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Phytochemical analysis and toxicity of *Casuarina equisetifola* (whistling pine) To *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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Abstract
Qualitative phytochemical analysis of *Casuarina equisetifolia* (whistling pine) leaf was carried out using three solvents; petroleum ether, ethanol and water. Subsequently, laboratory investigation was conducted on the toxicity of the leaf extracts to *Sitophilus zeamais*. Six concentrations of the extracts ranging from 0.625%- 20% including a control were used. Aliquot of each concentration (1.0mL) was evenly dispensed onto No. 1 Whatman filter paper in petri-dishes placed in a Completely Randomized Design (CRD). Ten unsexed active adults of *S. zeamais* were introduced into each petri-dish. Qualitative phytochemical analysis revealed the presence of Saponins, flavonoids, Cyanogenic glycosides and Anthracene glycosides in all extracts, while tannins were present in only ethanol and aqueous extracts. Similarly, alkaloids were present in the aqueous extract only. Results of the toxicity study showed that dosage-related mortality responses were observed after 12 hours of exposure to different extracts of the plant. Hence, mortality increased with increase in concentration of the plant. The plant extracts caused mortalities of 80%, 77.5% and 85% at highest concentrations of aqeous, ethanolic and petroleum ether respectively while the lowest concentrations caused 37.5%, 35% and 35% respectively. Statistical analysis showed that these extracts were not significantly different (P>0.742) from each other. The LD_{50} values of these extracts were respectively 16.81µg/ml, 18.59µg/ml and 6.07µg/ml for aqeous, ethanolic and petroleum ether. This study suggests that plant extracts of *Casuarina equisetifolia* could perhaps be good alternatives to synthetic pesticides which adversely affect man especially when used on food crops. It is similarly suggested that the active phytochemicals could be the saponins, flavonoids, cyanogenic glycosides or anthracine glycosides acting individually or synergistically.

Keywords: *Bactrocera cucurbitae*, biopesticides, abamectin, lufenuron, comparative efficacy.

Introduction
Sitophilus zeamais which belong to the family Curculionidae, is a cosmopolitan pest of sound and whole-some grain in both tropical and temperate regions of the world [1]. Adult weevils and larvae feed on undamaged grains and reduce them to powdery form. Insect infestation of storage grain cereal and products may cause serious nutritional and economic losses which can be diverse and intense [2]. Globally, 5-15% of cereals loss during storage is reported but in developing countries like Nigeria, losses of 30-50% or even 100% are uncommon [3]. Most efforts at protecting grains during storage in time past were concentrated on the use of synthetic chemical insecticides. In Nigeria, various chemicals are conventionally used for the control of stored pests of maize such as pirimiphos-methyl (Acetellic dust 2%), dichlorvos, carbofuran and dioxicarb. Also fumigation with phostoxin® tablets under hermetic storage condition has been recommended [1]. However, apart from their prohibitively high costs to the peasant farmers, synthetic insecticides have other limitations including their residual effects, high mammalian toxicity, pest resistance and health hazards [4].

Current research efforts on chemical product development are being focused more on ecologically tolerable control measures including the use of inert materials, plant powder, oils and extracts. There is also increase in awareness that plants possess chemicals which naturally protect them from pests and pathogens. The tropical region is well endowed with a wide variety of these floristic species with defensive chemicals and quite a number of them have been used traditionally in protecting maize against weevil attack. Plants respond to herbivory through various morphological, biochemicals, and molecular mechanisms to counter/offset the effects of herbivore attack. The biochemical mechanisms of defense against the herbivores are wide-ranging, highly dynamic, and are mediated both by direct and indirect defenses. The defensive compounds are either produced constitutively or in response to plant damage, and affect feeding, growth, and survival of herbivores. In addition, plants also release volatile organic compounds that attract the natural enemies of the herbivores. These strategies either act independently or in conjunction with each other.

One of the earliest reports on the use of plant extracts against insects is credited to [5] who found that alkaloids like nicotine, anabasine, methyl anabasin and lupins extracted from Russian weed, *Anabasia aphylla* killed larvae of *Culex pipens* (L.) and other *Culex* species. Extensive researches have been conducted on the impact of oils extracts from other plants to control *S. zeamais*. Most naturally occurring botanical insecticides such as neem oil and other plant essential oils, defined as sources of characteristic plant odors, have long been used in Asia and most African countries for protection against grain insect pests. More recently, researchers in Africa have begun to assess their use as alternatives to fumigants and other chemical insecticides [6, 7]. However, there is dearth of information on the use of *Casuarina equisetifolia* plants against this pest. This plant is known to exhibit allelopathy [8]. Allelopathy is a biological

phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. These biochemicals are known as allelochemicals and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. Allelochemicals are a subset of secondary metabolites [9] which are not required for metabolism (i.e. growth, development and reproduction) of the allelopathic organism. Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivores [9]. The possible application of allelopathy in agriculture is the subject of much research [10]. Current research is focused on the effects of weeds on crops, crops on weeds, and crops on crops [10]. This research furthers the possibility of using allelochemicals as growth regulators and natural herbicides, to promote sustainable agriculture [11]. A number of such allelochemicals are commercially available or in the process of large-scale manufacture.

*Casuarina equisetifolia*is a drought and salt resistant tree from Australasia widely planted in the tropics and sub-tropics. In Florida, it is spreading on sandy shores and in areas disturbed by hurricanes. It forms dense stands and destroys reptiles breeding sites. It has two similar and closely related species. These species are *Casuarina glauca* and *Casuarina cunninghamiona.* However, *Casuarina equisetifolia* is the most common and widely distributed compared to the other species.

It is a tall fast growing monoecious and deciduous tree with a soft, wispy pine like appearance; it can attain a height of up to 30m or more and up to 1m in basal diameter with a symmetrical or irregular cornical crown [12]. It is often found growing together with trees common in coastal and lowland area.

The rapid growth of the tree and the fine quality wood it produces makes it one of the best and excellent firewood in the world [12]. It is also very important for control of erosion, especially on coastlands, sand dunes and on poor inland soil where it does well because of its ability to fix nitrogen. In the past, the wood for *Casuarina equisetifolia* was used extensively for making house parts, posts, fish hooks and various other tools and artifacts. It forms excellent ornamentals especially in beautification of streets, roads and highways (that is when trimmed into pleasant dense hedge).

C. equisetifolia barks contains 6-18% tannin and are used extensively in Madagascar for tanning purposes [13]. Also, the leaves and litter were found to be rich in phenolics and exhibited phytotoxic effects against the species, Bidens pinnata and Parthenium hysterophorus [14].

Casuarina equisetifolia has been shown to have therapeutic potentials; the leaf extracts exhibits anticancer properties [15]. The bark has an emetic effect, which includes vomiting or coughing to bring up phlegm [12]. The bark is also astringent for stomach, diarrhea, dysentery and nervous disorder. The seeds are anti-helmintic, antispasmodic and anti-diabetic [15]. In addition, extractions from the bark of this plant are useful in producing lotion for beri-beri and as a gargle to relieve sore throat. Another useful utilization of this plant is in the use of its wood ash in soap making.

However, pest control potentials of *Casuarina equisetifolia* remained largely untapped due to the use of broad spectrum synthetic insecticides and over utilization of other plants. Against this background, the present studies evaluated the qualitative phytochemical analysis and toxicity of *Casuarina equisetifolia* leaf extract against *Sitophilus zeamais*.

Materials and Methods

Collection and Identification of Plant Materials

Fresh leaves of *Casuarina equisetifolia* (whistling pine) were collected from the whistling pine trees located in the Department of Sociology Nnamdi Azikiwe University, Awka, Southeastern Nigeria. Their botanical identities were determined and authenticated by a taxonomist of the Department of Botany, Nnamdi Azikiwe University Awka. The plant materials were air-dried for five days and plucked off from their stem. The dried plant materials were then pulverized into fine powder using the manual hand grinder. The pulverized fine powder was then tied up in polyethylene bags before use.

Preparation of Extracts of Whistling Pine

Water Extraction

The pulverized fine powder (100g) of whistling pine was weighed into a Winchester bottle. Subsequently, 600ml of distilled water was added into the Winchester bottle containing the 100g of pulverized powder of whistling pine. The bottle was corked and shaken slightly to ensure proper soaking of the fine powder. The soaking was done for twenty four hours. Afterwards, the soaked powder was filtered using the Edman vaccum pump (Suction pump). The aqueous filtrate obtained was weighed and evaporated in a water bath at 40^{o}C to obtain the crude aqueous extract. The crude aqueous extract was also weighed and the yield was then determined.

Ethanolic Extraction

The pulverized powder was weighed (100g) and soaked in 600ml of 70% ethanol in a Winchester bottle. The soaking was allowed for twenty four hours. Afterwards, the soaked powder was filtered using the vacuum pump, the ethanolic filtrated was weighed and then evaporated in a water bath to obtain the crude ethanolic extract. The crude ethanolic extract was then weighed and its yield was determined.

Petroleum ether Extraction

The same procedures employed above were used except that 100g of the pulverized powder of whistling pine was soaked in 600ml of petroleum ether for twenty four hours.

Percentage Yield

The percentage yield of the extracts was calculated as:

$$\text{Percentage yield} = \frac{\text{weight of the crude extract x}}{\text{Weight of the sample}} 100$$

Phytochemical Screening for Each Extract

Qualitative analysis were carried out on the crude extracts of aqueous, ethanolic and petroleum ether of *Casuarina equisetifolia* at the Department of Biochemistry Laboratory (06^{o}15’11.2N to 0706’53.3E) of the Faculty of Biosciences, Nnamdi Azikiwe University (6^{o}14’N, 6^{o}14.5’N to 7^{o}8.6’E, 7^{o}9’E), Awka(6^{o}25’N,7^{o}12’E), Anambra State, Nigeria. This screening was done to detect the presence or absence of some phytochemicals in the leaf extracts of *Casuarina equisetifolia.* The phytochemical screening was done according to the method of [16].

Pest Used For the Toxicity Study

Sitophilus zeamais (maize weevil) were procured from the insectary at the laboratory of Parasitology and Entomology Department Nnamdi Azikiwe University, Awka, South-eastern Nigeria. These strains of the insect were reared on a standard maize variety, White Mangu Jos (preferred substrate) obtained from Eke Awka market, Awka. The maize was heat sterilized in an oven set at 50 °C for two hours to kill further any existing infestation. When cooled, 200g of the maize were measured into one-liter capacity Kilner jars and fifty adult insects were introduced into each jar. The insects were then allowed to reproduce inside transparent plastic buckets. Thereafter, the top end of the plastic bucket was covered with muslin cloth and secured firmly with rubber bands to allow for ventilation and free air circulation. The edges of the bench were sprinkled with vegetable oil (to prevent the crawling of other insects into the culture jars) and all culture jars placed on them. The culture was allowed to stand under ambient conditions to obtain enough F_1 progeny.

Formulation of the Leaf Extracts

Serial dilutions of all extracts (aqueous extract ethanolic extract and petroleum ether extract) were prepared separately in acetone. Acetone was used as a medium or vehicle to permeate the crude extracts into the *Sitophilus zeamais* (maize weevil). Each of the crude extracts were serially diluted with acetone in a 20ml syringe to give varying concentrations of 20%, 10%, 5%, and 2.5% 1.25% and 0.625% equivalent to 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml (adopted from [17].

Laboratory Invitro Bioassay

No. 1 Whatmann filter paper (90mm in diameter) was placed in each of the Petri-dishes (9cm in diameter) used for the experiment. The various dosage levels of the insecticide used included 20%, 10%, 5%, 2.5% 1.25% and 0.625% and each was replicated three times. Aliquots of 1ml of each concentration was evenly dispensed onto the filter paper and left for about one hour to ensure proper spreading of the solution. The acetone solvent was allowed to evaporate completely. Controls with only acetone were included and the experiment laid out in a completely randomized design (CRD). Subsequently, 10 unsexed two week old adults of *S. zeamais* were introduced into each Petri-dish by means of a mouth operated aspirator. Each of the Petri-dishes was covered with its lid to prevent escape of the insects. Mortality counts were taken for 24 hours.

Data Collection

A daily count of dead *Sitophilus zeamais* (maize weevil) were taken, the maize weevil was certified dead when probed with a pin on the abdomen and no response was shown. Data collected from the two days post-treatment observations were used to determine mortality. Percentage mortality of the adult beetles was determined as number of dead insects divided by total number of insects multiplied by 100.

Statistical Analysis

Log- probit regression analysis was carried out [18] for determining LD_{50}. The data collected were analyzed using simple factorial ANOVA model in SPSS version 17 for Windows statistical package [19]. Treatments with significant differences were compared and separated at 0.05% level of significance using LSD values at $P<0.05$.

Results

The result of the percentage yield of the various extracts used (water, ethanol and petroleum ether extracts) is shown in Table 1. Percent yield of all extract as shown in Table 1 is of the order, ethanolic extract yield > petroleum ether extract yield > water extract yield. The qualitative phytochemical screening of these plant extracts showed the presence and absence of certain phytochemicals in these extracts (Table 2). The phytochemicals present are mainly saponin, flavonoids, anthracene, cyanogenic glycoside and alkaloids.

The effect of 12 hour exposure time of different concentrations of the various crude extracts of whistling pine on *Sitophilus zeamais* are presented in Tables 3-5 respectively. The results showed that the aqueous, ethanol and petroleum ether extracts of *Casuarina equisetifolia* exhibited significant ($P<0.032$, 0.035 and 0.026 respectively) levels of individual toxicity to adults of *Sitophilus zeamais*. There were much greater responses at the higher concentrations of toxicants and mortality of 0.25% was observed in the control. Therefore, at 200µg/ml of these extracts, mortalities were respectively 80%, 77% and 85%. However, comparing the effect of the three extracts in causing mortality of *Sitophilus zeamais*, it was observed that aqueous, ethanolic and petroleum ether extracts of *Casuarina equisetifolia* caused mortalities of 59%, 57.9% and 67.5% respectively. Hence, there was no significant difference ($P> 0.472$) between these extracts.

The log-probit regression analysis revealed that after 12 hours of exposure, the LD_{50} value of the aqueous, ethanolic and petroleum ether extracts of *Casuarina equisetifolia* on the adults of *Sitophilus zeamais* were respectively16.81µg/ml, 18.59µg/ml and 6.07µg/ml.

Table 1: Different Extracts of Whistling pine and their percent yield

Extract	Percentage Yield (%)
Aqueous (water) extract	6.41
Ethanol extract	12.90
Petroleum ether extract	6.50

Table 2: Phytochemical Analysis of the different extracts of Whistling pine *Casuarina equisetifolia*

Phytochemicals	Ethanolic Extract	Aqueous Extract	Petroleum Extract
Saponins	+	+	+
Tanins	+	+	-
Flavonoids	+	+	+
Cyanogenic glucosides	+	+	+
Alkaloids	-	+	-
Anthracine glycosides	+	+	+

Table 3: Effect of 24 hour exposure to different concentrations of aqueous extract of *Casuarina equisetifolia* on *Sitophilus zeamais*

Dose (µg/ml)	Log dose	24 hour of exposure R1 R2	R3 R4	Mean Mortality	% Mortality	Probit
200	2.30	9 9	7 7	8.0±0.6	80.0	5.8416
100	2.00	6 9	8 5	7.0±0.9	70.0	5.5244
50	1.69	9 6	9 3	6.7±1.4	67.0	5.4399
25	1.39	5 8	6 3	5.5±1.0	55.0	5.1257
12.5	1.09	5 6	4 3	4.5±0.7	45.0	4.8743
6.25	0.79	5 4	3 3	3.75±0.5	37.5	4.6631
Control	0.0	0 0	0 0	0.0±0.0	0.0	0.0000

Means of four replicates (±s.e); P= 0.032

Table 4: Effect of 24 hour exposure to different concentrations of ethanolic extract of *Casuarina equisetifolia* on *Sitophilus zeamais*

Dose (µg/ml)	Log dose	24 hour of exposure R1 R2	R3 R4	Mean Mortality	% Mortality	Probit
200	2.30	7 9	5 10	7.75± 1.1	77.5	5.7554
100	2.00	8 7	7 5	6.75± 0.6	67.5	5.4538
50	1.69	8 3	7 7	6.25± 1.1	62.5	5.3180
25	1.39	5 8	3 7	5.75± 1.1	57.5	5.1891
12.5	1.09	5 6	4 4	4.75± 0.5	47.5	4.9373
6.25	0.79	4 3	3 4	3.50± 0.3	35.0	4.5462
Control	0.0	0 0	1 0	0.25± 0.01	2.50	3.0400

Means of four replicates (±s.e); P= 0.035

Table 5: Effect of 24 hours exposure to different concentrations of Petroleum ether extract of *Casuarina equisetifolia* on *Sitophilus zeamais*

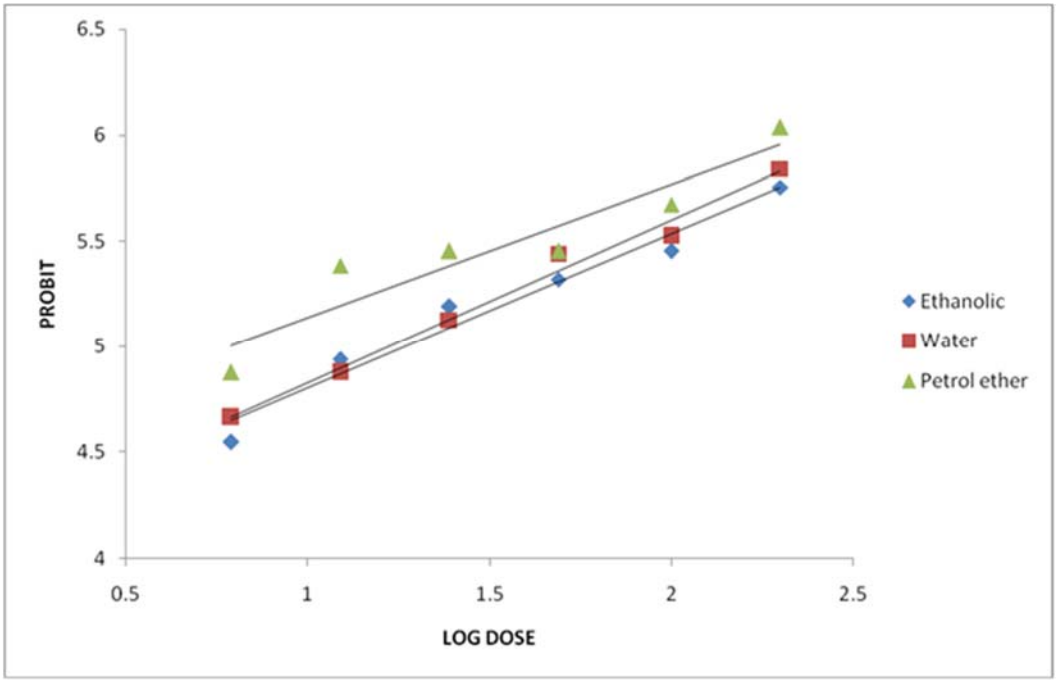
Dose (µg/ml)	Log dose	24 hour of exposure R1 R2	R3 R4	Mean Mortality	% Mortality	Probit
200	2.30	9 8	8 9	8.50± 0.2	85.0	6.0364
100	2.00	9 8	5 8	7.5± 0.6	75.0	5.6745
50	1.69	9 8	6 4	6.75± 1.1	67.5	5.4538
25	1.39	7 6	6 8	6.75± 0.5	67.5	5.4538
12.5	1.09	8 6	7 5	6.5± 0.7	65.0	5.3853
6.25	0.79	3 5	6 4	4.50± 0.7	35.0	4.5462
Control	0.0	1 0	0 1	0.25± 0.01	2.50	3.0400

Means of four replicates (±s.e); P= 0.026

Table 6: Mean percentage mortality of *Sitophilus zeamais* at different concentrations of different extracts of *Casuarina equisetifolia* after 24 hours of exposure

Dose (µg/ml)	Water	Ethanolic	Petroleum ether	Mean mortality	% Mean mortality
200	8.0± 0.6	7.75± 1.1	8.50± 0.2	8.08± 0.6	80.8
100	7.0± 0.9	6.75± 0.6	7.5± 0.6	7.08± 2.1	70.8
50	6.7± 1.4	6.25± 1.1	6.75± 1.1	6.57± 1.2	65.7
25	5.5±1.0	5.75± 1.1	6.75± 0.5	6.00± 0.9	60.0
12.5	4.5±0.7	4.75± 0.5	6.50± 0.7	5.25± 1.6	52.5
6.25	3.75± 0.5	3.50± 0.3	4.50± 0.7	3.92± 0.5	39.2
Mean mortality % mortality	**5.90± 0.8**	**5.79± 0.8**	**6.75± 0.6**		
Control	59.0	57.9	67.5	0.17± 0.0	
LSD= 0.509	0.0	0.25± 0.01	0.25± 0.01		

Means of four replicates (±s.e), P= 0.472



Regression equation:
Water extract: $y = 0.7296x + 4.0739$; LD_{50}= 16.81µg/ml; $R^2 = 0.9637$
Ethanolic: $y = 0.6311x + 4.5057$;LD_{50}=18.59µg/ml;$R^2 = 0.879$
Petroleum ether: $y = 0.7706x + 4.0555$;LD_{50}=6.07µg/ml; $R^2 = 0.9869$

Discussion

Studies were conducted on the phytochemistry and toxicity of whistling pine, *Casuarina equisetifolia*. Results from the phytochemical screening showed that each extract contained at least one of the paramount phytochemical or bioactive constituents. This agreed with [20] who reported some paramount bioactive constituent of plants. Similarly, results from phytochemical screening agreed with the reports of [12] that the leaf and bark extracts this plant contained valued amount of tannins which are used for a variety of uses such as dying of cloths, nets and lots more. It is therefore suggested that the pesticidal actions of whistling pine plant may be attributed to the presence of the phyto-chemicals present in the plant.

In the present study, the sequence of percent yield is however opposite of their strength in pesticidal activity as ethanolic extract showed a higher estimated lethal dose of (18.59µg/ml) followed by water extract (16.81µg/ml) and petroleum ether extract (6.07µg/ml). A study carried out by [21] showed that methanol extract of *Securidacalonge pedunculata* on *Sitophilus zeamais* had an estimated LD_{50} value between 34µl and 36µl. These doses were less toxic when compared to LD_{50}values of 18.59µg/ml, 16.81µg/ml, and 6.07µg/ml respectively for ethanol, aqueous and petroleum ether extracts of whistling pine on *Sitophilus zeamais*. From each of the regression analysis shown, coefficient of determination (r-square value) showed how much variability or percentage mortalities of the *Sitophilus zeamais* (maize weevil) that could be attributed to the logarithm of the doses. Hence, at the highest concentration of 200µg/ml of petroleum ether extract, r-square value showed that 85% mortality of the *Sitophilus zeamais* was attributed to the log of the doses used. In the same vein, for ethanolic extract, 77.5% mortality of the *Sitophilus zeamais* was attributed to the log of doses given. Similarly, for the mortality was attributed also to the log of doses.

Hence results obtained from toxicity study demonstrate the attractive potentials of the three extracts of *C. equisetifolia* in causing mortality in the population of maize weevil, *Sitophilus zeamais*. The results also showed that petroleum ether extract had the highest toxicity when compared with the other two extracts. This may perhaps be attributed to the presence of the phytochemicals it contained. However, statistical analysis showed that the three extracts were not significantly ($P > 0.05$) different, this suggests that the three extracts have similar activity when used as solvent in the extraction of whistling pine.

The search for the allelochemicals responsible for toxicity of *C. equisetifolia* could yield some result from the profile of the pytochemicals in the various extracts. The petroleum ether extract seemed to be the most potent of the three extracts. The chemicals identified to be present in the ether extracts were also present in the water and aqueous extracts. The toxic molecules could therefore be saponin, flavonoid, cyanogenic glucoside, anthracine glycoside or their combinations. Saponins have been shown to be toxic to insects possessing antifeedant and growth-regulatory properties. They are also able to disturb moulting and induce mortality. Their toxicity to insects is based on their interaction with cholesterol causing a disturbance in the synthesis of ecdysteroids. They are equally protease inhibitors or cytotoxic to some insects [22]. Their cytotoxic and haemolytic activities to mammals limit their use as insecticides. They are also limited by ready loss of molecules associated with the agluycone. Similarly, their hydrophilic properties limit penetration through the lipophilic barrier of insect cuticle [22]. The saponin 3 GLCA-28-AraRhaxyl-medicagenale isolated from the seed of *Medicago truncatula* had no effect on the worm, *Caenorhabditis elegans* and the bacteria *Escherichia coli*. However, it had an inhibitory effect on growth of *Saccharomyces cerevisiae* at concentration higher than 100µg/ml. The purified molecule was toxic to the

adults of rice weevils at concentrations down to 100µg/g of food. This was shown not to be sensitive to other insects tested such as coleopteran *Tribolium castaneum* and sta insect cultured cells [23]. Butanol fractions of the leaves of *Castanospermum austral* Cunn and Fraser were toxic to *Callosobruchus analis* [24] when given intravenously to higher animals and saponins components were found to be highly toxic. However, their toxicity is very much lower when administered orally [25]. It is noteworthy that many of the saponins of foods and feeding stuff are apparently without any significant oral toxicity [24].

Also, flavonoids have pesticidal activities, [26] reported that insects can discriminate among flavonoids and that the compounds can modulate the feeding and oviposition behavior of insects. In addition, [27] reported that pinocembrin, a widely distributed flavonoids could be used for insect control in a stimulo-deterrent diversionary strategy. Flavonoids isolated from the aqueous extracts of leaves of *Ricinus communis* L (Euphorbiaceae) showed insecticidal activity against *Callosbruchus chinensis* (Coleoptera: Bruchidae) [28]. Similarly, flavonoids isolates from aqueous foliar extracts of Annona sqamosa demonstrated anti-microbial activity against all the common microbial contaminants of pulses and caused 80% insecticidal activity against the store grain pest beetle, *C. chinensis* [29]. Flavonoid extracts of the plant, *Vitex negundo* and *Andrographis paniculata* showed larvicidal activity against mosquitoes (*Aedes aegypti* and *Anopheles stephensi* (Liston) [30]. [26] was of the opinion that flavonoid modulate the feeding and oviposition behavior of insects.

Cyanogenic glycosides have also been shown to be destructive to herbivores. When plant tissue is disrupted by herbivore, cyanogenic glycosides establish contact with B-glucosidase and alpha hydro-xynitrile lyase which hydrolyses the cyanogenic glycoside generating hydrogen cyanide which is toxic thereby providing plants with a defense against herbivores and other pathogenic organisms [31]. They are inhibitors of the electron transport chain. Cyanogenic glycosides function effectively as deterrents to herbivores [32] [33]. Tannins have a strong deleterious effect on phytophagous insects and affect the insect growth and development by binding to the proteins, reduce nutrient absorption efficiency, and cause midgut lesions [34]. Tannins are astringent (mouth puckering) bitter polyphenols and act as feeding deterrents to many insect pests. In addition, tannins also chelate the metal ions, thereby reducing their bioavailability to herbivores. When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. Condensed tannins are oligomeric or polymeric flavonoids, also known as proanthocyanidins. They have diverse structures and functions. They act as feeding deterrents against some insects such as, *Lymantria dispar* (L.), *Euproctis chrysorrhoea* (L.) and *O. brumata* [35]. Condensed tannins such as (+) -catechin, (+) - gallocatechin, and vanillin in leaves of *Quercus robur* L. inhibited winter moth larvae, *O. brumata* [35]. Procyanindin polymers have been found as feeding deterrent to *Aphis Craccivora* (Koch) in groundnut [36]. Condensed tannins from Alaska paper birch (coated on birch leaves at 3% dry weight.) reduced the pupal mass and survival of *Rheumaptera hastata* (L.) larvae [37].

Anthracene (anthraquinone) glycosides were present in the extracts of the three solvent systems and might have played important roles in the mortality of the insect. Anthraquinones are both larvicidal and insecticidal in their action [38, 39]. They are also considered as pesticides in a study by [40, 41, 42]. Saponins, flavonoids, cyanogenic glycosides and anthracene glycosides are pesticidal compounds and were present in the three different solvent system in the present study. Therefore, the pesticidal properties of the whisthling pine could be as a result of the presence of these compounds acting individually or synergistically.

Conclusion

Maize production in Nigeria and other parts of the developing world is left in the hands of peasant farmers who cannot afford high cost of synthetic insecticides for pest control. It may therefore follow from the present study, that *Casuarina equisetifolia* has considerable potentials in the control of maize weevils. Plant extracts have in the past shown to be effective against this storage pest. Among their benefits is that they are environmentally friendly, less expensive and less toxic systematically compared to synthetic insecticides.

To this end, since these phytochemical which are present in the leaf extract of plants have physiological actions on the human body [43], the phytochemicals present in the leaf extract of whistling pine (*Casuarina equisetifolia*) could be fully harnessed and used as alternative means to control pests especially *Sitophilus zeamais*, maize weevils which affects maize grains. This can in a large extent, reduce the low levels of chemical pesticide residues, resistance and toxicity to humans through their diet, as this, overtime can develop into various heath complications. Similarly, further studies can be carried out to determine if the pesticidal effect of the leaf extracts of whistling pine is due to a synergetic action of all the phytochemicals present or if it is attributed to a particular phytochemical. It is suggested from the present study that the active principle in the phytochemicals of *Casuarina equisetifolia* with toxicity against *Sitophilus zeamais* Motschulsky could be a saponin, flavonoid, cyanogenic glycoside or anthracene glycoside. Similarly, their toxicity could be due to synergistic effects of the three phtochemicals.

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