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Title of the Paper: Efficacy of Essential Oil of *Eucalyptus Maculata* on the Protein Profile of Rice Weevil, *Sitophilus Oryzae*(Coleoptera: Curculionidae)

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Abstract:

In developing countries like India synthetic pesticides are used to prevent huge post –harvest loss of food grains due to infestation by stored grain pest like rice weevil, *Sitophilus oryzae* . Different environmental and health concerns caused due to use of synthetic pesticides arose the need for use of biopesticides. *Eucalyptus* oil from different species has been shown to have insect repellency, antifungicidal activity, antimicrobial and antibacterial activity. In the present paper the mortality rate and change in protein profile of *S. oryzae* through use of essential oil of *Eucalyptus maculata* was investigated. The laboratory cultured *S. oryzae* at adult stage was exposed to various concentrations of the oil under laboratory condition at 27°C. The ability of the plant oil to protect rice grains was assessed in terms of mortality and change in protein profile of rice weevil at different concentrations. The result affirmed the efficacy of essential oil of *Eucalyptus maculata* against control of rice weevil showing good toxicity. It showed that essential oil of *Eucalyptus maculata* can be used as biopesticide instead of synthetic pesticides.

Key words: *Sitophilus oryzae*, *Eucalyptus* oil, toxicity, biopesticide

INTRODUCTION

In many countries of the world, agriculture still serve as the main source of livelihood. There is huge post harvest loss of food grains in the developing countries of the world due to insufficient and inadequate storage facility. Rice and wheat are heavily infested by stored grain pests. Rice is a carbohydrate rich food. It is the staple food to man in many countries. It is one of the food often infested by pests both in fields and in storage conditions. It is infected by various pests like *Sitotroga cerealella, Sitophilus oryzae, Sitophilus granarius, Rhyzopertha dominica, Oryzaephilus Mercator, Scirpophaga incertula, etc.*

The rice weevil, *Sitophilus oryzae* Linnaeus 1763(Coleoptera: Curculionidae) is serious pest of stored grains found all over the world. It is the most destructive stored grain pests which also feed upon wheat, maize, barley, jowar, etc. It causes huge economic losses in agriculture dependent countries like India by destroying large quantities of stored grains, legumes, etc. They infest the grains and make them unsuitable for consumption.

Adults rice weevils are 3 – 4mm long reddish brown to black in colour and have cylindrical body and head with long slender snout. They have four light coloured patches in elytra. Adult female

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rice weevil feed on the rice grain and bores about 1mm deep hole in kernel with her snout and lay a single oval white egg per day per grain. It lays about 150-400 eggs during its whole life. Incubation period is 4-9 days. After hatching, larva comes out and are legless grubs 5mm long. Larvae have horny brown head, white body and lives inside the kernel permanentlyand feeds on the endosperm within the kernel thus removing the proteins and vitamins of the grain. Larval period is 19 – 35 days. Pupation occurs inside the grain. They are yellowish white in colour. Pupation period is 5 – 7 days after which the adult beetle comes out of rice kernel by chewing its way out making a hole in the grain. Adult life span is 6 – 8 months to 2 years, 5 to 6 generations per year. Many synthetic pesticides have been formulated and used to control the infestation by rice weevils in storage conditions. . Chemical pesticides like organochlorines (lindane), organophosphates (malathion), carbamates (carbaryl), pyrethroids (deltamethrin) and fumigants such as methylbromide, phosphine and sulfuryl fluoride are used to control the infestation. But the continuous use of synthetic pesticides can cause negative impact on the health of man and animals. These insectides produced synthetically when are ingested indirectly through food can cause harm to man and animals including birth defects, damage to the nervous system; disruption of hormones and endocrine systems; respiratory disorders; skin and eye irritations; and various types of cancers. Therefore much research has been going on to find alternative source of using synthetic pesticides. The utilization of plant products to protect field crops and stored grains against insect attack has been done for a long time. Biopesticides or biological pesticides offer an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to humanhealth. The most commonly used biopesticides are living organisms, which are pathogenic for the pest of interest. At the end of 2001, there were approximately 195 registered biopesticide active ingredients and 780 products. **Biochemical pesticides** are naturally occurring substances that control pests by non-toxic mechanisms. Biochemical pesticides include substances, such as insect sex pheromones, that interfere with mating, as well as various scented plant extracts like essential oils that attract insect pests to traps.

Essential oils are concentrated volatile aromatic compounds extracted from various parts of stem, leaves, barks, roots of plants. These oils have anti-microbial effects, therapeutic effects and are often use in medicine, food and cosmetics and as insecticide by people for a long time. Plants use them to fight infection, contain hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal, viral, and animal foes. These oils are easy to extract, biodegradable and do not persist in soil and water. These oils possess a wide range of desirable properties for pest management and is regarded as non-toxic to humans. Among these oils, cinnamon oil, horseradish oil, eucalyptus oil have shown potent insecticidal effect to control pests.

It is found that eucalyptus essential oil is toxic to microbes including bacteria and fungi. Eucalyptus essential oil could therefore have a role to play in the protection of crops against mould, mildew and wood rot fungi. In addition, when applied in a vapour form, eucalyptus essential oil has potential to manage weeds, especially as its toxicity appears to be species-specific. Since eucalyptus oils are particularly strong when in vapour form,they could also be used commercially as a fumigant (gaseous pesticide) for stored products and impregnated into packaging to prevent insect infestation. Eucalyptus oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones; however, the exact composition and proportion of which varies with

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species (Brooker and Kleinig, 2006). The pesticidal activity of eucalyptus oils has been due to the components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, pcymene, eucamalol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene, and aromadendrene (Watanabe et al., 1993; Li et al., 1995, 1996; Cimanga et al., 2002; Duke, 2004; Batish et al., 2006; Su et al., 2006; Liu et al., 2008). The various components of eucalyptus essential oil act synergistically (and not additively) to bring the overall pesticidal activity (Cimanga et al., 2002). Among the various components of eucalyptus oil, 1,8-cineole is the most important one and, in fact, a characteristic compound of the genus Eucalyptus, and is largely responsible for a variety of its pesticidal properties (Duke, 2004). Thus Eucalyptus essential oil could have a large role in the control of pests like in rice weevil and provide an alternative to chemical pesticides. The objective of this study was to investigate the mortality rate and change in protein profile of adult rice weevil, *S. oryzae* through use of essential oil of *Eucalyptus maculata* . The species was chosen due to its world-wide pest status and the plant oil was selected for the experiment based on prior knowledge of its insecticidal activities against other insect pests.

MATERIALS AND METHODS

TEST INSECTS

Adult rice weevils were obtained from infested grains in a household of Gossaigaon area of Kokrajhar district, Assam. The culturing was done on whole grains of local rice for *Sitophilus oryzae* at room temperature in the laboratory of Zoology department, Gauhati University. About 250g of the healthy local rice grains was weighed and poured into the plastic container containing the infested rice with rice weevil proper ventilation was ensured and confinement of the insects in the container was taken care of to avoid mortality. After culturing, freshly emerge adults *Sitophilus oryzae* from the laboratory cultures were taken out and used for the experiment.

ESSENTIAL OIL

The essential oil was extracted from fresh leaves of *Eucalyptus maculata* collected from Gauhati University campus and hydro distilled in all glass Clevenger apparatus, which was made of a glass distillation flask with a thick round neck condenser and graduated measuring tube with a collecting tap at the end. In carrying out the distillation process. 120g of *Eucalyptus maculate* leaves was weighed and cut into small pieces and put into a distillation flask and 300ml of water added. The apparatus was set up using clamp on a heating mantle and heated for a period of 5 hours at 40˚to 50˚C. The volatile oil deposited on water was then collected through the attached graduated measuring tube by opening the tap.Four concentrations of *Eucalyptus o*il was prepared by using Acetone as solvent-3000ppm, 2000ppm, 1000ppm, 100ppm. For the control, 1.5ml of Acetone was used during each different concentrations of the essential oil used during the experiment.

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BIOASSAY

PROTEIN ESTIMATION

Protein estimation was done using Lowry et al(1971) method. Freshly emerged adult *Sitophilus oryzae* from the cultures in the laboratory were used for the experiment. At first 6 filter papers were placed in 6 petridishes in three of which labeled as control I,II, III where 1.5ml of Acetone was poured using 1µl micropipette. In other 3 petridishes labeled treatment I, II, III and 1.5ml of concerned concentration of essential oil prepared was poured in the respective filter papers and then allowed to air dry for about 10 minutes. Then 20 adult rice weevil were introduced into the petri-dishes and then covered properly to prevent escaped of any rice weevil. The petri-dishes were then observed after interval of 1minute, 30min, 1 hour, 3 hours, 6 hours, 24 hours respectively. Mortality rates were recorded at every concentration. Adults were assumed dead when probed with sharp needle. After 24hours of treatment and recording the observations, the insects in each labeled petridishes were weighted using electric weighting balance. Rice weevils in respective petridishes were then crushed in pastle-mortar in 5ml of phosphate buffer and kept in centrifuge tubes. In labelled upendroff tubes, the homogenates were centrifuged in cooling centrifuged at 10000rpm for 10min and supernatant was taken and pellets were discarded. The above extraction was repeated for 3 more times.The combined supernatants were collected in 6 test tubes and 5ml of phosphate buffer was added to each to make the volume upto 25ml. From above 1ml of each extract was taken in another 6 test tubes and 1ml of 20% TCA was added to each extracts. After half an hour, the above mixtures were again centrifuged at 10000 rpm for 10 min and after discarding the supernatents, pellets were washed in acetone and then gain centrifuged at 10000 rpm for 10min. After discarding the supernatant, pellets were dissolved in 0.1N sodium hydroxide and mixed well to dissolve pellets properly. 1ml of above solutions were taken and 5ml of freshly prepared alkaline $CuSO_4$ solution was added to each, mixed well and kept 10min. Then 0.5ml of Folins reagent was added and kept for 30min after shaking to mix the contents in the test tubes to allow appearance of colour . After appearance of colour, absorbancy was taken at 660nm using digital spectrophotometer and noted down for comparison with standard BSA curve to know the protein concentration in the samples.

RESULTS

The change in protein profile was examined and was found that absorbancy increases with increase in the concentrations of the essential oil(Table 2). This shows that there is increase in the concentration of protein with increasing concentration of the oil indicating change in the protein profile of the pest. The results obtained at different concentrations of oil treatment and control used showed that with increasing concentrations of the essential oil, the insecticidal activity also increases. The highest toxicity was seen in 3000ppm concentration of *Eucalyptus* oil.

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NO. OF *SITOPHILUS ORYZAE* USED	PETRIPLATE	CONC. AND AMT. OF EUCALYPTUS OIL	AMT. OF ACETONE USED	OBSERVATIONS						ABSOR-BANCY AT 660nm
				TIME AND NO. OF MORTALITY						
				1 MIN	30 MIN	1 HOUR	3 HOUR	6 HOUR	24 HOUR	
20	Control I		1.5 ml	-	-	-	-	-	0	0.056
	Control II		1.5ml	-	-	-	-	-	1	0.052
	Control III		1.5ml	-	-	-	-	-	0	0.055
	Treatment I	3000ppm (3µl/ml) 1.5ml each petriplate		-	-	-	-	-	1	0.065
	Treatment II			-	-	-	-	-	2	0.074
	Treatment III			-	-	-	-	-	1	0.069
20	Control I		1.5ml	-	-	-	--	-	1	0.058
	Control II		1.5ml	-	-	-	-	-	1	0.054
	Control III		1.5ml	-	-	-	-	-	0	0.050
	Treatment I	2000ppm (2µl/ml) 1.5ml each petriplate		-	-	-		-	1	0.062
	Treatment II			-	-	-	-	-	0	0.058
	Treatment III			-	-	-	-	-	0	0.064
20	Control I		1.5ml	-	-	-	-	-	1	0.056
	Control II		1,5ml	-		-	-	-	0	0.052
	Control III		1,5ml	-	-	-	-	-	0	0.054
	Treatment I	1000ppm (1µl/ml) 1.5ml each petriplate		-	-	-	-	-	1	0.058
	Treatment II			-	-	-	-	-	0	0.057
	Treatment III			-	-	-	-	-	0	0.052
20	Control I		1.5ml	-	-	-	-	-	0	0.055
	Control II		1.5ml	-	-	-	-	-	0	0.052
	Control III		1.5ml	-	-	-	-	-	0	0.058
	Treatment I	100ppm (1µl/10ml) 1.5ml each petriplate		-	-	-	-	-	2	0.055
	Treatment II			-	-	-	-	-	0	0.052
	Treatment III			-	-	-	-	-	0	0.057

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-Figure 1: STANDARD BSA GRAPH SHOWING ABSORBANCY IN Y-AXIS VS BSA CONCENTRATION IN X-AXIS

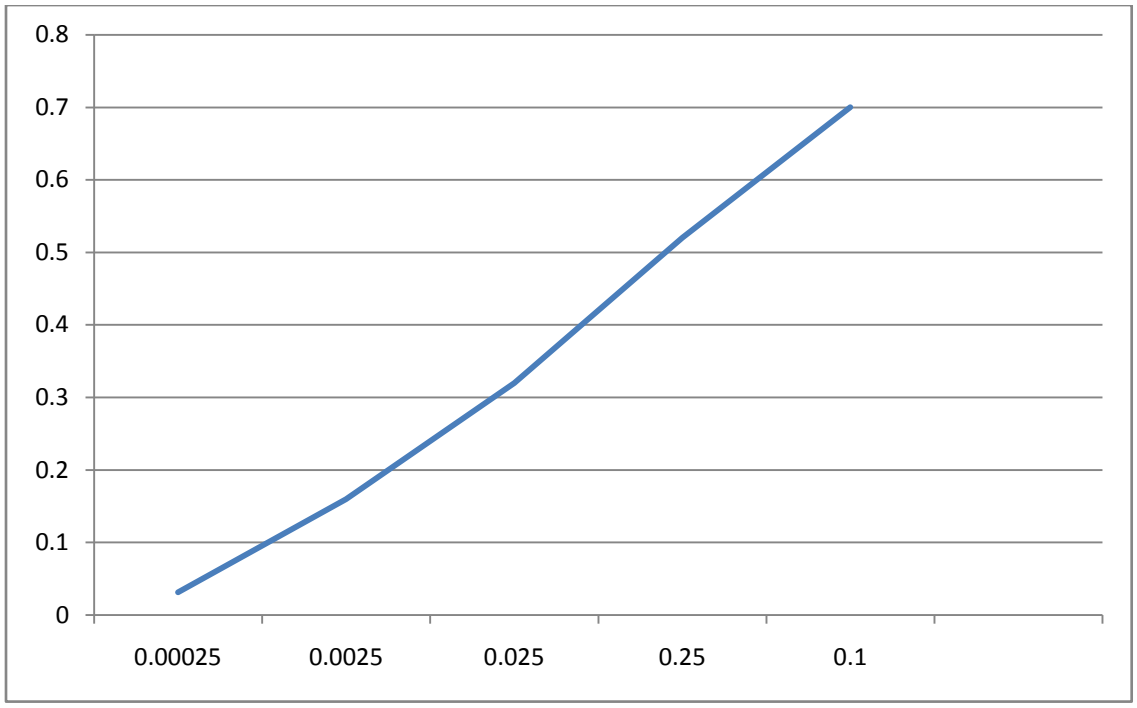

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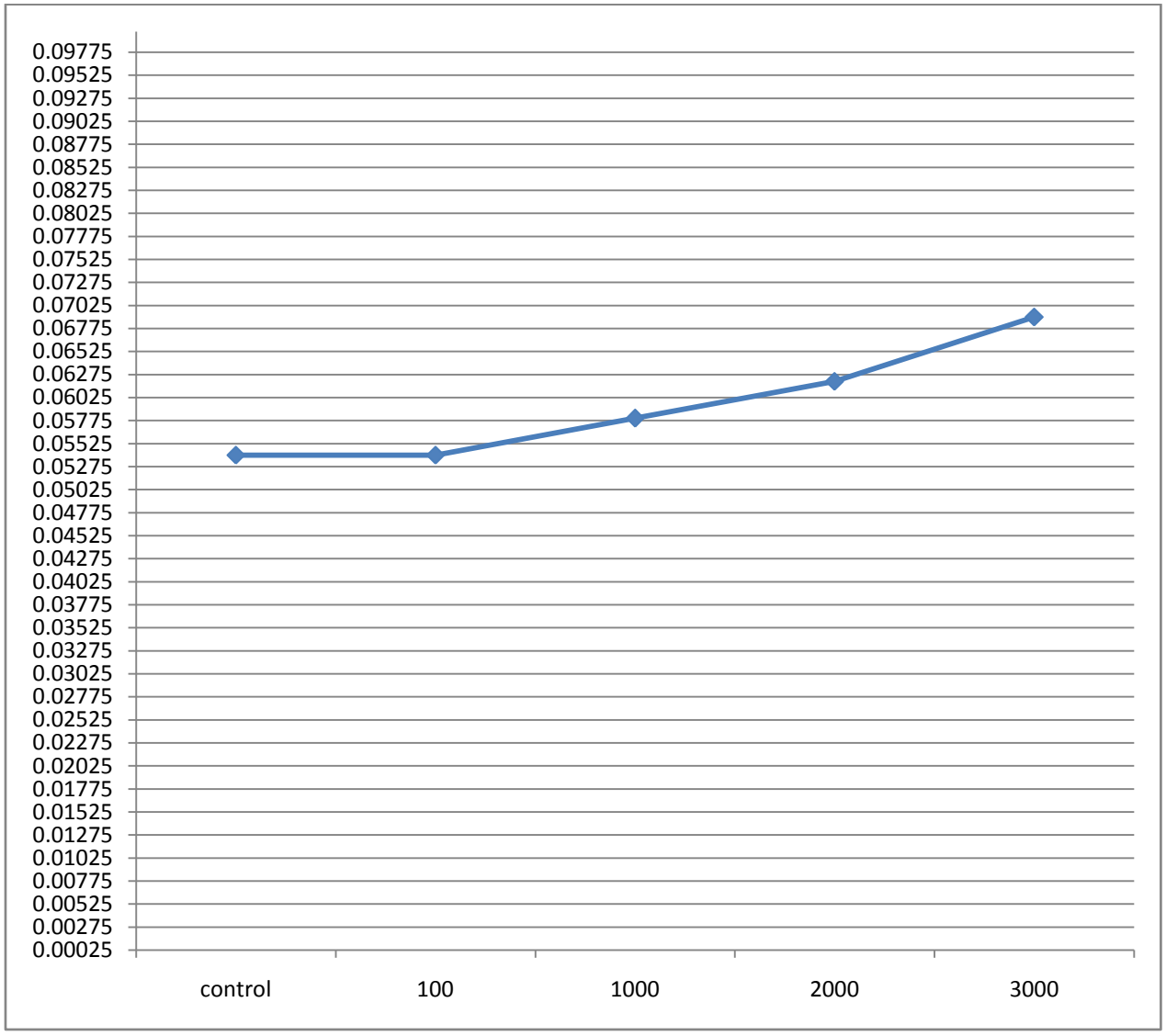
Figure 2: GRAPH SHOWING THE TOXICITY EFFECT OF DIFFERENT CONCENTRATION OF *EUCALYPTUS* OIL AFTER 24 HOURS TREATMENT AGAINST *SITOPHILUS ORYZAE* (CHANGE IN ABSORBANCE IN Y-AXIS VS APPLIED CONCENTRATION (ppm) IN X-AXIS)



It was observed that with increase in concentration the oil the insects moved away from the area of application of the oil. After 24 hours some of the insects died showing moderate toxicity of the essential oil.

DISCUSSION

The essential oil selected for the in-vitro experiment was four concentrations of Eucalyptus oil against *S. oryzae*. The result showed that with increase in concentration of the essential oil the toxicity also increases and the highest toxicity was seen in 3000ppm concentration of Eucalyptus oil. Franz et al(2012) also found *C. citrates* oil and *Z. officinales* oil showing toxic effect on *S.*

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oryzae Mishra et al(2011) found laung oil also another essential oil having toxic effect on rice weevil. Khani et al(2012) observed peppermint oil effectiveness in controlling rice weevil. Other workers also found effectiveness of essential oils at various concentrations against S. oryzae like Nalini et al in poduthalia oil, pippal oil, kalihari oil; C. *roseus* oil by Majeed(2011); *O. basicum* oil, *E. globules* by Mishra et al(2012). *E. citriodora* show toxicity against *Sitophilus zeamais* have been found out by Tinkeu et al. (2004). *E. intertexta, E. sargentii and E. camaldulensis* also show toxic effect and was found to kill 1–7 days adults of *Callosobruchus maculatus, Sitophilus oryzae and Tribolium castaneum* as found by Negahban and Moharramipour (2007). *E. nicholii, E. codonocarpa, E. blakelyi Sitophilus oryzae a*lso insecticidal activity against *Tribolium castaneum* and *Rhyzopertha dominica* was found out by Lee et al. (2004). E. saligna show repellent activity against *Sitophilus zeamais* and *Tribolium confusum* was found by Tapondjou et al. (2005). Thus different species of Eucalyptus have insecticidal activity against various insect pests. We found out from our experiment that *Eucalyptus maculata* oil also have insecticidal effect and show repellency against *S. oryzae*. Higher concentrations show more toxicity.

CONCLUSION

From the obtained result and previous works of various workers we can conclude that *Eucalyptus maculata* oil which is an essential oil show repellency against *S. oryzae* and can be used as insecticide in controlling rice weevil in post harvest rice grains. Since this oil is a plant extract it should show no harmful effect on consumption of rice treated with this oil by man.
Since the essential oils come natural resources and can be used as an alternative to control rice weevil as contact toxic, fumigant, insect repellent, antifeedant, etc.

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