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Influence of solvent medium on the bioefficacy of ten plant materials against adult African rice gall midge (AfRGM-*Orseolia oryzivora*)

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Abstract
A study conducted at Africa Rice Center, International Institute for Tropical Agriculture, Ibadan, Nigeria aimed to evaluate ten botanicals extracted in n-hexane, methanol and chloroform against African rice gall midge (AfRGM). The experiment was laid out in 10 x 3 factorial arrangements fitted into completely randomized design (CRD) with carbofuran and water as checks in three replications. The level of infestation by AfRGM significantly reduce in botanicals treated-plant particularly, on rice treated with *Ageratum conyzoides* and *Chromolaena odorata* extracted in n-hexane. *A. conyzoides* extracted in n-hexane (5.94 tons/ha) produced a significantly higher yield compared to other treatments, resulting into 73.91% yield increase over the control. There was a negative and significant correlation (r= - 0.60) between tiller infestation and grain yield, as well as other agronomic characters. Application of *A. conyzoides* extracted in hexane, exhibited better control of AfRGM and enhanced higher grain yield without adversely affecting agronomic traits.

Keywords: AfRGM infestation, botanicals, solvent, rice, *Ageratum conyzoides*

1. Introduction
The African rice gall midge (AfRGM), *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae), is an indigenous insect pest of rice in Africa [1, 2]. AfRGM was initially considered a minor pest of rice until late 1970s when an outrageous outbreak occurred in southern Burkina Faso. In late 1980s, it was reported in central and southeast Nigeria claiming total yield in worst affected fields [3, 4]. Larval AfRGM causes severe damage on rice plants during the vegetative stages (Seedling to panicle initiation) in which tube-like 'silver shoot' or 'onion leaf' galls which prevent panicle production are produced. The larvae feed on the growing primordial of the rice tiller to generate the development of the tubular gall which is irreversibly damaged causing profuse tillering and stunting of the plant. Severe yield losses are reported from countries where AfRGM is endemic and these vary significantly depending on the climatic zone, ecosystem and level of cropping intensification. Yield loss could be as high as 100% in endemic areas [5].

Managing AfRGM in Nigeria has been dominated by the use of conventional insecticides [6]. These insecticides have not given the desired control as they are not environment-friendly [7]. The adverse effect of this measure and the high economic impact of the AfRGM have led to much attention on use of botanical insecticides in the recent decades because plants produce a diversity of biologically active substances that affect the growth and development of other organisms and can provide protection against herbivores to influence their feeding, settling, oviposition, growth and development, and/or fertility. The use of botanicals, which are considered to be environmentally friendly, does not only reduce application of synthetic insecticides, but also reduce the cost of pest management. Furthermore, the efficacy of botanical extracts is dependent on the quality of bioactive compounds it possesses, which in turn is dependent firstly on the use of appropriate solvents amidst other factors [8]. Consequently, extraction solvents have effects on the extraction yields as well as the constituents and contents of extracted bioactive compounds, which in turn affect the biological activity of the extract significantly [9, 10]. For instance, [9] reported methanol as the most effective solvent, that extracted optimal bioactive phytochemical yield of *Severinia buxifolia* relative to distilled water, ethanol, chloroform, dichloromethane and acetone tested.

Therefore, the study evaluated ten plants extracted in n-hexane, methanol and chloroform for bio-efficacy against AfRGM**.**

2. Materials and Methods

The experiment was carried out at the Africa Rice Center (AfricaRice) research station at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Latitude 3°54′32″ E and Longitude 7°29′15″N). The study was conducted in cages and placed in the lowland Paddy Screen house on the institute.

2.1 Stock culture of AfRGM

Orseolia oryzivora culture was raised on ITA-306 (FARO-37), a susceptible variety.in the screenhouse of AfricaRice Center, Ibadan according to [11] with a little modification. The AfRGM population used for initial infestation was obtained from the Entomology Unit, AfricaRice Center, Ibadan. ITA-306 was planted in seed boxes 50 x 60 x 7 cm and NPK 15-15-15 fertilizer was applied at 60 kg/ha. At two weeks after seeding (2WAS), the seed boxes were placed in an AfRGM oviposition cage where 20 mated females of *O. oryzivora* collected from the stock culture using a glass vial were introduced onto the seedlings for continuous oviposition. The seed boxes containing the seedlings and the eggs were later transferred into the emergence cage where the seedlings were then sprayed with sufficient water using Hill Master^R handheld sprayer every 2 hours for five days to aid larval entry into the rice tillers. Newly emerged adults were thereafter collected to infest fresh batches of susceptible rice seedlings in order to maintain a clean and healthy culture.

2.2 Collection of plant materials

Ten plant species belonging to six different families (Poaceae, Asteraceae, Caricaeae, Lamiaceae, Meliaceae and Euphorbiaceae) that have been reported to possess insecticidal properties were selected and used for the study. These include- *Cymbopogon citratus* (DC.), *Chromolaena odorata* L., *Ageratum conyzoides* L., *Carica papaya* L., *Hoslundia opposita* Vahl., *Azadirachta indica* A. Juss, *Ocimum gratissimum* L., *Tithonia diversifolia* Hemsl., *Vernonia amygdalina* Del., *Jatropha curcas* L.). Fresh leaves of *Ageratum conyzoides* and *Chromolaena odorata* were collected within IITA campus at EH N6 with latitude N07^o 29.405 and longitude E003^o 54.021, elevation 199 m and EHS6 with latitude N07^o 29.372 and longitude E003^o 54.036, elevation 204 m. Leaves of *Ocimum gratissimum* were collected from the botanical garden of the Institute, while other leaf materials were collected in and around the IITA campus.

2.3 Preparation of extracts for spraying.

Fresh leaves of each of the selected plant materials were weighed using the electric weighing scale. The leaves were blended separately and 200 g each of the blended leaf materials were soaked in n-hexane, methanol and chloroform (500 mL each) separately for 24 hours at room temperature (28±4 °C). The different solvent extracts obtained were filtered using Whatman No.1 filter paper and the filtrates were exposed to air for 24 hours to allow evaporation of the solvents. The extracts left behind were thereafter stored in glass jars at 4^{o}C until needed for the experiment. The botanical extracts were prepared five days before the start of its application.

2.4 Experimental design

The experiment was a 10 x 3 factorial fitted into Completely Randomized Design (CRD) with three replicates. The two factors were ten botanicals: *Cymbopogon citratus, Chromolaena odorata, Ageratum conyzoides, Carica papaya, Hoslundia opposita, Azadirachta indica, Ocimum gratissimum, Tithonia diversifolia, Vernonia amygdalina* and *Jatropha curcas* and three solvent mediums: methane, chloroform and n-hexane making a total of 30 treatment combinations. The rice variety used was ITA-306 And carbofuran (1.5 kg a. i. ha^{-1}) was used as check and the unsprayed plot was the control.

2.5 Rice Seeding and treatment application

In order to raise the seedlings, seed boxes, 50 x 60 x 7 cm, were each were filled with paddy topsoil into which ITA-306 was seeded. 96 pots (11-liter buckets) were filled with paddy soil and were placed in the planting cages measuring 1 x 1 x 1.3 m. Four seedlings were transplanted from the seed boxes into each of the planting pots at 14 days after sowing (DAS). At 7 days after transplanting, the four seedlings per pot were thinned to one per stand, while dead rice seedlings were replaced at 3 WAS. Twelve (12) mls of botanical extracts were applied to the seedlings using Hill Master^R hand sprayer, after which six 1-3-day-old adult midges (1:1; male: female) were introduced from the stock culture to each planting cage containing rice seedlings. The rice seedlings were sprayed daily with sufficient water from the base of the rice plant to run off, using hand sprayer to aid the entry and survival of the larvae in the rice culms. At 3 WAS, the extracts were applied at 10% concentration once a week for five weeks till 55 DAS, the rice early booting stage. The potted seedlings were wetted with 500 ml of water once in two days, while manual weeding was carried out once in a week from 3 WAP till the end of the experiment.

2.6 Damage assessment of AfRGM on the rice variety

Scoring of rice galls on each treatment was done at 21, 28, 35, 42, 49, 56, 63 and 70 days after infestation (DAI). Scoring was carried out through physical observation of the rice stems for the presence of onion leaf shoot symptoms. The number of onion leaves were counted and recorded as infested rice tiller and percentage tiller infestation of AfRGM was calculated using

$$X = \frac{\text{Number of infested tiller}}{\text{Total number of tillers}} X\,100$$

Where X is the percentage tiller infestation of AfRGM.
The following agronomic parameters were also measured: plant height (cm), number of leaves, panicle weight, number of tillers, number of panicles, panicle length and yield (ton/ha).

2.7 Data analyses

Data on tiller infestation, agronomic characters including yield were subjected to analysis of variance at 5% probability using the General Statistical Software package [12], while Student's Newman Kuels Test (SNK) was used to separate significant means. Tiller infestation and related agronomic characters were correlated with grain yield.

3. Results

The results indicate that the level of tiller infestations varied depending plant materials used and extraction solvent. At 21 days after treatment, tiller infestation obtained on rice seedling treated with *A. conyzoides* and *A. indica* extracted in methanol, *C. odorata* and *C. citratus* in chloroform, and *V. amygdalina* in hexane were significantly lower compared to others. However, infestation seemed to increase as the rice plants grew (Table 1). *Ageratum conyzoides* and *C. odorata* in n-hexane, *O. gratissimum* in chloroform and carbofuran recorded no tiller infestation i.e. 0.00% consistently throughout the rice growth stages which was the best relative to others. This is followed by *C. citratus* extracted in chloroform (2.68%), *C. odorata* in chloroform (2.72%), *A. conyzoides* in methanol (3.04%), *C. citratus* in methanol (3.26%), and *V. amygdalina* in hexane (3.46%) relative to untreated plants (57.11%), which recorded the highest tiller infestation (Figure 1).

Botanicals extracted in three different solvents did not significantly have negative impact on the plant height, number of tillers and number of panicles produced, as well as panicle length were not adversely affected by solvent extraction (Table 2). Rice plants treated with *A. conyzoides* extracted in hexane and carbofuran were comparable producing higher agronomic traits compared to other treatments. In the control, however, significant lower values were obtained for number of panicles, panicle length and grain weight. Although number of panicles produced and panicle length in plots treated *A. conyzoides* extracted in chloroform (4.86 and 29.35cm), methanol (4.87 and 29.45 cm), and *C. odorata* (4.85 and 29.72 cm) extracted in hexane were statically similar, they were significantly higher than *T. diversifolia* extracted in chloroform (2.22 and 13.21cm) which recorded the lowest values. Highest grain yield was recorded in plots treated with *A. conyzoides* extracted in hexane (5.94 tons/ha), followed by *A. conyzoides* in methanol (3.92 tons/ha) and chloroform (3.89 tons/ha) relative to the untreated control (1.55 tons/ha) (Table 2). A yield increase of 73.91% was obtained on rice plants treated with *A. conyzoides* in hexane over the control.

In addition, mean tiller infestation was negatively and significantly related to rice height, number of panicles produced, panicle length and grain yield with gall midge accounting for 36% of grain yield reduction (Table 3).

Table 1: Mean tiller infestation recorded on ITA-306 rice variety treated with different solvent extracts at different days after artificial infestation of African Rice Gall Midge in the Africa Rice screenhouse

		Tiller infestation (%)						
Botanical	**Solvent**	**21 DAT**	**28 DAT**	**35 DAT**	**42 DAT**	**49 DAT**	**56 DAT**	**63 DAT**
Ageratum conyzoides	Chloro	5.761k	4.89jk	4.44t	3.66jk	2.79o	8.56op	13.30l
	Hex	0.00l	0.00m	0.00x	0.00l	0.00p	0.00w	0.00t
	Met	0.00l	4.85jkl	0.52w	0.44l	0.42p	8.56p	6.44p
Carica papaya	Chloro	9.43hi	13.89h	15.78m	14.76gh	16.96j	10.91m	6.22p
	Hex	5.35k	13.81h	20.67h	19.91f	33.63e	21.96i	13.00lm
	Met	5.77k	7.94ij	17.43k	16.49g	22.76h	5.08r	17.43k
Chromolaena odorata	Chloro	0.00l	4.07jklm	0.00x	0.00l	0.49p	6.55q	7.96o
	Hex	0.00l	0.00m	0.00x	0.00l	0.00p	0.00w	0.00t
	Met	12.76dfg	3.38jklm	5.38s	4.72j	9.72l	14.03l	0.46tu
Cymbopogon citratus	Chloro	0.00l	1.51klm	2.83u	5.50j	4.34n	4.28s	4.36r
	Hex	5.56k	7.35ij	1.69v	0.00l	2.42o	1.20u	0.53t
	Met	6.98jk	13.76h	16.59l	10.08i	16.73j	8.43p	22.64g
Hoslundia opposita	Chloro	17.65a	14.25h	13.47o	12.43hi	11.15k	6.52q	19.10j
	Hex	19.65a	33.45cd	14.13n	23.12ef	21.01i	24.67f	20.80h
	Met	14.84cde	52.04b	19.35i	21.92ef	30.29f	23.07h	23.17f
Jatropha curcas	Chloro	6.53jk	30.61de	40.76b	39.50b	42.86c	19.66j	20.89h
	Hex	7.51ijk	13.36h	10.72p	10.18i	5.70m	2.82t	3.11s
	Met	5.56k	2.32klm	2.96u	12.54hi	1.27p	0.77v	12.58m
Azadirachta indica	Chloro	19.44ab	32.26cd	8.65q	28.48d	23.27h	23.77g	19.35ij
	Hex	10.97gh	29.7de	17.88j	44.90a	36.54d	36.52c	38.21b
	Met	0.00l	23.97fg	23.01f	22.28ef	20.09i	8.97o	8.16o
Ocimum gratissimum	Chloro	0.00l	0.00m	0.00x	0.00l	0.00p	0.00w	0.00t
	Hex	7.47ijk	24.13fg	23.99e	19.93f	17.36j	15.51k	11.84n
	Met	15.93c	10.63hi	6.38r	9.94i	19.85i	37.36b	26.32d
Tithonia diversifolia	Chloro	8.82hij	25.33fg	21.58g	21.23f	36.28d	31.49d	30.76c
	Hex	10.57gh	28.12ef	16.86l	16.78g	17.95j	27.63e	22.50g
	Met	14.89cd	25.06fg	30.33d	24.85e	16.70j	23.12h	19.72i
Vernonia amygdalina	Chloro	12.26fg	35.87c	33.93c	33.43c	54.30b	24.84f	24.21e
	Hex	0.00l	4.86jk	0.00x	0.00l	3.96n	10.47n	4.94q
	Met	12.61dfg	22.13g	21.28g	20.49f	28.52g	23.55g	24.49e
Check	Carbofuran	0.00l	0.00m	0.00x	0.00l	0.00p	0.00w	0.00t
	Control	13.62def	68.71a	44.70a	33.76c	59.32a	93.22a	86.47a
SE		1.03	1.85	0.20	1.56	0.65	0.22	0.27
CV (%)		13.20	10.70	1.50	10.60	3.80	1.30	1.70

Means followed by the same letters are not significantly different from one another ($p<0.05$). Hex=n-hexane, chloro=chloroform, Met=methanol, SE= Standard error, Cv=Coefficient of variation, DAT = Day after Treatment

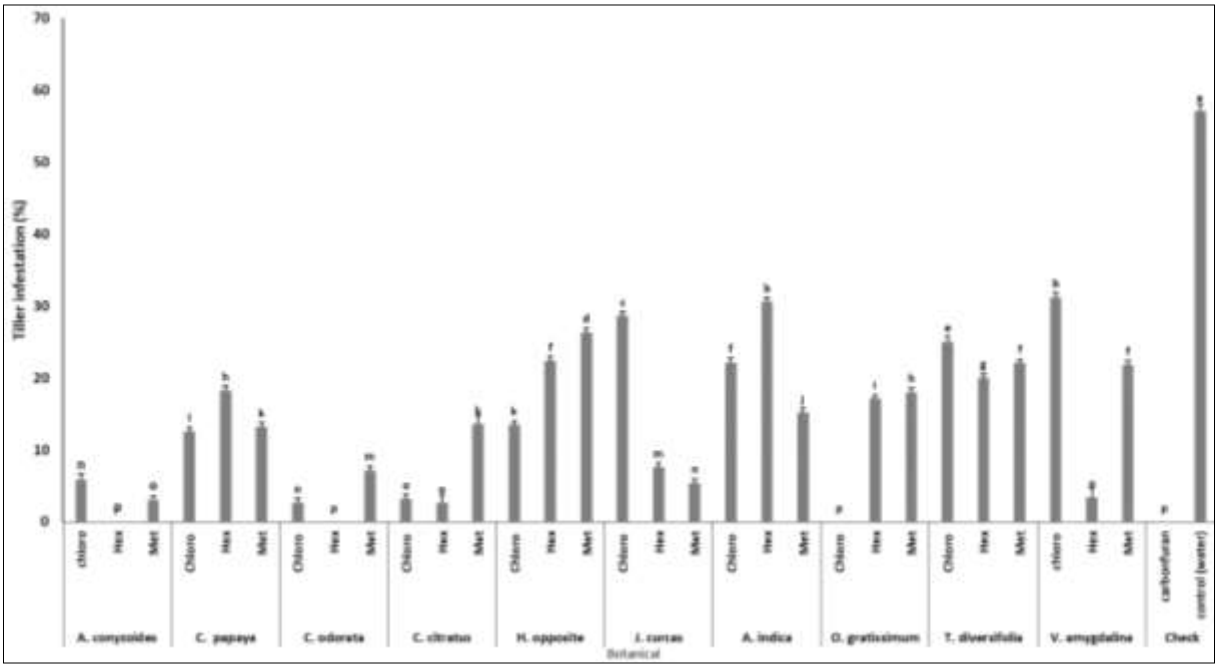

Fig 1: Mean tiller infestation induced by adult African Rice Gall Midge on ITA-360 variety treated with ten different solvent extracts after artificial infestation of the midge. Hex=n-hexane, chloro=chloroform, and Met=methanol

Table 2: Yield and some agronomic characters of ITA-306 rice variety treated with different solvent extracts after artificial infestation of African Rice Gall Midge in the Africa Rice screenhouse

Botanical	Solvent	Plant height (cm)	Number of Tiller	Panicle Number	Panicle length (cm)	Grain Yield. (tons/ha)	YDI (%)
Ageratum conyzoides	Chloro	85.62abc	18.3abc	4.86b	29.35b	3.89bc	60.15
	Hex	89.27a	20.18ab	5.10a	32.91a	5.94a	73.91
	Met	83.46a-d	18.3a-d	4.87b	29.45b	3.92b	60.46
Carica papaya	Chloro	76.87a-g	9.61f-i	2.83i	15.35d-g	2.44n	36.48
	Hex	76.22b-g	8.39hi	3.20g	16.25def	2.55l	39.22
	Met	78.16a-f	11.10c-i	2.96hi	16.12def	2.88i	46.18
Chromolaena odorata	Chloro	75.70b-g	23.30a	4.22c	24.19c	3.29f	52.89
	Hex	78.84a-f	16.51a-f	4.85b	29.72b	3.86c	59.84
	Met	72.75c-g	15.9a-g	4.19c	24.34c	3.21g	51.71
Cymbopogon citratus	Chloro	78.26a-f	9.1ghi	2.63j	14.61efg	2.26p	31.42
	Hex	78.98a-f	10.28f-i	2.85i	15.45d-g	2.62k	40.84
	Met	79.02a-f	13.22b-h	3.05gh	16.33def	2.63k	41.06
Holundia opposita	Chloro	79.83a-f	7.40hi	3.46f	16.67def	2.56l	36.45
	Hex	65.08ghi	20.76a	3.26g	16.23def	2.49m	37.75
	Met	84.86abc	8.37hi	3.21g	15.71d-g	2.33o	33.48
Jatropha curcas	Chloro	64.38ghi	8.43hi	3.19g	16.33def	3.27f	52.60
	Hex	73.9bc-g	6.58hi	3.58ef	17.15de	2.59kl	40.15
	Met	68.17fgh	5.98hi	3.21g	16.2def	2.34o	33.76
Azadirachta indica	Chloro	74.53b-g	7.90hi	3.58ef	17.49d	2.93hi	47.10
	Hex	78.64a-f	16.14a-g	3.61f	17.22de	2.92hi	46.92
	Met	82.37a-e	4.13i	3.49f	17.15de	2.63k	41.06
Ocimum gratissimum	Chloro	79.21a-f	16.15a-g	3.92d	28.68b	2.96h	47.64
	Hex	72.57c-g	17.97a-e	3.76e	23.00c	3.39e	54.28
	Met	69.26e-h	6.74hi	3.59ef	17.53d	2.73j	43.22
Tithonia diversifolia	Chloro	56.13i	6.69hi	2.22lm	13.21g	2.46mn	37.00
	Hex	59.8hi	8.46hi	2.59j	13.33g	1.76q	11.93
	Met	74.69b-g	16.78a-f	2.47jk	14.93d-g	2.63k	41.06
Vernonia amygdalina	chloro	71.59d-g	10.23f-i	2.14m	14.13fg	2.56l	39.45
	Hex	74.49b-g	7.92hi	2.36kl	14.88d-g	3.47d	55.33
	Met	74.17b-g	5.82hi	2.24lm	14.22fg	2.49m	37.75
Check	Carbofuran	86.45ab	22.68a	5.14a	33.07a	5.95a	73.94
	Control	69.29e-h	10.25f-i	1.35n	11.12h	1.55r	-
SE		4.33	2.71	9.62	0.95	0.02	-
CV (%)		5.70	22.30	2.80	5.00	0.80	-

Means followed by the same letters are not significantly different from one another ($p<0.05$). Hex=n-hexane, chloro=chloroform, Met=methanol, SE= Standard error, CV=Coefficient of variation, YDI= Yield data index

Table 3: Pearson's correlation coefficients of the relationship between African rice gall midge infestation and some agronomic parameters of rice

Parameter	Tiller infestation (%)
Number of Panicles	-0.65*
Plant Height	-0.42*
Number of Tillers	0.30
Panicle Length	-0.63*
Grain Weight	-0.60*

* Significant at p=0.05; values without asterisk are not significant

4. Discussion

Synthetic insecticides have contributed greatly to plant protection, but they have also had negative impacts on ecology and health. As a result, there has been an increasing demand for cheaper, safer and more environment-friendly alternatives, such as botanical insecticides [13]. Several plant species have been extensively studied for discovery and development of new sources of botanical insecticides and antifeedants [14, 15, 16, 17, 18, 19]. To acquire quality bioactive molecules, appropriate solvents are first selected, after which extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques are then determined [8].

As revealed through this study, chloroform, n-hexane and methanol, which are among the common and effective solvents, tested in [15] and [8] influenced the activity of the botanical extracts against *O. oryzivora* across the days of infestation. Since the highest tiller infestation was recorded in the untreated control plots, it is indicated that the extracts were effective against the AfRGM, although to varying degrees. However, the three most effective extracts, for which no tiller infestation situation was obtained were *C. odorata* extracted in n-hexane, *A. conyzoides* extracted in hexane and *O. gratissimum* extracted in chloroform. The three extracts were comparable to the synthetic insecticide (carbofuran) used in terms of efficacy. Previous studies have shown that these plants, extracted in different solvents, were effective against some insect pests and are useful for pest control [16, 17, 19].

The biological activity obtained for *A. conyzoides* against *O. oryzivora* might be due to wide range of chemical compounds reported in the plant. *A. conyzoides* as well as other plants in the family Asteraceae have been reported to contain certain secondary metabolites such as flavonoids, alkaloids, cumarins, chromenes, benzofurans, tannins and terpenoids used in defense against biotic stresses [20], [21] identified extracts of three members of family Asteraceae - *Sonchus oleraceus*, *Ageratum conyzoides* and *Ambrosia maritima* as effective against *Tribolium castaneum* and attributed the bioactivity to inherent chemical compounds common to them. Similarly, from previous work in Nigeria, many chemical compounds identified from this plant have been found to possess varying insecticidal activities [22]. For instance, *A. conyzoides* extracts was useful against *Leucinodes orbonalis* on eggplant resulting in higher yield [23], possesses impressive larvicidal activity against *Culex quinquefasciatus* among other plant materials tested [24], and with *Hyptis suaveolens* ethanolic extracts active against many larval insect vectors of public health importance [25].

At 9 WAP, rice plants treated with *A. conyzoides* extracted in n-hexane and carbofuran were of similar heights and number of tillers produced were higher than other treatments. Also, number of leaves produced by rice plants treated with *A. conyzoides* extracted in chloroform, n-hexane and carbofuran did not differ significantly. Many authors have previously reported the comparativeness of *A. conyzoides* with synthetic insecticides especially when extracted in n-hexane [17, 19]. Particularly, *A. conyzoides* extracted in n-hexane is known to partition certain flavonoids which are classified as antifeedant and growth inhibitors in insects, probably because they interfere with endocrine regulation [26]. Similar activity of the hexane extract has been reported by [27] against *Culex quinquefasciatus*, and [28] against *Diaphania. hyalinata, Musca domestica, Periplaneta americana* and *Rhyzopertha dominica.* As it has been observed in this study, the differential susceptibility of different insect species, would always elicit variations in insecticidal activity of different solvent extracts against insect pests is expected [28].

5. Conclusion

This result indicates that botanical extracts can be used in the control of AfRGM as alternatives to carbofuran, the synthetic insecticide particularly *A. conyzoides* and *C. odorata* extracted in hexane and *O. gratissimum* in chloroform which exhibited an effective control against adult AfRGM though in a controlled environment. It is therefore, suggested that these botanical extracts be further investigated in an endemic rice field after which they can be incorporated into the Integrated Pest Management (IPM) of AfRGM.

6. Declaration of competing interest

The authors declare that they have no competing interests.

7. Authors' contributions

1. **Ayangbemi B.T:** Experimental design and field work.
2. **Pitan O.R:** Project supervision.
3. **Nwilene F.E:** Experimental setup with provision of facilities.
4. **Atayese M.O:** Interpretation of statistical analysis.
5. **Onifade O.S:** Proof reading.
6. **Adeoti A.:** Statistical analysis.

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