Determination of the Biopesticidal and Antimicrobial activity of *Khaya Senegalensis* Seed Oil Grown in Jos North Local Government Area of Plateau State, Nigeris.

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Abstract

Khaya senegalensis seed oil was evaluated for its effectiveness to control *Callosobruchus maculatus* on stored cowpea. The seed oil was extracted using steam distillation method of extraction. There was almost complete adult mortality of *C. maculatus* within 24 hrs after treatment with the seed oil at 1, 2 and 3ml/100g of cowpea, lower concentrations 0.2ml, 0.5ml, 0.8ml, required longer time for a notable effect on *C.maculatus* for all parameters examined, *K. senegalensis* seed oil has shown to have control over *C. maculatus*, suggesting that *K. senegalensis* seed oil has high potential for use as a botanical resource for control of *C. maculatus*. The antimicrobial activity was determined using Agar diffusion method with Gentamicin 10mg/ml serving as standard. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined using Tween 80 as diluents. The extract does not show appreciable antioxidant properties and the antibacterial analysis shows that the activity of the oil on organism isolate increase with increasing concentraton the oil inhibited *Staphylococcus aureus, Salmonella typhi and E.coli.* However the oil displayed bactericidal activities on organism isolate throughout the concentration. The effectiveness of the oil displayed bactericidal activities on organism isolate throughout the concentration. The effectiveness of the oil on organism isolate justified the claim among the traditional communities for treatment of ailment such as typhoid, skin rashes, boils and abscesses as plausible.

Keywords: *Callosobruchus Maculatus*, Cowpea, Pest, Biopesticide, Antibacterial.

Introduction

The high cost of synthetic pesticides, the danger of pesticides misuse and of toxic residues in food has resulted in a rapid development and assessment of botanicals as alternatives for chemical control of stored product pest (Shaya *et al.,* 1997). In many storage systems, use of fumigants is the most economic tool for managing stored grain insect pests; however, storage pests are fast ell and Wilson, 1995; Chaudhry, 1995). Since there are increasing drawbacks of the continued use of today's conventional fumigants, efforts are needed in the development of new compounds to replace those currently used (Bell and Wilson, 1995; Chaudhry, 1995; Lee *et al.,*). The toxicity of essential of essential oils extracted from plants to stored product insects has been of special interest during the last decade (Tunc *et al.,* 2000). Most constituent of the essential oils are mono-terpenoids that are secondary plant

chemicals. The mono-terpenoids are of interest to industrial markets because of other potent biological activities in addition to their toxicity to insects (Kubo *et al.,* 1994; Shaaya *et al.,* 1997). The use of oils as fumigants or contact insecticides to protect grains, especially legumes against storage insects is a traditional practice in many countries in Asia and Africa. This method is convenient and inexpensive for the protection of stored seeds in households and on small farms (Shaaya *et al.,* 1997). Essential oils have a low toxicity to warm-blooded animals, high volatility and toxicity to stored grain insects pests (Regnault-Roger and Hamraqui, 1993; Shaaya *et al.,* 1994).

Oil is any neutral, non-polar chemical substance that is a viscous liquid at ambient temperature and is both hydrophilic and lipophilic. Oils have high carbon and hydrogen content and are usually flammable and slippery(Wikipedia, accessed 2016)

This research reports the antimicrobial activity and toxicity of *Khaya senegalensis* seed oil against *Callosobrochus maculatus* which is a major pest of stored cowpea causing severe damage to cowpea throughout the world

Materials and Methods

Sample Preparation and Preparation

Khaya senegalensis seeds were collected by picking from tree shades in the department of Chemistry, University of Jos, Nigeria. The seeds were gathered, sorted, cracked and dried at room temperature for seven days after which they were grounded to powder and the seed oil extracted by steam distillation.

Freshly harvested and air dried cowpea were used for the experiment. Hundred grams of cowpea were weighed into three kilner jars. The concentration of *K.senegalensis* seed oil used for this experiment were 1.2 ml/100g and 3.0 ml/100g of cowpea. To each 100g of cowpea in a labeled kilner jar, concentration was measured and placed into triplicates of the concentrations per treatment. All the kilner jar was shaken vigorously to ensure proper mixing after which they were infested with the weevils.

Ten pairs of adult *C.maculatus* were infested into each kilner jar according to treatments. The jars covered were arranged on a laboratory bench in a completely randomized design (CRD) in triplicates per concentration per treatment at room temperature for 12 weeks.

Data Collection

Mortality counts were recorded after 24hrs, 48hrs, and 72hrs post exposure for all the treatments, during which dead insects are removed. Egg counts were carried out 14 days post treatment. This was done by random draw of 20 cowpeas from each jar in each concentration per treatment. The number of adults produced in each concentration per treatment was counted from 28-35 days and from 56-63 days post infection for the f_1 and f_2 generations respectively. During which newly emerged adults were sieved out and counted in each case.

Statistical Analysis

The data collected were subjected to ANOVA and the means were separated using SNK (Student New-man Keuls). The percentage damage and percentage weight loss were determined by using the thousand grain mass (TGM) method. From each jar of all treatments, 100 grains were randomly taken and separated into holed (damaged) and whole (undamaged) grains. The grains in each category were counted used and the numbers used to calculate the percentage damage.

$$\text{Damage (\%)} = \frac{\text{Damage grains} \times 100}{\text{Total grains}}$$

For percentage weight loss, the grains in each of the above categories were weighed, and the weights used to calculate the percentage weight loss:

$$\text{Weight loss (\%)} = \frac{(\text{und}) - (\text{Dnu}) \times 100}{\text{U}(\text{nd} + \text{nu})}$$

Where;

U – Weight of undamaged grains

D – Weight of damaged grains

nd – number of damaged grains

nu – number of undamaged grains

After 12 weeks, the grains were tested for viability or germination. From each jar, 10 grains were randomly taken and placed in petri dishes lined with moistened filter paper. These were left on the laboratory bench at room temperature for 7 days after which the percentage germination was determined

Preparation of Culture Media

Mueller Hinton agar (5.0g) was dissolved in 250ml of water and sterilized by autoclaving at 121^0C for 15 minutes. The cork borer of diameter 4mm was used to bore five holes on the plate that was inoculated with a

single strain organism isolate and about 0.2m of diluted extract extract concentration was introduced into the holes with respect to the labels on the plate from the highest to the lowest concentration. The plate was allowed to stand for 1 hour to ensure ore diffusion before incubation at 37^0C for 24 hours.

Preparation of *Khaya Senegalensis* Seed Oil for Sensitivity Test

The oil (3ml) was measured in a pipette into a small bottle and 10ml of Tween 80 solution was added to it. The bottle was closed and shaken vigorously to ensure mixing. Double serial dilution was done with this to obtain 300mg, 150mg, 75mg, 37.5mg and 18.75mg of the oil concentration respectively, these various concentrations was introduced in the plate containing prepared culture media using Mueller Hinton agar. Gentamicin 80mg was used as a standard control. The plate was left for 1 hour at room temperature to allow for the diffusion and then incubated at 37^0C for 24 hours. The zones around the holes show inhibition and was measured in mm using a transparent plastic rule line.

Minimum Inhibitory Concentration (Mic)

The MIC of the extract was determine using small bottles, five for each organism isolate for the 300, 150, 75, 37.5, and 18.75mg different concentrations. 0.1 ml of *E.coli, Staphylococcus aureus* and *Salmonella typhi* was inoculated into the bottles containing diluted extract of the various concentrations and incubated at 37^0C for 24 hours.

Minimum Bactericidal Concentration (Mbc)

The culture media was prepared using Mueller Hinton agar 5.0g of the agar was dissolved in 250ml distilled water and autoclave to sterilize at 121^0C for 15 minutes. At the concentration in which the organism isolate shows positive test (there was no growth in the bottles) small quantity was picked into a small culture media aseptically using a flame ware loop and then incubated at 37^0C for 24 hours in the incubator this was done to subculture the isolate.

Results and Discussion

Table 1: Adult mortality of *C.maculatus* after treatment of cowpea grain with *K.senegalensis* seed oil

Sample no	Treatments(ml)	24 hours	48 hours	72 hours
1	0.2	0.00	0.00	2.00±0.12
2	0.5	0.00	2.00±0.23	4.00±0.14
3	0.8	4.00±0.54	6.00±0.73	-
4	1.0	10.00±0.50	-	-
5	2.0	10.00±0.72	-	-
6	3.0	9.44±0.32	-	-

Table 2: Effect of *K.senegalensis* seed oil on the number of emerged progeny of *C.maculatus*

Sample no.	**Treatments (ml)**	**24DAT**	**58DAT**
1.	0.2	34.42±0.21	6.00±0.40
2.	0.5	23.10±0.66	3.22±0.01
3.	0.8	17.83±0.33	2.10±0.21
4.	1.0	13.36±0.01	1.52±0.03
5.	2.0	10.00±0.08	0.83±0.01
6.	3.0	5.67±0.01	0.33±0.00

DAT – Days after treatment

Table 3: Effect of *K.senegalensis* seed oil on percent damage and weight loss of cowpea grains

Sample no.	Treatments (ml)	Damage(%)	Weight loss(%)
1	0.2	1.44±0.0	0.76±0.03
2	0.5	1.20±0.02	0.62±0.00
3	0.8	0.88±0.04	0.43±0.02
4	1.0	0.80±0.06	0.41±0.01
5	2.0	0.60±0.07	0.40±0.00
6	3.0	0.30±0.06	0.36±0.01

Table 4: Effect of *K.senegalensis* seed oil on germination of cowpea grains

Sample no.	Treatments (ml)	Germination count
1	0.2	72.43±0.01
2	0.5	87.22±0.02
3	0.8	89.00±0.05
4	1.0	89.02±0.00
5	2.0	90.67±0.02
6	3.0	100.00±0.02

Table 5: Susceptibility of the plant extract (*K.senegalensis*) on the test organisms' zone of inhibition (mm) concentration (mg/ml)

est organisms	300	150	75	37.5	18.75	+ve C
E.coli	22	20	16	13	8	21
Salmonella typhi	20	18	18	12	-	22
S.aureus	24	20	18	16	14	23

KEY:

- **=** Number, zone of inhibition.

+ve C = Positive control = 10mg/ml

Table 6: Minimum inhibitory concentration (MIC) of *K.senegalensis* on the test organisms

Test organisms	300	150	75	37.5	18.75	MIC
E.coli	-	-	-	-	-	18.75
Salmonella typhi	-	-	+	+	+	150
S.aureus	-	-	-	+	+	75

KEY:

- **=** No growth,
- += growth

Table 7: Minimum bactericidal concentration (MBC) of *K.senegalensis* on the test organisms

Test organisms	300	150	75	37.5	18.75	MBC
E.coli	+	+	+	+	+	>300
Salmonella typhi	+	+	+	+	+	>300
S.aureus	+	+	+	+	+	300

KEY:

12mm and below = resistance

13mm and 14mm = moderately sensitive

15mm and above = sensitive

Table 8: Gas chromatography-mass spectrometry results

Compound name	Chemical	Molecular Formula	R-Time weight
2-octenal	$C_8H_{14}O$	126	1013
Dodecanoic acid	$C_{12}H_{24}O_2$	200	1570
Tridecanoic acid	$C_{13}H_{26}O_2$	214	1670
2, 6, 10-trimethyldecane	$C_{15}H_{35}$	212	1320
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1769
2- methylhexacosane	$C_{27}H_{56}$	380	2641
9, 19-cycloergost-24(28)-en-3-ol	$C_{30}H_{50}O$	426	2760

Urs-12-ene	$C_{30}H_{50}$	410	2685
4, 22-stigmas tadiene-3-one	$C_{29}H_{46}O$	410	2722
2,2,4-trimethyl-3-(3,7,11,15-tertaenyl)-cyclohexanol	$C_{30}H_{52}O$	428	2075

The results of the mortality count are presented in table 1. The seed oil of *K.senegalensis* killed nearly all adults of *C.maculatus* within 24 hours.

The progeny emergence result is presented on table 2. The treatment with seed oil significantly ($p<0.05$) supposed progeny emergence for two generations (28 DAT and 56 DAT). This indicates that even though there was high oviposition, the treatment either prevented the eggs from hatching or prevented the larvae and Probably the pupae from completing their development in the cowpea grains. In addition to action by physical properties of the oil coating, blocking respiration, rather than by a specific chemical effect. The larvae hatching from the eggs of *C.maculatus* must penetrate the seed to survive, but are unable to do this unless the egg is firmly attached to the seed surface. Eggs on oil-treated seeds are less firmly attached than on the controls, suggesting that the oil may inhibit successful larval penetration into the seed (Don pedro, 1989). Application of oil to *C.maculatus* eggs might occlude the funnel, and thus lead to the death of the developing insect by asphyxiation.

The result of germination tests is presented in table 4. The seed oil concentration at ($p<0.05$) reduced germination. The seed oil reduced the damage and weight loss of infested cowpea grains. Thus seed oil of *K.senegalensis* may be more suitable for protecting grains for consumption, less so for seeds for planting as it appears to affect the viability of seed.

Medicinal plant constitutes an effective source of both traditional and modern medicines, but assessment of antimicrobial potential of the sources is essential. The present study found a very promising and readily available source (*Khaya senegalensis)* for treating infections caused by some bacteria. This is particularly significant because drug resistant to human pathogens has been increasing not only in the developing countries but throughout the world to indiscriminate use of antibiotic (Barie, 2012).

The seed oil was effective against effective enteric bacteria such as *E.coli, salmonella typhi and staphylococcus aureus* from the investigation carried out on the antibacterial activity of the oil as shown in tables 5, 6 and 7, the oil was found to have higher activity with increasing concentration both on Gram negative and Gram positive bacteria. The minimum inhibitory concentration (MIC) result show that the oil inhibited *E.coli, salmonella typhi and staphylococcus aureus* at the concentration of 31.25mg/ml. The extract kill the organisms isolate throughout the concentration, Therefore the minimum bactericidal concentration (MBC) is 31.25mg/ml.

From table 8, the GC-MS analysis result indicated the presence of urs-12-ene (C_{30}) and dodecanoic acid (C_{12}) etc. The extract does not show appreciable antioxidantproperties.

Conclusion

In medicine, leaves and bark of k. senegalensis are used for treatment of stomach upset in both humans and livestock (Datziel 1948). Its use in crop protection dates back as early as 1900 when it was used for preserving seeds for annual planting (Thomas 1910: Meek 1931). The oil is used as a dry skin lotion in the middle Belt of Nigeria (Personal communication).

Therefore, K. senegalensis products are less harmful to humans than most conventional insecticides. Studies have reported that plant oils are readily biodegradable and less detrimental to non- target organisms than synthetic pesticides(Tunc *et al*., 2000; Lale 2002).

Shaaya et al., (1997) indicated that there is adsorption of plant oils by the treated commodity. They suggested however that the higher concentration and longer exposure periods were needed to achieve a similar level of mortality for a given species than required when oils were applied in space fumigation.

K. senegalensis is widely used in traditional medicine to combat and cure various ailments and found to be rich in secondary metabolites. The presence of tannins, saponins, alkaloids, flavanoids and oxalate in the plant may be attributed to their curative properties. The exploitation of these pharmacological properties is imperative because these support the claim among traditional communities for its potential as therapeutic agents for treatment of urinary tract infection (UTI), Respiratory Tract Infection (RTI), Typhoid, Skin rash and Abscesses.

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