MEANS (ESTERASE ACTIVITY)	
	Beta-esterase	Alpha esterase
200	0.191 cd	0.105 ef
400	0.308 b	0.181 f
800	0.272 b	0.158 de
1600	0.293 b	0.159 de
3200	0.293 b	0.185 cd
5000	0.247 bc	0.163 de
control	0.387 a	0.072 f

* In a column, treatment means having a common letter are not significantly different at 5% level of DMRT.

Summary

S. zeamais, was treated with different concentrations of turmeric oil, sweetflag oil, and Margosan-O. The toxicity of the three oil derivatives against the weevil were noted. It revealed percent mortality to be directly proportional to the concentration of the oil. Turmeric oil had the highest percentage in inflicting death (85.45%), and Margosan-O the lowest (16.03%). Sweetflag oil registered a 53.57% in inflicting death to the insect. The surviving insects were homogenized and their enzyme extracts were subjected to total protein content, amylase, acetylcholinesterase, and esterase assays using spectrophotometry.

With regards to total protein content, the general trend was the decrease in content as the concentration of the oil increased. Insects treated with turmeric had the lowest protein content, followed by those treated with sweetflag oil, and Margosan-O. The decrease in total protein content may be attributed to the increase in food consumption which negatively affects protein synthesis.

Amylase activity decreased in all three oil derivatives, as the concentration increased. The reduction in amylase activity can be correlated with the strength of the antifeedant properties of the oils. Turmeric oil had the strongest antifeedant property and lowest amylase activity, trailed by sweetflag oil and Margosan-O.

The same trend was observed in ACTH production, except in Margosan-O which showed otherwise. The production of ACTH was inhibited most by turmeric oil, then by sweetflag oil and Margosan-O, respectively. Margosan-O increased ACTH production. It is hypothesized that the insect had developed the capacity to detoxify the neem-based insecticide.

Lastly, increase in esterase activity was generally observed. Esterases using beta-napthyl esters as substrates showed increased activity compared with esterase using alpha-napthyl esters in all three oil derivatives. In the three oil derivatives, Margosan-O induced the highest esterase activity and turmeric oil the lowest. Since the action of esterase is in the detoxification processes, the increase in toxic components of Margosan-O, which is less potent to the insect, enhanced the activity of esterase. On the other hand, due to the strength of the toxin in turmeric oil, reduced esterase activity occurred through inhibition.

The study showed that the three botanical extracts have the potential of being effective in controlling pests with turmeric oil as the most potent and effective among the three oil derivatives. Considering that in previous studies, only a small amount can already cause repellency, in this study, a higher concentration was needed to reduce physiological effects on protein activity, indicating that these botanicals are effective in pest control aside from being cheap. The three botanicals tested are good substitutes for chemical pesticides and vital in pest control management of stored grain pests, like *S.zeamais*.

References Cited

Abraham, C.C. s1972. Relative efficiency of some plant products in controlling infestation by *Sitotrega cereallela* Olivar (Seloctridae:Lepidoptera) infesting stored paddy in Kerala. Bulletin of Grain Technology. 10(4):263-266.

Ahmad, s. and A.J. Forgash 1976. Non-oxidative enzymes in the metabolism of insecticides, Drug Metab. Rev.. 5:141-164.

Amoranto, R.Jr. 1989. Spectrophotometric analysis of the effects of azadirachtin of amylase activity and total proteins in rice weevil, *Sitophilus oryzae* L., corn weevil, *Sitophilus zeamais* Mots. and the flour beetle, *Tribolium castaneum* Herbst. Undergraduate Thesis, U.P.L.B., Philippines.

Ascher, K.R.S. and I. Ishaaya 1973. Antifeeding and protease and amylase inhibiting activity of fentin acetate in *Spodoptera littoralis* larvae. Pesticide Biochemistry and Physiology, 3:326-336.

Augustinson, K.B. 1959. Electrophoretic studies on blood plasma esterases. Acta Chemical Scand., 13:571-592.

Augustinson, K.B. 1961. Multiple forms of esterase in vertebrate blood plasma. Ann. N.Y. Acad. Sci., 94:944-970.

Brattsten, L.B. 1979. Ecological significance of mixed-function oxidations. Drug Metab. Rev., 10:35-58.

Chopra, R.N. et al. 1965. Chromatographic estimation of asarones in Indian *Acorus calamus* L.oil (Tetrapoid variety). Jour Chromat., 17:195-196.

Dauterman, W.C. 1976. Extra microsomal metabolism of insecticides. Insecticide Biochemistry and Physiology, ed. C.F. wilkinson. New York: Plenum Publishing Co., pp. 149-176.

Dauterman, W.C. 1985. Insect metabolism: extrachromosomal. Comprehensive Insect Physiology, Biochemistry, and Pharmacology, eds. G.A. Kerkut and L.I. Gilbert. New York: Pergamon Press, Vol. 12, pp. 713-730.

Dixit, R.S.1956. Evaluation of *Acorus calamus* Linn. - An insecticidal plant of India. Jour. Sci Industri. Res., 15c(1):16-22.

Gayapa, R.G. 1989. Spectrophotometric analysis of the physiological effects of neem oil on amylase and non-specific esterase activities in the rice weevil, *Sitophilus oryzae* corn weevil *Sitophilus zeamais* mots. and the red flour beetle, *Tribolium castaneum* Herbst.

Undergraduate Thesis, U.P.L.B., Philippines.

Gomez, L.A., et al. 1983. Influence of nutritional characteristics of selected corn genotypes on food utilization by the rice weevil (Coleoptera:Circulionidae). J. Econ. Entomol., 76:728-732.

Habacon, C.J. 1989. Physiological effects of azadirachtin on acetylcholinesterase and non-specific esterases in rice weevil, *Sitophilus oryzae* L.,corn weevil *Sitophilus zeamais* and the red flour beetle, *Tribolium castaneum* Herbst. Undergraduate Thesis, U.P.L.B., Philippines.

Hama, H. 1983. Resistance to insecticides due to reduced sensitivity of acetylcholinesterase. Pest resistance to Pesticides, eds. G.P. Georgiou and T. Saito. New York: Plenum Publishing Co., pp. 299-332.

Heyde, J. R. C. Saxena, and H. Schmutterrer 1984. Neem oil and extracts as potential insecticides for control of hemipterous rice pests. Proceedings of the 2nd International Neem Conference, pp. 377-390.

Heyde, J.V.D., R.C. Saxena and H. Schmutterrer 1985. Effects of neem derivatives on growth and fecundity of the rice pest *Nephotettix virescens* (Homoptera:Cicadellidae). J. Plant Dist. Prot., 92:346-354.

Heymann, E. 1980. Carboxylesterases and amidases. Enzymatic Basis of Detoxification, ed. W.B. Jacoby. New York: Academic Press. Vol. 2:291-323.

Imms, G. 1951. General textbook of Entomology. Britain: Buttler and Tanner Ltd., pp. 519-520.

Israel, P. and G. Vedamurthy 1953. Indigenous plant products as insecticides. Rice Newsletter, 1(4):3-5.

Jacobson, M. 1981. Neem research in the U.S. Dept. of agriculture: chemical and cultural aspects. Natural Pesticides from the Neem Tree (*Azadiractha indica* A. Juss), eds. Schmutterrer, H., Ascher, K.R.S. and Rembold, H. Eschborn, West Germany: GTZ Press, p.33.

Jacobson, M. 1986. The neem tree: Natural resistance par excellence. Natural Resistance of Plants to Pests: Roles of Allelochemicals, eds. Green, M.B. and Hedin, P.A. Washington, D.C.: American Chemical Society, p. 220.

Jilani, G. and M.M. Malik 1973. Studies on neem plants as repellent against stored grain pests. Pak. J. Sci. Ind. Res., pp. 16,251.

Jilani, G. 1984. Use of botanical materials for protection of stored foodgrains against insect

pests. Planning Workshop on Use of Plant Materials for Pest Control, I.R.R.I., Los Banos, Laguna, Phillipines, pp. 1-22.

Jilani, G., R.C. Saxena, and B.P. Rueda 1988. Repellent and growth-inhibiting effects of turmeric oil, sweetflag oil, neem oil and Margosan-O on red flour beetle (Coleoptera:Tenebrionodae). Ent. Soc. of America, 1(4):1226-1230.

Jilani, G.,and R.C. Saxena 1989. Repellent and antifeedant effects of turmeric oil, sweetflag oil, neem oil and Margosan-O against *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Econ. Entomol., Vol. 82.

Jilani, G. and H.C.F. Su 1983. Laboratory studies on several plant materials as insect repellants for protection of cereal grains. J. Econ. Entomol., 76(1):154-157.

Junge, W. 1984. Carboxylesterase. Methods in Enzymatic Analysis, 3rd ed. Verlay Chenie, Miami, Vol. 4:8-14.

Junge, W. and H. Klees 1984. Arylesterase. Methods in Enzymatic Analysis, 3rd ed. Verlay Chenie, Miami, Vol. 4:8-14.

Ketkar, C.M. 1976. Modified neem cake manurial project. Utilization of Neem (*Azadirachta indica*) and its By-products. Bombay, India: Khadi Village Industries Commission.

Mima, H. 1959. Purification and stabilization of turmerone by thiorea adduct formation. Pharmaceutical applications of Inclusion Compounds III. Yakugaku asshi, 79:664-649.

Mironov, V.S. !940. *Acorus calamus* as an insecticidal and repellent preparation. Med. Parasit., U.S.S.R., Vol. 9:409.

Morallo-Rejesus, B. and P.A. Javier 1976. Laboratory assessment of damage caused by *Sitophilus spp.* and *Rhizopertha dominica* in stored cereals. Grain Quality Improvement. Malaysia, pp.220-223.

Mukerjea, T.D. and R. Govind 1959. A plant insecticide-*Acorus calamus* Linn.. Indian Jour, Ent., 21(3):194-205.

Neem Newsletter 1988. Margosan-O. New Delhi: Ind. Agri. Res. Inst. 5(1):5.

Osterbaan, R.A. and H.S. Jansz 1965. Cholinesterases, esterases and lipases. Comprehensive Biochemistry, eds. M. Florkin and E.H. Stolz. New York: Elsevier Publishing Co., Vol. 16, pp. 1-54.

Qadri, S.S.H., G. Usha and K. Jabeen 1984. Sub-acute dermal toxicity of Neemrich-100 to rats. Intl. Pest Control, pp. 26, 18.

Salisbury, F.B. and C. Ross 1969. Plant Physiology. California: Wadsworth Publishing Co., Inc., pp. 224-245.

Salzer, V.J. 1977. The analysis of essential oils and extracts (oleoresins) from seasonings. CRC Critical Review in Food Sciences and Nutrition, pp. 345-372.

Saxena, B.P. and J.B. Srivastava 1972. Effects of *Acorus calamus* L. oil vapours on *Dysderans koenigil* F. Indian Jour. of Experimental Biology, 10:891-898.

Shankar, T.N.B. 1980. Indian Jour. Exp. Biol., 18:73.

Smissaert, H.R. 1964. Cholinesterase inhibition in spider mites susceptible and resistant to organophosphates. Science, 143:129-131.

Sreenivasamurthy, V. and K. Krishnamurthy 1959. Food Science (Mysore), p.284.

Su, H.C.F., H. Robert and G. Jilani 1982. Isolation, purification and characterization of insect repellents from *Curcuma longa* (L.). J. Agri. Food Chem., 30:290-292.

Subramaniam, T.V. 1949. Sweetflag (*Acorus calamus*), a potential source of valuable insecticide. Jour. of the Bombay National History Society, 48(2):300-341.

Terra, W.R., C. Ferreira and A.G. De Bianchi 1979. Distribution of digestive enzymes among endo-and ectoperitrophic spaces and midgut cells of Rhynchosciara and its physiological significance. Jour. of Insect Physiology, 25:4870494.

Teotia, T.P.S. and G.C. Tewari 1977. Insecticidal properties of drupes of Dharek (*Melia azaderach*) and rhizomes of Sweetflag (*Acorus calamus*) against adult *Sitotroga cereatella* Olive.. Indian Jour. of Entomology, 39:222-227.

Watt. J.M. and M.G. Breyer-Brandwijk 1962. The medicinal and poisonous plants of Southern and Eastern Africa. Edinburgh, Scotland: E. and S. Livingstone, Ltd., p. 1062.

Wilkinson, C.F. 1976. Insecticide Biochemistry and Physiology. New York: Plenum Publishing Co., p. 786.

Yadava, R.L. 1971. Use of essential oil of *Acorus calamus* L. as an insecticide against the pulse beetle *Bruchus chinensis* L. Zeitseriff fur Angewandte Entomologie, 68(3):289-294.

Zhang, X. and S. H. Zhao 1983. Experiments on some substances from plants for the control of rice weevils. Journal of Grain Storage, 1:1.