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Insecticidal Activity of *Ageratum conyzoides* (Asteraceae) Aqueous Extracts against the Grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae)

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Authors' contributions

This study was carried out in collaboration among all authors. Authors SRA and MY designed the study. Author DTI collected the data, performed the statistical analysis and wrote the first draft of the manuscript. Authors SRA and MY reviewed and refined the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Ageratum conyzoides (Asteraceae) is an annual plant with insecticidal activity against some insect pests. The present study was designed to evaluate the insecticidal activity of aqueous extracts (infusion and maceration) of *A. conyzoides* against the pest grasshopper *Zonocerus variegatus* (Orthoptera, Pyrgomorphidae). Concentrations of 0, 10, 30, 100 & 300 µg/ml were prepared and administrated through injection to grasshoppers. Mortality rate was recorded for adult grasshoppers each day until the 9th day of treatment. Results revealed a significant increase (p < 0.05) of mortality rate with increasing concentrations of the two plant extracts. Plant extracts at 300 µg/ml

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induced 100.00 ± 0.00 percent mortality of adults *Z. variegatus* after 4 and 5 days for *A. conyzoides* infusion and maceration respectively. No mortality was registered after treatment with 0 & 10 µg/ml of the two plant extracts. Male mortality rate tended to be higher than that of female at the same concentration. Also, infusion tended to significantly increase ($p < 0.05$) mortality rate than maceration at the same concentration. Phytochemicals screening have shown that the presence of saponins, tannins, triterpenoids and alkaloids in the *A. conyzoides* aqueous extracts may be responsible for the insecticidal activity of this plant. These phytochemicals may therefore be exploited and serve as suitable alternatives to synthetic insecticides against the grasshopper *Z. variegatus*.

Keywords: Ageratum conyzoides; infusion; maceration; insecticidal activity; Zonocerus variegatus.

1. INTRODUCTION

For several centuries, pest grasshoppers have attracted a lot of attention for biological studies and control [1]. Swarms of desert locust *Shistocerca gregaria* (Orthoptera, Acrididae) were recently observed in many countries of East Africa, representing a threat to food security and livelihoods [2]. Because these pest grasshoppers can cause severe damage on food crops, more attention should then be concentrated on them. *Zonocerus variegatus* (Linnaeus, 1758) is a polyphagous insect pest of food crops which prefers man made environments living at the expense of human activity [3,4]. They are often collected on cassava leaves (*Manihut esculenta*), bitter leaves (*Vernonia amygdalina*) and on the weed *Chromolaena odorata* [4]. The degradation of forest vegetation and the presence of the weed *C. odorata* in fallows also favour swarms of *Z. variegatus* [3]. In West Region of Cameroun, this grasshopper specie is found in the field during the dry season (November-March) and the wet season (May-July) [1]. In Center Region of Cameroun, adults of *Z. variegatus* are observed in March and from mid-June to mid-December [5].

Since classical insecticides have shown to have negative effects on human health and environment, many plants extracts and essential oils derived from plants are being used against pest insects like grasshoppers. *Cestrum parqui* leaves fractions have been used against the grasshopper *S. gregaria* [6]; natural plant essential oils of Garlic (*Allium sativum*), Eucalyptus (*Eucalyptus globulus*) and Mint (*Mintha pipereta*) have been used against the grasshopper *Heteracris littoralis* [7]; *Annona squamosal*, *Ailanthus altissina*, *Calotropis pocera*, *Syzygium aromaticum* and *Azadirachta indica* organic extracts have been used against the pest grasshopper *Chrotogonus trachypterus* [8]. *Ageratum conyzoides* (Asteraceae) or "goat weed" possess some insecticidal and pesticidal activity against various types of insects. Extracts and essential oil derived from this plant have been used against many insects like grasshopper (*S. gregaria*), domestic fly (*Musca domestica*), cowpea weevil (*Callosobruchus maculatus*), pulse beetle (*Callosobruchus chinensis*), mustard aphid (*Lipaphis erysimi*) and mosquitoes (*Aedes aegypti* and *Anopheles stephensi*) [9]. From the above literature, it became necessary to test the insecticidal activity of *A. conyzoides* against some other pest insects. Thus, the present study was designed to evaluate the insecticidal activity of *A. conyzoides* against the pest grasshopper *Z. variegatus*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves and stem of *A. conyzoides* were collected from Bamendou, West Region of Cameroon. Reference number 6575/SRFK/Cam was giving after authentication at the Cameroon National Herbarium in Yaounde. The plant was then dried, powdered and kept in air tight containers for the preparation of the different extracts.

2.2 Preparation of Plant Extracts

Infusion and maceration were the different aqueous extracts prepared.

2.2.1 Infusion

For the infusion, 100 g of dried powder was mixed in 1 litre of hot distilled water heated at 100°C. After decantation, the mixture was filtered using a sieve of 150 µ of diameter, cotton and coffee paper no. 4. The filtrate obtained was then concentrated in a hot air oven at 45°C for 48 hours.

2.2.2 Maceration

Maceration was prepared with 100 g of dried powder mixed in 1 litre of distilled water at room temperature for 36 hours. The mixture was stirred twice a day using spatula. After 36 hours, the mixture was filtered using a sieve of 150 µ of diameter, cotton and coffee paper no. 4. The filtrate obtained was also concentrated at 45°C in a hot air oven for 48 hours.

The dried aqueous infused and macerated extracts obtained were then kept in a refrigerator at 4°C until use.

2.2.3 Preparation of extract solutions at different concentrations

For the two extracts (infusion and maceration), a stock solution of 10 mg/ml was prepared and microdilutions were done from it. The concentrations of 0, 10, 30, 100 and 300 µg/ml were prepared by adding distilled water.

2.3 Phytochemicals Analysis

The phytochemical screening was carried out on the dried aqueous extracts using standard procedure to identify the constituents as described by [10]. Alkaloids, flavonoids, cardiac glycosides, phlobatannins, saponins, steroids, tannins and triterpenoids were the different classes of secondary metabolites tested.

2.4 Biological Material

Adult male and female grasshoppers of *Z. variegatus* were collected from field at Yaounde (Center Region of Cameroon) in August 2018 and brought to the Laboratory of the Research Unit of Biology and Applied Ecology (RUBEA) of the University of Dschang (West Region of Cameroon). The grasshoppers were reared in cages and fed with fresh leaves of bitter leaves (*Vernonia amygdalina*). A total of 135 adult male and 135 adult female grasshoppers were used for this study.

2.5 Administration of Extract

After five (5) days of acclimatization, grasshoppers were separated into three (3) groups (A, B and C). The screening of plant extracts was tested by topical application method [8]. Group A (control group) grasshoppers were made up of ten (10) grasshoppers (5 males and 5 females) and receive by injection 0.1 ml of distilled water. Grasshoppers in groups B and C were separated into four (4) subgroups (B1, B2, B3, B4 and C1, C2, C3, C4) of respectively ten (10) grasshoppers (5 males and 5 females) each. The grasshoppers in group B were respectively injected with 10, 30, 100 and 300 µg/ml of *A. conyzoides* infused extract while those in group C were respectively injected with 10, 30, 100 and 300 µg/ml of *A. conyzoides* macerated extract. Mortality counts in both treatments were recorded for three (3) replications every day and for a total of nine (9) days.

2.6 Statistical Analysis

The data related to the mortality rate were expressed as Mean ± Standard Deviation (S.D). Statistical significance among groups was done using One Way ANOVA followed by Duncan Multiple Range Test. Statistical significance between the two (2) technics of extractions (infusion and maceration) was done using Student Independent Samples test. Statistical significance was set at $p < 0.05$. SPSS version 20.0 computer software was used to analyse the data. Mortality rate was calculated as follows:

> Mortality rate = (Cumulated number of death individuals / Total number of initial individuals) x 100.

3. RESULTS AND DISCUSSION

3.1 Phytochemicals Screening of *Ageratum Conyzoides* Aqueous Extracts

Different phytochemicals tested are shown in Table 1. From all the phytochemicals tested, *A. conyzoides* infusion contained saponins, triterpenoids and phlobatannins while *A. conyzoides* maceration contained alkaloids, tannins and cardiac glycosides. Though the two extracts (infusion and maceration) were aqueous extracts, different phytochemicals were found depending on the type of extraction. These results clearly suggest that the presence of a phytochemical in an extract depends on the type of the extraction. Terpenoids, tannins and saponins were found in the aqueous crude extracts of *A. conyzoides* [11]. Also, flavonoids, triterpenes, steroids, alkaloids, phenols, cardiac glycosides were found in *A. conyzoides* [12,13,14,15].

Table 1. Phytochemicals screening of aqueous extracts of *Ageratum conyzoides*

Phytochemicals	Infusion	Maceration
Alkaloids	-	+
Flavonoids	-	-
Cardiac glycosides	-	+
Phlobatannins	+	-
Saponins	+	-
Steroids	-	-
Tannins	-	+
Triterpenoids	+	-

+ = present; - = absent

3.2 Mortality Rate

3.2.1 Daily mortality rate of *Zonocerus variegatus*

The Daily evolution of the mortality rate of *Z. variegatus* treated with *A. conyzoides* aqueous extracts is presented in Table 2. This Table reveals that at 300 µg/ml, plant extracts induced 100.00 ± 0.00 percent mortality of adults *Z. variegatus* after 4 and 5 days for *A. conyzoides* infusion and maceration respectively. Mortality rate was nil (0.00 ± 0.00) in control group and in group treated with 10 µg/ml of the two aqueous plant extracts after 9 days of treatment. Until the day 9, no 100% mortality was recorded for others concentrations. Nevertheless, the mortality rate was higher in groups treated with infusion than maceration. At 100 µg/ml, *A. conyzoides* infusion induced 96.67 ± 5.77 percent mortality while *A. conyzoides* maceration induced 66.67± 11.55 percent mortality after 9 days of treatment. These results indicated that mortality rate is related to the concentration and to the type of extract. Also, the mortality rate increased with days of treatment but in some cases, this percent mortality tended to be constant. At 30 µg/ml, *A. conyzoides* infusion induced 3.33 ± 5.77 percent mortality in day 5, 6 and 7. Percent mortality of 30.00 ± 0.00 were recorded in day 4, 5, 6 and 7 after treatment with *A. conyzoides* maceration at 100 µg/ml. This constant percent mortality could be attributed to the moulting process because old removed shells were observed in grasshopper cages. During moulting process, the grasshopper may release some hormones which influence the insecticidal activity of different extracts. Injection of 30 mg/ml of *Cestrum parqui* (Solanaceae) extract to the 7 days L5 larvae of the grasshopper *S. gregaria* did not induce mortality due to exuviation process during moulting cycle [6].

3.2.2 Males and females mortality rate of *Zonocerus variegatus*

Adults male and female mortality rate is given in Table 3. The two extracts at 300 µg/ml caused 100.00 ± 00.00 percent mortality of male and female adults *Z. variegatus*. *Ageratum conyzoides* infusion also caused 100.00 ± 00.00 percent mortality of adult males of *Z. variegatus*. The mortality rate of males and females was nil (0.00 ± 0.00) in control group and in groups treated with 10 µg/ml of *A. conyzoides* infusion and maceration. In groups treated with 30 and 100 µg/ml of the two extracts, mortality rate of males was higher than those of female grasshoppers *Z. variegatus*. At 100 µg/ml, male mortality rate was 100.00 ± 00.00 percent for infusion and 86.67 ± 11.55 percent for maceration. At this same concentration (100 µg/ml), female mortality rate was 93.33 ± 11.55 percent for infusion and 46.67 ± 11.55 percent for maceration. Studies carried out by [8] revealed that LC50 values of males were lower than those of females for all the five plants tested (*Azadirachta indica*, *Annoma squamosal*, *Ailanthus altissima*, *Calotropis procera* and *Syzygium aromaticum*) on the adult grasshoppers *C. trachyptera* (Orthoptera, Acrididae). These lower LC50 values of males indicate that males were more sensitive to the plants extracts than females which therefore lead to higher male mortality rate compared to female mortality rate at the same concentrations of plants extracts. The higher mortality rate of male grasshoppers could be attributed firstly to the different hormonal metabolisms taking place in the grasshopper, and secondly to the weight of male grasshoppers. Male grasshoppers are generally smaller than female, making them to be more sensitive to the effects of plants extracts.

3.2.3 Effects of plant extracts on mortality rate of *Zonocerus variegatus*

Effects of aqueous extracts of *A. conyzoides* on the mortality rate of adults *Z. variegatus* is given in Table 4. This Table shows that the mortality rate of adult grasshoppers significantly ($p < 0.05$) increased with increasing concentrations of the two aqueous plant extracts. No significant differences ($p > 0.05$) were observed in control group and groups treated with 10 µg/ml of *A. conyzoides* infusion and maceration. The same observation was made in groups treated with 100 and 300 µg/ml of *A. conyzoides* infusion. At 30 µg/ml, infusion induced 30.00 ± 0.00 percent mortality while maceration induced 13.33 ± 5.77

Table 2. Daily mortality rates of adults of *Zonocerus variegatus* treated with aqueous extracts of *Ageratum conyzoides*

Treatments	C (µg /ml)	Mortality rate (%)								
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Control	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Infusion	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.33± 5.77	3.33± 5.77	3.33± 5.77	13.33± 5.77	30.00± 0.00
	100	16.67 ± 5.77	26.67 ± 5.77	40.00± 0.00	50.00 ± 0.00	73.33 ± 11.55	83.33± 11.55	93.33± 11.55	96.67± 5.77	96.67± 5.77
	300	50.00 ± 0.00	86.67± 5.77	96.67± 5.77	100.00 ± 0.00	/	/	/	/	/
Maceration	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.33± 5.77	10.00± 0.00	10.00± 0.00	13.33± 5.77
	100	0.00 ± 0.00	0.00 ± 0.00	10.00± 5.77	30.00 ± 0.00	30.00 ± 0.00	30.00± 0.00	30.00± 0.00	36.67± 11.55	66.67± 11.55
	300	50.00 ± 0.00	73.33± 5.77	93.33± 5.77	96.67± 5.77	100.00 ± 0.00	/	/	/	/

Data are Mean ± S.D. C: Concentration (µg/ml)

Table 3. Mortality rate of adult males and females of *Zonocerus variegatus* treated with aqueous extracts of *Ageratum conyzoides* after 9 days

C (µg/ml)	n	Infusion		Maceration	
		♂	♀	♂	♀
0	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
30	5	46.67 ± 11.55	13.33 ± 11.55	20.00 ± 0.00	6.67 ± 11.55
100	5	100.00 ± 00.00	93.33 ± 11.55	86.67 ± 11.55	46.67 ± 11.55
300	5	100.00 ± 00.00	100.00 ± 00.00	100.00 ± 00.00	100.00 ± 00.00

Data are Mean ± S.D. C: Concentration (µg/ml); n: Number of individuals; ♂: Male; ♀: Female

Table 4. Effects of aqueous extracts of *Ageratum conyzoides* on mortality rate of adults *Zonocerus variegatus*

C (µg/ml)	n	Percent mortality	
		Infusion	Maceration
0	10	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{d}
10	10	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{d}
30	10	30.00 ± 0.00 ^{Ab}	13.33 ± 5.77 ^{Bc}
100	10	96.67 ± 5.77 ^{Aa}	66.67 ± 11.55 ^{Bb}
300	10	100.00 ± 00.00 ^{a}	100.00 ± 00.00 ^{a}

Data are Mean ± S.D. ^{a,b,c,d}…Means within a column followed by the same letter are not significantly different (Duncan test, $p < 0.05$). ^{A,B}…Means within the same line followed by the same letter are not significantly different (Student t test, $p < 0.05$). C: Concentration (µg/ml); n: Number of individuals

percent mortality. At 100 µg/ml, infusion induced 96.67 ± 5.77 percent mortality while maceration induced 66.67 ± 11.55 percent mortality. Considering the two plant extracts at these concentrations (30 and 100 µg/ml), infusion seems to significantly ($p < 0.05$) induced higher percent mortality than maceration. This could be due to the effects of phytochemicals present in each plant extracts.

Biological activities of plant extracts for insects control have been related to various phytochemicals including terpenes, phenols, alkaloids and phenylpropenes [16,17]. *Ageratum conyzoides* infusion contain saponins, triterpenoids (terpenes) and phlobatannins while *A. conyzoides* maceration contain alkaloids, tannins (phenols) and cardiac glycosides. Tannins are capable to deactivate digestive enzymes by forming a tannin-protein complex, difficult to digest by the grasshopper *S. gregaria* [18]. Various terpenoids (tetra-cyclic terpenes and tetranortriterpenoids) contained in *A. indica* (neem) are responsible for it antifeedent activity [8]. *Syzygium cumini* seed extracts possessed significant effect on central nervous system (CNS) due to the presence of saponins [19]. It had been reported that saponin molecules played an important role in larvicidal activity of the *S. cumini* extract against the yellow fever mosquito *A. aegypti* [20]. Also, saponins contained in *C. parqui* have shown high solubility in *S. gregaria* haemolymph and was responsible for the insecticidal activity against this grasshopper [6]. *A. conyzoides* contains pyrrolizidine alkaloids, a class of hepatotoxic, carcinogenic, genetotoxic, teratogenic and sometimes pneumotoxic phytochemical [21]. Pyrrolizidine alkaloids of *Calotropis procera* are known for their high toxicity against *Locusta migritoria nymph* (Orthopera, Acrididae) [8]. *Zonocerus variegatus* feed deliberately on plants containing these pyrrolizidine alkaloids which are easily digested, stored in the non-toxic form by this grasshopper and used for the defense mechanism [22]. Nevertheless, studies carried out by [23] have shown that alkaloids in excessive amount could be poisonous. Previous studies have shown that oil of *A. conyzoides* exerted acute toxicity on adult cowpea weevil (*C. maculatus*) and possess high nymphal mortality (91%) against the nymphs of *S. gregaria* while hexane extract of the whole plant showed activity against *M. domestica* larvae [9]. The presence of saponins and triterpenoids may be therefore responsible for the relatively high mortality rate of adult grasshopper *Z. variegatus* after treatment with *A. conyzoides* infusion. The mortality rate of adult grasshopper *Z. variegatus* after treatment with *A. conyzoides* maceration may be more attributed to the presence of tannins than the presence of alkaloids because the effect of these

alkaloids may be influenced by the transformation of pyrrolizidine alkaloids by this grasshopper.

4. CONCLUSION

Phytochemicals screening of *A. conyzoides* aqueous extracts confirm the presence of phytochemicals which have insecticidal activity that could be exploited in the production of biopesticides against the pest grasshopper *Z. variegatus*. The presence of saponins and triterpenoids may be responsible for the insecticidal activity of *A. conyzoides* infusion while the presence of tannins and alkaloids may be responsible for the insecticidal activity of *A. conyzoides* maceration. We would like to suggest quantitative isolation of these active phytochemicals for further study in order to clearly describe their insecticidal mechanism against grasshoppers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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