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ACTIVITY OF EXTRACTIVES FROM *Albizia anthelmintica* BRONGU. AND *Teclea trichocarpa* ENGL. AS BIORATIONAL ALTERNATIVES TO CONTROL THE MAIZE WEEVIL (*Sitophilus zeamais*)

Mwangi S. K. E.[1], **Keriko M. J.**[1], **Tarus K. P.***[2], **Machocho K. A.**[3], **Chhabra C. S.**[3], **Wanyonyi A.**[3]

[1]Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000 Nairobi, Kenya.

[2]Department of Chemistry and Biochemistry, Chepkoilel University College (Moi University), P.O. Box 1125, Eldoret, Kenya.

[3]Department of Chemistry, Kenyatta University, P.O. Box 43844, Nairobi, Kenya.

Abstract

Organic solvent extractives and isolated compounds of two Kenyan plants *Albizia anthelmintica* and *Teclea trichocarpa* were evaluated for insecticidal activity against maize weevil, *Sitophilus zeamais* Motchulsky, and for brine shrimp lethality. Hexane extract of the leaves of *Teclea trichocarpa* displayed mild brine shrimp toxicity (LD_{50} =153.2 μg/ml), while the other extracts showed no significant toxicity (LD_{50}> 240 μg/ml). Both hexane and dichloromethane extracts of leaves of *Teclea trichocarpa* showed the highest mean percentage adulticidal activity at almost all doses. Three-acridone alkaloids melicopicine **1**, normelicopicine **2**, arborinine **3** and the furoquinoline, skimmianine **4** were isolated from *Teclea trichocarpa* and had previously been reported from the other *Teclea* species were noted to have low mortality of between 10% and 22% at 0.1% w/w of the compounds against *Sitophylus zeamais*. The other metabolites were the two terpenoids, β-sitosterol **5** which showed higher activity (12.5±2.5) than α-amyrin **6**. The results are discussed with regard to the use of the two plants as suitable and sustainable alternatives to synthetic insecticide in maize grain storage.

Key words: brine shrimp; *Albizia anthelmintica*; *Teclea trichocarpa*; adulticidal activity; cytotoxicity; maize weevil, *Sitophilus zeamais*

[1]Corresponding author E-Mail address: taruskosgei@gmail.com
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Introduction

Sufficient production of food grains pose a big challenge to man. A variety of techniques have been applied to meet the challenge. One of the strategies is to improve efficiency in grain production and post harvest practices to ensure that food losses are minimized if not eliminated and that the grains produced is of good quality and safe for human consumption. Prophylactic methods have not constrained the pests to acceptable levels. Synthetic pesticides have been used against post-harvest pests. However, the persistence, resistance, the cost and availability of these conventional insecticide and potential health hazard both to the consumers and to the environment have necessitated continued use of local plant products (Negahban, *et al*., (2007). Although these botanicals have been in use since time immemorial their efficacy, safety and their active principles deserve more attention (Balandrin *et al*., 1985; Bakkali *et al*., 2008). Plants have been screened for repellency and protectants against maize weevil (Hassanali and Lwande, 1989; Hassanali *et al.*, 1990; Lwande *et al*., 1983, Ndungu *et al.*, 1999 and Bekele *et al*., 1996) With the rising concern for environmental safety there has been a renewed interest in the use of naturally occurring substances as pesticides, including plant bioactive compounds. Many natural insecticides have active control agents for a variety of insect pests. Maize weevil (*Sitophilus zeamais* Motchulsky) is one of the most damaging insect pests of stored maize grains and there are also some reports on the resistance of the maize weevil against many synthetic insecticides. Therefore, searching for new botanical insecticides for controlling the maize weevil is still essential. In present work, two Kenyan native plants' extracts were investigated for their insecticidal property. The bioactive compounds of the selected plants' extracts were purified, identified and confirmed for their insecticidal effectiveness against maize weevil.

Albizia anthelmintica Brongu. and *Teclea trichocarpa* Eng. belong to the plant families Mimosaceae and Rutaceae, respectively. Traditionally, *A. anthelmintica* is widely used in East Africa by resource poor smallholder farmers and pastrolists to treat their livestock against internal parasites and against grain pests (Kokwaro, 1993). Several members of this genus have shown broad range of activities, ranging from antibacterial to pest control. Water extract of *A. julibrissin* has shown insect feeding deterrence against *Tribolium castana* on leaf disc with a mean deterrence of 50% (Doskotch *et al*., 1977). Essential oil of *A. lebbek* bark showed insect repellant activity (12.5% against *Apis florea*) (Gupta, 1987), while *A. mollis* bark showed insecticide activity against tobacco beetle (Sievers, 1949). Inhabitants of some arid and semi-arid region particularly from North Rift (Kerio Valley), Kenya are known to use the root bark of *A. anthelmintica* as grain protectant during storage. *Teclea trichocarpa* is reported to be used by traditional healers belonging to the Akamba tribe for malaria treatment and as anthelmintic, while the Giriama tribe steam the leaves and inhale the vapour as a cure for fever (Watt and Breyer-Brandwijk, 1962). The plant bark of the plant showed antifeedant activity against the African armyworm, *Spodoptera exempta* (Lwande *et al*., 1983).

Materials and methods

General

Melting points were determined on an electro thermal melting point apparatus and expressed in degree centigrade (^oC). IR spectra were taken in KBr pellets and recorded on a Shimazdu (model FT-IR-8400 CE) with absorption given in wave numbers (cm^{-1}). NMR spectra were recorded at room temperature on a Bruker DPX- 400 NMR. The spectra were recorded in $CDCl_3$ as the solvent and TMS as the internal standard. The chemical shifts reported in δ (ppm) units relative to TMS signal. TLC was performed on aluminium sheets pre-coated with silica gel 60 F_{254} (Merck) with a 0.2 mm layer thickness, Preparative TLC was done using normal phase silica gel (F_{254} Merck) pre-coated on aluminium plate (20 x 20 cm) and a layer thickness of 0.25 mm. Spots on chromatograms were examined under UV light (254 and 366 nm), and by anisaldehyde and dragendorff's visualization reagents. VLC column were packed with thin

layer chromatography silica gel 60 (6-35 microns mesh, ASTM) and column chromatography on silica gel 60 (0.040-0.063 mm 230-400 mesh, Merck). Solvents were laboratory grade and were obtained from Kobian Kenya Ltd and were double distilled before use.

Plant materials

The root barks of *A. anthelmintica* were collected from Kiptoro area, Keiyo District, Elgeiyo Marakwet County in August 2009. Leaves of *T. trichocarpa* were collected from Maina, Marakwet District in August 2010. Authentication was done by a plant taxonomist, Botany Department, Mr. Karimi, Kenyatta University. Reference specimens of the plants have been deposited at the Herbarium at Kenyatta University, Nairobi and given voucher specimen numbers SM/KU/AA/08/3 and SM/KU/TT/08/3 for *A. albizia* and *T. trichocarpa*, respectively.

Extraction, fractionation and isolation

The air-dried, powdered root bark (2.7 kg) and leaves, (2.0 kg) of *A. anthelmintica* and *T. trichocarpa*, respectively, were sequentially extracted with 7.5 litres each of hexane, dichloromethane (CH_2Cl_2) and methanol exhaustively at room temperature. Each extract was concentrated in vacuo under reduced pressure at 45^{o}C using a rotatory evaporator and the yields and percentage yields are presented in Table 1. Hexane and dichloromethane extracts were selected for preliminary screening because of their low toxicity against brine shrimp.

Leaves of *T. trichocarpa* yielded a yellow paste of hexane extract (25.0 g) and a green paste of dichloromethane extracts (48.5 g). These were subjected to vacuum liquid chromatography (VLC) separation on silica gel 60 each at a time, eluted with *n*-hexane with increasing amount of CH_2Cl_2 and later increasing amount of methanol in CH_2Cl_2 up to 1:5, 55 and 65 fractions, respectively, were collected and from TLC analysis similar fractions pooled together. UV active spots on TLC were considered for further separation. From the hexane extract of *Teclea trichocarpa*, fraction (31-37) 2128.9 mg that eluted with *n*-hexane: CH_2Cl_2 (1:4) was further chromatographed on sephadex and eluted with a mixture of CH_2Cl_2 and methanol (1:1) to give 32 sub-fractions, sub fraction 31-32 on crystallization in methanol afforded α-amyrin [**6**] (32.7 mg). Fractions 34-38 (8034.1 mg) that eluted with 2:3 (*n*-hexane: CH_2Cl_2) was loaded onto VLC and eluted with hexane and increasing amount of CH_2Cl_2 and then increasing amount of methanol. Twenty-eight sub fractions were obtained from which fraction 13-21 was further chromatographed on silica gel and eluted with 2:3 (*n*-hexane: CH_2Cl_2) this yielded β-sitosterol [**5**].

From VLC of the CH_2Cl_2 extract, fraction 34-40 (1014 mg) was loaded onto sephadex column and eluted with CH_2Cl_2: methanol (1:1) to give 16 sub fractions. Sub fraction 3-4 was subjected to column chromatography and eluted with ethyl acetate: CH_2Cl_2 (1:3). This afforded 38.1 mg of melicopicine [**1**]. Sub Fraction 5-18 showed UV active spots, column chromatography of this fraction eluted with CH_2Cl_2: ethyl acetate (2:1) mixture gave 25 fractions from which sub fractions 9-11, 12-15 and 21-25 were followed. Sub fractions 9-11 were subjected to preparative thin layer chromatography, this afforded skimmianine [**4**] (22.2 mg), sub fractions 21-25 was subjected to preparative thin layer chromatography. This afforded two compounds Melicopicine [**1**] (64.8 mg), and normelicopicine [**2**] (46.9 mg). Fraction 41-42 from VLC was subjected to column chromatography and eluted with ethyl acetate: CH_2Cl_2 (1:9). The sub fraction (5-8) that eluted with CH_2Cl_2: ethyl acetate (1:1) afforded yellow needle like compound, arborinine [**3**] (99.0 mg) on partitioning between methanol and CH_2Cl_2 (2:1). Sub fraction (18-27) that eluted with CH_2Cl_2: ethyl acetate (2:3) which on crystallizing in methanol yielded orange crystals [**7**] this was not characterized due to lack of enough spectra data.

Toxicity testing against the Brine shrimp

Hatching shrimp

Brine shrimp eggs, *Artemia salina* leach were hatched in artificial seawater prepared by

dissolving 38 g of sea salt (Sigma chemicals Co., UK) in 1L of distilled water. After 48 hrs incubation at room temperature (22-29^{o}C), the larvae (nauplii) were attracted to one side of the vessel with a light source and collected with pipette. Nauplii were separated from eggs by aliquoting them three times in small beakers containing seawater.

Brine Shrimp Test

The bioactivity of the extracts was monitored by the brine shrimp lethality test (Meyer et al., 1982). Samples were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial sea salt water so that final concentration of DMSO did not exceed 0.05%. Fifty microlitres of sea salt water was placed in all the 96-well microtitre plates. Fifty microlitres of 4000 ppm of the plant extract was placed in the row one and a two-fold dilution carried out down the column. The last row left with sea salt water and DMSO only served as the drug free control. Hundred microlitres of suspension of nauplii containing 10 larvae was added into each well and incubated for 24 h. the plates were then examined under a microscope (12.5 x) and the number of dead napulii in each well counted and recorded. Lethality concentrations fifties (LC_{50} values) for each assay were calculated by taking average of three experiments using a Finney probit analysis program on an IBM computer (McLaughlin et al., 1991).

Adulticidal

Sitophilus zeamais culture

Adult *S. zeamais* was obtained from a laboratory colony reared under ambient conditions with natural photoperiods on untreated (insecticide-free) maize obtained from farmers stock in Keiyo District, and maintained at National Agricultural Research Laboratories (NARL), Kenya. The food media used was whole maize grains. Fifty pairs of mixed sex *S. zeamais* were introduced following the methods of Bekele and Hassanali (2001) into 1 litre glass jars containing 400 g weevil susceptible maize grains (Bende white). The mouths of the jars were then covered with nylon mesh held in place with rubber bands. Freshly emerged adults of *S. zeamais* were then used for the experiments (Asawalam and Emosairue, 2006).

Assessments

Bioassay test was carried in the laboratory to determine the efficacy of the botanicals under different dosage levels against *Sitophilus zeamais*. Three rates of each plant extracts, a synthetic insecticide Actellic super 2% dust at
0.05 % w/w and untreated control were used as treatment to show adulticidal activity against maize weevil. For pure compounds and blend mixtures the concentrations were double, equal and half that of positive control (Actellic super). The test samples were mixed with talc thoroughly and the dust were admixed with 50 g of maize held in jam jags covered with ventilated lids. To ensure a thorough admixture, the grain was put in plastic jam jag, dust applied and top lid replaced. The grain was then swirled within the jag until a proper admixture was realized. Ten pairs of 5-day old *S. zeamais* adults were introduced into treated and untreated maize grains and confined by perforated lids placed over muslin cloth that was held in place by a rubber band. The design of the experiment was completely randomized design (CRD) with three replications. The treatments were kept on a laboratory room temperature for seven days before mortality was assessed. Percentage mean mortality for *S. zeamais* was recorded after seven days exposure period (Bekele *et al*., 1996).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) procedure (SAS, 2000) and significantly different ($P>0.05$) means were separated by using Tukey's studentised range (HSD) test.

Results and Discussion

The potential of using the *Albizia anthelmintica* and *Teclea trichocarpa* extracts and thereof bioactive components as protectant for stored maize grains against maize weevil, and toxicity against brine shrimp, were the main objectives for this study. On extraction with various organic solvents, the yields were as shown in Table 1.

Table 1: Plant material with percentage yield of extracts (dry weights).

Plant	*A. anthelmintica*			*T. trichocarpa*			
Extract	Hexane	CH_2Cl_2	MeOH	Hexane	CH_2Cl_2	EtOAc	MeOH
Yields (g)	8.2	29.6	142.3	26.0	52.4	41.6	199.2
Percentage yields	0.3	1	4.9	1.3	2.6	2.1	10

From the preliminary adulticidal test against maize weevil the methanol and ethyl acetate extracts showed no activity. Therefore, hexane and dichloromethane crude extracts were further studied. The hexane and dichloromethane crude extracts of *A. anthelmintica* and *T. trichocarpa* were tested for their toxicity against brine shrimp lethality assay. The results are shown in Table 2.

Table 2: The mean LD_{99} and LD_{50} values ± s.d. for plant extracts screened against brine shrimp (*Artemia salina*, Leach).

Plants extract	A*a*BH	A*a*BD	T*t*LH	T*tLD*
LD_{99}	16.5 ± 0.7	24.4 ± 1.7	19.7 ± 2.5	9.7 ± 1.8
LD_{50}	692.9 ± 0.3	375.5 ± 0.6	153.2 ± 1.0	279.9 ± 0.7

A*a*BH = *A. anthelmintica* bark, hexane extract; A*a*BD = *A. anthelmintica* bark, dichloromethane extract
T*t*LH = *T. trichocarpa* leaves, hexane extract; T*tLD* = *T. trichocarpa* leaves, dichloromethane extract

The hexane extracts of *T. trichocarpa* leaves with LD_{50} values of 153.2 µg/ml was considered active, while CH_2Cl_2 extracts of *T. trichocarpa* leaves showed mild toxicity against brine shrimp with LD_{50} values of 279.9 µg/ml. The hexane and CH_2Cl_2 extracts of *A. anthelmintica* bark showed low toxicity against brine shrimp with LD_{50} values of 693 and 376 µg/ml, respectively (Table 2). A Crude is considered active up to a concentration of 240 µg/ml (Meyer *et al*., 1982). Since brine shrimp test is an indicator of toxicity, various pharmacological actions, and pesticidal effects (Meyer *et al*., 1982), it was deduced that both hexane and CH_2Cl_2 extracts of *T. trichocarpa* had the better bioactivity against brine shrimp. The crude extracts (hexane and dichloromethane) of *A. anthelmintica* and *T. trichocarpa* were subjected to adulticidal test against maize weevil. The effects of different doses of hexane and CH_2Cl_2 extracts on maize weevil after seven days were determined and LD_{50} values computed and the results are summarized in Table 3.

Table 3: Percent mortality of adult *S. zeamais* on maize grains treated with different concentrations of hexane and CH_2Cl_2 extracts from *A. anthelmintica* and *T. trichocarpa* against maize weevil (*S. zeamais*)

Plants Extract	100 ppm	200 ppm	400 ppm	600 ppm	800 ppm
*Aa*BH	5.0 ± 5.0 c	10.0 ± 5.0 c	35.0 ± 5.0 c	45.0 ± 5.0 c	55.0 ± 5.0 b
*Aa*BD	15.0 ± 5.0 bc	15.0 ± 0.5 c	60.0 ± 0.0 d	65.0 ± 5.0 b	80.0 ± 10.0 a
*Tt*LH	25.0 ± 5.0 b	75.0 ± 5.0 b	75.0 ± 5.0 b	100.0 ± 0.0 a	100.0 ± 0.0 a
*Tt*LD	25.0 ± 5.0 b	40.0 ± 0.0 d	45.0 ± 5.0 c	70.0± 10.0 b	95.0 ± 5.0 a
Actellic super	75.0 ± 5.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Negative control	5.0 ± 5.0 c				

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test).

From the results in Table 3, it is evident that adulticidal activities are dose dependent for both plant extracts. The most active extracts, with the highest mean adulticidal activity at almost all doses were hexane and CH_2Cl_2 extracts of *T. trichocarpa* leaves, showing 100% and 95% adulticidal at 600 and 800 ppm, respectively, which were comparable to the positive control, actellic super, a synthetic pesticide at the recommended rate of 0.05%, which is in the market today. *A. anthelmintica* extracts (*Aa*BD and *Aa*BH) displayed lower adulticidal activity, with activity of between 10 and 15% comparable to treatment free (negative control). However, above 400 ppm activity was registered with *Aa*BD at 800 ppm exhibiting 80% mortality that was comparable to the actellic super at 95% confidence level.

The fact that, the crude extracts at high concentration had significant mean percentage adulticidal against maize weevil is interesting and led support to the traditional use of this plant material as grain protectant against destructive pests. Both extracts represents an attractive candidate for field evaluation as a protectant of stored maize. It is also expected that, the crude plant extract could offer suitable and sustainable alternative to synthetic pesticide. However, conclusive recommendation of their use can only be made after exhaustive analysis of the effect of these crude extracts on the quality of grain and safety.

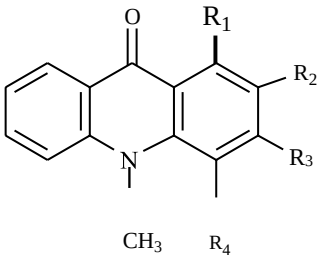
Although the trend of adulticidal effects are dose dependent for both plant extracts, the low adulticidal activity of *A. anthelmintica* could partly be explained by the circumstances that may be it is used in protection, not for their insecticidal effect but may be due to feeding and ovipositional deterrence or even as insect growth regulators. The other reason is that the local communities use a mixture of plants for grain protection, and this mixture could be active due to synergistic effects.

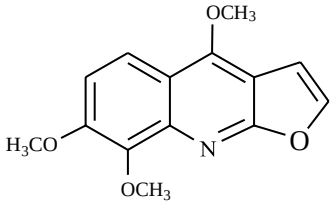
Examining Tables 2 and 3, the brine shrimp lethality and adulticidal activity results for the crude extracts, respectively, the extracts of *Teclea trichocarpa* showed higher toxicity as well as adulticidal activity against maize weevil when compared to *A. anthelmintica*. It was evident that toxicity against brine shrimp may be a basis of deducing an active adulticidal extract.

The TLC profile of *T. trichocarpa* revealed the presence of several UV active and fluorescing compounds in the crude extracts. Chromatographic separation of the hexane and dichloromethane extracts afforded two terpenoids (α-amyrin and β–sitosterol) and four alkaloids; melicopicine, arborinine, normelicopicine (acridone alkaloids) and skimmianine (furoquinoline alkaloid). The

structures of the compounds were characterized and identified by their IR., ^{1}H NMR and ^{13}C NMR, and comparing with data of authentic samples α-amyrin (Mahato and Kundu, 1994), arborinine (Bergenthal *et al*., 1979), melicopicine (Rasoanaivo *et al*., 1999), normelicopicine (Muriithi *et al*., 2002), β–sitosterol (Knight, 1974) and skimmianine (Funayama, and Cordell, 1984) and are shown in Figure 1.

Figure 1: Compounds isolated from *Teclea trichocarpa*



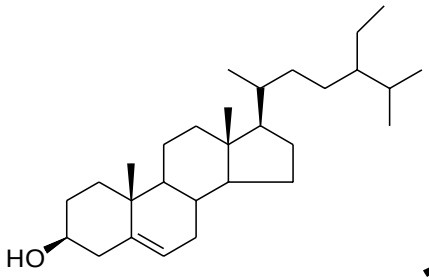


Skimmianine (4)

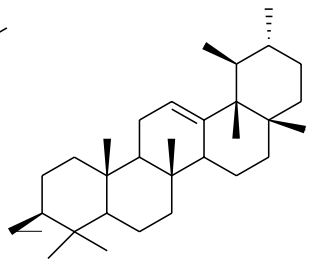
Melicopicine (1): (R_1, R_2, R_3, R_4 = OCH_3)

Normelicopicine (2): (R_1= OH, R_2, R_3, R_4 = OCH_3)

Arborinine (3): (R_1=OH, R_2, R_3 = OCH_3; R_4 = H)



β-Sitosterol (**5**)



α-Amyrin **6**

The six compounds thus isolated from hexane and CH_2Cl_2 extracts were tested against maize weevil (adulticidal) at different doses. Actellic super, a synthetic insecticide, was used as positive control and no treatment was included as the negative control. Results are summarized in Table 4.

From the results in Table 4, the mean percentage adulticidal was dose dependent. However, all the compounds showed low activities at both 0.1 and 0.05 w/w, being between 10% to 22% at 0.1% w/w when compared to actellic super. The adulticidal activity of the three-acridone alkaloids melicopicine [**1**], normelicopicine [**2**] and arborinine [**3**] were noted to be low with the mortality being between 10% to 22% at 0.1% w/w of the compound. Comparing the two terpenoids, 3β–sitosterol [**5**] showed higher activity (12.5±2.5) than α-amyrin [**6**] (5.0±0.0) at 0.05 w/w and were significantly different ($p < 0.05$). Although the two compounds share a common biosynthetic pathway, the difference in activity may be attributed to their structural difference. β–sitosterol has also been reported to show weak feeding inhibitory activities against the larvae of *Chilo partellus* (Tsanuo, 1992). This compound could be a better protectant against destructive pests due to its feeding inhibitory and adulticidal activities.

Table 4: Mean percentage adulticidal ± s.d. of isolated compounds from *T. trichocarpa* against maize weevil.

Compounds	Mean percentage adulticidal at different concentration in % w/w	
	% 0.1 w/w	% 0.05 w/w
Melicopicine [**1**]	12.5 ± 2.5 c	2.5 ± 2.5 c
Normelicopicine [**2**]	15.0 ± 0.0 a	7.5 ± 2.5 ac
Arborinine [**3**]	22.5 ± 2.5 a	10.0 ± 0.0 a
Skimmianine [**4**]	17.5 ± 2.5 a	7.5 ± 2.5 ac
β–Sitosterol [**5**]	20.0 ± 0.0 a	12.5 ± 2.5 a
α–Amyrin [**6**]	10.0 ± 5.0 ac	5.0 ± 0.0 c
Actellic super	95.0 ± 0.0 b	87.5 ± 2.5 b
Negative control	5.0 ± 0.0 c	

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test).

The isolated compounds were less active than the crude extracts, from which they were isolated, an indication of possible loss of synergism in the isolation process. In order to ascertain these observations, pure isolated compounds were blended in the same ratio and subjected to adulticidal test. The adulticidal assay results at different dosage of thereof blended mixture of isolated compounds; actellic super (positive control) and drug free (negative control) are summarized in Table 5.

Table 5: Mean percentage adulticidal ± s.d. of the blended compounds from *Teclea trichocarpa* against maize weevil.

Blended Compounds	Mean percentage adulticidal at different concentration in % w/w	
	0.1% w/w	0.05% w/w
Skimmianine/arborinine	17.5 ± 2.5 a	10.0 ± 5.0 a
α-Amyrin/normelicopicine	20.0 ± 0.0 a	15.0 ± 5.0 a
Arborinine/melicopicine	22.5 ± 2.5 a	17.5 ± 2.5 a
α-Amyrin/arborinine	17.5 ± 2.5 a	12.5 ± 2.5 a
3β–sitosterol/arborinine	95.0 ± 0.0 b	87.5 ± 2.5 b
Actellic super	5.0 ± 0.0 c	
Negative control	100	

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test).

From the results in Table 5, it is evident that the adulticidal activities are concentration dependent. However, comparing these results with those presented in Table 4, mixture of α- amyrin/ normelicopicine, and skimmianine/ arborinine, at higher concentration showed higher activity than corresponding pure compounds, implying some synergism. Whether this implies, a mixture of terpenoids and alkaloids or different types of alkaloids are more effective remains to be investigated. Arborinine/normelicopicine and β–sitosterol/ arborinine mixtures showed lower activity than corresponding pure compounds, implying there was loss of activity (antagonist). Mixture of α- amyrin and arborinine did not show significant change in activity at high concentration but at 0.05% w/w there was increased activity, implying synergism is in play.

Although all the test mixtures used were in the ratio of 1: 1, there occurrence in the crude extracts of the plant is not in these ratios hence their effects could differ. Similarly, the isolated compounds were not the only compounds present in the crude extracts as evidenced from TLC analysis and therefore, it is evident adulticidal activity is caused by additive effect of most constituent components with different levels of activity. The results provide a scientific rationale for the use of *T. trichocarpa* in post- harvest protection.

Conclusion

These natural plant products may improve efficiency in post-harvest practices as a strategy of providing people with sufficient and healthy food in an ecologically sustainable manner. Being natural, protectants from plant materials would be easily degraded by biological factors, whereby cases of pollution and poisoning would be reduced. Improving grain storage would mean less hunger, improved nutrition for mankind, a higher standard of living and a sounder economy for the nation.

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