THE POTENTIAL FOR NATURAL PRODUCTS AS GRAIN PROTECTANTS AGAINST STORED PRODUCT INSECTS

By

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I. INTRODUCTION

Botanical insecticides are natural products that are derived from plants. Prior to the second world war, pyrethrum, rotenone and nicotine commanded at least 20% or more of the commercial insecticide market (Isman, 1994). Currently only a handful of botanicals are approved for use in the United States of America, among them pyrethrum is the predominant one.

Insecticides used on or around grain are usually classified into five broad groups: knockdown agents, surface sprays, structural treatments, grain protectants, and fumigants (Snelson, 1981). The area of interest in this study is grain protectants. Grain protectants are insecticides, which when applied to grain prevent insect infestations from becoming established. In practice grain protectants will control light infestations present at the time of treatment, but, because this will accelerate the selection of resistant strains, it should be avoided. Storing grain in Oklahoma involves risk due to severe insect and mold problems, with losses exceeding $50 million each year (Cuperus et al., 1995). The most important factors affecting stored grain insect and mold population dynamics are grain moisture content, temperature, the time the grain is susceptible and accessibility to pests.

Currently synthetic insecticides are a method for insect pest control (Arthur 1994, Arthur 1998). However there is an acknowledged interest in replacing toxic chemicals with less hazardous means of control. Development of genetically resistant strains of stored grain pests, adverse environmental impacts and a growing demand for chemical free food products are aspects of this problem (McLaughlin 1994). Insect resistance to widely and repeatedly used insecticides such as malathion is widespread and very high (Zettler & Beeman 1995). In Oklahoma the red flour beetle and lesser grain borer have shown high resistance to malathion. Moderate resistance was exhibited for Reldan and light to moderate resistance to phosphine gas by lesser grain borer (Zettler & Cuperus 1990, Cuperus et al., 1995). Resistant strains develop through the survival and reproduction of individuals carrying a genome altered by one or more of many mechanisms that allow survival after exposure to an insecticide (Bratsten et al, 1986)

The use of plants as insecticides has been practiced for many hundred years. Extracts from plants that had been tested for their insecticidal activity are the essential oils and organic solvent extracts. Edible oils are locally used to prevent stored grains from insect attack in many countries of Asia and Africa (Shaaya et al., 1997). Outstanding examples of older biopesticides are nicotine, rotenone, and the natural pyrethrums, but the most prominent botanicals in recent years are the neem products, derived from the neem tree, *Azadirachta indica* A. Juss., family Meliaceae.

Neem is widely distributed in tropical countries and is considered as a multipurpose tree especially in rural areas. Almost every part of the tree is known to have some use. The limonoid compound from the Indian neem tree, azadirachtin, is a naturally occurring substance that belongs to an organic molecule class called tetranortriterpenoids. This tetranortriterpenoid plant limonoid has potent insect antifeedant and growth disrupting properties (Schumutterer et al, 1981, Schumutterer and Ascher, 1984,).

Interest in botanical insecticides, especially in neem based products, has grown over the last decade as more insecticides are lost due to environmental and food safety problems. Currently several Indian companies or institutions commercially produce neem- based insecticidal formulations for the control of cutworms, whiteflies and other insect pests. In the U.S., the Environmental Protection Agency (EPA) has granted registration for "Margosan-O" (W.R. Grace & Co., Cambridge, MA, USA), an azadirachtin –enriched formulation, for use on non-food crops and ornamentals. Recently other products like Ecozin and Amazin have been registered for field crops insect control (PNN Notification: 2000-16).

Until now the only botanically based insecticides used on a large scale on stored commodities are pyrethrins, which are extracted from chrysanthemum flowers. (Weaver and Subramanyam, 2000). The majority of the food processing uses of natural pyrethrins is in combination with the synergist piperonyl butoxide. Piperonyl butoxide is a pale yellow, odorless oil, which is resistant to light and hydrolysis unlike pyrethrins, which are unstable to sunlight and degraded rapidly by alkalis. Hence the lethal action of the pyrthrins is increased by the addition of the synergist piperonyl butoxide (Snelson 1981).

Many of the botanicals tested against stored-product insects have moderate insticidal activity at best. Nearly 25% of the 866 plants discussed in Prakash and Rao (1997) were tested against stored–product insects. Among recently tested products for the control of stored -product pests is crude cedar oil. Crude cedar oil is extracted from eastern red cedar, *Juniperus viriniana* (L).

Botanical insecticides such as neem, pyrethrins and essential oils of many plants kill storage pests when applied at high levels but not equal in effectiveness to synthetic insecticides when applied at low levels (Sehgal and Ujagir, 1990). Initial research with proprietary materials suggests that diatomaceous earth (DE) a naturally occurring insecticidal desiccant can act as a synergist for botanical insecticides. DE is effective on grain by itself but is not desirable because the levels that are needed for food insect control reduces the test weight and value of the grain.

The objectives of this study were:

1. To evaluate the efficacy of natural products from three broad catagories as grain protectants against four-stored product insect species; lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L), red flour beetle, *Tribolium castanum* (Herbst), and Indian meal moth, *Plodia interpunctella*.

2. To examine potential synergistic effect of natural products combined with diatomaceous earth, or DE.

II. LITERATURE REVIEW

Stored product insects and their importance

Insects that infest stored agricultural commodities cause substantial economic and quality loss. The worldwide average post harvest loss is considered to be close to 10%. (Pedersen 1978), and in the United States the yearly post harvest loss is estimated to be more than $5 billion dollars (Pimentel 1991).

Hundreds of species of insects and mites are known to infest stored commodities (Gorham 1991). The Oklahoma stored grain system is composed of 36% on –farm storage and 64 % commercial storage (Anderson 1988) and a survey of Oklahoma grain producers and elevator managers found that both groups ranked insects as their greatest storage problem (Cuperus et al. 1990, Kenkel et al. 1994). Stored grain insects cause the major grain damage in Oklahoma (Noyes et al., 1991). Internal feeding grain insects, like the lesser grain borer and rice weevil, penetrate and destroy whole sound kernels, causing insect damaged kernels (IDK). External feeding grain insects feed on broken grain kernels, and cause less damage than primary insects. They include the rusty grain beetle, flat grain beetle, saw-toothed grain beetle, red flour beetles, the confused flour beetle, Indian meal moth and others.

High grain temperatures and moisture, along with dockage and broken kernels, provide conditions that accelerate mold and insect development. Maximum grain moisture content for aerated grain ranges from 7 to 14 percent according the grain type and storage time (Krischik, et. al., 1995). Insect infestation magnifies mold problems in grain by exposing otherwise hidden endosperm surfaces to molds, transporting mold spores to new areas in the grain, and encouraging mold germination including those that produce mycotoxins. Insect and mold metabolic activity can raise grain temperatures to 110°F (43°C). Beside direct feeding and encouraging mold problems, insects lower the quality of the grain by contaminating it with cast skins, fecal material, webbing and body parts. Insect feeding can also contribute to lower test weight and presence of musty odors. The Grain inspection, Packers and Stockyards Administration (GIPSA) labels grain as sample grade, if the grain has an unacceptable odor or contains excessive amounts of stones animal filth, toxic substances, or other inferior conditions (Krischik, et. al., 1995). During the late 1800s, the growth of the grain markets and production in the U. S. generated a need for standard system of inspecting and grading grain. GIPSA is an agency with in the United States Department of Agriculture, and its responsibility is to administrate a national inspection and weighing program for grain, oil seeds, pulses, and others. The Food and Drug Admistation (FDA) is a regulatory agency of the federal government. Insect contamination is an integral part of the inspection by FDA. Reason for inspecting could be regulatory or commercial purpose. Where private companies or marketing authorities are involved in the handling of grain and storage products, these organizations are concerned with the inspection of their particular cereal or manufactured goods.

It is important to control insect population size before insect feeding and mold germination irrevocably damages the grain. Grain should be inspected every 21 days when its temperature exceeds 60°F (15°C) (Krischik, et. al., 1995). Plastic pitfall traps should be checked for the species type and number of insects. The number of insects found in a trap should be recorded and charts constructed so that changes in population size can be easily noticed. Increase in number of insects indicates that management tactics need to be changed to prevent levels of infestation that damage the grain. Screening or sieving, examining kernels for damage, checking grain for webbing and odor can also be done to inspect grain.

Insect infestation in most grain storage facilities is managed through the use of insecticides. The behavioral and biological characteristics of the groups of stored-grain insects are important to management decisions. For example, an insecticide treatment incorporated in the surface part of a grain mass may provide adequate control of the Indian meal moth, but such an application will not control a secondary beetle or weevil problem that already exists in the core of the grain mass. Cleaning grain to remove fine material will greatly contribute to controlling infestations of several secondary beetles, but this action is of only limited value for reducing infestations of weevils or other internal feeders.

In Oklahoma residual grain protectants give short- term protection because of chemical breakdown and resistance (Noyes et al., 1991), and this leads to rapid fall insect build up. On the other hand numerous residual insecticide applications and fumigation's occur during transportation and storage (Cuperus et al., 1990). Such treatments result in low profitability and increase risk of workers to insecticide exposure hazards. Data collected in 1995 from domestic elevators and storage's showed that 71% of the wheat sampled had malathion residues (USDA 1995). Food safety concerns play an important role in postharvest management. National and regional surveys have indicated that 75% of the consumers are very concerned with the safety of the food supply (Kenkel.et.al., 1994). In addition to insecticide residue problems in treated grain, insecticide resistance is becoming an increasing concern. Studies indicate that insects have developed resistance to all major classes of insecticides and will develop resistance to future insecticides as long as present application techniques and use patterns prevail (Brattsten et al., 1986.) Resistance is the genetically based ability of insects to tolerate a dose lethal to normal populations. Resistance to widely and repeatedly used insecticides such as Malathion is widespread and very high in stored product insects (Zettler & Beeman 1995). Therefore, Oklahoma State University no longer recommends malathion for grain treatment because of high insect resistance and chemical break down in warm and relatively moist grain (Cuperus & Noyes 2001).

Controlling established infestations of stored-grain insects is often difficult. Usually preventing infestations is more profitable and successful than eliminating problems that have already developed. Preventive steps include sanitation, application of an insecticide to the empty storage, proper cleaning and drying of grain, application of protectant insecticides when extended storage is planned, and adequate aeration for temperature and moisture management.

Following are brief summaries of the biology of insects used in this research.

Sitophilus oryzae (Rice weevil)

The rice weevil (*Sitophilus oryzae*) is a primary stored- product pest and can be found in both tropical and temperate areas on cereal grains. It belongs to the family Curculionidae, which is very large and many of its members are pests of stored product or timber, either as larvae or adults. The rice weevil has functional wings and can fly. It has small round pits on the surface of the thorax, and red yellow markings on the forewings. It is less tolerant to low temperature than granary weevil, which is only common in temperate zone. It is often confused with the maize weevil, but it is somewhat smaller than maize weevil usually 2-3mm in size and both species can only be reliably distinguished by examining the genitalia (Halstead 1963, Hidayat et al 1996).

As eggs are laid inside the kernel both larvae and adults of rice weevil feed inside the kernel too. Hence it is grouped as internal feeder. This weevil is an important insect pest in the other parts of the world but it is seldom found in Oklahoma grain storage's, since it requires for its development grain moisture content more than 12% (Cuperus et. al., 1995). Most grain is stored in Oklahoma at around 11.5% moisture content or less.

Dispersal of this pest depends on flight as well as passive transport in trade. This insect pest can also attack the grain before harvest. It attacks wheat, barley, paddy, rice, millet, sorghum and maize, among whole grains, but it is of less importance in milled grains. The usual method of controlling grain that is already attacked by rice weevil is fumigation. Preventive methods such as sanitation can limit the incidence of attack by this stored product insect.

Rhyzopertha dominica **(Lesser grain borer)**

The Lesser grain borer *Rhyzopertha dominica* (F.) is a serious primary stored product pest, which is small and highly destructive. This pest belongs to the family of Bostrichidae and is related to certain wood boring insect species (Borror et al 1989). Lesser grain borer is considered to be native to India (Schwardt 1933). Beside Lesser grain borer, only *Prostephanus truncatus*, the larger grain borer, which attacks maize grain, and tubers in the southern states of USA, Central and South America (Shires

1979), and *Dinoderus minitus* the bamboo borer, which attacks bamboo, grain, flour and tobacco are of any significance as pests of stored products.

The female lays her egg in clusters within cracks and crevices in the grain or singly amongst the grain dust (Potter 1935). The newly hatched white cylindrical larva, either enters the grain directly or crawl and feeds in the grain mass until it reaches third or fourth larval instar and then bore in to the kernel. Both larvae and adults are voracious feeders and leave fragmented kernels and powdery residue. The beetle is a strong flyer and cosmopolitan in its distribution (Potter 1935).

The life cycle of *R.dominica* can be completed over 20-38° C, although the optimum for increase lies between 32° C and 35° C (Birch 1953). In contrast with grain weevils, oviposition is not inhibited until moisture content is less than 9%. The first instar larva is susceptible to desiccation at high temperatures and low humidities, especially if access to the grain is difficult.

R. dominica adults move freely from one storage to another but within a grain mass, the species disperses rather slowly and is generally found in the driest part of the mass. Field infestations are rare presumably because of the limited ability of the adults to cling to surfaces and to penetrate undamaged grains.

The lesser grain borer is well adapted to breeding in warm, dry grain and hence of particular importance in countries such as Arabia, India and Australia. It is most important in wheat, rice and millet and less important in milled products. In Oklahoma according a survey of storage bins on farm between 1982 and 1985, lesser grain borer was the most abundant primary insect pest in sampled grain and causes the most damage to grain (Cuperus et. al., 1986). This insect pest and others have been controlled mainly by residual treatments of malathion, chlorpyrifos - methyl and less frequently with dechlorovos insecticide strip. Where control failures occurred, fumigation treatment with phosphine is done. Later in a study carried out in Oklahoma, discriminating dose tests for lesser grain borer showed all the tested strains were resistant to malathion, dichlorvos, and to chlorphyrifos-methyl, and 8 of the 21 tested strains were resistant to phosphine (Zettler & Cuperus 1990).

Tribolium castneum **(The red flour beetle)**

The red flour beetle, *Tribolium castneum* (Herbst), is a reddish –brown beetle, which is small (2.3-4.4) mm, and with three distinctive segmented club antennae. Width of the eyes (seen from below) about equal to distance between eyes. It belongs to the family of Tenebrionidae, where most members of this family are saprophagous and up to now almost 80 species have been recorded as stored product pests.

Eggs are laid singly amongst the substrate and they are usually covered with particles of flour, which adhere to the sticky egg membrane (Good 1936). The cylindrical larvae are white to yellow. Have rather obvious protuberances, or horns, on the terminal segment. Larvae pass through 6 or more instars while feeding among the flour portion of the product. The pupae, which are yellow when young and brown when mature, may be found loose amongst the substrate or in cracks and crevices of buildings or machinery's. The sexes can be distinguished by a pair of small but clearly developed appendages on the last abdominal segment of the female pupa.

The life cycle can be completed over 20-42° C but the optimum for increases lies between 32 and 35° C (Howe 1956, 1962).

Adult *T .castaneum* fly readily when the temperature is warm but in practice the distribution of this pest depends on passive transport in trade. Red flour beetle is a cosmopolitan pest and the most frequently found of all stored product beetles. Adults can live a year or more. Females lay hundreds of eggs in here lifetime. Red flour beetle is generally a secondary pest of wide range of cereals, cereal products, legumes, oilseeds and cakes, nuts, spies and animal products, and can be regarded as an extremely successful omnivore that is particularly abundant in warm and dry conditions. Quinones secreted by the adults produce an unpleasant musty taint. The grain industry generally refers to a complex of secondary grain beetles as "bran bugs."

Plodia interpunctella (The Indian meal moth)

The Indian meal moth, *Plodia inerpunctella* (Hubner) belongs to the family Pyralidae (subfamily Phycitinae), a common insect pest of stored product world wide, and is classed as secondary stored-product insect pest. The insect tends to survive most on damaged or broken grains, dried fruits and vegetables or otherwise processed produce. Larval feeding leads to a real loss of weight and quality in the produce, since the germinal layer is preferentially eaten and seeds can not germinate later. Indian meal moth is capable of living away from grain stores. This species has short-lived adults that lay up to several hundreds of small eggs loosely on the top of the produce. The minimum life cycle is 26 days. Adults are non-feeding and short lived (Krischik, et. al., 1995). Feeding larvae spin silk that holds together the food particles. This can cause a mat like covering on the upper part of the produce and facilitate heating and molding of the grain. Where the insect inhabits a food processing plant, sheets of larval silk can cause machinery to seize up or to be otherwise damaged. Silks can be difficult and expensive to remove from the grain.

Integrated pest management (IPM) and botanical insecticides

In the United States, stored products are attacked by insects as well as molds, which produce mycotoxins. Damage from insects and molds includes weight loss, grade loss, market discounts, potential mycotoxin contamination, and loss of national and international markets, which together result in at least a 10% loss of value (Harein et al. 1985). As damaged grain moves through the marketing system, concerns and regulatory issues are raised in relation to standards set by the GIPSA, the FDA. At the same time the use of insecticides to control insect damage also causes concern over food safety and insecticide residues.

The use of synthetic insecticides usually protects grain but impose strong selection pressures that can result in the development of resistance. The most important resistance mechanisms are enhancement of the capacity to metabolically detoxify insecticides and alterations in target sites that prevent insecticides from binding to them. Hence insect control methods must incorporate strategies to minimize resistance (Brattsten et. al., 1986). The most promising approach to tackle resistance and other problems with synthetic insecticides is application of integrated pest management (IPM). IPM is a systematic approach to pest regulation that emphasizes increased sampling to assess pest infestation levels and promote improved decision making so that control costs can be reduced and social, economic and environmental benefits can be maximized (Phillips et al., 2000). Stored products can provide an excellent opportunity to develop integrated pest management (IPM). Program managers have the ability to control temperature, moisture content, storage time, market destination, and pest movement into facilities. Temperature, time and moisture content regulate the population dynamics of insects and molds in storage (Cuperus et. al., 1995).

Insect sampling devices such as plastic pitfall traps used in bulk products and insect food/pheromone traps used in food warehouses permit the monitoring of the product for changes in insect population size and species. There are many management strategies, including sanitation, residual grain protectants, moisture control through drying, grain cleaning, grain spreaders, biological agents, and fumigants. When these management practices are integrated, safe grain storage is the result. But none of these practices alone ensures safe storage.

One way of integrated management is replacing the existing hazards synthetic insecticides with safer natural products like pyrethrum, diatomaceous earth and other natural products, to insure the safety of the consumers and to avoid environmental pollution. Diatomaceous earth (DE) is among inert materials that have been tested for stored grain pest control by numerous investigators (Parkin 1944; LaHue1965, 1967,1978; White et al., 1966; Redlinger and Womack 1996; McGaughey 1972; Desmarchelier and Dines 1987; Aldryhim 1990, 1993; Subramanyam et al.1994; McLauglin 1994; Xie et al. 1995). Since its approval for insect control in 1991,diatomaceous earth is already in use in the U.S. for bulk grain treatment. Diatomaceous earth kills insects with its abrasive qualities disrupting the insect water barrier and by removing or absorbing the epicuticular lipid layers causing excessive water loss through the cuticle (Mewis et al. 2001). DE does not leave unacceptable pesticide residues, as do some contact insecticides. If diatomaceous earth is identified as an unknown foreign substance in an inspection, the grain can be labeled as sample grade, the lowest designation. The sample grade designation results in grain suitable only for uses outside the food channel (Andreson et al. 1989). Consequently, it is advisable to identify the diatomaceous earth when the grain is submitted for grading. Use of dusts to control insect pests in storage is not new. Sand and clay and ash have been used for decades (Ebeling, 1971). Diatomaceous earth is amorphous dust and is no longer considered hazardous to human health in contrast to crystalline dusts if it is used correctly and exposure time is short (Ferch et al., 1987). Unfortunately, when DE is applied at rates that result in good insect control the application is more expensive than use of synthetic chemical protectants and the grain loses value because the DE reduces bulk density (test weight). Thus the grain industry in US has not widely adopted DE as grain protectant (Phillips. T. Personal communication). Initial research with proprietary materials suggests that diatomaceous earth (DE) a naturally occurring insecticidal desiccant can act at low rate as a synergist for botanical insecticide.

Presently the only botanical insecticides registered by EPA for application in the grain system is pyrethrum (Weaver and Subramanyam, 2000). Pyrethrum refers to the oleoresin produced by solvent extraction of chrysanthemum flowers *Chrysanthemum cineraraefolium* (Thev.) family Asteraceae. The majority of pyrethrum is produced in Kenya and Tanzania. (Henrick 1995, Weaver and Subramanyam, 2000) The active principles are a series of four neurotoxic esters (Pyrethrins) that block voltage dependent sodium channels in neuromembranes (Isman, 1994). Pyrethrum has a rapid knock down effect on flying insects and this has maintained pyrethrum as one of the best ingredients for use in pressurized household insecticide sprays (Isman, 1995). The major draw back to the use of pyrethrum is that it is unstable when exposed to ultraviolet component of sunlight and degraded rapidly by alkalis. Hence pyrethrum loses effectiveness quickly when used out doors. Pyrethrum has low mammalian toxicity. Rat oral LD_{50} value for technical grade pyrethrum is 1500 mg/kg (Isman, 1994). The lethal action of the pyrethrins is increased by the addition of the synergist piperonyl butoxide (Snelson 1981).

One of the most prominent botanicals in recent years is the Neem product. The neem tree *Azadirachta indica* A. Juss., family Meliaceae, is native to the Indian subcontinent. It is widely cultivated in Africa, Central and South America, the Caribbean islands, and Australia. It has been an age-old practice in rural India to mix dried Neem leaves with stored grain to control stored product insects (Koul et al., 1990). The predominant insecticidally active ingredient in neem is the limonoid azadirachtin. It had been isolated from neem leaves and seeds in 1968 by Morgan and Coworkers at Keele University in England (Ascher 1993). This group claimed that azadirachtin is also found in a closely related species, *Melia azedarach*. Azadirachtin is dually important for its insect regulating and antifeedant effects on insects (Saxena 1987, Schmutterer 1990).

The useful properties of azadirachtin in insect management are repellence, feeding and oviposition deterrence, insect growth regulator activity, low mammalian toxicity and low persistence in the environment (Koul et al. 1990, Schmutterer 1990). From the viewpoint of practical insect pest control the effect on insect development is the most important. The hypotheses that exist on the mode of action are an interference with the neuroendocrine controlling ecdysone and juvenile hormone synthesis. But also an inhibition of ecdysone release from the hormone- producing gland (Calliphora). Azadirachtin is a chitin synthesis inhibitor (Schmutterer, 1988). Good results are obtained with azadirchtin containing seed extracts under field conditions (Schmutterer, 1988). Currently in US. the Environmental Protection Agency (EPA) has granted registration for Neem based products like "Margosan-O," The neem extract and Ecozin for the control of insects on ornamentals and field crops. The residual effect just lasts 4-8 days (Schmutterer, 1988).

Essential oils are obtained by steam distillation of plant tissue. Essential oils and solvent extracts of them can be used as admixtures directly on stored products. Among extracts of that have been tested recently for storage pest control is cedar oil (Gebre-Amlak et al, unpublished data) from the eastern red cedar tree, *Juniperus virginiana*. Essential oils have been evaluated as well for repellent effect using simple bioassays (Xie et al. 1995).

Objectives

This study was carried out with the overall objective of evaluating the efficacy of botanical natural products with and without combination with DE as grain protectants against four-stored product insect species: lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L), red flour beetle, *Tribolium castanum* (Herbst), and Indian mealmoth, *Plodia interpunctella*. The specific objectives were as follows

1. To evaluate a new neem based biopesticide against four-stored product insect species.
2. Examine the potential synergistic effect of neem extract, cedar oil, and pyrethrum combined with DE against the four species.
3. Evaluate a proprietary grain protectant against the four species of stored product insects.

III. MATERIALS & METHODS

Test insects: -All experiments were conducted in the laboratory using established colonies of insects. All adults obtained from laboratory cultures were maintained at 30 ± 1°C and 70 ± 5% RH. Rice weevils and lesser grain borers used in this study were reared on whole kernels of hard red winter wheat with 5% brewer's yeast added. Grain moisture content for rearing rice weevil and lesser grain borer was 13% and 12.5% respectively. Red flour beetles were reared on whole-wheat flour and 5% brewer yeast and the Indian meal moths were reared on a mixture of 1: 1: 2: 0.75 of chick starter and grower crumbles, 15% mash egg crumble, yellow corn meal and glycerin, respectively. Indian meal moth eggs were collected by inverting a jar that contained adults 1 to 3 days old over a 20-mesh screen in a pert dish.

Test materials: - Among products tested in this study was Amazin 3% EC (Amvac, 4100 E, Washington BLVD., Los Angeles, CA 90023), which is a neem based botanical insecticide. The active ingredient in this product is 3% azadirachtin and 97% inert ingredient. The neem extract was tested at different rates to evaluate its efficacy as grain protectant. EPA registers this neem extract only for mushroom insect pest control (PNN Notification: 2000-16). The above mentioned neem extract product was tested in this study alone and in combination with DE (Protect- It, Hedley Technologies Inc. 5160 Explorer Drive, Unite 20, Mississauga, Ontario, L4 W4 T7). One of the candidate products in this study was crude cedar oil and was tested either mixed with DE or alone. Cedar oil was steam-distilled from cedar heartwood by a local producer. Another product tested in this study was a mixture of DE and pyrethrum. The pyrethrum we used was provided by MGK (MCLaughlin Gormley King Co. 8810 Tenth Avenue, North

Minneapolis MN. 55427 USA) and product name was Evergreen Grower's Spray (EGS), which is an organically approved formulation of pyrethrin that does not contain synergyst PBO. The EGS we received was 5% ai, which is equivalent to 50,000 ppm. Two proprietary materials were also tested in this study. All Natural 6 was a neem-based product combined with diatomaceous earth (DE) originating from Mexico. All Natural 6 also contained a plant extract from China with an LD_{50} of 3000mg/kg and an edible oil from Canada. All Natural 7 is the same like All Natural 6 except that it doesn't contain a neem extract. These products are not registered and have been provided as experimental samples by Dr. Zlatko Korunic, Hedley Technologies Inc., 14 Greenwich Drive, Guelph, Ontario, Canada NIH 8B8.

Sample preparation :- Experiments were conducted with 100g of wheat in 236.5 ml round glass jars. To eliminate the possibility of previously established pest infestation in the wheat, the wheat was frozen for one week prior to use and then cleaned on a seed cleaner. Mixing was either carried out in a 3.8 litter jar or by directly adding to each 236.5 ml treatment jars and, by adding the calculated amount of solution prepared or water was dripped inside to the side of the jar and rotated to side with the required amount of grain, and turned and mixed thoroughly. The mixing process was the same for all treatments.

Grain for the combination treatments were first treated with the liquid or EC treatment (either neem extract, pyrethrum, or cedar oil) and were left for 24 hours to dry. Grain for the control and DE treatments was moistened with the same amount of water or the solvent, in the case of cedar oil the solvent was hexane, and was left over night to dry. DE was added to the assigned jars 24 hour after treatment. Test insects were added to the treated grain and to the controls 24 hours after treatment. Fifty adult beetles collected within 7 to 21 days of emergence, or 0.006gm eggs of Indian meal moths was added into the assigned treated grain jar. Jar rings, wire screen and one piece of filter paper on each side of the screen were placed on the jars. These lids allow air and moisture circulation into the jars, while prohibiting insect migration. Whole grain of hard red wheat was used for rice weevil and lesser grain borer but for Red flour beetles and Indian meal moth eggs, 5g of the treated wheat grain was crushed after treatment and put back into the jars to facilitate feeding of the free-living larvae of these species.

Table1 Experiments conducted

Expt.#	Objective	Test material	Specices tested
1	Evaluating efficacy of a commercial neem extract at different dosage rates for the control of four storage pests (part one)	Neem extract Table (2, 3) At 200,100, 50, 10 ppm level and control.	*S. oryzae,* *R. dominica,* *T. castneum* *P.interpunctella*
2	Evaluating effect of low dose commercial neem extract for the control two external feeder storage pests (part two).	Neem extract Table (4, 5) At 10, 5, 1, 0.5 ppm level and control.	*P.interpunctella,* *T. castneum*
3	Evaluating effect of low does commercial neem extract for the control of two external feeder storage pests (part 3)	Neem extract Table (6) At 1, 0.5, 0.1, 0.01, 0.001 ppm level and control	*P.interpunctella,* *T. castneum*
4	Determining potential synergitic effect of commercial neem extrat and DE. (part one)	Neem extrac + DE Table (7, 8) Neem extract 5, 10 ppm, DE 80ppm, Neem extract 5 ppm+ DE 80ppm, Neem extract 10 ppm+ DE 80ppm and control	*S. oryzae,* *R. dominica,*
5	Determining potential synergitic effect of commercial neem extrat and DE .(part 2)	Neem extrac + DE Table(9, 10) Neem extract 10ppm, DE 80, 100, 150ppm, 10ppm neem extrac.+ DE 80ppm, 10ppm neem extrac.+ DE 100ppm, 10ppm neem extrac.+ DE 150ppm, and control	*S. oryzae,* *R. dominica,*
6	Determining potential synergitic effect of commercial Cedar and DE against four storage pests.	Cedar + DE Table (11, 12) Cedar 0.3 % , Cedar 0.3% + DE 80ppm, Cedar 0.3 % + DE 150ppm and control.	*S. oryzae,* *R. dominica,* *T. castneum* *P.interpunctella*
7	Evaluating potential synergitic effect of pyrethrum and DE against three storage beetles	Pyrethrum + DE Table (13, 14) 3ppm EGS, 3ppm EGS+80 ppm DE, DE 80, 150 ppm and control.	*S. oryzae,* *R. dominica,* *T. castneum*
8	Comparison of two properitary materials with DE against four storage pests.	N6, N7 and DE Table (15, 16) N6 80ppm, N7 80 ppm, DE 80, 300 ppm and control.	*S. oryzae,* *R. dominica,* *T. castneum.* *P.interpunctella*

Experimental Design and Data analysis

The experimental design for all experiments was a randomized complete block design (RCBD). The number of treatments varied for each experiment but they all included the untreated check with four replications. Due to lack of enough shelves in the environmental chamber, all jars from the four replications were on the same shelf. This eliminates the very slight differences, which could exist between shelves, but there might be some difference between jars near the door of the growth chamber and those jars at rear end of the chamber.

Initial assessment for rice weevil, lesser grain borer, and red flour beetle species were carried out one week after treatment. During the initial assessment the adult beetles were removed from the jars and assessed for mortality. The second assessment for the beetles was seven weeks after treatment when the number of F1 progeny was counted. Indian meal moth assessment was on the 5th, 6th, and 7th weeks after treatment in which newly emerged adult moths were counted.

Data collected from this experiment was analyzed using SAS (SAS Institute Inc.1994). All data were subjected to analysis of variance (ANOVA) followed by a protected least significance difference test (PLSD) for comparison of means to analysis. Proportional data were first transformed by the angular transformation prior to ANOVA. Means and standard errors of untransformed data are reported for all experiments.

IV. RESULTS & DISCUSSION

i. Neem

The results obtained for the adult mortality test seven days after treatment with neem is shown on Table 2. In all the treatments for the three test beetles the mortality test for the neem extract did not show any significant difference than the control, except for the neem extract at 200ppm level against rice weevil, which showed high significant deference from the control.

In the progeny development test, there were high significant differences between the treated and the untreated grain in all the test insects as shown on table 3. Significant difference was also observed among treatments on the percent suppression of progeny development for rice weevil and lesser grain borer. No significant difference was observed among the different rates of the neem extract for red flour beetle and Indian meal moth, but all treatments had shown good suppression of these two pests. Especially on Indian meal moth no progeny development was observed on all the treatments except on the untreated check. Grain was thoroughly investigated during the final data collection to check which stage of Indian meal moth was controlled by the candidate botanical insecticide. During the thorough observation of the grain sign of many dead early larval stages of the insect and larval excrement were found but no late larval stages or adult stages were observed. Hence sign of growth regulating effect of this product was observed on all the test species and this is in agreement with the growth regulating property of neem (Koul et al. 1990, Schmutterer, 1990). Though the progeny suppression was high in the test insects of the internal feeders, but the number of progeny developed seven weeks after treatment was still high at the lower neem doses. Hence testing mixtures of natural products with DE was the second step for the control of internal feeders. Since Neem extract showed good suppression of the progeny development on Indian meal moth and red flour beetles further tests were designed to determine the minimum dosage rate of Neem extract for the control Indian meal moth and red flour beetle.

Result for the second experiment is shown on Table 4. In this experiment no adult Indian meal moth development was observed in the treated grain but there was larval development in all the treatments seven weeks after treatment and statistically there was significant difference between the treated and the untreated concerning the larval development. Larvae on the treated grain were not healthy looking as that of the control. Since there is no statistically significant difference between the treated, the best minimum dosage rate would be 0.5mg/kg of neem extract. The result for effect of low dose of neem extract against red flour beetle is shown on Table 5. The result for this experiment indicates adult mortality test one week after treatment did not show any statistical significant difference between the treated and the check. On the other hand, significant differences were observed between the other treatments and the control on the progeny development test and no significant difference in progeny production among other treatments of neem extract tested in this experiment were observed. All of the treatments showed good suppression of the insect. Hence the minimum effective dosage rate in this test would be 1ppm. But it was necessary to repeat and see the result of these experiments with rates less than 0.5 ppm of neem extract. In the third experiment the data collected for adult moth emergence seven weeks after treatment (see Table 6), treatments of 1ppm and 0.5ppm were statistically significantly different than the other treatments and the untreated check. While treatment 0.1ppm was not significantly different from treatment 0.01ppm & 0.001ppm or the control. On the other hand for some reason unknown the treatment of 0.001ppm, which was the lowest dosage rate did not show significant difference from treatment 1ppm and 0.5ppm or the control. Since 1ppm neem extract and 0.5ppm neem extract were statistically significantly different from all other treatments and the lowest dosage rate is treatment is 0.5ppm neem extract and since this has been observed on the first experiment as well 0.5ppm is chosen as the lowest dosage rate of neem extract for Indian meal moth management.

Further test to find the lower dosage rate of the neem extract for the control of red flour beetle was carried out. The result for the successive experiment is shown on Table 6. Treatments of 1ppm and 0.5ppm were statistically significantly different from the rest of the treatments and the control, while the treatment 0.1ppm was in the second place in suppressing the progeny. Treatments 0.01ppm & 0.001ppm were not significantly different from the control. As there is no statistically significant difference between treatment 1ppm and 0.5ppm the best minimum dosage rate for the neem extract in this experiment is treatment is 0.5 ppm azadirachtin concentration.

The results from the neem extract experiment of this study suggests that neem- based insecticides like the amazin 3% EC (Amvac) has the potential to control externally feeding storage pests like red flour beetle and Indian meal moth at the rate as low as 0.5ppm and at the same time can suppress internal feeding storage pests population when used at higher doses.

Adult mortality of beetles was very low to nil in most of the cases seven days after treatment. In the case of rice weevil adult mortality was likely to be dose dependent. While on the rest of the test beetles dose did not have any effect on adult mortality. Neem based product has to be applied against the most sensitive life stage or larval instars of the target insect, as there are remarkable differences in sensitivity during metamorphosis (Schmutterer, 1988).

Progeny development suppression was dosage dependent on all test species and promising results were obtained especially with external feeders where all life stages of the insect species occur loose outside the kernel and are easily exposed to the treated product. In the case of internal feeders, better progeny development suppression was observed on lesser grain borer where the early immature stages are found out side the kernel.

In experiment 2 Indian meal moth larval development was recorded on the treated grain but no adult development was observed. The above might be related to the growth regulating effect of neem. In this case the larvae may live up to several weeks, as they are unable to molt (Schmutterer 1990).

Since the larvae are unable to molt there was no adult development. Hence farther reproduction is checked and the neem treated grain will stay free from external feeders like Indian meal moth for certain period as long as there is no external source of infestation.

ii. Neem extract + DE

The result obtained from the mortality test Table 7 for lesser grain borer showed there was no significant difference between the combinations and DE 80ppm alone. The neem extract at the 5 ppm and 10ppm level was not significantly different from the control, or from all other treatments for lesser grain borer. The mortality test one-week after treatment for rice weevil showed the combinations were significantly different from neem extract alone but DE was not significantly different from the combinations or the neem extract treatments. The neem extract treatments were not significantly different from the control. In general the percent mortality obtained from any of the treatments was low for both species.

Progeny development Table 8 for lesser grain borer was reduced mostly by the combination neem extract 10ppm + DE 80ppm, followed by neem extract 10ppm alone which did not show difference from both neem extract 10ppm combination or neem extract 5ppm combination with DE. Next was neem extract 5ppm alone followed by the control. On the progeny suppression for rice weevil, the highest suppression was observed again by the combination with neem extract 10ppm + DE 80ppm and neem extract at 5 ppm level alone. Followed by neem extract 5ppm + DE 80ppm and DE 80ppm which didn't show any difference both from the superior combination in this experiment or from neem extract 10ppm alone. In both species all treatments showed significant difference from the control or the untreated check. For a reason unknown to the researcher neem extract at 5ppm level had performed better than neem extract at 10ppm level in this experiment. Hence to improve the performance of our combination we intended further testing by increasing the rate of DE up to 100- 150ppm.

The result obtained from the mortality test Table 9 for rice weevil showed there was a statistically significant difference between the treated and the untreated except for the 10 ppm neem extract and DE 100 ppm which did not show difference from one another and the control. Superior result was obtained by neem extract 10 ppm +DE 150 ppm followed by DE150 ppm, which was not statistically different either from neem extract 10 ppm +DE 150ppm or neem extract 10ppm+ DE 100ppm and the neem extract 10 ppm +DE 80 ppm. On the other hand neem extract 10ppm+ DE 100ppm was not significantly different from the neem extract 10 ppm +DE 80 ppm or DE 80 ppm. DE 80ppm was not significantly different from DE 100ppm or neem extract 10ppm. Neem extract 10ppm and DE 100ppm were not significantly different from the untreated check in this experiment. (For lesser grain borer the result obtained for the mortality test (Table 8) showed statistical significant difference between the treated and the untreated except for DE 80ppm, even though the maximum percent mortality was 8 % by DE 150ppm). Generally the adult percent mortality was very low for all treatments. As to the progeny development (Table 10) for rice weevil all treatments showed statistically significant difference than the control except neem extract 10ppm. In this experiment the rest of the treatments did not show any statistical significant differences among themselves. Result on the progeny development for lesser grain borer showed all the combinations were statistically significantly different from the control. While treatment DE 80ppm did not show any statistical difference from the control and treatment DE 100 ppm and DE 150 ppm did not show statistical difference from the control or the combination neem extract 10ppm + DE 80ppm.

When looking at treatments, which were common for both experiments the percent mortality for the second experiment was higher than the one obtained in the first experiment for rice weevil. But the data statistically showed consistence for both test insects.

The combined effect of neem extract and DE was observed in both experiments. It looks rather additivity than synergism and the percent mortality had increased even though it looked that most of the job is done by DE in the case of rice weevil as well as lesser grain borers on the adult mortality test.

The reduction of offspring by the combination treatments was not better than the one obtained by the DE treatments as far as rice weevil is concerned. A much better offspring reduction was observed on lesser grain borer by the combinations than by DE or the neem extract treatment alone. Even though we have not proved any synergetic effect, the neem extract and DE could suppress the progeny development of lesser grain borer much higher than neem extract and DE alone.

We need to do more experiments by increasing the dose of each product until we obtain total reduction. Or we have to give up and look for other botanicals which can work much better with DE and give higher reduction or total reduction of the F 1 progeny on internal feeder storage pests.

iii. Cedar oil

The result obtained from the mortality test (Table 11) for rice weevil and lesser grain showed that both combinations Cedar 0.3 % + DE 150 ppm, and Cedar 0.3% + DE 80 ppm were statistically significantly different from the control and Cedar 0.3% alone. The highest percent mortality was 100% seven days after treatment for rice weevil followed by 94.5% respectively. For lesser grain borer the highest percent kill was 94.5% followed by 91% respectively. For red flour beetle most of the treatments were not statistically significantly different from the control or did not show any percent kill except Cedar 0.3 % +DE 80ppm which gave statistically significant difference from the other treatments with the amount of 2 % kill.

As to the progeny development (Table 12) for rice weevil all treatments were statistically significantly different from the control and both combinations Cedar 0.3 % + DE 150 ppm, and Cedar 0.3% + DE 80 ppm were significantly different from the control and cedar 0.3% alone. The mean progeny count for the grain treated with Cedar 0.3 % + DE 150ppm was as low as 0.5. The result for the progeny development for lesser grain borer showed that all treatments were significantly different from the control, and there was no statistical significant difference between cedar 0.3% alone and the two combinations. In general the mean progeny count for all treatments as compared to the control was very low and was with in the range of (0.5-5.0). The result for the progeny development for red flour beetle showed that all treatments were significantly different from the control. Both combinations showed the highest progeny suppression. The lowest mean progeny count was obtained for red flour beetle from grain treated with Cedar 0.3 % + DE 150ppm and was 5.8 seven weeks after treatment. When we look at the progeny development of Indian meal moth, both combination showed 100% suppression of the F1 generation of this insect pest and were statistically significantly different from the control and cedar 0.3 %. On the other hand cedar 0.3 % alone had the highest progeny count of Indian meal moth in this experiment.

The Cedar + DE experiment result showed that the two combinations gave the highest adult mortality on internal feeder test insects while red four beetle was resistant to the two combinations as well as to cedar 0.3 % alone. This could be from the repellent action of cedar oil to red flour beetle (Assefa et al., unpublished data).

When we look at the mortality test all test spies showed high resistance to cedar oil 0.3 %, which is similar to the result by Gebere Amlak et al (unpublished). On the other hand the combinations showed above 90% to 100% adult mortality on both internal feeders and this is in agreement with our assumption about the synergetic effect of DE when combined with natural biopesticides.

Both combinations showed high inhibition of progeny development on all the tested insects. In the case of the internal feeder test insects the progeny inhibition could be due to the initial high adult mortality after treatment. For red flour beetle where there was no initial adult mortality, the high inhibition of progeny development by the combinations followed by cedar oil 0.3% alone is unclear from the current study . In situations where no adult mortality was observed initially the progeny inhibition might be from inhibition of oviposition or egg viability or larval survival.

The greatest finding in this study is that cedar 0.3 % + DE 80 ppm, which was not statistically different from cedar 0.3 % + DE 150ppm in progeny inhibition could be used in the management of the above listed test insects. For lesser grain borer management using combination of cedar + DE might not be necessary since Cedar 0.3 % alone equally inhibited its progeny development.

iv. Pyrethrum + DE

Resulting mortality from pyrethrum DE combination treatments to the three test beetles are shown in (Table 13). Mortality test for lesser grain borer showed that treatment D was superior and gave 99.0 % kill followed by treatment C which gave 56% kill seven days after treatment. Both combinations were statistically different from all other treatments and the control. Treatment B, which was pyrethrum alone, was in the third place and all other treatments (E, F) were not statistically different from the control. Mean mortality percentage for rice weevil as shown on Table 13 indicates that the two combinations were statistically significantly different from all other treatments and the control. There was no statistically significant difference among the two combinations in the percent for rice weevil. Treatment B and E was not statistically different from the control. Treatment F was not statistically different from treatment B. The percent kill obtained by the two combinations against red flour beetle was very low as compared to the other test beetles, Treatment D having 12 % mortality was statistically significantly different from all other treatments except treatment C, which in turn was not significantly different from the control. The mean progeny development for the three test beetles is presented in Table 14. Lesser grain borer had the highest mean mortality by the pyrethrum +DE combinations and also had the highest progeny suppression by the combinations among all the test insects. No statistically significant difference was observed between the combinations and pyrethrum alone. Although statistically different from the control, high numbers of mean progeny were observed on the two DE treatments (E, F). For rice weevil the mean progeny count for the combinations treatments was statistically significantly different from all the other treatments and the control. All other treatments were also different from the control and among themselves except treatment B and E, which were not statistically different from each other. For red flour beetle the mean progeny development suppression by treatment C, and D was very high and was close to one hundred percent on both pyrethrum DE combinations. Treatment B did not show any statistical significant difference from the combinations or the two DE treatments and all treatments were statistically significantly different from the control.

The result from this study showed 99% adult mortality for lesser grain borer exposed for one week to wheat treated by treatment D which is a pyrethrum + DE combination. Lesser grain borer was the most susceptible test species followed by rice weevil where both combinations gave > 50% mortality, with red flour beetle being the least susceptible (Table 12). In general the two combinations out-performed all other treatments in all the test insects. Hence the data for the mortality test is in agreement with our assumption that the synergetic effect of DE when combined with natural biopesticides is much higher than the sum of activity of the individual test products.

The pyrethrum + DE combinations caused a significant reduction in the number of progeny produced compared to the other treatments and the control in all the test insects (Table 13). The highest progeny reduction was observed on lesser grain borer where there were > 99 % progeny reduction followed by red flour beetle, which gave > 98 % reduction. The least progeny reduction was observed on rice weevil, which were around > 93 %. In the case of the internal feeder test insects the high progeny reduction could be due to the initial high to medium adult mortality after treatment. For red flour beetle where the initial adult mortality was low the high reduction of the progeny development by the combinations followed by 3ppm EGS pyrethrins alone is unclear from the current study. In the case in which no adult mortality was observed initially the progeny inhibition might be from inhibition of oviposition or egg viability or larval survival.

Since red four beetles are external feeders, in which all the life stages are exposed to the product applied the high progeny reduction is most likely from the inhibition of the susceptible life stages of the insect especially the early immature stages inhibition.

v. Proprietary material

In the experiment with the proprietary materials (Table 15) the result for the mortality test 1 week after treatment showed that both N6 and N7 were significantly different from the control on all the tested insects. DE 300ppm was also significantly different than the control for rice weevil and lesser grain borer, while on red flour beetle DE 300 did not show statistical significant difference either from N6 or the control. DE 80ppm on rice weevil was statistically significantly different from the control but on the rest of the test insects didn't show any statistical significant difference from the control. The result shows that the two proprietary products gave 100% control on both internal feeders. On the other hand on red flour beetle N7 followed by N6 were significantly different from the other treatment and the control.

As to the mean progeny development seven weeks after treatment on rice weevil there was no significant difference between N6, N7, and DE 300 (Table16). But there was no insect development observed on N6 and N7 treated grain, while there were few internal feeder insects development was observed on the grain treated with DE 300. The above three treatments were statistically significantly different from DE 80ppm and the control. On lesser grain borer both N6 and N7 were highly significantly different from the other treatments and no insect was observed on the grains treated with those products seven weeks after treatment. DE 300ppm was in the second place in this experiment for lesser grain borer control and it was significantly different from the rest of the treatments followed by DE80ppm. In the case of red flour beetles, no insects were observed on grain treated with N7 and N6 and DE 300ppm seven weeks after treatment. The above listed treatments were significantly different from DE 80ppm and the untreated check. In the Indian meal moth control experiment all treatments showed significant difference from the control. No progeny development of Indian meal moths was observed on the treated grain.

Both N6 and N7 provided very good performance on both the mortality test and progeny development test on all the species tested. Hence this experiment has proved the alternative hypothesis to be true for the internal feeders. The whole idea of using mixtures of botanical insecticides with DE is to improve the performance of the natural product by the synergistic effect of the two products. The proprietary products received from a private company in Canada gave a positive indication in favor of this assumption although all components were not tested separately. We know for sure that N6 and N7 contain DE and another botanical extract, from China and edible oil from Canada and N6 in addition to the above listed products also contain azadirachtin. The 80 ppm DE was not very effective against rice weevil and lesser grain borer, but the 80 ppm N6 and N7 were effective. The two combinations had given excellent performance on both external as well as internal feeder test insects. The greater achievement with these products was the 100 % adult mortality, which was observed on both internal feeder test insects. Botanical insecticides rarely give 100% adult mortality after treatment as do some synthetic insecticides. Total suppression of the F1 generation was observed on both internal and external feeders. One reason for the total suppression of the F1 generation could be the 100 % adult mortality. This clearly shows that adult mortality is very important for F1 generation suppression in the case of internal feeders. When we look at the result obtained from the external feeder test insect (red flour beetle), there was 100 % F 1 generation suppression, but adult mortality was low. This is telling us may be that adult mortality is not an important property to control external feeders since immatures were also exposed to the natural insecticide which was applied to the grain and these are the most sensitive life stage of the insect (Schmutterer, 1988).

V. SUMMARY & CONCLUSION

Result from the first part of this study suggests the neem based botanical insecticide tested has the potential to control external feeding storage pests like Indian meal moth and red flour beetle at rates as low as 0.5 ppm. At the same time neem can suppress progeny development of internal feeding storage insect pests. Among the internal feeders, better progeny suppression was observed on lesser grain borer than on rice weevil. The reason is likely due to the growth regulating effect of neem being expressed on early larval instars of lesser grain borer which normally occurs lose outside the kernel.

The reduction of offspring by combination treatments of neem extract and DE was not better than that obtained by DE treatments as far as rice weevil was concerned. A much better offspring reduction was observed on lesser grain borer with the combinations than by DE or the neem extract treatment alone.

When we look at the Cedar + DE experiment, the result for mortality test on all the test species showed high tolerance to cedar oil at 0.3 %. Combinations showed above 90% to 100% adult mortality on both internal feeders and this was in agreement with our assumption about the synergetic effect of DE when combined with natural biopesticides. The greatest finding in this study was that cedar oil at 0.3 % + DE at 80 ppm, was not statistically different from cedar oil 0.3 % + DE 150ppm in progeny inhibition could be used in the management of all the listed test insects. For lesser grain borer management using combination of cedar oil + DE might not be necessary since cedar oil 0.3 % alone equally inhibited progeny development.

Results from the pyrethrum + DE combinations experiment showed significant reduction in the number of progeny produced by combinations as compared to the control and to the other treatments, except 3-ppm EGS (Pyrethrin), which also showed equally significant reduction on lesser grain borer and red flour beetles. Highest progeny reduction was observed on lesser grain borer (> 99 %) followed by red flour beetle (> 98 %). The least progeny reduction was observed on rice weevil, (> 93 %). In the case of the internal feeder test insects, the high progeny reduction could be due to the initial high to medium adult mortality after treatment. Although the progeny reduction by the combinations was high for lesser grain borer and red flour beetles no statistically significant difference was observed between the combinations and 3-ppm EGS (Pyrethrin). Hence the only test species for which management might be recommend 3 ppm EGS (Pyrethrin) + 80ppm DE would be rice weevil.

The present study further supports evidence that DE; can act as a synergist for botanical insecticides. This study has proved neem-based biopesticides, can be used for management of storage pests like Indian meal moth and red flour beetle at rates as low as 0.5ppm. We have proved also that combination of cedar oil 0.3 % with DE 80ppm could also be used for management of storage pests. Good control was also achieved with 3 ppm EGS (Pyrethrin) + 80ppm DE against rice weevil and other test insects.

The proprietary materials provided 100% adult mortality on both internal feeders and total F1 progeny reduction on all test insects.

VI. **Tables**

Table 2. Mean percent mortality of adult beetles one week after exposure on wheat treated with commercial formulation of a neem extract.

Treatments	Mean percent mortality ± (SE)*		
	Rice weevil	Red flour beetle	Lesser grain borer
Control	0.0 (0.0) b	0.0 (0.0) a	2.0 (1.0) a
10ppm	0.0 (0.0) b	0.5 (0.0) a	1.0 (1.0) a
50ppm	0.0 (0.0) b	0.5 (0.0) a	0.0 (0.0) a
100ppm	4.0 (2.0) b	4.0 (0.0) a	1.5 (1.0) a
200ppm	90.0 (5.0) a	2.0 (1.0) a	2.5 (2.0) a
F (df)	55.49 (3, 4)	0.71 (3, 4)	1.16 (3, 4)
P	0.0001	0.6627	0.3921

*Means in the same column with the same letter are not significantly different. LSD test (P<0.05)

Table 3. Adult progeny produced by four species on grain treated with commercial neem extract .

Treatments	Mean number of progeny ± (SE)*			
	Rice weevil	Red flour beetle	Lesser grain borer	Indian meal moth
Control	907.5 (61. 1) a	118.0 (14.7) a	646. 75 (41.5) a	22.5 (1.7) a
10ppm	685.5 (59.9) b	3.75 (1.4) b	313. 0 (21.7) b	0.0 (0.0) b
50ppm	415.5 (32. 7) c	5.25 (1.3) b	119.0 (12.2) c	0.0 (0.0) b
100ppm	249.8 (39.4) d	8.25 (2.5) b	19.0 (2.9) d	0.0 (0.0) b
200ppm	8.5 (2.9) e	2.0 (1.1) b	9.5 (1.8) d	0.0 (0.0) b
F (df)	51.18 (3, 4)	32.93 (3, 4)	80.54 (3, 4)	99.61 (3, 4)
P	0.0001	0.0001	0.0001	0.0001

*Means in the same column with the same letter are not significantly different.
LSD test (P<0.05)

Table 4. Effect of low doses of neem extract applied to wheat on progeny production of Indian meal moth.

Treatments (Neem extract)	Mean number of Indian meal moth adults and larvae ± (SE)* Indian meal moth adult	 Indian meal moth larvae
10ppm	0.0 (0.0) b	5.75 (1.1)
5ppm	0.0 (0.0) b	8.5 (2.5)
1ppm	0.0 (0.0) b	10.25 (0.8)
0.5ppm	0.0 (0.0) b	9.25 (4.8)
Control	9.5 (0.5) a	18.5 (4.8)
F (df)	21.77 (3, 4)	2.23 (3, 4)
P	0.0001	0.1067

*Means in the same column with the same letter are not significantly different.
LSD test (P< 0.05)

Table 5. Effect of low doses of neem extract applied to wheat on adult mortality and progeny production of red flour beetle.

Treatments (Neem extract)	Red flour beetles	
	Mean Percent Dead ± (SE)*	Mean number of progeny ± (SE)*
10ppm	10.0 (3.0) a	1.5 (0.29) b
5ppm	9.5 (1.0) a	0.75 (0.25) b
1ppm	4.5 (3.0) a	1.75 (0.75) b
Control	4.0 (0.8) a	98.25 (1.75) a
F (df)	2.80 (3, 3)	1024.24 (3, 3)
P	0.0802	0.0001

*Means in the same column with the same letter are not significantly different. LSD ($P< 0.05$)

Table 6. Effect of low doses of neem extract applied to wheat on progeny production of Indian meal moth and red flour beetle

Treatments (Neem extract)	Mean number of progeny ± (SE)*	
	Indian meal moth adult	Red flour beetle
1ppm	0.25 (0.25) c	14.5 (3.52) c
0.5ppm	3.5 (1.85) c	19.5 (5.38) c
0.1ppm	13.5 (1.04) ab	61.5 (6.51) b
0.01ppm	20.75 (4.97) a	112. 75 (16.35) a
0.001ppm	8.25 (1.80) bc	102. 75 (6.28) a
Control	13.75 (2.81) ab	109.75 (20.86) a
F (df)	4.59 (3, 5)	10.67 (3, 5)
P	0.0054	0.0001

*Means in the same column with the same letter are not significantly different LSD test (P< 0.05).

Table 7. Effect of Neem extract + DE experiment adult beetles mean percent mortality part one.

Treatments	Mean percent mortality one week after treatment ± (SE)*	
	Lesser grain borer	Rice Weevil
Control	2.0 (1.2) b	3.5 (2.3) c
DE 80ppm	11.5 (4.1) a	17.0 (1.9) a b
Neem ex. 10 ppm + DE 80 ppm	10.0 (2.9) a	28.5 (0.96) a
Neem ex. 5 ppm+ DE 80 ppm	7.0 (1.7) a	22.5 (2.5) a
Neem ex. 10 ppm	4.5 (1.3) a b	10.5 (4.5) b c
Neem ex. 5 ppm	1.5 (0.96) b	7.0 (2.38) b c
F (df)	4.0 (3, 5)	4.03 (3, 5)
P	0.0100	0.0098

*Means in the same column with the same letter are not significantly different.

LSD test ($P < 0.05$)

Table 8. Mean progeny development for neem extract + DE experiment part one.

Treatments	Mean progeny development ± (SE)*	
	Lesser grain borer	Rice weevil
Control	577.5 (41.5) a	410.0 (64.0) a
DE 80ppm	311.25 (41.9) b	159.3 (24.4) bc
Neem ex. 10ppm + DE 80ppm	85.75 (7.9) e	83.5 (14.6) c
Neem ex. 5ppm + DE 80ppm	174.25 (21.0) cd	186.8 (24.0) bc
Neem ex. 10ppm	148.0 (13.9) de	254.8 (26.5) b
Neem ex. 5ppm	241.5 (14.2) bc	147.8 (27.4) c
F (df)	27.35 (3, 5)	6.76 (3, 5)
P	0.0001	0.0002

*Means in the same column with the same letter are not significantly different. LSD test (P< 0,05).

Table 9. Adult beetles mean percent mortality experiment for neem extract + DE test.

Treatments	Mean percent mortality ± (SE)*	
	Rice weevil	Lesser grain borer
Control	13.5 (4.1) f	0.0 (0.0) c
10ppm neem ex. + DE 80 ppm	63.9 (7.9) bcd	6.0 (0.8) a
10ppm neem ex. + DE 100 ppm	76.0 (10.4) abc	4.0 (1.2) ab
10ppm neem ex. + DE 150 ppm	93.6 (3.9) a	7.0 (2.6) ab
10ppm neem ex.	28.5 (11.9) ef	1.0 (0.5) bc
DE 80 ppm	54.0 (7.7) cde	1.5 (0.9) a b c
DE 100ppm	39.5 (7.6) def	4.0 (1.6) a b
DE 150 ppm	82.0 (12.4) ab	8.0 (3.4) a
F (df)	6.32 (3, 7)	2.18 (3, 7)
P	0.0002	0.0640

*Means in the same column with the same letter are not significantly different.
LSD test (P< 0.05)

Table 10. Mean progeny produced seven weeks after treatment for neem extract + DE experiment part two.

Treatments	Mean number of progeny + (SE)*	
	Rice weevil	Lesser grain borer
Control	487 (25.4) a	1013.5 (130.0) a
10ppm neem ex. + DE 80 ppm	147.8 (18.9) b	777.0 (46.4) b
10ppm neem ex. + DE 100 ppm	129.0 (20.9) b	572.5 (49.9) c
10ppm neem ex. + DE 150 ppm	110.5 (33.8) b	513.3 (44.0) c
10ppm neem ex.	565,0 (118.9) a	676.3 (80.1) a b
DE 80 ppm	183.0 (17.6) b	1020.5 (21.2) a
DE 100ppm	160.3 (21.12) b	824.0 (77.4) a b
DE 150 ppm	147.3 (47.8) b	821.0 (20.4) a b
F (df)	9.26 (3, 7)	5.47 (3, 7)
P	0.0001	0.0005

*Means in the same column with the same letter are not significantly different

LSD test (P< 0.05).

Table 11. Adult beetles mean percent mortality for Cedar oil + DE test.

Treatments	Mean percent mortality ± (SE)*		
	Rice weevil	Lesser grain borer	Red flower beetle
Control	0.5 (0.5) c	0.5 (0.5) b	0.0 (0.0)
Cedar 0.3 %	1.5 (1.0) c	9.5 (4.6) b	0.0 (0.0)
Cedar 0.3 % + DE 80 ppm	94.5 (2.0) b	91.0 (3.0) a	2.0 (1.0)
Cedar 0.3 % + DE 150 ppm	100 (0.0) a	94.5 (3.0) a	0.0 (0.0)
F (df)	270.68 (3, 3)	40.41 (3, 3)	1.81 (3, 3)
P	0.0001	0.0001	0.2038

*Means in the same column with the same letter are not significantly different. LSD test ($P< 0.05$).

Table 12. Mean progeny produced 7 weeks after treatment cedar + DE

Treatments	Mean progeny development ± (SE)*			
	Rice weevil	Lesser grain borer	Red flour beetle	Indian meal moth
Control	341.5 (15.5) a	541.8 (42.7) a	160.3 (11.9)a	8.3 (2.0) b
Cedar 0.3 %	143.3 (32.5) b	5.0 (1.5) b	34.8 (8.9) b	20.5 (2.5) a
Cedar 0.3 % + DE 80ppm	40.8 (30.5) c	0.5 (0.3) b	18.0 (6.4) c	0.0 (0.0) c
Cedar 0.3 % + DE 150ppm	0.5 (0.5) c	1.3 (0.95) b	5.8 (1.6) c	0.0 (0.0) c
F (df)	15.87 (3, 3)	79.93 (3, 3)	34.94 (3, 3)	15.04 (3, 3)
P	0.0001	0.0001	0.0001	0.0003

*Means in the same column with the same letter are not significantly different

LSD test ($P< 0.05$).

Table 13. Adult beetles mean percent mortality Pyrethrum +DE.

Treatments	Mean % mortality ± (SE)*		
	Lesser grain borer	Rice weevil	Red flour beetle
A. Control	0.0 (0.0) d	0.0 (0.0) c	1.0 (1.0) c b
B. 3 ppm EGS (Pyrethrins)	14.0 (1.4) c	6.0 (2.1) b c	0.5 (0.5) c
C. 3 ppm EGS + 80ppm DE	56.0 (13.7) b	58.5 (10.0) a	6.0 (1.9) b a
D. 3ppm EGS + 150ppm DE	99.0 (1.0) a	54.0 (16.0) a	12.0 (5.8) a
E. 80ppm DE	0.0 (0.0) d	0.0 (1.0) c	0.5 (0.5) c
F. 150 ppm DE	0.0 (0.0) d	16.0 (3.1) b	2.0 (1.1) b c
F (df)	49.65 (3,5)	11.0 (3,5)	2.86 (3,5)
P	0.0001	0.0001	0.0178

*Means in the same column with the same letter are not significantly different LSD test (P< 0.05).

Table 14. Mean progeny produced 7 weeks after treatment pyrethrum + DE

Treatments	Mean number of F1 progeny ± (SE)*		
	Lesser grain borer	Rice weevil	Red flour beetle
A. Control	506.3 (59.7) a	375.3 (24.3) a	99.3 (12.0) a
B. 3 ppm EGS (Pyrethrins)	3.0 (0.7) c	209.8 (45.4) b	12.5 (1.4) bc
C. 3 ppm EGS + 80ppm DE	0.0 (0.0) c	36.8 (5.7) d	1.3 (0.5) c
D. 3ppm EGS + 150ppm DE	0.75 (0.0) c	25.8 (7.4) d	0.3 (0.3) c
E. 80ppm DE	348.0 (49.3) b	202.8 (34.0) b	29.5 (11.2) b
F. 150 ppm DE	272.3 (59.5) b	119.5 (16.2) c	24.8 (1.8) b
F (df)	29.19 (3, 5)	15.15 (3, 5)	20.6 (3, 5)
P	0.0001	0.0001	0.0001

*Means in the same column with the same letter are not significantly different LSD test ($P < 0.05$).

Table 15. Mean percent mortality of adult beetles one week after exposure on grain treated with proprietary materials N6 and N7.

Treatments	Mean percent mortality ± (SE)*		
	Rice weevil	Lesser grain borer	Red flour beetle
N7	100 (0.0) a	100 (0.0) a	38.5 (9.2) a
N6	100 (0.0) a	100 (0.0) a	10.5 (5.0) b
DE 300	98.5 (1.5) a	4.5 (1.3) b	1.5 (1.5) bc
DE 80	37.5 (5.6) b	0.0 (0.0) c	0.5 (0.5) c
Control	1.0 (0.5) c	0.5 (0.5) c	0.0 (0.0) c
F (df)	140.02 (3, 4)	878.97 (3, 4)	9.14 (3, 4)
P	0.0001	0.0001	0.0005

*Means in the same column with the same letter are not significantly different LSD test (P< 0.05).

Table 16. Adult progeny produced by four species on grain treated with preparatory material N6 and N7.

Treatments	Mean number of progeny ± (SE)*			
	Rice weevil	Lesser grain borer	Red flour beetle	Indian meal moth
N7	0.25 (0.25) c	0.0 (0.0) d	0.0 (0.0) c	0.0 (0.0) b
N6	0.0 (0.0) c	0.0 (0.0) d	0.0 (0.0) c	0.0 (0.0) b
DE 300	66.75 (8.4) c	85.75 (2.98) c	0.0 (0.0) c	0.0 (0.0) b
DE 80	306.5 (22.1) b	429.0 (23.7) b	10.75 (4.0) b	0.0 (0.0) b
Control	633.0 (117) a	819.75 (23.0) a	28.0 (4.7) a	9.0 (0.41) a
F (df)	14.36 (3, 4)	450.82 (3, 4)	9.86 (3, 4)	278.14 (3, 4)
P	0.0001	0.0001	0.0004	0.0001

*Means in the same column with the same letter are not significantly different.
LSD test (P<0.05)

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