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Comparative Efficacy of Aqueous Extracts of *Tithonia diversifolia*[Asteraceae] and *Vernonia amygdalina* [Asteraceae] Leaves in the Management of *Sitophilus zeam*ais Infestation in Stored Maize

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Abstract: *Impact of aqueous extracts of leaves of Tithonia diversifolia (Mexican sunflower) and Vernonia amygdalina (bitter leaf) on aspects of Sitophilus zeamais bionomics were investigated at concentrations of 0.5, 1.0 and 1.5 % under fluctuating ambient temperature and relative humidity in Makurdi, Benue State, Nigeria. T. diversifolia recorded higher toxicity (43.0 %) at concentration of 1.5 % after 96 hours of exposure. The plant failed to match the efficacy of permethrin insecticide (P < 0.05) that killed 70 % of the weevils in 24 hours, causing 100 % mortality after 72 hours at a lower concentration of 0.5 %. However, at concentrations of 1.0 and 1.5 %, hot water leaf extract of T. diversifolia in particular, matched the efficacy of the conventional synthetic insecticide (P > 0.05) in deterring oviposition in female S. zeamais. Although leaf extracts of V. amygadalina showed certain level of insecticidal activities against the weevil, the study suggests that the potentials of T. diversifolia should be exploited in the development of plant-derived insecticides against S.zeamais infestation in stored maize. Hot water extract was more effective than cold water extract in treatment application. In the study, there is need for increased research for plant-derived control agents that will effectively protect stored maize against weevil infestation.*

Keywords*: Sitophilus zeamais, Tithonia diversifolia, Vernonia_amygdalina, bionomics, infestation*.

I. Introduction

Maize or Corn (Zea mays L.) is an important staple food crop [16] and a major component of many cropping systems. Its importance as food, livestock feed and industrial raw material cannot be over-emphasized [27] [17]. It can adapt to a wide range of agro-ecological environments [22], giving it an advantage over some other cereals.

Climate change may worsen global food crises [9] [22] and will undoubtedly exacerbate decreased maize production caused by the activities of the notorious post harvest pest, Sitophilus zeamais. Grain damage and loss in maize during storage to the maize weevil is a very serious problem for farmers and traders [24]. [13] [14] estimated annual loss of stored maize due to S. zeamais infestation to be 30-50 % in tropical Africa. Maize weevil infestation results in substantial reduction in the quantity and quality of the grain [1] and these culminate in outright rejection of the product at the local and international markets [19]. Damaged and depreciated maize grains are unsuitable for human consumption and cannot be used for agricultural and commercial purposes [5].

Conventional synthetic chemicals (such as permethrin) can significantly reduce S. zeamais infestation in stored maize, but health, environmental and economic considerations favor the use of botanical insecticides in the control of the pest [18] [20] [21] [6]. Many Nigerian medicinal plants and spices have been investigated as pest control agents against Sitophilus zeanais [3] with varying degrees of efficacy. Plant-derived insecticides are effective, safe, easy to apply, economical and eco-friendly [12]. Potentials of Tithonia diversifolia (Mexican sunflower) and Vernonia amygdalina (bitter leaf) has been reported by [6] [5]. Therefore, the major objectives of this study were to assess (in relation to a conventional synthetic chemical) the two plant species for (i) toxicity against adult S. zeamais and (ii) oviposition deterrence in the female weevil. Identifying easy to apply and eco-friendly plant materials that will effectively protect against weevil infestation and damage in stored maize at economically justified concentration will be a major contribution to concerted international efforts at finding substitutes to synthetic insecticides.

II. Materials And Methods

2.1 Insect Culture and Plant Materials

The maize weevil, Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) population was received from the Department of Crop production, University of Agriculture, Makurdi, Benue State, Nigeria. The weevils were reared on the susceptible maize variety, Bende White under fluctuating ambient temperature and relative humidity. The experiment was conducted at the Advanced Research Laboratory of Department of Biological Sciences, University of Agriculture, Makurdi.

The plant leaves on the other hand were obtained from mature stands of Tithonia diversifolia and Vernonia amygdalina at the University of Agriculture, Makurdi. They were washed separately, dried under room temperature for a period of two weeks, ground into fine powder using a micro speed electric grinder and sieved using a 300 µm mesh. Twenty grams of each powdered material were separately harmonized for five minutes in 100 ml of hot (70 ^{0}C) sterile water [7] to obtain an aqueous extract for the experiment. A cold water aqueous extract was also obtained using same method except that water temperature differed. The liquid contents of hot and cold water extracts were separately filtered using Whatman No 2 filter paper. The resulting filtrate 20 % (w/v) was used for the bioassays.

2.2 Test Maize Variety

The test variety, LNTP-YC_5F_2 used for the study is known to be susceptible to S. zeamais_infestation [5] and was obtained from the Maize Breeding Unit of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Susceptible variety eliminates the effect of varietal resistance. The experimental grains were cleaned, disinfested and standardized using the method of [23]. Two weeks acclimatization of the maize grains stabilized moisture content at 12-13 % [1].

2.3 Toxicity and Ovipostion Deterrence Assays

Hot and cold water leaf extracts of the plant species were tested for toxicity against the adult Sitophilus zeamais at concentrations of 0.5, 1.0 and 1.5 %. Filtrates of 0.1, 0.2 and 0.3 g/ml in 20 g of maize grains yielded consecutively, the listed concentrations in percent. Each water extract (at a particular concentration) for each plant species was separated and thoroughly mixed with 20 g maize grains in a plastic container aided by a glass rod. The grains were then air-dried for two hours prior to weevil introduction. Permethrin emusifiable concentrate, a conventional synthetic insecticide at concentrations of 0.5, 1.0 and 1.5% (w/w) was included in the assay as check to effectively assess the performance of the plant species. An untreated control was also included. Five pairs of newly-emerged (1-5 days old) adult weevils sexed morphologically using the method of [11] were introduced into each container. The containers were covered with muslin cloth (strongly fixed with cut-edge of container lid) for aeration and to prevent exit of the weevils and entry of unwanted organisms. Each set up was replicated four times and arranged in completely randomized block design on a laboratory bench at fluctuating ambient temperature and relative humidity. Mortality count of adult weevils was done daily up to 96 hours and percent mortality was recorded.

The infested grains were left for seven days under same experiment condition during which the weevils fed and laid eggs [1]. After seven days post infestation, both dead (those that did not respond to probe with a pin) and life weevils were removed and discarded. The total number of eggs laid in a no choice condition was counted on ten randomly selected grains using the method of [10]. Grains were examined under a dissecting microscope to locate the gelatinous egg plugs which stained cherry red. It was assumed that each plug covered only one egg and each egg was covered by one plug. Feeding puncture and mechanical injuries were distinguished from the gelatinous egg plug by their shiny surface [5]

2.4 Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using SPSS statistic 17.0 software. Mean separation was achieved using Fischer's Least Significant Difference at 5 % probability level.

III. Results

Table 1 presents results of the toxicity of the two plant leaves extracts compared to a synthetic insecticide against the adult Sitophilus zeamais. The results showed that percent mortality of the maize weevil varied among treatments, type of treatment extract, concentration levels and period of exposure. At 0.5 % concentration, there was no significant difference ($P > 0.05$) in adult mortalities caused by the plant treatments, type of treatment extract and post treatment period. Hot and cold water extracts of Tithonia diversifolia and Vernonia amygdalina both had same result (zero mortality) with unprotected grains at concentrations of 0.5 %. At the same time 0.5 %, permethrin emusifiable concentrate killed 70 % of the weevils in 24 hours, reaching 100 % after 72 hours of exposure. Weevil mortality increased with treatment concentration and period of exposure. Hot water extracts of both plant species killed significantly ($P > 0.05$) more weevils than cold water extracts. However, between the plant species, leaf extracts of T. diversifolia were more toxic to S. zeamais adults killing 43 % in 96 hours at treatment concentration of 1.5 %. However, over short duration of exposure (< 5 days), this plant species did not match the efficacy of the synthetic insecticide in killing weevils. On the contrary, leaf extracts of T. diversifolia killed significantly ($P < 0.05$) more weevils when compared to unprotected grains.

Table 1: Effect of Plant Leaf Extracts on Mortality (%) of Adult Sitophilus zeamais

Treatment	Concentration (%)	Post treatment period (Hours)*			
		24	48	72	96
T. diversifolia					
Hot Water Extract	0.5	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	1.0	0.00±0.00a	0.00±0.00a	**20.00±0.20b**	**40.00±2.40b**
	1.5	0.00±0.00a	**10.00±2.50**c	**23.30±4.70b**	**43.00±10.40b**
Cold Water Extract	0.5	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	1.0	0.00±0.00a	0.00±0.00a	**20.00±3.50d**	**30.00±6.70b**
	1.5	0.00±0.00a	0.00±0.00a	**20.00±0.90d**	**30.00±3.50b**
V. amygdalina					
Hot Water Extract	0.5	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	1.0	0.00±0.00a	0.00±0.00a	0.00±0.00a	16.67±11.28c
	1.5	0.00±0.00a	**3.30±10.46d**	**13.33±0.40c**	**26.63±5.20c**
Cold Water Extract	0.5	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	1.0	0.00±0.00a	0.00±0.00a	0.00±0.25a	10.00±0.00c
	1.5	0.00±0.00a	0.00±0.00a	**10.00±0.00e**	**16.67±0.73c**
Permethrin					
	0.5	70.00±0.00b	90.00±0.00b	100.00±0.00b	100.00±0.00b
	1.0	100.00±0.00b	100.00±0.00b	100.00±0.00c	100.00±0.00d
	1.5	100.00±0.00b	100.00±0.00e	100.00±0.00d	100.00±0.00d
Untreated control		0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

*All values are means of four replicates followed by standard error of the means. Mean separation was done treatment-type concentration versus treatment-type concentration. Means of treatment followed by the same letter within each column are not significantly different ($P > 0.05$) by Fischer's Least Significant Difference. E.C. = Emulsifiable concentrate.

Table 2 presents the result of comparative effect of leaf extracts of the two plant species on oviposition of adult Sitophilus zeamais. At concentrations of 0.5 and 1.0 %, cold water extracts of T. diversifolia deterred oviposition significantly ($P < 0.05$) more than cold water extract of V. amygdalina. Meanwhile, at concentrations of 0.5 and 1.5 %, significant differences ($P > 0.05$) were not recorded in oviposition deterrence by hot water extracts of the plants species. However, at 1.0 %, hot water extract of T. diversifolia significantly ($P < 0.05$) deterred oviposition more than hot water extract of V. amygdalina. At higher concentrations of 1.0 and 1.5 %, hot water extract of T. diversifolia in particular, matched the efficacy of permethrin insecticide in deterring oviposition among adult female S. zeamais. When compared with the resulst in unprotected grains, both hot and cold water leaf extracts of the two plants species deterred oviposition significantly ($P < 0.05$).

Table 2: Effect of Plant Leaf Extracts on Oviposition of Female Sitophilus zeamais

Treatment	Concentration (%)	Number of eggs laid on ten grains*
T. diversifolia		
Hot water extract	0.5	3.00±1.29a
	1.0	0.00±0.00a
	1.5	0.00±0.00a
Cold water extract	0.5	2.33±1.50e
	1.0	1.00±0.50g
	1.5	0.00±0.00j
V.amygdalina		
Hot water extract	0.5	4.00±0.32a
	1.0	1.67±0.15b
	1.5	0.00±0.00a
Cold water extract	0.5	5.67±2.72f
	1.0	2.00±0.50h
	1.5	0.00±0.00j
Permethrin E.C.	0.5	0.00±0.00b
	1.0	0.00±0.00a
	1.5	0.00±0.00aj
Untreated control	-	13.33±0.10d

*All values are means of four replicates followed by standard error of the means. Mean separation was done treatment-type concentration versus treatment-type concentration. Means of treatment followed by the same letter are not significantly different (P > 0.05) by Fischer's Least Significant Difference. E.C. = Emulsifiable concentrate.

IV. Discussion

The comparative effect of aqueous extract of Tithonia diversifolia and Vernonia amygdalina leaves on aspects of the biology of the maize weevil, Sitophilus zeamais has been investigated. Hot water extract of T. diversifolia showed higher insecticidal activity against S. zeamais. Its toxic effect (at 1.5 %) was significantly (P > 0.05) more felt after 48 hours of exposure, killing 43 % of the weevils in 96 hours. Although considerable, this result did not match the efficacy of the permethrin chemical in causing weevil mortality at short duration of exposure.

Furthermore, that analysis of the result showed that hot water leaf extract (at 1.0 and 1.5 %) of T. diversifolia matched the efficacy of the conventional synthetic insecticide, permethrin in suppressing oviposition in female weevils suggests that the plant species can be exploited in the development of botanical insecticides. [5] reported that extract from T. diversifolia protected crops from termites. Extracts from T. diversifolia have also been reported to contain chemicals that control insects [2]. Therefore, it is not strange that hot water leaf extract of T. diversifolia considerably killed weevils in this study. Toxicity to adult maize weevils and oviposition deterrence in the females caused by leaf extracts of V. amygdalina in the study agrees with the findings of [6] that assessed the potentials of the plant as a grain protectant against S. zeamais attack. [15] reported that leaf of V. amygdalina contains hydrocyanic, lactoric and oxalic acids and it is possible that these components are active against S. zeamais. Insecticides may for a long time continue to be the most dependable pest control measure in the battle against pests of agricultural and medical importance. Concerted efforts are being made at the international level to discover plant materials that will effectively protect stored products against pest infestation [3]

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