RESEARCH PROGRAMME

Susceptibility of *Tribolium castenum* to essential oils of citrus fruit peel, castor oil, eucalyptus oil, and mentha oil

AGR690 DISSERTATION –II SYNOPSIS REPORT

Submitted to the

LOVELY PROFESSIONAL UNIVERSITY,
PHAGWARA, PUNJAB, INDIA

In partial fulfilment of the requirements for the award of degree of

MASTER OF SCIENCE
IN
(ENTOMOLOGY)
BY

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LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

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Nov, 2017

CERTIFICATE

I hereby declare that the Synopsis entitled **"Susceptibility of *Tribolium castenum* to essential oils of citrus fruit peel, castor oil, eucalyptus oil, and mentha oil**" is an authentic record of my work and carried out at Lovely Professional University as requirement for the degree of **Master of Science** in the discipline of **Entomology**, under the guidance of Dr Ravinder Nath, Asst. Professor,,School of Agriculture (Entomology) and no part of this Synopsis has been submitted for any other degree and diploma.

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M.Sc. Entomology

CERTIFICATE

This is to certify that synopsis titled **"Susceptibility of *Tribolium castenum* to essential oils of citrus fruit peel, castor oil, eucalyptus oil, and mentha oil"** submitted in partial fulfilment of the requirement for the award of degree of **Master of Science** in the discipline of **Entomology,** is a research work carried out by **Gurlal Singh (Registration No. 11615779)** under my supervision and that no part of this synopsis has been submitted for any other degree or diploma.

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Introduction:

Indian economy is heavily dependent on agriculture sector as more than 60% of its population is dependent on it . Production of food grain in India has raised at rate of recorded1.20% ot of around 260 MMT in year 2014-2015(1st is rice at 104 MMT and 2nd is wheat at 96 MMT) . Due to increase in the production of cereals as well as pulses, the demand and need of storage houses have increased significantly.

In India every year 12 to 16 MTT of food grains have been lost due to poor storage , harvesting and handling practices. According to the World Bank, this much amount can feed the one third of poor population of entire country annually. These losses costs upto 50,000 crores reported by Singh, 2010).. The losses during storage are quantity losses as well as quality losses.. Around 500 insect spp. Are reported as stored grain pests and 100 out of them cause economic losses.

The major insect pests of stored grain are:-

- Red flour beetle
- Rice moth
- Rice weevil
- Khapra beetle
- Pulse beetle
- Indian meal moth
- Lesser Grain Borer
- Potato Tuber Moth
- Cigarette beetle
- Grain weevil
- Warehouse moth

Insects attack the grains during maturity and harvesting in the field, during handling or storage.

Management strategies for stored grain insect pests

Sanitation

Insect infestations mostly developed from pests that are already present in or around farms and storage warehouses . A good sanitation program can reduce the chances of having severe insect infestations:-

- Grain handling equipment must be kept clean. Avoid using contaminated equipment.
- Even Transport machinery is properly cleaned of all old grains.
- Moisture content in grains must be 20-27% at the time of harvesting which is brought down to 12-14% before storing them.

Curative Measures

- Use of low (13-20*C) and high (>35*C) temperatures
- Mixing activated clay or inert dust.
- Use of controlled atmosphere. (increasing Carbon dioxide and Decreasing Oxygen levels)

Fumigation

Several factors assures successful fumigation:-
• Grains are to be leveled in the container for penetration of fumigant.
• All the cracks and holes must be clogged and filled in order to prevent leakage of poisonous fumigant gas

Types Of Fumigaton:

- Shed
- Probe
- Bulk

CHEMICAL PROTECTION

1. By **prophylactic control** measures, For crawling insect spraying effective prophylactic chemicals such as:
   Deltamethrin 2.5% WP at recommended dose
   Malathion 50% EC at recommended dose

2. By **curative control** measures, the insects are killed by using fumigants like:-
   Aluminum phosphide (phosphine) at recommended dose
   Methyl bromide at recommended dose

Use of Plant Extracts as insecticides.

Even though the chemical insecticides are still most commonly used for storage pest control all over the world, lots of research work has been done in recent decades about using plant extracts like neem extracts to use as botanical insecticide. The main reason of this sudden shift is due to:-

- Development of resistance in storage insects against chemical insecticide like Malathion Dichlorovos etc.
- Also botanical insecticides are much safer and has no residual effects on the stored grains

From early times Inert dusts and dry soils were used to dehydrate the insects in order to control them(Levinson et al 1989). One way or other, this technique is still in practice. The use of harmful conventional insecticide like methyl bromide is reduced for fumigation because it's gas is producing holes in ozone layer (Zhang 2004). Insecticides or botanicals derived from plants are safer for the environment and their extract and ois are highly used and researched for their insecticidal properties.(Devi and Devi 2011). Some plant extracts like neem has shown their repelling and mortality producing abilities against various insect species(Clemente 2003) and some of these botanicals can be used to controle storage infections without any deterioration of grain quality (Dubey et al. 2010).

Similarly, in this study we will be trying to examine the effect of few plant extracts on the most common storage insect of India, ***Triboium castenum.***

Objectives of Study:

1. Screening of Red flour beetle for insecticidal properties of four plants from Punjab.
2. Selective bioassay – mortality and lethal concentration (LC_{50}) of certain plant extracts under in vitro conditions.
3. Probit analysis of the Treatments

Plant Extracts to be used:-

- Citrus peel
- Caster leaves (Ricinus communis)
- Menthol leaves (Mentha longifolia)
- Eucalyptus leaves (Eucalyptus globulus)

Test Subject's Introduction:

Red Flour Beetle :- *Tribolium castaneum*

Order : Coleoptera

Distribution and status: All over India And Worldwide

Host :

Wheat-flour, dry fruits, pulses and prepared cereal foods, such as cornflakes.

Description and characters:

Shape and Size: Small, flat, slender, elongated

Colour: reddish brown beetles

Length : ⅛ inch

Identifying keys: Smooth bodies, punctures on thorax and elytra with small longitudinal ridges.

Nature of Damage:

As it is the secondary pest, it cannot feed on the whole grain. So it infest on food which is broken and previously infested stock. Heavy infestation produce foul smell and turn grains into powder. It contaminates the food with its extrecta, dead larvae and pupas making it unfit to eat or sell.

Life history: They lay eggs on grain and both larval stages and adults feed on it. Pupation occurs within the grain bulk. Their life cycle depends on temperatures,(6 weeks in summers and longer in winters). Up to 10 generations per year is possible and adults are can live up to 1 year.

MATERIALS AND METHODS

Rearing of insects

A sample of rice grains infested with Red flour Beetle was collected from the FCI warehouse situated in Bariwala, Sri Muktsar Sahib. In order to get homogenous population, adults of *T. castaneum* were released in glass and plastic jars and kept in incubator at 30°C and 60% Rh. Wheat grains and flour was sterilized at 60°C before use and then was provided as culture medium. Mouth of jars was cover ed with muslin cloth with rubber bands to avoid entry of any other pest from surrounding (Zia *et al.*, 2011). Beetles were kept in the culture media for 6 days for oviposition and then removed with sieve and brush. The flour having their eggs was again put back in the jars.

Population hatched from these eggs after a month was a homogeneous culture and will be used in the experiment. Wheat meal is exchanged after every 15 Days.

Extraction of essential oil

The peels of citrus cultivars were collected from University Juice Shops while leaves of menthol, and eucalyptus were collected from the field. The caster leaves were collected from roadside as it is the major weed in Jalandher area. Once collected, materials were initially washed with tap water then dried in shade for over a month and thereafter grinded to form a powder Grinding of plant material was done in electric grinder. A total of 50 g fine powder of each plant was allowed to mix with 100 ml of acetone. The Mechanical shaker, adjusted at 120 rpm was used to shake all the ingredients for 24 hours. After this period, the initial filtrate was obtained by using filter paper. Solvent was then allowed to evaporate using waterbath to get pure plant extracts at 60*C for about 2 hrs. These extracts were then put into glass vialsand kept in deep freeze for preservation at -23*C temperature.

Bioassay; In Vitro

Each experiment will be carried out using a Petri dish and same for filter paper. Different concentrations of oil (2, 5, and 10%) will be applied on the filter paper and it wil be allowed to dry. Twenty adults of Red flour beetle will be introduced in each covered Petriplate having same amount of food (5gm). Mortality rate of adults was recorded after 24hrs and then 7 days of exposure to treatments. Similer method was used for larval stage but mortality reading was taken after 24,and 72 h of exposure to treatments following Upadhyay and Gayatri (2007).

The dishes were then incubated without light. Each petriplate was observed for data after every 24hrs

Data analysis;

Mortality: Death Rate of Individuals after treatment:

Calculation:

Mortality % = Number of Insects Died/ Number of insects Treated X 100

LC50:
It is the concentration of the toxic substance required to kill the 50% of target population

Probit Analysis

A probit-mortality curve will be drawn for probit analysis. After Finney (1967), the LD50 of each test sample will be calculated statistically through the respective probit regression equation. Probit is statistical analysis used in toxicological studies to find out the relative toxicity of Toxins to living organisms. As the response is binomial and the relationship between the response and the concentrations is sigmoid. This analysis transforms the curve from sigmoid to linear and then regression is run on the relation.

After that output of the probit analysis is used for comparison of chemical concentration needed to produce the similer response in target.

Review of Literature

Seyed et al 2016 reported about Susceptibility of Red floor beetle larvae to oil extract of Citrus peels and diethyl maleate(synergist). Combination of Citrus peel with the synergist DEM after 24 and 48 h of exposure reduced the LC50 values as compared to citrus peel extract alone. With the increase in the concentration of Citrus extract and exposure time , larval mortality also increased.

H Bilal et al 2015 worked on Insecticidal as well as Repellent Potential of Citrus Essential Oils Against Red floor beetle . Oils extract of citrus seeds were tested on different growth stages of *beetle.* On adults LC50 of different citrus seed oils were 5.5%, 7.7%, 10.8% and 11.8% for rough lemon, freutrall early, kinnow and red blood orange respectively. Citrus reticulate was on top in killing both adults and grubs.

Mohd. Sagheer et al (2014) tested the Repellent effects of acetone based medicinal plants extracts like Funnel Flower, Clove (and Ajvain against Rust-Red Flour Beetle,. Analysis was done by half-filter paper disc and at various concentrations was done. Trials showed significant repellent effects as *Ajvain* (76.7%) followed by Clove 76.5% and funnel flower. Hence making ajvain and clove equally effective.

Jaya et al 2012 studied the Insecticidal properties of *Ageratum conyzoides* L *a*nd Pignut essential oils to kill storage grain pest Red floor beetle in both **In Vitro** and **In Vivo** conditions.
Extracted oils of Pignut and *A. conyzoides* resulted in 100 % death rate of the beetle at 250 ppm. The extracted oils of both plants had no harmful effect on seed germination and development of seedling thus showing no phytotoxicity in them

M Nadeem et al 2012 came out with the report on Management of Tribolium castaneum (Hbst.) Using Neem and Tumha leaves. Different doses of Neem and Tummha. fruit ethanol extracts were tested on Red flour Beetle. The effect of neem seed extract at tested concentrations against the test insect was dose dependent; as the 64%, 55%, and 36% where mortality recorded at 10%, 7.5%, and 2.5% concentrations, respectively.

S Ashouri et al (2009) studied the tnsect killing capacity of black pepper and red pepper powder on adults of Lesser grain borer and Grain Weevil
The powders of both plants mixed with wheat grains as direct mixtures at different doses of black pepper as well as for red pepper to determine mortality of Fl generation. Results stated that black pepper at 0.5% leads to 100% mortality of *Grain weevil* within a week, also Lesser grain Borer adults died completely @ 5% level after 14DAT.

Mohd Esmail et al 2008 researched on Effect of Neem, Basil and Chilli Powders on the Larval Instar of the *Tribolium castaneum (Herbst*). Larvae mortality, anti feedant, number of (3rd) larvae were used as parameters of efficacy. The sorghum seeds were used as food in this study. Each petriplate was treated with the powder of each product at concentrations: 5% and 10% (w/w). 20 larvae of *T. castaneum* were usedin each treatment. Observation was done every 48 hrs for 2 weeks for mortality and at the end of the two weeks for antifeedant property was studied. Result showed that both neem and chilli at the concentration of 5% and 10% were better than basil concentrations. Neem at 5% gave the best results.

Rahman 2007 worked on Efficacy of commonly used insecticide on the red flour beetle. Wheat samples containing beetles were collected from 9 locations of Bangladesh and collected insects were reared. Strains of the red flour beetle, *Tribolium castaneum* collected from eight storagehouses and a silo, were tested for susceptibility to malathion, dichlorvos, fenitrothion, pirimiphos-methyl and phosphine. The results of dose tests of the red flour beetle showed that adults were resistant against all test insecticides..
Ghatail and Tangail beetle population showed highest degree of resistance against **malathion**, Sheddirganj populations against **dichlorvos** and beetles from tangail against **phosphine**.

By M.F Khan and S. M Ahmed 2002 worked on the Toxicity of Neem Fruit Extract and Cypermethrin Against red flour beetle and *Papilio demoleus* Linn. *Tribolium confusum* adults were treated to Neem fruit extract (NFE) and cypermethrin fumigation while *Papalio demoleus.* 2nd instar larvae were topically treated with the selected dose. The mortality counts after an exposure of 24 hours. The LD50 values of Neem and cypermethrin against beetle was 222.887 µg/cm2 and 0.258 µg/cm2, respectively, Also,The log probit regression equation was calculated.

Adil Shareif Mohamed Khair 2002 studied the Biology of the Red-flour beetle in different cereal flours.

Chemical analysis of the four cereal flours was carried out at the Biochemistry Laboratory. The objectives were to study the biology of the red flour beetle., when reared on flours of sorghum, wheat, millet and rice.
Life-cycle studies of beetle at laboratory conditions shown that, egg incubation, larval, pupal and total developmental period in sorghum flour were significantly different. The shortest durations was recorded in June (higher temp.) and the longest in September and October (lower temperature).
Millet turned out to be the most favourable and rice was the least favourable for the development of beetle. Flour had a significant effect on the body weight, the mean weight of larva, pupa and adult were less for insects in rice than on wheat.

Generally, the study showed the Red floor beetle can survive in all four types of the cereal flours but wheat is most succeptible to it.

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