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BIOCIDAL ACTIVITY OF SELECTED BOTANICALS And *Beauveria bassiana* ON ORIENTAL FRUIT FLY, *Bactrocera dorsalis* (DIPTERA; TEPHRITIDAE)

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ABSTRACT

The effectiveness of aqueous extracts of four plants(Azadirachta indica, Piper guineese, Moringa oleifera and Aframomum melegueta) and aqueous formulation of Beauveria bassiana were evaluated against larvae and pupating larvae of Bactrocera dorsalis (Hendel) (Diptera;Tephritidae) in the laboratory. Bioassays were conducted on B. dorsalis larvae for contact and residual effects of these four botanicals and effects of soil treatments with botanicals and B. bassiana against B. dorsalis pupating larvae were assessed. Larval mortality on bioassays was recorded at 20 min intervals for 1440 minutes while adult emergence and pupating larvae mortality on treated soil were recorded after 21 days. All the extracts significantly ($p<0.05$) showed contact and residual effects on B. dorsalis larvae. Piper guineese recorded 100% larval mortality and was the most effective for contact toxicity while A. melegueta and A. indica recorded 87% larval mortality for residual action on B. dorsalis larvae. Effects of the extracts and B dorsalis on treated soil against pupating larvae revealed that A melegueta was the most effective among all the treatments. A. melegueta recorded 100% mortality of the pupating larvae, followed by B. bassiana recording 83.3% pupating larvae mortality with 17% adult emergence. The efficacy of A. melegueta was comparable with cypermethrin against B. dorsalis on treated soil while B. bassiana was more effective than other plant extracts. Therefore, the use of aqueous seed extracts of A. melegueta, A.indica, P.guineense and B. bassiana for soil applications could be incorporated in Integrated Pest Management component for the management of African invader fly in fruits orchards.

Key words: *Bactrocera dorsalis*, aqueous extracts, fungal pathogens, soil treatment, control

INTRODUCTION

Fruit production and export is one of the main growing agricultural sectors in Africa, contributing both income and employment (Ekesi *et al.*, 2005). Mangoes, citrus, apple, papaya, coffee and avocado are among the common fruits grown for export that targets large markets in Europe and the Middle East. Apart from export fruits, other indigenous fruit like *Irvingia* spp (African bush mango), *Chrysophyllum albidum* African star apple) and *Dacryodes edulis* (African bush pear) are huge source of income to many African countries at local and regional trade. According to Ekesi *et al.(* 2005) profitable fruit production in Africa is seriously hindered by fruit fly infestation which includes species of *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi), *C. rosa* (Karsch), *C. anonae* (Graham), *C. capitata* (Weidemann) and a recently introduced *Bactrocera* spp of Asian origin (Ekesi *et al.*, 2005). Currently, the persistent spread of *B. dorsalis* especially in the subtropical and tropical regions of the world threatens the commercial fruit industry by increasing costs of production and control in addition to quarantine restrictions (Aketarawong *et al.*, 2014). *B. dorsalis* attacks over 250 different types of commercial fruits and vegetables in different continents of the world, especially Africa and Asia.

Lux *et al*. (2003) reported that study conducted across Kenya, Tanzania, Sudan, Uganda, Côte d'Ivoire, Nigeria, Namibia, South Africa and the Indian Ocean Islands revealed that about 40% of the 1.9 million tonnes of mangoes produced annually are lost to fruit fly damage. Fruit fly infestation rates varied among countries and seasons, ranging from 5 to 70% (Lux *et a*l., 2003).

During the fruit fly development, third-instar larvae of most species drop from fruits to the ground and burrow into the soil to change forms through the process called pupation (White and Elson-Harris, 1992). A major part of a fruit fly control and eradication programme includes soil treatment with insecticides beneath host trees to kill fruit fly larvae and puparia (CDFA, 1993; Roessler, 1989; Saul *et al.*, 1983). Integrated pest management (IPM) approaches such as the use of kairomones, protein baits, and fruit bagging, might prove effective in controlling *B. dorsalis* and thus minimize the use of conventional pesticide us (Sharma *et al.*, 2014). Biological control by entomopathogenic microorganisms represents one of the most effective options among the non-chemical pest control measures (Ruiu, 2015; Shawer, *et al.*, 2018; Fanning *et al.*, 2018).

The use of entomopathogenic fungi (EPF) in biological control is increasing recently due to more awareness of environment and food safety concern and increasing resistance of most insect pests to conventional insecticides (Shahid *et al*., 2012). Entomopathogenic fungi are effective against many insects' orders such as Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera (Ramanujam ***et al***., 2014). The use of entomopathogenic fungi (EPF) to reduce pest population density and crop damage plays a vital role in sustainable pest management programs (Mantzoukas, 2020)

Beauveria bassiana (Bals.) and *Metarhizium anisopliae* (Met.) Sorokin are entomopathogenic fungi, of Hyphomycetes group, that are natural inhabitants of soil, they are found infecting a wide range of insect species that spend at least one stage of their life cycle in the soil. They are also found in agricultural crops as epizooties on defoliator lepidopteran larval populations (Jorge *et al*., 2006).

According to Ekesi *et al.* (2002, 2003) several isolates of entomopathogenic hyphomycetes fungi have been screened and used for soil inoculation against pupating larvae and puparia as an alternative to soil application of chemical insecticides. Ekesi *et al.* (2005) also reported that three formulations (oil, aqueous and granular) of *M. anisopliae* were more effective than diazinon in reducing adult emergence of three fruit fly species. Moreover, several plant extracts have proved to be toxic against all the developmental stages of several insect species both in the laboratory and field. Ugwu (2016) reported that hexane and ethanol extracts of *Azadirachta indica* A Juss, *Piper guineense* Schum and Thonn, *Eugenia aromatic*a (L) Baill. and *Jatropha.curcas* Linn. were very effective against larvae and adult *Callosobrunchus maculatus* on stored cowpea This study therefore evaluated the efficacy of some selected aqueous plant extracts (*Azadirachta indica* A.Juss, *Piper guineese* Schum and Thonn, *Moringa oleifera* Lam and *Aframomum melegueta* (Roskoe) K. Schum and *Beuveraia bassiana* against larvae and pupating larvae of *B. dorsalis* and their potential in reducing adult emergence.

MATERIALS AND METHODS

Study Area

The study was conducted in the Biology Laboratory of the Federal College of Forestry Ibadan. Four plant seed extracts were evaluated in this study and they include*; A. melegueta, P. guineense, M. oleifera* and *A. indica.* Dried *A. melegueta* and *P. guineense* seeds were purchased from a local market (Oje) in Ibadan while Mature *M. oleifera* pods and *A. indica* fruits were collected from Forestry Research Institute of Nigeria, Ibadan.

Source of Plant Materials, Processing and Extraction

Seeds from pods and fruits were extracted and air dried for four weeks. Air dried seeds were ground into powder using an electric blender (Binatone blender/grinder BLG.450). Extraction of botanicals was done by adding 100 g each of the powdered samples of plant materials in 200 ml of warm water (60ºC). The mixture was vortexed manually at intervals for 30 min and allowed to stay for 48 hours under laboratory conditions before sieving with muslin cloth to obtain the extracts.

Isolation of Entomopathogenic Fungi (*Beauvaria bassiana*) from the Soil

Soil samples collected from *Gmelina arborea* plantation in the Federal College of Forestry Ibadan, Oyo state, Nigeria were put in petri dishes and moistened with water; Five third instar *Galleria mellonella* larvae collected from the culture reared with artificial diet in the laboratory were added to each soil sample in the petri dishes of 100 mm x 15 mm. The set up was allowed to incubate for about 14 days at room temperature after which cadavers were collected at intervals and processed for mycosis. The mycosed insects were prepared by dipping the cadaver into 2% sodium hypo chloride for 2-3 seconds and thereafter in 70 % ethanol for 1-2 minutes to facilitate wetting of the specimen and remove any contaminant. This was followed by rinsing twice with distilled water to remove to remove all ethanol. The cadaver was placed in a sterile petri dish with sterile moist filter paper and the petri dish was sealed using a Para film and incubate at room temperature for five days. The pure culture of *B. basiana* was plated on potato dextrose agar (PDA) following standard procedure and watched for sporulation. The spores were harvested and kept for later use.

Toxicity and residual bioassay

Extracts were evaluated for residual action by applying 1ml of each extracts on petri dishes lined with Whatman filter paper (90 mm). Petri dishes were left for 10 minutes to drain off before five third instar larvae of *B. dorsalis* were separately introduced into each petri dish. The contact toxicity of the extracts were assessed by applying 0.1 ml of each extracts using 20µl micro pipettes on the dorsal cavity of the *B. dorsalis* third instar larvae

Soil treatment with extracts and *B. bassiana*

Soil samples were collected from Federal College Forestry Ibadan experimental farm and weighed 100 g to each plastic cage of 14 cm x 9 cm x 7cm. *B. bassiana* liquid formulation was prepared by mixing 10 g of the powder in 10 ml of distilled water. The soil was then treated with aqueous extracts at 20 ml/100 g of soil, liquid formulation of *B. bassiana* at 10 ml / 100g of soil. Cypermethrin was introduced as a positive control and was applied at 5ml/liter of water/ 100 g of soil. Ten (10) pupating *B. dorsalis* larvae were introduced into each cage containing treated and untreated soil (soil) and observed for adult emergence. All the experiments were arranged in Completely Randomized Design (CRD) and the treatments were replicated thrice

Data Collection and Analysis

Data on the mortality of *B. dorsalis* larvae were recorded at 20 minutes intervals for 24 hours for both contact and residual bioassays while adult emergence were recorded on the treated soil after three weeks (21) days. The pupating larvae mortality was inferred by calculating from the number of adult emergence after 21 days. Data collected were subjected to square root transformation before analysis of variance (ANOVA) and significant means were separated by Duncan multiple range test at 5% level of significance using ASSISTAT statistical software 7.6 beta.

RESULTS

Some of the extracts gave an effective kill of the *B. dorsalis* larvae as time progressed for the contact toxicity. There were no significant differences ($p > 0.05$) among the treatments at 20 min, 40 min, 80 min and 24 h of application on larvae mortality (Table 1). However, there were significant differences ($p < 0.05$) on the larval mortality among the treatments at 60 min and 120 min of post treatment application. Similar trend was observed for the residual action of the treatments on the larval mortality (Table 2). Larval mortality commenced from 20 min of post treatment for the *M. oleifera* while other treatments began action from 40 min post treatments.

The percentage contact toxicity revealed that all the extracts significantly ($p < 0.01$) enhanced *B. dorsalis* larval mortality compared to control (Fig. 1). *P. guineense* showed equal contact toxicity with Cypermethrin, both gave 100 % mortality of *B. dorsalis* larvae. *P. guineense* with 100 % larval mortality was the most effective among the extracts for contact toxicity , followed by *A. melegueta* with 73.4% larval mortality (Fig.1). There was no significant difference ($p > 0.05$) between the effects of *A. melegueta* and *M. oleifera* extracts on the mortality of *B. dorsalis* larvae. *A. melegueta* and *A. indica* extracts were more effective than other extracts for the on *B. dorsalis.* They both recorded

86.6% larval mortality, followed by *P. guineense* (80.0 %) after 24 h of observation. There were no significant ($p > 0.05$) differences among the extracts and cypermethrin on the mortality of *B. dorsalis*. All the extracts and *Beuvaria bassiana* formulation were effective against *B. dorsalis* pupating larvae on the treated soil (Fig. 2).

A. melegueta was the most effective among the aqueous extracts tested against *B. dorsalis* pupating larvae on the treated soil. It recorded 100.0 % mortality with zero emergence of adults *B. dorsalis* and its efficacy was comparable with *Cypermethrin*. This was followed by *Beuvaria bassiana* which recorded 83.3% mortality of *B. dorsalis* pupating larvae with 16.7% adult emergence. There were no significant differences ($p > 0.05$) on the effects of Cypermethrin, *A. melegueta and B. bassiana* on treated soil against *B.dorsalis*.

Table 1: Contact effects of the aqueous plant extracts on *Bactrocera dorsalis* larvae

Treatments	Time interval (minutes)					
	20	**40**	**60**	**80**	**120**	**1440**
M.oleifera	0.67	0.00	0.33^{ab}	0.33	0.00^{c}	1.38
A. indica	0.00	0.33	0.00^{b}	1.00	0.00^{c}	0.80
P.guineese	0.00	0.67	0.80^{a}	0.47	1.41^{a}	0.47
A. melegueta	0.00	0.67	1.00^{a}	0.67	1.14^{a}	0.33
Cypermethrin	0.67	1.52	1.28^{a}	0.00	0.33^{b}	0.00
Control	0.00	0.00	0.00^{b}	0.00	0.00^{c}	0.33

Means with the same letter within the same column do not differ statistically by DMRT

Table 2. Residual effects of aqueous plant extracts on *Bactrocera dorsalis* larvae

Treatments	Time interval (minutes)					
	20	**40**	**60**	**80**	**120**	**1440**
M.oleifera	0.67	0.33^{b}	0.33^{ab}	1.00	1.00	0.67^{a}
A. indica	0.00	0.33^{b}	1.00^{a}	0.67	1.00	1.14^{a}
P.guineese	0.00	1.00^{a}	0.67^{ab}	0.80	0.80	0.00^{b}
A. melegueta	0.00	1.00^{a}	1.00^{a}	0.67	0.80	0.67^{a}
Cypermethrin	0.00	1.00^{a}	1.00^{a}	1.28	1.28	0.00^{b}
Control	0.00	0.00^{b}	0.00^{a}	0.00	0.00	0.00^{b}

Means with the same letter within the same column do not differ statistically by DMRT

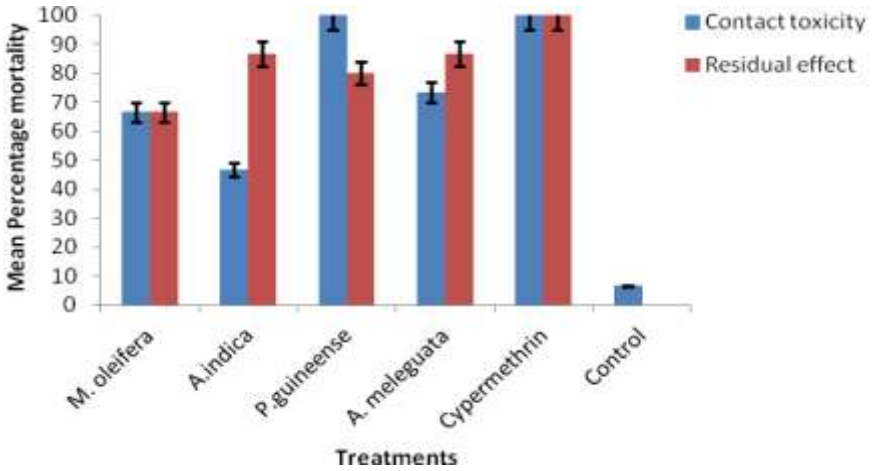

Figure 1: Effects of botanicals on mean percentage mortality of *Bactrocera dorsalis* larvae after 1440 minutes post treatments.

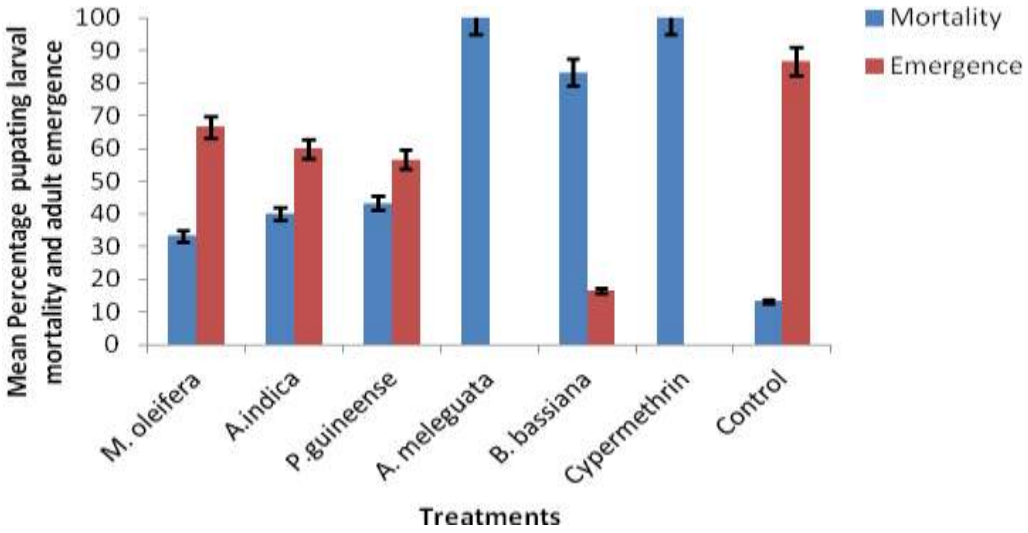

Figure 2: Effect of the soil treatments on mean percentage mortality of *B dorsalis* pupating larvae and adult emergence

DISCUSSION

The result of the laboratory bioassays of the aqueous plant extracts evaluated shows that the mode of action of the extracts could be by contact effects and or through residual action. *P. guineense* showed higher contact effect on the *B. dorsalis* larvae than the residual effects which indicates that *P. guineense* mode of action is more of contact than residual.

Oparaeke *et al* .(2005) reported that visual observations after direct spraying of *P. guineenses* extracts against *C. tomentosicollis* and *Maruca* larvae on cowpea plants indicated that the extract first had a 'hallucination' effect on these pests and then caused their death within 10–15 min of contact with the extracts. Similarly, Idoko and Adesina (2012) reported that application of *P. guineense* powder on cowpea grains against *Callosobrunchus maculatus* caused adults mortality, inhibited oviposition by female and suppressed F1 progeny emergence and attributed its effect to contact toxicity.

Olaiya *et al*. (1987) earlier reported that the mode of action of the phytochemical present in *P. guineense* is contact toxicity. They further claimed that the powder may also cause physical abrasion to the cuticle of bruchids with a resultant loss of body fluids or blockage of spiracle. . *A.meleguata* and *Azadritchata indica* recorded higher larvae mortality on the residual activity than other plant extracts evaluated indicating their higher efficacy over the other extracts. *A. indica* (neem) has proved to be a very effective biopesticides against several insect species both in the field and in the laboratory. Ivbijaro and Agbaje M (1986) and Schmutter (1995) have reported the potential of neem products for the control of field insect pests of egg plant and okra.

Aliero (2003) and Nzanza and Mashela (2012), reported that neem extracts are effective for the control of mosquito larvae, aphids and whitefly. Neem based extracts has been reported to be even more effective than synthetic insecticides against several insect species. Basedow *et al*. (2002) reported that *A. indica* - based products were effective or even more effective than synthetic insecticides for the control of aphids and white flies. Similarly Ugwu *et al.* (2012) reported that *A. indica* seed extracts showed higher efficacy than the cypermethrin in controlling *S. derogata* on okra. The efficacy of neem based product could be attributed to their molecular component. According to Hossain *et al*. (2013), neem extract is composed of a complex mixture of molecules, together with normal hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides. The neem molecules act on various phases of an insect's life cycle, making it difficult for pests to resist the physiological effects of neem extract (Mordue-Lunt and Nisbet, 2000).

The study also confirmed the efficacy of the selected plant extracts and *B. bassiana* against *B. dorsalis* pupating larvae and their potential in reducing adult emergence. The variation on the effectiveness of aqueous plant extracts and the liquid formulation of *B. bassiana* against the *B. invadens* pupating larvae and in reduction of adult emergence can be attributed to their diverse mode of action and residual effects. This corroborates earlier reports (Saxena 1989; Isman *et al.,* 1990; Koul *et al.,* 1990; Schmutterer, 1990) that the biological activities of *A. melegueta* extracts comprises feeding and oviposition deterrence, repellency, growth disruption, reduced fitness and sterility. Similarly, Adenike *et al.* (2014) reported that powders and extracts of the *A. meleguata* significantly reduced adult emergence of *Sitotroga cerealella* and increased their developmental period on two paddy varieties.

B. bassiana was effective against pupating larvae causing mortality and reduced adult emergence in this study. This corroborates the earlier report of Munoz (2000) who found mortality levels between 20 and 98.7% when 16 strains of *B. bassiana* were evaluated against *C. capitata* adults and Swiergiel *et al.* (2016) who submitted that the commercial strain *B.bassiana* isolate was virulent to apple sawfly under laboratory conditions.

Similarly, Erler and Ozgur Ates (2015) reported that the Entomopathogenic fungi (EPF), *B. bassiana* strain PPRI 5339 and *M. anisopliae* strain F52, were effective against *Polyphylla fullo* (June beetle) larvae and comparable in efficacy to the standard pesticide, chlorpyrifos-ethyl. According to Butt *et al.* (2001) the use of microbial control agents (MCAs) especially EPF, with no or low-hazard effects on human health and environment is attractive alternative to chemical pesticides. Berón and Díaz (2005) earlier reported that different isolates of *B. bassiana* were generally more virulent to most soil-dwelling insect pests than *M. anisopliae*. Integration of EPF in the integrated pest management (IPM) strategy for control of *Bactrocera dorsalis* can reduce dependence on synthetic insecticides and boost the levels of control especially against pupating larvae in the soil. The EPF are used as ready-made components of IPM because of their matching or synergistic insecticidal activity with other control elements like predators and parasitoids (Roy and Pell, 2000; Goettel *et al.,* 2000; Lacey *et al*., 2001; Wraight *et al.,* 2001; Goettel *et al.,* 2010). Commercial products based on *B. bassiana* and *M. anisopliae* are currently in use in some parts of the world like Europe, United States and Australia. Moreover, *B. bassiana* and *M. anisopliae* strains has been used successfully in controlling different insect pests under field conditions (Puterka 1999; Lababidi 2002; Lacey *et al.,* 2001, 2011). The efficacy of *B. bassiana* could have been influenced by the prevailing environmental condition of the soil at the time of the study. This corroborates the report by Vidal and Fargues (2007) that environmental factors like temperature, humidity and sunlight play intense role on field persistence of EPF (especially, at the beginning of their growth, sporulation, and infection to the cuticle of host insect pests) and this period lasts 3 to 8 h for many EPF. This implies that efficacy of EPF can be determined by the prevailing atmospheric condition at the time of application.

CONCLUSION

Based on the results of this study, it was concluded that the plant extracts tested were all effective against *B. dorsalis* larvae under laboratory condition. *P. guineense* proved higher contact toxicity over other extracts and was comparable to Cypermethrin. *A. meleguata* and *Azadrichta. indica* demonstrated equal residual effect on the *B. dorsalis* larval mortality. However, *.A. meleguata* and *B. bassiana* were more effective against *B.dorsalis* pupating larvae in the treated soil. These plants extracts and *B. bassiana* (EPF) may provide possible alternatives to synthetic insecticides for the control of *B. dorsalis.* Their use in combination with good agricultural practices may reduce the use of chemical pesticides and provide an aspect within an integrated pest management (IPM) system. Thus soil treatment with *A. meleguata*, *A. indica*, *P. guineenses* and *B. bassiana* targeting pupating larvae could be incorporated in IPM component for the management of *B.dorsalis* in fruits

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