Efficacy of Indian spices against *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae) as Third Generation Insecticides

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CERTIFICATE

I feel great pleasure in certifying that the thesis entitled **"Efficacy of Indian Spices against *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae) as Third Generation Insecticides"** embodies a record of the results of investigations carried out by **Mrs. Archana Saxena** under my guidance. I am satisfied with the analysis of data, interpretation of results and the conclusions drawn.

I recommend the submission of thesis.

Date : **26-04-2016**

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I feel blessed to complete this thesis close to my retirement from government college Kota Rajasthan.

(ARCHANA SAXENA)

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CHAPTER-1

INTRODUCTION

Before 1947, few synthetic insecticides used in crop protection were stomach poisons based on heavy metals such as lead and arsenic, which killed only if eaten, were known as first – generation insecticides. Some botanical extracts, such as rotenone and pyrethrum, both of which quickly degrade in the environment, were also used.

After Second World War varieties of artificially synthesised compounds were recognised. These, were effective in killing insects by mere physical contact known as second- generation insecticides, beginning with DDT in 1947 and these often killed natural enemies more efficiently than they killed targeted pest, are known as wide spectrum insecticides. They indirectly increased the cost of application, pest resurgence and ability to develop resistance to insecticides (Mcintosh 1940).

Resistance may be defined as ability of a strain of insects to tolerate doses of an insecticide, which in the normal course would kill the majority of a population of the same species. Phosphine resistant populations of *T. castaneum* were treated with methyl bromide to control infestation **(**Irshad and Iqubal 1994**).** But the use of methyl bromide is being restricted and will be phased out by 2015 because of its potential to damage the ozone layer. The problem of resistance to chemical insecticides in the insect pests increased manifolds. To overcome the problem high doses of insecticides were applied. Generally chemical insecticides like melathion, fenitrothion, permethion, deltamethrin, cypermethrin have been used as grain protectant for stored grain. These are hazardous, being toxic to the flora and fauna of the ecosystem and also leading to abiotic and biotic environmental polluation. Insecticide t were entering in the food chains and bio-magnification took place at different trophic levels. Residues of insecticides left behind polluted the air, water and soil. This led to serious health problems in mankind.

Uncontrolled use of these chemicals causes great environmental hazard due to their persistent nature, increased risk of neuro toxic, carcinogenic, teratogenic and mutagenic effects in non-target animals, acute residual toxicity, ability to create hormonal imbalance, spermatotoxicity. (Khatter 2011)

Reducing conflicts of natural enemies with insecticides is a major focus of Integrated Pest Management. There are two potential solutions: using insecticides that have intrinsically selective action or using application systems that are ecologically selective.

Systemic insecticides enter into plant tissues and available only to insects that feed on crop tissue or sap, doing no harm to natural enemies resting or walking on plants. Insect growth regulators are chemicals that mimic insect hormone preventing natural moulting are narrow spectrum insecticides known as third –generation insecticides.

There have been numerous reports on the occurrence of plant metabolites having JH activity. The compounds isolated from paper made from Balsam fir (*Abies balsamea*) and acetone extracts of *Cassia occidentalis*, were identified as juvabion and dehydrojuvabion. If insect undergoing metamorphosis are treated with JH or JH analog, they moult into intermediates which are immature and half adults, which do not gain reproductive competence. The insect growth regulators from plants mimic the JH action and could be exploited for insect pest management. (Gaur and Kumar 2010)

Pest status of *Tribolium castaneum* as a stored grain and milled product of wheat has been well established. Information's regarding developmental stages and life history helps in manoeuvring infestation led by *T. castaneum* (Herbst). The adult insect is of tapering form, tiny, flat, reddish brown beetle with antennae ending in abrupt clubs. Adults and grub are active whole year and these stages cause damage. From eggs to adult stage is completed in 50 days. An adult female lays about 300 eggs in pits, cracks and crevices in the soil or in cavity in the grain. When eggs are freshly laid, they are very small and white in colour, but they become bright red before hatching. Hatching takes 3 to 8 days, more eggs hatch in low humidity than higher. The grub covered with fine hairs is yellowish white and it has six legs. The young caterpillar makes, it way into the grain through some crack or hole in it. Larval instars are six in number and larval period lasts for 21 -28 days. When it is fully developed, it starts pupating, at the expiry of 3 to 10 days, adult comes up. Adults are 3.5mm long and 1.2mm in width. A generation of this insect takes 80 to 125 days to cover life span subjected to the effect of climate. 4 to 7 generations of them may evolve in a year. These beetles have chewing mouth parts but do not bite or sting. It spends whole life outside the grain kernels. It has high reproductive potential and breed through the year in warm area. *T. castaneum* pest status is considered as secondary, requiring prior infestation by an internal feeder.

Adults and larvae cause serious damage to stored grains. The larvae feeds upon endosperm of seed, leaving only the seed coat. The seed thus completely loses its viability as well as its nutritive value. So the grain is rendered unfit for human consumption.

Larvae also produce a large quantity of whitish powdery excreta, which makes the grain more dusty and imparts an unpleasant smell. The use of insecticides is first line of defence in controlling the insect pests.

Increased public awareness regarding human safety and environmental damage due to insecticides also diverted attention towards the use of plant Plants produce secondary metabolites , many of which have insecticidal properties, are eco- friendly alternative to synthetic insecticides products in stored-grain insect pest management. Essential oils are potential alternatives to current stored-grain fumigants and repellents because of their low toxicity to warm-blooded mammals and their high volatility. Besides preventing qualitative and quantitative loses they do not leave the toxic residues in the food grains.

The toxic principles in botanicals are mostly alkaloids-heterocylic nitrogenous compounds with marked physiological activity e.g. Nicotine, rotenone, sabadilla, rynia and pyrethrum .Neem *Azadirachata indica* has aroused worldwide interest. Neem owes its toxic attributes due to presence of azadirachtin, meliantrial, salanin, nimbandiol etc, among which azadirachtin is most potent.

Spices are characterized by flavoured odour due to presence of aromatic oil. They can be easily extracted into pure form. Their volatile constituents can influence insect behaviour and can be used as potential fumigant. These are even exempted from pesticide registration. This special regulatory status combined with the wide availability of essential oils from the flavour and fragrance industries, has made it possible to fast track commercialization of essential oil-based pesticides.

LD_{50} value against the pest insect for spices in question were evaluated .Also their insecticidal property in combination was studied. Experiments for residual effect and repellency were also conducted against adults of *T.castaneum*.

Black pepper (*Piper guineense*) caused100% mortality in red flour beetle (Ajayi and Olonisakin, 2011), Turmeric and cinnamon has been proved to work as repellent and shown toxic effect against lesser grain borer, granary weevil and red flour beetle (Shayesteh and Ashouri,2010)

Phenyl propene, eugenol, are the major constituent of essential oils obtained from both clove buds (90%) and clove leaves (45-60%), and is the active ingredient in a number of home as insecticides. Cinnamon bark oil contain 60-82% of cinnamaldehyde as its main constituent (Devi and Devi, 2013). The total essential oil content of plants is generally very low and rarely exceeds 1% but in some cases, for example clove (*Syzygium aromaticum*) and nutmeg (*Myristica fragrans*), it reaches more than 10%. Essential oils are hydrophobic and soluble in alcohol.

The insecticidal value of *Myristica fragrans* (Haryadi and Rahayu. 2002) has been established against stored grain pests.

Monoterpenes can be some of the best and safest alternatives to synthetic insecticides (Popovic *et al* 2013). The monoterpenes can penetrate through breathing and quickly intervene in physiological functions of insect. These compounds can also act directly as neurotoxic compounds, affecting acetylcholinesterase activity including hyperactivity, seizures, and tremors followed by knock down, which are very similar to those produced by the pyrethroid insecticides.

The long-standing cultivation of cloves and cinnamon in Southeast Asia and their worldwide use as culinary spices has led to their ready availability and relatively low cost, attractive features for their development and marketing as natural insecticides.

Research on spices as grain protectant in India is scanty. The current trend is the search for and use of alternative methods to manage pests, which, in the economic context, are effective without presenting the risks associated with the use of conventional pesticides.

Therefore the present research work was undertaken to evaluate efficacy of ethanol extracts of spices against *Tribolium castaneum* a notorious pest of wheat round the globe.

CHAPTER-2

LITERATURE REVIEWED ON THE SUBJECT

Eagleson (1942) reported 35 vegitable oils mixed with pyrethrins in kerocene, sesameic oil was synergistic when tested against housefly due to seseamin content.Furthermore it is stated that 3,4 methyl dioxphenyl group of sesamine is essential for synergistic activit.

Mcintosh (1947) tested colloidal D.D.T. suspension by dipping and spraying against *Tribolium castaneum*. Colloidal D.D.T. was less toxic than crystalline suspension. Toxicity is measured by amount of poison retained and not by concentration of suspension. Coarse suspensions were more effective than finer ones.

Dyte and Blackman (1970) tested strains of *Tribolium castaneum* from eleven countries for resistance to Lindane and Malathion.Strains from cargoes being unloaded in English and Scottish ports were tested. Eleven were resistant to Lindane out of thirteen and fifteen out of seventeen tested were resistant to malathion.

Dhingra and Swarop (1979) reported that irrespective to ratio Karenj oil synergised carbyl and showed additative effect with DDT, Lindane and Malathion. Their combinations against *Tribolium castaneum* appeared additive but antagonism was observed with melathion. Piperonyl butaoxide formulations with lindane, pyrithrins at different ratio revealed synergistic against Cylas farmicarius.

Jilani and Helen (1983) evaluated three plant materials that are common in Pakistan rhizome of *Curcuma longa* (turmeric), leaves of *Azadirchata indica* (neem) and leaves of *Trigonella foenum-graecum* (fenugreek) for their repelling against adults of three spices of stored products insects, *Tribolium castaneum*, *Sitophlius granaries* and *Rhizopertha domonica*. Petrolium ether extract was more effective than acetone and ethanol extracts.

Mariappan and Saxena(1984) in laboratory tested synergistic action of custard apple oil and neem oil against rice green hopper (*Neptholettix virescens*) and rice fungus virus RTV.

Saxena (1987) revealed that (PBO) Peperonyl butaoxide synergised DDT but antagonised Melathion and Pyrethrin when tested against *Sitophlius oryzae*.

Collins (1990) was of opinion that the use of PBO synergised Pyrethroids would result in control failure of pyrethroids resistant strain of *Tribolium castaneum* in the fields. However the resistance of pyrethroids would not jeopardise the use of organophosphates

Arthur (1992) determined different formulations of Cyfluthrin on unpainted and painted steel for control of melathion resistant red flour beetle *Tribolium castaneum*. Both insecticides remain effective up to one year on painted steel giving 99.8% mortality. Determining the degree to which such changes impair, the performance of chemical under commercial applications are essential step in development of discreet pest management strategies. Non toxic vegetable oils emerged as a best substitute for PBO in mixture containing deltmethrine as toxic agent against *Tribolium castaneum*. This reduces hazards caused by use of chemical alone for pest management.

Irshad and Iqubal (1994) observed that phosphine resistance in important stored grain insect pests in Pakistan.Resistance to phosphine were only found in *Triboium castaneum* and *Tribolium granarium*.

Huang *et al* (1997) reported that nutmeg oil in concentration of 1.05g/100gm of wheat, totally suppressed F-1populationof *Tribolium castaneum*. Larvae were more susceptible than adults to contact toxicity. Extracts were obtained by steam distillation and tested for contact toxicity.

Pemonge *et al* (1997) investigated seeds and leaves extract of *Trigonella-foenum-graecum* appeared to be moderately toxic to larvae of *Tribolium castaneum* (LD50-= 18% in diet) Seeds affect fertility in both sexes. Topical application of extract produced a mortality high in both insects (at 6 and 30mg/insect).

Huang and Ho (1998) observed that a methylene chloride extract of spice cinnamon (*Cinnamomum aromaticum*) was having insecticidal properties against *Tribolium castaneum* and *Sitophlius zeamais* Motsch. The contact and antifeedant effect of cinnamaldehyde were tested against adults and larvae. Hence it is a potential grain protectant.

Verma and Dubey (1999) said that various plant products affect nerve axons and synapsis (pyrethrins, nicotines), muscles (ryanodines), respiration (rotenone), hormonal balance (phyto ecdysomes), reproduction (basarone) and behaviour (attractants, repellants, antifeedants). There is amounting social pressure against use of toxic chemicals to control insectpests, plants are richest source of renewable bio active organic chemicals.

Suzuki *et al* (2000) with the help of chromatograms obtained by silica gel chromatography and uv absorption detector identified glycolipids in clove, nutmeg and red pepper. Peaks in clove indicate that it may contain new and plural neutral glycosphingolipids.

Tripathi and verma (2000) evaluated the efficacy of powdered black pepper (*Piper nigrum*) against *Rhizopertha dominica* .Dose of 3g pepper per 100g wheat resulted in 100% mortality after 15 days, as did 2.5g after 25 days

Haryadi and Rahayu (2002)) evaluated mixture of acetone extracts from black pepper (*P. nigrum*) and nutmeg (*M. fragrans*) seeds for their insecticidal effect on maize weevil (*S.zeamais*) under laboratory conditions. Extracts in ratio 0.0, 0.1 and 0.2% incorporated into the diet significantly reduced number of F1 offsprings, prolonged the development period and development index. Extract of black pepper alone has relatively better insecticidal properties than nutmeg.

Nakatani (2003) searched for active antioxidant and antimicrobial constituents of spices and herbs. Antioxidants included phenolic diterpines from rosemary and sage, phenolic carboxylic acids and glycosides from oregano and marjoram, diaryl heptanoids from ginger, biphenyls and flavonoids from thyme and phenolic amides from pepper and chili pepper. Antimicrobials included monoterpenes in nutmeg and clove.

Waqas *et al* (2004) collected different strains of *Tribolium castaneum* from various locations in Sheikhupura district of Punjab, Pakistan, were reared in laboratory for getting individuals of uniform age under controlled conditions of humidity and tempreture. These insects were given dose of 200ppm, 300ppm and 400ppm concentration of phosphine gas for 1, 3, 5, 7 and 14 days exposure period, gave almost 100% mortality of *T. castaneum*. Higher concentration (300ppm and400ppm) gave 100% mortality by exposure of 7 days.

Haq *et al* (2005) assessed repellency and toxicity potential of leaves of *Eucalyptus, Bonganivellea glabra, Azadirachata indica, Saraca indica and Ricinus communis* in the grain to protect against insect infestation. When crushed leaves were used in 5% ratio attained 50% control.Use of plant leaves besides being cost effective, may also abate the environment pollution and reduced health hazards

Jbiolon *et al* (2006) tested significant insecticidal activity of crude methanol extract of *P. harmala, A. liva, A. baetica* and *R. raphanistrum,* against *Triboluim castaneum* larvae and adults. In pupae plant extract did not induce mortality. *P. harmala* is rich source of beta-carboline alkaloid as harmol, harmine and harmaline .In control larval duration is 7-1 days, when treated with *P.harmala* larval period extended 8-1,8-4 days. Extracts were mixed with the diet at concentration of 10% and could be useful for managing *Tribolium castaneum* population.

Pimentel *et al* (2006) reported that resistance to fumigants has been frequent in insect pest of stored products and is a main obstacle in controlling them. The phosphine resistance bioassay followed the standard method recommended by Agriculture Organisation of the United Nation (FAO). The respiration rate (CO_2 production) and the instantaneous rate of increase (ri) of each population were correlated to the resistance ratio at LC_{50}. In *T.castaneum* it ranged from 1.0 to 186.2-fold. Lower respiration rate showed a higher resistance ratio, possibly related to phosphine resistance. Susceptibile population had low rate of reproduction

Zhou *et al (*2006) in the west of Hubei, China first reported reproduction inhibition rates of methanol extracts of *Cypripedium japonicum* (stem and leaves) and *Curculiogo orchioides* against *Tribolium castaneum,* 60 days after treatment were around 50%inhibition. *Spiraea prunifolia* (flower) extract against adults 30days after the treatment there were over 60% reproductive inhibition recorded All these plants extracts caused no weight gain, pupation delay, abnormal pupae and pupae death.

Tribolium castaneum third instar larvae showed antifeedant activities against these plants.

Ataur Rahman and Talukder (2007) explained that malathion resistance in *Tribolium castaneum* is widespread and stable in natural populations even in absence of pesticide exposure. Samples collected at central storage depots (CDS) and local storage depots (LSD). LC_{50} level tested after 72hr exposure. A few local populations were more sensitive.

Abdel-latief (2007) investigated expression of chemoreceptor gene using quantification real time RT-PCR analysis in the antennae, mouth parts, in adult and larvae of *T.castaneum*. It is mediated by G-protine-coupled receptors (GPCR). 62 Gustatory and 26 olfactory (Grs and Ors) receptors were identified so far. Chemoreceptor genes was observed in head tissues that contains the brain and suboesophageal gangalion(SOG). Such a finding in *T.castaneum* reported, which is evolutionarily related to other insect species.

Chaubey (2007) isolated essential oils from *Cuminum cyminum* (Umbelliferae), *Piper nigrum* (Pipeaceae) and *Foeniculum vulgare* (Umbelliferae) and then evaluated their repellency, contact toxicity and developmental inhibitrory activites against *Tribolium castaneum*. At 0.2 % concentration (vol/vol) repellent activity was observed. LC_{50} against larval stages of insect was 15.02, 14.02 and 17.48 micro l were recorded respectively for all the three extracts. For adults it was little higher 16.26, 15.26 and 18.55 micro l recorded for *P.cyminum, P. nigrum* and *F. vulgare* respectively. These essential oils reduced oviposition potential of *T.castaneum*. Development is inhibited with vapours of these oils, fumigation also resulted in deformaties in various stage of the insect.

Gupta and Singh (2007) mentioned that egg laying in untreated adults *Tribolium castaneum* started on fourth day (6.66 eggs per female) after emergence of adults. Mean total eggs laid by female in 20 days were 206.6 (100%). At 1 and 2% treatment with neem leaf powder, egg laying significantly reduced (38.00 to 13.2%) respectively. Methnol mint leaf powder also reduced egg laying (79.86 to 33.97%) at

1 and 2 % treatment respectively. *T.castaneum* reared at 2% tulsi leaf powder laid only 60.46 % eggs in 20 days.

Su *et al.* (2007) evaluated radical-scavenging activities against cations ABTS, DPPH peroxyl and hydroxyl radicals from Black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf extracts with 50% acetone and 80% methanol. Total phenolic content and chelating activity were also determined. Acetone extract was more effective, indicating that solvent may alter the antioxidant activity for selected botanicals, including spices and herbs.

Upadhyay and Jaiswal (2007) examined repellent, insecticidal and developmental inhibitory activities of the essential oils of *Piper nigrum* L against *Tribolium castaneum* (Herbst). 0.2% concentration of *P. nigrum* repelled *T. castaneum* in filter paper test. The LC_{50} values for larvae and adults were calculated, appear to be 14.022 µl and 15.262 µl respectively. Adult mortality increased while adult emerg ence decreased with increase in the concentration of essential oils. EC_{50} dose of *P. nigrum* oil reduce larval transformation into pupae to 50% was found to be 6.919 µl.

Ahmadi *et al* (2008) examined essential oils of *Rosmarius offcinalis* L. and *Artemesia sieberi Besser* collected from Tehran province for their potent fumigant toxicity against stored product beetle, *Tribolium castaneum* (Herbst). Dry leaves of plant were subjected to hydrodistilation. The mortatlity of 1-3 days old adults increased with concentration from 2.50 to 8.93 micro l/l air for *R. officianalis* and 3.57 to 28.57 micro l/l air for *A. sieberi* respectively. Results indicate that *T. castaneum* was most sensitive, hence further research on their fumigant potential for commercial use against stored –product insect is warranted.

Arabi and Sefidkon (2008) tested fumigant toxicity of essential oil extracted from *Tanacetum polycephaaselum* L. (*Asteraceae*) against adults of two stored product insects, *Tribolium castaneum* and *Callosobruchus maculatus*. Dry aerial parts of the plant were subjected to hydrodistilation . Fumigant toxicity was tested against 1-7 day old adults with five replications at 25± 1^0C and 65± 5% RH under dark condition. For *T.castaneum* LT_{50} values ranged from 7.43h for lowest dose 32 micro l/l air to 5.2h for highest dose 483 micro l/lair. The LT_{50} for *C.maculatus* reported decreased to 8.0h for lower dose and 6.2h for highest dose. It is a safe pesticide or model for new synthetic pesticides to control stored- product pests.

Azevedo *et al* (2008) said that seeds and grains are targets of insect pests which causes great serious problem in warehouses. Losses are estimate in circa of 10% of the world production. Neem seeds oil bioactivity was observed against *Triblolium castaneum*. Peanut seeds were treated with neem oil concentration of 0.0, 0.5, 1.0, 1.5 and 2.0% (volume/seed mass). Insect mortality, offspring number (larvae, pupae and adult) and number of punched seeds were evaluated in four stored period (30, 60, 90 and 120 days) Friedman test ($p <= 0.05$) was used for variance analysis and Student Newman Keuls test ($p<=0.05$) for means comparison. Neem presented low efficiency on mortality of adult insects. The effect of neem on biological development was observed.

Bukahari *et al* (2008) investigated antioxidant properties of fenugreek seeds. It is due to presence of many active phytochemicals including vitamins, flavionides, terpenoides, iron, beta-carotene, saponins and phytic acides. Antioxidant activity of fenugreek extract was assessed by free radical scavenging by DPPH reducing powder. Phenolic compounds may contribute directly to antioxidant action.

Jablion *et al* (2008) investigated bio-insecticidal effect of methanol extracts of *Centaurium erythaea*, *Peganum harmala*, *Ajuga iva*, *Aristolochoal baetica* and *Raphanus raphanistrum* against *Tribolium castaneum*, plants extracts inhibit growth of larvae, *C.erythaea* was the most toxic with 63% mortality 10 days after treatment, followed by *P.harmala* with 58% reduction in the emergence rate. *P. harmala* extend the larval period when compared to control. *Ajuga iva* and *Aristolochoal baetica* inhibited F1 progeny production. Larvae fed on treated diet had lower alpha-amylase activity. These plant extracts could be useful to reduce seed damage caused by this pest species.

Priyanka *et al* (2008) conducted an experiment to study synergism and insecticidal resistance of insecticides, commonly used against *Triboium castaneum*. Treatment comprised : insecticides malathion,dichlorvos and deltamethrin.The decreasing order of synergism reaveled role of esterases and mixed function of oxidases in detoxification of melathion. Synergistic activity revealing inhibition of esterases activity in dichlorvos resistance. Synergism also revealing partial inhibition of carboxyl esterases in dichlorvos degradation. The level of deltamethrin resistance could be reduced by all the three synergists.

Alam *et al* (2009) evaluated residual film toxicity, fumigant toxicity and repellent effect of methanol extract of root of *Calotropis gigantean* (Linn) and its chloroform and petroleum ether (40-60 degree C) soluble fractions against several instar larvae and adult *Tribolium castaneum*. Methanol extract showed lowest LD50 valuae against several stages and 50 adults (121.59,147.73,146.84,202.98,358.42 and 300.03 micro gm/cm^2 respectively) Whereas LD50valuae of petroleum ether extract were (407.69, 485.46,437.38, 502.23, 551.26 , 625.36 and 411.84 micro gm/cm2 from first to sixth instar larvae and adult .Chloroform extract recorded 291.83, 299.29, 382.98, 745.18, 637.71, 1259.71 and 739.87 micro gm/cm^2 respectively. The order of toxicity on *T. Castaneum* was methanol extract>petroleum ether> chloroform fraction. No fumigant toxicity of test sample was found and show mild to moderate range.

Farjana Nikkon Habib *et al* (2009) tested crude extracts of flower of *Tagetes erecta* Linn for insecticidal activity against *Tribolium castaneum* (Herbst) The chloroform extracts showed highest toxicity against both larvae and adults followed by petroleum ether fraction and ethanol extract. The LC_{50} value of chloroform fraction against first to sixth instar larvae were 11.64, 14.23, 19.26, 29.02, 36.66, 59.51 micro gm/cm^2(72hr) respectively and for adults value was 65.93 micro gm/cm^2 (72hr). No mortality was observed in control.

Gul and Safdar (2009) recorded that *Cinnamon* contain ash 2.4%,crude protein3.5%,crude fat 4%,fiber33%,moisture5.1% and carbohydrates52%.*Cinnamon* contains higher amount of potassium and no sodium at all.

Haridasan and Gokuldas (2009) prepared petroleum ether leaf extract of *Vitex negundo* (VPE) and methanol (VME). Effects were observed on adult emergence of *Tribolium castaneum* during 24, 30, 40 and 50 days of treatment during different period of exposure, and compared with control. VPE at 10% concentration exhibited the highest percentage of inhibition or lowest adult emergence index.

Islam *et al* (2009) investigated biological activity of essential oil extracted from coriander, *Coriandrum sativum* L. (Apiaceae) against eggs, larvae and adults of *Tribolium castaneum* (Herbst). Air tight glass chambers showed vapour toxicity and strong repellency on filter paper arena test towards all stages. Responses varied significantly ($p<0.001$) within insect stages doses and exposures. At 12 micro g/ml dose mortality reached 95%, but for 12-, 14- and 16-days larvae, mortalities were 65, 74 and 82% respectively. Fumigated larval stages reaching pupal to adult stage, decreased significantly ($p=0.001$).Oil fumigation yields 100% mortality for *T. Castaneum* larvae, pupae and adults at 0.08 micro g/ml dosage. Again eggs toxicity increased with exposure time and concentration .Overall repellency was 90% at a dose of 12 micron g/ml.

Pugazhvendan *et al* (2009) mentioned that stored food grains face severe damage due to infestation by insects. The damages are ranging from 5-30%of the total agriculture produce worldwide. *Tribolium castaneum* has been found in association with a wide range of commodites including grain, flour, peas, nuts, dried fruits and spices. It also infesting groundnut kernels and pods. Milled grain products remain its preferred food. This insect causes substantial loss in storage because of its high reproductive potential and can breed throughout the year in warm area. Synthetic pesticides use is discouraged because of their adverse effect on human health and environment. Highest repellent activity was observed in *T.purprea* powder and EPI value for *T.purprea* in 0.5mg was -0.11and-0.56 at 1 and 6hr respectively and in 1.0mg concentration, it was -0.45 and -0.80 at 1hr and 6hr respectively against *Tribolium castaneum.*

Sahaf and Moharramipour (2009) extracted oils from *Carum copticum* and *Vitex pseudo-negundo* using hydro distillation and evaluated antifeedant activity agains *T.castaneum* Data recorded for relative growth rate (RGR), relative consumption rate (RCR),efficiency of coversion of ingested food (ECI) and feeding deterrence index(FDI).Essential oils(100-1500ppm) were spread evenly on the flour discs.When solvent evoparated 10 insects were introduced,after72h nutritional indices were calculated. *C.copticum* decreased RGR, RCR and ECI at levels significantly higher than *V.pseudo-negundo* but FDI increased.Antifeedant activity of *C.copticum* was more effective than *V.pseudo-negundo.*

Sharma *et al* (2009) tested three plant product s viz.turmeric,garlic and neem against *Tribolium castaneum* infesting stored basmati rice, under laboratory conditions. After six month of treatment, mortality was highest in the stored grain treated with turmeric powder 2.0% concentration. Alcoholic acidity, free fatty acids and uric acids content decrease or increase with mortality rate. Optimum cooking time of rice does not change during storage period. Order of effectiveness was turmeric powder> neem powder>turmeric grits>garlic powder>garlic cloves> neem seed powder.

Shukla *et al* (2009) extracted essential oils from fruit of *Myristica fragrans* and *Illicium verum* by hydrodistilation method and its toxic and developmental inhibitory activities were determined against *Tribolium castaneum* adults and larvae. Median lethal concentration against larvae were 12.63micro l and 18.43 micro l against adults it was recorded as 14.23 micro l and 19.87 micro l for *Myristica fragran* by the dose of 6, .08 micro l and 11.97 micro l of *Myristica fragrans* and *Illicium verum* respectively because they inhibit pupal transformation from larvae.

Tripathi *et al* (2009) said that insect pest causes major damage to stored grain and foodstuffs, reducing weight of product, quality and market value. Eight spices commonly used to add flavour alone or in combinations were evaluated for their contact and fumigant toxicity, repellency and effect upon progeny development against *Callosobruchus maculates* and *Tribolium castaneum* Herbst. Powders of large cardamom, turmeric and ginger showed contact toxicity against two insects. Green cardamon was found to have fumigant toxicity. Clove powder at 1.5g/50g dose showed 100% repellency against adult *T.castaneum*. These powders also caused inhibition of progeny production at dose of 5g/100g.1:1 Ratio of turmeric and green cardamom showed both contact and fumigant toxicity, while mixture of clove and large cardamom showed repellency and inhibited progeny development in *T.castaneum*.

Wang *et al* (2009) estimated the contact toxicity of monoterpenes of 3-carene, 1, 8-cineole, beta-pinene, terpinene and terpinolene on the repellent, contact toxicity of *Tribolium castaneum* adults and sustaining fumigant toxicity of *Sitophilus zeamaise* adults were examined. Monoterpene of 1, 8-cineole in 20microLml-1 and beta pinene in 20 microLml-1 exhibited highest percent of repellency, but even 4micro Lml-1 concentration of 3-carene exhibited lower percentage of repellency. Contact toxicity of beta-pinene LC_{50} value ranging between 31.44 and 62.07 micro g mg-1 and for 3-careen LC_{50} lies between 66.58 and 93.68 micro g mg-1. Fumigant toxicity for 24, 48, 72 and 96hr, when LC_{50} value of terpene and terpinolene was not changed, mortality was reduced to 71.8% after 96hr where as beta-terpene and 3-carene 35.0 and 31.4% reduction were observed, respectively. Monoterpenes could be screened for the control of storage pests.

Yang FengLian *et al* (2009) evaluated insecticidal activity of polyethylene glycol (PEG) coated nanoparticles loaded with garlic essential oil against *Tribolium castaneum*. Oil loading capacity could reach 80% at PEG (10%). Nano particle were round and good dispersion, < 240nm in diameter, with dynamic light scattering ability. There is slow and persistant realease of active components through nano particles. Control efficacy remained over 80% against *T.castaneum* after five months. In contrast in control efficacy of free garlic essential oil at similar concentration (640mg/kg) was reported only 11%.

Abdel-Sattar *et al (*2010) stated that fruit and leaves of essential oils of *Schinus molle* showed insect repellent and insecticidal activity against *Tribolium castaneum* and *Trogoderma granarium*. Hydrocarbons dominated the oils composition with monoterpenes occurring in the largest amounts in leaves and fruits, 74.84% and 80.43% respectively, p-Cymene was identified as major component in both the oils. The high yield and efficacy of *S.molle* provide leads for active insecticidal agents .

Ahmed *et al* (2010) conducted laboratory experiment to evaluate effectiveness of leaf powder of Spider plant ,Basil, Hyptis, Sugar apple,Desert date and Negro coffee and synthetic insecticide *Primrose-methyl* (Actellic Dust) in controlling *Tribolium castaneum* Herbst in stored millet grain(Variety-Sosat). *Premrose-methyl* treatment mortality was 3.17 after 22h after treatment and 1.83 at 72h after treatment, significantly higher than all the plant materials, with complete suppression of larval development and adult emergence at 15 and 45 days after treatment, desert date and sugar apple caused adult mortality of 1 and 1.29 at 22h and 72h respectively. Grain weight loss of 1.98% and grain damage of 22.2% recorded. Although, 5.0gm 7.5gm concentration of plant powders were equally effective in reducing larval development and adult emergence. Use of 7.5gm/25gm is recommended for maximum protection against *T.castaneum*.

Ashouri and Shayesteh (2010) mixed black pepper seed powder and red pepper fruit powder in 20gm of wheat as direct admixture at five different doses 0, 0.15, 0.2, 0.27, 0.37 and 0.5% (w/w) for black pepper and 0.0, 0.5, 1.5, 3 and 5% for red pepper, to assess mortality and reduction in F 1 progeny. Treated grains were infested with 20 adults. At 0.5% conc 100% mortality of *S.granarius* in first five days and *R. dominica* at 5% showed 100% mortality after 16 days. But red pepper did not cause complete mortality after 14 days. Both plant material caused F1progeny reduction.

Farzana Parveen *et al* (2010) tested toxicity and residual effect of yellow-berried nightshade, *S. kolanum surrattense* leaves extract against red flour beetle *Tribolium castaneum* under laboratory conditions. The lowest mortality 15% was observed at minium dose 2.4 microl/cm^2 and the highest 100% observed at 16.8microl/cm^2 after 24h.The LD$_{50}$ of *S.surrattense* was found 8.02micro/cm^2. Residual effect observed, during 1st day, mortality was lowest 49.4% at minimum 2.4microl/cm^2 and highest mortality was 91.6% at12.0 microl/cm^2. During 7th day lowest was 5.0% at 2.4microl/cm^2 and highest 60.5% at 12.0 microl/cm^2. During 8th day lowest 0.4% at 2.4 *S. surrattense* could be useful for managing populations of *T. castaneum*.

Gandhi *et al* (2010) tested pulverized leaves of *Punica granatum* (Pomegranate) and *Murray koenigii* for their efficacy against the stored pest *Tribolium castaneum*. Five different concentrations ranging from 0.05 to 1.0gm/10 gm of wheat grains were tested for their insecticidal and seed protective properties .Powders of leaves resulted in high mortality and delay in development and reduction of population. Number of days (8-27) required for 100% mortality was decreased with increased concentration. Damage from infestation reduced to 5 and 6% over a period of 50 days, hence giving protective effect of the plant, extended to 85 to 90% in *P.granatum* and *M.koenigii* over control.

Gaur and Kumar (2010) treated final stadium larvae of red flour beetle, *Tribolium castaneum* with acetone extract of *Cassia occidentalis,Commenlia benghalensis, Polygonum lanceolatum, Momrdica charantia* caused juvenilizing effects such as supernumerary moults, ecdysial stasis, larval-pupaul, and pupal-adult intermediates, similar to effects produced by exogenous application of juvenile horomone (J H) or its analogs. Growth regulators from plants extract mimic the J H action and could be exploited for pest management.

Harvansh *et al* (2010) measured antifeedent activity of leaves extract of *Cleistanthus collinus* against third instar larvae of cotton worm and adulticidal activity and repellency test for *T.castaneum*. LD_{50} and LD_{90} were 3mg/ml and 5mg/ml respectively. P-value is 0.005 considered significant. The repellency percent was noted according to the concentration (mg/ml) 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 between the time interval 0 to 10 hr was 0%, 10%, 20%, 30%, 40%, 60%, 70%, 80% and 90%.

Kim *et al* (2010) analysed insecticidal activity and repellency of *Origanum vulgaris* L.oil against *Tribolium castaneum*. All constituents were identified, the main components were carvacrol (67.2%).p-cymene (16.2%), gamma-terpinene (5.5%), thymol (4.9%) and linalool (2.1%). Fumigation was more effective in closed conditions (LD_{50}=0.055mg/cm^3) than in open conditions (LD_{50}>0.353mg/cm^3). Repellency observed at 0.03mg/cm^2, it depends upon both time and concentration.

Mondal and Khalequzzaman (2010) evaluated four compounds (trans-anethol, thymol, eugenol and cinnamaldehyde) occurring naturally in the plant essential oils for contact and fumigant toxicity against adult *Tribolium castaneum* and 10 days and 18 days old larvae. Insecticidal activity varied with concentration and exposure time. Most sensitive period was 10 days old larvae. Cinnamaldehyde and eugenol were highly effective against *T.castaneum* when applied for 48hr at lowest dose. At highest dose level of 0.288mg $cm\text{-}^2$ and lowest exposure time 6hr, trans-anethol achieved 100% mortality of 10 days larvae as contact toxicity and fumigant toxicity of eugenol achieved only 36.66 and 30% of 10days old larvae and *T.castaneum*. Against 18days old larvae, eugenol and cinnamaldehyde achived 100% mortality for 48hr exposure even with the highest exposure dose volume.

Shayeshteh and Ashouri (2010) conducted tests to find out the repellency of four powderd spices, Black pepper (*Piper nigum*), chilli pepper(*Capsicum annuum*), cinnamon (*Cinnamomum aromaticum*) and turmeric (*Curcuma longa*), against three stored-product insects, *Rhyzopertha dominica*, *Sitophilus granarius* and *Tribolium castaneum*. Repellency potential was measured by observing visual movement from treated grains.A cylinder of 6cm diameter and 15cm high with 2cm perforations, with a mesh bottom was placed in the center of a container 15 cm in diameter and 15cm high The powdered spices are poured into 200g of wheat mass with long funnel at concentration of 0, 0.25, 0.75, 1.5 and 2.5% on((w/w) basis. Twenty insects of each species were introduced into the container at room conditions. At highest concentration *S. granarius* were repelled faster, followed by *T. castaneum* and *R.dominica*. Wheat treated with cinnamon powder were most repellent to adult *S.granarius* 92.5% after 1h, chilli pepper treatment for *T. castaneum*72.5% after 6h and black pepper treatment for *R.dominica* 58.75% after24h.

Yang *et al* (2010) studied the fumigant activity of garlic essential oil and its major components, diallyl disulfide, diallyl trisulfide and diallyl sulfide against stored product insect *Tribolium castaneum*. Diallyl trisulfide among all had strongest fumigant activity. The concentration of 8micro l/l obtained 100% mortality, at exposure time of 4-days but 6-7 days exposure time was required for garlic essential oil. There was no side effect on seed germination. Progeny production was totally suppressed at 4microl/l at 10% or 50% filling ratio.

Zhou Lin *et al* (2010) bio-assayed population inhibition, due to total alkaloid from *Tripterygium wilfordii* Hook against *Sitophilus zeamais* and *Tribolium castaneum*.In 33 days of treatment with dose of 500mg/kg alkaloid, mortality of *S.zeamais* was above 90.83% and inhibition rate of the population of F1 generation fed on the poisonous wheat was above96.70%.When F1 generation fed on normal wheat was also 80.10% with the dose of 500mg/kg alkaloid. With the dose of 100-1000mg/kg, the lethal effect on *T.castaneum* was not significantly differ when compared with that of powder used alone, but inhibition effect on its population size was significant. The inhibition rate of the population size was 54.94% with the dose of 250mg/kg alkaloid and reached 98.02% with the dose of 500mg/kg alkaloid.

Abbasipour *et al* (2011) tested extract from *Datura stramonium* L. against *Tribolium castaneum* for toxic and antifeedant activity. Extract made with 150ml absolute ethanol at 45^0C.Various nutritional indices, such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion rate of ingested food (ECR) and feeding deterrence index (FDI). Flour disk were kept at 27± 1^0C and 60± 5% RH. 10μl extract applied evenly and then 10 adults were introduced into each treatment. After 72h nutritional indices were calculated. LC_{50} and LC_{90} of *D.stramonium* were 2936 and 15373 mg/1 respectively. All indices were reduced and food consumed also decreased.

Abo El-Sad *et al* (2011) estimated that in the essential oils from clove bud *Syzygium aromaticum* L. contained eugenol (48.92%) caryophyllene (18.53%).alpha – caryophyllene (3.255%), eugenol acetate(23%), cis-13docosenamide (3.21%) were present. *Tribollium castaneum* beetle showed dramatic repellent activity by 1.0, 0.8 and 0.2% clove oil 4, 8 and 10 min respectively. RT_{50} and RT_{95} were 1.1 and 8.0 min at 0.2% and 0.4 and 2.6 min at 0.8% respectively. At 100 micro L/L mortality was 75, 80, 100% after 6 ,7 and 8 days exposure period with LC $_{50}$ 17 micro L/L air.

Ajayi and Olonisakin (2011) determined that toxicity of following essential oils caused 100% mortality in both adult and larvae of *Tribolium castaneum* at higher dose. The LC_{50} of clove was 0.40 (adults), 0.46 (larvae), for African pepper 0.21 (adult), 0.54 (larvae) and Ethopian pepper1.78 (adult), 0.067 (larvae) per 20 gms of grains.

Arthur *et al* (2011) evaluated Catmint oil and hydrogenated catmint as repellents against adult *Tribolium castaneum* and *Tribolium confusum* by visual assessment of distribution and video recording to determine pattern of movement of individual insects. It was observed that hydrogenated catmint oil was more effective than the pure catmint oil. There was no significant difference between two species, when repellency was measured. Avoidance movement and change in direction observed when encountered with repellent. Repellency can be more accurately assessed by single insect behaviour recording than in groups.

Caballero-Gallardo *et al* (2011) said that essential oils and plant extracts used as a tool in pest management. Their contents were tested for repellent activity against *T.castaneum*. Main components found were (>10%) methylchavicol, limomeme/alpha-pinene, carvone/limonene, benzyl acetate/linalool/benzylbenzoate, andalpha-pinene, for *Tagetes lucida, Lepechinia betonicifolia, Lippa alba, Cananga odorata* and *Rosmarius officinalis* respectively. All these plants cultivated in Colombia are sources of repellent against *Tribolium castaneum*

Chowdhury *et al* (2011) elucidated repellent activity and toxicity of extract of leaves of *Vitex negundo*. L against *Tribolium castaneum*. Mortality of methanol extract were recorded at 24hr, 48 and 72hr post exposure period at nine different concentrations, by filter paper method, Repellency was assessed up to 5hr.Two bioactive compounds, 22, 23-dihydro-alpha-spinasterol-beta –D-glucoside and salicylic acid were isolated from methanolic leaves extract, caused 100% repellency in the strains of T. castaneum. It has great potential as a herbal insecticide against *T.castaneum*.

Ebadollahi (2011) studied antifeedant effect of *Eucalyptus globules* and *Lavendula stoechas* against adults *Tribolium castaneum* in laboratory conditions and applied sublethal doses. LC_{15} to LC_{35} killed 15% to 35% of the populations when these concentrations were used for antifeedant effect The fumigation of sublethal doses was done for 24hr exposure time. Decrease of flour weight was calculated after 48 h.Moreover these oils had dose-dependant antifeedant activity,and may be towards other insect pests as well.

Ignatowicz and Olejarsk (2011)said that Control of phosphine resistance by manipulating biolobical factors may be obtained by:((a) limiting the immigration of strains that are resistant to phosphine and(b) reducing the number of pest generations per year. Operational factors can be easily manipulated by following ways: (a) use of methyl bromide to control phosphine resistant strains (b)use of phosphine in well-sealed enclosures only, (c) use of IPM economic thresholds (d) use non-chemical methods (natural enemies, hygiene, physical factors etc). (e) use of contact insecticides. Inadequate exposure periods over many years are likely the reason for resistance to phosphine in some strainsof stored pests.

Khatter (2011) evaluated the potential of insect growth regulator on the growth and development of *Tribolium confusum* by *Azardirachtin*, a neem derative which is a chitin inhibitor. Its activity was tested topically, at four doses (0.5, 1.0, 1.5 and 2micro g insect-1) on adults, change in fecundity, hatchablity and viability of eggs, longitivity and morphometric of oocytes were recorded. The width of follicles not affected significantly by this treatment. Hatching rate of eggs are not affected in F-1generation but it affected viability of these eggs laid. (F=7.5, DF=99, p.0.0001). Duration of embryonic and post embryonic development period increase with concentration 0 to 2 micro g of dose of *Azardirachtin.*

Liska *et al* (2011) tested bioactivity of 1, 8-cineol, camphor and eugenol composition of essential oils from aromatic plants, to mitigate progeny in *Tribolium castaneum*. Thirty adults of red flour beetle 2-4 week old, mixed gender were put in a glass container volume 350ml filled with mixture of rough flour and dry yeast. Treatment was performed according to Huang et al. Compound 1, 8-cineole, camphor and eugenol were applied in three concentrations (120, 300 and 600 microl/350ml-1 vol) After 50 days of rearing in controlled conditions, adults of F1 generations were counted. At lowest concentration (120 microl/350ml-1vol).The progeny ranged between 174.25 to 221, 50 without statistic differences among tested compounds. At higher concentration 600microl/350ml-1vol lowest impact appear in progeny of *T. castaneum* had camphor (191.00) which was lower related to 1,8- cineole(72.25) and eugenol (112.00). This indicate camphor have no impact upon number of progeny.

Liu ZhiLong *et al* (2011) mentioned insecticidal properties of several Chinese medicinal herbs, ethanol extract of *Aconitum episcopale* roots possessed significant feeding deterrence against the red flour beetle, *Tribolium castaneum*.Deterrent were identified as chasmanine, crassicauline A, karacoline and sachaconitine, talatisamine and yunaconitine exhibited feeding deterrent activity. Chasmanine, talatisamine, karcoline and sachaconitine expressed feeding deterrent against *T.castaneum* with EC_{50} values of 297.0, 342.8, 395.3, 427.8ppm respectively. For yunaconitine and crassicauline EC_{50} against *T.castaneum* recorded as 653.4 and 1134.5ppm.

Mahmoudv *et al* (2011) assessed fumigant toxicity of essential oils extracted from *Rosmerinus officinalis* L, *Mentha pulegium* L, *Zataria multiflora*,and *Citrus sinesis* L, *Osbek var hamlin* against *Tribolium castaneum, Sitophilus granarius, Callosobruchus maculataus*. Values of *C. sinensis var hamlin* against *T. castaneum, S.granarius* and *C maculataus* were 391.28, 367.75 and 223.48 micro L L-1 air after 24 h and 362.40, 20.45 and 207.17 micro L L-1air after 48 h respectively. *T. castaneum* were most sensitive, these essential oils have good fumigant toxicity on stored- product pests.

Mishra *et al* (2011) studied effect of volatile compounds of essential oil of *Citrus reticulata* fruit peels against *Sitophilus oryzae* and *Tribolium castaneum*. Results indicated that oil of *C.reticulata* showed toxic effect against these stored grain pests. The LC_{50} against the larva of *T.castaneum* was 18.733 microl at 48 hr exposure. The LC_{50} against *T.castaneum* and *S.oryzae* were 21.698 micro l and 19.336 microl at 48 hr exposure, respectively.

Muhammad *et al* (2011) evaluated efficacy of plant extract of *Amaranthus hybridus(*Chaoli),*Calotropis procera* (AK), *Salsola baryosma* (Khar boot) and *Cumium cyminum* (zeera) against *Tribolium castaneum* under laboratory conditions. Direct mortality observed at 5%, 10%, 15% and 20% concentration of *A. hybridus,C. Procera,S. baryosma* and *C. cyminum* in petri dishes. Acetone was used as a solvent and 30 adults of *T.castaneum* were introduced, 2.89%, 1.11%, 1.00% and 0.78% mortality reported respectively. High concentration decreases larval emergence, pupal and adults emergence time duration was increased. *C.cyminum* exhibited maximum repellency (90%) and least by *A.hybridus* .

Naseem and Khan (2011) conducted experiments to determine efficacy of oil extracted from *Eucalyptus* camaldulensis and *Piper nigrum* in various concentrations that is 20, 40 and 60% for the suppression control of *Tribolium castaneum* under laboratory conditions.The incubator was maintained at **30+or-2 degree C and 65+or-**5% R.H. Higher concentration ,resulted in maximum repellency at maximum exposure period compared with minimum concentration at minimum exposure period. *E camaldulensis* proved more effective than *P.nigrum* at all the concentrations, at all post treatment interval.

Osman *et al* (2011) calculated residual effects of *Manikara zapota* (L) P. Royen plant against *T. castaneum*. Four doses,i.e.,123 8.5,619.25,309.6 and 154.8 micro g/cm^2 of ethyl acetate extract of stem bark applied on larvae and adult beetles. Maximum residual toxicity observed with LD50 of 228.8, 281.1, 413.4, 423.7, 455.2, 498.7 and 526.5 micro g/cm^2 for first, second, third, fourth, fifth, sixth instar larvae and adults respectively. Earlier instar were more sensitive among all.The effectiveness of extract was increased with the increase of exposure time and after 72hs exposure.

Parle and Khanna (2011) observed that a drop of Clove oil is 400 times more powerful as antioxidant than worlf berries or blue berries. It is also anti- fungal, anti-viral, anti-microbial, anti-diabetic, anti-thrombotic and posses great insect repellent properties. Eugenol is the main constituent

Qadir *et al* (2011) presented research work to evaluate, toxicity of chlorinated, organophosphorylated and pyrophosphated oil against *Tribolium castaneum*. Kerocene, castor, taramera (*Eruca sativa* seeds oil) and turpentine oils were chlorinated, phosphorylated and converted into pyrophosphate to assess their toxicity against *T.castaneum*. Experiment showed LC_{50} value of 6.5, 6.0, 4.5 and 5.7% for chlorinated, kerocene, castor, taramera and turpentine respectively. While phosphorylated kerocene, castor, taramera and turpentine oils showed LC_{50} values of 4.5, 4.0, 4.5 and 3.7% respectively against *T. castaneum* adults. Pyrophosphorylated, kerocene, castor, taramera and turpentine oils showed LC_{50} values of 2.3, 1.9, 1.9 and 2.0% respectively.

Santos *et al* (2011) stated that ever- growing concern of human health and environmental safety, alternate method of fumigation, other than methyl bromide and phosphine are need of time. Allyl isothiocynate (AITC) is the main component of mustard oil, is a potential fumigant and its pesticide activity is reported. Fumigant toxicity of 18 populations of adult *Tribolium castaneum* were assessed, all were found susceptible to AITC, despite of variation in body mass, respiration rate and fitness among populations, even developmental stages- eggs, larvae and pupue were similarly susceptible. Malformation were observed in larvae and adults. Results indicate that AITC is an alternate fumigant against stored product insects.

Stefanazzi *et al* (2011) evaluated bioactivity of essential oils from *Tageta terniflora* Kunth, *Cymobopogon citrates* Stapf and *Elyonurus muticus* Kuntz against stored-grain pests. Contact and fumigant toxicity were observed with *T.terniflora* against both the insects. All larvae and adult showed repellency. *Cymobopogon citrates* reduced the relative growth rate and efficiency of conversion of ingested food in *T.castaneum* larvae. *T.terniflora* produced a feeding stimulant effect in *T.castaneum* adults and feeding deterrent action against *S.oryzae* adults.

Suthisuf *et al* (2011) tested contact toxicity of mixture of rhizome of *Alpinia conchigera* Giff, *Zingiber zerumbet* Smith, *Curcuma zedoaria* Berg, major compounds (camphene, camphor, 1, 8-cineol, alpha humulene alpha pinene, beta pinene and terpinen-4-of) from essential oils and synthetic essential oils, comprised of of pure compounds in the same ratio as occur naturally, feeding reduction and repellency impact againt *Sitophlius Szeamais* and *Tribolium castaneum* were also assessed. *T.castaneum* had similar sensitivity (35-58micro g/mg) for all the three oils but less sensitivity than *S.zeamais*. LD_{50} value of synthetic oils were almost corresponding to extracted essential oils. Both the insects were sensitive to terpinen in contact toxicity tests. In antifeedant tests flour consumption decreases and terpnen-4 only showed repellent activity against both insect.

Suthisut *et al* (2011) assessed fumigant toxicity of essential oils from rhizomes of *Alpinia conchigera,Zingiber zerumbet,Curcuma zedoaria* and their major compounds;camphene,camphor, 1, 8-cineole, alpha-humulene, isoborneol, alpha pinene, beta-pinene and terpinen-4-ol against adults of *Sitophilus zeamais, Tribolium castaneum, Anisopteromalus calandrae* and *Trichogramma deion* larvae. Last two insect are parasitoids are commonly used to control store-product weevils and moth. Dose of 0, 37, 74, 148, 296, 444, 593 micro L/L in air after 12, 24, 48h for *S.zeamais, T.castaneum* and *A.calandrae,* and 24hr for *T.deion* were evaluated. *A. conchigera* oils were more toxic than other two plants. *S.zeamais* adults (LC_{50} -85microL/L in air) were more tolerant to *A. Conchigera* than *T.castaneum*(LC_{50} 73 microL/L in air) after 48h exposure.Synthetic oils were more toxic than plant extract. *Z. zerumbet* oils(LC_{50} 26 micro L/Lin air) and *C.zedoaria* oils(LC_{50} 25micro L/L in air) were significantly more toxic to adults where as *T.deion* larvae were more sensitive to *A.conchigera* oils than *Z.zerumbet* and *C.zedoaria* oils. *T. castaneum* was more susceptible than *S.zeamais* to the pure compounds. Terpinen-4-ol was highly toxic to both insects.

Sutton *et al* (2011) exposed to aerosol formulations of either 1% active ingreadients (AL) pyrethrin (synerzised with piperonyl butaoxide) +36.6% (AL) methoprene or 3%(AL) pyrethrin +33.6% (AL) methoprene. Insecticides mixture were prepared to give 100 to1 ratio of active ingredient pyrethrine to methoprene. Four week old larvae of *Tribolium castaneum* introduced every two weeks, up to sixteen weeks post exposure to the aerosol. Less than 2% larvae of *T.castaneum* emerged as normal adults from any kind of treated surfaces regardless to concentration of pyrethrin. No exposed larvae were able to advance further development and die. However if *T.confusum* larvae exposed to 3% pyerthrine + methopre adult emergence reduced. *T.castaneum* larvae were more susceptible to *T. confusum*.

Ukeh and Umoetok (2011) observed that secondary plant compounds are recognised as important components of plant defence system against herbivores and pathogens.Five monoterpenoids (R)-linalool, 1, 8-cineole, (S)-2-heptyl acetate. (S)-2-heptanol and citral, which are naturnal compounds of essential oils of *Aframomum melegueta* (K.Schum) *Zingiber offcinale* (Roscop), were tested at ratio that occur naturally as a repellent against *T.castaneum* and *R.dominica* (F) in a four way olfactometer. (R)-linalool and(S)-2-heptanol were strong repellent compounds than the others. Linalool showed good repellent activity and *T.castaneum* stays only 1.22 min in the test arm as compared to control arm (2.78) min. *R.dominica* stays 0.89 min in test arm and 2.87min in control arm, when tested with other compounds both the insects were significantly repelled ($P<0.05$), hence can be used as safer repellent or fumigants.

Upadhyay and Ahmad (2011) explained that plant products show enormous toxicity against several stored product pests and provide prolonged protection to the grains. Due to high mortality of adult insect (*T.castaneum*) besides reduced oviposition and hatching. Essential oil of black pepper significantly suppressed survival of larvae and pupae and heavily cut down adult emergence. Repellency was maximum in clove 0.05micro L concentration.

Abbasipour *et al (*2012*)* observed that potency of *Cinnamomum zeylaincum* as a fumigant toxicant is higher on *E.kuchniella* (LC_{50} 287micro L/LI of air)than *Callosobruchus macutatus* (LC_{50} 443.17micro L/L air) and *Tribolium castaneum* is

(LC_{50} 755.9 micro l/l air).Increase with concentration and exposure time highest mortality observed during 12hr to24hr at concentration 385.56mg/ml. Major content is cinnamaldehyde is (97.38%), has highest rate as oviposition deterrent in newly emerged adults just after fumigation. Number of eggs laid are counted to find out rate of deterrence.

Arthur and Fontenot (2012) evaluated the juvenile hormone analog methoprene and chitin synthesis inhibitor novaluron by exposing late stage larvae of *Tribolium castaneum* or *Tribolium confusum* to it. Larvae exposed to it in food material on concrete, on plywood and on floor tile. Larvae of *T.castenum* were more susceptible than *T.confusum* to both methropene and navaluron on all surfaces. When progeny of treated were studied, it showed emergence of malformed adults and fewer adults in number.

Caballero *et al* (2012) stated that natural products present less risk to humans and environment. Essential oils isolated from *Cymbopogon martini* (palmrosa). *Cymbopogon flexuous* (lemongrass) and *Lippia origanoides* (wild oregano) against *T,castaneum*, using the area of preference and contact toxicity on filter paper methods respectively. The repellent action decrease in order *C.martini*> *C. Flexuous*> *L. Origanoides* .

Cao Jie *et al* (2012) while screening of Chinese medicinal herbs and local wild plants found that extract of *Boschniaka himalaica* tubes, possess strong feeding deterrent activity against *Tribloium castaneum*. The constituent compounds were isolated and identified as- 3 beta-acetoxyurs-12-en-28-oic acid, 3 beta-acetoxyurs-28, 13 olide and (±) pinoresinol monoglucoside etc. Compounds exhibited feeding deterrent activity against *Tribolium castaneum* with LD_{50} valuaes of 378, 940 and 609ppm, respectively.

Chaubey (2012) found that terpenes are present in all essential oils. Pure volatile compound of terpene group up: alpga-pinene and beta-caryophyllene have ben evaluated for their repellent,acute toxicity and developmential inhibitory activites alone or in binary combination against *Tribolium castaneum,* in repellency assay adults significantly repelled even at 0.025% concentration. Fumigation of larvae and adults caused lethality LC_{50} of alpha-pinene was 0.998 and for beta caryophyllene was 1.624 microl/cm^3 and against larvae were 1.379 and 1.949 microl/cm^3 respectively. In binary combination, the LC_{50} values against adult and larvae were found 1.277 and 1.438 microl/cm^3. Fumigation with two sublethal concentration 40 and 80% of 24h exposure LC_{50} alone or in binary combination reduced oviposition potential, inhibited pupation and adult emergence in larvae. Alpha-pinene is more effective but both are synergistic and thus used as efficient insecticidal tool against *T.castaneum*.

Haouas *et al* (2012) evaluated toxic properties of essential oils extracted from three species of *Chrysanthemum coronarium, C. fuscatum, C.grandiflorum, C. grandiflorum* was rich in sesquiterpenoids, while *C. Fuscatum, C.coronarium* rich in monotrrpenoides. The spathulenol and caryophyllene oxide, alpha- pinene, myrcene, alpha humulene, beta-caryophylene are the common constituents of essential oils. *C.grandiflorum* leaves extract inhibited the relative growth rate, efficiency of conversion of ingested foods was 50.69%, relative consumption rate, caused antifeedant effect 66.43% and high mortality 80% of *Tribolium confusum* larvae. Topical application caused insect mortality that remains 27% after 7 days of treatment by *C.coronarium* flower extract has contact and fumigant toxicity with mortality of 9 and 13% respectively. These oils may help poor farmers who store small amount of grains.

Hussian and Tipu (2012) worked together to evaluate persistence and insecticidal activity of a commercial biological insecticide, *Spinosad* based on fermentation product of *Actinomycetales bacterium,Saccharopolyspora spinosa* and two plant extracts, namely *Azadirachta* and *Nelarium* against *Tribolium castaneum*. Five concentration of *Spinosad* i.e 0.5, 1.0, 1, 5, 2,0 and 2.5% and exposure time was 24, 48, 72 and 168hr. Maximum mortality against *Tribolium castaneum*,55% at 2,5% dose at 168h exposure time was obtained and minimum 16.66% at 0.5% concentration and 24h exposure time. Filter paper dip method was used. Neem showed 45% mortality at 168h exposure at 2.5% concentration and 0.5% minimum mortality 16.67% at 24h exposure followed by Kanair with 38% mortality at maximum application rate and exposure time and 15% mortality at minimum concentration and exposure time.

Jahromi *et al* (2012) said that use of synthetic pesticides may create toxicity to non-target orgarisms, development of resistance and residues in treated product, are big problems in storage. The application of repellents is a better choice Percentage repellecy (PR) of *Sirinol (*garlic emulsion) was assessed against *Lasioderma serricrome* and *T.castaneum* using three techniques, petri-dish,Y-shape olfactometer tube and leaky glass. Insects were exposed to 0, 0.5, 1.5 and 10% concentration of *Sirinol* and PR is determined. Quantities were equal to 58.56, 42.58 and 26.29% for *L.serricrome* and 70.99, 55.47 and 38.72 for *T.castaneum* in petri-dish, Y-shaped olfactometer tube and leaky glass techniques respectively. PR for *Sirinol* were more significantly different between the time of 12 and 72hr.

Jagadeesan *et al* (2012) observed that heritable high level resistance to phosphine in stored grain pests is a serious concern among growing countries around the world. Three strains susceptible (QTC4), weakly resistant (QTC1012) and strongly resistant(QTC931) collected from field samples. Resistance is due to single major gene, in the weakly (3.2x) resistant strain, strongly resistant (431x) strain, it confers 12-20x resistance itself. Strong synergistic epistatic interaction between the the genes is responsible for high resistance. Phosphine resistance is not sexed linked as it is incompletely recessive autosomal trait. This effect is not consistent and apparently masked, can be noticed by level of response towards phosphine.

Khani and Asghari (2012) tested volatile toxicity of oils extracted from, *Mentha longifolia, Pulicaria gnaphalodes* and flowers of *Aschiliea wilhelmsii* against *T.castaneum* and *C.maculatus*. Major compounds in *M.longifolia* were pepriteone (43.9%) tripal (14.3%). oxathiane (9.3%), d-limonene (4.3%) and piperitone oxide (5.9%). In *P.gnaphalodes*, the major compounds were chrysanthenyl acetate (22.38%), 2L-4Ldihydroxy eicosane (18.9%), verbenol 916.9%), beta pinene (6.43%) and 1, 8 cienole (5.6%). In *A. wilhelmsii* the major compounds were 1, 8 cienol (13.3%), carnol (8.26%) alpha pinene (6%) and p-cymene (6%). *C.maculatus* was more susceptible to the plant products than *T. castaneum* .

Lu Jian Hua *et al* (2012) anhydrous diethyl ether extract, of *Alpinia offcianarum* rhizome was prepared. Its repellent and fumigant activity was investigated against *Tribolium castaneum* adults. It has around 80% repellency at tested concentration

(1:10, v/v) during 48 hr exposure time and 75% mortality at 80 microl/air after 48h exposure. These naturally occurring plants extract could be useful for managing populations of *T.castaneum*.

Opit *et al* (2012) evaluated adults of nine different populations of *T.castaneum* and five populations of *R. dominica*, collected from different geographical locations in Oklahoma. Dose assays determined eight out of the nine and all five *R.dominica*, contained phosphine-resistant individuals. LC_{99} were 3ppm for susceptible at 337ppm for resistant *T.castaneum* and 2ppm for susceptible and 3,430 ppm for resistant *R.dominica* populations. There is substantial increase in phosphine resistance in major stored wheat pests in the past 21 years.

Wijayartna *et al* (2012) using larvae of *Tribolium castaneum*,to test residual efficacy of the juvenial hormone analog methoprene, Larvae were exposed to unsealed concrete or varnished wood treated with liquid formulations held at different temperatures. At 20, 30, 35 degree centigrade for 0-24wk, their rpercentage of adult emergence on concrete increased with time but on varnished wood no adult emerged from exposed larvae. Methoprene was stable for 48hr at 65 degree centigrade and wheat held at 46 degree centigrade. Residual persistence depends upon substrate methoprene applied than temperature variations.

Devi and Devi (2013) screened various spices to find out their insecticidal potential and anti ovipositional properties against *Sitophilus oryzae*, a serious stored food grain pest. Bioefficacy of powders and hexane extracts were determined by assessing the toxicity, effect on F1 progeny, contact toxicity persistence and seed viability. Responses varied with spices, dosage and exposure time. Mace (Nutmeg) and pepper, at 1% level resulted total mortality by one week followed by nutmeg and clove with 100% mortality and cinnamon and star anise with 90% mortality at 5% concentration. 1000 ppm showed insecticidal activity, with pepper extract recording 100% mortality by 5 days. Clove oil resulting in 92% mortality, 51.63% nutmeg, 66.6% cinnamon, 79% in case of mace. Hexane extracts of star anise, cinnamon and clove at 0.59 microl/cm^2 on filter paper discs induced 100% mortality by 72 hour. Spices offered protection to wheat up to 9 months without affecting seed germination thereby showing promise as grain protectants.

Fauad (2013) evaluated toxicity and repellent activity of essential oils of camphor (*Eucalyptus globules*), castor (*Ricinus communis*), cinnamon (*Cinnamoum zylancium*), clove (*Syzygium aromaticum*) and mustard (*Brassica rapa)* against *B,incarnates* adults. 0.5, 1.00, 2.00 and 4.00% concentrations were applied. All repelled B. incarnates except castor oil. Percentage of repellency was higher for cinnamon at 2.00 and 4.00% conc followed by clove,camphor and mustard. Bud extract of *Trachyspecmum ammi and S.aromaticum* against *Tribolium castaneum. T.ammi* had (7.17%) greater knockown effect,followed by 7.0% by *S.* Fawad *et al*. (2014) examined seed extract contact toxicity of *Nigella sativa* and flower *aromaticum* and *N.sativa* 3.5% respectively. This indicates definite potential of these extracts as bio- pesticides in future, for food storage.

Hariharasudhan and Kalaiarasu (2013) stated that spices and herbs in addition to their flavour and fragrance valued for their microbial and medicinal qualities. By analyzing the total bacterial population of egg, meat and fish against clove (*Syzygium aromaticum*) extract at different time intervals, found that extract was highly inhibitory for microbial growth at 48 hour.

Kapoor *et al.* (2013) using Clevenger and Soxhlet apparatus extracted essential oils and oleoresins (ethanol, ethyl acetate and iso-propyl alcohol) of *Myristica fragrance*. Sabinene (29.4%), betapinene (10.6%), alphapinene (10.1%), terpine-4-ol (9.6%) as major components and several other minor components showed their presence, when Gas chromatography-mass spectrometry analysis was done. Inhibition of primary and secondary oxidation measured. It can be used as natural food preservative.

Khan *et al* (2013) conducted experiments for evaluation of toxic aspect of *Allium sativum, Azardirachta indica* and *Euaclyptus globules* against *Tribolium castaneum*. Acetone extracts of these plants showed contact and repellent activities. Various concentration (5, 10 and 15%) were applied on the filter papers, 15 days old beetles were released and mortality was evaluated after fixed intervals(24, 48, 72, 96, 120, 144 and 168h) repellent action was checked after fixed period of (24, 48 and 72h) Results revealed that *Euaclyptus globules*(9.41%) more effective followed by *Allium sativum* (9.20%), *Azardirachta indica* (7.48%). Repellent action confirmed *E. Globules* (76.29%) to be more potent than *A.indica* (63.08%) and *A.sativum* (59.625).

Khan *et al* (2013) tested contact action and repellent effects of acetone based leaf extracts of *Murraya exotica*, *Murraya koenigii* and *Nicotina tabacum* against adult stage of *Tribolium castaneum*. Diffrent concentration of 5, 10 and15 were employed and knockdown effects were checked after a period of 24, 48, 72, 96, 120, 144and 168 hours. *N. tabacum* (12.95%) proved more effective than *M.exotica* (9.53%) *M.koengii* (4.31%) respectively. Repllent effect exhibited different trend *M.koengii* (51.96%), *M.exotica* (70.61%) and *N.tabacum* (60.98%). This reflected their potential to be used as alternate to synthetic insecticides.

Kumar *et al.* (2013) explored anti-lice properties of three different extracts of *Myristica fragrans* Houtt seeds. Alcoholic, aqueous and hydro alcoholic extracts at five different concentrations such as 5%, 10%, 15%, 20%, 25% were prepared. Results were comparable to standard drug benzoate at similar concentrations. Hydro alcoholic extract was more potent among all.

Licciardello *et al* (2013) observed that cereal- based foods may be infested by insects even during their packaging, distribution, transportation and storage in warehouse or in retail stores. Very few studies have assessed insect-repellent packaging materials for foods. This work is aimed at assessing the repellent efficacy of novel functional packaging materials containing three essential oils: citronell, oregano and rosemary. Concentrations higher than 0.005 microL/cm^2 showed potential in terms of repellent activities against the *Tribolium castaneum*, results ranging from 53% to87% for citronella and rosemary respectively. Without affecting packed product, insect attack preventation is an ecofriendly and economical advantage.

Popovic *et al* (2013) obtained adult *Tribolium castaneum* from cultures in dark at *25*±1^{0}C and 70-80% r.h, reared on flour and fed with known concentration of essential oils of nine plants. They all are rich in monoterpene alcohol carvacol and ketonic components, showed insecticidal and fumigant activity. *C.grandulosa* at concentration of 1.14% showed powerful toxic and repellent effect, with very high mortality rate after24h(56.67%).

Akhtar and Muleta (2014) carried out experiment to evaluate antifungal potential of (*Allium sativum, Zingiber officinale, Cinnamomum zeylancium* and *Capsicum annuum*) aqueous and ethanolic extracts of four spices, against post- harvest spoilage fungi isolated from diseased fruits.Fungus identified were *Rhizopus sp*,(26.45%),followed by *Penicillium sp(*19.93%) *Aspergillus sp* (10.86%) and *Fusarium sp*(9.06%). *C zeylancium* was found to be most effective against *Penicillium sp* in ethanolic extract followed by aqueous extract of *A. sativum* reducing the severity of disease.

Fawad *et al*. (2014) examined seed extract contact toxicity of *Nigella sativa* and flower *aromaticum* and *N.sativa* 3.5% respectively. This indicates definite potential of these extracts as bio- pesticides in future, for food storage.

Jeong and Byung (2014) evaluated anti-cholinestrase activity of the ethyl acetate fraction of the methanol extract of *Myristica fragrans Hautt* seeds. This is the first report of significant anti cholinesterase activity of nutmeg.

Kasim *et al* (2014) evaluated that nine major volatile compounds were present in the extract of cinnamon such as, alcohol, aldehydes, alkenes, carboxylic acids, ether, esters and ketones etc. It is a safe repellent to ants than chemical repellent N.N.diethyl-3methyl benzamide.

Martins *et al. (*2014) identified that myristicin an allybenzene is a major active component of various spices such as nutmeg and cinnamon,plants from Umbelliferae family or in some essential oils of clove or marjoram . Myristicin is not genotoxic and yet induced apoptotic mechanism in human leukaemia K562 cells by alterations in the mitochondrial membrane potential, cytochrome- c release, caspase-3 activation, PARP-cleavage and DNA fragmentation. Also analysed alterations in expression of 84 genes associated with DNA response pathway by myristicin..

Przygodzka *et al*.(2014) investigated antioxidant capacity of selected spices in ethanol and ethanol/water(1:1v/v) extracts.Total phenolics (TPC) and total flavonoids (TF) were determined.High antioxidant capacity(clove, cinnamon and allspice) middle(star anise and nutmeg) and low (anise, ginger, fennel, cardamom, white pepper and coriander).

Sami and Shakoori (2014) identified role of neem derived compounds (saponins and azadirachtin) on the digestive cellulose hydrolyzing enzyme activity of *T.castaneum, Oxya chinensis* and A*ulacophora foveicolis*. Cellular proteins isolated from insects gut and salivary glands were tested for cellulose hydrolyzing activity on substrate agar plate.Saponins isolated from tissues (neem) and used for enzyme inhibition studies .It was able to inhibit beta-1,4-endoglucase enzyme activity in *T.castaneum*. Repellency test for *T.castaneum* revealed that saponins and azardirachtin were able to repel the insect.

Thakur *et al.* (2014) performed qualitative phytochemical analysis of Mace, also known as flower of nutmeg, showed presence of tannins, saponins, flavonoids, terpenoids, phenolics carbohydrates as well as proteins and amino acids. The present study evaluates the quantitative phytochemicals, total phenolic content and antioxidant potential of methanolic extracts of Mace.

Brari and Thakur (2015) evaluated contact and fumigant activity of *Cinnamomum zylanicum* essential oil and its two constituents viz. cinnamaldehyde and linalool against *Callasobruchus maculatus* and *Sitophlius oryzae*. Dose of 1.2mg/cm^2 caused 98 and 80% mortality in *C. maculatus* and *S.oryzae* adults respectively, after 24hrs of treatment. Dose of 0.3% mg/cm^2 cinnamaldehyde caused 100% mortality in *C.maculatus* where as similar dose of linalool caused 89%mortality in *S.oryzae* after exposure of 12hr. Contact and fumigant toxicity is higher in *C.maculatus* than *S.oryzae* adults. Cinnamaldehyde showed higher toxicity than linalool.

Meena *et al* (2015) observed that Black Pepper seeds powder 0.5% exhibited maximum adult mortality,65% and 29.75% in *Rhyzopertha dominica* adults after40 and 60 DAT recorded in 48 hr exposure period. Insecticidal activity of different grain proctants viz.*Capsicum annum*, *Azadirachta indica*, *Curcuma longa* and *Oscimum basilicum*.

Vijay kumar *et al* (2015) determined biological activities of spices namely turmeric, chilli, coriander, fennel seeds, black pepper, ginger, fenugreek garlic and cumin against *Tribloium castaneum*. All spices showed significant effect on adult mortality. Toxic effect followed- black pepper> cumins> garlic> fennel seed> ginger> fenugreek> untreated control.

CHAPTER-3

MATERIALS AND METHOD

TABLE- 1, 2, 3

PHOTO PLATE 1-5

Rearing of culture of test insect:

The culture of *Tribolium castaneum* was carried out in infested kernel of wheat. Beetles were reared at temp. 28± 2^0C and 65±5% RH at dark place. After 2 weeks of oviposition, dead as well as alive adults were removed, eggs laden broken wheat Kernel were maintained and recultured to produced newly emerged adults of same generation for further use. Fresh wheat grains were washed with distilled water and dried in sunlight. Then they were refrigerated for few hours to completely wipe out traces of any type of infestation. Sterilized glass jars were taken and half filled with wheat. Part of the grain was broken before filling and it was observed that it facilitated multiplication of the insect. Now hundred beetles were introduced in each jar and the mouth was covered with muslin cloth with the help of rubber bands. The culture was kept in dessicators in wooden cupboard. Use of electric bulbs in winter and wet sponges and jute in summers were made to maintain temperature at 28± 2^0C and relative humidity 65±5%. As the insect population increased in culture jar, fresh wheat was added and partial culture was shifted in new jars. The temperature and humidity conditions were monitored by help of hygrometer cum-thermometer. Adults and larvae were obtained from this laboratory culture of test insect, *Tribolium castaneum.*

Preparation of spice extracts:

Five spices were used for preparation of extracts viz. *Cinnamomum zeylancium* (cinnamon), *Myristica fragrans* (nutmeg), *Piper nigrum* (black pepper) *Syzygium aromaticum* (clove) and *Trigonella foenum-graecum* (fenugreek). Bark of cinnamon,fruit of nutmeg,bud of clove,seedsof pepper and fenugreek were dried and grinded then extracts were prepared by soxhlet method using ethanol as a first solvent. The extracts were dried by heating upon water bath till the solvent was completely evaporated and dry residue of spices kept in airtight bottles in the refrigerator at 4^0 **centigrate** temperature until further use. Dilution of extract was done with the help of acetone, to make solution of required concentration. (conc-10 %)

Preparation of Mixtures:

A mixtures of cinnamon extract and other spice extract in the ratio 1:1 (v/v) were made. Thus four mixtures were prepared to test the phenomenon of joint action, against *T.castaneun* in the research laboratory of department of Zoology, Government college Kota (Raj.)

Study of Adulticidal/Larvicidal effect:

Small culture tubes were washed, dried and sterilized. In each tube 20 gms of wheat grain was taken. The spice extract were added to the wheat by help of micropipette and mixed well with a glass rod to ensure adequate coating of grains with the spice extract until the acetone was completely evaporated. Twenty adults/10 larvae of the test insect were released and the mouth of the culture tube was covered with a muslin cloth by help of rubber bands. The inside of culture tube was covered by petroleum jelly just above the level of wheat to prevent insects from crawling on the sides. After 24 hours of treatment, mortality counts were made. The mortality range was found out arranging several preliminary trials to obtain mortality between 10-90%. Each extract was used at five different dose levels and replicated in three sets under ambient laboratory conditions. Side by side control having untreated 20 gms. of wheat and 20 adults/10 larvae was also run in parallel.

Study of joint toxicity:

Another important aspect of the experiment was joint use of cinnamon with other spices extract separately to find out synergism or antagonism. Synergism is increase in bioactivity of two compounds to produce an effect greater than the one expected from a simple algebraic summation of the effects of two compounds individually administered. Generally the word Potentiation is used when two toxic materials applied together elicit a response greater than expected from, sum of individual toxicants. If two materials elicit lower response than expected from the sum of two individuals it is known as antagonism, negative synergism/Potentiation.

The same procedure was followed as for the study of adulticidal effect except that instead of single spice extract mixture of two spices were mixed with wheat grain and then the mortality effect was observed after 24 hours of treatment.

Repellent Action:

Six inches petri dish were taken. Each petridish was lined by Whatman filter paper no.1 and it is marked into two equal half. One half of the paper is sprayed by acetone and the other half is sprayed by 0.25% concentrated solution of each spice extract. Treated and untreated halves of filter paper were rejoined using clear adhesive tape at the back side of and then place in petridish. 20 adults of test insect were introduce in the central area of the petridish and acetone was allowed to evaporated, then it was covered by a second petridish. Each treatment was replicated three times and treatments were arranged in randomized fashion. After 1 hour the adults in treated and untreated area were counted respectively. For each spice extract three observations were taken, a control was also run parallel. Adults *T. castaneum* were found to be repelled by very low concentration of spice extracts.

Study of residual toxicity:

For study of residual toxicity 500 gms of sterilized wheat seed was taken in a glass jar and the extract were mixed well with the wheat grain. Only that dose which had near about 90-100% adulticidal effect were taken. The treated wheat was then stored in airtight bottles. The test for residual toxicity was done on 10^{th}, 30^{th}, 60^{th}, 90^{th}, and 120^{th} day after treatment of the wheat. For the test 20 gms of treated wheat was taken in a sterilized culture tube. 10 adults of test insect were released into the tube and the mouth was covered by muslin cloth tied by help of rubber band. Mortality count was made after 72 hours. Each test was replicated thrice. Control with untreated wheat was also run.

Table -1

Spice extracts used and their adulticidal effect at various dose level against *Tribolium castaneum*

S.N.	Common name of spice	Botanical name of the spice	Part of the plant used	No 0f adult insects	Corrected mortality at different doses (v/w)*						
					10µl	**15 µl**	**20 µl**	**30 µl**	**40 µl**	**50 µl**	**60.00 µl**
1	Ciannmon	*Cinnamomum zeylancium*	Bark	60	13.22	21.36	39.66	43.73	51.86	57.97	60.00
2	Nutmeg	*Myristica fragrans*	Fruit	60	12.00	16.00	28.00	32.00	34.00	44.00	50.00
3	Black pepper	*Piper nigrum*	Seeds	60	20	26.32	30.53	38.93	53.68	55.79	60.00
4	Clove	*Syzygium aromaticum*	Bud	60	8.28	24.83	28.97	37.24	45.52	51.72	55.86
5	Fenugreek	*Trigonella foenum-graceum*	Seeds	60	10.00	14.00	20.00	26.00	38.00	50.00	54.00

* 24hr post treatment data

Table-2

Spice extracts and their larvicidal impact at various dose level against *Tribolium castaneum*

S.N.	Common name of spice	Botanical name of the spice	Part of the plant used	No 0f adult insects	Corrected mortality at different doses (v/w)*						
					10µl	**15 µl**	**20 µl**	**30 µl**	**40 µl**	**50 µl**	**60.00 µl**
1	Ciannmon	*Cinnamomum zeylancium*	Bark	30	5.36	11.79	21.43	25.71	27.86	30.00	30.00
2	Nutmeg	*Myristica fragrans*	Fruit	30	1.07	6.43	9.64	16.07	21.43	24.64	27.86
3	Black pepper	*Piper nigrum*	Seeds	30	4.44	8.89	15.56	23.33	25.56	28.89	30.00
4	Clove	*Syzygium aromaticum*	Bud	30	4.00	5.00	12.00	17.00	25.00	26.00	28.00
5	Fenugreek	*Trigonella foenum-graceum*	Seeds	30	00	3.10	9.31	12.41	18.62	22.76	24.83

* 24 hrs post treatment data

Table-3

Joint Action: Mortality data of spice extracts in combination at different dose level against *Tribolium castaneum* adults (30)

S. No.	Combinations of spices	Ratio (µl/µl)	Corrected Mortality*					
			10 µl	15 µl	20 µl	30 µl	40 µl	50 µl
1	Cinnamon + Fenugreek	1:1	2	4	5	7	8	9
2	Cinnamon + Black pepper	1:1	4	5	8	11	14	20
3	Cinnamon + Nutmeg	1:1	5	7	8	13	14	16
4	Cinnamon + Clove	1:1	6	8	10	12	16	19

* 24 hrs post treatment data

TRIBOLIUM CASTANEUM ADULT

TRIBOLIUM CASTANEUM LARVAE

CULTURE OF TRIBOLIUM CASTANEUM

SETS OF EXPERIMENTS

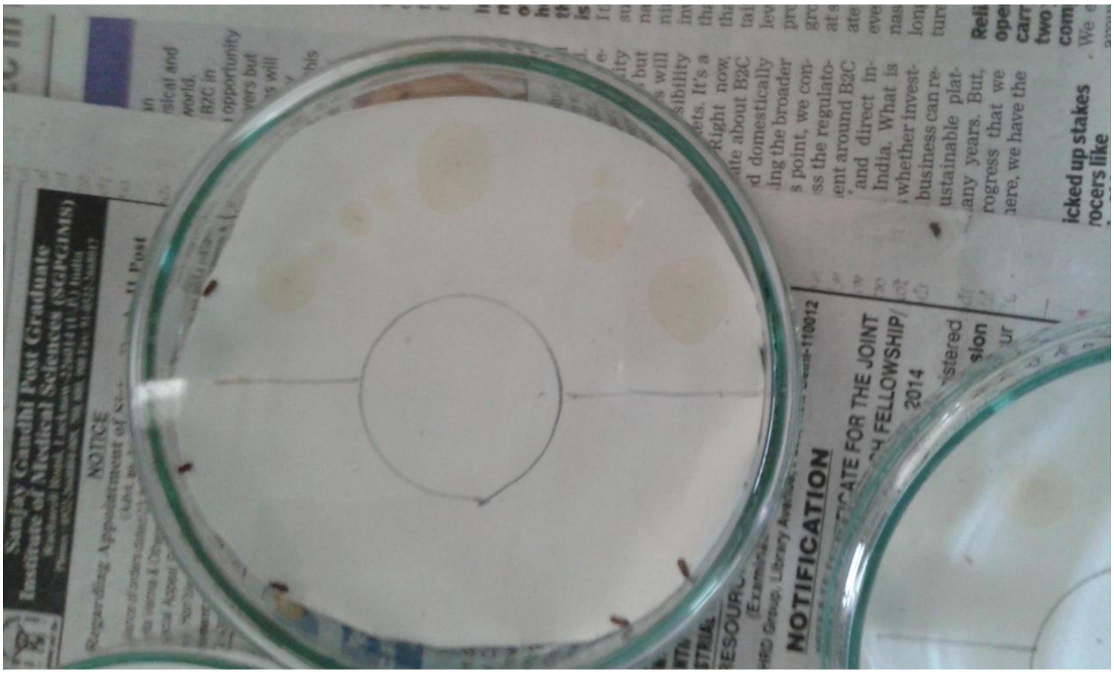
EXTRACT OF PIPER NIGRUM SEEDS & MYRISTICA FRAGRANS FRUITS

EXTRACT OFTRIGONELLA SEEDS & CINNAMOMUM BARK

MYRISTICA FRAGRANS FRUITS

TRIGONELLA FOENUM-GRAECUM SEEDS

SYZYGIUM AROMATICUM BUDS



REPELLENCY IMPACT OF SYZYGIUM AROMATICUM EXTRACT

CHAPTER-4

CALCULATION

LD_{50} (Adulticidal effect):

The percentage mortality was calculated by summing up the number of dead insects of all three replicates of each concentration and by calculating the average of mortality. Then observed mortality percentage was found out. The mortality percentage was corrected by following Abbott's (1925) formula:

$$P = \frac{P_1 - C}{100 - C} \times 100$$

P - corrected mortality percentage

P_1 - observed mortality percentage

C - mortality percentage in control

The mortality and insecticide concentration data was subject to probit analysis (Finney 1942) so as to calculate their LD_{50} values in the following manner:

1. Log values of insecticide doses were found out.
2. Mortality percentage were corrected by Abbott's (1925) formula.
3. Empirical probits of corrected percentage mortality were fount out from probit tables.
4. A regression line was drawn between log of dose x and empirical probits.
5. By drawing an eye – fitted line, the values of expected 'Y' were found out.
6. Working probits 'y' were seen, for corrected percentage mortalities (P) against expected probits (Y).
7. The weighting coefficients (w) were directly read against expected probits 'Y'.
8. The product of weighing coefficient 'w' and 'n' (number of insects released in one dose) was calculated.
9. 'nwx' was calculated by multiplying 'nw' with log of dose (x) in each column.
10. The 'nwx^2' was calculated by multiplying 'nwx' with 'x'.
11. The value of 'nwy' was calculated by multiplying 'nw' with working probit 'y'
12. Value of 'nwy' was multiplied with 'x' to find out value of 'nwxy'.
13. By summing up the values of 'nw', 'nwx', 'nwx^2, 'nwy' and 'nwxy' in each column, the summation was obtained $\sum nw, \sum nwx, \sum nwx^2, \sum nwy$ and $\sum nwxy.$

14. Value of $\bar{y}$ was found out by the formula $\bar{y} = \sum nwy / \sum nw.$

15. Similarly value of $\bar{x}$ was found out by the formula $\bar{x} = \sum nw \times / \sum nw$.

16. The values of $\sum xy$ and $\sum x^2$ were found out as follows

$$\sum xy = \sum nwxy - \frac{\sum nwx \times \sum nwy}{\sum nw}$$

And $$\sum x^2 = \sum nwx^2 - \frac{(\sum nwx)^2}{\sum nw}$$

17. Regression coefficient (b) was calculated by following formula :

$$b = \sum xy / \sum x^2$$

18. The values of $\bar{x}, \bar{y}, Y$ and b were placed in the regression equation : Y = $\bar{y}, + b(x - \bar{x})$ (where Y = 5)

19. The value of 'x' was found out by regression equation.

20. By finding out the value of antilog of 'x' LC_{50} value of insecticide was estimated

21. Taking cinnamon as standard insecticide than relative toxicity of various botanical insecticides are calculation by following formula:

Fiducial limits = $y^{+}t\sqrt{(Vy)}$ and $y - t\sqrt{(Vy)}$

Where y	=	expected probit
Vy	=	variance of y
t	=	normal deviate for level of probability

Determination of joint toxicity:

The joint action of insecticides was calculation by Sun and Johnson (1960) method as follows:

Toxicity index of insecticide $$A = \frac{LD_{50} \text{ of } B \times 100}{LD_{50} \text{ of } A}$$

(using B as standard)

Toxicity index of insecticide B = $B = \frac{LD_{50} \text{ of } A \times 100}{LD_{50} \text{ of } B}$

(Using A as standard)

Actual toxicity index of mixture M $M = \frac{LD_{50} \text{ of } A \times 100}{LD_{50} \text{ of } M}$ =

(using A as standard)

The theoretical toxicity index of mixture M is equal to the sum of toxicity indices of A and B multiplied by their respective percentage in mixture. Thus

Theoretical toxicity coefficient = toxicity index of A × percentage of A in M

of a mixture M + toxicity of B × percentage of B in M

From actual and theoretical toxicity of the mixture the joint toxicity was calculated as follows.

Co toxicity coefficient of mixture $\frac{\text{Actual toxicity index of mixture} \times 100}{\text{Theoretical toxicity index of mixture}}$ =

Residual percent mortality and Regression Results of residual toxicity.

Repellency

Percentage of repellency $\frac{\text{Number of insects repelled} \times 100}{\text{Total No. of Insects}}$ =

Statistical analysis of repellency of spice extracts against *T. castaneum*

CHAPTER-5

OBSERVATIONS

TABLE 4–14

PROBIT GRAPHS 1–14

Table -4

Toxicity of spice extracts against Adults of *Tribolium castaneum* (Herbst)

S. No.	Spices	Heterogeneity	Regression equation y	LD_{50} µl/20 g. wheat	S_m	Fiducial limits
1	Cinnamon	23.1687	3.8005x± 0.50084	22.889	1.800	68.4924 to 4.7559
2	Nutmeg	4.6251	1.9658x ± 2.1413	26.1214	0.2526	72.7942 to 29.7195
3	Black pepper	18.9133	4.6198x ± 0.2447	35.7813	0.9228	19.5542 to 17.8105
4	Clove	3.8335	1.828x ± 2.8817	21.0937	0.288	23.6856 to 18.8291
5	Fenugreek	9.2102	0.9325x ± 2.8426	26.9711	0.276	30.0302 to 24.2251
Y = probit kill, x= log dose, S_m = Standard error, Significance P = 0.05						

Table -5

Toxicity of spice extracts against Larvae of *Tribolium castaneum* (Herbst)

S. No.	Spices	Heterogeneity	Regression equation y	LD_{50} μl/20 g. wheat	S_m	Fiducial limits
1	Cinnamon	6.9	59.222x ± 52.302	10.8767	222.06	11.031 to 11.2160
2	Nutmeg	0.7861	0.9926x ± 3.1200	19.2474	0.3856	16.599 to 22.3183
3	Black pepper	37.255	6.2626x ± 1.3086	9.2209	2.370	10.2112 to 17.9368
4	Clove	4.5393	1.4376x ± 2.9187	16.6155	0.3579	14.1880 to 19.4583
5	Fenugreek	23.00	5.9367x ± 0.41707	176.1916	1.606	11.6050 to 59.7645
Y = probit kill, x= log dose, S_m = Standard error, Significance P = 0.05						

TABLE -6

Joint Toxictiy of cinnamon with other spices in (1:1) combination against adults of *Tribolium castaneum* (Herbst)

S. No.	Spices in combination	Heterogeneity	Regression equation	LD_{50} µl/20g. wheat	s_m	Fiducial limits
1	Cinnamon+ Nutmeg	0.3120	Y=2.4417x±1.5827	43.8248	0.4204	29.2019 to 65.7702
2	Cinnamon+ Black pepper	1.2576	1.5596x±2.911425	37.1495	0.4407	28.9361 to 47.6943
3	Cinnamon + Clove	0.5645	2.4405x ± 1.64944	35.6252	0.4149	25.9071to 48.1816
4	Cinnamon + Fenugreek	0.2079	2.3118x± 1.29643	118.4571	0.4761	50.8045 to 0.000

Table-7

Co- Toxicity coefficients of combinations of spices and their joint action

S. No.	Toxicity of Mixture	Ratio	Co- toxicity coefficient of standard	Toxicity of mixture	Actual toxicity index of mixture	Theoretical toxicity index of mixture	Co- toxicity coefficient	Type of joint action
Cinnamon LD_{50} 22.889								
1	Cinnamon + nutmeg	1 : 1	100	43.8248	52.2078	93.7955	55.613	Antagonism
2	Cinnamon+ Black papper	1 : 1	100	37.1495	61.5889	81.972	75.1340	Antagonism
3	Cinnamon+ clove	1 : 1	100	35.62	64.2242	104.234	61.6115	Antagonism
4	Cinnamon+ Fenugrek	1 : 1	100	118.457	90.3150	93.5014	20.6574	Antagonism

Co- toxicity coefficient calculated by Sun and Johnson (1960) Formula.

Table -8

Residual toxicity: Mortality of *T.castaneum* by spices at various DAT

Compound	10 DAT	30 DAT	60 DAT	90 DAT	120 DAT
Cinnamon	22	16	12	9	6
Fenugreek	6	4	1	0	0
Black pepper	20	18	14	6	4
Nutmeg	14	11	8	5	0
Clove	22	17	15	7	3
Control	Nil	Nil	Nil	Nil	Nil

Dose : LD_{95} μl /20g. wheat Insect no – 30 adults (of three sets) Time : 72 hrs exposure.

DAT : Days after treatment.

Table -9

Residual toxicity : Percent mortality of *T. castaneum* by spices at various DAT

Percent Mortality					
Compound	10 DAT	30 DAT	60 DAT	90 DAT	120 DAT
Cinnamon	73.33	53.33	40.00	30.00	20.00
Fenugreek	20.00	13.33	3.33	0.00	0.00
Black pepper	66.67	60.00	46.67	20.00	13.33
Nutmeg	46.67	36.67	26.67	16.67	0.00
Clove	73.33	56.67	50.00	23.33	10.00

DAT : Days after treatment.

Table -10

Regression Results of residual toxicity

Compound	Intercept (y)	Reg. Coefficient	S.E.	P -value	Significance	R Square
Cinnamon	71.658	-0.457	0.061	0.00499	**	0.949
Fenugreek	18.926	-0.187	0.043	0.02303	**	0.861
Black pepper	73.959	-0.526	0.057	0.00263	**	0.966
Nutmeg	50.406	-0.404	0.024	0.00045	**	0.990
Clove	78.020	-0.570	0.054	0.00181	**	0.974
** Highly Significant						

Table -11

Repellency Data of spice extracts against *T. castaneum*

Extracts	No. of insects per trial	Insects present in untreated area in three sets			Insects present in treated area in three sets		
		a	b	c	a	b	c
Cinnamon	10	6	6	5	4	4	5
Fenugreek	10	3	4	4	7	6	6
Clove	10	7	7	6	3	3	4
Black Pepper	10	5	5	6	5	5	4
Nutmeg	10	4	6	6	3	4	4

Table No -12

Repellency percentage of spice extracts against *T. castaneum* in the three sets

Extracts	a	b	c
Cinnamon	60	60	50
Fenugreek	30	40	40
Clove	70	70	60
Black Pepper	50	50	60
Nutmeg	40	60	60

Table No -13

ANOVA Calculation----Angular Transformed (phi-value) Value of Repellency

Extracts	a	b	c
Cinnamon	50.77	50.77	45
Fenugreek	33.21	39.23	39.23
Clove	56.79	56.79	50.77
Black Pepper	45.00	45.00	50.77
Nutmeg	39.23	50.77	50.77

ANOVA (phi-value)

Source of Variation	SS	Df	MS	F	P-value	
Compounds	478.702	4	119.676	6.594	0.0072625	**
Error	181.492	10	18.149			
Total	660.194	14				

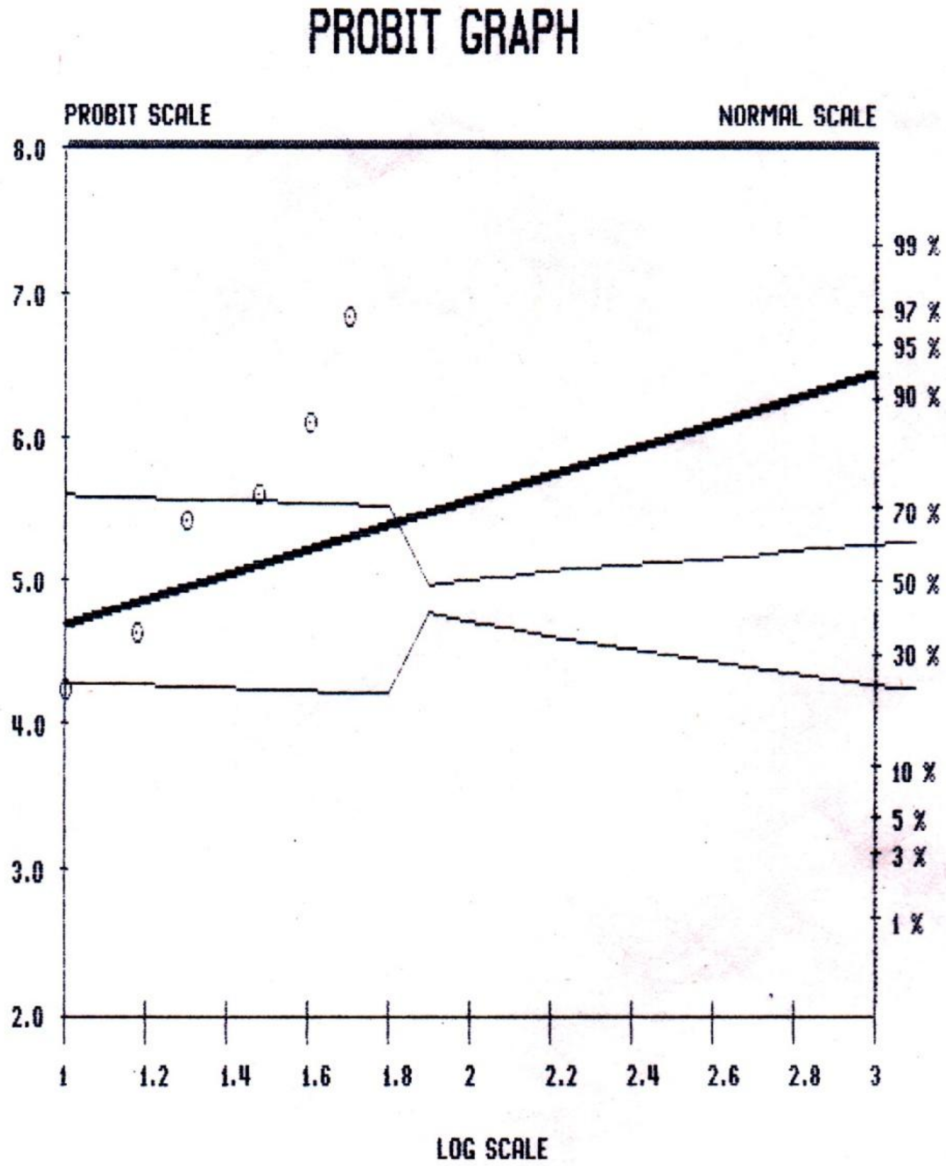
**Data highly significant

Table -14

Statistical analysis of repellency data of spice extracts against *T. castaneum*

Compound	*phi-value*	% value
Cinnamon	48.85	56.67
Fenugreek	37.22	36.67
Clove	54.78	66.67
Black Pepper	46.92	53.33
Nutmeg	46.92	53.33

G.M.	46.94	
S. Em.±	2.46	
C.D.5%	7.75	
C.V.%	9.08	

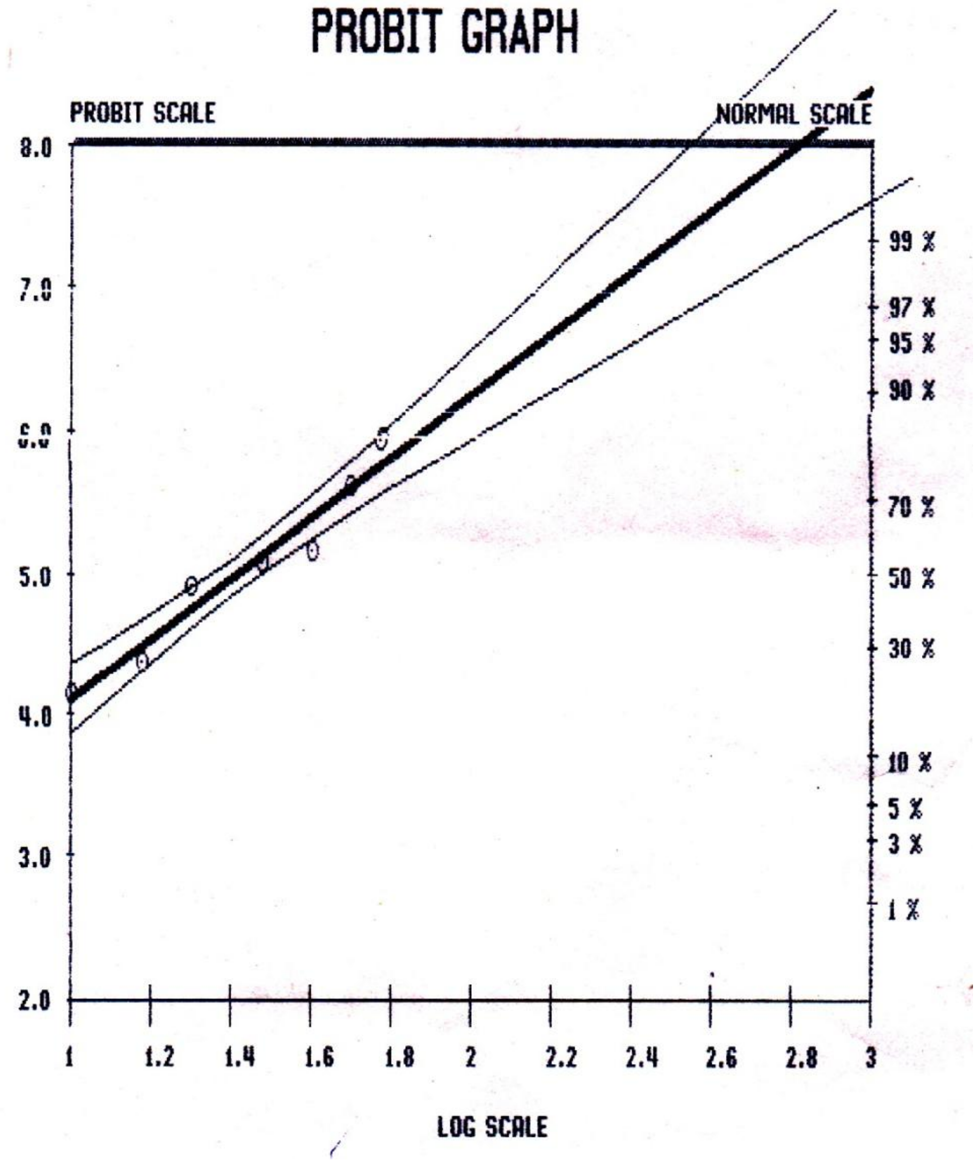


y-mean : 4.9179 x-mean : 1.2657

PROBIT EQUATION : $Y = 3.8005 \pm 0.8828436\ x$

No.-1

Probit graph of Cinnamon extract used against *T. castaneum* adults

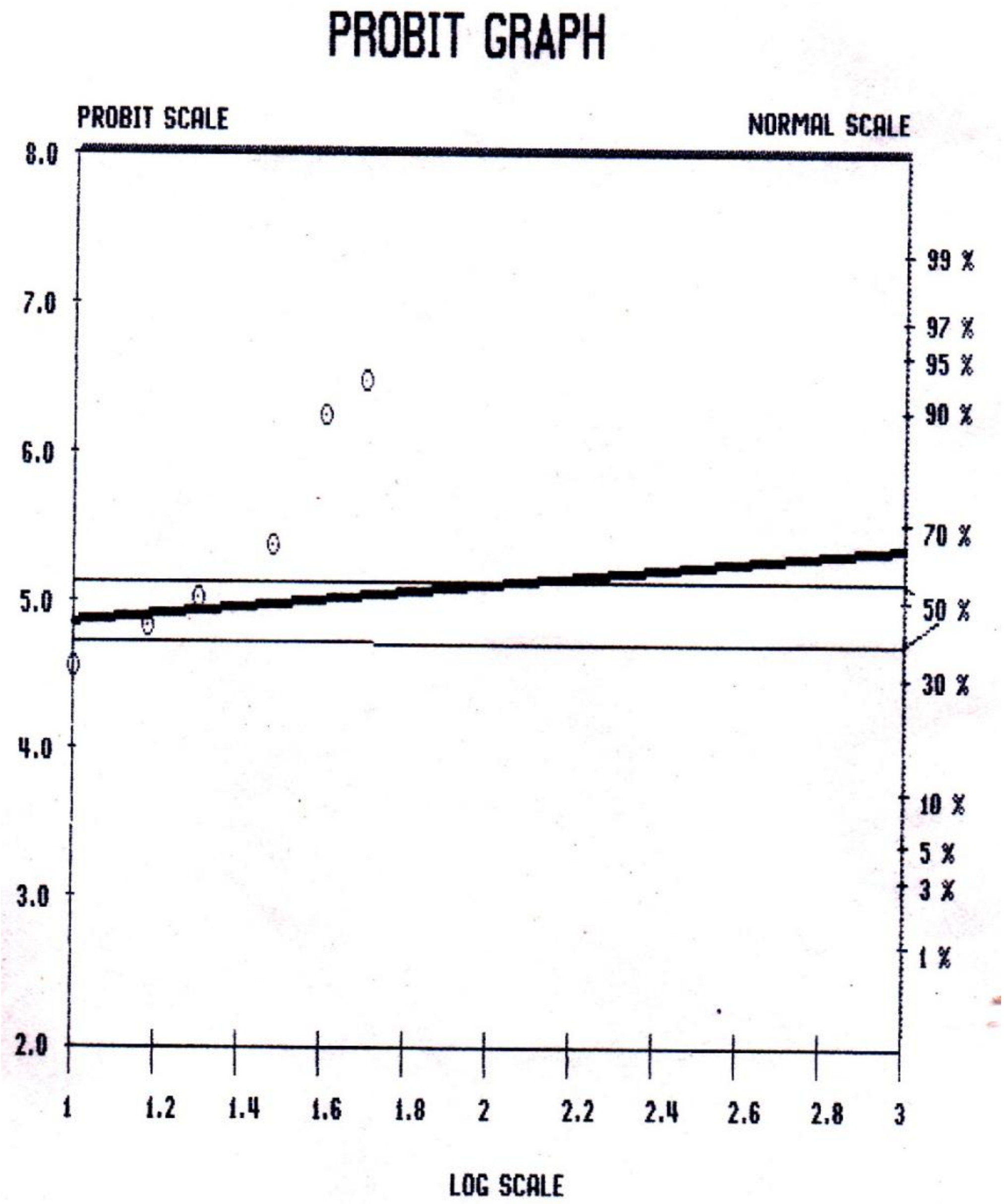


y-mean : 5.0429 x-mean : 1.4370

PROBIT EQUATION : Y = 1.9658 ± 2.1413066 x

No.-2

Probit graph of Nutmeg extract used against *T.castaneum* adults

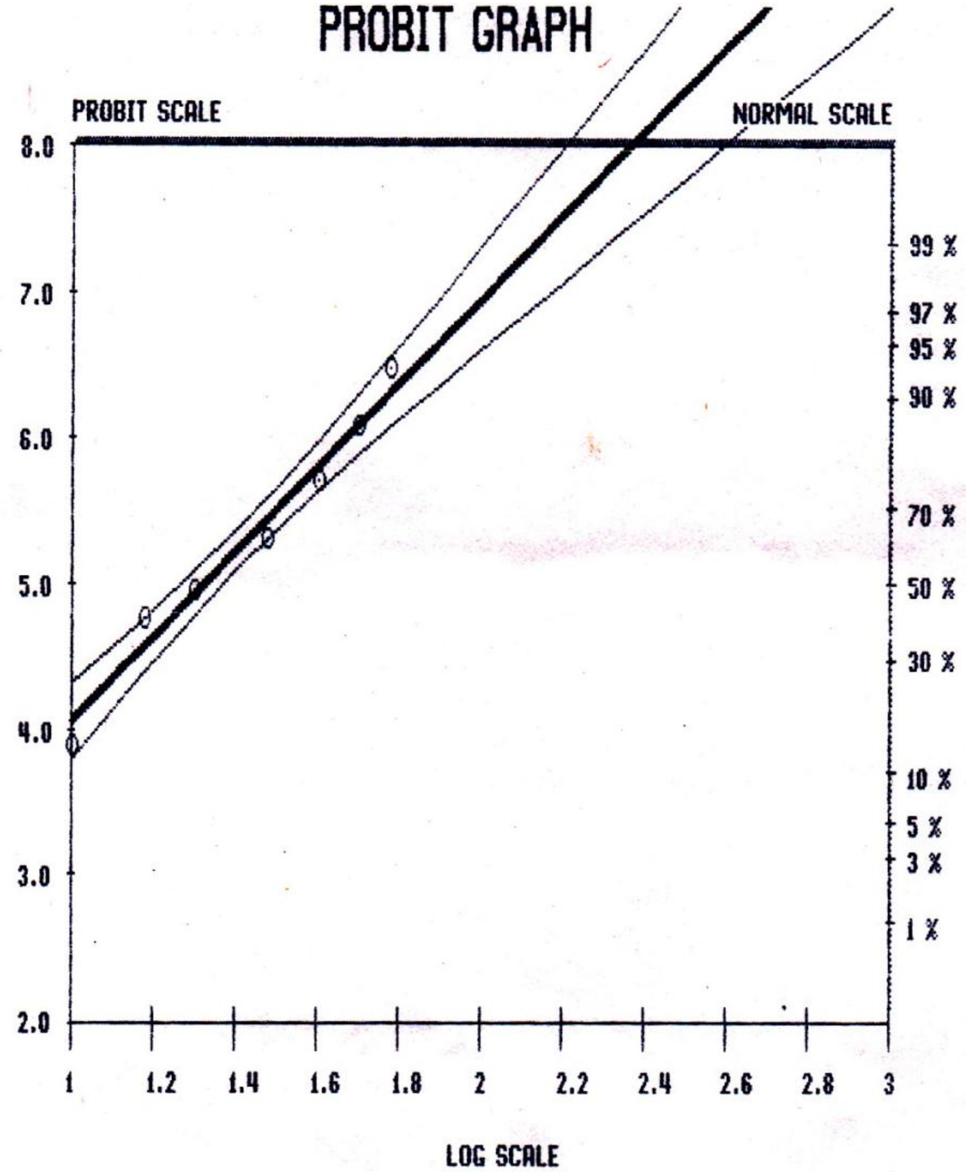


y-mean : 4.9307 x-mean : 1.2705

PROBIT EQUATION : Y = 4.6198 ± 0.2447078 x

No.3

Probit graph of Black pepper extract used against *T. castaneum* adults

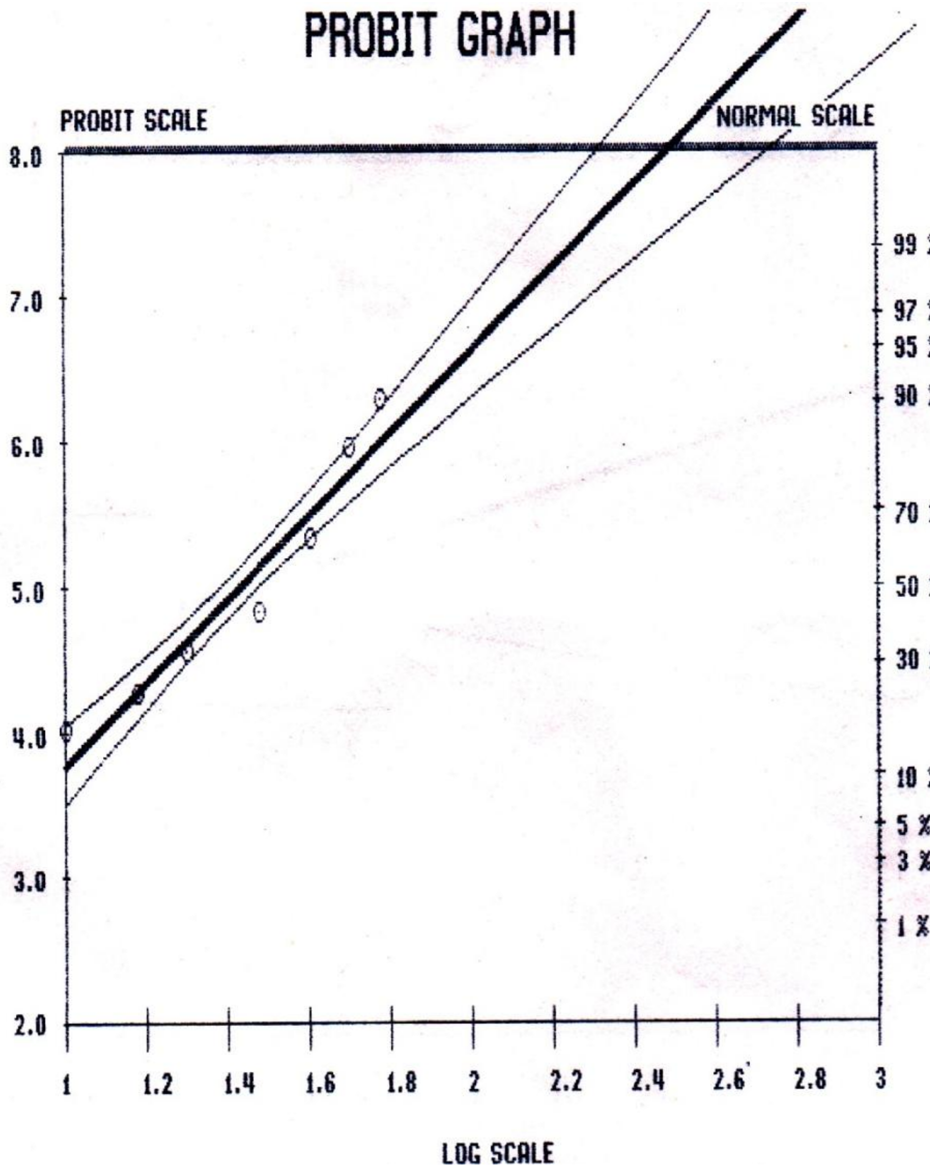


y-mean : 5.2288 x-mean : 1.4035

PROBIT EQUATION : Y = 1.1828 ± 2.8827672 x

No.4

Probit graph of Clove extract used against *T. castaneum* adults

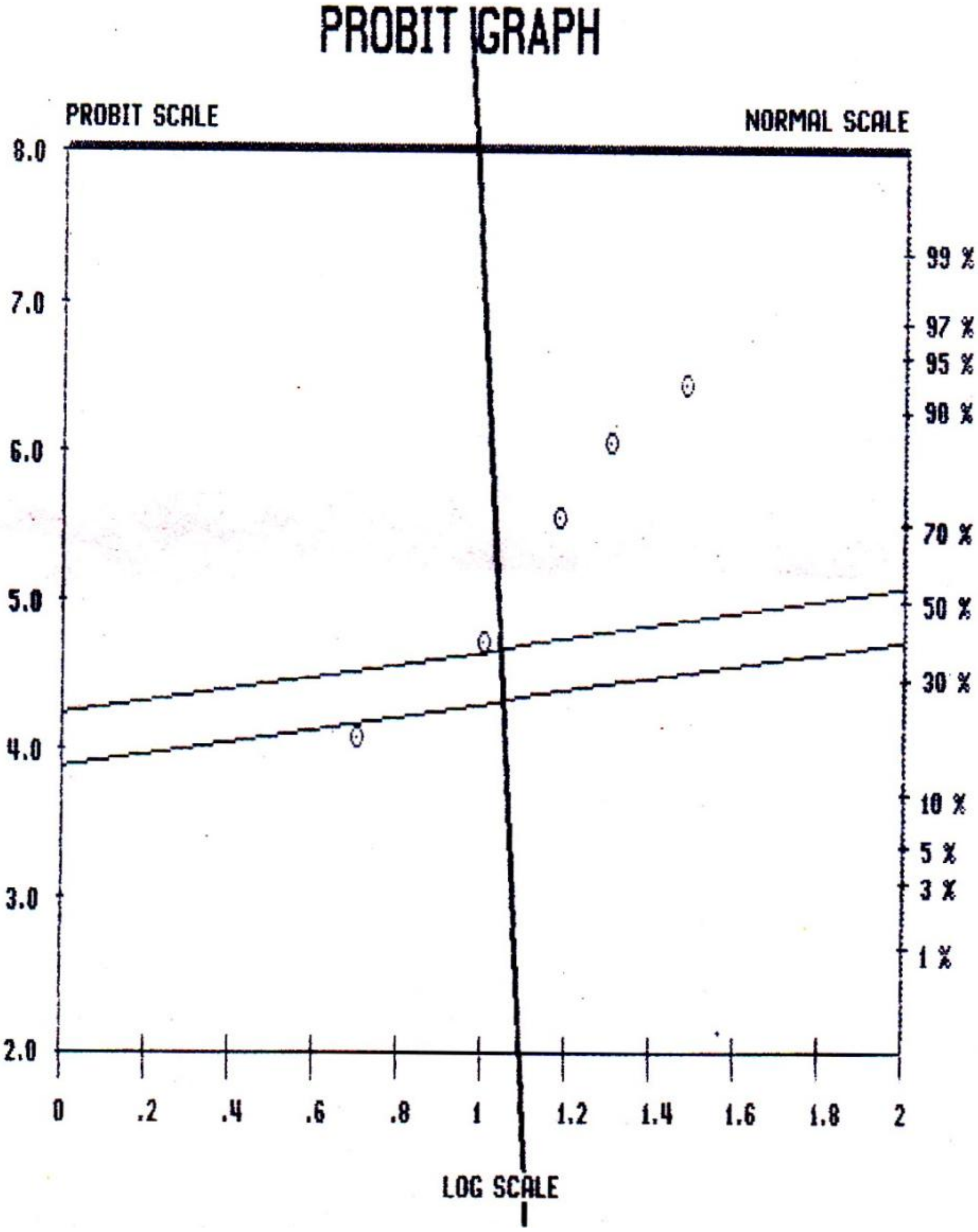


y-mean : 5.0282 x-mean : 1.4408

PROBIT EQUATION : $Y = 0.9325 \pm 2.8426483\ x$

No.5

Probit graph of Fenugreek extract used against *T. castaneum* adults

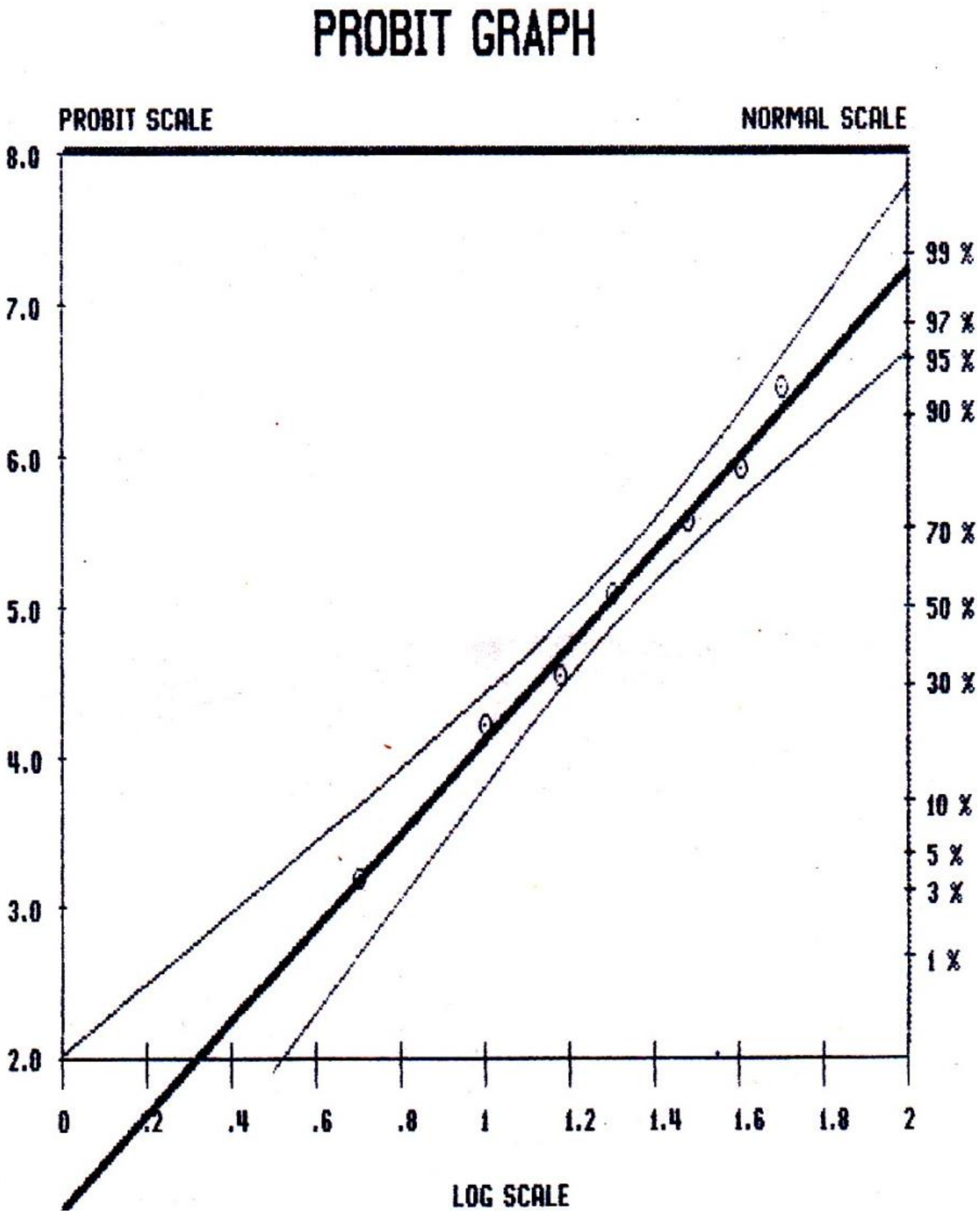


y-mean : 4.5030 x-mean : 1.0460

PROBIT EQUATION : Y = 59.2298 ± 52.3202133 x

No.6

Probit graph of Cinnamon extract used against *T. castaneum* larvae

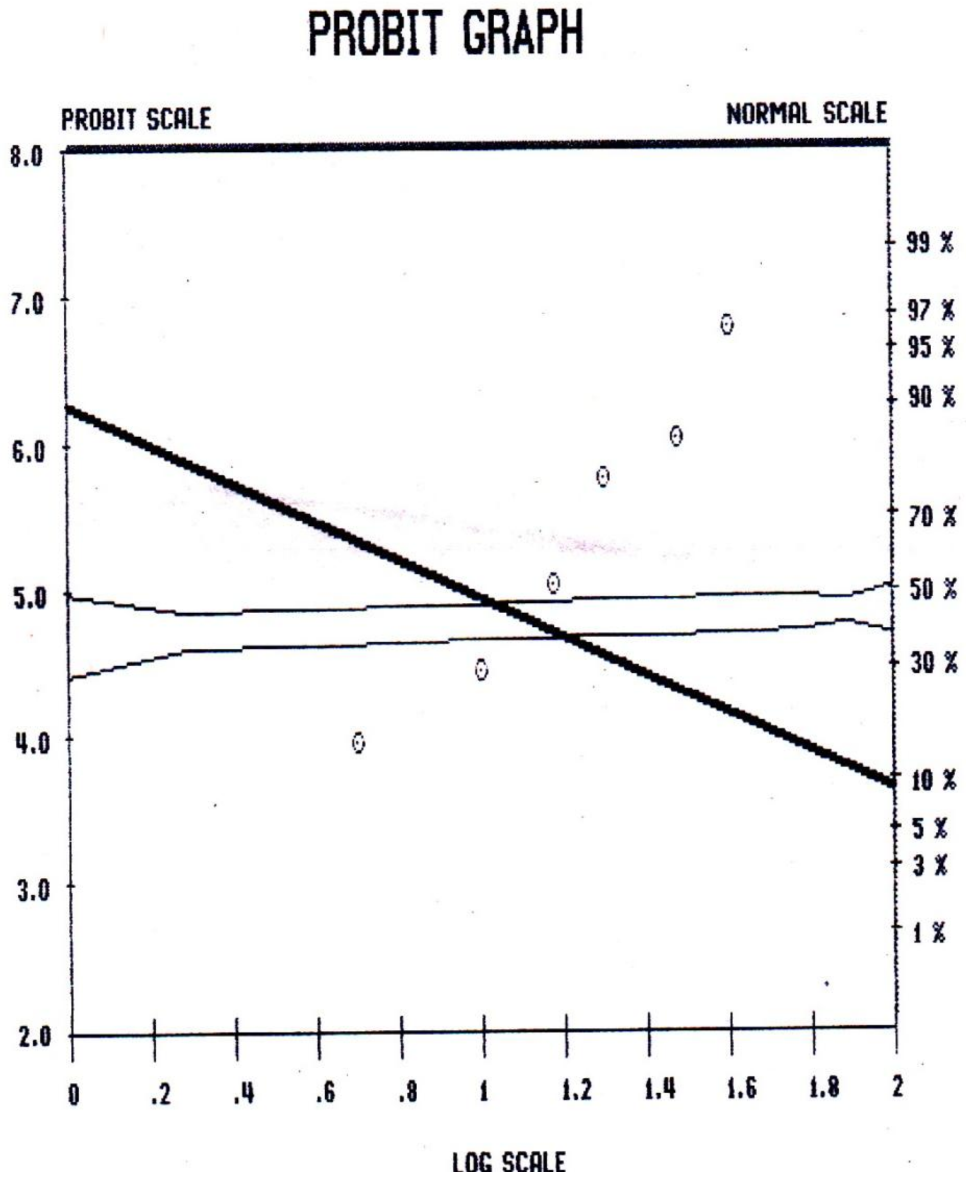


y-mean : 5.0858 x-mean : 1.3119

PROBIT EQUATION : Y = 0.9926 ± 3.1200907 x

No.7

Probit graph of Nutmeg extract used against *T. castaneum* larvae

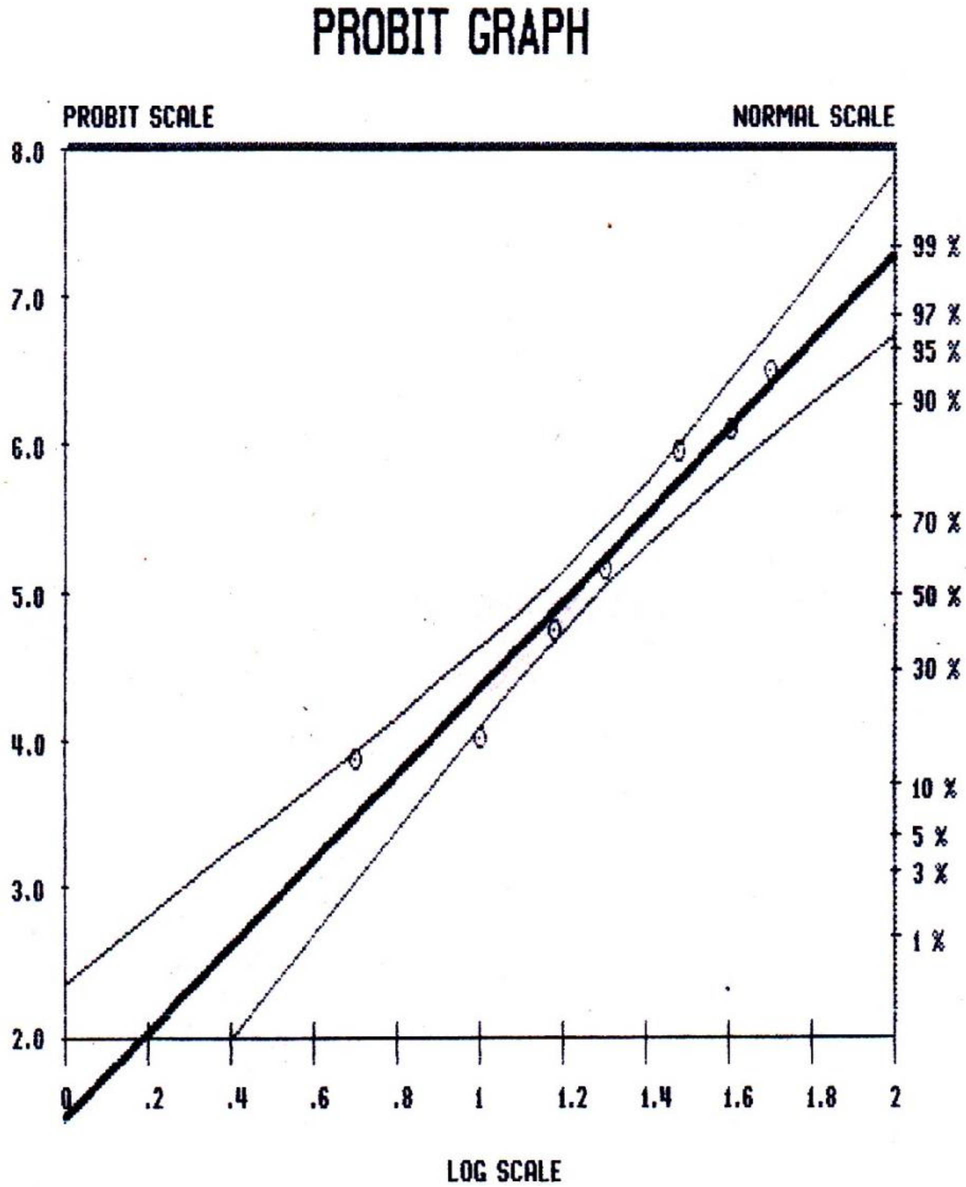


y-mean : 4.7943 x-mean : 1.1220

PROBIT EQUATION : Y = 6.2626 ± 1.3086798 x

No.8

Probit graph of Black pepper extract used against *T. castaneum* larvae

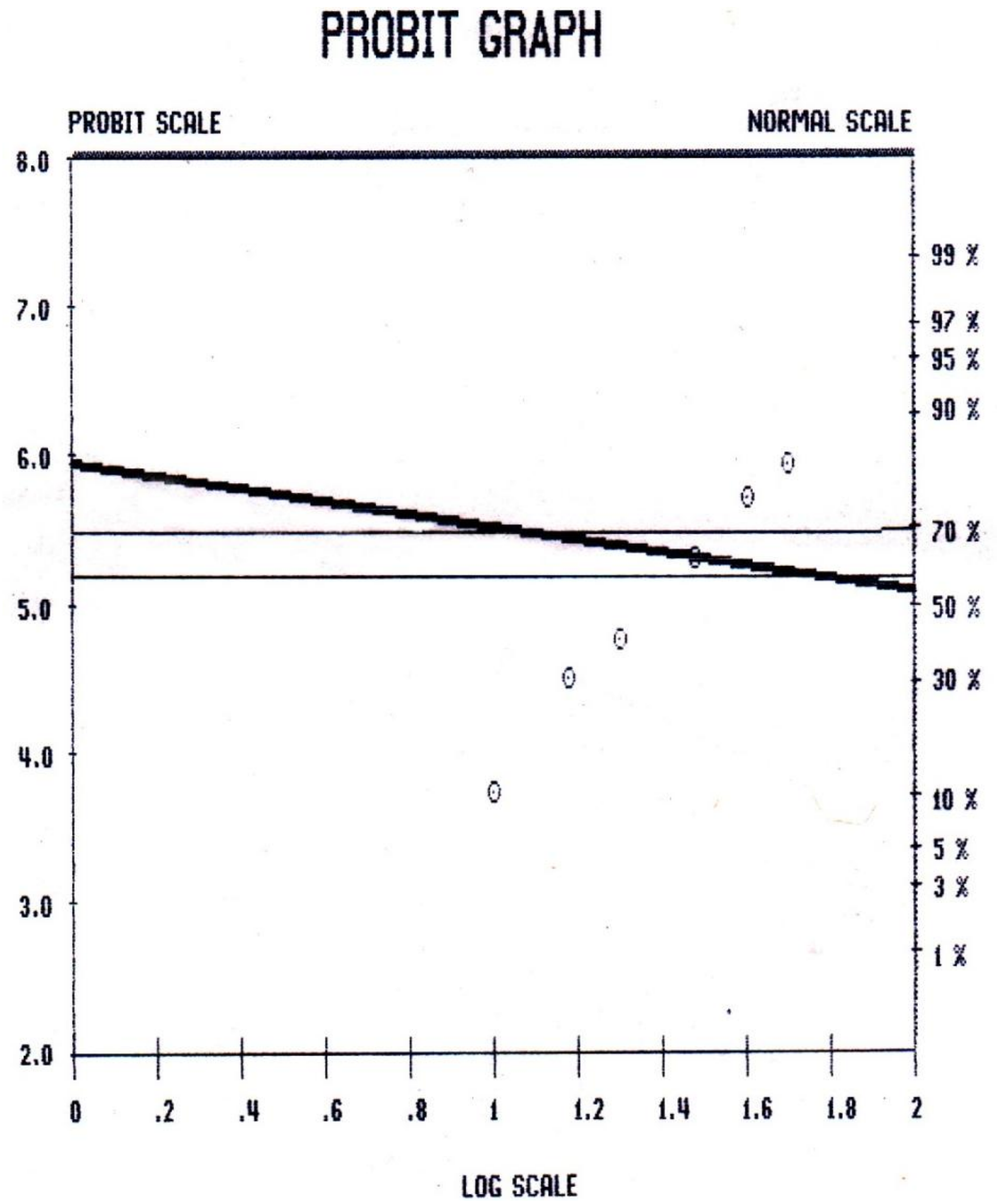


y-mean : 5.1564 x-mean : 1.2741

PROBIT EQUATION : Y = 1.4376 ± 2.9187810 x

No.9

Probit graph of Clove extract used against *T. castaneum* larvae

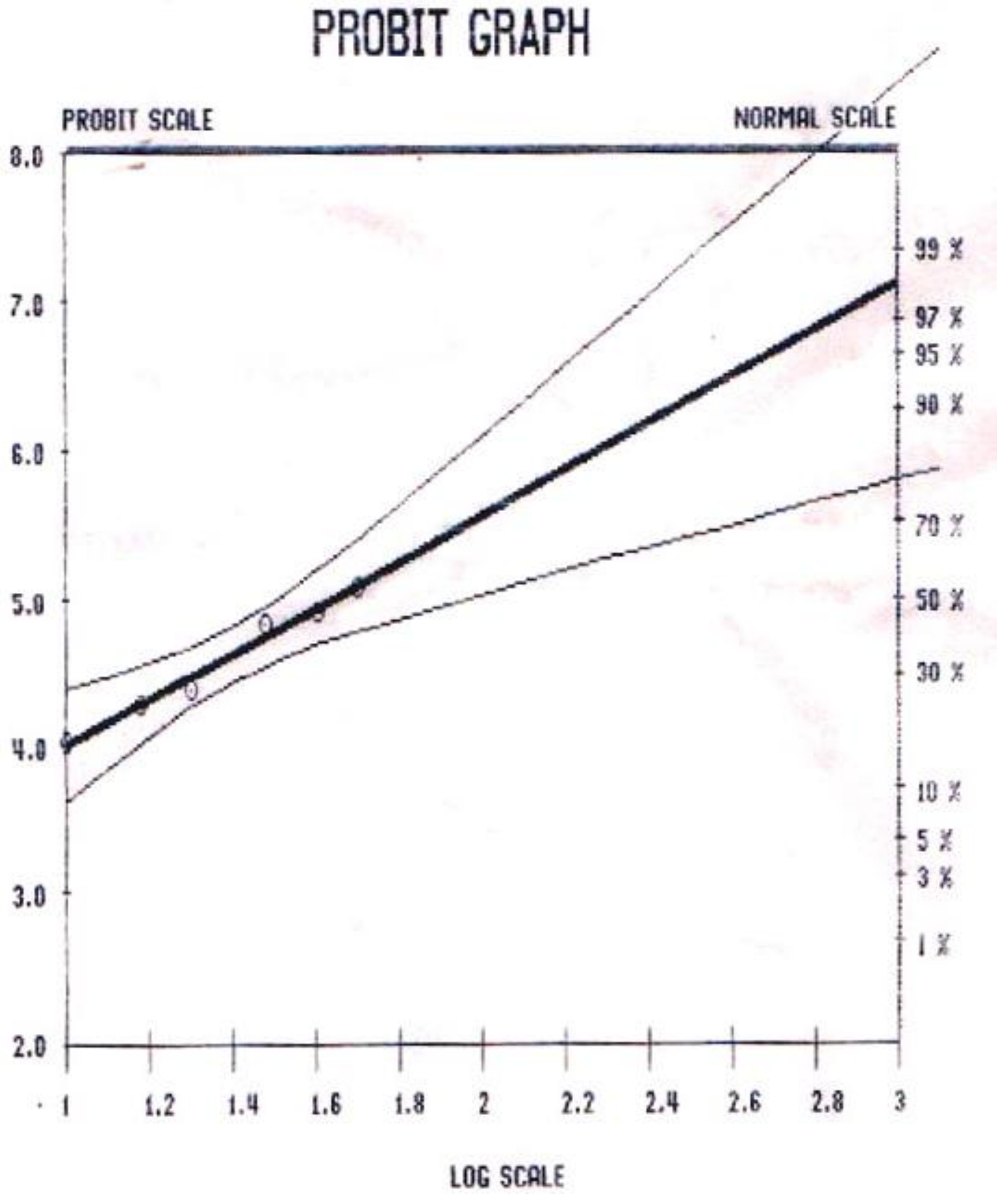


y-mean : 5.3408 x-mean : 1.4290

PROBIT EQUATION : Y = 5.9367 ± 0.4170728 x

No.10

Probit graph of Fenugreek extract used against *T. castaneum* larvae

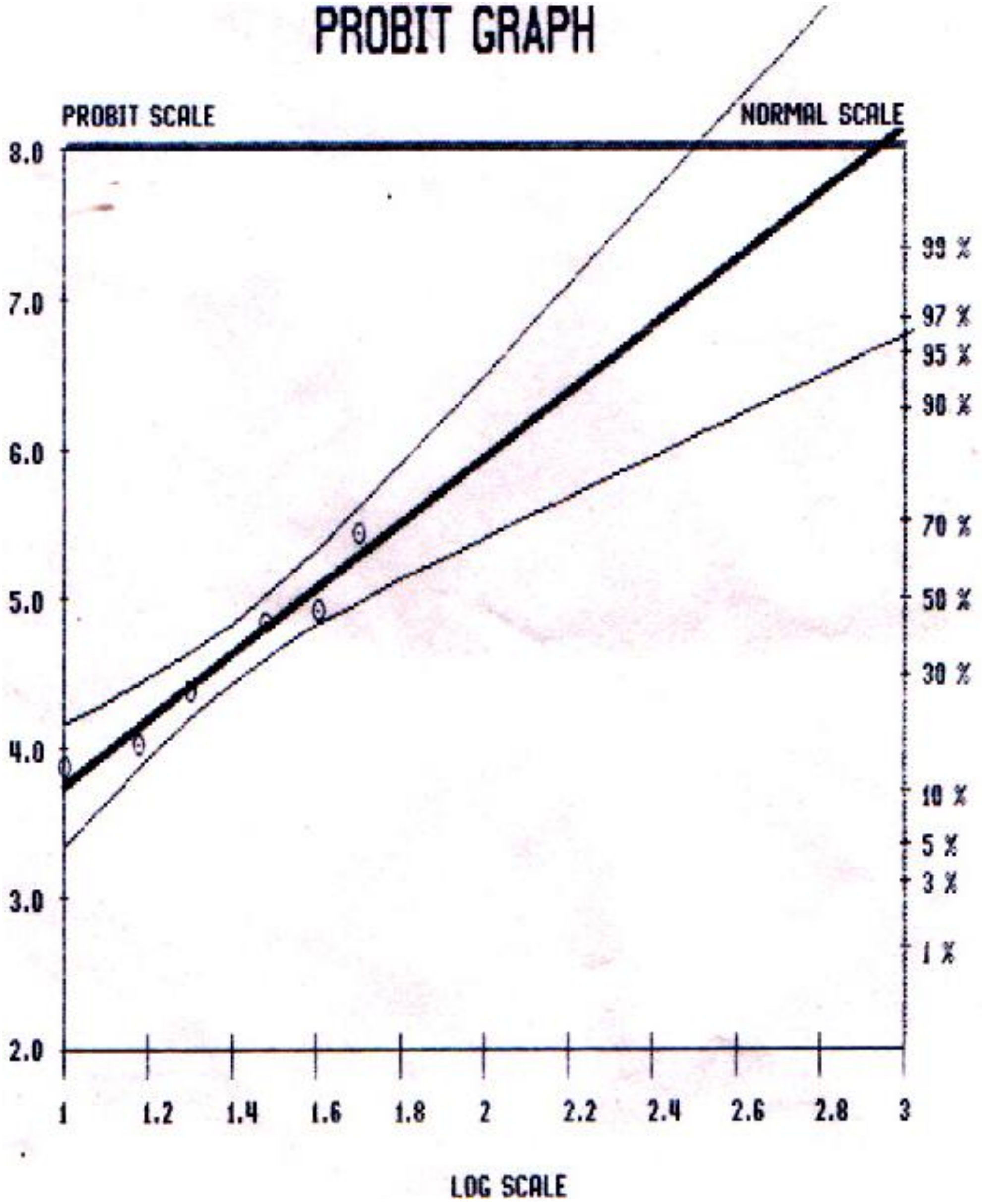


y-mean : 4.6306 x-mean : 1.4047

PROBIT EQUATION : Y = 2.4417 ± 1.5582790 x

No.11

Probit graph of Joint Action of Cinnamon + Nutmeg extract used against *T. castaneum* adults

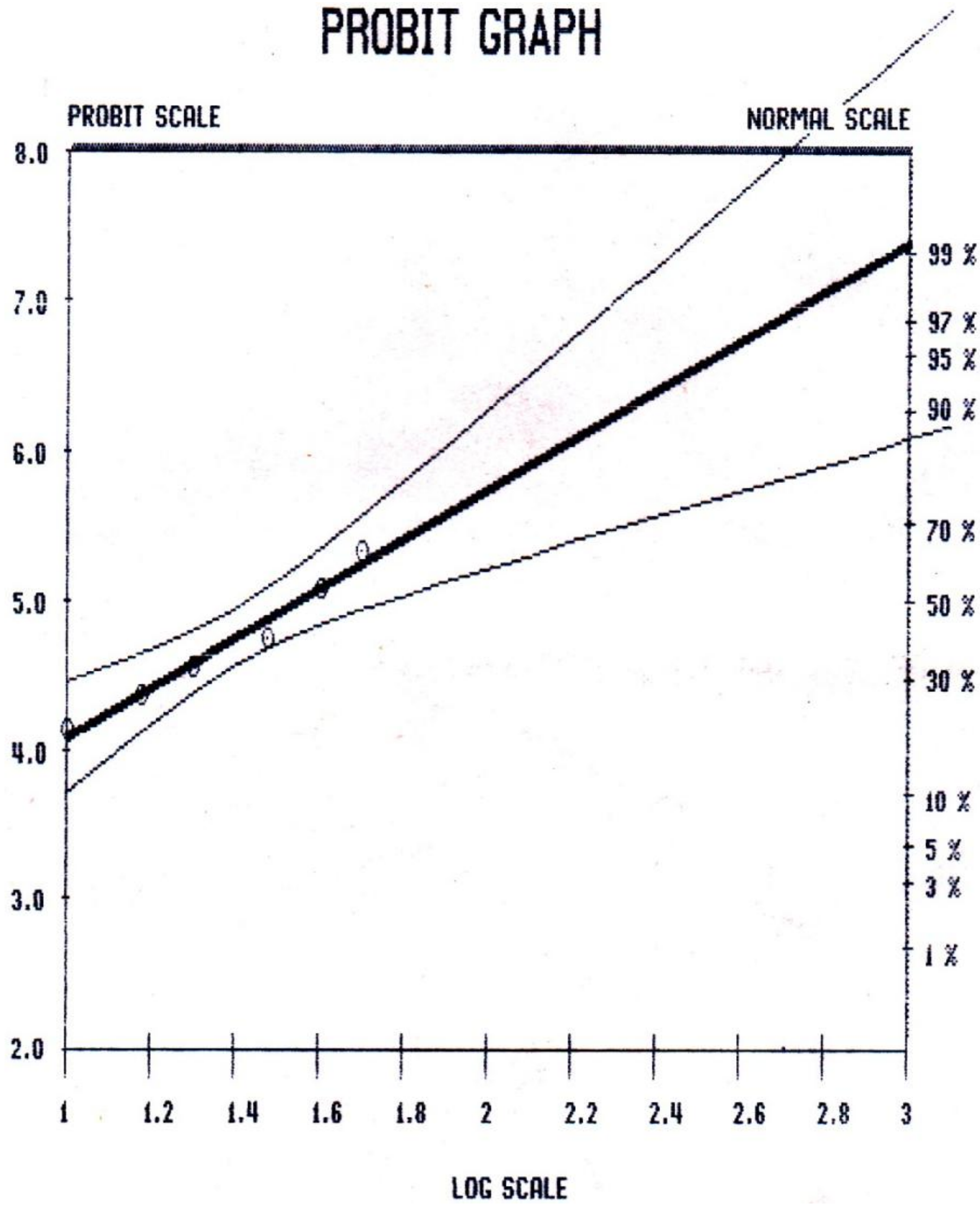


y-mean : 4.6631 x-mean : 1.4162

PROBIT EQUATION : Y = 1.5596 ± 2.1914256 x

No.12

Probit graph of Joint Action of Cinnamon + Black pepper extract used against *T. castaneum* adults

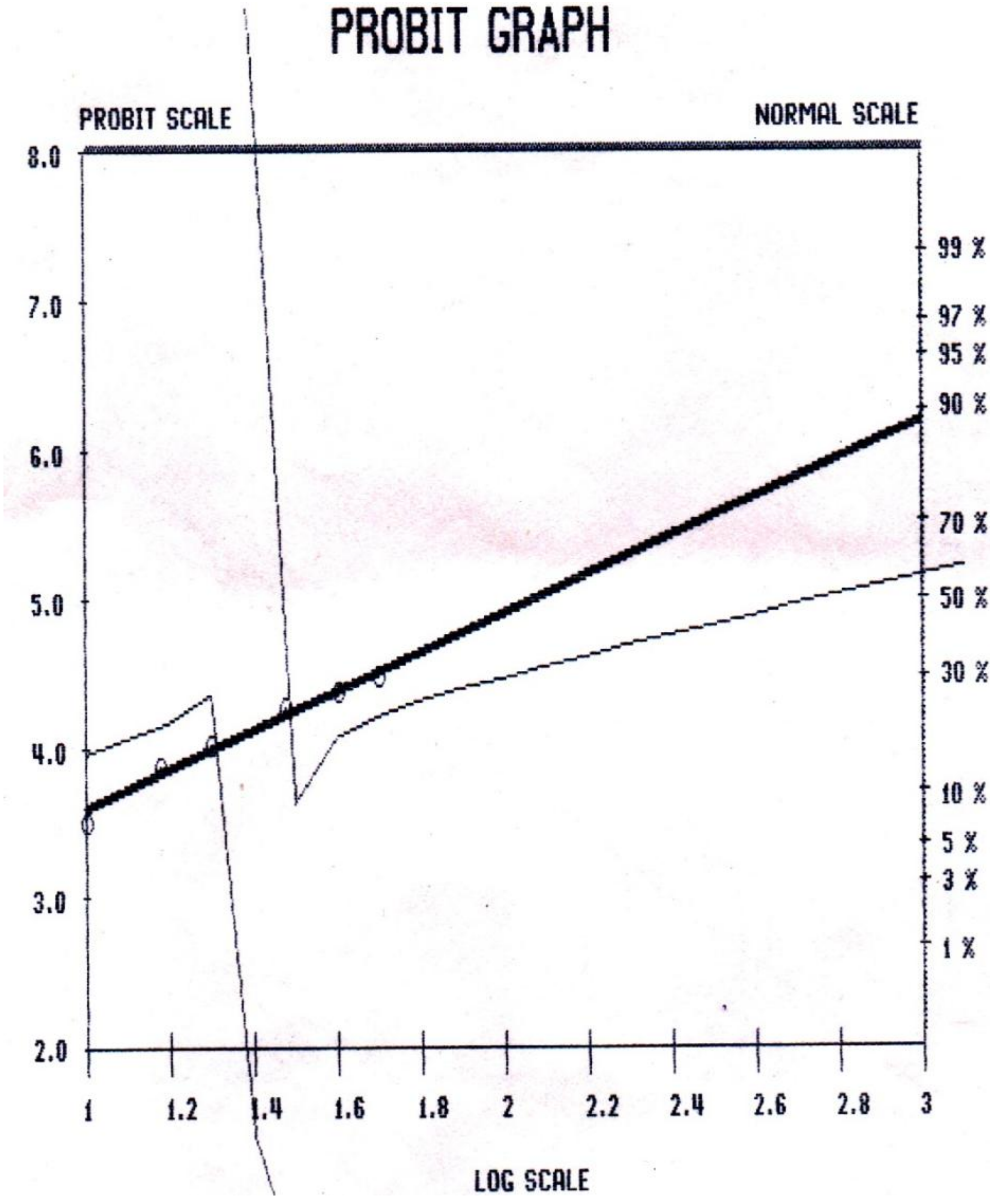


y-mean : 4.7458 x-mean : 1.3976

PROBIT EQUATION : $Y = 2.4405 \pm 1.6494449\ x$

No.13

Probit graph of Joint Action of Cinnamon + Clove extract used against *T. castaneum* adults



y-mean : 4.1647 x-mean : 1.4292

PROBIT EQUATION : Y = 2.3118 ± 1.2964308 x

No.14

Probit graph of Joint Action of Cinnamon + Fenugreek extract used against *T. castaneum* adults

CHAPTER-6

RESULT AND DISCUSSION

GRAPH 15-23

In the present investigations, the insecticidal activities of individual spice extracts were evaluated, in order to determine their effect on the mortality of adults and larvae, joint action, residual toxicity and repellent action against *Tribolium castaneum* (Herbst) in the laboratory experiments.

Toxicity of *Cinnamomum zeylancium* (cinnamon), *Myristica fragrans* (nutmeg), *Piper nigrum* (black pepper), *Syzygium aromaticum* (clove) and *Trigonella foenum-graecum* were assessed against *T. castaneum*. Ethanol extracts were prepared by soxhlet method. Acetone was used to make solutions of desired concentrations.

Spices extract contain a blend of volatile components of chemicals which exert toxic, deterrent, antifeedant and repellent effect on the *T.castaneum* adults and larvae. Laboratory experiments conducted, suggests a wide spectrum of action from these spices extract.

Spices possess several alkaloids, terpenoides, phenols and other secondary metabolites characterised by strong odour, volatility and have generally lower density than water.

Nutmeg extract had low potency than extract of cinnamon and clove. Black pepper also exhibited great insecticidal properties against *T.castaneum.* Fenugreek extract found to be least effective in all treatments.

These spices extract can play important role in checking *T.castaneum* infestation during storage of wheat. It is easiest way to control invasion of *T.castaneum* for small farmers and to adopt this simple technique. Spices can protect wheat up to nine months without affecting seed germination (Devi and Devi 2013).

Insecticidal activity remained different with different spice extracts at different doses level, exposure time kept 24 hrs in every set examined. 20 Adults/10 larvae of *T.castaneum* were introduced into 20gms of wheat sample in each trial.

The following toxicology studies were made:-

1-Toxicity of spices extract against *Tribolium castaneum* (Herbst) Adults.

2-larvicidal effect of spice extracts on *Tribolium castaneum*.

3-The Joint action of ethanol extract of Cinnamon with other spices extract against *Tribolium castaneum* (Herbst) adults.

4-Residual toxicity of the each spice extract.

5-Repellent action of individual spice extract was assessed.

Toxicity of Spice Extracts against *Tribolium castaneum* (Herbst) Adults

Assessment of lethal impact of spices against *Tribolium castaneum* (Herbst) adults, according to experimental data is as follows-

Ethanolic extracts of Cinnamon, nutmeg, black pepper, clove and fenugreek have insecticidal value against *Tribolium castaneum* adults.

In each experiment 20 gm of wheat grain was used in a triplicate set for every observation recorded.(Table 1)

The median lethal dose LD_{50} against adults of *T,castaneum* were recorded as- *Cinnamomum zeylancium* (cinnamon) 22.889 µl ,*Myristica fragrans* (nutmeg) 26.1214 µl ,*Piper nigrum* (black pepper) 35.7813 µl, *Syzygium aromatic* (clove) 21.0937 µl, and *Trigonella foenum-graecum* (fenugreek)26.9711 µl. (Table 4,Graphs 1-5 ,15)

In each experiment 20 gm of wheat grain was used in a triplicate set for every observation recorded.

Syzygium aromatic was found to be most effective against *Tribolium castaneum* adults.

Followed by *Cinnamomum zeylancium*, *Myristica fragrans* and *Trigonella foenum-graecum.*

LD_{50} value for *Piper nigrum* was 35.7813 µl, showing it as least effective among the five spices taken.

Data for *Piper nigrum* and *Cinnamomum zeylancium* were found to be hetrogenous and non-significant. (p=0.05%)

Syzygium aromatic, Myristica fragrans , and *Trigonella foenum-graecum* data were non-hetrogenous and significant.(p=0.05%)

Values of probit kill and working probit were almost corresponding ,hence validating the data collected.

Upper and lower fiducial limits of *Syzygium aromatic, Myristica fragrans* and *Trigonella foenum-graecum* fall in order as indicated in the graphical presentation.

For *Piper nigrum* standared error S_M also found to be 0.9225.

S_M for *Syzygium aromatic, Myristica fragrans* and *Trigonella foenum-graecum* were less than 0.3.

Parallel work of similar observations are listed below as follows:-

Devi and Devi (2013) screened various spices to find out their insecticidal potential against *Sitophilus oryzae*,a serious stored food grain pest. Mace and pepper, at 1% level resulted total mortality by one week followed by nutmeg and clove with 100% mortality and cinnamon and star anise with 90% mortality at 5% concentration .1000 ppm pepper extract recorded 100% mortality by 5 days .Clove oil resulting in 92% mortality, 51.63% nutmeg, 66.6% cinnamon, 79% in case of mace. Hexane extracts of star anise, cinnamon and clove at 0.59 microl/cm^2 on filter paper discs induced 100% mortality by 72 hour.

Abo El-Sad *et al* (2011) estimated that in the essential oils from clove bud *Syzygium aromaticum* L. contained eugenol (48.92%) caryophyllene (18.53%). alpha – caryophyllene (3.255%), eugenol acetate(23%), cis-13docosenamide (3.21%) were present. *Tribollium castaneum* beetle, at 100 micro L/L recorded 75,80,100% mortality after 6, 7 and 8 days exposure period with LC_{50}, 17 micro L/L air.

Akhtar and Muleta (2014) evaluated antifungal potential of (*Allium sativum, Zingiber officinale, Cinnamomum zeylancium* and *Capsicum annuum*) aqueous and ethanolic extracts of four spices, against post- harvest spoilage fungi, *Rhizopus sp*, (26.45%), followed by *Penicillium sp(*19.93%) *Aspergillus sp* (10.86%) and *Fusarium sp*(9.06%). *C zeylancium* was found to be most effective against *Penicillium sp* in ethanolic extract.

Brari and Thakur (2015) evaluated contact and fumigant activity of *Cinnamomum zylanicum* essential oil and its two constituents viz. cinnamaldehyde and linalool against *Callasobruchus maculatus* and *Sitophlius oryzae*. Dose of 1.2mg/cm^2 caused 98 and 80% mortality in *C. maculatus* and *S.oryzae* adults respectively, after 24hrs of treatment. Dose of 0.3%mg/cm^2 cinnamaldehyde caused 100% mortality in *C.maculatus* where as similar dose of linalool caused 89%mortality in *S.oryzae* after exposure of 12hr. Contact and fumigant toxicity is higher in *C.maculatus* than *S.oryzae* adults. Cinnamaldehyde showed higher toxicity than linalool.

Bukahari *et al* (2008) investigated antioxidant properties of fenugreek seeds. It is due to presence of many active phytochemicals including vitamins, flavionides, terpenoides, iron, beta-carotene, saponins and phytic acides. Phenolic compounds may contribute directly to antioxidant action.

Hariharasudhan and Kalaiarasu (2013)) by analyzing the total bacterial population of egg, meat and fish against clove (*Syzygium aromaticum*) extract at different time intervals, found that extract was highly inhibitory for microbial growth at 48 hour.

Haryadi and Rahayu (2002)) evaluated mixture of acetone extracts from black pepper(*P. nigrum*) and nutmeg (*M. fragrans*) seeds for their insecticidal effect on maize weevil(*S.zeamais*. Extracts in ratio 0.0,0.1and 0.2% incorporated into the diet significantly reduced number of F1 offsprings and prolonged the development period . Extract of black pepper alone has relatively better insecticidal properties than nutmeg extract

Huang and Ho (1998) observed that a methylene chloride extract of spice cinnamon (*Cinnamomum aromaticum*) was having insecticidal properties against *Tribolium castaneum* and *Sitophlius zeamais* Motsch.The contact and antifeedant effect of cinnamaldehyde were tested against adults and larvae.

Kumar *et al.* (2013) explored anti-lice properties of three different extracts of *Myristica fragrans* Houtt seeds. Alcoholic,aqueous and hydro alcoholic extracts at five different concentrations such as 5%, 10%, 15%,20%,25% were prepared .Results were comparable to standard drug benzoate at similar concentrations.Hydro alcoholic extract was more potent among all.

Liska *et al* (2011) tested bioactivity of 1,8-cineol,camphor and eugenol composition of essential oils from aromatic plants, to mitigate progeny in *Tribolium castaneum* . Compound 1,8-cineole,camphor and eugenol were applied at lowest concentration 120 micro l/350ml-1vol).The progeny ranged between174.25 to221,50 and higher concentration 600microl/350ml-1vol lowest impact appear in progeny of *T. Castaneum* had camphor (191.00) which was lower related to 1,8- cineole(72.25) and eugenol(112.00).This indicate camphor have no impact upon number of progeny.

Pemonge *et al* (1997) investigated that seeds and leaves extract of *Trigonella-foenum-graecum* appeared to be moderately toxic to larvae of *Tribolium castaneum* (LD50-= 18% in diet)Seeds affect fertility in both sexes.Topical application of extract produced a mortality high in both insects (30mg/insect).

Vijay Kumar *et al* (2015)determined biological activites of spices namely turmeric, chilli,coriander ,fennel seeds, black pepper, ginger, fenugreek garlic and cumin against *Tribloium castaneum*. All spices showed significant effect on adult mortality.Toxic effect followed-black pepper> cumins> garlic> fennel seed>ginger>fenugreek>untreated control.

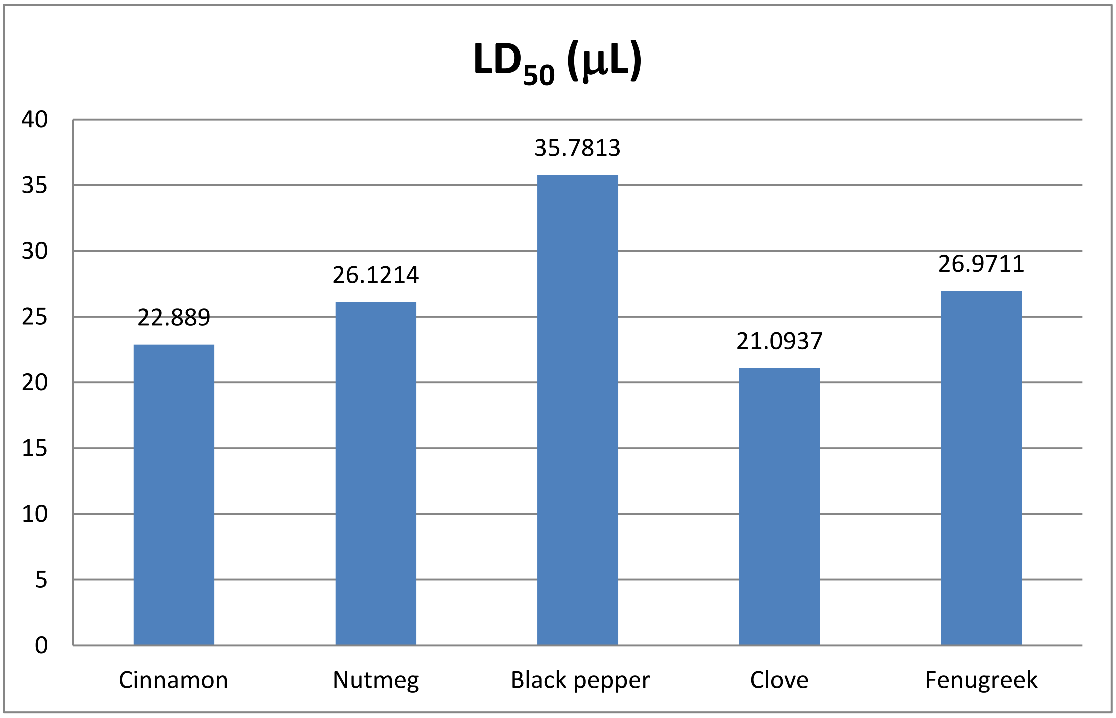
Thakur *et al.* (2014) performed qualitative phytochemical analysis of Mace, also known as flower of nutmeg, showed presence of tannins, saponins, flavonoids, terpenoids, phenolics carbohydrates as well as proteins and amino acids. Methanolic extracts of mace had 238.52 phenolic content.DPPH scavenging activity was 85.2% at 500 micro gram/ml concentration.

Tripathi *et al* (2009) evaluated spices for their contact and fumigant toxicity and effect upon progeny development against *Callosobruchus maculates* and *Tribolium castaneum* Herbst. Against adults of *T.castaneum* these powders also caused inhibition of progeny production at dose of 5g/100g At .1:1 ratio of turmeric and green cardamom showed both contact and fumigant toxicity.

Upadhyay and Ahmad (2011) explained that due to high mortality of adult insect,besides reduced oviposition and hatching, essential oil of black pepper significantly suppressed survival of larvae and adults of *Tribolium castaneum*.

Upadhyay and Jaiswal (2007) examined inhibitory activities of the essential oils of *Piper nigrum* L against *Tribolium castaneum* (Herbst)..The LC50 values for larvae and adults were calculated ,appear to be 14.022 micro l and 15.262 l respectively .Adult mortality increased while adult emergence decreased with increase in the concentration of essential oils. EC50 dose of *P.nigrum* oil reduced larval transformation into pupae to 50% was found to be 6.919 micro l.

Wang *et al* (2009) estimated the contact toxicity of monoterpenes of 3-carene,1,8-cineole,beta-pinene,terpinene and terpinolene on *Tribolium acstaneum* adults and sustaining fumigant toxicity of *Sitophilus zeamaise*.Contact toxicity of beta-pinene LC_{50} value ranged between 31.44 and 62.07 micro g mg-1 and for 3- careen LC_{50} lies between 66.58 and 93.68 micro g mg-1.Fumigant toxicity for 24,48 ,72 and 96hr , when LC_{50} value of terpene and terpinolene was not changed,mortality was reduced to 71.8% after 96hr wehereas for beta –terpene and 3-carene 35.0 and 31.4% reduction were observed,respectively. Monoterpenes could be screened for the control of storage pests.



LD_{50} value of spice extracts against *T.castaneum Adult* (15)

Larvicidal effect of spice extracts on

Tribolium castaneum (Herbst)

Assessment of lethal impact of spices against *Tribolium castaneum* (Herbst) larvae, according to experimental data is as follows-

Cinnamon, nutmeg, black pepper, clove and fenugreek, ethanolic ectracts had insecticidal value against *Tribolium castaneum* larvae.

In each experiment 20 gm of wheat grain was used in a triplicate set, for every observation recorded. Ten larvae were released in each culture tube. A control set was also run side by side. (Table 2)

Mortality data were recorded 24hrs post treatment of wheat with specific dose of each spice extract.

The median lethal dose (LD_{50}) against larvae of *T. castaneum* were recorded as– *Cinnamonum zeylancium* (cinnamon) 10.8767µl, *Myristica fragrans* (nutmeg) 19.2474 µl, *Piper nigrum* (black pepper) 9.2209 µl, *Syzygium aromatic* (clove) 16.6155 µl and *Trigonella foenum-graecum* (fenugreek) 176.1916 µl. (Table 5; Graphs 6-10,16)

Piper nigurm (black pepper) was found to be most effective against the larvae of *Tribolium castaneum* (LD_{50} 9.2209µl), followed by *Cinnamomum zeylancium* (cinnamon) (LD_{50} 10.8767 µl)

Data for *Piper nigrum* (black pepper) and *Cinnamomum zeylancium* (cinnamon) were found to be hetrogenous and non-significant. (p= 0.05%)

Trigonella foenum-greacum (fenugreek) was least effective against larvae of *T. Castaneum* with the LD_{50} 176.1916 μl

LD_{50} value for *Syzygium aromatic* (clove) and *Myristica Fragrans* (nutmeg) were found as 16.6155 μl and 19.2474 respectively.

No heterogeneity was found in nutmeg and clove, data were found to be significant (p=0.05%)

Larvae is more susceptible to cinnamon extract than adults of *T. castaneum*. (Graphs 17)

Toxicity of nutmeg against adults and larvae was almost in similar ranges 26.1214 ml and 19.2474 in adults and larvae respectively.

Black pepper LD_{50} value for larvae is far more less than adults when compared. In larvae is 9.2209 ml and for adult it is 35.7813 ml.

Motality data of clove for adults and larvae of *T. Castaneum* are significant and non-hetrogenous. LD_{50} 21.0931 ml for adult and LD_{50} 16.6155 for larvae is reported in experiment perform.

The results are in confirmation with findings of various researchers:-

Chaubey (2007). LC_{50} value against larval stages of *Tribolium castaneum* was 15.02,14.02 and 17.48 micro L were recorded respectively .For adults it was little higher 16.26,15.26 and 18.55 micro L recorded for the extracts of *P.cyminum*, *P.nigrum* and *F.vulgare* respectively. Vapours of these oils and fumigation resulted in deformities in various stage of the insect. Period of development was also extended all these oils inhibit various biosynthetic processes of insect.

Huang and Ho (1998) observed that a methylene chloride extract of spice cinnamon (*Cinnamomum aromaticum*) was having insecticidal properties against *Tribolium castaneum* and *Sitophlius zeamais* Motsch. The contact and antifeedant effect of cinnamaldehyde were tested against adults and larvae. Hence it is a potential grain protectant.

Huang *et al* (1997) reported that nutmeg oil in concentration of 1.05g/100gm of wheat, totally suppressed F-1populationof *Tribolium castaneum*. Larvae were more susceptible than adults to contact toxicity. Extracts were obtained by steam distillation and tested for contact toxicity.

Mondal and Khalequzzaman (2010) evaluated four compounds trans-anethol, thymol, eugenol and cinnamaldehyde and their contact and fumigant toxicity against *Tribolium castaneum's* 10 days and 18 days old larvae. Most sensitive period was 10 days old larvae. Cinnamaldehyde and eugenol were highly effective against *T.castaneum* when applied for 48hr at lowest dose. At highest dose level of 0.288mg cm-^{2} and lowest exposure time 6hr.Trans-anethol achieved 100% mortality of 10 days larvae as contact toxicity. Highest dose level 115.38 and 6.153 mg-1, thymol and eugenol achieved only 36.66 and 30% of 10days old larvae and *T.castaneum* as fumigant toxicity. Against 18days old larvae, eugenol and cinnamaldehyde achived 100% mortality for 48hr exposure even with the highest exposure dose volume.

Pemonge *et al* (1997) investigated that seeds and leaves extract of *Trigonella-foenum-graecum* appeared to be moderately toxic to larvae of *Tribolium castaneum* (LD_{50}

18% in diet) Seeds affect fertility in both the sexes. Topical application of (at 6 and 30mg/insect), extract produced a mortality.

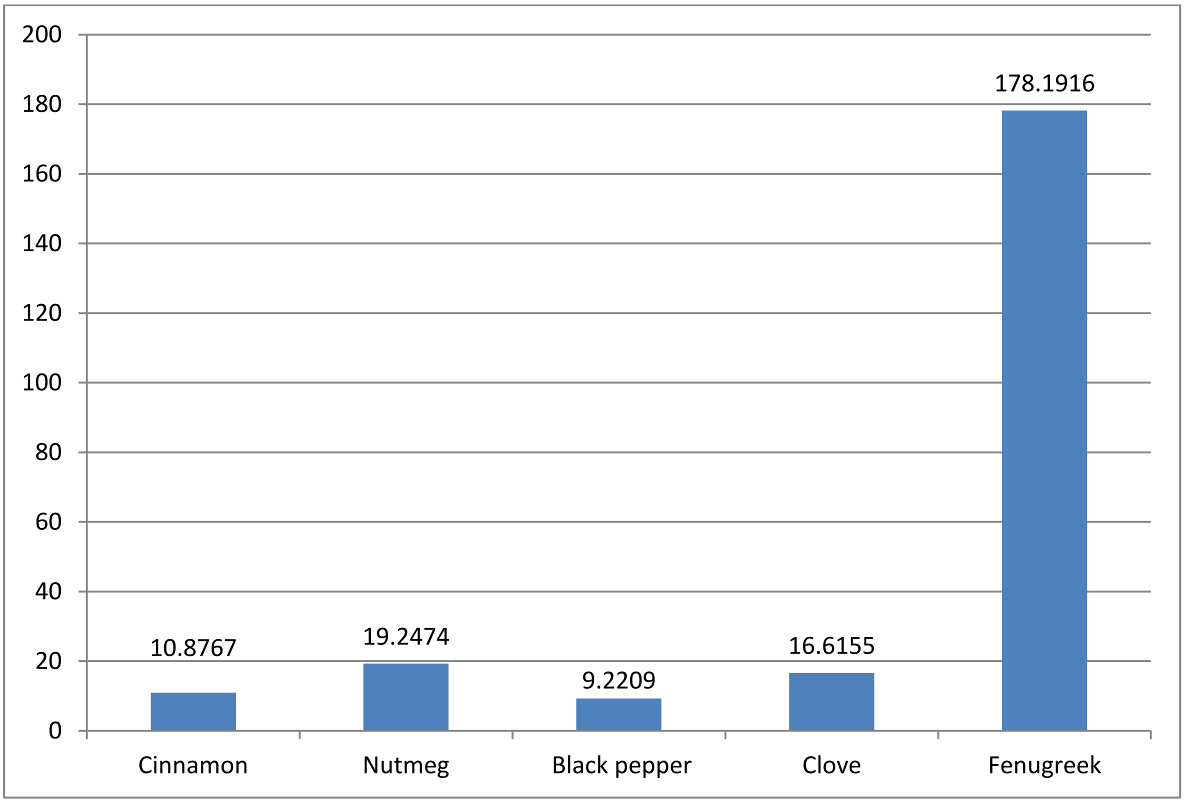
Upadhyay and Ahmad (2011) explained that besides high mortality of adult insects, reduced oviposition and hatching, essential oil of black pepper significantly suppressed survival of larvae and adults.

Upadhyay and Jaiswal (2007) investigated that insecticidal and developmental inhibitory activities of the essential oils of *Piper nigrum* L against *Tribolium castaneum* (Herbst). The LC_{50} values for larvae and adults were calculated, appear to be 14.022 micro l and 15.262 l respectively. EC_{50} dose of *P.nigrum* oil reduced larval transformation into pupae by 50% was found to be 6.919 micro l.

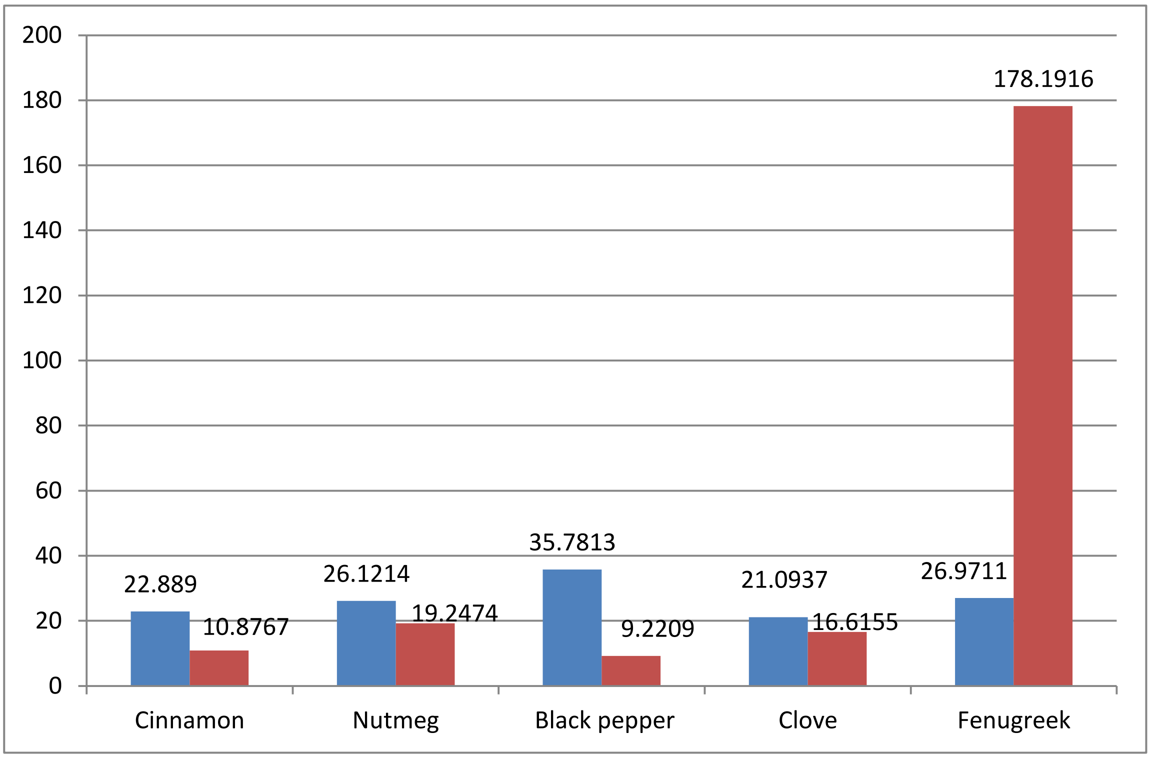
Alam *et al.* (2009) evaluated toxicity effect of methanol extract of root of *Calotropis gigantean* (Linn) and its chloroform and petroleum ether (40-60 degree C) soluble fractions against several instar larvae and adult *Tribolium castaneum*. Methanol extract showed lowest LD_{50} value against several stages and LD_{50} value for adults (121.59, 147.73, 146.84, 202.98, 358.42 and 300.03 micro gm/cm^2 respectively) Whereas LD_{50} value of petroleum ether extract were (407.69, 485.46, 437.38, 502.23, 551.26, 625.36 and 411.84 micro gm/cm^2 from first to sixth instar larvae and adult. Chloroform extract recorded 291.83, 299.29, 382.98, 745.18, 637.71, 1259.71 and 739.87 micro gm/cm^2 respectively. The order of toxicity on *T. castaneum* larvae was methanol extract > petroleum ether > chloroform.

Azevedo *et al* .(2008) treated Peanut seeds with neem oil concentration of 0.0, 0.5, 1.0, 1.5 and 2.0% (volume/seed mass) and mortality of *Tribolium castaneum* offspring number (larvae, pupae and adults) and number of punched seeds were evaluated in four stored period (30, 60, 90 and 120 days).The effect of neem on biological development was observed.

Osman *et al.* (2011) studied residual effect of *Manikara zapota* (L) P. Royan plant against *Tribolium castaneum* (Herbst). Ethyl acetate extract of stem bark were applied upon larvae and adult in four doses, i.e. 1238.5, 619.25, 309.6 and 154.8 micro g/cm^2. Effectiveness of the extract increased after 72hr exposure, maximum residual toxicity was observed with LD_{50} of 228.8, 281.1, 43.4, 423.7, 455.2, 495.7 and 526.5 micro g/cm^2 for first, second, third, fourth, fifth, sixth instar larvae and adults, respectively. Larval stages were more sensitive than adults.



LD_{50} value of spice extracts against larvae of *T.castaneum* (16)



Comparison of LD_{50} value of Adults and Larvae of *T.castaneum* against treatment of indiduval spices extracts (17)

The Joint action of ethanol extract of Cinnamon with other spices extract against *Tribolium castaneum* (Herbst) adults

The concept of joint action of insecticides as mixtures or combinations has many advantages. Insects have developed resistance to a number of chemical insecticides. Therefore, synergism is an important research tool in the laboratory to determine the mechanism of resistance involved in a particular population .Sometimes an insecticide becomes less effective against particular pest due to a prolonged and continuous use. The problem can be solved by using the less effective insecticide in a mixture with another effective insecticide. If the effect of the mixture is greater than simple algebraic summation of effect of the two compounds individually administered, then this phenomenon is called potentiation or synergism, a situation likely to be of practical and economic importance .If the two materials elicit lower response than expected sum of two individual insecticides ,it is known as negative potentiation or antagonism.

In present investigation *T. castaneum* was the test insect. The mortality counts recorded 24hrs post treatment were subjected to probit analysis (Finney 1942-81) to calculate LD_{50} value of mixture of spices and possibilities of any kind of joint action was explored. Co- toxicity coefficient was calculated with the Sun and Johnson's Formula (1960). Extract of cinnamon was taken as a standard and in ratio of 1:1 with other spice extract applied against test insect.

Following observations were made on the basis of data recorded:-

In (Table 3,6,7**)** are given the values of LD_{50} of various combinations of *Cinnamomum zeylancium* with other spices. The mixture of *Cinnamomum zeylancium* and *Syzygium* aromatica extract was most toxic and the combination of *Cinnamomum zeylancium* and *Trigonella foenum-graecum* were recorded as least toxic.

However no synergistic behaviour of extract of cinnamon with other spice extracts have been reported. This aspect of joint action has been investigated here.

The LD_{50} values of various combinations of cinnamon with other spices extracts in 1:1 ratio (v/v) and order of their joint toxicity were as follows-

Cinnamomum zeylancium and *Syzygium* 35.6252 µl> *Cinnamomum zeylancium* and *Piper nigrum* 37.1495 µl > *Cinnamomum zeylancium* and *Myristica fragrans* 43.8248 µl > *Cinnamomum zeylancium* and *Trigonella foenum-graecum* 118.457 µl.

There was absence of the heterogeneity in the present work as per the calculation done by Finney's probit analysis; hence all the data are significant.

Actual toxicity and Theoretical toxicity were calculated and then co toxicity coefficient. (Graphs11-14; 18-20)

Toxicity co-efficient of standard is considered 100 as per Sun and Johnson formula (1960) and individual toxicity of each sample should not be more than 100. If joint toxicity coefficient is more than 100 then it is synergistic combination but if less than100 then joint action is called antagonistic.

The co toxicity coefficients of the mixtures (Table-7) revealed that phenonmenon of antagonisum was reported in the laboratory experiments conducted for all the four combination of spices with cinnamon.

Their co toxicity co-efficient were in order- Cinnamon + Black pepper > Cinnamon + Clove > Cinnamon + Nutmeg > Cinnamon + Fenugreek, values recorded were- 75.1340 > 61.6115 > 55.613 > 20.6574.

However no synergistic behaviour of extract of cinnamon with other spice extracts has been reported.

All the combination results indicate antagonism. More than 100 value of co toxicity co-efficient only gives synergistic results (Sun and Johnson 1960)

There was absence of the heterogeneity in the present work as per the calculation done by Finney's probit analysis; hence all the data are significant.

These results are supported by the same pattern of experimentation done by various researchers as follows:-

Chaubey (2012) evaluted alpha-pinene and beta-caryophyllene, alone or in binary combination against *Tribolium castaneum*. LC_{50} of alpha-pinene was 0.998 and for beta caryophyllene was 1.624microl/cm against larvae were 1.379 and 1.949 microl/cm^3 for adults respectively. In binary combination, the LC_{50} values against adult and larvae were found 1.277 and 1.438 microl/cm^3. Alpha-pinene is more effective but both are synergistic and thus used as efficient insecticidal tool against *T.castaneum*.

Haryadi and Rahayu (2002) reported that mixture of acetone extracts of black pepper (*P. nigrum*) and nutmeg (*M. fragrans*) in ratio 0.1 and 0.2% reduced number of F1 offsprings and prolonged the development period in *Sitophilus zeamais*.

Sutton *et al.* (2011) exposed *T.confusum* larvae to 3% pyerthrine+methoprene. *T.castaneum* larvae were more susceptible than *T. confusum.*

Tripathi *et al* (2009) said that Green cardamon and Clove powder at 1.5g/50g dose showed 100% repellency against adult *T.castaneum*. These powder also caused inhibition of progeny production at dose of 5g/100g and 1:1 Ratio of turmeric and green cardamom showed both contact and fumigant toxicity ,while mixture of clove and large cardamom showed repellency and inhibited progeny development in *T.castaneum*.

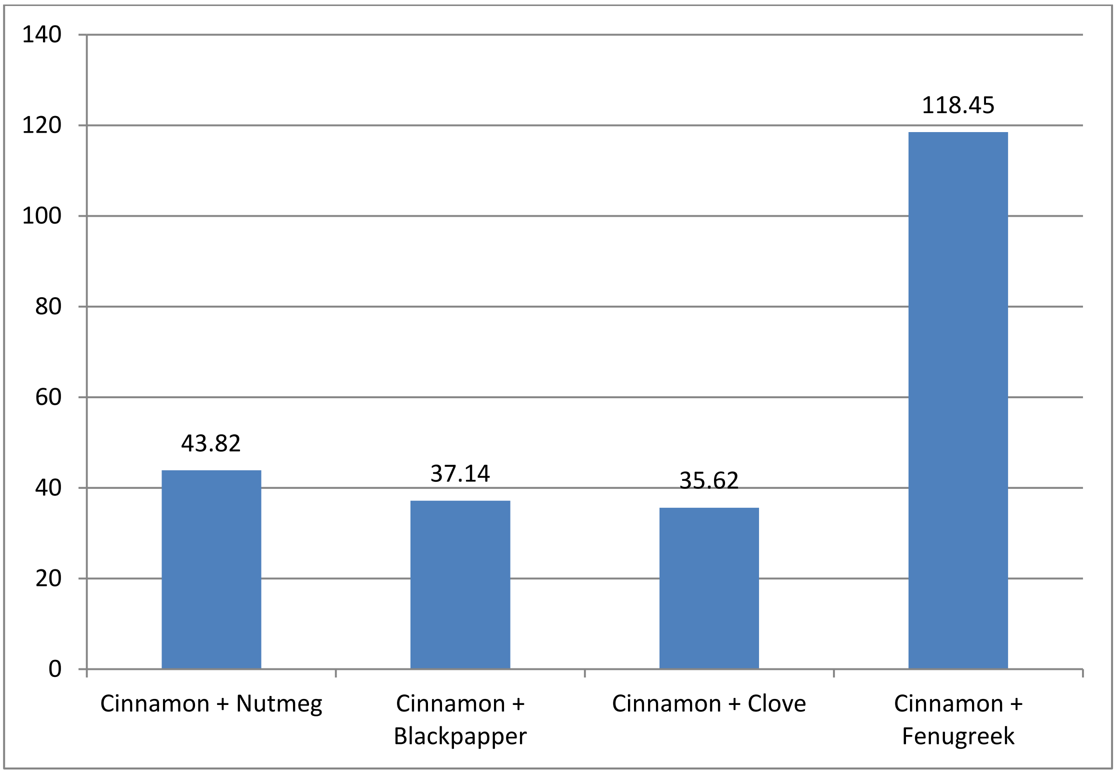
Collins (1990) was of opinion that the use of PBO synergised Pyrethroids would result in control failure of pyrethroids resistant strain of *Tribolium castaneum* in the fields.

Dhingra and Swarop (1979) reported that irrespective to ratio Karenj oil synergised carbyl and showed abdicative effect with DDT and Lindane against *Tribolium castaneum* but antagonism was observed with melathion. Piperonyl butaoxide formulations with lindane, pyrithrins at different ratio revealed synergistic against *Cylas farmicarius*

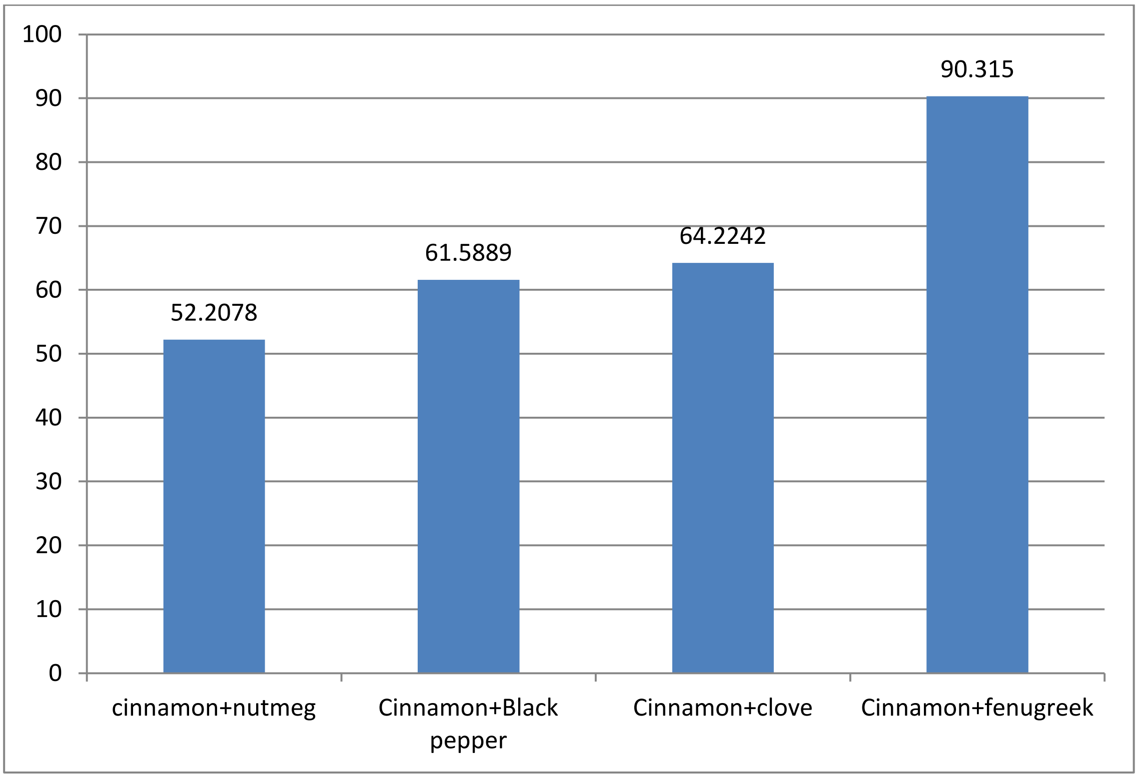
Eagleson (1942) reported 35 vegitable oils mixed with pyrethrins in kerocene, sesameic oil and 3, 4 methyl dioxyphenyl group of sesamine is essential for synergistic activity against house fly.

Mariappan *et al* (1984) in laboratory tested synergistic action of custard apple oil and neem oil against rice green hopper (*Neptholettix virescens*) and rice fungus virus RTV.

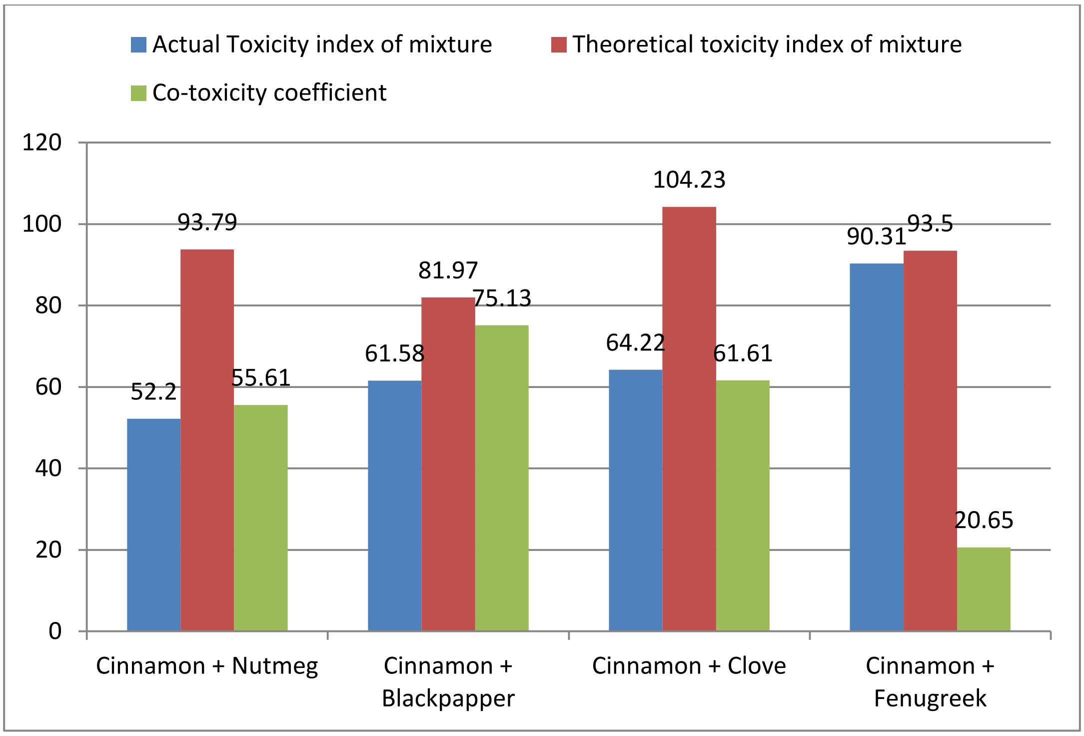
Saxena (1987) revealed that (PBO) Peperonyl butaoxide synergised DDT but antagonised Malathion and Pyrethrin when tested against *Sitophlius oryzae*.



Joint Toxicity of mixture LD_{50} (18)



Actual Toxicity Index of Mixture (19)



Actual Toxicity index of mixture, Theoretical toxicity index of mixture, Co-toxicity coefficient (20)

Residual Toxicity

Residual toxicity of the various extracts of spices was assessed to find out the period of time for which they could protect wheat grains against infestation by *Tribolium castaneum*. Mortality test were conducted at intervals to analyse the impact of remaining residues left in the grains at different days after treatment (DAT). Concentration of spice extracts giving 90% mortality were mixed with wheat grain (v/w) basis. Extracts were prepared in ethanol. With the help of acetone desired concentration of solution were made out of extract prepared.

After 10 days of post treatment, 20 gm of treated wheat taken and 10 adult *T. castaneum* were introduced. Mortality was recorded after 72 hr period of exposure. Similarly after 30.60, 90, 120 DAT (Days after Treatment) mortality data analysed by ANOVA. (Table 8-10; Graph 21)

Cinnamon remained most effective up to 120 days, black pepper and clove were also gave residual efficacy.

Nutmeg was effective up to 90 days of post treatment and gave 16.67% mortality.

Fenugreek was effective only up to 60 DAT, reflecting only 3.33% mortality.

The results of linear regression analysis revealed that, the regression co-efficient of all the five compounds are highly significant. Thus the corresponding regression lines fitted can be used for estimation of mortality percentage (Y) with respect to day's interval (D).

Many plant materials have been evaluated against *T. castaneum* and other Coleopteran storage pests for their residual toxicity as follows:-

Abo El-Sad *et al* (2011) estimated that in the essential oils from clove bud *Syzygium aromaticum* L. against *T. castaneum* beetle showed at 100 micro L/L mortality was 75, 80, 100% after 6 , 7 and 8 days exposure period .

Devi and Devi (2013) screened various spices against *Sitophilus oryzae*, a serious stored food grain pest. Bioefficacy of powders and hexane extracts were determined. Responses varied with spices, dosage and exposure time. Mace and pepper, at 1% level resulted total mortality by one week followed by nutmeg and clove with 100% mortality and cinnamon and star anise with 90% mortality at 5% concentration .1000 ppm showed insecticidal activity, with pepper extract recording 100% mortality by 5 days. Clove oil resulting in 92% mortality, 51.63% nutmeg, 66.6% cinnamon, 79% in case of mace.Hexane extracts of star anise, cinnamon and clove at 0.59 microl/cm^2 on filter paper discs induced 100% mortality by 72 hour. Spices offered protection to wheat up to 9 months without affecting seed germination thereby showing promise as grain protectants.

Meena *et al* (2015) investigated that 0.5% dose of black pepper seed powder against *Rhyzopertha dominica* exhibited maximum adult mortality ,65%.and 29.75% after 48hrs adults were realeased at 4 and 60 DAT respectively .

Tripathi and Verrma (2000) evaluated the efficacy of powdered black pepper (*Piper nigrum*) against *Rhizopertha dominica* .Dose of 3g pepper per 100g wheat resulted in 100% mortality after 15 days, as did 2.5g after 25 days.

Farzana Parveen *et al* (2010) tested toxicity and residual effect of yellow-berried nightshade, *S.kolanum surrattense* leaves extract against red flour beetle *T. castaneum* under laboratory conditions ,during 1st day, mortality was lowest 49.4% at minium 2.4 microl/cm^2 and highest mortality was 91.6% at 12.0 microl/cm^2. During 7th day lowest was 5.0% at 2.4micronl/cm2 and highest 60.5% at 12.0 micro l/cm2. During 8th day lowest 0.4% at 2.4 micro l/cm2 *S. surrattense* could be useful for managing populations of *T. Castaneum.*

Haridasan and Gokuldas (2009) prepaered petroleum ether leaf extract of *Vitex negundo* (VPE) and methanol(VME) effects were observed on adult emergence of *Tribolium castaneum* during 24,30,40 and 50 days of treatment during different period of exposure, and compared with control.VPE at 10% concentration exhibited the highest percentage of inhibition or lowest adult emergence index.

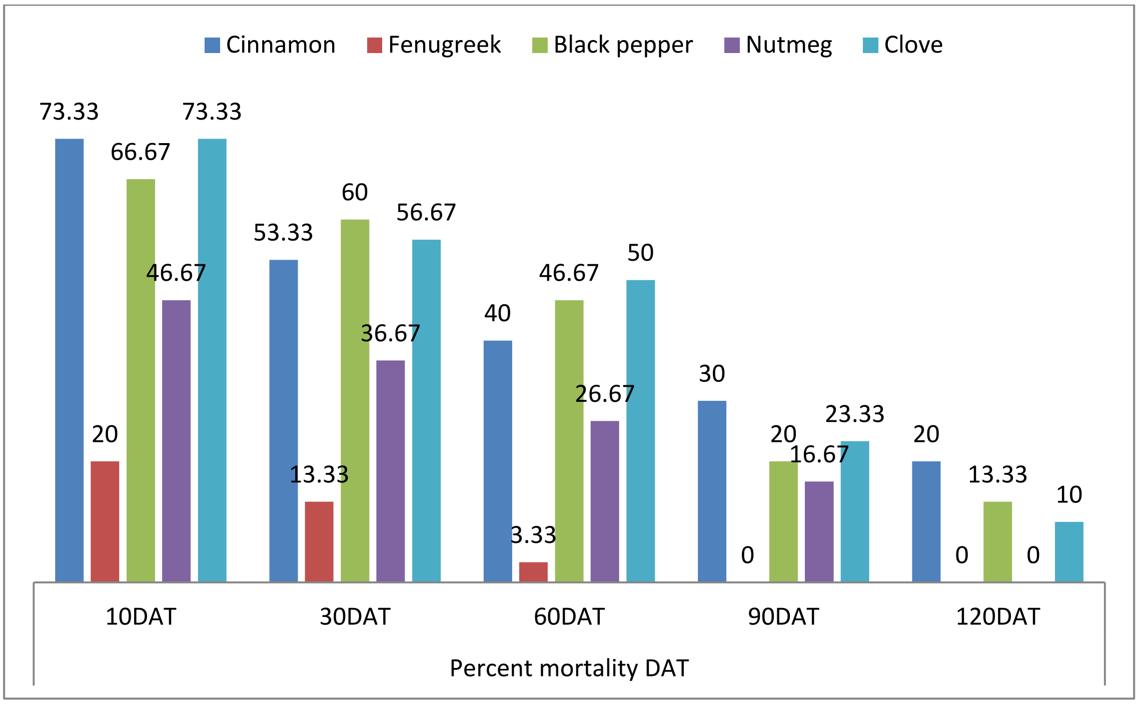
Khan *et al* (2013) tested acetone based leaf extracts of *Murraya exotica*,*Murraya koenigii* and *Nicotina tabacum* against adult stage of *Tribolium castaneum* .Diffrent concentration of 5,10,and15 were employed and knowdown effects were checked after a period of 24, 48, 72, 96, 120, 144 and 168 hours. *N .tabacum* (12.95%) proved more effective than *M.exotica* (9.53%) *M. koengii* (4.31%) respectively.

Waqas *et al* (2004) collected different strains of *Tribolium castaneum* These insects were given dose of 200 ppm, 300ppm and 400ppm concentration of phosphine gas for 1, 3, 5, 7 and 14 days exposure period, gave almost 100% mortality of *T.castaneum.* Higher concentration (300ppm and400ppm) gave 100% mortality by exposure of 7 days.

Zhou *et al* (2006) in the west of Hubei, China reported the reproduction inhibition rates of methanol extracts of *Cypripedium japonicum* (stem and leaves) and *Curculiogo orchioides* against *Tribolium castaneum* 60 days after treatment was over, it was 50%. *Spiraea prunifolia* (flower) extract against adult 30 days after the treatment was over,it was 60%. All these plants extracts caused no weight gain, pupation delay, abnormal pupae and pupae death. *Tribolium castaneum* third instar larvae showed antifeedant activities against these plants.

Zhou Lin (2010) bio assayed population inhibition, due to total alkaloid from *Tripterygium wilfordii* Hook against *Sitophilus zeamais* and *Tribolium castaneum* .In 33 days of treatment with dose of 500mg/kg alkaloid ,mortality of *S. zeamais* was above 90.83% and inhibition rate of the population of F1 generation fed on the poisonous wheat was above 96.70%. Population size was 54.94% with the dose of 250mg/kg alkaloid and reached 98.02% with the dose of 500mg/kg alkaloid.

This study is of great importance and utility in imparting recommendations for protection of major food grain of the world i.e. wheat seed and for saving national loss which will have easy and wide acceptability to the farmer for natural and much more safe eco-friendly nature of spices.



Residual Percent mortality (DAT) of *Tribolium castaneum* (21)

REPELLENCY

In the present investigation *Tribolium castaneum* was found to be repelled by extracts of spices at very low concentrations. Essential oils have low vapour density hence readily volatized. Extracts of cinnamon, clove, nutmeg, black pepper and fenugreek exhibited different trend in repellencey.

20µl acetone extract of a spice (0.25% on v/v basis) was applied evenly on the half of whatman no. 1 filter paper by pipette. The other half of filter paper remains untreated. In the center of petridish lined by rejoined filter paper, 10 adult *T. castaneum* were released.

Insects were first agitated and showed exceptional excitement and within no time tried to move towards non- treated area of filter paper. Number of insects present in treated and untreated area was recorded after one hour and repellency was calculated by ANOVA.

The results of ANOVA revealed that Clove extract was giving significantly highest repellency (66.67%), followed by cinnamon (56.67%).(Table 11-14; Graph 22, 23)

Other spices namely black pepper, nutmeg were performing significantly lower than clove extract, fenugreek extract was least repellent towards *T. castaneum* adults.

The results are in confirmation with findings of Chaubey (2007). He isolated essential oils from *Cuminum cyminum* (Umbelliferae), *Piper nigrum* (pipeaceae) and *Foeniculum vulgare* (Umbelliferae), and tested their repellency against *T. castaneum* at 0.2 % concentration (vol/vol).

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By eminent researches in the field of repellency remarkable work done is mentioned as follows:-

Upadhyay and Jaiswal (2007) said that *Piper nigrum* L at 0.2% concentration against *Tribolium castaneum* (Herbst) showed repellency in filter paper test.

Tripathi *et al* (2009) stated that Clove powder at 1.5g/50g dose showed 100% repellency against adult *T.castaneum*.while mixture of clove and large cardamom showed more repellency and inhibited progeny development in *T.castaneum*.

Shayeshteh and Ashouri (2010) tested four powdered spices, black pepper (*Piper nigum*), chilli pepper (*Capsicum annuum*), cinnamon (*Cinnamomum aromaticum*) and turmeric (*Curcuma longa*), against three stored-product insects, *Rhyzopertha dominica*, *Sitophilus granarius* and *Tribolium castaneum*. Wheat treated with cinnamon powder were most repellent to adult *S.granarius* 92.5% after 1hr,chilli pepper treatment for *T. castaneum* 72.5% after 6hr and black pepper treatment for *R.dominica* 58.75% after24hr.

Chaubey (2012) found that volatile compound of terpene group: alpha-pinene and beta-caryophyllene have ben evaluated for their repellent, acute toxicity and developmential inhibitory activites alone or in binary combination against *Tribolium castaneum*, in repellency assay adults significantly repelled even at 0.025% concentration.

Jilani and Helen (1983) evaluated *Curcuma longa* (turmeric), leaves of *Azadirchata indica* (neem) and leaves of *Trigonella foenum-graecum* (fenugreek) for their repelling action against adults of *T. castaneum*, *Sitophlius granaries* and *Rhizopertha domonica*. Petrolium ether extract was more effective than acetone and ethanol extracts.

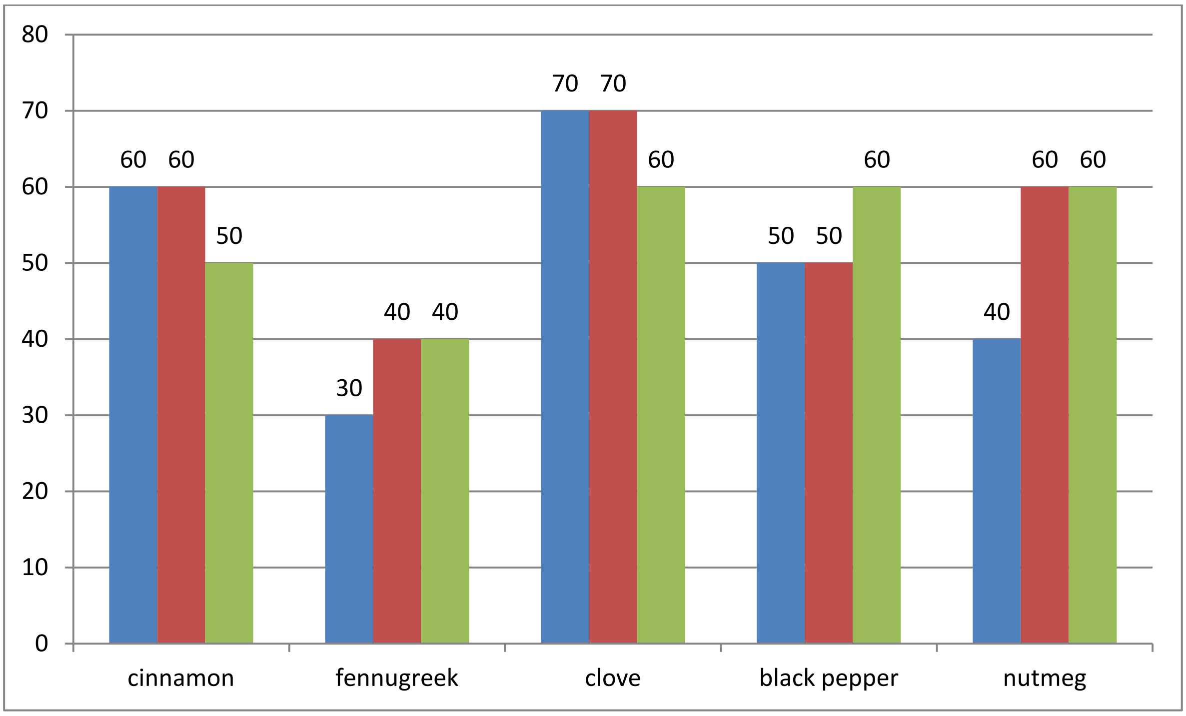
Kim Soonil *et al* (2010) analysed insecticidal activity and repellency of *Origanum vulgaris* L. oil, a spice, against *Tribolium castaneum*. Repellency was observed at $0.03 mg/cm^2$; it depends upon both time and concentration.

Naseem and Khan (2011) determined efficacy of oil extracted from *Eucalyptus camaldulensis* and *Piper nigrum* in various concentrations that is 20,40 and 60% for the suppression control of *Tribolium castaneum* under laboratory conditions.The incubator was maintained at 30± 2^0C and 65± 5%R.H. Higher concentration ,resulted in maximum repellency at maximum exposure period compared with minimum concentration at minimum exposure period. *E. camaldulensis* proved more effective than *P. nigrum* at all the concentrations, at all post treatment interval.

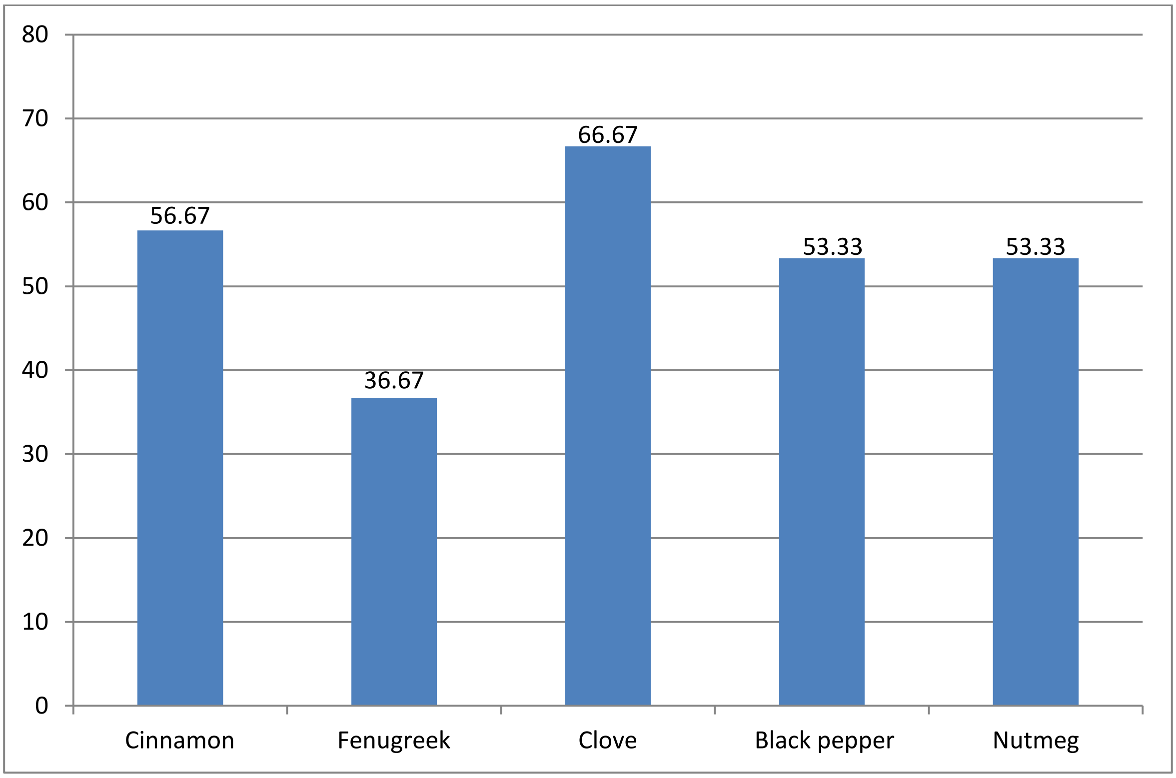
Sami and Shakoori (2014). isolated saponins from tissues (neem). Repellency test for *T.castaneum* revealed that saponins and azardirachtin were able to repel the insect.

Wang *et al* (2009) estimated the effects of monoterpenes of 3-carene, 1, 8-cineole, beta-pinene, terpinene and terpinolene as repellents, against *Tribolium acstaneum* adults. Monoterpene of 1, 8-cineole in 20microLml-1 and beta pinene in 20 microLml-1 exhibited highest percent of repellency.

Hence, the above discussion has proved that the spices extracts used in present investigation are significantly effective for control of the *Tribolium castaneum*. Spices can be useful tool for the people to protect grain from infestation and damage during storage .Today health conscious people in global market is turning towards total organic food devoid of any form of pesticide residue.



Repellency perentage of spice extracts against *T.castaneum* three sets (22)



Statistically calculated repellency percentage of spice extracts against *T. castaneum. (23)*

CHAPTER-7

ABSTRACT

SUMMARY (ABSTRACT)

The first generation insecticides were a few synthetic insecticides based on heavy metals such as lead and arsenic which killed only when eaten, were known as stomach poisons. Second generation insecticides were also artificially synthesised but they were killing insects by mere physical contact, DDT was an established insecticide after Second World War. Insect growth regulators are the chemicals that mimic insect growth hormones preventing natural moulting. They are narrow spectrum insecticides known as third generation insecticides. Insecticidal properties of spice extracts are still unexplored at a greater horizon. The increasing magnitude of hazards of synthetic insecticides has intensified the search for alternate compounds of lower mammalian toxicity and those which are bio-degradable and environmentally safe.

In view of these considerations the assaying for toxicity of *Cinnamomum zeylancium* (cinnamon), *Myristica fragrans* (nutmeg), *Piper nigrum* (black pepper), *Syzygium aromaticum* (clove) and *Trigonella foenum-graecum* (fenugreek) was initiated. Adulticidal and Larvicidal impact as well as their potential of Joint Action against *Tribolium castaneum*, were evaluated. *T. castaneum* causes substantial loss in the storage of grains. Repellency percentage and residual toxicity of spice extracts were also measured simultaneously.

Culture of *T.castaneum* was maintained at 28 ± 2^{0}C and 65±5% RH in the dark. Broken kernel of wheat were taken because it is a secondary pest. Ethanol extract of individual spices were prepared by soxhlet method and then each extract was dried in the hot water bath. Dry extracts were kept at 4^{0}C in the refrigerator for the further use. Acetone was used to make 5% test solution of each extract.

For investigation of adulticidal effect different doses of 10, 15, 20, 30, 40, 50 and 60µl of spice extract were applied to the 20 gms of wheat with the help of micropipette. Triplicate sets were arranged for each observation. Twenty adults of *Tribolium castaneum* were introduced into each test vial and mortality data were recorded 24 hrs post treatment. Experimental work was done in the research laboratory of zoology department, Government College, Kota, Rajasthan.

Mortality data were corrected by Abbott's formula(1925) then probit analysis done by Finney's method(1942) to calculate LD_{50} value of individual spice against *T.castaneum*. The median lethal dose LD_{50} of spice extracts against *T. castaneum* adults were recorded as follows:-

Cinnamomum zeylancium 22.889 µl, *Myristica fragrans* 26.1214 µl *Piper nigrum* 35.7813 µl, *Syzygium aromaticum* 21.0937 µl and *Trigonella foenum-graecum* 26.9711 µl .

Syzygium aromaticaum was found to be most effective against *T.castaneum.*

Syzygium aromaticaum, Myristica fragrans and *Trigonella foenum-graecum* data were non- heterogenous and significant. (p=0.05%)

Data for *Piper nigrum* and *Cinnamomum zeylancium* were found to heterogeneous and non significant.

Cinnamon, Nutmeg, Black pepper, Clove and Fenugreek had insecticidal value against Larvae of *T.castaneum*. Following major observations were reported in the research work undertaken:-

Black pepper was found to be most effective against larvae of *T. castaneum* with the LD_{50} value 9.2209 µl followed by cinnamon, LD_{50} value recorded was 10.8767 µl. LD_{50} value for Clove and Nutmeg were recorded 16.6155µl and 19.2474 µl respectively.

Fenugreek was found to be least effective against larvae of *T.castaneum* with LD_{50} value 176.1916ul µl.

Toxicity of Nutmeg against adults and larvae of *T.castaneum* is almost in similar range i.e. LD_{50} 26.1214 µl and LD_{50} 19.2474 µl respectively.

Mortality data of Clove for adults and larvae of *T.castaneum* were significant and non- heterogeneous. LD_{50} value of adults was 21.0931 µl and for larvae LD_{50} value was16.6155 µl.

Joint action of cinnamon with other spice extracts in 1:1 ratio (v/v) was determined experimentally against adults of *T. castaneum*.

The values of LD_{50} of various combinations of *Cinnamomum zeylancium* with other spices were reported on the basis of experiment conducted in the laboratory. The mixture of *Cinnamomum zeylancium* and *Syzygium aromaticum* extract was most toxic and the combination of *Cinnamomum zeylancium* and *Trigonella foenum-graecum* were recorded as least toxic among all the four combinatios examined.

LD_{50} of various combinations were found in this order- *Cinnamomum zeylancium* + *Syzygium* 35.6252 µl> *Cinnamomum zeylancium* + *Piper nigrum* 37.1495 µl > *Cinnamomum zeylancium* + *Myristica fragrans* 43.8248 µl > *Cinnamomum zeylancium* + *Trigonella foenum-graecum* 118.457 µl.

Their co-toxicity coefficient were in order- Cinnamon + Black pepper > Cinnamon + Clove > Cinnamon + Nutmeg > Cinnamon + Fenugreek, values recorded were- 75.1340 >61.6115 >55.6613 >20.6577.

None of the combination expressed synergism. All the combination results indicates antagonism. More than 100 value of co-toxicity coefficient only gives synergistic results as per the Sun and Johnson's Formula (1960) applied to calculate co toxicity coefficient.

There was absence of the heterogeneity in the present work as per the calculation done by Finney's probit analysis; hence all the data are significant

Residual toxicity of individual spice extract was measured on the basis of mortality data obtained at different DAT. (days after treatment) for test insect *T.castaneum*. Concentration of spice extracts giving 95% mortality were mixed with wheat grain on (v/w) basis.

After 10 days of post treatment, 20gms of treated wheat was taken and 10 adult *T.castaneum* were introduced, experiment was set in triplicate.

Mortality data were recorded after 72hr. period of exposure. Similar readings were taken after 30, 60, 90 and 120 DAT. Mortality data were analysed by ANOVA. Cinnamon remained most effective up to 120 days. Black pepper and Clove were also effective up to 120 days but to a lesser extent. Nutmeg was effective up to 90 days of post treatment. Fenugreek was effective only up to 60 DAT, reflecting only 3.33% mortality. The regression coefficients of all the five compounds are highly significant.

Repellent action of spice extracts against *T. castaneum* adults exhibited importance of these extracts in controlling wheat infestation by this pest. 20µl extract of a spice (0.25% conc on v/v basis) was applied evenly on the half of whatman filter paper no-1 by pipette, the other half of filter paper remains untreated. Petridish was lined by rejoined filter paper, and 10 adult *T. castaneum* were released in the centre. The petridish covered with another one and after an hour insects in treated and untreated area were counted. Experiment was replicated in three sets and control was run parallel.

The results of ANOVA revealed that clove extract was giving significantly highest repellency (66.67%), followed by cinnamon (56.67%). Other spices namely black pepper, nutmeg were performing significantly lower than clove extract. Fenugreek extract exhibited least repellency against *T.castaneum* adults.

We can conclude in the end on the basis of data obtained for adulticidal, larvicidal, residual efficacy and repellency, during the course of experimental research in the laboratory that pattern of toxicity of spice extracts against *T. castaneum* was observed as - clove> cinnamon> black pepper> nutmeg> fenugreek. The spices were highly/significantly effective.

It has long been known that spices posses insecticidal properties. The relative safety of botanical insecticides to man, helps in maintaing their continued use. Present studies is only first step towards exploring possibilities of usefulness of chosen spices in the future on commercial basis, as a source of third generation organic insecticides against *Tribolium castaneum*, a common persistent pest of stored wheat grain or its milled products.

CHAPTER-8

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ANNEXURE-1

RESEARCH PAPERS PUBLISHED

RESEARCH PAPERS PUBLISHED

1. Paper Entitled - Residual toxicity of spice extracts against *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae) has been published in the *Journal of Pestology* vol.xl (2): 27-28. 2016.

2. Lethal impact of some common spices against adults and larvae of *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae) has been published in the *Journal of Pestology* vol.xl (3): 53-54. 2016.

SPICES

BEETLE

LETHAL IMPACT OF SOME COMMON SPICES AGAINST ADULTS AND LARVAE OF *Tribolium castaneum* (HERBST)

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ABSTRACT

Lethality of ethanolic extracts of selected five spices were compared, regardless to the part of the plant they were obtained, against *Tribolium castaneum* adults and larvae. The toxicity level of cinnamon, fenugreek, black pepper, nutmeg and clove differed and reflected by the variation in the account of mortality estimated in the laboratory.

KEY WORDS: **Mortality, *Tribolium castaneum*, Spices, Adult and Larva.**

Introduction

Red flour beetle *Tribolium castaneum* is a serious insect pest of both raw grain and processed grain products. To counter negative effects of synthetic insecticides, essential oils of plant origin, emerged as valuable eco-friendly insecticides. Present study is an effort to observe the impact of ethanolic extracts of five spices-cinnamon, fenugreek, black pepper, nutmeg and clove, against adults and larvae of *T. castaneum*. They feed upon dust of broken grain kernels and spends entire life outside the grain.

Method and Methodology

Tribolium castaneum culture was reared upon broken kernel at 28 ± 2°C and 65 ± 5% relative humidity in the laboratory because it is a secondary pest. Ethanolic extracts of dried spices were prepared by soxhlet method. The required concentrations were prepared by dilution with acetone. In 20g of wheat sample 20 adults insects were introduced in glass tubes, then with the help of micro pipette, different dosage of each spice was applied. For each dose level experiments were set in triplicate. A control was run parallel. After 24 hour of exposure period mortality data were recorded. In 20g of wheat sample 10 larvae were introduced to measure larval mortality. Data were collected and analysed by Probit analysis (Finney 1981) to find out LD_{50} values of each spice against adults and larvae of *T. castaneum*.

Results and Discussion

Present studies revealed that clove extract was most effective with LD_{50} value 21.0937 ml against adults of *Tribolium castaneum* and data were found to be non heterogenous and significant. Cinnamon, Nutmeg and Fenugreek were also effective as insecticide against adults of *Tribolium castaneum*. Extract of black pepper with LD_{50} value 35.7813 ml against adults of *Tribolium castaneum* found to be heterogenous and non significant (*Table-1*).

Similarly data revealed that extract of clove with median lethal dose of LD_{50} value 16.6155ml found to be effective against larvae of *Tribolum castaneum* and data were non hetergenous and significant. LD_{50} value of Nutmeg obtained 19.244ml found to be significant. But Ld_{50} value for cinnamon was 10.87ml and for black pepper LD_{50} value recorded 9.220ml but data were found to be heterogenous and non significant. Fenugreek found to be least effective against larvae of *Tribolium castaneum* (*Table-2*).

Extracts of plant influence, insect pest of stored grain, in adult state and in larval form or at any other developmental stages as follows:

Osman (2011) said that larvae of *Tribolium*

Table 1

Toxicity of spices against Adults of *Tribolium castaneum* (Herbst)

Sr. No.	Spices	Heterogeneity x^2 (4)	Regression equation	LD_{50} (ul)/20 g.wheat	S_m	Fiducial limits
1	Cinnamon	23.1687	y= 3.8005± 0.50084	22.889	1.800	68.4924 to 4.7559
2	Nutmeg	4.6251	1.9658 ± 2.1413	26.1214	0.2526	72.7942 to 29.7195
3	Black pepper	18.9133	4.6198 ± 0.2447	35.7813	0.9228	19.5542 to 17.8105
4	Clove	3.8335	1.828 ± 2.8817	21.0937	0.288	18.8291 to 23.6856
5	Fenugreek	9.2102	0.9325 ± 2.8426	26.9711	0.276	24.2251 to 30.0302

Y = probit kill, x = log dose, S_m = Standard error, Significance P = 0.05.

Table 2

Toxicity of spices against Larvae of *Tribolium castaneum* (Herbst)

Sr. No.	Spices	Heterogeneity x^2 (4)	Regression equation	LD_{50} (µl)/20 g.wheat	S_m	Fiducial limits
1	Cinnamon	60.94	y =59.222 ± 52.302	10.8767	222.06	11.031 to 11.2160
2	Nutmeg	0.7861	0.9926 ± 3.1200	19.2474	0.3856	16.599 to 22.3183
3	Black pepper	37.255	6.2626 ± 1.3086	9.2209	2.370	10.2112 to 17.9368
4	Clove	4.5393	1.4376 ± 2.9187	16.6155	0.3579	14.1880 to 19.4583
5	Fenugreek	23.00	5.9367 ± 0.41707	176.1916	1.606	11.6050 to 59.7645

Y = probit kill, x = log dose, S_m = Standard error, Significance P = 0.05.

castaneum are sensitive against ethyl acetate extract of stem-bark of *Manakara zapota* at 72 hr exposure period. LD_{60} for sixth instar larvae is reported 498.7 mg/cm^2. Suthisut (2011) reported camphene, camphore, 1-8 cineol, alpha humilene, alpha pinene, beta pinene and terpins were present in extracts of *Alpinia conchigera, Zingiber zerumbet and Curcuma zedoaria. Tribolium castaneum* is sensitive to 30-58 mg/gm. They have anti feedent properties. Kim (2012) *Organo-vulgare* has p-cymene, gama terpine, thymol, linalool etc is a active fumigant against *Tribolium castaneum*. Linolool is an active nerve toxin. Liska (2012) determined that essential oils from aromatic plants contain bio-active molecules 1-8 cinoel, eugenol affected mitigation of number of F-1 generation of *Tribolium castaneum* Sami and Shakoori (2014) isolated saponins from neem,which inihibit beta-1, 4 endoglucase enzyme activity in *Tribolium castaneum*. Neem is a potent repellent.

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TOXICITY | SPICE EXTRACTS

RESIDUAL TOXICITY OF SPICE EXTRACTS AGAINST *Tribolium castaneum* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT

Essential oils of plant origin have insecticidal value and are eco-friendly alternative of conventional insecticides. Extracts of five selected spices were put to test against *Tribolium castaneum*, a major stored grain pest. For each spice-cinnamon, fenugreek, black pepper, nutmeg and clove etc, their residual toxicity was measured, 10, 30, 60, 90 & 120 days post treatment. Samples were tested in triplicate for 72 hr exposure periods and mortality in adult *T. castaneum* was assessed. Significant effectiveness was reported in cinnamon, black pepper, clove & nutmeg. Fenugreek was least effective.

KEY WORDS: **Spice extracts, Residual, Efficacy, *Tribolium castaneum*.**

Introduction

Safe storage of food grain under hygienic conditions is a sensitive problem, due to infestation by *Tribolium castaneum*, a cosmopolitan major pest of stored grain products. According to FAO about 10 to 25% loss of wheat is estimated annually, by insect pests and rodents all over the world. Great economic impact has been observed, due to reduction in nutritive value, quality of wheat grain, noxious smell and debris created by its populations.

Commercially available synthetic pesticides and their unchecked use and toxic residues, leads to health hazards and environmental pollution. Essential oils extracted from plants showed contact ,fumigant toxicity, repellency, antifeedent activites and also affected some biological parameters such as growth rate ,life span and reproduction insects. In the ethanol extract of selected spices – cinnamon, fenugreek, black pepper, nutmeg and clove, contains naturally occurring volatile compounds monoterpines and their derivatives, phenols esters, aldehydes alcohols and glycosides, having insecticidal value. Suzuki (2000) suggested clove, nutmeg and red pepper extracts have glycolipids and clove may contain glycosphingolipids and Nakatani (2003) mentioned that in nutmeg and clove monoterpines are present and Phenolic amides are present in black pepper and chilli pepper.

Material and Methods

Extracts of dried spices were prepared in ethanol by soxhlet and with the help of acetone, desired concentration of solutions were made out of stock solution. Culture of *Tribolium castaneum* was maintained at 28 ± $_0$C and 70±5% relative humidity. Beetles were reared upon broken wheat kernels. In the air tight jars, 250 gm of sun dried (broken kernel) wheat was kept and measured volume of dose(v/w) of each spice was added. After 10 days of post treatment, 20 gm of treated wheat was taken and 10 insects were introduced into it. Mortality was recorded after 72 hr period of exposure. Experiments were done in triplicate at each dose level. Mortality data were recorded for 10, 30, 60, 90, 120 days and data and were analysed by ANOVA.

Results and Discussion

The results of linear regression analysis revealed that, the regression co-efficients of all the five compounds are highly significant. Thus the corresponding regression lines fitted can be used for estimation of mortility percentage (Y) with respect to days interval (d). Residual efficacy of spices were reported in sequence: Cinnamon > Black pepper > Clove > Nutmeg > Fenugreek.

Present studies is in agreement with some other earlier studies as follows:

Table 1

Insect No 30 Adult **72 HR**

Compund	10 Days	30 Days	60 Days	90 Days	120 Days
Cinnamon	22	16	12	9	6
Fenugreek	6	4	1	0	0
Black pepper	20	18	14	6	4
Nutmeg	14	11	8	5	0
Clove	22	17	15	7	3
Control	Nil	Nil	Nil	Nil	Nil

Table 2

Per cent Mortality	Days				
Compund	10 Days	30 Days	60 Days	90 Days	120 Days
Cinnamon	73.33	53.33	40.00	30.00	20.00
Fenugreek	20.00	13.33	3.33	0.00	0.00
Black pepper	66.67	60.00	46.67	20.00	13.33
Nutmeg	46.67	36.67	26.67	16.67	0.00
E Clove	73.33	56.67	50.00	23.33	10.00

Zhou *et al.* (2006) evaluated that after 60 days of treatment of *Tribolium castaneum* with methnol extract of *Curculigo orchinoides* recorded 82.52% mortality. (Sahaf *et al.* 2008) elucidated that at concentration(3 microl/ml acetone) of *C.copticum* and *Vpseudo-negundo* oils causes 87.50 and 100% repellency on adult *Tribolium castaneum.* Persistence or half-life of the *C.copticum* was 36.24 days. After six months of treatment with extracts (2%) against *Tribolium castaneum*, effectiveness was reported in following order for tumeric > neem powder > garlic powder > garlic clove. (Sharma *et al*.2009). Clove powder at 1.5 g/50g of stored grain showed 100% repellency against *Tribolium castaneum* and 5g/100g resulted in inhibition of progeny production (Tripathi, 2009). Hexane extract of spices at 1.5% concentration and 1000ppm dose, by five days of application gave 92% mortality with clove oil, 51.63% with nutmeg and cinnamon extract gave 66.6% mortality against *Tribolium castaneum.* The spices offered protection for 9 months without affecting seed germination. (Devi and Devi 2013).

The advantage of using spices as grain protectant is that it adds fragrance to grains. Spices popularly consumed all over the world and above findings support its insecticidal value along with residual efficacy, oils can be extracted in pure form ,are volatile can be used as potential fumigants.

Table 3

Regression Results

Compund	Inter cept	reg. Coeff.	S.E.	P - value		R Square
Cinnamon	71.658	-0.457	0.061	0.00499	**	0.949
Fenugreek	18.926	-0.187	0.043	0.02303	**	0.861
Black pepper	73.959	-0.526	0.057	0.00263	**	0.966
Nutmeg	50.406	-0.404	0.024	0.00045	**	0.990
Clove	78.020	-0.570	0.054	0.00181	**	0.974

***Highly Significant*

Table 4

Compund	Regression Equations
Cinnamon	Y = 71.658 - 0.457 d
Fenugreek	Y = 18.926 - 0.187 d
Black pepper	Y = 73.959 - 0.526 d
Nutmeg	Y = 50.406 - 0.404 d
Clove	Y = 78.020 - 0.570 d

Y = Mortality percentage d= Days interval.

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