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Bio-efficacy of oil extract of *Eugenia aromatica* in the control of storage insect pests

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Abstract: ***The effect of ethanolic extract of essential oil of E. aromatica for the control of S. zeamais, S. oryzae , C. maculatus and T. castaneum was investigated in the laboratory. Essential oil was extracted from pulverized dry flower buds of E. aromatica using volatile or essential oil steam distillation apparatus.***

The oil was prepared into the following concentrations; 100%, 50%, 25%, 10%, 5%, 4%, 3%, 2%, and 1% respectively in ethanol. 16.4g of cowpea seeds, 15.05g of maize seeds, 5g of rice seeds and 3.25g of wheat seeds were put into separate petri dishes. 5males and 5females of the insect pests were introduced into the petri dishes containing the seeds. The essential oils were were sprayed using a hand sprayer into the petri dishes containing the insect pests at different levels of concentrations 100%, 50%, 10%, 5%, 4%, 3%, 2% and 1% respectively. Insect mortality rate was monitored and observed to fall between for 5minutes in Sitophilus zeamais and 45minutes in Tribolium castaneum. The essential oil applied at all levels significantly reduced the population of each insect pest of the studied storage crops.

Keywords: Extract, Storage pest

1. Introduction

Insect pests have caused so much damage to crops, farmers and to the world at large. That is why researchers have used so many ways and methods to control it. A large number of plant species from a wide range of families have been evaluated. Jacobson 1989 suggested that the most promising botanicals were to be found in the families *Meliaceae*, *Rutaceae*, *Asteraceae*, *Annonaceae*, *Lamiaceae* and *Canellaceae* .The plant species that have been investigated are frequently those used locally, within individual countries, as spices or in traditional medicine. According to Ofuya (2003), synthetic insecticides involves risks for human health and the environment especially when improperly used which may be common among uneducated rural farmers in Africa, Since the last decades, Plant- derived insecticide have been vigorously investigated worldwide, as a possible replacement for synthetic insecticide in stored products protection(Lale 2001).

Desirable characteristics of botanicals for use in pest control would probably be that, the plant is perennial, easy to grow and not expensive to produce, plants should also show no potential to become weed or host for plant pathogens themselves and should if possible offer complementary economic uses.

Research in recent has been turning more towards selective bio-rational pesticides, that is safer, cheaper and more easy to produce than synthetic insecticides. It has been reported Essential oil from plants have been proved to possess good potential for use as fumigants against stored product insects including storage bruchids (Papachristos and stamopoulos, 2002 and Tapondjou *et al*, 2002). Raja *et al*, 2000, reported that when jute bags treated with different plant leaves extract including *A. indica*, *V. negundo*, *C. collinus* and *J. Curas* and then used for cowpea seeds storage, the egg laying rates by the *C. maculatus* adult emergence and seed damage were reduced.

Kim *et al* (2003) showed the potent insecticidal activity of extract of cinnamomum cassia bark and oil, horseradish (*Cocholeria aroracia*) oil and mustard (*Brassica juneea*) oil against *C. chinensis*, within one day after *Eucalyptus* seed powder treatment cause the death of emerging adult of *C. chinensis*. plant products such as vegetable oils essential oils, volatile oils, crude extracts and powders have been tested against *C. maculatus*, (Lale, 2001, Boeke *et al*, 2002). Also dry powder made from *A. indica* seed, *A. juss*, buds of clove tree, *Eugenia aromatic*, baill, fruits of West Africa brown pepper, piper guineense, seed of "pepper fruit" tree, *Dennetia tripetala* baker and root bark of the "tooth ache plant", *Zanthozylum zanthozyloides* (Lam) waterm, applied at 2% of the weight of seed beetle in storage (Lale, 2001 Ogunwolu *et al 2001*; Adedire and Lajide, 2001, Ofuya and Salami, 2002).

Tapondjou *et al* (2002) showed that the dry ground leaves of *Chenopodium ambrosioides* inhibited F. progeny production and adult emergence of the *C. chinensis* and *C. maculatus*.

The use of plant materials, extracts, oils, serve as repellant against several insect such as weevils, flour beetles, bean- seed beetles, and potatoes moth etc. plant materials, extracts and oils also help to reduce the amount of synthetic pesticides needed thereby, decreasing the pesticides load in food grains. To investigate the effectiveness of essential oil of *Eugenia aromatica* on mortality rate of *Sitophilus oryzae*, *Sitophilus zeamais*, *Callosobruchus maculatus* and *Tribolium castaneum*.

The extracts and oils of plant materials have been found to be alternatives to conventional synthetic insecticides for the control of stored product insect pests (Lale, 2001 and Boeke *et al*, 2002). This is due to adverse effects of chemical fumigants used in stored products, for protection, in respect of ozone depletion, high mammalian toxicity, insect resistance and health hazard. It is now established that vegetable oil, essential oil extract from plants are very effective in controlling certain species of bruchids by their effect, such as melon seed oil (Okunola, 2003). The insecticidal properties possessed by some essential oils are due to their monoterpenoid contents, this include fatty acids, phenolics, alkaloids and terpenes, especially monoterpenes which are the bioactive consistuent of plant products (Lale, 2001). Botanical insecticides tend to have broad Spectrum activity. They are safe and relatively specific in their mode of action, easy to produce and use.

In this study, the biological control of *Sitophilus oryzae*, *Sitophilus zeamais*, *Callosobruchus maculatus* and *Tribolium castaneum* (red flour beetle) using oil extract of *Eugenia aromatica.*

2. Materials And Methods

The study site

Laboratory experiments were conducted at the department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo state.

Culturing of Insects

Adult *Sitophilus oryzae, Sitophilus zeamais, Callosobruchus maculatus* and *Tribolium castaneum* were obtained from infected grains in Ikare market Ikare Akoko, Ondo State.

The insects were cultured in the laboratory at room temperature.

The food media used for the insect culture were Rice for *Sitophilus oryzae*, maize for *Sitophilus zeamais*, cowpea for *Callosobruchus maculatus* and wheat for *Tribolium castaneum*.

About 50g of each food medium were weighed into a small covered plastic bucket. Twenty adult insects (ten males and ten females) were introduced into the culturing medium and covered tightly.

Preparation of plant materials

The extraction of plant oil was carried out at the Department of Crop, Soil and Pest Management laboratory, School of Agriculture, Federal University of Technology. Akure, Ondo State.

Dry flower buds of *Eugenia aromatica* were purchased from Local herbal market in Akure, Ondo State. The flower buds were further oven dried to a constant weight 400^0C. Thereafter, the dried buds were grinded into fine powder using Sonik Japan Blender, Model number: SB-738, Voltage: 220-240v, 50HZ 350W and then sieved to a particle size of 300*u*m with a British Laboratory test standard sieve (Serial number: 133032). The fine plant powder was kept in an air tight container until required.

Extraction of Essential oil from *E. aromatica*

The oil was extracted from dry powder of *E. aromatica* following by using volatile or essential oil steam distillation apparatus, which is made of 200ml capacity distillation flask with a thick round neck condenser and graduated measuring tube with a collecting tap at the end.

In carrying out the steam distillation process, 120g of *E. aromatica* was weighed into a distillation flask and 300ml of water added. The apparatus was set up using a clamp on a heating mantle and heated for a period of 4hours. The volatile oil deposited on water was then collected through the attached graduated measuring tube by opening the tap.

Method of Treatment

Freshly emerged adults of Sitophilus oryzae, Sitophilus zeamais, Callosobruchus maculatus and Tribolium castaneum from the cultures in the laboratory were removed and used accordingly for the experiments.

16.40g of cowpea seeds, 15.05g of maize seeds, 5g of rice seeds, and 3.25g of wheat seeds were put into separate petri dishes. Thereafter, 5males and 5 females of each insect were introduced into the petri dishes and each was suspended in different set up.

The essential oil of *Eugenia aromatica* was sprayed using a hand sprayer into the petri dishes containing the insect pests at different levels of concentration, 100%, 50%, 25%, 10 %, 5%, 4%, 3%, 2%, 1% level of concentration respectively in ethanol. Mortality rate was recorded for 5min, 10min, 15min, 20min, 30min, and 45min. Each treatment was replicated three times and after treatment, the seeds were reweighed.

In vitro experiment

Effect of concentrated *E. aromatica oil* on insect pests

5male and 5female of each pest were handpicked into different petri dishes, 100% concentrated oil of *E. aromatica* was dropped into each petri dish.

Mortality was monitored in each petri dish for 5min, 10min, 15min, 20min, 30min and 45min, thereafter all insects were removed.

Effect of Ethanol on insect pests

5ml of 98% ethanol was dropped into petri dishes containing 5male and 5female insect pest. Mortality was monitored in each petri dishes.

Re affirmation of effect of ethanol on insect pests

Several concentration of Ethanol was prepared which include: 98%, 50%, 25%, 10%, 5%, 4%, 3%, 2%, 1% to 0.1%. 0.1% of ethanol was mixed with essential oil of *E. aromatica* to prepare the following concentrations of 100%, 50%, 25%, 10%, 5%, 4%, 3%, 2% and 1% respectively.

3. Results And Discussions

Table 1 shows the mean mortality count of S*itophilus zeamais* treated with essential oil of *E. aromatica* at different level of concentration.

Treatment	Mean mortality count (minutes)		
	5mins	10mins	15mins
100%	6.6 ± 0.9b	4.67 ± 0.3a	3.33 ± 0.3b
50%	6.67 ± 0.9b	4.33 ± 0.9a	3.33 ± 0.9b
25%	5.67 ± 0.9b	4.00 ± 1.0a	2.00 ± 0.6ab
10%	5.33 ± 0.3ab	3.33 ± 0.9a	1.00 ± 0.6ab
5%	5.33 ± 0.3ab	3.67 ± 0.7a	1.67 ± s0.3ab
4%	4.67 ± 0.9ab	3.67 ± 0.7a	1.00 ± 0.6ab
3%	4.00 ± 0.6ab	3.67 ± 0.9a	1.00 ± 0.6ab
2%	2.33 ± 0.6a	3.33 ± 0.9a	1.00 ± 0.6ab
1%	2.00 ± 0.9a	2.33 ± 0.7a	0.00 ± 0.0

In a column, values with the same alphabets are not significantly different at 5% level of probability using Tukey's Honestly Significant Test.

Table 2 shows the mean mortality count of *Sitophilus oryzae* treated with essential oil of *E. aromatica* at different levels of concentration.

Treatment	Mean mortality count (minutes)		
	5mins	10mins	15mins
100%	6.33 ± 0.9b	5.00 ± 0.6a	4.33 ± 0.9b
50%	6.00 ± 0.6b	5.00 ± 0,6a	2.00 ± 0.6a
25%	6.33 ± 0.9b	4.67 ± 0.7a	2.00 ± 0.0a
10%	5.33 ± 0.9ab	4.00 ± 0.6a	1.00 ± 0.6a
5%	5.00 ± 0.6ab	4.00 ± 0.0.6a	1.00 ± 0.6a
4%	4.00 ± 0.6ab	3.67 ± 0.9a	0.67 ± 0.3a
3%	3.00 ± 0.6ab	4.00 ± 0.6a	0.67 ± 0.6a
2%	3.33 ± 0.9ab	3.33 ± 0.7a	0.33 ± 0.3a
1%	2.00 ± 0.6a	3.00 ± 0.6a	0.00 ± 0.00

In a column, values with the same alphabets are not significantly different at 5% level of probability using Tukey's Honestly Significant Test.

Table 3 shows the mean mortality count *of Callosobruchus maculatus* treated with essential oil of *E. aromatica* at different levels of concentration.

Treatment	Mean mortality count (minutes)		
	5mins	10mins	15mins
100%	6.33 ± 0.9b	4.33 ± 0.7a	4.67 ± 0.2b
50%	6.00 ± 0.6ab	4.00 ± 1.2a	3.00 ± 0.6bcd
25%	5.33 ± 0.9ab	4.00 ± 0.6a	3.33 ± 0.9cd
10%	5.33 ± 0.9ab	4.00 ± 0.6a	2.00 ± 0.6abc
5%	5.33 ± 0.7ab	4.00 ± 0.6a	1.33 ± 0.7abc
4%	4.00 ± 0.6ab	3.67 ± 1.2a	0.67 ± 0.3abc
3%	3.33 ± 0.7ab	3.33 ± 0.7a	0.67 ± 0.3abc
2%	3.00 ± 0.6ab	3.33 ± 0.3a	0.33 ± 0.3ab
1%	2.33 ± 1.2a	2.33 ± 1.5a	0.00 ± 0.0a

In a column, values with the same alphabets are not significantly different at 5% level of probability using Tukey's Honestly Significant Test.

Table 4 shows the mean mortality count of *Tribolium castaneum* treated with essential oil of *E. aromatica* at different levels of concentration.

Treatment	Mean mortality count (minutes)		
	5mins	10mins	15mins
100%	5.00 ± 0.6a	6.00 ± 0.6b	5.33 ± 0.9b
50%	4.33 ± 1.2a	4.67 ± 1.5ab	4.67 ± 0.7ab
25%	3.67 ± 1.5a	4.33 ± 0.3ab	4.67 ± 0.7ab
10%	3.33 ± 0.9a	4.33 ± 0.3ab	4.33 ± 0.9ab
5%	3.00 ± 0.6a	3.67 ± 0. 3ab	3.33 ± 0.9ab
4%	2.00 ± 0.6a	3.33 ± 0.9ab	2.33 ± 0.7ab
3%	2.23 ± 0.9a	3.00 ± 0.6ab	2.00 ± 0.6ab
2%	1.67 ± 0.9a	2.67 ± 0.3ab	2.00 ± 1.2ab
1%	1.67 ± 0.6a	2.33± 0.9a	1.00 ± 0.6a

In a column, values with the same alphabets are not significantly different at 5% level of probability using Tukey's Honestly Significant Test.

Table 5 shows the comparison of mean mortality count with 100% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	4.67	3.33	0	0
S. oryzae	6.33	0	5	4.33
C. maculatus	6.33	4.33	4.67	0
T. castaneum	0	5	0	6

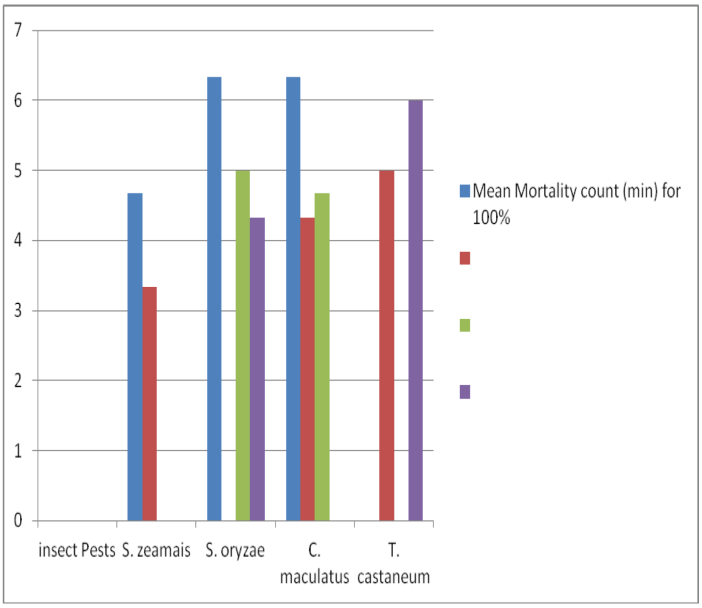

Figure 1: Graphical representation of comparison of mean mortality count with 100% concentration

Table 6 shows the comparison of mean mortality count with 50% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	4.33	3.33	0	0
S. oryzae	6	0	5	2
C. maculatus	6	4	3	0
T. castaneum	0	4.33	0	4.67

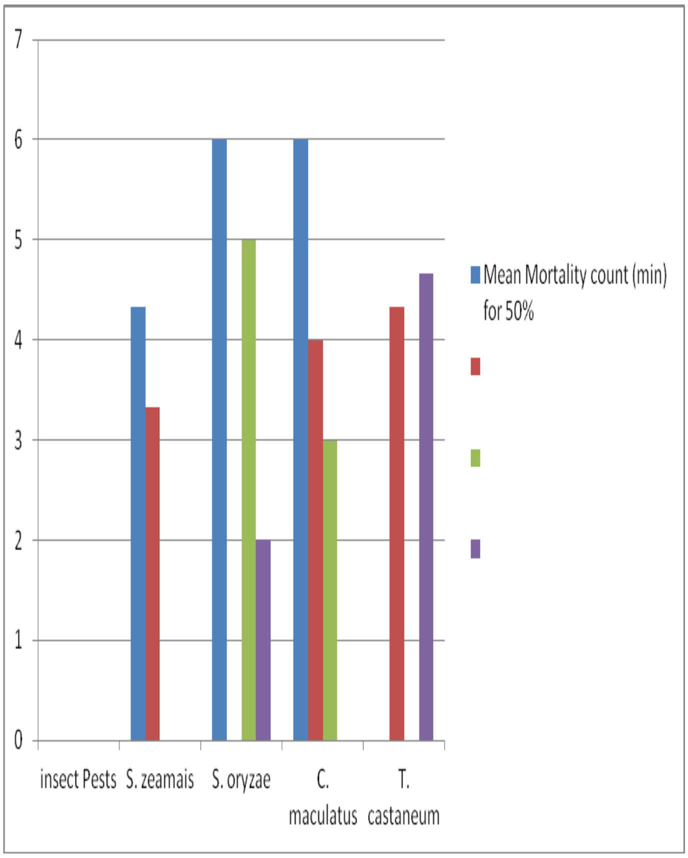

Figure 2: Graphical representation of comparison of mean mortality count with 50% concentration

Table 7 shows the comparison of mean mortality count with 25% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	4	2	0	0
S. oryzae	6.33	0	4.67	2
C. maculatus	5.33	4	3.33	0
T. castaneum	0	3.67	0	4.33

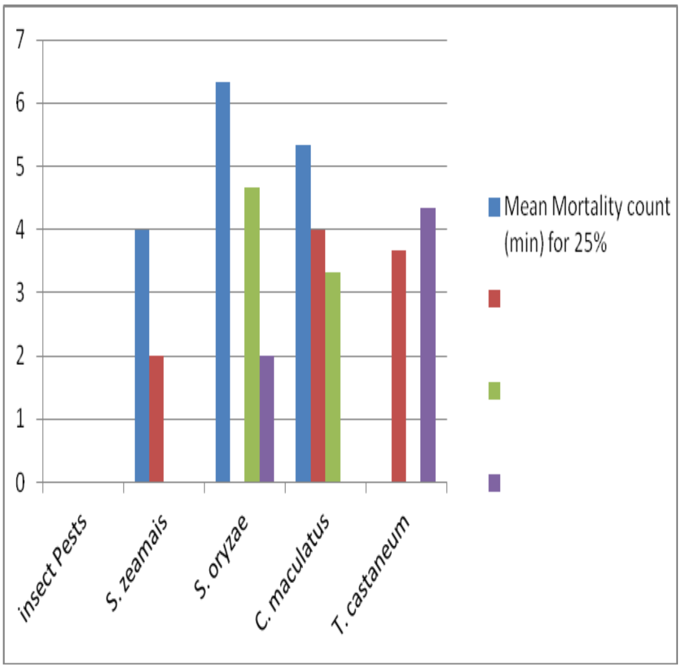

Figure 3: Graphical representation of comparison of mean mortality count with 25% concentration

Table 8 shows the comparison of mean mortality count with 10% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	3.33	1	0	0
S. oryzae	5.33	0	4	1
C. maculatus	5.33	4	2	0
T. castaneum	0	3.33	0	4.33

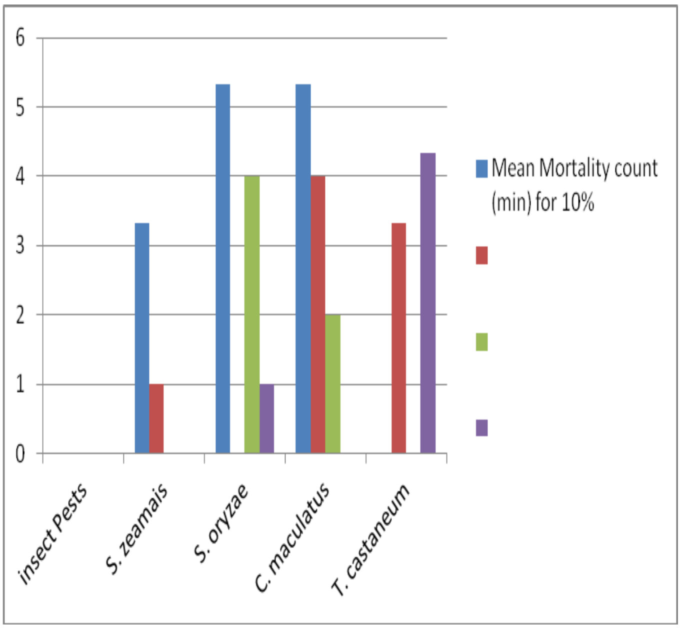

Figure 4: Graphical representation of comparison of mean mortality count with 10% concentration

Table 9 shows the comparison of mean mortality count with 5% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	3.67	1.67	0	0
S. oryzae	5	0	4	1
C. maculatus	5.33	4	1.33	0
T. castaneum	0	3	0	3.67

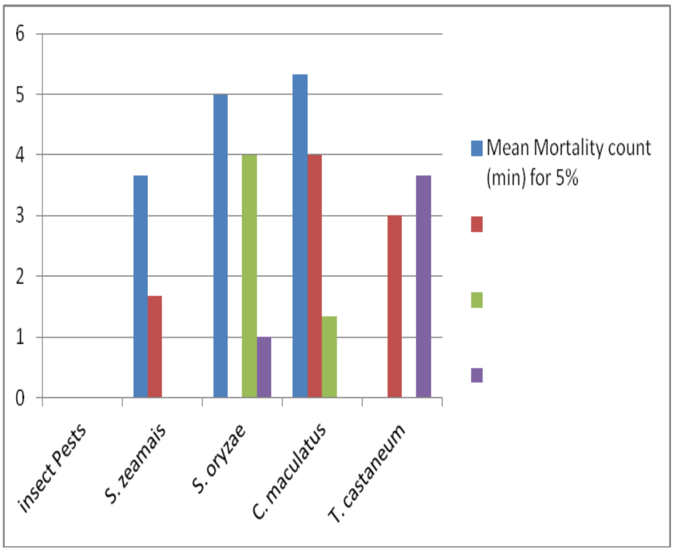

Figure 5: Graphical representation of comparison of mean mortality count with 5% concentration

Table 10 shows the comparison of mean mortality count with 4% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	3.67	1	0	0
S. oryzae	4	0	3.67	0.67
C. maculatus	4	3.67	0.67	0
T. castaneum	0	2	0	3.33

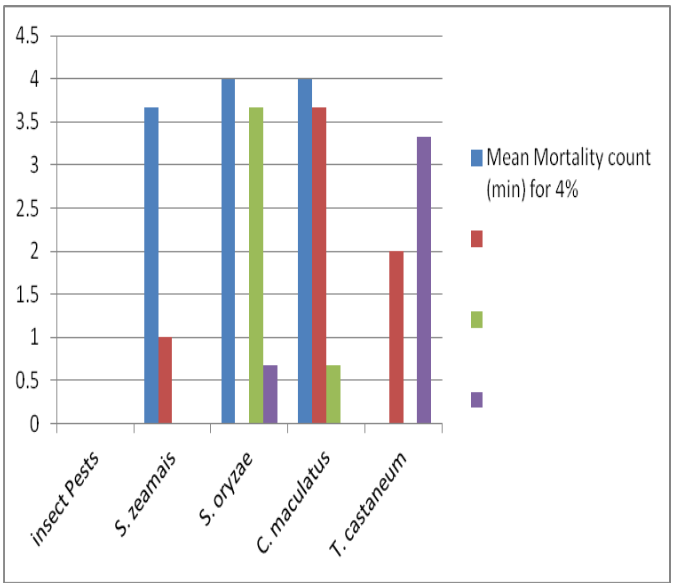

Figure 6: Graphical representation of comparison of mean mortality count with 4% concentration

Table 11 shows the comparison of mean mortality count with 3% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	3.67	1	0	0
S. oryzae	3	0	4	0.67
C. maculatus	3.33	3.33	0.67	0
T. castaneum	0	2.33	0	3

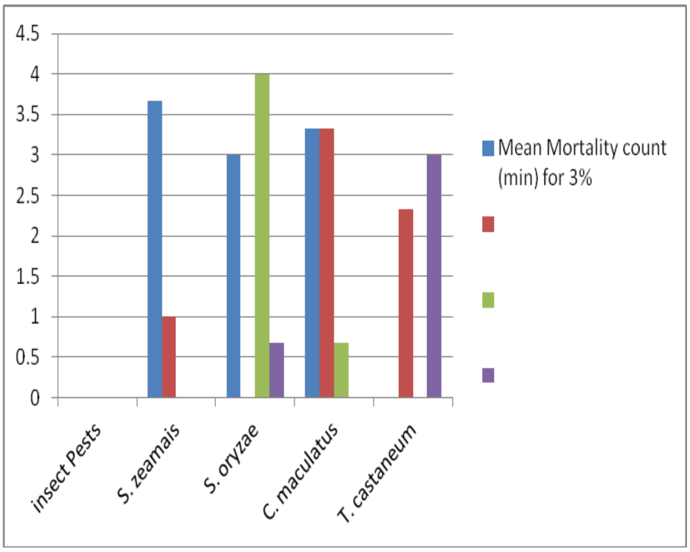

Figure 7: Graphical representation of comparison of mean mortality count with 3% concentration

Table 12 shows the comparison of mean mortality count with 2% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	3.33	1	0	0
S. oryzae	3.33	0	3.33	0.33
C. maculatus	3	3.33	0.33	0
T. castaneum	0	1.67	0	2.67

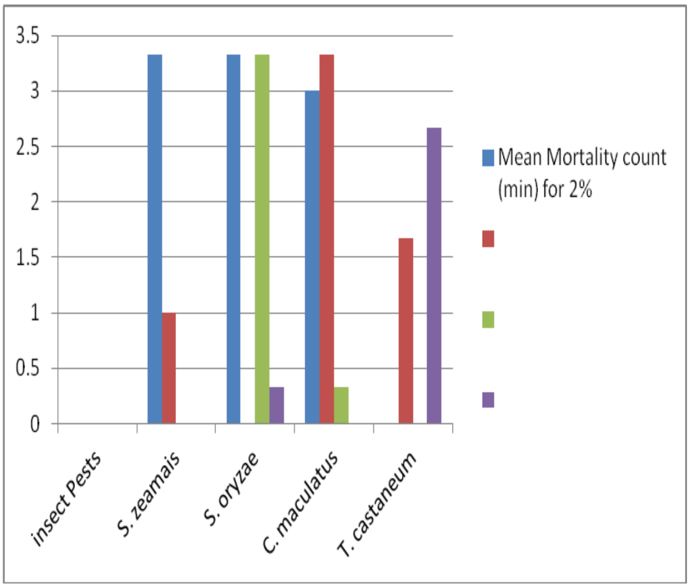

Figure 8: Graphical representation of comparison of mean mortality count with 2% concentration

Table 13 shows the comparison of mean mortality count with 1% concentration

	Mean Mortality count (min)			
Insect Pests	10mins	15mins	20mins	30mins
S. zeamais	2.33	0	0	0
S. oryzae	2	0	3	0
C. maculatus	2.33	2.33	0	0
T. castaneum	0	1.67	0	2.33

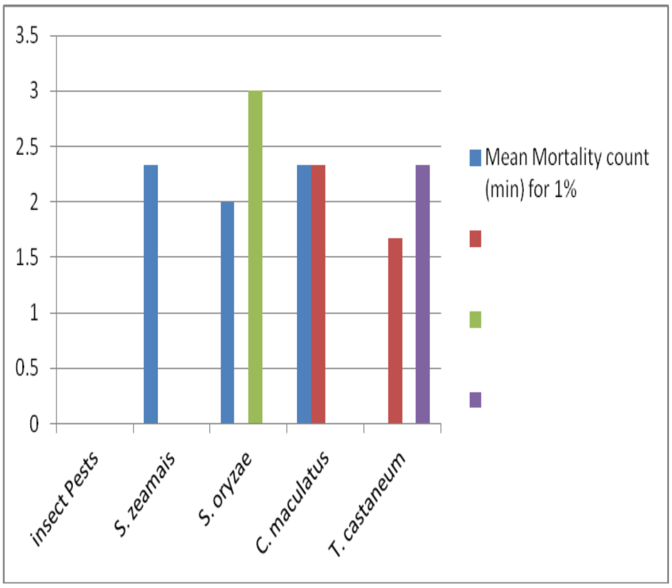

Figure 9: Graphical representation of comparison of mean mortality count with 1% concentration

Peasant farmers in many parts of Africa frequently mix plant materials with stored grains to prevent insect pest damage (Ofuya, 2003). The result obtained from this study suggests that ethanol extract of *E. aromatica* is effective as contact biorational against *S. zeamais, C. maculatus, S. oryzae* and *T. castaneum*. However, the effect of essential oil of *E. aromatica* on these insect pests is dependent on the levels of concentration of oil administered. The difference in the response by the different insect pest species could be attributed to the morphological and behavioural differences between the insect (Tanpondju *et al*; 2002).

All insects were susceptible to *E. aromatica* plant extract treatment, although susceptibility varies among insect species *with T. castaneum* being the most resistant. *E. aromatica* is known to have pungent smell and contains eugenol, sesquiterpene and caryophyline. The action of *E. aromatica* on these beetles could be as result of stomach poisoning through picking lethal dose of the plant extract by the beetles while feeding on whole or fragmented grains.

Therefore, the high toxic effect of *E. aromatica oil on S. zeamais* which is known to have thick exo-skeleton that should give them some level of resistance could probably be due to the feeding habits of these pests during which lethal dose of plant material must be taken up. The comparatively lower susceptibility of *C. maculatus, S. oryzae and T. castaneum* might be due to their feeding habit also. During the experiment, it was observed that about 90% of *T. castaneum* clustered round a spot thereby reducing the chance of making contact with the plant oil.

Results from this investigation revealed that extract from *E. aromatica* significantly ($P < 0.05$) reduced the population of all the storage pests. In comparing the mean mortality count of each insect pest treated with the different concentrations as shown in Table 5 -13, it was observed that; for *S. zeamais and S. oryzae*, the mean mortality count under 10mins and concentration levels between 4% and 100%, there was significant difference in their mean mortality count. Consequently, from 1% to 3% level of concentration, there was no significant difference in the mean mortality count.

Also, for *S. zeamais* and *C. maculatus*, the mean mortality count under 10mins and 20mins, there was significant difference in all level of concentration except for 1% concentration under 10mins as was also observed for *S. zeamais* and *T. castaneum*, the mean mortality count under 15mins of treatment, there was no significant difference in all levels of concentration.

Similarly, for *S. oryzae and C. maculatus* the mean mortality count under 10 mins and 20mins of treatment, there was no significant difference in all levels of concentration of 10mins but there was significance difference under 20mins for all level of concentration.

S. oryzae and *T. castaneum,* showed significant differences in the mean mortality count under 30mins of treatment between 1% and 100% level of concentration likewise *C. maculatus and T. castaneum* in the mean mortality count under 15mins of treatment.

Finally, the effect of essential oil on these four species of stored product pest was dependent on the level of concentration administered.

4. Conclusion

The result obtained from this study suggests that ethanolic extract of essential oil *of E. aromatica* was effective as contact biorational against *S. zeamais, C. maculatus, S. oryzae and T. castanuem, with S. zeamais* being the most susceptible followed by *C. maculatus and S. oryzae; T. castaneum* is the most resistant. However, the effect of essential oil of *E. aromatica* on these insect pests is dependent on the levels of concentration of oil administered.

5. Recommendation

Several measures have been adopted to curtail the problems of insect infestation. . (Okunola, 2003) had also suggested the use of oils extracted from plants for crop protection, since synthetic insecticide tends to be hazardous to man and its environment. The adoptive use of oil of *Eugenia aromatica* could also be intensified as a probable panacea to crop infestation. Thus its formulation on a larger scale could also be considered beyond laboratory evaluation.

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