

**EVALUATION OF CONTACT ACTIVITIES OF
LEAF EXTRACTS FROM FOUR PLANT SPECIES
AGAINST *Sitophilus zeamais* MOTSCHULSKY
(COLEOPTERA: CURCULIONDAE)**

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ABSTRACT

Experiments were conducted in the Biotechnology Central Laboratory, the Biological Sciences Laboratory and the Animal House of the Department of Biological Sciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, to test the insecticidal effects of ethanolic and aqueous leaf extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia*. Doses of 0.5ml, 1.0ml, 1.5ml, and 2.0ml of ethanolic leaf extracts and dose of about 0.5ml, 1.0ml and 3.0ml of aqueous extracts were applied to 20g of maize grains in which 5-13 adult *Sitophilus zeamais* were released for adult mortality tests. The results showed that the highest (100.00%) mortality of *Sitophilus zeamais* was obtained when a dose of 1.5ml of *Tithonia diversifolia* and 2.0ml of *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* was applied and the least (21.21%) mortality was recorded from treatments of 0.5ml of *Azadirachta indica*, while for aqueous extracts the highest mortality recorded was (21.66%)

and the least (0.00%) mortality of adult *Sitophilus zeamais*. The adult mortality of *Sitophilus zeamais* was non-significantly ($P>0.05$) different between the ethanolic extracts used and aqueous extracts used. The findings of this study also showed that ethanolic extracts were more effective than aqueous extracts used and higher dose of ethanolic leaf extracts of *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* were more effective in protecting maize grains against *Sitophilus zeamais* infestation. It was observed that stains were left on the grains applied with ethanol extracts. Therefore, more research work is recommended on providing a solution that ensures the removal of the stains left by the extract on the grain.

Key words: contact activities, leaf extracts, *Sitophilus zeamais* and *Tithonia diversifolia*

ABSTRAK

Kajian telah dijalankan di Makmal Pusat Bioteknologi, Makmal Sains Biologi dan Rumah Haiwan Jabatan Sains Biologi, Universiti Persekutuan Pertanian, Abeokuta, Ogun State, Nigeria, untuk menguji keberkesanan insektisid ekstrak daun etanol dan ekstrak daun berair *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* dan *Tithonia diversifolia*. Dos 0.5 ml, 1.0 ml, 1.5 ml dan 2.0 ml ekstrak daun beretanol dan dos 0.5 ml, 1.0 ml dan 3.0 ml ekstrak daun berair telah digunakan pada 20 g bijirin jagung yang telah dilepaskan 5-13 *Sitophilus zeamais* dewasa untuk mengkaji jumlah kematian dewasa. Hasil menunjukkan bahawa kematian yang paling tinggi (100.00%) *Sitophilus zeamais* telah diperolehi pada dos 1.5 ml *Tithonia diversifolia* dan 2.0 ml *Chromolaena odorata*, *Mallotusop positifolius* dan *Tithonia diversifolia* telah digunakan dan menunjukkan jumlah kematian yang lebih sedikit (21,21%) pada dos 0.5 ml daripada *Azadirachta indica*, manakala bagi ekstrak akueus kematian yang tertinggi dicatatkan adalah (21,66%) dan yang paling kurang (0.00%) kematian serangga dewasa *Sitophilus zeamais*. Kematian serangga dewasa daripada *Sitophilus zeamais* adalah tidak ketara ($P>0.05$) yang berbeza antara ekstrak beretanol

dan ekstrak berair yang digunakan. Hasil kajian ini juga menunjukkan bahwa ekstrak beretanol lebih berkesan daripada ekstrak berair yang digunakan dan dos ekstrak beretanol yang lebih tinggi daripada ekstrak daun beretanol pada *Chromolaena odorata*, *Mallotus oppositifolius* dan *Tithonia diversifolia* lebih berkesan dalam melindungi bijirin jagung terhadap serangan *Sitophilus zeamais*. Terbukti bahawa keberkesanan pada bijirin digunakan dengan ekstrak etanol. Oleh itu, disarankan di masa hadapan kerja-kerja penyelidikan seterusnya adalah untuk memastikan penyingkiran kesan yang ditinggalkan oleh ekstrak beretanol pada tanaman bijirin.

Kata kunci: aktiviti sentuh, ekstrak daun, *Sitophilus zeamais*

INTRODUCTION

Maize Weevil *Sitophilus zeamais* Motschulsky (Coleoptera : Curculionidae) is one of the most serious, internal feeding pests of maize grain. *S. zeamais* are found in all warm and tropical parts of the world (Dobie et al., 1984). *Sitophilus zeamais* appears similar to the rice weevil (*Sitophilus oryzae*), but has more clearly marked spots on the wing covers, and is somewhat larger and is able to fly. The synthetic pesticides widely used for the control of stored – product pests are valued for their effectiveness, their relatively long shelf life, and the ease with which they can be transported, stored and applied. It is now obvious, however, that pesticides cause serious problems which include toxicity to humans with an estimated three million or more cases of pesticide poisoning annually, 20,000 of which prove fatal; pollution of soils, water and air, with as yet unknown long-term consequences for human, wildlife and the environment; and the development of pesticide resistance necessitating the use of larger doses at increasing cost to the farmer and to the society as a whole.

Additionally, other factors are now perceived to make the use of alternative control methods for stored products protection (SPP) more attractive. The limited external reserves and poor currency exchange rates of many developing countries limit the quantity of pesticides that can be imported. A reduction in the level or complete removal of subsidies on synthetic pesticides has made them inaccessible to the

majority of farmers and agricultural products merchants in many developing countries. In areas where the majority of farmers are illiterate, the misuse of pesticides is a frequent occurrence (Lale, 2002). As a result, there has been a significant shift both in research and practice to the possible utilization of products from locally available plant species. Although these products may not possess the great potency of synthetic pesticides, they are not frequently associated with harmful residues in stored produce. They are also relatively cheap and most do not require sophisticated technology for their application. The tropical region of the world is endowed with a wealth of diverse floral species of medicinal and/or pesticides value of which only a small proportion has been screened due to a lack of appropriate technology and funds. There is currently a renewed interest in screening plant species for biological activity (Lale, 2002).

MATERIALS AND METHODS

Research Locations

The experimental work was conducted in the Biotechnology Central laboratory, the Biological Sciences Laboratory and the Animal House of the Department of Biological Sciences, Federal University of Agriculture, Abeokuta. (FUNAAB), Ogun state, Nigeria. Stock of *Sitophilus zeamais* was gotten from Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun state. The insects were kept in Bama glass bottle, until they were required for use. Plants of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia*, were gotten from the wild and the Botanical Garden of FUNAAB, the leaves were air dried and then transferred into the oven for proper drying. The plants parts used were blended using a miller, and kept in the desiccator. Maize grains were purchased from these markets (Kuto, Osiele and Lafenwa) in Abeokuta metropolis and later preserved in the refrigerator for about two weeks to ensure disinfestations. Figs 1-4 show the different leaves used.



Figs. 1-4. Leaves of: 1. *Azadirachta indica*; 2. *Chromolaena odorata*; 3. *Mallotus oppositifolius*; 4. *Tithonia diversifolia*.

Ethanol Extract Formulation

15g of the powder from the leaves of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* were soaked in 100ml of (99.7%–100%) ethanol in small conical flasks for about 72 hours and filtered using funnel and cotton wool. The extracts were kept in labeled bottles in the fridge until required for use.

Aqueous Extract Formulation

15g of the powder from the leaves of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia*, was soaked in 100ml of distilled water in small conical flasks for about 72 hours and filtered using funnel and cotton wool. The extracts were kept in bottles in the fridge until required for use.

Effect of Leaf Extracts on *Sitophilus zeamais* Mortality

To 20g of sorted grains, 0.5ml of aqueous extracts and ethanol extracts of leaf materials were added and mixed with the grain in small plastic bowls. This was exposed for some minutes, after which about 5-13 adult weevils were introduced into the plastic bowls. Other doses of 1.0ml, 1.5ml and 2.0 ml of leaf extracts and 0.5ml, 1.0ml and 3.0ml of aqueous extracts were also used. The upper lids of the small plastic bowls were perforated with holes to allow air circulation, mesh cloth and cover was used to cover the bowls and observation was done after 24 hours, on the second day and fourth day after treatment with extracts. The old weevils were removed and new ones were introduced and the observation was repeated. Mortality of adult *Sitophilus zeamais* were recorded. Control tests were setup by introducing the adult weevils into grains treated with 99.7%–100% ethanol. Also, another experiment containing untreated seeds only was setup. Each treatment was replicated twice. These set-ups were kept inside a wooden box surrounded with net to prevent rat attack. Data collected were subjected to one way Analysis of bvariance (ANOVA) and when ANOVA result was significant, the Tukey's test was used to separate the mean at $P < 0.05$.

RESULT

Mortality of adult *Sitophilus zeamais* that were exposed by contact to ethanolic extracts used

The effect of ethanolic leaf extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia*, on the 4th day after treatment is shown in Table 1. The Table shows that *C. odorata* ethanol leaf extract, *Mallotus oppositifolius* ethanol leaf extract and *T. diversifolia* ethanol leaf extract resulted in highest [100.00%] mortality of adult *S. zeamais* than ethanol leaf extract of *A. indica* which gave [70.00%] when a dose of about 2ml was applied, while there was [68.11%] mortality of adult *S. zeamais* in the control (grains treated with ethanol only) and [8.68%] mortality of *S. zeamais* in the untreated grain. The adult mortality was non-significantly ($P > 0.05$) different between the ethanolic extracts used.

Table 1. Effect of Ethanolic Leaf Extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* on the 4th day on Adult Mortality of *S. zeamais*.

Plant Type	Dose (ml)	Mean Mortality (Percent)	
<i>A. indica</i>	0.5	21.21	32.26±11.40
	1.0	70.00	
	1.5	60.38	
	2.0	70.00	
<i>C. odorata</i>	0.5	30.00	36.97±13.97
	1.0	30.00	
	1.5	86.36	
	2.0	100.00	
<i>M. oppositifolius</i>	0.5	48.89	27.99±9.89
	1.0	60.60	
	1.5	81.81	
	2.0	100.00	
<i>T. diversifolia</i>	0.5	56.25	31.63±11.18
	1.0	66.36	
	1.5	100.00	
	2.0	100.00	
Control	0.5 – 2.0	68.11	
Untreated	0.00	8.68	

Mortality of adult *Sitophilus zeamais* that were exposed by contact to aqueous extracts from leaves used

The effect of aqueous extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* on adult mortality of *S. zeamais* on the 2nd day, 4th day and 24 hours after treatment is shown in Table 2. The table shows that after 24 hours of applying 3.0ml of aqueous extracts, *C. odorata* aqueous extracts resulted in higher [11.11%] mortality of adult *S. zeamais* than *A. indica* [0.00%], *M. oppositifolius* [0.00%] and *T. diversifolia*

[0.00%], while on the 2nd day after treatment with 1.0ml of aqueous extracts, *M. oppositifolius* resulted in higher [21.66%] mortality of adult *S. zeamais* than *A. indica* [0.00%], *C. odorata* [0.00%] and *T. diversifolia* [0.00%], and on the 4th day after treatment with 0.5ml of aqueous extracts, *M. oppositifolius* also resulted in higher [16.66%] mortality than *A. indica* [11.25%], *T. diversifolia* [5.55%] and *C. odorata* [0.00%]. There was 5.27% mortality in the untreated grain. The adult mortality was non-significantly ($P > 0.05$) different between the aqueous extracts used.

Table 2. Effect of Aqueous Leaf Extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* on Adult Mortality of *S. zeamais*

Plant Type	Dose (ml)	Mean Mortality (Percent)	
<i>A. indica</i>	0.5	11.25	5.86±2.39
	1.0	0.00	
	3.0	0.00	
<i>C. odorata</i>	0.5	0.00	8.98±3.66
	1.0	0.00	
	3.0	11.11	
<i>M. oppositifolius</i>	0.5	16.66	40±5.47
	1.0	21.66	
	3.0	0.00	
		13.	
<i>T. diversifolia</i>	0.5	5.55	4.53±1.85
	1.0	0.00	
	3.0	0.00	
Untreated	0.00	5.27	

0.5ml for 4th day after treatment

1.0ml for 2nd day after treatment

3.0ml for 24 hours after treatment

Table 3. Mean mortality (+S.E) of *Sitophilus zeamais* caused by leaf extracts used at all doses (0.5ml, 1.0ml, 1.5ml, 2.0ml and 3.0ml).

Plant Type	Ethanol Extract	Aqueous Extract
<i>A. indica</i>	55.39 ± 11.40 ^a	3.75 ± 2.39 ^a
<i>C. odorata</i>	66.10 ± 13.97 ^a	3.67 ± 3.66 ^a
<i>M. oppositifolius</i>	72.82 ± 9.89 ^a	7.22 ± 5.47 ^a
<i>T. diversifolia</i>	80.65 ± 11.18 ^a	1.85 ± 1.85 ^a

Mean values in the same column having the same superscript are not significantly different, P> 0.05.

Efficacy of ethanolic and aqueous extracts from leaves used on *Sitophilus zeamais*

The mean mortality of adult *S. zeamais* caused by ethanolic and aqueous extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* at doses of 0.5ml, 1.0ml, 1.5ml and 2.0ml for ethanolic extract and 0.5ml, 1.0ml and 3.0ml for aqueous extract is shown in Table 3. The table shows that ethanolic extract of *T. diversifolia* resulted in highest [80.65%] mortality rate of adult *S. zeamais*, followed by *M. oppositifolius* [72.82%], *C. odorata* [66.10%] and *A. indica* [55.39%], while the aqueous extracts of *M. oppositifolius* resulted in higher [7.22%] mortality, followed by *A. indica* [3.75%], *C. odorata* [3.67%] and *T. diversifolia* [1.85%]. This indicates that ethanolic extract of *T. diversifolia* is the most effective because it had the highest [80.65%] mortality of adult *S. zeamais* than *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia*. The aqueous extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* had little or no effect on adult *S. zeamais*.

DISCUSSION

The results obtained from this study suggested that ethanol leaf extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* were effective against *S.*

zeamais than aqueous extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia*. This agrees with the results obtained by Potenza *et al.*, (2005) where they showed that the acetonic extracts of *Ruta graveolens*, and *Dieffenbachia brasiliensis* resulted in 80.0 and 75.0% mortality for adult *S. zeamais* where as the aqueous extracts of *R. graveolens* and *D. brasiliensis* resulted in 12.00% mortality. The findings of this work revealed that the effectiveness of the ethanolic leaf extract was dependent on the dosage. This is in line with the results obtained by Adedire and Akinkurolere (2005) who reported that the effectiveness of ethanol extracts was dependent on dosage and exposure periods.

The action of *Tithonia diversifolia* on the insect could be as a result of stomach poisoning through picking lethal doses of the ethanol leaf extracts while feeding on whole or fragmented grain, as it was observed during the experiment that some insects were found dead on the grain. This agrees with the findings of Ahmed *et al.*, (1984); Lajide *et al.*, (1998); Adedire and Akinkurolere (2005) on the action of *Eugenia aromatica* on beetles. The high toxic effect of *Tithonia diversifolia* might be as a result of the feeding habits of insects during which deadly dose of the ethanol extract might have been taken up, and this is in conformity with the findings of Wasserman and Asami (1985) who reported that the high toxic effect of *Eugenia aromatica* on *S. zeamais* which is known to have thick exoskeleton that should give them some level of resistance, could probably be due to the feeding habits of these pests during which lethal dose of the plant material might have been taken up.

Also, when the grains were treated with 1.5ml of *M. oppositifolius* and *C. odorata* ethanolic leaf extracts and 2.0ml of *Azadirachta indica* ethanolic leaf extracts, higher percentage of mortality was recorded but it is not as high as the percentage mortality recorded when a dose of 1.5ml ethanol extract of *Tithonia diversifolia* was used.

The result obtained from this study is in agreement with the reports of Niber (1994) and Adedire and Lajide (2003) who reported that some tropical plants could be mixed with grains in storage in order to protect them from storage beetles.

CONCLUSION

The findings of this study have shown that the aqueous leaf extracts of *Azadirachta indica*, *Chromolaena odorata*, *Mallotus oppositifolius* and *Tithonia diversifolia* had little or no effect on *S. zeamais*, while ethanolic leaf extracts of *Tithonia diversifolia*, *Mallotus oppositifolius* and *Chromolaena odorata* have a great potency in controlling *S. zeamais*. Farmers should employ the locally available plants in keeping their maize grains free from *S. zeamais* attack in the store. However, discoloration is seen in the grains treated with ethanol extracts, which could abridge its acceptability by purchasers. More research work is hereby recommended to provide a solution that ensures the removal of the stain left by the extract on the grains.

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