World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Preliminary assessment of larvicidal activity of *Ageratum conyzoides* (L.) flower and Spinosad against *Aedes aegypti* (Diptera: Culicidae): A vector of dengue fever

Ernest O. Dawodu [1, *], Samuel A. Babarinde [2], Olukayode J. Adelaja [3], Ismail A. Ayegboyin [2], Tolulope E. Ajetomobi [2] and Omolara R. Salami [2]

[1] *Department of Agricultural Science and Technology, Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti, Nigeria.*
[2] *Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.*
[3] *Department of Zoology, University of Ilorin, Ilorin, Nigeria.*

World Journal of Advanced Research and Reviews, 2025, 26(02), 1365-1371

Publication history: Received on 30 March 2025; revised on 09 May 2025; accepted on 11 May 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.2.1785

Abstract

The larvicidal potentials of aqueous extract of *Ageratum conyzoides* L. (Asteraceae) flower was compared with Spinosad against 4th or 5th instar *Aedes aegypti* (Diptera: Culicidae) at 1, 2, 3 and 4 percent volume per volume (v/v). Dichlorvos and distilled water served as positive and negative control, respectively. Mortality data collected at 0.5, 1.0, 1.5, 2.5 and 3.0 hours after treatment (HAT) were subjected to analysis of variance. The lethal time (LT50 and LT90) were estimated using probit analysis. At 2.0-3.0 HAT, Dichlorvos was consistently superior (90.00%) to other treatments, while Spinosad evoked 49.45-85.40% mortality compared to 49.50-69.55% mortality observed in 4% v/v *A. conyzoides*. There was no significant difference in the LT50 of *A. conyzoides* applied at 4% v/v and Spinosad. *Ageratum conyzoides* showed lower toxicity capability against *A. aegypti* than Dichlorvos.

Keywords: Mosquito; Larvae; *Ageratum conyzoides*; Vector; *Aedes Aegypti*; Dengue Fever

1. Introduction

In many tropical and subtropical countries of the world, mosquitoes (Diptera: Culicidae) serve as vectors of life-threatening diseases such as malaria, lymphatic, filariasis, dengue fever, Japanese encephalitis, chikungunya, Zika and yellow fever among others [1, 2]. Apart from the favourable weather conditions that favour the biology of mosquitoes, diverse conducive habitats is another factor that is contributory to their success. Agricultural practices such as the use of irrigation, the use of ponds for fish farming and the storage of water in tanks for livestock provide suitable breeding grounds for mosquitoes [3]. Besides, abandoned containers, tree trunks, uncompleted buildings can serve as breeding sites. Mosquito habitat varies for each species and can include natural areas such as rain puddles and ponds, decomposing material such as wet leaf matter, ditches and marshes [4]. Vector's control strategies have traditionally focused on killing mosquitoes using a variety of insecticides. Environmental management (through reduction or removal of mosquito breeding sites) has often been used alongside chemical or [microbiological ovicides, larvicides, and pupicides [5, 6, 7) in areas where endemic mosquito-borne diseases occur. The use of synthetic insecticides has to be regulated given that the development of resistance to insecticides is widespread [8, 9, 10, 11, 12]. Dichlorvos is still being used in some developing countries despite its barn in some developed countries. It is a popular mosquitocide sold in markets with different brand names and in different packages. Although, Spinosad has been identified as a mosquitocide in some developed countries, the need for the evaluation of geographical influence on its efficacy against different species of mosquitoes is pertinent [13]. Since the issue of resistance has become a major concern in the management of disease vectors, Spinosad has been proposed as a reliable alternative in places where organophosphates

* Corresponding author: Ernest O. Dawodu

resistance in mosquitoes have been reported [14]. Incidentally, not many farming families in the rural resource-poor setups currently have access to affordable brands of Spinosad. Therefore, attempts are focused on the exploration of botanicals as sources of mosquitocidal compounds.

Ageratum conyzoides is a weed with notable ethnobotanical potentials [15]. Its antimicrobial activities have been reported [16]. To a very great extent, published works on the pesticidal potentials of *A. conyzoides* focused on the whole plant or its leaves. For instance, both the essential oil as well as the major component of the oil, namely Precocene, have been reported to have antijuvenile hormonal activity. The oil exerted acute toxicity on *Callosobruchus maculatus* upon fumigation [17], while [18] reported its bioactivity against house fly, *Musca domestica*. Various insects which have been susceptible to the weed have been documented [19].

Several authors have reported the insecticidal potentials of Ageratum species against different species of mosquitoes. For instance, [20] evaluated the larvicidal activity of the essential oil of *Ageratum conyzoides* aerial parts and its major constituents against *Aedes albopictus*. The toxicity of the extracts of the leaves against *Anopheles stephensi* has been reported [21] while the toxicity of the volatile oils of the leaves against Culex species has also been reported [22]. The work of [23] seems outstanding since they evaluated the mosquitocidal potential of the essential oils and crude extracts obtained from the leaves and the flowers. Incidentally, majority of the reported cases of the screening of the products from *A. conyzoides* did not compare their efficacy with Spinosad, a bio-rational microbe-based insecticide, or any synthetic insecticide. The study which compared the mosquito larvicidal potential of three botanical oils including that of *A. conyzoides* with synthetic insecticide was on Endosulfan and not Spinosad and Dichlorvos [24].

Essential oils and inorganic extracts have recently received renewed attention as biorational alternatives to over-dependence on synthetic mosquitocides. Although, they displayed outstanding efficacy, they would practically be difficult for resource-poor populace to adopt due to the technicality involved in their extraction. The present study evaluates a cost-effective formulation with reduced tendency of technological bottleneck for adoption. Therefore, the aim of the study was to evaluate the comparative larvicidal potentials of the aqueous extract of *A. conyzoides* flower and Spinosad against the dengue fever mosquito, *Aedes aegypti*.

2. Materials and methods

2.1. Research site

The research was carried out at the Entomology Unit of the Department of Crop and Environmental Protection Laboratory, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria.

2.2. Field collected laboratory maintained *Aedes aegypti* larvae

The larvae of *Aedes aegypti* were collected at the premises of Adegolu Poultry House, Faculty of Agricultural Sciences Teaching and Research Farm, LAUTECH, Ogbomoso. The Field Collected Laboratory Maintained (FCLM) larvae were carefully transported from the collection site to the laboratory and were sorted by separating 4lh or 5th larval instar of *A. aegypti* larvae from other species and predators. The larvae were identified using standard morphological keys [25]. The Field Collected Laboratory Maintained (FCLM) mosquito larvae were allowed to adapt to the laboratory environment by placing them in clean water and left for 12h; after which the active larvae were selected for the bioassay.

2.3. Preparation of *Ageratum conyzoides* aqueous extract

Ageratum conyzoides flowers used for the study were harvested from the Faculty of Agricultural Sciences Teaching and Research Farm, LAUTECH, Ogbomoso, Nigeria. The flowering weeds were carefully uprooted and the flowers were manually severed and air-dried until crisps, under ambient conditions for 16 days. The dried flowers were pulverized, and 50 g of the powder was weighed into a round bottom flask with 500 ml distilled water and soaked for 24h. Thereafter, the extract was sieved with a muslin cloth and the filtrate was kept in a bottle as the stock solution. The process was repeated to obtain sufficient volume of the extract for the bioassay.

2.4. Larvicidal Activity of *Ageratum conyzoides* aqueous extract

The larvicidal activity assay of the extract against *A. aegypti* larvae was based on the procedure described by the World Health Organization [26]. Serial dilutions of the stock solution were made with distilled water to obtain 1.0, 2.0, 3.0, 4.0% (v/v) in 50 ml extract-distilled water mixture separate transparent 200 ml-capacity plastic cups. A portion (10 g) of Spinosad (Spinter® dust 12.5% a. i.) was mixed with 50 ml of distilled water and 0.5 ml of the Spinosad solution was added to 50 ml of distilled water in 200 ml-capacity plastic cups.

One ml of Dichlorvos (2% EC) was dissolved in 500 ml distilled water to obtain a stock solution of Dichlorvos, out of which 0.5 ml dissolved in 50 ml distilled water was used as the positive control. Distilled water (50 ml) served as the negative control. Ten active FCLM 4th-5th instar larvae of *A. aegypti* were carefully introduced into each cup and were not fed throughout the experimental period. Larval mortality was recorded at 0.5, 1.0, 1.5 2.0, 2.5 and 3.0 Hours After Treatment (HAT), percentage mortality (PM) was calculated using the formula below:

$$\text{PM} = \frac{\text{Number of dead larvae}}{\text{Number of Assayed larvae}} x\ 100$$

The experiment was replicated four times.

2.5. Experimental design and data analysis

The experiments were set up in completely randomized design and data were subjected to analysis of variance (ANOVA). Significant means were separated using Studentized Newman Keuls (SNK) at 5% significance level. Thereafter, probit analysis was used to determine the lethal time (LT50 and LT90) for each of the treatments. All statistical analyses were carried out with the aid of SPSS Software version 16.

3. Results

There was no mortality in distilled water throughout the experimental duration. When *A. aegypti* larvae were exposed to the treatment at 0.5 HAT, there was no mortality in all *A. conyzoides* concentrations and Spinosad, but Dichlorvos caused 45.00% mortality. At 1.0 HAT, Dichlorvos caused significantly ($F=118.945$; $df=6, 27$; $p<0.0001$) higher mortality (52.00%) than 22.50 and 13.80% mortality observed in Spinosad and 4% *A. conyzoides*, respectively. The same trend was observed at 1.5 HAT, where 80.80% mortality observed in Dichlorvos was significantly ($F=158.886$; $df=6, 27$; $p<0.0001$) higher than 36.50, 22.50 and 26.20% larval mortality observed in Spinosad, *A. conyzoides* at 3% and 4%, respectively. At 2.0-3.0 HAT, Dichlorvos was consistently superior (with 90.00% mortality) to other treatments, while Spinosad evoked 49.45-85.40% mortality compared to 49.50-69.55% mortality observed in 4% *A. conyzoides* (Table 1).

Table 1 Comparative toxicity of *Ageratum conyzoides* flower extract, spinosad and Dichlorvos against *Aedes aegypti* larvae

	Mortality at Hours after treatment					
Treatments	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**
Ageratum conyzoides at 1% v/v	0.00 ±0.00a	0.00 ±0.00a	0.00 ±0.00a	4.60±4.60a	20.45±2.05b	28.25±1.65b
Ageratum conyzoides at 2% v/v	0.00 ±0.00a	0.00 ±0.00a	9.20±5.31a	18.40±0.00b	22.50±2.36b	28.25±1.65b
Ageratum conyzoides at 3% v/v	0.00 ±0.00a	4.60±4.60ab	22.50±2.36b	33.05±2.57c	43.50±3.69c	52.55±4.56c
Ageratum conyzoides at 4% v/v	0.00 ±0.00a	13.80±4.60bc	26.20±3.02b	43.50±3.69d	50.85±2.40c	69.55±2.05d
Spinosad	0.00 ±0.00a	22.50±2.36c	36.05±3.15c	49.45±4.40d	74.15±5.62d	85.40±4.60e
Dichlorvos	45.00±2.36b	52.50±3.88d	80.80±5.31d	90.0±0.00e	90.0±0.00e	90.0±0.00e
Negative control	0.00±0.00a	o. oo±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
ANOVA Result	df=6,27 F=91.956 P<0.001	df=6,27 F=118.945 P<0.001	df=6,27 F=158.886 P<0.001	df=6,27 F=149.091 P<0.001	df=6,27 F=178.125 P<0.001	df=6,27 F=299.865 P<0.001

Values are means four replicates ± SE. Means followed by same letter of alphabet within the column are not significantly different using SNK at 5% significance level.

The result of probit analysis followed the same trend as the analysis of variance. When *A. conyzoides* was applied at 1% v/v, the LT50 value [0.555 (0.511-0.636) h] was higher than the value obtained in application of *A. conyzoides* at 4%

[0.401 (0.358-0.454) h] and the value obtained when Spinosad [0.276 (0.200-0.360) h] and Dichlorvos was applied [0.181(0.001-0.034) h]. That indicates that application of *A. conyzoides* at 4% v/v performed at par with Spinosad, but not Dichlorvos. The LT90 values of Dichlorvos, Spinosad and *A. conyzoides* applied at 4% v/v were similar with the overlap of their fiducial limits (Table 2).

Table 2 LT_{50} and LT_{90} for *Aedes aegypti* exposed to *Ageratum conyzoides* flower extracts, spinosad, and Dichlorvos

Treatments	LT50(LFL-UFL)	LT90(LFL-UFL)	Slope	X2	df	P	Intercept	SE
Ageratum conyzoides at 1% v/v	0.555(0.511-0.636)	0.760(0.669-0.937)	-8.030	2.817	4	0.589	-3.479	0.433
Ageratum conyzoides at 2% v/v	0.620(0.545-0.751)	1.004(0.845-1.298)	-10.832	4.713	4	0.318	-2.064	0.191
Ageratum conyzoides at 3% v/v	0.542(0.382-1.570)	1.034(0.668-3.951)	-12.102	19.361	4	0.001	-1.413	0.117
Ageratum conyzoides at 4% v/v	0.401(0.358-0.454)	0.835(0.733-0.987)	-11.388	5.160	4	0.271	-1.181	0.`104
Spinosad	0.276(0.200-0.360)	0.648(0.523-0.906)	-9.907	9.868	4	0.043	-0.953	0.096
Dichlorvos	0.181(0.001-0.034)	0.419(0.268-0.760)	6.013	11.253	4	0.024	0.387	0.064

LFL: Lower fiducial limit; UFL = Upper fiducial limit

4. Discussion

The results of this study indicate that *A. conyzoides* flower extract possessed toxicity potential against *A. aegypti*. The toxicity was dependent on concentration and exposure period. In earlier botanical studies, toxicity of botanical formulation was dependent on dose/concentration and exposure period [27, 28, 29, 30]. The increase in mortality with dosage agrees with [31] which reported that the toxicity of *Afromomum melegueta* leaf and seed extracts against Anopheles species increased with an increase in concentration. The basis for the progression in the observed toxicity was that the experimental insects had no escape route from the treatments and were exposed to the toxicants via contact in the experimental unit, which could consequently affect the physiology of the larvae. Besides, since the experimental larvae were not fed, they orally picked lethal phytochemicals from the extracts added to the water where they were confined to.

Earlier studies reported the phytochemical components of *A. conyzoides* flower to contain terpenes, tannin, alkaloids, chromenes sterols and flavonoids from different parts of the plant [16, 32]. The observed bioactivity could have been due to the bioactive compounds present in the flower. This postulation agrees with previous authors who reported that the bioactivity of botanicals was related to the inherent bioactive compounds. According to [20], the larvicidal properties of the essential oil of *A. conyzoides* against *A. albopictus* was attributed to Precocene II and Precocene I, the major compounds of the oil. Also, another group of researchers related the toxicity of *Clerodendrum phlomidis* Linn. F. against *A. stephensi* to Pectolinaringenin, a compound isolated from the plant [33]. *Aedes Aegypti* larvae were vulnerable to the bioactive compounds in the assayed *A. conyzoides* flower extract, Spinosad and Dichlorvos, with contact, systemic and stomach toxicity being the possible mechanism of action, because the treatments were directly applied into the habitant of FCLM *A. aegypti* larvae.

The results of the probit analysis indicated that *A. conyzoides* flower extract applied at 4% v/v compared with spinosad. However, Dichlorvos was superior to other treatments. The ANOVA results also affirmed the superior toxicity of Dichlorvos over other treatments. This agrees with the reports of [20] who reported the superior bioactivity of Chlorpyrifos compared with the essential oil of *A. conyzoides* against *A. albopictus*. Incidentally, [34] reported that the toxicity of *Ocimum gratissimum* essential oil applied at 50 µL/L air in a fumigant bioassay performed at par with Chlorpyrifos against *Trogoderma granarium*; while the efficacy of the synthetic pesticide surpassed the toxicity of the lower doses of the essential oil. That implies that the toxicity of Chlorpyrifos is influenced by the method of assay and the species of insects. In an earlier study to evaluate the efficacy of spinosad against three species of mosquitos (*Aedes aegypti*, *Anopheles stephensi*, and *Culex pipiens*) under laboratory condition, Spinosad was particularly effective against

larval Aedes and Culex, with a less marked activity against anophelines [35]. The toxicity of spinosad reported in the present study indicates that the Nigerian strain of *A. aegypti* was susceptible to Spinosad.

Despite the superior efficacy of Dichlorvos, its ecological safety and cost implications are major constraints to its recommendation to resource-poor rural dwellers. Therefore, since *A. conyzoides* is often regarded as weed in many farming systems, its flower can be harvested and processed as *A. aegypti* larvicide. This ethnobotanical potential of *A. conyzoides* can also serve as a weed management scheme in *A. conyzoides*-prone agrarian settlements. With the result of this finding, *A. conyzoides* has also been established as a potential component of Integrated Pest Management of *A. aegypti*.

5. Conclusion

The effect of extracts from Ageratum conizoides was determined on the 4th and 5th instar *Aedes aegypti*, with Spinosad and Dichlorvos as source of comparison. Mortality of larvae was recorded at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hours after treatment (HAT). Data was subjected to analysis of variance. Probit analysis was carried out at estimated lethal time of (LT50 and LT90). At 2.0-3.0 HAT, Dichlorvos was consistently superior (90.00%) to other treatments, while Spinosad caused 49.45-85.40% mortality compared to 49.50-69.55% mortality observed in 4% v/v *A. conyzoides*. The toxicant effect exhibited by *A. conizoides* showed that it could be a veritable source for the control of *A. aegypti* although, Dichlorvos was more toxic.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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