Original Research Article

Insect pest profile of leaf amaranth (*Amaranthus hybridus* L) in a single organic cropping system and prevention of damageherbivory using oil-based extracts of *Alium sativum* L, *Xylopia aethiopica* Dunal and *Eucalyptus globolus* L

ABSTRACT

Pest profile of *Amaranthus hybridus* was recorded in a single organic agro-ecosystem in Southwestern Nigeria between November-December in 2016 and January-February 2017, during the dry season. Activities of different pests were monitored to identify those responsible for the most significant damage. OilVegetable oiil-based extracts of *Alium sativum*, *Xylopia aethiopica* and *Eucalyptus globolus* were prepared and applied on *A. hybridus* as protectants against herbivory by phytophagous insects and damage to foliage was were assessed. Thereafter, the extracts were rated based on the mean percentage damage (MPD) recorded in different plots in relation to the treatments. A total of nine pests were recorded from three insect Orders namely, Orthoptera (6362.5%), Coleoptera (1312.5%) and Lepidoptera (25%) **(Calculation not 100%)** and they were grouped into Major, Minor or Occassional pests based on their activities. Two lepidopterans, *Spoladea recurvalis* and *Psara basalis* (Family: Crambidae) were responsible for the most significant damage. All the extracts reduced damage with statistically significantly difference (P<0.05) compared with the control. The MPD in *X. aethiopica*-, *A. sativum*- and *E. globolus*-treated plots and the control plots were

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10.9%, 8%, 14% and 31.2% respectively, when the amaranth was due for harvest in the first trial. The MPD to the amaranth in the treated plots during the second trial was between 13.6% and 16.3% when harvest was due while the MPD in the control was 54.9%. The performances of *E. globolus* and *X. aethiopica* were comparable and they were relatively more effective in protecting *A. hybridus* against phytophagous pest attacks.

Keywords: *Amaranthus hybridus,* Herbivory, Phytophagous insects, Damage, plant extracts

INTRODUCTION

Amaranthus hybridus is widely cultivated in Nigeria for its edible leaf which contains significant amounts of dietary proteins, vitamins and minerals (Akubugwo et al., 2007). It is well adaptable to the climatic conditions in different agro-ecological zones in Nigeria. In the South West, which is the major production hub, dry season amaranth cultivations are restricted to wetland areas or locations with proximity to water for irrigation. It is a rapid source of income for subsistent and poor-resource farmers because of its relatively short production cycle (14-21 days), simple method of cultivation and high market demands. During the dry season, supply of leaf amaranth often falls short of the demand, the price becomes relatively high (Emokare et al., 2007) and there are periods when amaranth is completely unavailable in the market.

Insect pests seriously undermine vegetable production in Nigeria, particularly when they are cultivated for their foliage. Pest density is often high and attacks are severe in the dry season due to relative scarcity of alternative hosts. Amaranth is attacked by a myriad of insect pests in a succession that depends on how long the crop is left in the field (cultivated for leaf or seed) before harvesting. The insect pests that are responsible for the most economic damage to leaf amaranth in the Southwest belong to Lepidoptera and Orthoptera Orders (Joseph et al., 2016; Borisade and Uwaidem, 2017a). When leaf amaranth foliage has fully developed, sometimes losses of up to 100% can be incurred within one week in pest endemic areas if appropriate pest control action is not initiated.

The major Lepidoptera pests of amaranth, *Spoladea recurvalis* and *Psara basalis* lay their eggs on the abaxial parts of early foliage at night, about one week after the appearance of the first foliage, thereby concealing infestation or potential development of pests on the amaranth (James et al., 2010). The eggs hatch into the larvae in about seven to fourteen days, which feed voraciously on the foliage. Major damage often occur between 15-21 days after sowing, although earlier attacks are possible. Apart from the feeding activities that ‘skeletonize’ the leaves, bulk of the produce is often contaminated with frass and excrements that further reduce quality. *Psara basalis* especially produce characteristic webbings on the leaves, which makes the crop completely unmarketable (Borisade and Uwaidem, 2017a). Grasshoppers and Katydids and many other phytophagous insects that move into the field are also responsible for damage.

The use of chemical insecticides in vegetable pest management and the unsafe levels of pesticide residues that are left in fresh vegetables are of ~~a~~ serious concern (Akan, et al., 2013). Increasingly and from time to time, chemical pesticides are being reviewed and unregistered for use in the management of vegetable pests, considering their toxicity to non-targets and levels of persistence in the environment. Chemical pesticides may be especially unsafe for pest management in the Nigerian leaf amaranth production system, where the production cycle of 14-21 days is far less than the half-life of the active ingredients in majority of the pesticides in use.

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Chemical pesticides of the Organochloride groups and those containing DDT, which are forbidden in the management of pests in food crops are found in agrochemical retail outlets in Nigeria, and they are being used in the management of vegetable pests by subsistent farmers. Thus, there is the need to reduce dependence on inorganic chemicals in the control of leaf amaranth pests by seeking alternative environment-friendly options.

Plants contain organic chemical constituents that protect them against herbivory and disease pathogens and many of these constituents have great potentials for pest management. Garlic (*Alium sativum*)*, Xylopia aethiopica and Eucalyptus globolus* are widely distributed tropical plants containing extractable bioactive compounds, which have been employed in pest control in different studies (*Ebadollahi,* et al., 2017: Moshi and Matoju, 2017). *Alium sativum* contains alicin, which is repellent or toxic to eggs, developmental stages, and adults of many economic pests (Huang et al., 2017). *Xylopia aethiopica* and *E. globolus* are also known to contain essential oils reported to show repellency, ovitoxicity and adulticidal effects against insect pests (Kouninki et. al., 2007). However, many of the promising evaluations on the use of extracts of these plants for crop protection were limited to ~~store pests~~ in *invitro* bioassays. Efficacy of botanical extracts in field pest management is expected to vary under variable interacting abiotic environmental factors: temperature and relative humidity, often encountered under field conditions. In the field, pests are not confined by limited space, a factor which may become a challenge against plant extracts that are relatively slow in action.

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Thus, the aim of this study was to record occurrence of pests on *Amaranthus hybridus* within a single organic agro-ecosystem in South-Western Nigeria and evaluate the propensity of oil extracts of *A. sativum*, *X. aethiopica* and *E. globolus* to prevent damage.

MATERIALS AND METHODS

Description of experimental site

The study was carried out at Ekiti State University Teaching and Research Farms, Ado-Ekiti, Nigeria (7.6124° N and 5.2731° E), from November to December 2016 and repeated between January and February 2017 during the dry season under irrigation system. The study area has an average temperature of 25 °C with wide fluctuations between day and night. The wet season is usually from April –October, with bimodal rainfall pattern which peaks in June and October, while the dry season is from November to March. The study area has a history of severe attacks on dry season amaranth.

Land preparation and experimental design

The land was cleared and plant debris were ~~packed~~ removed before the preparation of beds. The size of each bed was two square meter and a space of 0.5 m was left between the beds. The experiment was a R~~r~~andomized C~~c~~omplete B~~b~~lock D~~d~~esign (RCBD) with three replications. Thus, the field consisted of nine blocks with three beds each, for the treatments and three additional blocks assigned to control. Three other separate blocks were created for the assessment of pest profiles. About 4 kg of poultry manure was spread on each bed and mixed with the top layer of the soil. Thereafter, the beds were irrigated, at least once in two days for a period of ten days to facilitate the decomposition of the poultry manure before sowing. Each block was about 10 m apart to eliminate the influence of a treatment over the other.

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Calculation of seed rate

Crop Density, CD (=number of seeds to be sown per square meter) was determined by measuring the weight of seeds equivalent to an estimated value using the proposed formula for standardizing the seed rate of amaranth, (Uwaidem and Borisade, (2017b), here summarized.: $E\ (g) = \frac{W}{NS} \times \frac{R}{1}$, ? (Where is the equation? It might have been deleted during downloading of the revised manuscript.) where E = Equivalent weight (g), W=Weight of 1 g amaranth seed, NS = Counted number of amaranth seed g^{-1}, R= Required number of plants per bed. One seed of the amaranth used in the current study weighed 0.000441g. Thus, considering an approximate plant density of 500 stands m^{-2}, 0.22 g of the amaranth seeds were sown on each bed.

Sowing and post-planting management

Dry sand was passed through 0.5 mm mesh and 100 g of the fine sand was mixed with the seed for even seed distribution during sowing. A plastic container with a tight fitting lid (100 ml) was modified for sowing the seeds by creating pin-sized perforations (~ 0.5 mm) on the lid. The sand-seed mixture was poured into the plastic and used for broadcasting the seeds. The beds were watered as required using a **Watering** watering **Can** can during afternoon periods until the amaranth was due for harvest.

Preparation of vegetable oil-based plant extracts of plants.

Five hundred grams of fresh bulbs of *A. sativum* and dry fruits of *X. aethiopica* were chopped manually using a knife and poured separately into one litre-glass jar with a tight fitting lid. Five hundred ml of vegetable oil was poured into each jar to submerge the contents and kept at -4°C for one hour. Thereafter, the contents of the jar: (*X. aethiopica* fruits + vegetable oil) or (*A. sativum* bulbs + vegetable oil) were blended to form an oily paste. Fresh *E. globulus* leaves (500 g) were harvested in the morning and shredded using a knife. The sliced leaves were poured into one litre-glass jar and 500 ml vegetable oil was poured to cover the leaves. The glass jars were transferred into Microwave (Model LG i-wave, MS2021F). Microwaving was done at the Medium-High Power in three 10 minute-sessions, followed by 25 minutes power-off after each session. The oil was separated by vacuum filtration at 4 °C and stored in air tight bottles at 4°C. These were used as the stock plant extract in subsequent assays. **This procedure is wrong for oil extraction.**

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Assessment of pest profile and nature of damage

Visual survey of insect pests on the amaranth was commenced at six days after sowing and this continued until maturity. Scheduled daily visits to the field was done in the morning (6:00-9:00 am), afternoon (12:00 noon-3:00 pm) and evening (6:00 pm-8:00 pm), to scout for insect pests. Insect samples were collected and brought ~~in~~to the Agricultural Entomology Laboratory of the Crop Protection Unit, Faculty of Agricultural Sciences, Ekiti State University, Nigeria for identification. The nature of damage and severity of the activities of the pests were visually assessed on the plant. ~~Camera shot of~~ **damage** Damage to foliage was photographed ~~processed into a~~ in JPEG format. ~~picture presented as a photographic data~~. The pests were classified into three groups: Major, Minor and occasional pests, based on their occurrence, density and severity of damage to the crop.

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Application of extracts and assessment of damage

The plant extracts were randomly assigned to different blocks and the blocks were labelled. At ten days after sowing, 50 ml of the extract of each plant was mixed with 200 ml water. The ~~resultant~~ mixtures were emulsifiable without the addition of a surfactant. They were sprayed on the amaranth in each block using a hand operated

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Knapsack Sprayer (1 Liter capacity). **state the volume)** until leaves were dripping. The control plots were sprayed with a mixture containing 50 ml vegetable oil + 200 ml distilled water. The spraying was repeated after five days and damage assessment was conducted at 24 days after sowing, when the leaf amaranth had reached the acceptable maturity standard for local market sales. Sampling to assess damage was done with a quadrat (Area = 20 cm^2) thrown randomly at five different positions on each bed and the total number of stands of amaranth within the quadrat area as well as the damaged were counted. The criteria used for damage assessment was based on the local consumers acceptable quality standards for leaf amaranth and the reasons for rejection (Borisade and Uwaidem, 2017a). These were summarized: (a) amaranth stands showing 2-3 skeletonized leaves (b) the presence of insect faecal contamination **or** of frass (c) signs of webbings and folded leaves. The recorded number of damaged amaranth stands within the quadrats **were** was averaged and multiplied by the total area of the block. Thereafter, the value was expressed as a percentage of the total number of plants in a block: (Is this the equation? It might have been changed during the downloading of the revised manuscript.)

Percentage damage per block = $\frac{Number\ of\ damaged\ amaranth\ stands}{Estimated\ total\ number\ of\ amaranth\ per\ block} \times \frac{100}{1}$

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RESULTS

Pest profile of *Amaranthus hybridus*

The pest profile of leaf amaranth within the single organic agro-ecosystem is shown in Table 1. Nine pests from three Orders: Orthoptera, Coleoptera and Lepidoptera were recorded during the first and the second amaranth production cycles. Only the adults of the majority of the Orthopterans, such as the Burrowing cricket (*Velarifictorus micado*), Slant-faced grasshopper (*Orphulella speciosa*), Variegated grasshopper (*Zonocerus variegatus*) and the Green-striped grasshopper (*Chortophaga viridifasciata*) occurred on the amaranth. Their frequency of occurrence was relatively low and they were few in number. Thus, they were classified as occasional pests, causing non-economically important damage in the current evaluation. The nymphs and adults of the Angle-winged katydid (*Microcentrum rhombifolium*), occurred frequently on the amaranth and they were found voraciously feeding on the leaves causing potentially economic damage. It was therefore classified as a major pest. Actively flying adults of two types of moth, *Psara basalis* and the beet webworm moth (*Spoladea recurvalis*) as well as their larvae occurred at all the sampling periods. The adults of these lepidopterans occurred most frequently in the evening while a few **was** were found resting under the leaves during the day. The larvae were voracious feeders and they were responsible for the most significant damage to the leaves (figure 1).

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Assessment of damage and performance rating of extracts.

Table 2. shows the mean percentage damage (MPD) to the **leafs** leaves of *A. hybridus* sprayed with emulsifiable oil extracts of *X. aethiopica, A. sativum* and *E.*

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globolus at 5, 10 and 15 days after application. There were significant variabilities (P=0.014) in

Table 1. Pest profile of *Amaranthus hybridus within* a single organic system in Southwestern Nigeria

Common name	Scientific name	Order	Family	Recorded life stage responsible for damage	Pest status (Based on occurrence, numbers & crop damage activities)
Angle-Wing Katydid	*Microcentrum rhombifolium*	Orthoptera	Tettigoniidae	Adult and Nymph	Potential/Major
Crickets	*Velarifictorus micado*	Orthoptera	Gryllidae	Adult	Occassional/Minor
green stripped grasshopper	*Chortophaga viridifasciata*	Orthoptera	Acrididae	Adult	Occassional/Minor
Slant-faced grasshopper	*Orphulella speciosa*	Orthoptera	Acrididae	Adult	Minor
Variegated grasshopper	*Zonocerus variagatus*	Orthoptera	Pyrgomorphidae	Adult	Occassional/Minor
Darkling beetle	*Lagria villosa*	Coleoptera	Lagriidae	Adult	Occassional/Minor
Moth	*Psara basalis*	Lepidoptera	Crambidae	Larvae	Major
Beet web worm	*Spoladea recurvalis*	Lepidoptera	Crambidae	Larvae	Major

Table 2. Mean percentage damage to *Amaranthus hybridus* treated with oil extracts of *X. aethiopica*, *A. sativum* and *E. globolus*

FIRST TRIAL				
Days after treatment	*X. aethiopica*	*A. sativum*	*E. globolus*	Control (Vegetable oil)
5	4.28^{a}	1.19^{a}	1.23^{a}	25.37^{b}
10	$9.59^{a,b}$	$25.26^{a,c}$	3.94^{b}	41.27^{c}
15	$10.19^{a,b}$	8.01^{a}	$14.00^{a,b}$	31.42^{b}
SECOND TRIAL				
Days after treatment	*X. aethiopica*	*A. sativum*	*E. globolus*	Control (Vegetable oil)
5	2.38^{a}	4.76^{a}	3.51^{a}	30.69^{b}
10	6.80^{a}	35.00^{b}	10.32^{a}	32.24^{b}
15	16.30^{a}	18.75^{a}	13.57^{a}	54.88^{b}

Values in the same row and sub-table not sharing the same superscript are significantly different at $p < .05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction

Figure 1. Characteristic damage caused by Lepidopteran pests of *Amaranth*, *Psara basalis* and *Spoladea recurvalis*

the MPD in relation to the extracts as well as the sampling periods. The MPD in the control was significantly the highest in the first and the second trials. At 5 days post-treatment during the first field trial, the MPD recorded in the *X. aethiopica*-, *A. sativum*- and *E. globolus*-treated plots were not significantly different, being 4.28%, 1.19% and 1.23% respectively, while the MPD in the control plot was 25.37%. The effect of these extracts were also not significantly different in the second trial at five days post treatment (MPD in treatment, 8.49-19.5%; MPD in control=30.69%). At 10 days and 15 days post-treatment, the MPD in the *X. aethiopica*-treated plots were not significantly higher, 9.59 % and 10.19% respectively in the first trial. The lowest MPD were recorded in the *X. aethiopica*- and *A. sativum*- treated plots at 15 days in the first trial being, 10.19% and 8.01% respectively and without statistically significant difference. However, significantly higher MPD were recorded in the control at these sampling periods and the values were 31.42% and 54.88% respectively.

Based on the pooled values of MPD recorded at the three sampling periods in the two successive trials, the extracts were grouped according to their overall performance using Tukey's Honestly Significant Difference (HSD) (Table 3). The performances of *E. globolus* and *X. aethiopica* were comparable and they were relatively more effective in protecting *A. hybridus* against phytophagous pest attacks.

Table 3. Ranking of oil extracts of *X. aethiopica*, *A. sativum* and *E. globolus* based on the mean percentage damage recorded on treated *Amaranthus hybridus*.

Tukey HSD[a,b]			Subset		
	Plant Extracts	N	1	2	3
	E. globolus	18	7.7617		
	X. aethiopica	18	8.2556		
	A. Sativum	18		15.4956	
	Control	18			35.9778
	Sig.		.997	1.000	1.000

Means for groups in homogeneous subsets are displayed based on observed means. The error term is Mean Square (Error) = 52.881.

a. Uses Harmonic Mean Sample Size = 18.000.

b. Alpha = .05.

DISCUSSION

The study has described the pest profile of leaf amaranth within a single organic agro-ecosystem, where 63% of the recorded pests were Orthopterans, 25% were Lepidopterans belonging to the Family Crambidae and 13% Coleoptera. The range of pests being reported are among those described in earlier studies in other parts of Southwestern Nigeria (Ezeh *et al*., 2015; Oke *et al*., 2015) except the Darkling beetle which has not been widely associated with leaf amaranth. Leaf amaranth pests within a single organic agro-ecological region was evaluated in this study and it is expected that the pest profile of crops in agro-ecological regions that share resemblances in temperature, humidity, vegetation patterns and cropping systems would be similar.

Biodiversity of insects pests associated with indigenous leaf amaranth species in Nigeria is increasing (Oke *et al*., 2015). It is therefore useful to update data on

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Incidentally, the insects classified as major pests in this study comprised those breeding on the amaranth. The results suggested that economically important pests of amaranth are essentially those that are capable of breeding on the crop ~~or at a proximity to the crop~~ and capable of completing their life cycle or reaching their pestiferous life-stage before the host plant is due for harvest, except where migrant pests are probably involved. Attack on crops can be ~~much~~ very severe when more than one of the life-stages of the pest are responsible for damage, such as the Katydid or when the habit of the pest inflict qualitative damage in addition to quantitative losses caused by their direct feeding. For example, contamination of leaves with frass, webbings and excrements was peculiar to *S. recurvalis* and *P.*

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basalis. Different instar larvae and adults of the two Lepidopterans-*P. basalis* and *S. recurvalis* were present, indicating their breeding on the amaranth and they were responsible for the most significant damage whereas, mainly the adults of the Orthopterans and the Coleopteran were found on the amaranth. Similar reports on the pest status of *P. basalis* and *S. recurvalis* showed they are serious pests of leaf amaranth in different agro-ecological regions in Nigeria and other parts of West Africa (James et al., 2010).

The oil extracts of the three plants significantly reduced vegetative damage to *A. hybridus* compared to the control and the results have demonstrated their potentials for use in the management of vegetable pests at the level of subsistent farming. The method of extraction described is simple, easily transferable and could be adopted by poor resource **can** farmers. The procedure can also be applied to other plants with volatile bioactive components. The three plant materials contain volatile bioactive substances, which may potentially be lost depending on the method of extraction used. Deep freezing of the plant materials before milling and reduction processes was done to minimize adverse effect of temperature during milling on loss of heat-labile, volatile constituents. Direct blending of the plant materials with vegetable oil was also done to trap oil-soluble volatiles during the milling process.

The levels of damage recorded at five days post-application of the three extracts were not significantly different statistically. However, between 5-10 days post-treatment, the MPD increased significantly where *A. sativum* extract was applied. Abiotic interactions (temperature, UV and relative humidity) (Kumar and Poehling, 2006) are capable of influencing persistence of organic pesticides rapidly, through their effects on evaporation and chemical decomposition in the field, indirectly affecting overall efficacy. This may be responsible for the increased damage recorded during sampling at 10 days post-treatment.

Insect pests are known to locate their hosts through visual and olfactory cues (Bruce et al., 2005) and plant extracts with strong odour may interfere with the capability of pests to accurately locate their targets. However, when the effect of the odour of the plant extracts subside, there are possibilities that more pests would successfully locate their food source. It may also be possible that the extracts were toxic to some of the pests or offered some antixenosis resistance to the plant- that probably diminished over time. More studies are needed in the development of stable formulations capable of yielding consistent results under a dynamic or marginal abiotic influences in the field.

The extracted plants; *X. aethiopica, A. sativum* and *E. globolus* have been applied into various uses in folk medicine, pharmacy as well as food components (Konning *et al*., 2004; Tattelman, 2005). Although concentrations of these plant materials that may be toxic to humans are yet to be established and the amounts detectable on treated plants have not been evaluated, they are not expected to cause bio-toxicity or environmental contamination problems when applied on edible vegetables. They can be considered as relatively safe compared with inorganic pesticides.

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Conclusion

This study compared the effect of the extracts at a single dose and the MPD to the treated plants over time was used to assess efficacy. More studies are needed to quantify the actual concentrations of bio-active constituents in the plant materials. The effects of the extracts against each of the identified pests need to be studied separately, to evaluate their modes of action, including repellency, toxicity to adults and developmental stages and antixenosis effects. However, the current results are useful primary information in the design of further *invitro* and field studies.

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