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Comparative Insecticidal Activity of Aqueous and Ethanol Extracts of Some Plants against *Bactrocera dorsalis.* Hendel Larvae .

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ABSTRACT

A bioassay was carried out at Biology and Entomology laboratory of the Federal College of Forestry Ibadan to compare the insecticidal potential of aqueous and ethanol extracts of five plants (*Aframomum melegueta*, *Azadirachta indica*, *Moringa oleifera*, *Anonna muricata* and *Piper guineense)* against *Bactrocera dorsalis* (oriental fruit fly) larvae. Extracts were applied on third instar larvae of *B. dorsalis* at 50% w/v for aqueous and at 75% concentration for ethanol. Larvae mortalities were documented at 20 minutes intervals for 24 hours, data collected were subjected to Analysis of Variance (ANOVA) and significant means were separated using Turkey's Honestly Significant Difference (THSD). Results revealed that both aqueous and ethanol extracts of all plants evaluated had contact and residual toxicity on *B. dorsalis* larvae at varied rates. The percentage mortality varied from 53.2% - 100% for the aqueous extracts while ethanol extracts ranged from 66.6%- 100% for both contact and residual toxicity. Aqueous and ethanol extracts of *A. muricata* was the most effective with equal efficacy of 100% larval mortality for residual actions. Ethanol extracts of *M. oleifera* was significantly ($p<0.05$) more efficacious than its aqueous extracts for both residual and contact toxicity while ethanol extracts of other plants were slightly more efficacious than their aqueous extracts for both contact and residual actions. Extracts from both solvents proved potent against *B. dorsalis* larvae and most of them were as effective as cypermethrin under laboratory conditions. Thus, aqueous extraction of plants for pest management should be adopted since the processing is easy, less expensive and required no technical expertise.

Keywords: Toxicity, oriental fruit fly, ethanol extracts, aqueous extracts, larvae,

Introduction

The oriental fruit fly ,*Bactrocera dorsalis* Diptera: Tephritidae) is a quarantine pests with significant economic importance attacking many fruits and vegetables across the globe. Production of quality fruits on commercial basis is constrained worldwide by fruit fly attack especially by the *Bactrocera species* which causes severe damage that may lead to overall loss of the crops if not controlled (Mwatawala *et al.*, 2006).

Bactrocera dorsalis complex constitute more than 75 species, and is one of the most important pest complexes in global agriculture (Clarke *et al*. 2005). *Bactrocera dorsalis* are highly polyphagous in nature and are the most highly invasive species among the *Bactrocera* complex, thus are considered as pest of major biosecurity concern (CABI 2015, EPPO 2015). Fruit fly attack especially by *B. dorsalis* complex are major threat to mango production for export in Africa. Apart from the fact that *B. dorsalis* affects fruit quality, it reduces export market

due to stringent restriction measures impose by importing countries (Ekesi *et al*., 2016; Jaleel and Lü, 2019; Jaleel *et al.*, 2018a). Several management and control strategies have been tried to address the menace *of B. dorsalis*, which include the use of; insecticide, biological, cultural, sterile male technique and host of others (Vargas *et al.* 2015). Application of pesticides directly below the host plants targeting the pupariating larvae and puparia is a vital aspect in fruit fly eradication and control strategies (Ekesi, *et al.*, 2003).Various research work have documented the management of *Bactrocera* species using synthetic (chemical) pesticides (Jin *et al.*, 2011; Nadeem *et al.*, 2014), however, pesticides residues in fruits are of great concern for human health, there is now a shift from sole reliance on organophospate insecticide (Amaro and Godinho, 2012; Vargas et. al. 2015).

In addition incessant use of chemical pesticides has ledto negative effects such as pollution, health hazards and loss of biodiversity, whereas the utilization of botanical pesticides results into healthy environment and sustainable agriculture (Shabana *et al.*, 2017). Moreover, farmers that are involved in export trade have been affected negatively when they use synthetic pesticides in the production of horticultural crops (Nashwa and Abo-Elyousr, 2012). Loss of market and income has been recorded by growers and exporter in developing countries due to detection of banned pesticides or their traces above the regulatory residue limits (Lengai *et al.*, 2020). The utilization of botanical pesticide in pest management is important and are currently advocated due to their efficacy, biodegradability, varied modes of action such as repellent action,, inhibition, protein denaturation, contact toxicity, anti feedant and other activities based on the type of compounds in the botanical and pest (Lengai *et al.*, 2020), low toxicity and availability of source materials (Neeraj *et al.*, 2017).

The use of botanical pesticides are popular in organic farming where organically produced food command higher prices (Srijita, 2015). Therefore, the use of botanical pesticides in crop production are recently gaining recognition because the produced crops are safe for human consumption and lately market for organically produced food is becoming lucrative among buyersthat can afford it (Misra, 2014).Several studies have reported the pesticide potential of several known plant species and those that are yet to be exploited. (Erenso and Berhe 2016, Jawalkar *et al.*, 2016). *Azadirachta indica* (neem), *Tanacetum cinerariifolium* (pyrethrum), *Schoenocaulon officinale (*sabadilla), *Nicotiana tabacum* (tobacco) and *Ryania speciosa* (ryania) plants are among the known botanical pesticides that are commercially available and have been used widely in the pest management (Arnason *et al.*, 2012).

Several solutions are used for the extraction of active compounds from plants for the control of different pest and diseases. However, some of these solvent are expensive, not readily available require technical expertise and sophisticated equipment during the extraction process of which are not usually at the disposal of most small holder farmers. To provide farmers with fast and cheap access to botanical extracts for crop pest control, this study compared the insecticidal activities of aqueous and ethanol extracts of *Aframomum melegueta*(alligator pepper), *Azadirachta indica* (neem*), Moringa oleifera (*moringa),*Anonna muricata* (soursop) and

Piper guineense (African black pepper)against *B. dorsalis* larvae under laboratory conditions.

Materials and Methods

The study was conducted at the Biology and Entomology Laboratory of the Federal College of Forestry (FCF) Ibadan under ambient laboratory conditions of 27 ± 2°C temperature and 65 -80% relative humidity and L12: D12 photoperiod.

Five plant species were evaluated for their contact and residual toxicity on *B. dorsalis* larvae and are shown in Table 1. Plant materials like *Piper guineense* (black pepper) and the *Aframomum melegueta(danielli*) (alligator pepper)were purchased from local market in Ibadan while *Azadirachta indica (*neem), *Moringa oleifera* (moringa), *Anonna muricata* (soursop) were sourced from their mother trees at the Forestry Research Institute of Nigeria premises . The seeds of the *A. indica,,A. muricata* and *M.oleifera* were extracted from their fruits and pods respectively and were air dried for three weeks under laboratory conditions. Dry seeds of all the plant materials were further oven dried at 40°C for 2 hours and then blended separately with a high-speed electric blender (Binatone) to obtain fine powders.

The extraction of aqueous extracts from plant materials was done by soaking 200 g each of the powdered samples of plant materials in separate conical flask containing warm water (60ºC) with a ratio of 1: 2 (50% w/v). The mixture was vortexed manually at 30 minutes intervals for three hours and allowed to stay for 48 hours under laboratory conditions, the supernatant was then filtered with 90mmWhatman filter paper(Ugwu, 2020). Ethanol extraction was done by weighing two hundred grams (200g) of powdered samples separately into a seed extraction chamber. Two hundred and fifty milliliter (250ml) of ethanol wasdispensed to each of the samples in a flask , extracted for 6hours and ethanol was latter distilled off from the flask using quick fit pressure equalizing funnels. All extracts were preserved in small air-tight bottles inside a refrigerator until when used.

Populations of *B. dorsalis* were raised on mango fruits in the laboratory at ambient temperature of 25 ± 2°C , 70 -80%relative humidity and 12: 12 photo period. The infested mango fruits were gathered from the mango plantation at the National Horticultural Research Institute (NIHORT) Ibadan and were stored in plastic cages in the Biology and Entomology Laboratory of Federal College of Forestry Ibadan and observed for the emergence of adult flies. The identities of the emerged adults were confirmed using identification key by Ekesi and Billah,(2007) and were paired in another cage for mating and oviposition. At the emergence of the first instar larvae, they were fed with sliced fresh mango fruits every two days and the culture was retained til the end of the experiment.

Aqueous and ethanol extracts were evaluated for contact and residual actions. The contact toxicity of the extracts were evaluated by applying 0.1 ml of each extracts using 20µl micro pipettes on the dorsal cavity of the *B. dorsalis* third instar larvae. Ethanol extracts was applied at 75% concentration (dilution)while aqueous extracts was applied as extracted (undiluted). The residual actions of the extracts was assessed byapplying 1ml of each extracts on petri dishes lined with 90mm Whatman filter paper. Petri dishes were kept for 10 minutes to drain off before five third - instar*B. dorsalis* larvae from the

raised culture were introduced separately into each petri dish.Cypermethrin was applied at the rate of 1ml/liter of water as standard check for both contact and residual assay following the same procedure and distilled water served as control. All assays were replicated three times in a Completely Randomized Design (CRD) at 5% level of probability.

The larval mortality was recorded at 20 minutes intervals for both contact and residual effects until 24 hours. Data collected were subjected to Analysis of Variance (ANOVA) and significant means were separated at 5% level using Turkey's Honestly Significant Difference (HSD).

Table 1. Plant species and their parts used as botanical insecticides against *B. dorsalis* larvae

Plant species	Common name	Family	Plant parts used
Aframomum meleguata(danielli)K .Schum	Alligator pepper	Zingiberaceae	seed
Azadirachta indica	Neem	Meliaceae	seed
Moringa oleifera	Moringa(Drumstick tree)	Moringaceae	seed
Anona muricata	Soursop	Annonaceae	seed
Piper guineense	African black pepper	Piperaceae	seed

Results and Discussion

Contact toxicity of aqueous and ethanol extracts on *Bactrocera dorsalis* larvae

Aqueous and ethanol extracts of the plant materials assessed demonstrated contact toxicity on the third instar *B. dorsalis* larvae under laboratory conditions. Larval mortality was observed from 20 minutes on aqueous extracts of *P. guineense* and from 40 minutes on ethanol extracts of other plants to 1440 minutes post treatments (Table 2).

Aqueous extracts of *P. guineense* recorded 0.67 mean mortality at 20 minutes post exposure. At 40 minutes of post exposure both aqueous and ethanol extracts of *P. guineense* recorded larval mortality with mean values of 0.33 and 0.67 respectively while only the ethanol extract of other plants recorded larval mortalities at varied rates. This corroborate the finding by Ugwu and Bobadoye (2020), when mortality of *B. dorsalis* larvae due to contact toxicity of ethanol extracts of *A. indica* , *A. melegueta* , *Moringa oleifera* and *Jatropha curcas* was reported to commenced at 40 minutes post exposure during laboratory assay . Synthetic insecticides (cypermethrin) exhibited strong toxicity achieving highest larvae mortality before 80 minutes of post exposure. All the botanicals significantly ($p<0.05$) caused larval mortality compared with the control where none of the larvae died(Table.2).

Residual toxicity of aqueous and ethanol extracts on *Bactrocera dorsalis* larvae

The residual toxicity of aqueous and ethanol extracts of the various plant followed aparallel

form with their contact toxicity. The ethanol extracts of all the plants assessed recorded larval mortality from 40 minutes post exposure (Table 3). The residual toxicity of aqueous extracts of from the various plants commenced from 60 minutes post exposure on*M. oleifera* and started with other extracts from 80 minute post exposure with *A. muricata* recording significantly($p<001$) higher larval mortality compared to other extracts. The residual toxicity of the various plant extracts increased with increase in exposure duration and continued until 24 hours. Synthetic insecticide commence the residual action from 20 minutes and caused total larval mortality at 80 post exposure.

However, no larval mortality was observed from the control assay until the termination of the experiment. Different researchers have reported that different botanical extracts have high residual toxicity on insect larvae and adults both in the field and laboratory. A recent study by Ugwu (2021) reported that the residual effects of *P. guineense* and *A. indica* on adult *Phytolyma fusca* were found comparable to synthetic insecticide(cypermethrin). Similarly, Ugwu and Bobadoye (2020) reported high residual toxicity of aqueous and ethanol extracts of *A. indica, J. curcas, M. oleifera and A. melegueta* against *B. dorsalis* pupariating larvae under laboratory assay.

Anikwe (2013) also reported the high residual action of six different plant extracts against *Sahlbergella singularis (*brown cocoa mired) in a laboratory bioassay.

Table 2. Sequential contact toxicity of aqueous and ethanol extracts of various plants on the *B. dorsalis* larvae for 24 hours

	Time of exposure (minutes)/mean mortality													
	20		**40**		**60**		**80**		**100**		**120**		**1440**	
Treatments	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**
A.meleguata	0.00c	0.00b	0.00b	1.33a	0.33b	1.00ab	0.67b	1.67a	0.67b	0.67	1.00ab	0.33	2.00a	0.00b
A.indica	0.00c	0.00b	0.00b	1.00ab	0.00b	0.67ab	0.00b	0.33b	0.67b	0.00	1.33ab	0.67	1.67a	1.33a
M.oleifera	0.00c	0.00b	0.00b	1.00ab	0.00b	0.67ab	0.00b	1.33a	0.00b	1.00	1.67a	0.33	1.00ab	0.67ab
A. muricata	0.00c	0.00b	0.00b	0.33bc	0.33b	0.67ab	1.33a	1.33a	0.33b	1.00	0.67bc	0.67	2.00a	1.00a
P. guineense	0.67b	0.00b	0.33b	0.67ab	0.00b	0.67ab	0.33b	1.67a	1.67a	0.33	0.33cd	0.33	1.00ab	0.00b
Cypermethrin	2.33a	2.33a	1.33a	1.33a	1.67a	1.67a	0.00b	0.00b	0.00b	0.00	0.00d	0.00	0.00b	0.00b
Control	0.00c	0.00b	0.00b	0.00c	0.00b	0.00b	0.00b	0.00b	0.00b	0.00	0.00d	0.00	0.00b	0.00b

Mean values with different letters are significantly different from each other at 5% level of probability by Turkey's Honestly Significant Difference (HSD). *AQ = aqueous extract, ET= ethanol extracts

Table 3. Sequential Residual toxicity of aqueous and ethanol extracts of various plants on the *B. dorsalis* larvae for 24 hours

	Time of exposure(minutes) /mean mortality													
	20		40		60		80		100		120		1440	
Treatments	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**	**AQ**	**ET**
A.meleguata	0.00b	0.00b	0.00b	0.67ab	0.00b	1.33b	0.67ab	0.67a	0.67ab	1.67a	0.67ab	0.33a	2.33a	0.33b
A.indica	0.00b	0.00b	0.00b	0.00b	0.00b	0.67b	0.33ab	0.33a	0.00b	1.33ab	1.0ab	1.00a	1.33ab	0.00b
M.oleifera	0.00b	0.00b	0.00b	0.67ab	0.33ab	1.00b	0.00b	0.323a	0.67ab	1.00ab	1.33ab	0.33a	1.33ab	1.67a
A. muricata	0.00b	0.00b	0.00b	0.67ab	0.00b	1.00b	1.00a	0.33a	1.67a	1.00ab	2.0a	0.67a	1.33ab	1.33a
P. guineense	0.00b	0.00b	0.00b	1.33a	0.00b	2.00a	0.00b	0.67a	1.33a	0.67ab	1.67a	0.33a	0.67bc	0.00b
Cypermethrin	2.33a	2.33a	1.67a	1.67a	0.67a	0.67b	0.33ab	0.33a	0.00b	0.00b	0.00b	0.00a	0.00c	0.00b
Control	0.00b	0.00b	0.00c	0.00b	0.00b	0.00c	0.00b	0.00a	0.00b	0.00b	0.00b	0.00a	0.00c	0.00b

Mean values with different letters are significantly different from each other at 5% level of probability by Turkey's Honestly Significant Difference (HSD). *AQ = aqueous extract, ET= ethanol extracts

Comparison of contact toxicity of aqueous and ethanol extracts of various plants on percentage mortality of *Bactrocera dorsalis* larvae after 24 hours post treatment

Both aqueous and ethanol extract of all the plant materials proved very effective against *B. dorsalis* larvae for the contact toxicity by 24 hours post treatment (Fig. 1). There were no significant differences ($p > 0.05$) between the aqueous and ethanol extracts of most plant materials tested on the percentage mortality of *B. dorsalis* larvae by 24 hours post treatment.

The percentage mortality of only ethanol extracts of *M. oleifera* was significantly($p<0.01$) higher than its aqueous extracts at 24 hours post treatment. However, ethanol extracts of all the plant materials were slightly more effective than their aqueous extracts. This results support

the findings by Huerta*et al.* (2010) who reported that ethanol leaf extracts of *Schinus molle* was moreefficacious than the aqueous extracts of the same concentration against elm leaf beetle recording higher mortality of the insects. Similarly, Ugwu, (2021) reported that ethanol extracts of *A. indica, J. curcas, P. guineese and A.melegueta* were more effective than their aqueous extracts against adult *P. fusca* both in the laboratory and field.

The aqueous and ethanol extracts of *A. melegueta* and *A. muricata* were comparable to synthetic insecticide (cypermethrin) recording 93% -100 % larval mortality. However, only ethanol extracts of *M.oleifera* and *P. guinnense* were comparable to the contact toxicity of cypermethrin causing100% larval mortality in this study. Botanical extracts of these plants have been documented to be very efficacious against several insect pests both in the field and laboratory. Petroleum ether seed extracts of *A. muricata* was found effective than Lambdacyhalothrin against *Maruca vitrata* and *Megalurothrips sjostedti* on cowpea in the field(Ugwu, 2020). Similarly, Padma et al. (1998) reported that *Annona muricata* based product were more effective than synthetic insecticides in the control of different order of insect pests.

The study by Idoko and Adesina (2012) revealed that *P. guineense* powder has contact effects on *Callosobruchus maculatus*(cowpea beetles) causing adults mortality and inhibiting oviposition by female insects.

The contact toxicity of both aqueous and ethanol extracts of *A. indica* were not comparable to cypermethrin in this study. This result is in contrast with earlier studies where *A. indica* extracts were stated to be very effective, even more efficient than cypermerthrin against several insect species. For instance, Basedow *et al.* (2002) reported that *A. indica*–based products were more effective than synthetic insecticides against aphids and white flies in the field. Similarly Ojo and Ugwu (2012) disclosed that ethanol extracts of *A. indica seeds* was more efficient than cypermethrin against field insect pests of *Adansonia digitata* seedlings. The reticent contact toxicity of both aqueous and ethanol extracts of *A. indica* on *B.dorsalis* larvae in this study could be attributed to their mode of action implying that *A.indica* act indirectly as a very effective insecticide through other means rather than contact toxicity. According to Ahmad *et al.* (2019) *A. indica* seed powder were found ineffective against *Tribolium castaneum* (red flour beetle) larval development and pupation and ascribed it to the fact that *A. indica* (neem) plants indirectly act by preventing lavae from feeding which affect the morphological development. Various studies have revealed that neem productspossess repellent potential than contact toxicity. Echereobia *et al.* (2010) reported that *A. indica* proved to possess high repellency potentials against *Podagrica* species . According to Gridisa and Grsic (2013) the mode of action of *A. indica* include disruption of the nervous system, repellency, feeding deterrence , oviposition hinderance, deterrance of egg hatching and moulting.

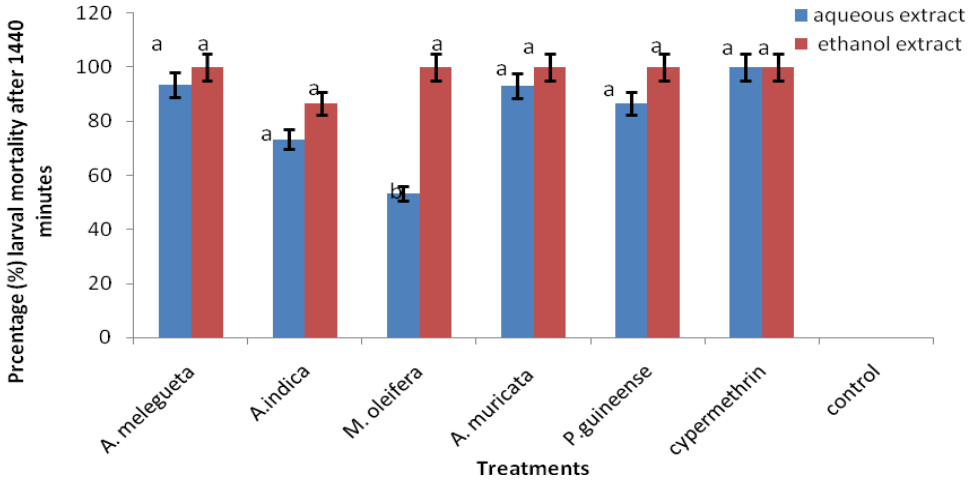

Fig. 1. Percentage larval mortality of *B. dorsalis* for the contact toxicity of the extracts at 24 hours post treatments

Comparison of residual toxicity of aqueous and ethanol extracts of various plants on percentage mortality of *Bactrocera dorsalis* larvae 24 hours post treatment.

The aqueous and ethanol extract of all the plant materials showed residual effects against *B. dorsalis* causing 53%- 100% larval mortality in 24 hours post observation (Fig.2). There were no significant differences ($p > 0.05$) between the aqueous and ethanol extracts of *A. melegueta*, *A. muricata* and *A. indica* on the overall percentage mortality of *B. dorsalis* larvae at 24 hours post treatment. The percentage mortality of ethanol extracts of *M. oleifera* and *P.guineense* were significantly($p<0.01$) higher than their aqueous extracts at 24 hours post treatment. However, ethanol extracts of *A. melegueta* and *A. indica* were slightly more effective than their aqueous extracts. Ethanol and aqueous extracts of *A. muricata* exhibited equal residual effects on *B. dorsalis* causing 100% mortality. *Annona muricata* have been reported to possess high insecticidal properties against several insect pests both in the field and storage. The bio-activity of *A. muricata* has been attributed to Annonaceous acetogins, Muri-catenol, Annomuricatin, Javoricin, montanacin, montecristin, and coronin,donhexocin which prevents development of insect pests (Jaramilloa *et al.*, 2000) .

The results of this study have proved that aqueous and ethanol extracts of the test plants exhibited residual action on *B. dorsalis* larvaeat varied levels. Studies have shown that the insecticidal activities of plant materials varied according to solvent used for extraction and parts of plant used. According to Overgaard *et al*. (2014) insecticidal activity of*Zanthoxylum heitzii*(indian prickly ash) against the *Anopheles gambiae* varied according to plant part, extraction method, solvent, and mosquito strain. Similarly Mostafa *et al*. (2012) reported that different solvent extracts of six plants (tamarind neemcucumber), gum trees, mahagony, and guava)leaves showed different toxicity effects against red flour beetle *(Tribolium castaneum*)indicating that the hexane extracts

of these plants showed more toxic effect than their methanol and aqueous extracts.

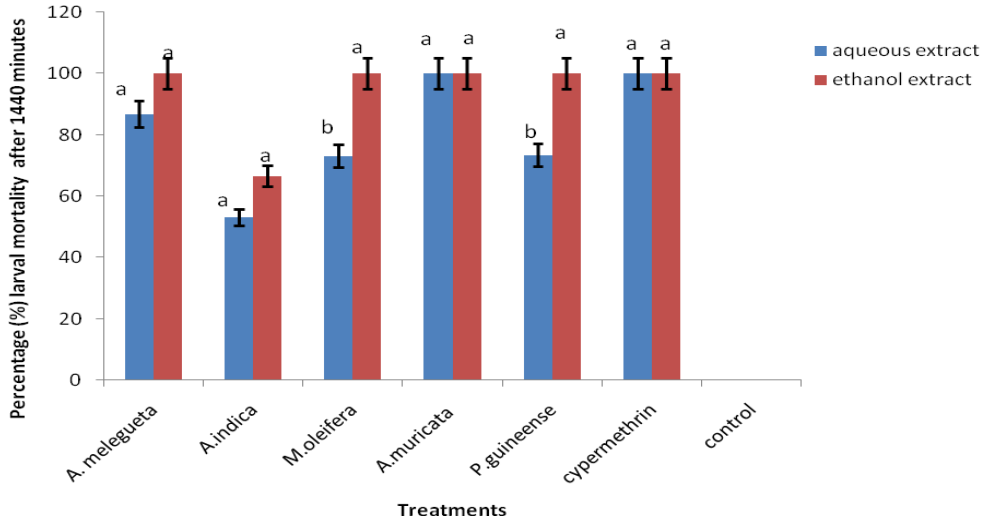

Fig. 2.Percentage larval mortality of *B. dorsalis* for the residual effects of the extracts at 24 hours post treatments

Conclusion

The study have established the contact toxicity and residual actions of aqueous and ethanol extracts from *A. muricata*, *P. guineense*, *A melegueta*, *M. oleifera* and *A.indica* against *B. dorsalis* larvae under laboratory conditions. Ethanol extracts of *M. oleifera* was significantly moreefficient than its counterpart aqueous extracts for both contact toxicity and residual actions.

Although, ethanol extracts of other test plants were slightly more effective than their counterpart aqueous extracts, both solvent are potent for extraction of plant materials for insect pests management. Therefore, the use of aqueous extracts of these plant materials should be adopted by small holder farmers for the management *B.dorsalis* in orchards via soil application targeting pupariating larvae since the process is easy, less expensive, required no complicated equipment and less technical skills.

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