



Use of *Blumea alata*, *Bidens pilosa* and *Chenopodium ambrosioides* as Mosquito Repellents and Mosquitocides

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ABSTRACT

The evaluation of the efficacies of mosquito repellents and mosquitocides of *Blumea alata*, *Bidens pilosa* and *Chenopodium ambrosioides*, plants used by some of the indigenous rural people of Hurungwe District, Mashonaland West Province, Zimbabwe, were carried out during the period: November 2006 to June 2007. The active constituents were extracted by dry distillation of the dried, powdered leaves of the three plants. The oily organic phases of the distillates were separated from the aqueous phases, and then used in repellence experiments on laboratory-reared *Aedes aegypti* mosquitoes. The results revealed that the oils of *B. alata*, *B. pilosa* and *C. ambrosioides* extracts were effective mosquito repellents. Evaluation of the pesticides involved burning the dried, powdered leaves of the three plants on glowing charcoal in a clay pot to produce smoke that knocked down or killed laboratory-reared *A. aegypti* mosquitoes. The mosquitocidal action of the smoke that was generated from each of the three samples of plant leaves indicated that their mosquitocidal efficacies were high and the plants could be used successfully as bases for the production of mosquitocides.

Key words Medicinal plants; mosquitocides; mosquito repellents

INTRODUCTION

Mosquito repellency of plants were well known before the advent of synthetic chemicals [1, 2], but the most commonly used mosquito repellents are synthetic chemicals that mostly contain Deet in their formulations. However, Deet is associated with disadvantages that stem principally from its activity as a solvent of paints, vanishes, and some plastic and synthetic fabrics [3]. Other disadvantages of Deet arise from concerns over the human toxicity reactions after its application and these vary from mild to severe [4]. The most successfully used mosquitocide is DDT. However, some countries have discontinued its use as a result of environmental concerns. The interest in anti-mosquito products of plant origin is being revived because of the drawbacks associated with the continued application of synthetic compounds, some of which have led to widespread development of insecticide resistance [5]. Extracts of several plants have been studied as possible mosquito repellents revealing the existence of natural repellents with good efficacy [4]. Some people mainly in rural areas of many tropical countries including Zimbabwe burn plant materials using glowing charcoal to produce smoke which repels or kills mosquitoes and flies [2][6-14]. In this study we report on some investigations which we have carried out on the potential of *Blumea alata*, *Bidens pilosa*, and *Chenopodium ambrosioides* as mosquito repellents and mosquitocides. These plants are widely used in Zimbabwe, particularly in the District of Hurungwe in Mashonaland West Province [2].

Blumea alata

Blumea alata belongs to the *Asteraceae* family. Its leaves may be rubbed on to skin to repel mosquitoes and flies, or they may be dried at room temperature and ground to powder and placed in a loose porous cloth and hung in the house to repel mosquitoes and flies, or the leaf powder may be burnt on hot metal or glowing charcoal to repel or kill mosquitoes and flies. Its essential oils have been reported to exhibit antibacterial and antifungal activities [15], the main constituents being terpinen-4-ol (27.6 %), germacrene-D (15.4 %), sabinene (8.0 %) and α -terpine (5.5 %) [16].

Bidens pilosa

Bidens pilosa is an annual herb that belongs to the *Asteraceae* family. Its medicinal uses are quite extensive. Infusions have been used as a tonic and stimulant. Decoctions are drunk for coughs and juice of leaves is dropped into the eye for conjunctivitis. Young shoots are chewed for the relief of rheumatism and strong decoctions of leaves are drunk for relief of inflammation. The plant is used for rheumatism, heartburn, coughs, and fits in children. It is also used to repel pests in stored grain as well as aphids, ants, beetle, cabbage root fly, caterpillars, crickets, mites and termites. The plant is believed to contain relatively high omega-3-fatty acids including α -linolenic acid, eicosapentanoic acid, and docosahexaenoic acid. These are said to elevate high-density lipoprotein, which may be linked to coronary diseases and hypertension treatment [17]. Other active constituents include chalcones, aurone glycosides, volatile oils, acylated okanin glycoside, gallic and oxalic acids and phenolic astringents. Some of these, particularly the volatile oils, are considered responsible for the mosquito repellency.

Chenopodium ambrosioides

Chenopodium ambrosioides (Shona name: Mbanda) is a member of the *Chenopodiaceae* family which originated from Central America. It is an annual herb that grows to a height of 40 cm with toothed, oval leaves that grow to a length of 4 cm and a width of 1 cm. The plant has a very strong odour and produces small green flowers and seeds which are very small, green when fresh, but black when dry. The plant is used to treat worm infections in humans and livestock. In Zimbabwe it is also used to treat convulsions. The constituents of its essential oils include ascaridole (68-80 %), isoascaridole, p-cymene, limonene, and x-terpinene [18].

MATERIALS AND METHODS

Mosquito repellence

a) Plant extracts

Fresh leaves of each of *B. alata*, *B. pilosa* and *C. ambrosioides* were collected from Hurungwe District, Mashonaland West Province, Zimbabwe, in January 2007, and dried in the shade at room temperature for approximately 2 weeks, then ground to powder using a mill in the Department of Chemistry, University of Zimbabwe. The powder of each was placed in a 500 cm³ distillation flask and the distillation apparatus set up. The distillation flask was heated over a Bunsen burner collecting the distillates into 50 cm³ conical flasks. Oils were separated from the aqueous phases using separation funnels yielding *B. alata* (16.0 cm³), *B. pilosa* (19.0 cm³), and *C. ambrosioides* (17.5 cm³), which were kept at 4°C until shortly before bioassaying when 15 cm³ of each dissolved in ethanol and the volume made up to 50 cm³ in volumetric flasks.

b) Repellent bioassay

Three different treatment methods (determination of landing time, dose finding experiments and determination of protection time) were used to study the repellent activity of the dry distilled extracts of the three test plants against laboratory-reared *Aedes aegypti* female mosquitoes after they were applied to the human skin. *A. aegypti* mosquitoes bite during the day, hence the experiments were carried out between 08.00 h and 16.00 h.

c) Preparation of extract mixtures

The extract solutions in ethanol were mixed in equal proportions by volume to give composite mixtures which were used to investigate the possibility of synergism in their repellence activities. Thus 7 cm³ solutions of each pair of *B. alata*, *B. pilosa*, and *C. ambrosioides* were mixed to give 14 cm³ composite mixtures of each of *B. alata/B. pilosa* (Ba/Bp), *B. alata/C. ambrosioides* (Ba/Ca), and *B. pilosa/C. ambrosioides* (Bp/Ca).

d) Preparation of mosquitoes

Female laboratory-reared *Aedes aegypti* mosquitoes (40 in number), free from pathogens, were placed in a 5-litre capacity cage with a mosquito netting on top and a sleeve on the side that was used to introduce the mosquitoes into the cage. The mosquitoes were fed on 10 % sucrose solution but starved for 24 hours prior to the experiment.

e) Repellent test procedure

Laboratory repellent tests were conducted using the human-bait technique of the WHO [18] standard method with modifications by the Blair Research Institute, Ministry of Health and Child Welfare [20]. Three human volunteers were used in the experiments. An area of 5 cm x 5 cm (25 cm²) was marked and cut open on each glove which was used for the experiments. The edges of the cut area were lined with masking tape and 40 randomly selected mosquitoes were placed in the 5-litre cage via the sleeve.

f) Determination of landing time

Landing time is the average time required for the first mosquito to land on the exposed area and attempt to take a blood meal. An untreated hand was exposed to the mosquitoes to determine the landing time which was then recorded. The procedure was repeated 10 times and the average landing time calculated.

Average landing time = $(30+25+34+29+33+40+37+26+36+30)/10 = 32$ seconds.

g) Exposure time

The repellence was monitored at 30-minute intervals, each volunteer putting the test hand in the cage for the first 3 minutes of every half hour [4].

h) Repellence test procedure

The repellence of the volatile oils was evaluated using the human-bait technique [18], the testing period lasting for 6 hours. *Aedes aegypti* being a day biter, the tests were carried out between 08.00h and 16.00 h, at 25-30°C and relative humidity of 60-80 %. Repellence was calculated according to the number of mosquitoes that were prevented from landing compared to the number of mosquitoes that landed on the control [3]: % Repellence = $[(C-T)/C] \times 100$

Where C is the number of mosquitoes that landed on the control and T is the number of mosquitoes that landed on the treated areas of the volunteer.

i). Dose finding experiments

A series of repellence experiments was run starting with the application of a 0.5 cm³ dose of the plant extract solution in ethanol (Section a). The 0.5 cm³ dose was applied on to the open area of the hand and the % repellence during the 3-minute exposure time was determined. The dose was increased by 0.5 cm³ for each succeeding experiment, calculating the % repellence after each dose, until a dose that gave 100 % repellence during the 3-minute exposure time was achieved (Figure 1). This dose was the minimum amount of extract that gave complete protection from mosquito landing and was used in all experiments. The results indicated that the minimum dose for *B. alata* and *C. ambrosioides* was 2 cm³ and that for *B. pilosa* was 2.5 cm³. Hence in all experiments involving *B. alata* and *C. ambrosioides*, the dose used was 2 cm³ and that for *B. pilosa* was 2.5 cm³. The procedure was repeated for repellent mixtures, giving 2 cm³ as the dose required for each of the pairs of mixtures: *B. alata*/*C. ambrosioides*, *B. alata*/*B. pilosa* and *B. pilosa*/*C. ambrosioides* (Figure 2), and this was used in all experiments involving the respective mixtures.

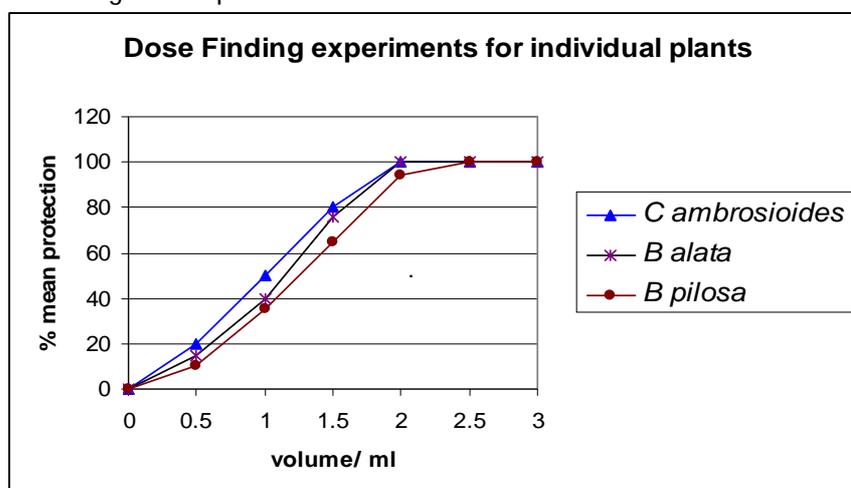


Figure 1

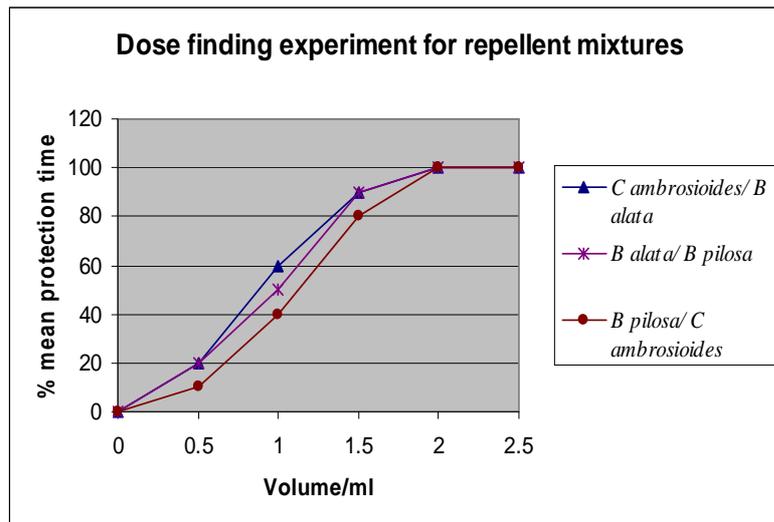


Figure 2

Mosquitocides

The powdered leaves (Section a) were used in the evaluation of the potential of the three plants as mosquitocides, using 50 individual 5-8 day old laboratory-reared female *A. aegypti* mosquitoes for each replicate test. Ten were placed in each of the 3 standard plastic cups. Ten of the remaining 20 were used for the control and the other ten for the standard. The plastic cups were covered with mosquito netting on top. The control was treated similarly to the test experiment except that there was no addition of plant powder to the glowing charcoal.

(i) Mosquitocides test procedure

The inhabitants of Hurungwe District, Mashonaland West Province, Zimbabwe, burn the plant materials on glowing charcoal or on hot metal sheets to produce smoke that will act as mosquitocide. In this project the leaf powders were burnt on glowing charcoal to release the mosquitocides. Three replicate cups were placed around glowing charcoal making sure that the direct heat from the charcoal did not affect them. The leaf powder was placed on the glowing charcoal and the set up was quickly covered using a 1m³ cardboard box for 3 minutes, and recorded the number of knocked down mosquitoes, maintaining the recorded knocked down mosquitoes at room temperature. Cotton wool previously soaked in 10% sucrose was placed on top of the cups which were then put in a box covered with a cloth and left for 24 hours. The number of mosquitoes which died was recorded and the percentage mortality was calculated in both the exposure and control cups. The experimental procedure was repeated with the other replicate experiments changing the position of the cups.

(ii) The percentage mortality

The percentage mortality was calculated using the expression:

$$\% \text{ Mortality} = [(\text{Number of dead mosquitoes}) / (\text{Total number of mosquitoes})] \times 100.$$

The percentage number of dead mosquitoes in the control experiment was represented by C and the percentage number of dead mosquitoes in the exposure experiment was represented by E. If the value of C that was calculated was between or equal to 5% and 20% then a corrected value for exposure mortality was calculated using Abbott's formula [21]:

Corrected exposure mortality = $[(E-C) / (100-C)] \times 100$. Mortality rates less than 80% after 24 hours post-exposure time indicate insect resistance. Rates between 80-90% suggest the possibility of resistance that needs to be clarified and above 98% indicate insect susceptibility [22].

(iii) The percentage knockdown

The percentage knockdown was calculated using the expression:

$$\% \text{ Knockdown} = [(C-T) / (10-T)] \times 100$$

where C is the number of mosquitoes knocked down in the exposure experiment, T is the total number of mosquitoes knocked down in the control experiment. The percentage knock down was calculated for all the replicates so that the mean % knock down could be determined. Commercial

mosquito coils which contain transfluthrin as active ingredient was used as the standard, and the results compared using the t-test.

(iv) Dose-finding experiments for mosquitocides

A series of knockdown experiments was run starting with the application of a 0.2 g dose of the plant leaf powder onto glowing charcoal and exposing 10 mosquitoes in a cup for an exposure time of 3 minutes, the experiment being done in replicates, and the number of knocked down mosquitoes recorded. In the next set of replicate experiments the dose was increased by 0.2 g to 0.4 g and the procedure repeated, recording the number of knocked down mosquitoes. The procedure was repeated, increasing the dose by 0.2 to 0.6 g, then to 0.8 g, and finally to 1.0 g. calculating the % knock down after each dose, until a dose that gave 100 % knockdown after the 3-minute exposure time was achieved (Figure 3). This dose was the minimum amount of powder that gave complete knockdown of mosquitoes and was used in all experiments. The results indicated that the minimum dose for *B. alata* and *C. ambrosioides* was 0.8 g and that for *B. pilosa* was 1.0 g. Hence in all experiments involving *B. alata* and *C. ambrosioides*, the dose used was 0.8 g and that for *B. pilosa* was 1.0 g of powder.

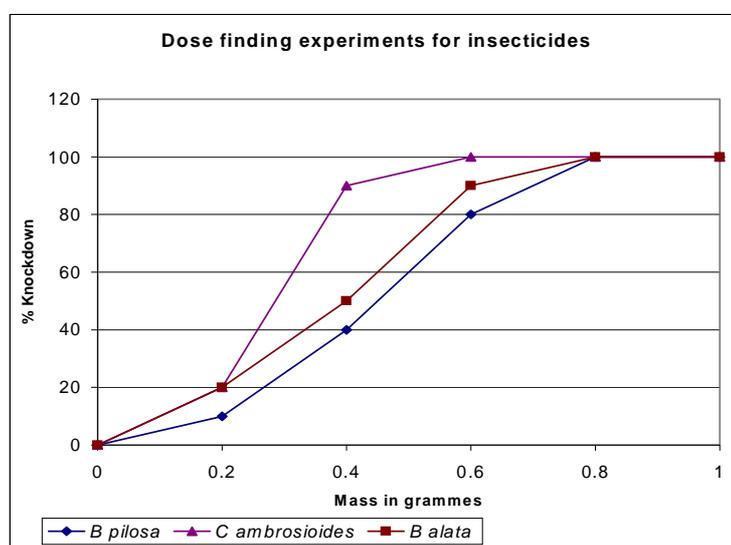


Figure 3

RESULTS AND DISCUSSION

Table 1: Percentage mean repellency of plant extracts against *Aedes aegypti* mosquitoes

Time in Hours	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
<i>C. ambrosioides</i>	100	100	98	100	96	89	88	72	66	58	35	32	18
<i>B. pilosa</i> (<i>Bp</i>)	100	100	94	96	79	62	60	51	39	22	24	18	15
<i>B. alata</i> (<i>Ba</i>)	100	100	100	98	100	92	86	75	69	60	42	28	22
<i>Ca/Ba</i>	100	100	98	100	100	98	94	85	78	72	58	42	34
<i>Ca/Bp</i>	100	100	100	96	100	92	92	78	62	56	44	35	30
<i>Ba/Bp</i>	100	100	100	100	98	94	84	78	69	54	36	30	26
DEET	100	100	100	100	100	100	100	100	100	100	100	100	100
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0

The results for the mean protection times in Table 1 indicate that *Chenopodium ambrosioides* would give 100% protection for 1.5 h, *Bidens pilosa* for 1.0 h, whilst *Blumea alata* gives 2.0 h. While 100% protection would be the desirable, a product that gives 70% protection would still be considered useful. In that scenario, *C. ambrosioides* would be considered an efficient repellent for 3.5 h, *B. pilosa* for 2.0 h, and *B. alata* for 3.5 h.

The mixture of *B. alata*- *C. ambrosioides* gives 100 % protection for 2.0 h, *B. alata*-*B. pilosa* for 1.5 h and *B. pilosa* - *C. ambrosioides* for 2.0 h. On the basis of a 70% cut-off, *B. alata* - *C. ambrosioides* would

be an efficient repellent for 4.5 h. Thus *B. alata* and *C. ambrosioides* have reinforced one another's efficiency from the 3.5 h for each of the two to the 4.5 for the mixture. There might be a case for the two acting in synergism, notwithstanding the unpronounced effect of either extract on the question of the 100 % efficiency where the 100 % efficiency of *B. alata* is 1.5 h and that for *C. ambrosioides* is 2.0 h but the mixture maintains the 100 % efficiency at 2.0 h. Thus, the efficiency of the mixture is clearly superior.

In the case of *B. alata* - *B. pilosa* mixture, the 100 % efficiency for the mixture is up to 1.5 h whereas that for *B. alata* is up to 2.0 h and that for *B. pilosa* is at 0.5 h and the effect of the 70 % efficiency for the individual extracts on that of the mixture does not appear particularly attractive. However, the mixture gives better results than either of the two up to 4.5 h. The two do not appear to favourably reinforce one another. The same argument would appear valid for the *B. pilosa* - *C. ambrosioides* mixture. Thus, a clear case for synergism has been established for the *B. alata* - *C. ambrosioides* mixture only. In mixtures containing *B. pilosa*, *B. pilosa* appears to lower the efficacy of the other extract. This observation would tend to mitigate against the tendency of some traditional healers to mix different herbs in an attempt to foster synergism between the different constituents.

The table indicates that *C. ambrosioides* gave complete repellence up to 1.5 hours, *B. alata* for 2.0 hours and *B. pilosa* for 0.5 hour. The mean protection dropped to 88 % for *C. ambrosioides*, 86 % for *B. alata* and 51 % for *B. pilosa* 3.0 hours after application. The differences between *C. ambrosioides* and *B. alata* protection times were not significant after 3.5 hours, but the differences between these two and *B. pilosa* which had dropped to 51 % after 3.5 hours were significant.

The mixtures *B. alata*-*C. ambrosioides* (*Ba/Ca*), *B. alata*-*B. pilosa* (*Ba/Bp*), and *B. alata*-*C. ambrosioides* (*Ba/Ca*) provided 95 % protection for the first 1.5 hours, and dropped to below 70 % after 4.5 hours for the *Ba/Ca* mixture, 3.5 hours for the *Bp/Ca* and *Ba/Bp* mixtures. The differences in protection time were not significant within the first 1.5 hours but became significant between 1.5 and 5.0 hours post application, the *Ba/Ca* mixture providing greater protection than the other two mixtures over the entire period.

B. alata, *C. ambrosioides*, and *B. alata* / *C. ambrosioides* mixture provided similar protection up to 1.5 hours post application. The repellence of the mixture was still similar to that of *B. alata* up to 2.0 hours, but after 2.5 hours the mixture provided significantly higher repellence than the other two repellents. It provided complete protection for 2.5 hours, thus giving higher mean protection time than either of the single repellents: 2.0 hours for *B. alata* and 1.5 hours for *C. ambrosioides*. There were statistical differences between the protection time of *B. alata* and that of *B. alata* / *C. ambrosioides* mixture at 95 % level ($t = 5.9$, $df = 12$) and also with *C. ambrosioides* ($t = 6.85$, $df = 12$). Thus the relationship between the two extracts is probably synergistic. The difference in repellence between the mixture and the individual plant extracts was statistically significant (F test, $p =$ smaller than 0.05). The mean repellence for the mixture fell to below 70 % after 4.5 hours post application, where as *B. alata* provided 75 % protection for 3.5 hours and *C. ambrosioides* provided 72 % protection for 3.5 hours.

There were no significant differences between the repellencies of *B. pilosa*, *C. ambrosioides* and *B. pilosa* / *C. ambrosioides* mixture during the first 1.5 hours post application. The repellence of *B. pilosa* dropped to less than 70 % after 2.0 hours when *C. ambrosioides* was still giving 96 % protection and the *B. pilosa* / *C. ambrosioides* mixture was giving 100 % protection. The repellence of *C. ambrosioides* only dropped to less than 72 % after 3.5 hours and the *B. pilosa* / *C. ambrosioides* mixture was still providing a mean protection of 78 %, though it then fell to below 70 %. The differences between the two individual repellents and the mixture were significant at 95 % ($t = 4.44$, $df = 12$), indicating possible synergism between *B. pilosa* and *C. ambrosioides* extracts, though to a lesser extent than that for the *B. alata* and *C. ambrosioides* extracts.

The differences between *B. alata*, *B. pilosa* and *B. alata* / *B. pilosa* mixture repellencies was not significant during the first 1.5 hours post application, with the mixture and *B. alata* giving 100 % repellence each, and *B. pilosa* giving 96 %. The repellence of *B. pilosa* dropped to below 70 % after 2.0 hours post application when *B. alata* was giving 100 % protection and the mixture was giving 98 % protection. The mixture gave mean repellence of 78 % up to 3.5 hours when *B. alata* gave 75 % up

to 3.5 hours. There after, they both fell to below 70 %. The extracts of *B. alata* and *B. pilosa* gave different repellence behaviour although the two plants are members of the same Asteraceae family. Plants of the same family may have different repellent properties and variations in plant constituent composition may occur due to geographical locations [2, 4, 23].

Percentage knockdown

Table 2: Percentage mean knockdown against time

Sample/time in minutes	0	10	20	30	40	50
<i>C. ambrosioides</i>	0	20	95	100	100	100
<i>B. pilosa</i>	0	25	87.5	100	100	100
<i>B. alata</i>	0	32.5	92.5	100	100	100
Control	0	0	0	0	0	0

Table 2 reveals that *B. alata*, *B. pilosa* and *C. ambrosioides* are effective mosquitocides. They all exhibit 100 % knockdown of the test mosquitoes within 30 minutes of application, with mean mortality: *B. alata* (90 %), *B. pilosa* (90 %) and *C. ambrosioides* (92.5 %).

CONCLUSION

Thus the results indicate that plant based mosquito repellents, generally giving an average of 2.5 hours repellence time, are effective for shorter periods compared to synthetic repellents which are known to give up to 8 hours protection. However, the study indicates that the plant based products may be enhanced by using them in the form of mixtures of different plant extracts. Thus, the mixture of *B. alata* and *C. ambrosioides* provided 72 % protection for 4.5 hours, compared to the individual plant extracts which gave protection above 70 % for a maximum of 3.5 hours and the differences between mixtures and individual plant extracts were generally significant. The three plants also showed mosquitocidal properties. Their knockdown as well as the mortality rates suggests that they can effectively be used as mosquitocides. The effectiveness of the plant mixtures as mosquitocides will in future be included in the test protocols so as to establish if there is the possibility of synergism or potentiation in their activities.

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Kazembe and Nkomo

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