Original Research Article

BIOASSAY OF *Azadirachta indica* AND *Jatropha curcas* OILS ON *Aphis craccivora*

ABSTACT

Aphis craccivora is a major pest of *Vignaunguiculata* due to its economic importance to the crop. A single bioassay was conducted and repeated ten times using live insects, botanicals and an olfactometer which contained the test compound in one arm and the remaining 3 arms served as a control in a four-armed olfactometer in order to determine its effectiveness as a repellent. The treatment odour source contained either 10µL Azadirachta indica or Jatrophacurcas oil while the control arms contained no solvent tested against adult A.craccivora. The data obtained included the time spent by A. craccivora in the different arms of the olfactometer and the number of visits to each arm. Each of the data was analyzed using paired t-test. The results indicated that both oils significantly ($p<0.05$) repelled A. craccivora as less time was spent in the plant oil-treated arms. Similarly, more visits were made by A. craccivora to the control arms. This showed the efficacy of both oil extracts as a repellent to A. craccivora. (Word count: 169)

Key words; *Aphis craccivora*, olfactometer, *Azadirachta indica*, *Jatropha curcas*,*Vigna unguiculata*, repellent

1.0INTRODUCTION

Cowpea is the most important member of the Fabaceae family in Africa because of its nutritive value (IITA, 2009; Haruna and Usman, 2013). The seed of cowpea is the highest contributor to the overall protein requirement of rural and urban poor diets (Agbogidi, 2010). It is a deep-rooted and warm season crop. It is more drought tolerant than soybean, does well in sandy soils and is better adapted to a temperature of 25^{o}C-30^{o}C (Awodun, 2007; Nwofia*et al*., 2015).

The production capacity of the crop in Nigeria was 2.95 million tonnes, cultivated in 3.8 million Hectares of land in 2013, making Nigeria the largest producer of the crop in the World (FAO, 2014). Cowpea has the ability to fix nitrogen to improve marginal lands and serve as a cover crop in a tropical cropping system (Dujge*et al*., 2008; IITA, 2009). Cowpea can fix about 240kg/ha of atmospheric nitrogen for succeeding crops grown in rotation with it (Nwofia*et al*., 2015), to make use of 60-70kg/ha available nitrogen (Nwofia*et al*., 2015).

Cowpea has *Aphis craccivora* as one of the major field pests, due to its economic importance to the crop (Van Emden and Harrington, 2007; Lozier *et al*., 2008; Martin and Brown, 2008). Adult *A. craccivora*has a medium size of 1.50mm-2.28mm long. The adult aphid is a shiny black pear shaped, while the nymph is gray in colour (Barari*et al*., 2005; Blackman and Eastop, 2007; Rajendra and Mamta, 2013). It can be distinguished from other species by their first segment of hind tarsus having two hairs (Cour d'aciere*et al*., 2007; Rajendra and Mamta, 2013). It has blackish dorsum of abdomen, usually completely scleroitic and a pale area around the siphunculi with a darkcontinous median patch (Poulios*et al*., 2007; Mehrparvar*et al*., 2012; Rajendra and Mamta, 2013). It feeds on the under surface of young leaves, young stem tissues and on pod of mature plants (Smith and Boyko, 2007; Soffan and Aldawood, 2014). Their large population directly feeding on cowpea caused stunted growth in the plant, leaf distortion, premature defoliation and seedlings death (Blackman and Eastop, 2007; Mehrparvar*et al*., 2012). It has been reported, that the most widely harmful effect of the insect is its transmission of Cowpea Aphid-borne Mosaic Virus (CAMV) (Shannag and Ja'far, 2007; Van Emden and Harrington, 2007).

The problems associated with the use of synthetic pesticides has necessitated the search for user and environment- friendly/ecological tolerable methods of control which are safe, affordable (cheap), locally available and provide adequate supply to meet the insecticide shortage (Zhao*etal*., 2006; Gokce*et al*., 2010). Jackai and

Adalla(1997) opined that insect pest problem on cowpea persist at least in part because of lack of diversity in control measures.

The use of botanicals notably, *Jatrophacurcas* and *Azadirachta indica* remains one of the most viable options for control of crop pests (Dubey *et al*., 2008; Chaieb, 2010; Devappa*et al*., 2012). The generic name "Jatropha" is derived from the Greek words "Jatros" meaning "Doctor" and "Trophe" meaning "Nutrition" hence the common name "Physic nut" (Nayak and Patel, 2010; Roy *et al*., 2016).Several studies have attributed the potency (toxicity) of *J. curcas* to its rich content of phorbol esters (Jing *et al*., 2005; Gaur, 2009; Makkar *et al*., 2012; Roy *et al*., 2016). Phorbol-12-myristate-13-acetate (PMA) is the most common phorbol ester (Goel *et al*., 2007). The seed contains 25-35 % crude oil while the kernel contains about 50-60 % oil (King *et al*., 2009; Makkar and Becker, 2009; Makkar *et al*., 2011a). The "oil contains 21% saturated fatty acids and 79 % unsaturated fatty acids" (Makkar and Becker, 2009; Harry-Asobara and Samson, 2014). The word Azadirachta is derived from the Persian azaddirakt "meaning noble tree" and it belongs to the same family with Mahogany called Meliaceae (Ogbuewu*et al*., 2011; Asif, 2013).There are two species in the genus, Azadirachta; *A. indica* A. Juss, native to Indian sub-continent and *A. excelsa*Kack, confined to Phillipines and Indonesia. Another morphologically similar species of trees to *A. indica* is *Melia azadirachta* (Boadu *et al*., 2011; Lokeshwar *et al*., 2011). Rajkumar *et al*. (2011) reported "47 of triterpenoids and steroids isolated from neem (*A. indica*) and 15 non-terpenoid and non-steroid constituents from various parts of neem". Azadirachtin has been reported, as the most active ingredient in *A. indica* (Lakshhi, 2009;Ogbuewu*et al*., 2011; Jafari *et al*., 2013; Amit and Das, 2018; Traore *et al*., 2019). Azadirachtin A have been reported to be the most active of all azadirachtin compounds (FAO, 2006). Many researchers have reported, the seeds of *A. indica*and *J. curcas*to contain the highest amount of azadirachtin (Fig1)and phorbol esters (Fig 2) respectively (Martinez-Herrera *et al*., 2006; Devappa *et al*., 2011; Diabate *et al*., 2014). Both *J. curcas* and *A. indica* have insecticidal properties of antifeedant, anti-oviposition, deterrent, ovicidal and fecundity inhibition to insect pests of crops (Devappa *et al*., 2010b; Habou *et al*., 2011; Jide-Ojo *et al*., 2013).The use of essential oils with strong odour enhance olfactory sensation which are easily manipulated and use as spray to repel insect pests of crops to sprayon them (Zewde and Jembere, 2010; Wekesa, 2011; Masry *et al*., 2020). *J. curcas* display potent cytotoxicity and anti-tumor activity (Makker and Becker, 2010; Nayak and Patel, 2010; Federation of Free Farmers, 2012; Srinivasan *et al*., 2019). The cytotoxicity is the consequence of its ability to inhibit protein synthesis (Devappa *et al*., 2010a; Devappa *et al*., 2012; Okbatinas and Haile, 2017). Aqueous extracts of Jatropha are very bitter due to its saponin content and there have high insecticidal activity owing to their interaction with cholesterol that impedes ecdysteroid synthesis (Boucheltaet *al*., 2005; Chaieb, 2010; Ojumoolaet *al*., 2018). Saponin ingestion cause stunting and reduce energy value in insect (Nesseimet *al*., 2012). When the *Jatropha curcas* oil, are inserted in the cell membrane of the insect, the enzymes, Protein Kinase C (PKC), is hindered of its performance, by the phorbol esters (Goel *et al*.,2007; Millard and Leclaire, 2008; Insanuet *al*., 2012; Ugwu and Mokwunye, 2019). The phorbol esters interfere with the normal message transfer system in the midgut cell, thereby preventing normal signal transduction that regulates the physiological/biochemical reactions stopping cell growth and differentiation (Luo *et al*., 2007; Nesseim*et al*., 2012; Ukatu*et al*., 2021). This eventually may leads to cell turbulence and death in some insects (Oluwale-Abulude, 2007; Aiyelaagbe and Gloer, 2008; Makkar *et al*., 2011b; Bayih*et al*., 2018).

The cultivation of cowpea is highly challenged by pests attack. Chemical (synthetic insecticides) control is one of the most widely used management approaches despite the fact that it has adverse effects on the ecosystem and human health. There is urgent need for alternative means of control of pest, which are environment- friendly, effective and could reduce the cost of productionand thereby increasing yield of the crop. The objective of this study is to evaluate the repellency activity of the *A. indica* and *J. curcas* oils against *Aphis craccivora***.**

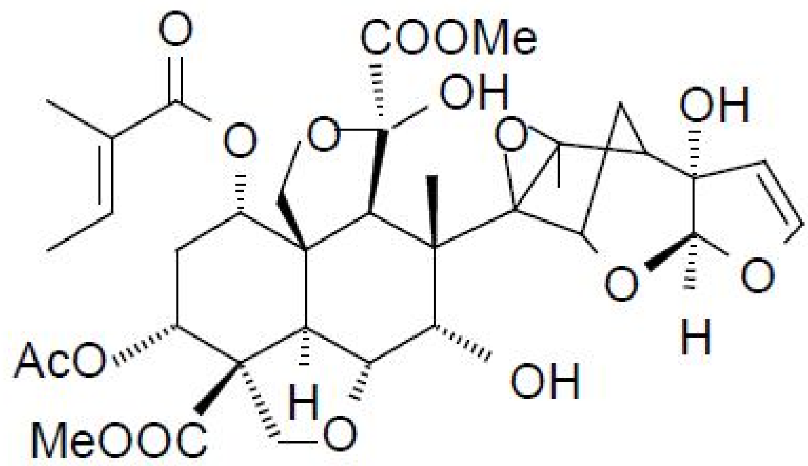

FIG 1: Chemical structure of azadirachtin A (FAO, 2006)

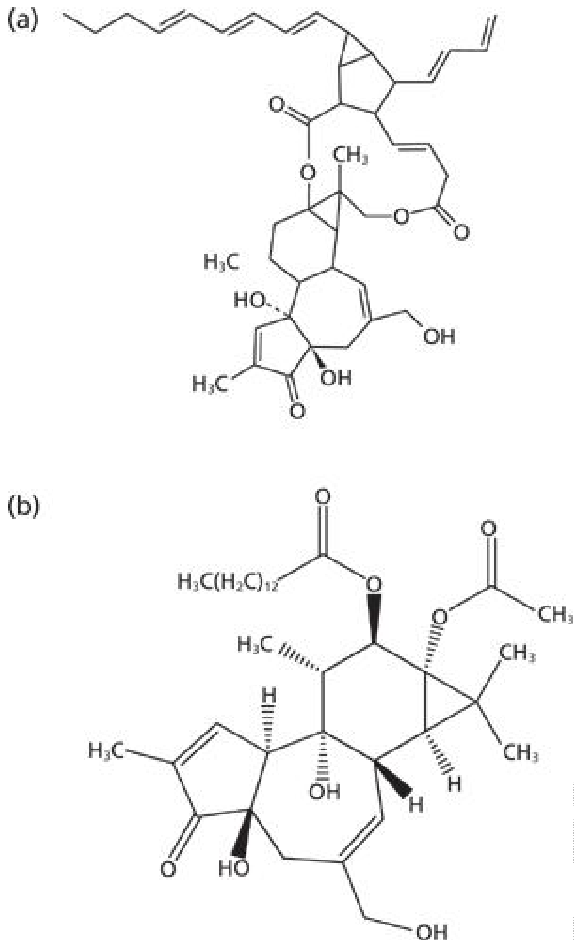

FIG 2:Chemical structures of phorbol esters reported to be present in *Jatrophacurcas*; (a) DHPB and (b) PMA (Visawanathan*et al.,* 2012).

NOTE:

DHPB=12-deoxy-16-hydrophorbol-13, 16 diester or

12-deoxy-16-hydroxyphorbol- 4'-[12', 14'-butadienyl]-6'-[16', 18', 20'-nonatrienyl]-bicyclo [3.1.0] hexane-(13-0)-2'-[carboxylate]-(16-0)-3'-[butenoic]ate

PMA=Phorbol-12-myristate-13-acetate

2.0 MATERIALS AND METHODS

2.1 Plant materials collection and extraction of oil

Mature seeds of neem, *Azadirachta indica* were collected from Murtala Muhammed High way; IBB way and Atimbo Road in Calabar, Cross River State. The *Jatropha curcas* seeds were obtained from IjeguYala of Cross River State. The seeds, were washed thoroughly with tap water and dried in a shade for three days.*Jatrophacurcas*and *A. indica* seeds were cracked manually using mortar and pestle but separately to obtain their kernels (Plates 1 and 2 respectively) into two different containers. The dried kernels of both plants were heatedat the temperature of 50^{o}C for 5 minutesin anoven prior to the extraction period in order to enhance the quantity of the oils (Warra, 2011). The kernels were now packed into two clean white muslin clothes and tied with rope. Then the kernels were subjected to mechanical press to extract the oils.

The sediment in each of the oils was allowed to settle down for one week after which, they were separated from the oils to make the oil available for use.Cowpea seeds (Vita 7 variety) used for the experiments were obtained from "International Institute of Tropical Agriculture (IITA)", Ibadan, Nigeria.

2.2 Field establishment for aphid infestation.

TopSoil was collected from the University of Calabar Botanical Garden and was air-dried. The air-dried soil was properly sieved to remove all the large particles using a wire mesh of 0.4cm x 0.4cm. Eight perforated plastic buckets were used in the experiment.Three kilogramme (3kg) of the air-dried and sieved soil was weighed into each perforated plastic bucket of 4500cm^2. All the plastic buckets containing the soil were watered to field capacity and left overnight to drain, before planting the Vita 7

PLATE 1: Cowpea plants being raised at the botanical garden for *Aphis craccivora* infestation (Mag=×0.1)

variety of cowpea the following morning. The cowpea plants were allowed to grow for 4 weeks when there was abundant aphid on the cowpea (Plate 1). Mature*Aphis craccivora*werecollected for the laboratory experiment, from four weeks old cowpea plant.

2.3Repellence bioassay test

Behavioural bioassay was performed in an Olfactometer modified after Pettersson(1970). Laboratory work was conducted by involving the responses of active cowpea aphids, *A.craccivora* to *A. indica* and *J. curcas* in 4-ways Olfactometer (Plate 2).A single choice bioassays was conducted and repeated ten times using live insect, botanicals and Olfactometer which contained the test compound in one arm and the remaining 3 arms served as controls (Ukeh and Umoetok, 2011).The treatments odour source contained either 10µl *Azadirachta indica* or J*atropha curcas*oilwhile the control arms contained no solvent tested against adult *Aphis craccivora.* All sourceswere impregnated into filter discs.

2.4 Data collection

Laboratory work; Computer progrmme was used for collecting and analyzing "Behavior" data with the four armed Olfactometer (OLFA programme) (Ukeh*et al*., 2009; Ukeh and Umoetok, 2011). The data that were recorded include ; the time spent by the *Aphis craccivora*in the different arm of the olfactometer and the number of visit into each arm (Ukeh*et al*., 2009)

2.5 Data analysis

The time spent in each Olfactometer arm and the number of visit made by *Aphis craccivora* in the laboratory experiment was analyzed using paired t-test (IBM Corp., 2011). The mean of the control arms was tested against the treatment arm.

PLATE 2: *Aphis craccivora*feeding on the cowpea stem (Mag=×2)

3.0RESULTS AND DISCUSSION

3.1 Repellent effect of plant oils on *Aphis craccivora*

The effects of *Azadirachta indica* and J*atrophacurcas*oils on the time spent and number of entries made by *Aphiscraccivora*in the treated and control arms of the olfactometer are presented in Table 1.The results indicated that both plant oils significantly ($p \leq 0.05$) repelled *A. craccivora*as less time was spent in the plant oil treated arms. Similarly, more number of visits were made by *A. craccivora*to the control arms (4.23, 4.06)than plant oil treated arms (0.51, 0.11).

TABLE 1

The repellent activity of *Azadirachta indica*and*Jatropha curcas*oils

Treatments	Mean Time spent(min)	Mean no. of entries
J. curcas		
Test arm	0.11±0.02	0.10±0.01
Control arm	4.06±0.44	1.87±0.17
P-value	0.05	0.05
A. indica		
Test arm	0.51±0.39	0.40±0.32
Control arm	4.23±0.34	2.33±0.84
P-value	0.05	0.05

3.2 DISCUSSION

These results imply that the use of oils with strong odour enhance olfactory sensation which are easily manipulated and use as spray to repel insect pests of crops to spray on them (Zewde and Jembere, 2010; Wekesa, 2011; Traore *et al*., 2019). *Jatrophacurcas* display potent cytotoxicity and anti-tumor activity (Makker and Becker, 2010; Nayak and Patel, 2010; Federation of Free Farmers, 2012). The cytotoxicity is the consequence of its ability to inhibit protein synthesis (Devappa *et al*., 2010a; Devappa *et al*., 2012; Amit and Das, 2018). Aqueous extracts of Jatropha are very bitter due to its saponin content and there have high insecticidal activity owing to their interaction with cholesterol that impedes ecdysteroid synthesis (Boucheltaet *al*., 2005; Chaieb, 2010; Masryet *al*., 2020). Saponin ingestion cause stunting and reduce energy value in insect (Nesseimet *al*., 2012). When the *Jatropha curcas* oil, are inserted in the cell membrane of the insect, the enzymes, Protein Kinase C (PKC), is hindered of its performance, by the phorbol esters (Goel *et al*.,2007; Millard and Leclaire, 2008; Insanuet *al*., 2012). The phorbol esters interfere with the normal message transfer system in the midgut cell, thereby preventing normal signal transduction that regulates the physiological/biochemical reactions stopping cell growth and differentiation (Luo *et al*., 2007; Nesseimet *al*., 2012; Masry *et al*., 2020). This eventually may leads to cell turbulence and death in some insects (Oluwale-Abulude, 2007; Aiyelaagbe and Gloer, 2008; Makkar *et al*., 2011b).

3.3 CONCLUSION

The research showed that seed oil extracts of *J. curcas* and *A. indica* were able to control *A. craccivora* on cowpea and can be effectively use as repellent of the pest. *Jatropha curcas* and *A. indica* are effective bio-insecticides in the management of*A.craccivora* of cowpea and may serve as replacement for synthetic insecticides that are currently being used for controlling this insect pest of cowpea.

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