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Table of Content

Review on bio-intensive management of African bollworm, Helicoverpa armigera (Hub.): Botanicals and semiochemicals perspectives 1
Tariku Tesfaye Edosa

Degradation assessment of wetlands under different uses: implications on soil quality and productivity 10
O.A. Osinuga and C.O. Oyegoke

Natural occurrence of *Diadiplosis megalamellae* (Barnes) in mealybugs on roses in Kenya 18
Anouk H. J. Hoogendoorn, Ruth Murunde, Evans Otieno and Henry Wainwright

Banana field resistance to insect-vector transmission of bacterial wilt caused by *Xanthomonas campestris p.v musacearum* 24
B. Mudonyi, L. Vanhaelewyn, G. Tusiime, F. Ssekiwoko, J. Kubiriba, W. K. Tushemereirwe and C. M. Changa

Evaluation of sugarcane hybrid clones for cane and sugar yield in Nigeria 34
Mohammed A. K., Ishaq M. N., Gana A. K. and Agboire S.

Status and distribution of soil available micronutrients along a hillslope at Ekpri Ibami in Akamkpa Local Government Area of Cross River State, Nigeria 40
John Kingsley, Ayito Esther O., A. U. Akpan-Idiok and O. D. Effiom

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Review

Review on bio-intensive management of African bollworm, *Helicoverpa armigera* (Hub.): Botanicals and semiochemicals perspectives

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African bollworm, *Helicoverpa armigera* (Hübner), is a serious pest of cereals, sorghum, cotton, pepper, maize, sunflower, flax and niger. To control this insect pest, doses of insecticides have been used. The use of indiscriminate synthetic insecticides causes adverse effect like environmental pollution, human and animal health hazards, and development of pesticide resistance. To alleviate these bottleneck problems, botanicals and semiochemicals are the effective controlling methods and believed to be safe to environment and human health. For many years, many plants species having pesticidal effects have been tested against the insect pest; however, some of them are commercially not produced. And also semiochemicals are the worthy tools, especially in the insect pest monitoring, aggregation and mating disruption of the pest. The manipulation of these management options need to get attention in the development of integrated pest management strategies. To do so, compiling information regarding the research so far done in the area is important. Therefore, this paper emphasized on the review of the research has been done concerning botanicals and semiochemicals to control *H. armigera*. Thus, in this review paper, different botanicals that affect the insect pest through their juvenile hormone and growth regulatory activity, antifeedant action, larvicidal action, ovipositional deterrence, ovicidal and pupicidal effects have been reviewed. Additionally, semiochemicals which include pheromones, kairomones and allomones of *H. armigera* were included.

Key words: African bollworm, botanicals, semiochemicals, bio intensive management.

INTRODUCTION

African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is polyphagous insect pest that attacks a number of economically important crops such as cereals, sorghum, cotton, pepper, sunflower, safflower, flax and niger seed. During the pest out breaks, the larvae damage leaves, tender, shoots, apical tips, flower buds and pods. It causes considerable yield loss both in quality and quantity, thus leading to various socio economic problems.

H. armigera are estimated at approximately US$5 billion on different crops worldwide (Kanpur, 2005). Yield loss by this pest varies from country to country as well as

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crop to crop. For instance, in India, 29.93 to 31.28% yield loss was recorded on chickpea (Dinesh et al., 2017). In Ethiopia, estimated yield loss on chickpea ranged from 21 to 36% (Geletu and Million, 1996), on faba bean it ranged from 3.5 to 57.5% pod damage, while on field pea it ranged from 32 to 42% yield loss (Kemal and Tibebu, 1994).

Therefore, to control this polyphagous insect pest, growers are forced to use indiscriminate synthetic insecticides repetitively which leads to deleterious effects of pesticides on the environment, human and animal health and causes insecticide resistance development in some species. Hence, to minimize these problems, there is an urgent need for the development of environment-friendly to suppress the pest population and effective monitoring method and thus making it less abundant and less damaging than it would be otherwise. More specifically, the major target of this review, botanicals and semiochemicals are one of the promising options in the management of the pest (Grechanova, 1986). Consequently, these management options play paramount role in developing an IPM which will be sustainable management of this insect pest. To do so, compiling information regarding the research so far done in the area is important. To date, organized information concerning botanicals and semiochemicals is not available. Therefore, the objective of this pepper is to review the research work carried out on botanicals and semiochemicals with particular emphasis on *H. armigera* and their role in agriculture.

Life cycle *H. armigera*

The female can lay several hundred eggs mainly at night on leaves, flowers, and pods. The oviposition period lasts for 5 to 24 days. The incubation period depends on temperature and varies from 2 to 5 days. Duration of larval development depends not only on the temperature, but also on the nature and quality of the host plants, for instance, 15.2 days on maize and 23.8 days on tomato. The number of larval instars varies from 5 to 7 and pupate in the soil. The pre-pupal period lasts from 1 to 4 days. In non-diapausing pupae, the pupal period ranges from about 6 days. The diapausing period for pupa may last several months. Pale colored adults are produced from pupae exposed to temperatures exceeding 30°C. In captivity, longevity varies from 1 to 23 days for males and 5 to 28 days for females (Zhudong et al., 2004). The total life time from egg to adult ranges from 23 to 34 days (Figure 1)

Adverse weather conditions of winter and summer induce diapause in *H. armigera*. The winter diapause resulted from exposure of the larvae to short photoperiods and low temperatures (Ken and Kenji, 2002). For example, in Australia, *H. armigera* undergoes diapause during winters when the temperatures are low. The other condition that causes diapause is exposure of larvae to very high temperatures (43°C for 8 h daily) during summer (Fitt and Cotter, 2005; Zalucki and Malcolm, 2002).

Host range and nature of damage

H. armigera is or pests of cotton, pigeon pea, chickpea, sunflower, tomato, maize, sorghum, pearl millet, okra, *Phaseolus* species, vegetables, tobacco, linseed, a number of fruits (Prunus, Citrus, etc.), and forest trees, grapevine, apple, strawberries, finger millet, etc. Adult female can lay several thousand eggs, so numbers can build up rapidly, often resulting in severe crop damage caused by the feeding caterpillars. The larvae cause severe damage to reproductive and vegetative tissues of agricultural and horticultural crops. Caterpillars could bore into flower buds, fruits, bolls or inflorescence (Yadav and Patel, 2015).

Behaviors of *H. armigera*

Like other insect pests, *H. armigera* has its own behaviors through its life cycle. These behaviors form a continuum throughout the life table of the insect behavior from mating to the location and selection of appropriate host plants by females and subsequent feeding activities of larvae. Therefore, those behaviors play great role in monitoring, forecasting and drawing effective management methods.

Mating behavior

The most known mating behavior of *H. armigera* is the response of male to female pheromons. Female of the insect pest produces pheromones and some volatile substances to attract males for the sake of mating (Hetan et al., 2017). Hence, in monitoring the insect pest, use the pheromones which are extracted from the females is and effective method. Studies of male responsiveness to pheromone have been conducted in the field and the number of males attracted was recorded (Blanco et al., 2010; Rothschild, 1978).

Host selection and oviposition

The host selection process of phytophagous insects is regarded as a catenary process or linked sequence of behaviors involving a series of behavioral responses by the female to cues from the plant or its environment. Presence of flowers, plant height and application of soil fertilizer positively influence host plant selection. Specifically, the presence of flowers greatly increased a

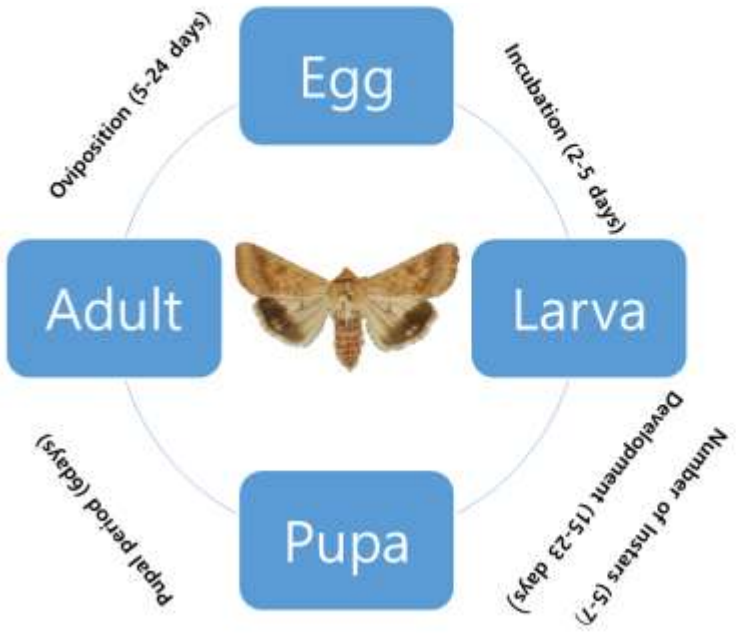

Figure 1. Life cycle of *H. armigera*.

plant's attractiveness to oviposition. (Firempong and Zalucki, 1989). The females' ability to locate and utilize wide ranging crops for laying eggs, the females ability to select an egg laying site on or close to the flowering fruiting structures of the crops and voracious feeding habit of larvae help the insect to adapt and cause severe damage to crop. Therefore, it is beneficial in the pest management strategies either to counteract or exploit by understanding all aspects of female oviposition and larval feeding behaviors.

Sensory receptors and electrophysiology

Adults and larvae of *H. armigera* perceive their environment via range of sensory receptors like olfactory and contact chemoreceptor on the antennae, ovipositor, tarsi and proboscis and mechanoreceptors on various parts of the body. Larvae of the insects also have gustatory organ located on the epipharynx which is used in distinguishing host from non host plants (Chapman, 1982; Stadler, 1984).

BOTANICALS IN THE MANAGEMENT OF *H. ARMIGERA*

Botanical insecticides are naturally occurring chemical extracted or derived from plants or minerals. Plants have their own defense mechanisms against insect attacks via repellents and even insecticidal effects. More than 2400 plant species around the world are known to posses' pest control properties. Using these plant species, about 2402 pests have been known to be controlled (Singh, 2000). In Ethiopia, about 30 plant species are recoded as pesticidal plant to control African bollworm (Tesfahun et al., 2000). Some of the promising pesticidal plants along with their known effects against *H. armigera* known in literature are shown in Table 1.

The common mode of action of plant extracts repels the insect pests, deters them from feeding and oviposition, disrupts the normal behavior and physiology of the insect and even toxic to the developmental stage and acting also as synergist in combination with other environmental friendly insecticides.

JUVENILE HORMONE AND GROWTH REGULATORY ACTIVITY

Juvenile hormone and growth regulatory properties play a great role in controlling of insect pests, especially *H. armigera*.

Dashpande et al. (1998) indicated that acetone extracts of *Catharanthus roseus* at 100, 250, and 500 ppm concentration significantly reduced the larval weight of 2nd, 3rd, and 4th instars of *H. armigera*. This affects the development of larva to adult and thus resulted in decrease of the population of the insect. The metanolic leaf extracts of *Persea indica* is shown to have negative effects against *H. armigera* larvae (Gomez et al., 1992). On the other hand, Solsoloy and Rejesus (1993) observed the juvenile hormone from *Pyschic nut*; *Jatropha curcas* effects on this insect larva in diet mediated bioassay.

Table 1. Botanical pesticides tested against *Helicoverpa armigera*.

Activity	Test material	Remarks
Antifeedant and growth inhibitors	Limonoids (citrus seeds)	Limonin, nomilin and obacunone
Ecdysis inhibitor	*Azadrachtin* (Neem)	Conc.<1ppminhibted growth rate by more than 50%
Ecdysis inhibitor	Plumbagin (*Plumbago capensis)*	ED50=150-350 ppm for 1st and 2nd instars in artificial diet bioassay
Growth inhibitors	Limonite (*Melia azadrach*)	Inhibited molting and reduced growth rate
Antifeedant	Abyssinin(*Besama abyssinica*)	Most potential anti feedant
Juvenile hormone	Petroleome ether extract (*Tribulus terrestris*)	Dose dependent response
Growth and development	*Catharanthus roseus*	Effective at 100 ppm
Toxic	*Pntaclethra macroloba* seed extracts	Reduced larval viability and growth
Growth inhibitors	*Petunia paradii*	50% growth reduction at 400 ppm
Insecticidal	*Persia indica*	Deterrence attributed activity
Pesticidal	Aqueous extracts (Syringo leaves)	Effective at 10 g/100 ml diet
Growth inhibitors	*Azadrachtin*	Decreased food utilization efficiency
Fd and Rep	Neem limonoids	Post ingested toxic effect
Larvicidal	*Azadrachtin and plumbagin*	Larval mortality in plumbagin
Growth and development	Neem leaf extracts	Toxic and morphogenic effect
Quiescent stage	Neem seed powder	NSP ED 50 =2.902%in soil
Antifeedant	Seed extract (*Trichilia havanansis)*	Highest activity with acetonic extract against 5th instar
Ovicidal and larvicidal	Neem seed kernel extract	Significant activity
Ovicidal and ovipositional deterrence	Neem seed kernel extract	Dose dependent action
Larvicidal and ovicidal	*Melia azadrach* stem extract	Methanolic extract showed activity
Growth inhibitor and antifeedant	Extract *(Melia dubia*)	Dichloroetane and methanol extract showed activity
Antifeedant and L	Aromatic oils (*Tagetes minuta)*	Antifeedal activity and mortality
Growth inhibitor and tox	Leaf extract (*Eucalyptus)*	-
Toxic	Seeds of *Cinnamomum camphora*	Cinnamomin LC50=1839 ppm
Toxic	Ergostanoids (*Petunia parodii*)	Petuniasterone steroid

Sources: Gunasekaran et al. (1985); Dashpande et al. (1988); Choi and Boo (1989); Babu et al. (1996); Koul et al. (1997); Sharma et al. (1997); Lopez-Olguin et al. (1989); Gupta and Jeyakumr (1999); Gupta (1998); Rani et al. (1999); Koul et al. (2000) and Rao et al. (2000)

Petroleum ether extract of weed *Tribulus terrestris* is also reported to affect adult emergence and larval mortality against late instars larvae of *H. armigera* (Gunasekaran et al., 1985). The crude ethanol extract of *Algea elaeagnoidea*, *Algea odorata* and *Algea roxyburghiana* actively inhibits the growth of *H. armigera* (Koul et al., 1997).

Antifeedant action

Antifeedant do not cause mortality of the insect directly, but lower their feeding potential and make them vulnerable to other mortality factors. An antifeedants are a behaviour-modifying substance that acts directly on the chemosensilla, thereby results in the feeding deterrence (Isman, 1994). There are many antifeedants from plant species against *H. armigera*.

Aqueous extracts from wild species of pigeon pea have significant antifeedant effect on *H. armigera* larvae (Shanower et al., 1997). In addition to this, acetate extracts seed of *Trichilia*

Table 2. Some recommendations of neem based pesticides for *H. armigera* management.

Neem product	Crops	Dose rate range
Formation containing Azadrachtin 0.3% (300 ppm)	Cotton	0.5-3.75 L
	Chick pea	1.2-1.5 L
	Cow pea	1.6-2 L
	Field bean	0.8-1.5 L
Formulation containing Azadrachtin 0.15% (1500 ppm)	Cotton	0.5-5 L
	Tomato	3.25 L
	Field bean	2 L
Formulation containing Azadrachtin 0.3% (3000 ppm)	Cotton	2.5 L
Formulation containing Azadrachtin 0.5% (5000 ppm)	Cotton	0.375-1 L
	Tea	0.2 L
	Tobacco	0.2 L
Fresh leaves of *A. indica*	Beans	350 g/L of water
NSKE with soap mixture	Chick pea	NSKE 50% with 1% soap

Source: Mohammed et al. (2004).

havenensis gave antifeedant activity at 5000 ppm against 5th instars of the insect larvae. The mixture of havenensin-1,7-diacetate and havenesin-3,7-diacetate compounds fractionated by chromatography and showed the maximum antifeedant activity at 1000 ppm under choice and non choice feeding assays (Lopez et al., 1998).

Neem has diverse mechanism of biological effects on the insects, while antifeedant and growth retardant effects are the major important effects (Mohammed et al., 2004). Lulie and Raja (2012) obtained significantly lower pod damage using neem seed extracts in chickpea when compared with untreated plots. Some recommendations of neem based pesticides for *H. armigera* are listed in Table 2.

Apart from the extracts, essential oil owing volatile nature has also been evaluated for antifeedanty. Rao et al. (2000) reported that oil of *Tagetes minuta* gave the highest antifeedant activity (86.3) at 0.5% concentration on the second instars *H. armigera* larvae on cotton leaves.

Larvicidal action

Most of the studies on biological activity of plants have shown that early instars are the most susceptible stage to larvicidal activity of botanicals (Choi and Boo, 1989). This suggests the sensitivity of early instars larvae as target stages of pest for their possible application in pest management.

The methanolic extract of *Malia azadrach* stems at 7.5% concentration was observed to be larvicidal and ovicidal action against *H. armigera* (Rani et al., 1999). Sundararajan (2001) also evaluated the effect of leaf methanolic extract of *Alstonia venenata*, *Ailanthus excels*, *Abutilan indicum,* and *Azima tetrachantha* under laboratory conditions against *H. armigera* on tomato. *A. venenata* and *A. tetrachantha* gave up to 73 and 51% larval mortality, respectively. Neem product of RD-9 Replin (1 and 2%), Neemark (0.5 and 0.75%) and Neemarch 20EC (0.1 and 0.15%) have been tested against young larvae of the insect pest in cotton and resulted in mortality levels of 70, 70, and 66.7%, respectively (Dhawan and Simwat, 1995). Ankita and Sangeet (2017) tested *Nigella sativa* extract and it performed well against *H. armigera* causing 72.99% mortality.

Manoharan and Uthamasany (1993) found that addition of *Azadrachta indica* oil to endosulfan and phosalone increased the mortality of the insect larvae by 16.7 and 25% as compared with insecticides alone. Babu et al. (2000) and Singh et al. (2013) reported that the synergistic effect of methanolic neem seed kernel extract is very effective in larval mortality and also delayed the metamorphosis of the pest.

SEMIOCHEMICALS

The term semiochemicals was proposed by Law and Regnier (1971), for chemicals converting signals between organisms. The chemicals under semiochemicals influence and regulate insect behavior and physiology.

Dethier et al. (1960) classified the semiochemicals on the basis of the chemical stimuli and response as arrestants, locomatory stimulants, attractants, repellants, feeding, mating, ovipositional stimulants or deterrents.

There are two major groups of semiochemicals, the pheromone and allelochemicals. Pheromone affects intraspecifically the behavior and physiology of another individual of the same species, while the allelochemicals act interspecifically between members of different species.

Pheromones

Monitoring the population of a highly mobile and polyphagous pest like *H. armigera* requires holistic approach to the component of cropping system. Pheromones have the potential advantage of low mammalian toxicity, no development of pesticidal resistance and inexpensive method of control unlike that of conventional pesticides. Pheromones technology for *H. armigera* assists in pest population monitoring as well as in other methods including mass trapping, enhancing biocontrol impact, pesticidal resistance, monitoring and mating disruption (Mohammed et al., 2004). Z-11-hexadecan-1-01 and Z-9- hexadecane-1-01 resulted in two to three-fold reductions in pesticides application (Zebtiz, 1997).

Pheromone dispensers are made up of rubber and cork septa found to catch more males than cigarette and filter pepper when loaded at a rate of 1 mg per septa than with lower concentrations (Krishna et al., 1998). The efficacy of funnel, sleeve and sticky traps was tested in the fields of Tikamgarh, in India (Rai et al., 2000).

Pest monitoring by pheromone

Sex pheromone traps have been widely used for decision making, intervention, using insecticides. Most of the research on the use of pheromone technology for trapping of *H. armigera* has so far been in China, India, Israel, New Zealand and Russia (Dunkelblum et al., 1980; Kehat and Dunkulbum, 1993; Parasad et al., 1993; Natarajan et al., 2002). Use of pheromones for monitoring *H. armigera* in tomato and maize field in Newzealand result in reduced crop in inspection time and more accurate timely insecticide application (Walker and Camegon, 1999). Short term forecast for 5 days could be worked out based on moth catches of *H. armigera* in Azerbaidzhan, USSR and time release of *Tricogramma* species against the pest. A total of 30 to 40 males of *H. armigera* trapped in 3 days were found to be reliable indicator of exceeding threshold level of 3 to 5 larvae/100 cotton plants in Tadzhik, USSR indicating the need for intervention measure (Grechanova, 1986). In addition to this, Reddy and Manjunatha (2000) suggested that integrated pest management should be initiated against *H. armigera* when 7 moths per trap per night are observed. In Ethiopia, on station monitoring of adults *H. armigera* as a key pest of cotton has been undertaken by pheromone trap at the cotton research center in Melka werer. The pheromone trap catch for 3 years indicated that higher *Helicoverpa* catches were observed in August and September and these catches were consistent with the field count of *H. armigera* eggs and larvae (Mohammed et al., 2004) (Figure 2).

ENHANCING OF BIOLOGICAL IMPACTS

Pheromone trapping for insect biological control agent is a valuable tool that can help to determine and improve parasitoids purpose. Sex pheromone of *H. armigera* was found to attract the eggs parasitoids, *Trichogramma chilonis.* Hosny (1988) reported that number of predators under pheromone treated cotton field is three times than that treated by insecticide under the same crop (Table 3).

Kairomones

These are natural chemicals present in plants or animal hosts that direct the insect pest towards the suitable site for feeding. Kairomones can be used mainly for two purposes: controlling the insect pests and for enhancing the performance of beneficial insects. As phytophagous insects use kairomone to locate and recognize host plants for feeding and oviposition, they can be used directly to disrupt the location and recognition of host plants, for mass trapping and indirectly for forecasting and monitoring pests (Singh, 2008). Additionally, the use of kairomone manipulates and enhances interaction of beneficial insects with their hosts or prey (Tamoghna and Nithya, 2017). The great potential utility at this time appears to be for aggregating natural parasitoids and predators in the targeted location.

Studies of parasitism rates of *H. armigera* eggs by *T. chilonis* in Petri dishes in response to different treatments of *H. armigera* by kairomones were made by Lewis et al. (1975). The results indicated that kairomonal compounds from *H. armigera* moth scales increased parasitization when applied over target sites. An analysis of *H. armigera* and *Corcyra cephalonica* moth scales for possible kairomonal substances using gas chromatography indicated the presence of hexatriacontane, nonacosane, docosane, pentacosane and heptadecane. The result strongly indicates the role of moth scale extracts in enhancing the parasitization rate of *T. chilonis* on *H. armigera* eggs. Laboratory observations on parasitism rates by *T. chilonis* in response to scale extract treatments reveal the importance of kairomones.

Allomones

These pheromones released by individuals of one species and influence the behavior of other species in

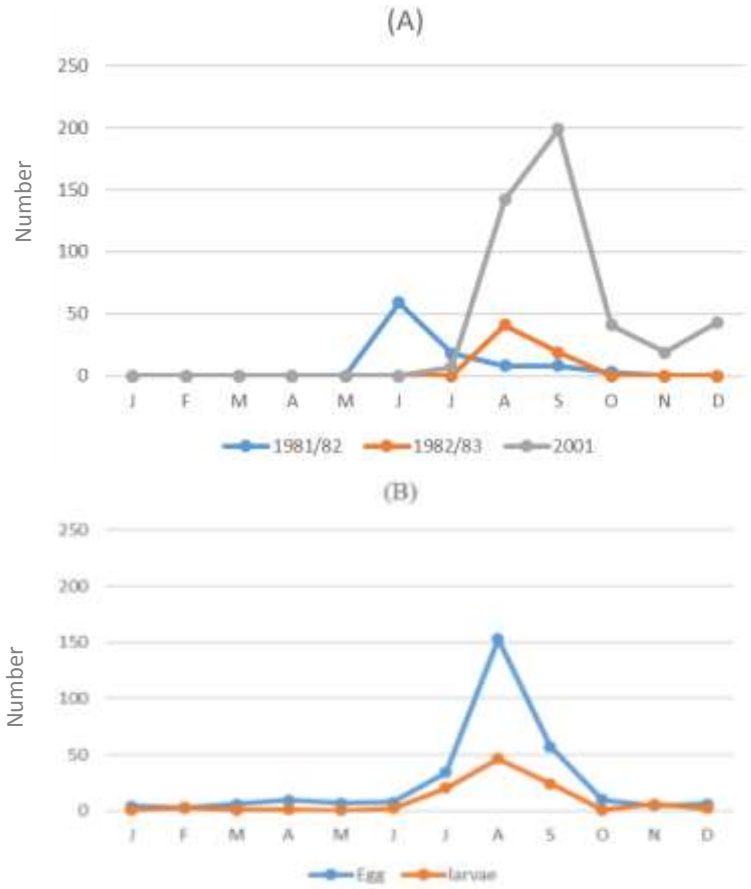

Figure 2. Monthly total catches of *Helicoverpa armigera* moths, egg and larvae Melka Wera, Ethiopia. (A) Catches of moths using pheromone traps. (B) Average number eggs and larvae (based on 400 cotton plants sampled). These figures illustrate that the peak moth catch during 1981/1982 was in June month, whereas during 1982/1983 and 2001 in August. The peak observed by pheromone trap of moths and eggs and larvae sampled from cotton plants shows that the pheromone trap is effective in monitoring *H. armigera.*

Table 3. Number of predator insects per hectare sampled by D-vac suction in cotton fields treated with pheromone compared with insecticide treated fields, Egypt.

Natural enemy	Mean number per plot	
	Insecticide treated	Pheromone treated
Coccinellid adults	33	122
Peaderus adults	17	322
Scymnus adults	33	55
Chysoperla adults	100	689
Chysoperla larvae	17	67
Orius adults	550	1145
Total	749	2400

Source: Hosny (1988).

manner of favorable to emitter, e.g. defense secretions (Torbjörn, 2001; Tamoghna and Nithya, 2017).

Allomones offer great potential as oviposition and feeding disruption and important to host plants as key mechanism of defense against phytophagous insects like *H. armigera* by employing the allomones in various ways for improving crop breeding program (Pickett, 1988) (Figure 2).

CONCLUSION AND RECOMMENDATIONS

To effectively manage any insect pest and prevent their further expansion, there is a need to study the detailed biological and ecological aspects of the insect populations. Before application of any control measure, complementary investigation of morphological, ecological, and biological aspects of the insect are desirable. Follow-up studies should be carried out on the distribution pattern and preferred host plants. Therefore, semiochemicals are an essential tool for monitoring and detecting the insect. There is seldom published information on sex pheromone composition against *H. armigera*. Therefore, effectiveness needs to be verified with different ratio combinations to increase the efficacy of sex pheromones.

Different plant species that have insecticidal, antifeedant, repellent, and ovicidal effects have been evaluated against *H. armigera*. Therefore, from the base of their effectiveness and environmental friendly, more plant species need to be evaluated. In addition, the mechanism by which they cause mortality requires study to enable commercialization. Plant-insect interaction is an under researched area for this insect pests.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Degradation assessment of wetlands under different uses: implications on soil quality and productivity

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The assessment of degradation status of wetland soils under five different land use types (LUTs) in Ogun state, Nigeria were studied. The laboratory study was conducted to determine the physical, chemical and biological properties of these wetlands and the results obtained were compared with the food and agriculture organization (FAO) standard indicators and criteria for land degradation assessment. The textural composition of the soil ranged from sandy loam to sandy clay loam. Total porosity was generally low with the mean value of 40.5%. The pH ranged from moderately acidic to slightly alkaline with fallow soil having the highest value. Available phosphorus was low across the LUTs. Total nitrogen was predominantly low in most of the cultivated soils to moderate in the fallow soils. Cation exchange capacity (CEC) was low, while the exchangeable sodium percentage (ESP) was high (>5) in all the LUTs. The organic matter ranged from low to moderate indicating low nutrients status of the soil. The soils were classified and placed in the order Alfisols. The degradation results showed that most of the cultivated wetlands were highly degraded compared to the reference (fallow) soils which were slightly degraded. It is opined that soil conservation practices like the use of inorganic fertilizers, organic manure, and composts should be intensified in these fragile low fertile wetlands. Also, there should be a periodic monitoring of the fertility status of the wetlands from the time it is first open for cultivation to subsequent uses.

Key words: Conservation, deforestation, environment, soil management, vegetation.

INTRODUCTION

Globally, soil degradation is one of the greatest challenges facing the developing countries in the tropics and sub-tropics (IFAD, 2010). Its extent and effect on agriculture and the larger environment is more severe now than ever before and cannot be ignored because most soils cannot continue to support crops production and yield maximally (Lal, 2015).

Some estimates provide that between 1950 and 2010 degradation decreased soil ecosystem services by 60% (Leon and Osorio, 2014). Soil degradation always leads to political and social instability due to its enormous impact. It is associated with increased deforestation rate,

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intensive and continuous use of marginal and fragile soil, accelerated runoff and erosion, natural waters pollution, and green house gases emission (Adaikwu et al., 2012).

Soil degradation is defined to be the physical and chemical deterioration of soil or reduction in soil quality which has pronounced implication on agricultural productivity by not able to support plant and animal growth optimally due decline in the levels of available moisture, available nutrients and biological activity (Ernst, 1995). It can also mean a reduction of the biological and economic productivity potentials of rain-fed cropland, irrigated cropland or range, pasture and forested land by one or a combination of processes such as displacement of soil materials by wind and water erosion, deterioration of soil physical and chemical properties as a result of long-term loss of natural vegetations due to misuse and lack of proper management (Amalu, 1998; Lal, 2009).

Agriculture and wetlands are closely linked together, and this relation between agriculture and wetlands has developed against wetlands up to date (Gopal, 2000). The conversion of wetlands into agricultural land and intensive agricultural activities around them has caused the degradation and destruction of the wetlands. Presently, productivity levels of soils have remained stagnant and even dropped despite the introduction of new crop varieties and germplasms, and an increase in quantity of agrochemicals application. This situation has been attributed largely to declining soil fertility (Ogbodo et al., 2012).

There is therefore need to have a comprehensive and useful information on the influence of different land uses on wetland degradation and agricultural productivity in Ogun State, Nigeria. Information on the extent to which the wetland has been misused overtime in this area is also required. The aim of this study is to assess the degree of degradation of selected wetlands under different uses in the study area using the food and agriculture organization (FAO) standard indicators and criteria for soil degradation assessment with a view to making modest and practical recommendations on the rehabilitation and proper management of degraded wetland soils.

MATERIALS AND METHODS

Description of the study area

The study area is the Odeda Local Government Area, Ogun State located at point Latitudes 07°10'N and 07°30'N and between Longitudes 03°15'E and 03°50'E. It shares boundaries with Oyo State to the north and east, Abeokuta South and North Local Government Areas to the west, and Obafemi-Owode Local Government Area to the south (Figure 1). The area has bimodal rainfall patterns, with peaks between June to July and September to October. This is followed by a short period of dry season that is usually between November and February. It has an annual rainfall of about 1113 mm and it is located in the guinea and derived savanna belt. The mean relative humidity of the area is high (above 70%) with the peak between May and October and the annual mean temperature is 27°C. The major land use types (LUTs) in the study area were arable crop, cash crop production and non-agricultural uses (such as residential, industrial, and roads construction). The wetlands have been ploughed at different intervals over times, and also agrochemicals both pesticides and fertilizers have been applied to the soil.

Field work

Five land use types (LUTs) which are rice (LUT 1), yam (LUT 2), oil palm (LUT 3), built-up sites (LUT 4) and fallow land (LUT 5) were evaluated and within each of the chosen LUTs, an area of 3 ha was demarcated, and bulk samples consisting of ten surface (0 to 15 cm) and ten subsurface (15 to 30 cm) samples were randomly collected and place in a properly labeled bags for physical, chemical and biological analyses. Core samples were also collected with the use of core samplers for bulk density determination. The general site description was described after the FAO guidelines for site and profile descriptions (FAO, 2006). Attributes like the climate, vegetation, land use, slope, drainage type, soil surface form, micro relief and depths to ground water table (GWT) were described and recorded. The soils classification was according to Soil Survey Staff (2014). The land use histories of the study area were obtained through field observations and interviews/interactions with the local farmers.

Laboratory methods

After the soil samples were air-dried for days, they were crushed and sieved using a 2 mm screens. The samples were then analyzed for the following parameters: Particle size analysis was done through hydrometer method (Bouyoucos, 1951), saturated hydraulic conductivity (Ksat) was determined using a constant head method and bulk density by core method. The soil porosity was estimated from the bulk density data at an assumed particle density of 2.65 g/cm^3. Soil pH in water (1:1) using glass electrodes pH meter (Mclean, 1965). Total nitrogen was determined by the macrokjeldahl digestion method of Jackson (1962), available P was after (Bray and Kurtz, 1945) extraction using Bray-I extract followed by molybdenum blue colorimetry. Exchangeable cations were extracted with 1M NH_4OAC (pH 7.0), K and Na were determined using flame photometer while Ca and Mg were by atomic absorption spectrophotometer (Sparks, 1996). Organic carbon was after dichromate wet oxidation method (Walkley and Black, 1934), and the organic matter content was got by multiplying a factor of percent organic carbon by 1.724. Cation exchange capacity (CEC) was determined by neutral, 1N Ammonium acetate method. Base saturation was computed by dividing the sum of exchangeable bases by CEC and multiplying by 100, while Exchangeable Sodium Percentage (ESP) was calculated by dividing the exchangeable sodium by the CEC.

Soil degradation assessment

The degradation status of the wetlands across different land use types (LUTs) was assessed by field observation and the standard indicators, and criteria for land degradation assessment (Table 1) by FAO (1979) using the obtained laboratory results. The four degrees of degradation level identified includes:

1. Slightly degraded soil, where productivity is between 75 – 100%.
2. Moderately degraded soil, where productivity is between 50 – 75%.
3. Highly degraded soil, where productivity is between 25 – 50%.
4. Very highly degraded, where productivity is between 0 – 25%.

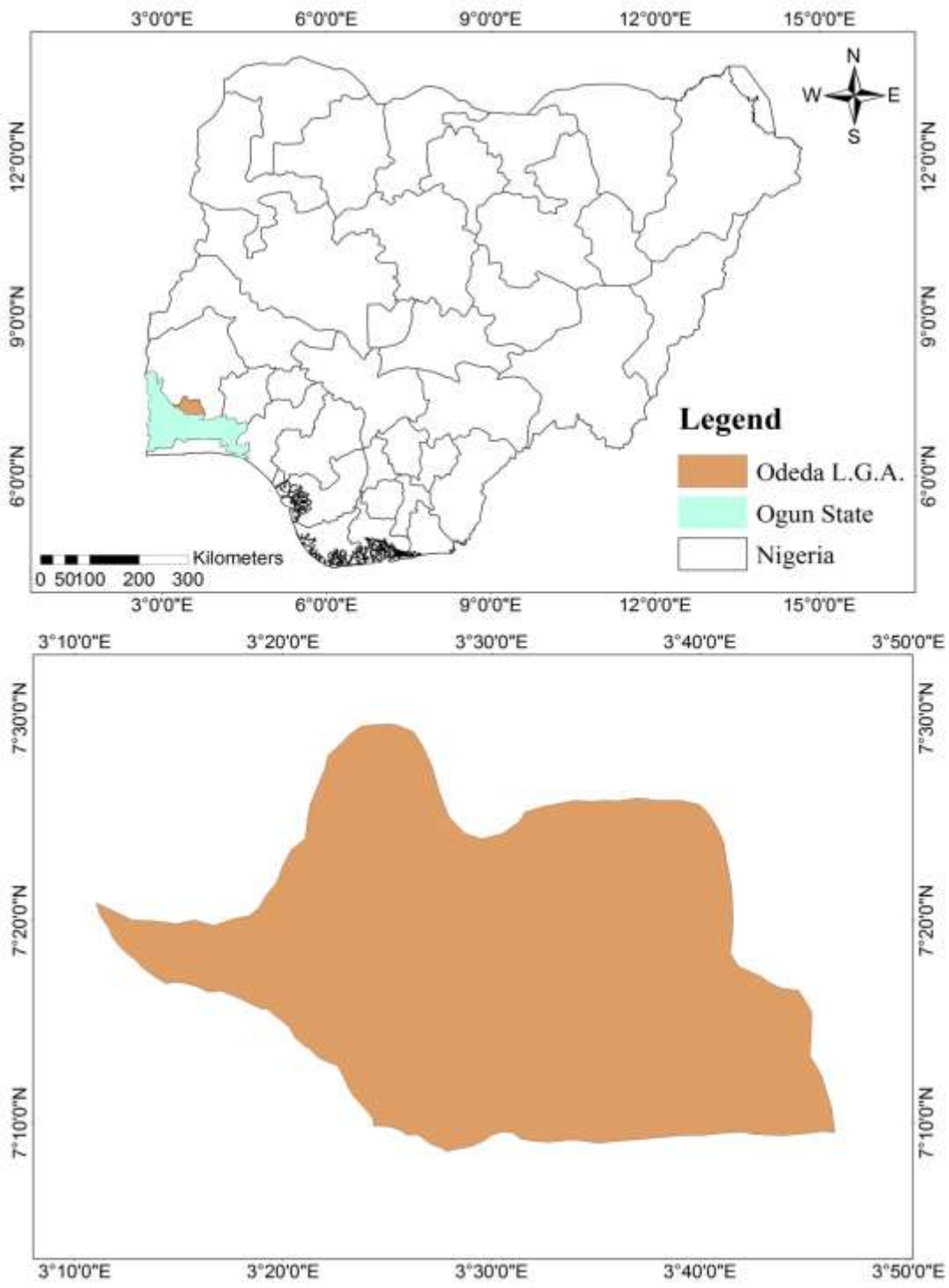

Figure 1. Map of Ogun State Nigeria showing the study area.

Statistical methods

The results were subjected to analysis of variance (ANOVA) to know the effect of different land use types on soil properties/quality and the means were separated with the New Duncan Multiple Range Test (DMRT) at $p<0.05$ (Table 1).

RESULTS

Soil physical properties

The physical properties of the top and sub soils of the

Table 1. Indicators and criteria for land degradation assessment.

Indicators	Degree of degradation			
	1	2	3	4
Soil bulk density (g/cm^3)	<1.5	1.5-2.5	2.5-5	>5
Hydraulic conductivity (cm/hr)	<0.13	0.13-0.15	0.15-2.0	2.0-6.3
Content of nitrogen (%)	>0.13	0.10-0.13	0.08-0.10	<0.08
Content of Phosphorus (mgkg^{-1})	>8	7-8	6-7	<6
Content of potassium (cmolkg^{-1})	>0.16	0.14-0.16	0.12-0.14	<0.12
Content of ESP (Increase by % of CEC)	<10	10-25	25-50	>50
Content of base saturation (%)	<2.5	2.5-5	5-10	>10
Content of organic matter (%)	>2.5	2.0-2.5	1.0-2.0	<1.0

Source: FAO (1979).
Key: Class 1 = Slightly Degraded, Class 2 = Moderately Degraded, Class 3 = Highly Degraded, Class 4 = Very Highly Degraded.

Table 2. Soil physical properties in the study area.

Soil properties	Depth (cm)	LUT 1	LUT 2	LUT 3	LUT 4	LUT 5
Sand (g/kg)	0-15	554	514	604	584	564
	15-30	544	504	584	554	544
Silt (g/kg)	0-15	204	314	204	114	244
	15-30	224	294	194	164	234
Clay (g/kg)	0-15	242	172	192	302	192
	15-30	232	202	222	282	222
BD (g/cm^3)	0-15	1.68	1.57	1.46	1.72	1.44
	15-30	1.64	1.59	1.51	1.70	1.47
Porosity (%)	0-15	36.6	40.8	44.9	35.1	45.7
	15-30	38.1	40.0	43.0	35.8	44.5
Ksat. (cm/hr)	0-15	3.37	1.60	1.12	3.47	0.94
	15-30	2.95	1.74	1.26	3.31	1.14
Textural Class	0-15	SCL	L	SL	SCL	SL
	15-30	SCL	L	SCL	SCL	SCL

BD = Bulk Density; Ksat. = Saturated Hydraulic Conductivity; SCL = Sandy clay loam, SL = Sandy loam, L = Loam.

different land use types were showed in Table 2. The particle size distribution result indicates different textural composition of the wetlands. The textural classes are the intrinsic soil properties that are sufficiently permanent and are often used to characterize the physical make up of soils (Hillel, 1980). The highest mean for clay content (302 g/kg) was recorded in built up land and was significantly different ($p<0.05$) from others, while the oil palm has highest sand content (604 g/kg). The sand content dominates the textural class, and this might be due to erosion from the upland into the wetland and turning of the soil overtime. The lowest bulk density (1.44 g/cm^3) was recorded on the fallow soil followed by 1.46 g/cm^3 in oil palm. The high soil bulk density in cultivated soils is due to intensive agricultural practices, low organic matter content and compaction of top soil as a result of overgrazing (Lal, 1986; Ceyhun, 2009). Soil bulk density increase depicts an increasing loss of soil binder materials, reduced soil biological activity, especially earthworms and plant roots, and due to the land use

Table 3. Soil chemical and biological properties in the study area.

Soil properties	Depth (cm)	LUT 1	LUT 2	LUT 3	LUT 4	LUT 5
pH (H_2O)	0-15	6.1	6.2	6.3	5.7	7.4
	15-30	6.0	5.8	6.1	5.6	7.2
Total N (%)	0-15	0.13	0.11	0.17	0.09	0.25
	15-30	0.11	0.09	0.14	0.08	0.21
Av. P (mg/kg)	0-15	4.87	4.76	5.61	4.59	5.85
	15-30	4.61	4.64	5.53	4.52	5.72
K (cmol/kg)	0-15	0.30	0.32	0.44	0.22	0.61
	15-30	0.28	0.34	0.30	0.21	0.53
Na (cmol/kg)	0-15	0.58	0.50	0.54	0.80	0.49
	15-30	0.57	0.52	0.51	0.69	0.48
Ca (cmol/kg)	0-15	3.31	3.28	3.53	2.72	3.63
	15-30	3.14	3.08	3.40	2.45	3.56
Mg (cmol/kg)	0-15	1.34	1.29	1.37	2.04	1.26
	15-30	1.23	1.20	1.22	1.97	1.17
CEC (cmol/kg)	0-15	6.52	6.61	6.85	6.27	7.11
	15-30	6.33	5.99	6.52	6.05	7.24
BS (%)	0-15	84.8	81.5	85.8	92.2	84.2
	15-30	82.5	85.8	83.3	87.9	84.7
ESP	0-15	10.49	9.28	9.18	13.84	8.18
	15-30	10.92	10.12	9.39	12.99	8.36
OC (%)	0-15	1.13	1.07	1.42	0.86	1.98
	15-30	0.93	0.84	1.29	0.71	1.65
OM (%)	0-15	1.95	1.84	2.45	1.48	3.41
	15-30	1.60	1.45	2.22	1.22	2.84

change and significant reduction of clay and silt and instead of increasing the amount of sand in the soil texture (Gholami et al., 2014). Saturated hydraulic conductivity result correlates with that of the bulk density. The effect of land use types on total porosity (Table 2) showed significant difference ($p<0.05$). Total porosity was low with mean value of 40.5%, highest in fallow soil (45.7%) and lowest in built up soil (35.1%). The low value in built up soil is attributed to the high bulk density. The results of the study agrees with the findings of Senjobi and Ogunkunle (2011) and Adaikwu et al. (2012) who affirmed that the use to which a land is put influences the soil physical quality indicators which are used for soil degradation assessment (Table 2).

Soil chemical and biological properties

The chemical and biological properties of the soils across the land use types were presented in Table 3. The laboratory analyses showed that pH varies from moderately acid (5.6) to slightly alkaline (7.4), and are significantly different at $p<0.05$. Decrease in soil pH from the cultivated land might be due to exhaustion of basic cations or higher microbial oxidation that creates organic acids causing soil pH reduction (Chauhan *et al.,* 2014). The total nitrogen content also varies from low (0.08%) to moderate (0.25%) with the fallow having the higher content than the rest LUTs. The trend is a pointer to nutrient loss in the farms due to continuous cultivation as

Table 4. Soil quality indicators degradation assessment.

Land use types	Depth (cm)	Physical		Chemical					Biological
		BD (g/cm3)	Ksat. (cm/hr)	N (%)	P (mg/kg)	K (cmol/kg)	BS (%)	ESP	OM (%)
LUT 1	0-15	MD	VHD	MD	VHD	SD	VHD	VHD	MD
	15-30	MD	VHD	MD	VHD	SD	VHD	VHD	HD
LUT 2	0-15	MD	HD	MD	VHD	SD	VHD	HD	HD
	15-30	MD	HD	HD	VHD	SD	VHD	VHD	HD
LUT 3	0-15	SD	HD	SD	VHD	SD	VHD	HD	MD
	15-30	MD	HD	SD	VHD	SD	VHD	HD	MD
LUT 4	0-15	MD	VHD	HD	VHD	SD	VHD	VHD	HD
	15-30	MD	VHD	HD	VHD	SD	VHD	VHD	HD
LUT 5	0-15	SD	HD	SD	VHD	SD	VHD	HD	SD
	15-30	SD	HD	SD	VHD	SD	VHD	HD	SD

well as nutrient loss during harvesting. Asongwe et al. (2016) affirmed that nitrogen is highly mobile and easily lost in wetlands especially those that are dominated by sand fraction.

Available phosphorus was generally low in all the LUTs been less than 15 mg/kg despite application of inorganic fertilizer (NPK) in the cultivated soils. The fallow soil had the highest value but result was not significantly different ($p<0.05$) across the LUTs. The exchangeable potassium (K) and calcium (Ca) were low in all the LUTs, but were higher in oil palm and in fallow soils. This could be due to the level of soil organic matter (SOM) and nutrient recycling respectively. Sodium (Na) and Magnesium (Mg) contents were also low across the LUTs except for built up soil which has higher values. This indicated the decrease in the Ca content and increased content of Mg. The exchangeable bases were significantly different at $p<0.05$ in all the LUTs.

The CEC of the soil was generally low but higher under fallow compared to other LUTs. The CEC increased in the sub soil under fallow which indicates better nutrient recycling. The CEC values obtained depends on the pH and SOM contents. The base saturation values obtained were high in all the LUTs and were not significant different from one another. The exchangeable sodium percentage (ESP) values were high been greater than 5%, and the results were differs significantly ($p<0.05$) across the LUTs. The implication of this is decline in Ca which can creates a collapse in soil structure and decrease in permeability (CUCE, 2007; Hazelton and Murphy, 2007).

The organic matter (OM) content of the soil ranged from low to moderate (1.22 to 3.41%). The OM decreases with depth in all the LUTs. The result corroborates the findings of Bhunia et al. (2016). Increased long-term cultivation significantly ($p>0.05$) decreased soil organic matter content in the cultivated soils and this has crucial implication on soil physical and chemical properties. The organic matter content of the soils was significantly higher in fallow soil (3.41%) than the cultivated soils. The low organic matter obtained may be partly due the effect of high temperature and relative humidity in the area which haste rapid mineralization of organic matter (Table 3).

Soil degradation assessment

The soil parameters used for the physical degradation assessment of the soils of the LUTs studied indicated that the soils were predominantly moderately degraded (MD) with respect to BD in all the LUTs (Table 4), except for surface (0 to 15 cm) soil of oil palm and fallow soil which were slightly degraded (SD), FAO (1979). The BD of soil is greatly influenced by the OM content. The correlation between BD, clay and OM was significant. This connotes that the lower BD in the cultivated soils compared with the fallow were indications of lower clay content and OM in the former. The continuous cultivation of the soils can modify the soil BD and the pore size distribution since the operation loosens, granulates and crushes the soil particles. On the other hand, the saturated hydraulic conductivity (Ksat.) rating showed that the soils were highly degraded (HD) in LUT 2, LUT 3 and LUT 5, while LUT 1 and LUT 4 were very highly degraded (VHD). The difference in the result could be from differences in the BD of each LUT soil.

The soils chemical degradation of the studied area revealed different degrees of degradation with respect to the parameters assessed. For instance, with respect to nitrogen content, the degradation status of the soils ranged from SD to HD, FAO (1979). The soils under oil

palm and fallow conditions were SD, while the soils under rice and yam (surface) were MD. The yam subsoil as well as the built up were all HD with respect to N content.

Nitrogen is a key nutrient which was used as a good soil quality indicator and listed as the one of the most important of all the 16 essential plant nutrient elements needed for plant growth, development and reproduction and also the most easily limiting or deficient throughout the world particularly in the tropics (Agbede, 2009). Available phosphorus was VHD in all the studied LUTs despite fertilizers use in the cultivated soils. The degradation degree with respect to potassium showed that all the LUTs were SD. The fallow soil recorded high percentage when compared with other LUTs. The degradation rating of base saturation (BS) showed that the soils were VHD. The soils were HD to VHD with respect to exchangeable sodium percentage (ESP). The soils were HD at the depth of 0 – 15 cm in LUT 2, but HD at both depths in LUT 3 and LUT 5, while the rest LUTs were VHD.

The biological degradation of the surface and subsurface soils ranged from SD to HD with respect to OM content (Table 4) FAO, (1979). The fallow soil was SD, the rice and oil palm soils were MD. The yam and built up soils were HD. This is an indication of very high biological degradation, which is typical of tropical soils. It can also be as a result of top soil removal during clearing for building construction and agricultural purposes. The OM depletion may rise from crop uptake exacerbated by continuous cropping of the wetlands without adequate measures of nutrient replacement either through the use of inorganic fertilizer or other forms of soil conservation measures. Harpstead (1973) reported the low OM content is a phenomenon associated with the tropical soils due to high temperatures that rapidly breakdown OM and inhibit nitrogen fixation by rhizo-bacteria (Table 4).

Conclusion

An investigation study was conducted to assess the degree of degradation of the wetlands in selected land use types of Odeda Local Government Area of Ogun State, Nigeria. The main objective of the study was to assess the degradation degree of the soils in the study area, using the standard indicators and criteria for land degradation assessment of FAO (1979). The results of the study showed the different textural composition of the wetlands. The soils were low in terms of major soil nutrients. The soils were classified as Alfisols according to the provisions of Soil Survey Staff (2014) on the basis of the physical, chemical and biological properties of the soils. The result of soil degradation assessment in these wetlands ranged from SD to VHD soils. On a comparative basis, most of the soils that were under cultivation (LUT 1 – LUT 4) showed higher degree of degradation compared to the fallow soil (LUT 5). The study revealed that 22.5% of the soils were slightly degraded, 17.5% were moderately degraded, and 23.8% were highly degraded while 36.2% were very highly degraded. Thus, this in turn affects the productivity of the soils negatively thereby leading to food insecurity. Base on the findings of this study, it is recommended that application of mineral fertilizer nutrients especially nitrogen and phosphorus is necessary, the use of organic manure such as cow dung and poultry dropping should be adopted to improve the productivity of these degraded soils. Farmers should be encouraged to leave crop residues on their farms and incorporate same during tillage rather than burning them. Also, monitoring the fertility status of the wetlands at regular intervals is very paramount.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Natural occurrence of *Diadiplosis megalamellae* (Barnes) in mealybugs on roses in Kenya

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Over the last decade there has been an increasing adoption of Integrated Pest Management on rose farms in Kenya. As a consequence, there has been a rise in secondary pests on rose plants, including in particular the citrus mealybug *Planococcus citri* (Risso). On cut flowerrose farms in Kenya, the presence of the predatory midge *Diadiplosis megalamellae* (Barnes) (Diptera: Cecidomyiidae) was observed. Therefore, a survey was carried out to quantify the occurrence of *D. megalamellae* and the association with mealybug infestations in commercial cut flower rose crops in Kenya. Four farms in four different regions of Kenya and eight rose varieties were surveyed. The midge *D. megalamellae* was present on farms located in Naivasha, Nairobi and Thika, but was absent in Nanyuki region. The midge *D. megalamellae* was found mainly in *P. citri* mealybug colonies and, although in much lower numbers, in the long tailed mealybug *Pseudococcus longispinus* (Targioni Tozzetti) colonies. The number of mealybugs was positively correlated with the number of number of *D. megalamellae* larvae suggesting increased multiplication of the *D. megalamellae* when the pest is present in larger numbers. The number of mealybugs increased with an increase in altitude at which a rose farm was located but there were no *D. megalamellae* present at the high altitude farm. The reasons for differences in mealybug population between farms is discussed along with further work needed, however, as an indigenous Kenyan predator, this midge offers potential for mealybug biocontrol on rose farms in Kenya.

Key words: Pseudococcidae, Cecidomyiidae, biological control, predatory midge.

INTRODUCTION

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera:Pseudococcidae), is a highly polyphagous pest that was initially associated with citrus. By now their host range has been reported to include at least 27 different plant families, including economically important indoor ornamentals, vegetables, and fruits (Tingle and Copland, 1988; Gill et al., 2013). This pest increasingly occurs in greenhouse ornamentals and is becoming a prominent problem in cut flower rose crops (Messelink, 2014). With the increasing adoption of Integrated Pest Management (IPM) and the reduced application of broad-spectrum pesticides, *P. citri* has become a major pest in rose crops in Kenya. Plants infested by *P. citri* exhibit yellow, distorted, and wilted leaves, premature leaf drop and stunted growth (Hill, 2008). This deformation leads to reduced photosynthesis and thereby to reduced yield.

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Roses are intensively grown and the high nutrient dosages supplied, especially high nitrogen, are known to increase mealybug pressure in a variety of species (Hogendorp et al., 2006). Female mealybugs are wingless, unlike males, and can produce five or more generations per year (Hill, 2008). Eggs of *P. citri* are laid in female ovisacs that can contain 300 eggs at a temperature of 18°C (Copland et al., 1985). Depending mostly on temperature, mealybugs have three nymphal stages lasting 16 to 38 days to develop to adults. Mealybugs are covered with white waxy particles and secrete honeydew. This is the main reason for the low efficiency of pesticides as they are unable to penetrate through the waxy layers (Al-Ali, 1969).

The importance of biological control of mealybugs has long been recognized and there has been strong interest in biological control solutions in the horticultural industry in Kenya for over a decade (Wainwright and Labuschagne, 2009). In Europe, three biocontrol agents are used against mealybugs, namely the ladybug *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae), the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and the endoparasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). Both *C. montrouzieri* larvae and adults feed on mealybugs. *C. montrouzieri* larvae are covered with white wax threads, causing them to resemble mealybugs. The adults can fly and find new mealybug colonies, while larvae find their prey by physical contact. In Kenya, *C. montrouzieri* was introduced in 1924 and has established (Booth and Pope, 1986). Despite numerous commercial trials in ornamentals in recent years *C. montrouzieri* is not used as a biological control agent in Kenya. This is mainly due to the high production costs and because it cannot be used as a preventive measure, as adult *C. montrouzieri* will disperse when mealybug population is low. The adult lacewing *C. carnea* is not predacious, but the larvae feed ferociously on many pests. In Europe, America and Asia, it is a common predator of aphids (Hemiptera: Aphididae) and can be used to control mealybugs (Rashid et al., 2012). The lacewing *C. carnea* is not indigenous to Kenya, and at the moment it is not commercially available for mealybug biological control in Kenya. The endoparasitoid *A. pseudococci* is a specialist parasitoid of *Planococcus* and *Pseudococcus* species mealybugs. The parasitoid lays one egg in a mealybug, the hatched larvae will feed on the mealybug whilst developing. The parasitoid *A. pseudococci* is indigenous in Kenya (Tanga et al., 2015), but is not commercially available in Kenya. However, since it is indigenous and used as a biocontrol agent in other parts of the world, it has the potential to be exploited as a suitable biocontrol agent in Kenya.

In addition, the authors recently found the predatory midge *Diadiplosis megalamellae* (Barnes) (Diptera: Cecidomyiidae) feeding on *P. citri* and on *Pseudococcus longispinus* (Hemiptera: Pseudococcidae) in Kenya. Cecidomyiids are commonly known as gall midges because the larvae of many species feed within plant tissues inducing the formation of noticeable galls. Moreover, the family also includes several less-noticeable genera such as *Diadiplosis,* whose larvae are predators of organisms such as scale insects (Hemiptera: Coccoidea) (Gagné, 1994). In a recent study Hayon et al. (2016) collected five gall midge species using mealybug sentinel traps in Israel: *Diadiplosis donaldi* (Harris, 1968), *Diadiplosis multifila* (Felt), *Dicrodiplosis manihoti* (Harris), *Lestodiplosis* species, and *Trisopsis tyroglyphi* (Barnes). Two species in the Cecidomyiidae family that are currently commercialized as biocontrol agents are *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) and *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae), which are used against aphids and tetranychid mites, respectively (Harris, 2004). The midge *D. megalamellae* is an indigenous species, only collected in tropical Africa (East Africa, Zaire, and West Africa) and preys on both *Planococcus* and *Pseudococcus* mealybug species (Harris, 2004). At the moment, little information is available on the predatory midge *D. megalamellae* (Barnes) occurrence in Kenya or its ability to control mealybugs. As emphasized by van Lenteren (2012), the most critical phases in any biological control programme are the steps where selection of natural enemies takes place. As an initial step in the development of a biocontrol strategy for mealybugs in roses, a survey was conducted in commercial rose crops to quantify the occurrence of *D. megalamellae*. This research provides evidence of the potential this predatory midge may offer for the control of mealybugs in roses.

MATERIALS AND METHODS

To determine the occurrence of *D. megalamellae* in cut flower roses in Kenya, a multiple location survey was conducted in the four major cut flower growing regions: Thika (Farm A), Nairobi (Farm B), Naivasha (Farm C), and Nanyuki (Farm D), in June 2018 (Figure 1). Most rose cut flower farms in Kenya are located at altitudes between 1,500 and 2,200 m so large areas of Kenya do not produce roses. Each farm, consisting of two greenhouses (100 × 100 m^2) with two rose varieties, were observed for mealybug infestation and predatory midge occurrence and sampled for the study (Table 1). To quantify *D. megalamellae* numbers, 92 rose plants that had mealybugs were sampled randomly in the greenhouse, the mealybugs were carefully brushed off by using a camel brush, placed into a vial (30 ml) and taken to the laboratory (Real IPM, Kenya). The mealybug colonies were removed and the number of *D. megalamellae* eggs, larvae and pupae were recorded (Figure 2). In addition, the altitude and mealybug species were recorded for each farm.

The identification of *D. megalamellae* (Barnes, 1939) was undertaken by Keith M. Harris (Private communication, 2017) based on a sample of adults (24 males and 10 females) and larvae (>40 third and earlier instars) (Harris, 1968).

Statistical Analysis

Data obtained from the surveys, region altitude, mealybug

Figure 1. Kenyan counties in which mealybug samples were collected:Kiambo (Farm A), Nairobi (Farm B), Nakuru (Farm C), Laikipia (Farm D).
Source: Adapted from Lewis (2016).

Table 1. Mean number of mealybug colonies, number of midge eggs, larvae and pupae in these mealybug colonies on four different rose farms in Kenya. Mealybug species and farm altitude in metersare also shown.

Farm	Mealybug species	Mealybug colonies (Mean ± SE)	Midge eggs (Mean ± SE)	Midge larvae (Mean ± SE)	Midge pupa (Mean ± SE)	Farm altitude (m)
Farm A	*P. citri*	3.81 ± 0.24^{a}	0.17 ± 0.07^{a}	0.68 ± 0.10^{b}	0.31 ± 0.07^{c}	1,525
Farm B	*P. longispinus*	7.84 ± 0.78^{b}	0.00 ± 0.00^{a}	0.05 ± 0.03^{a}	0.01 ± 0.01^{a}	1,973
Farm C	*P. citri*	9.40 ± 0.59^{c}	0.42 ± 0.15^{b}	2.38 ± 0.26^{c}	0.14 ± 0.04^{b}	1,905
Farm D	*P. citri*	10.74 ± 0.84^{c}	0.00 ±0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2,335
$F_{(3,732)}$ value	-	21.05	5.89	64.93	11.70	-
P value	-	<0.001	<0.001	<0.001	<0.001	-

Means within each column followed by the same letter are not significantly different (P>0.05). SE: Standard error.

species, and number of *D. megalamellae* eggs, larvae, and pupae, were recorded in Microsoft Excel spreadsheets, and subjected to analysis of variance with a General Linear Model (GLM). Regression analysis of the number of mealybug number (independent variable) against the number of *D. megalamellae* larvae (dependent variable) was undertaken with the exclusion of

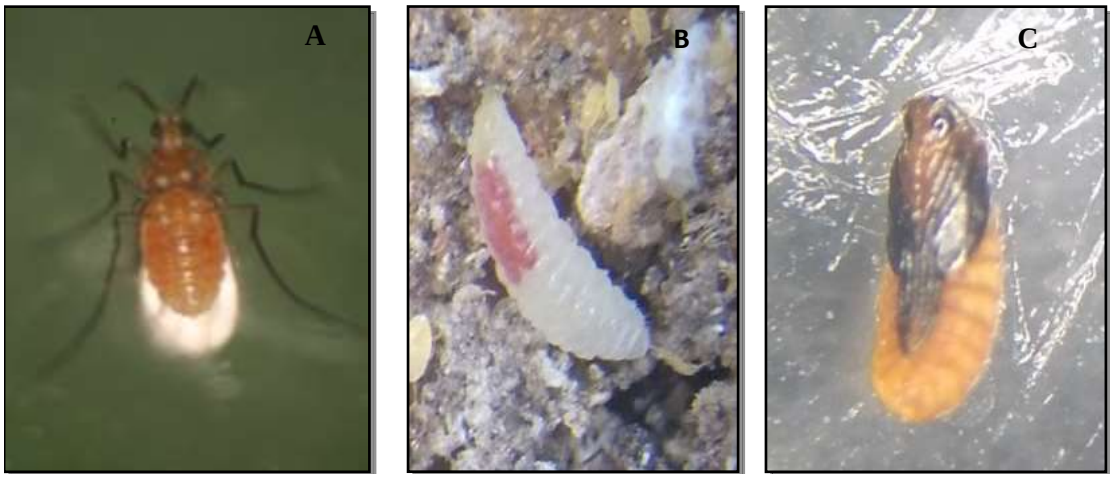

Figure 2. *Diadiplosis megalamellae* (Barnes) life cycle stages. (A) Adult (ventral view); (B) Larva (dorsal view), (D) Pupa (ventral view).

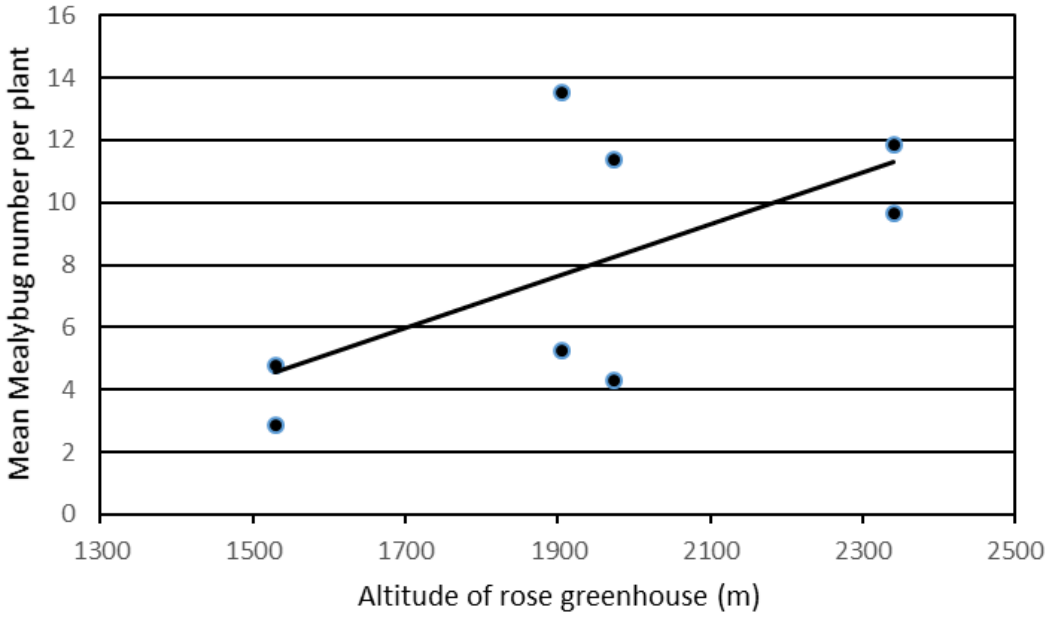

Figure 3. Linear regression of mean citrus mealybug colony number per rose plant against altitude at which the rose farm was located. $Y = 0.0083x - 8.213$; $R^2 = 0.392$; $F_{(1,7)} = 3.86$; $P = 0.097$.

farm D where no *D. megalamellae* was observed. An additional regression analysis of mealybug against the larvae against farm altitude was undertaken using the mean data from all varieties and locations (Figure 3). All analysis was performed using Genstat software and means separated by LSD-test and Tukey's test at $P \leq 0.05$.

RESULTS

On Farm A, C, and D, the mealybug *P. citri* was observed, whilst on Farm B the mealybug *P. longispinus* was found feeding on rose plants. The number of mealybug colonies was significantly different between farms ($P<0.001$). The highest number of mealybug colonies per plant were recorded on Farm D, and the least on Farm A (Table 1). The highest number of midge eggs and midge larvae was observed on Farm C, whilst the highest number of midge pupae was found on Farm A (Table 1). On Farm D, no *D. megalamellae* eggs, larvae and pupae were observed.

The number of mealybug colonies present on each farm was also influenced by rose variety ($P<0.001$). The highest mealybug colony number was found on Madam Red, whereas the lowest mealybug colony number was

Table 2. Mean number of mealybug colonies on infested rose plants and the number of midge eggs, larvae and pupae in these mealybugcolonies on eight rose varieties.

Rose variety	Farm	Mealybug colonies (Mean ± SE)	Midge eggs (Mean ± SE)	Midge larvae (Mean ± SE)	Midge pupa (Mean ± SE)
Nightingale	Farm A	2.87 ± 0.25^{a}	0.12 ± 0.06^{a}	0.71 ± 0.14^{b}	0.41 ± 0.13^{c}
Aqua	Farm B	4.28 ± 0.56^{a}	0.00 ±0.00^{a}	0.10 ± 0.05^{a}	0.00 ±0.00^{a}
Proud	Farm A	4.75 ± 0.38^{a}	0.23 ± 0.12^{a}	0.65 ± 0.14^{b}	0.21 ± 0.07^{b}
Pegasso	Farm C	5.26 ± 0.35^{a}	0.04 ± 0.02^{a}	1.04 ± 0.26^{c}	0.12 ± 0.05^{ab}
Maritim	Farm D	9.64 ± 0.77^{b}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
Sweet Sara	Farm B	11.39 ± 1.36^{bc}	0.00 ±0.00^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
Confidential	Farm D	11.84 ± 1.48^{bc}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
Madam Red	Farm C	13.53 ± 0.95^{c}	0.79 ± 0.29^{b}	3.72 ± 0.39^{d}	0.15 ± 0.07^{a}
$F_{(7,728)}$ value		21.75	5.74	47.10	5.9
P value		<0.001	<0.001	<0.001	<0.001

Means within each column followed by the same letter are not significantly different (P>0.05). The respective farms where these varieties were grown are indicated as A-D. SE: Standard error.

found on Nightingale (Table 2). The number of midge eggs per colony was significantly higher on variety Madam Red compared to midge egg number on the varieties Nightingale, Proud and Pegasso. However, the number of midge eggs found on Nightingale, Proud and Pegasso varieties was not significantly higher than Aqua, Maritim, Sweet Sara, and Confidential varieties that had no midge eggs (P > 0.05).

On variety Madam Red a significantly higher number of midge larvae was found than on Pegasso, Nightingale and Proud varieties. On variety Aqua, the number of midges did not differ from the varieties Maritim, Sweet Sara, and Confidential, that had no midge larvae (P > 0.05).

The number of pupae was higher on variety Nightingale compared to the varieties Proud and Pegasso. However, the number of pupae on variety Proud did not differ from variety Pegasso, although the number of midge pupae on Pegasso did not differ from the remaining varieties including those that did not have any midge pupa (Table 2).

The regression analysis of the number of mealybug number (independent variable) against the number of *D. megalamellae* larvae (dependent variable) (y = 0.058x + 0.626, R2 = 0.0414, n=552, P < 0.001) showed there was a positive but weak relationship. The number of mealybugs increased with an increase in altitude at which a rose farm was located (Figure 3). At a lower altitude, a lower number of mealybugs was found, whereas at a higher altitude a higher number of mealybugs was present.

DISCUSSION

The predatory midge *D. megalamellae* may serve as a potential predator for the control of mealybugs in roses in Kenya. There was a significant positive correlation between the number of mealybug and the number of midge *D. megalamellae*. As the midge is feeding on the mealybug this relationship is to be expected. In addition, a high number of *D. megalamellae* pupae was associated with a lower number of mealybugs present on the farm. There was also a relationship between the number of mealybugs present and the altitude of the farm (Figure 3). It is expected that at a lower altitude, temperatures and mealybug growth are higher, in line with the study of Laflin and Parrella (2004) that studied the mealybug number on roses in California. However, in our study at higher altitude, where temperatures are lower, a higher number of mealybugs occurred. Under controlled conditions, the development of mealybugs in relation to temperature follows a sigmoid curve (Laflin and Parrella, 2004). However, extrapolation of such laboratory results to the farm is not straightforward, as other factors such as humidity, temperature over time, could well be as important. In addition, absence of *D. megalamellae* at the highest altitude (farm D) could have played a role in this outcome.

The population of mealybugs varied significantly between the four farms surveyed. The reasons for this are probably multifactorial but could include the management practices on each farm, differences in susceptibility of rose varieties to mealybugs, the chemicals applied, the climatic conditions and the presence of natural enemies. The presence of *P. longispinus* mealybugs on farm B further complicates the understanding of what factors influence mealybug and midge populations. Nymphs of *P. longispinus* mealybugs hatch immediately upon oviposition, which has led some observers to conclude mistakenly that female *P. longispinus* mealybugs are viviparous (Goolsby 1994). Consequently, the midge *D. megalamellae* may not be a suitable predator for long tailed mealybug; hence, the very

low presence of the midge on farm B. However, Charles (1981) does report *Diadiplosis koebelei* (Diptera: Cecidomyiidae) preying on *P. longispinus.* Future studies could test the suitability of *P. longispinus* mealybugs as prey for *D. megalamellae* and *D. koebelei*.

The presence of mealybugs on cut flower roses is a relatively recent development. It is slightly surprising that the predatory midge *D. megalamellae* has migrated into commercial crops of cut flower roses that have citrus mealybug in a period of just a few years. Furthermore, our survey has demonstrated that this is not a one-off event as the midge is present in three distinct geographical rose growing areas of Kenya, Thika, Naivasha and Nairobi. It may be possible to supplement the predation by the midge with augmentative applications in order to achieve biological control of citrus mealybug in cut flower rose production in Kenya. Hayon et al. (2016) pointed out that the advantages of predatory Cecidomyiidaeare to effectively locate mealybugs even in cryptic places, and that they appear to be less susceptible to intraguild predation than other natural enemies. Additionally, they suggest that predatory Cecidomyiidaeare not deterred by ants that guard colonies of mealybugs. The observation that *D. megalamellae* lays its eggs in the egg mass under the waxy layer of the citrus mealybug offers the predatory midge protection against conventional pesticides being used in rose cut flower production.

To conclude, the predatory midge *D. megalamellae* may be an important biocontrol agent for citrus mealybugs. However, there is little fundamental knowledge on the biology of this predatory midge and on its role in restraining mealybug populations and its potential in biocontrol programs. More information on its prey range, sensitivity to climate conditions and insecticides, is required for their successful deployment as a biocontrol agent in Kenya.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Banana field resistance to insect-vector transmission of bacterial wilt caused by *Xanthomonas campestris p.v musacearum*

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Banana, a major staple in East and Central Africa is constrained by banana Xanthomonas wilt (BXW) caused by *Xanthomonas campestris* pv*. musacearum* (Xcm). Xcm-infected plants are rapidly destroyed leading to 100% yield loss. Cultural controls are effective but laborious attracting laxity among farmers. This has led to epidemic resurgence in areas where BXW had been contained hence spread to new regions. Reliable control option would be planting Xcm-resistant varieties but extensive germplasm evaluation for their identification has not been conducted. Objective therefore was to determine existence of Xcm-resistance in banana by evaluating major banana cloneset representatives among indigenous cultivars plus introduced foreign Musa accessions. Potted plants were artificially inoculated with 0.5 ml (10^8CFU) of Xcm suspension. Promising selections from pot trial were later evaluated under natural transmission in field. Field trial plants were infected via insect vectors from spreader plants of highly susceptible cv Kayinja infected by spraying flowers with Xcm. Severity of Xcm-infection was semi-quantified using scales 1-5 and 0-5 for pot and field screening trials respectively. This enabled calculation of disease index as a measure of resistance for each genotype. High index implied highly susceptible banana genotype and low index resistant genotype. Findings 44 days after artificial inoculation showed wild banana *M. balbisiana* had 0.0 disease index thus highly resistant. All other banana genotypes tested under similar conditions had disease index of 100 thus susceptible. In field (insect vector transmission), disease index varied significantly among various genotypes evaluated, some susceptible while others; *M. balbisiana, Mbwazirume*, M9 and *M. Zebrina* resistant throughout 360 days of observation. We recommend that heritable traits that confer resistance in M*. balbisiana*, Mbwazirume, M9 and *M. zebrina* to Xcm be identified for utilization in genetic modification of farmer preferred bananas. Varieties Mbwazirume and M9 should be promoted for farmer growing to complement cultural controls against BXW.

Key words: *Xanthomonas campestris pv. musacearum,* banana Xanthomonas wilt, banana.

INTRODUCTION

Banana (*Musa* spp.) is an important staple food crop in Uganda where production is globally ranked second

largest after that of India (Biruma et al., 2007; Vurro et al., 2010). Since the emergence of Banana *Xanthomonas* wilt (BXW disease in 2001, it has devastated banana production undermining food and income security for more than 70% of Uganda's population that depend on banana industry (Tushemereirwe et al., 2004; Biruma et al., 2007). BXW that is caused by *Xanthomonas campestris pv. musacearum* (*Xcm*) (Yirgou and Bradbury, 1974) which leads to complete yield loss. Banana fruits on infected plants ripen prematurely; their pulp is hardened and/or rotten. Also suckers on mat of such plants that would give subsequent ratoon crop cycles and planting material needed for plantation expansion and /or establishing new ones also wilt and die (Smith *et al*., 2008). Unfortunately also the BXW that was initially reported since the 1960s to be confined in Ethiopia on enset (*Enset ventricosum*) a close relative of banana, (Shimelash et al., 2008) for unknown factors has spread rapidly throughout Eastern and Central Africa. Banana plantations in as far as Burundi, the Democratic Republic of Congo, Kenya, Rwanda, Tanzania and Uganda (Tushemereirwe et al., 2001; Ndungo et al., 2006; Biruma et al., 2007; Reeder et al., 2007; Carter et al., 2010) have been affected.

BXW symptoms appear on all plant parts and arise from internal blockage of the vascular tissue leading to incapacitated plant transport system. External symptoms involve initial drooping of leaves to entire plant wilting, death and eventual rotting of the entire stem (Karamura et al., 2008). There is mummification of the male bud characterized by fading of bract color from deep purple to dark brown or grey, shriveling of bracts and eventual drying up or rotting of the entire stalk. This is a typical characteristic of insect mediated (natural) transmission of *Xcm* (Karamura et al., 2008). Infection from the male bud of very susceptible varieties for example cultivar Pisang awak (Kayinja) (ABB) proceeds through the rachis, pseudostem down to the corm where it spreads to the adjoining suckers around the mother plant resulting in death of entire mat. However the other banana varieties; Bluggoe (ABB), Sukali Ndizi (AAB), East African highland banana (AAA) cultivars that have shriveled and less conspicuous flowers do not show this symptom. Infected fruits harden and develop a dark brown discoloration in the pulp. In addition, a cross-section through the fruit and pseudo stem reveals yellow pus-like bacterial ooze.

Recommended cultural practices are quite limited towards achieving long term acceptable control levels of BXW. These practices involve complete uprooting of diseased mats, burying of uprooted and chopped plant debris, use of clean garden tools and timely removal of male buds to prevent insect vector transmission (Ssekiwoko et al., 2010). Compliant farmers to BXW-control mobilization and sensitization campaigns have achieved 60-90% success (Kubiriba et al., 2012). These practices are quite laborious and are often associated with laxity tendencies among farmers. Consequently, there has been a repeat of epidemic resurgence in areas where it had been contained. The epidemic has also continued to advance into additional plantations in regions not previously affected by bacterial wilt. East African Highland Bananas (EAHBs) are the dominant cultivars grown by farmers in this region where BXW is endemic. EAHBs that are also clonally propagated aid dissemination of BXW through infected planting materials.

EAHBs that are triploids of AAA genotype presumably have a very narrow genetic base. They are very difficult to improve utilizing conventional breeding approaches because they are sterile and pathenocarpic. The practice of planting resistant banana varieties therefore seems to be nonrealistic BXW control option for the farmers in East Africa since all popular cultivars grown are unfortunately perceived to be highly susceptible to BXW. For example, Ssekiwoko et al. (2006) evaluated 42 indigenous EAHB cultivars and four wild banana relatives by artificial inoculation under screen house trial conditions. They reported that all these bananas except *M. balbisiana*, were susceptible to BXW. Since *M. balbisiana* is a diploid with BB genome, it is very interesting to establish whether presence of B genotype in banana triploids and tetraploids would consistently confer resistance to BXW. In present investigation the range of banana genotypes evaluated by artificial inoculation with *Xcm* under screen house trial conditions was broadened to include those that were not evaluated by Ssekiwoko et al. (2006). Introduced triploids and tetraploid bananas with B or BB genotypes available in the Uganda's National Banana germplasm collection that reportedly arose from hybridization between diploid species of *M. acuminata* (AA) and *M. balbisiana* (BB) were also evaluated. Finally, unlike for the study of Ssekiwoko et al. (2006) that was only via artificial inoculation of test banana plants under screen house conditions, representative banana genotype selections from those in the screen house trial were also further evaluated in the field for resistance to *Xcm* utilizing procedure of natural spread of *Xcm* by vector transmission.

MATERIALS AND METHODS

Banana genotypes evaluated in pot trial

To broaden the number of germplasm accessions evaluated for response to infection by *Xcm* under screen house conditions, 40

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Table 1. List of banana genotypes evaluated for resistance to BXW in pot trial.

Genome group	Name of genotype	Genotype category and ploidy level
A	Long tavoy	Foreign banana, Diploid (AA)
	M. ornata	Wild banana, Diploid (AA)
	M. zebrina	Wild banana, Diploid (AA)
	Pisang mas	Foreign banana, Diploid (AA)
	Pitu	Foreign banana, Diploid (AA)
	GCTC.V.215	Foreign banana, Triploid (AAA)
	KM5	Landrace, Triploid (AAA)
	M2	Hybrid banana, Triploid (AAA)
	M9	Hybrid banana, Triploid (AAA)
	Cavendish	Dessert banana, Triploid (AAA)
	Dumingi	Foreign banana, Triploid(AAA)
	Kafunze	Local banana, Triploid (AAA)
	Mbwazirume	Local banana, Triploid (AAA)
	Nakasabira	Local banana, Triploid (AAA)
	Pisang nangk	Foreign banana, Triploid (AAA)
	Red bogoya	Dessert banana, Triploid (AAA)
	Williams	Foreign banana, Triploid (AAA)
	IC2	Foreign banana, Tetraploid (AAAA)
B	*M. balbsiana*	Wild banana, Diploid (BB)
AB	Amou	Foreign banana, Triploid (AAB)
	Pisang rajabul	Foreign banana, (AAB)
	burroCEMSA	Foreign banana , Triploid (ABB)
	ITC	Foreign banana, Triploid (ABB)
	Saba	Foreign banana, Tripliloid (ABB)
	P.A. 03.22	Foreign banana, Tetraploid (AAAB)
	PV.03.44	Foreign banana, Tetraploid (AAAB)
	FHIA3	Foreign banana, Tetraploid (AABB)

plants for each of the 25 banana genotypes presented in Table 1 that had not been previously screened (Ssekiwoko et al., 2006) for resistance to BXW were included in the current pot trial. In addition, two matooke hybrids, M2 and M9 developed utilizing conventional breeding approach by scientists of the Banana Research Programme, at NARL, Kawanda were included in this study. The wild relative of banana, *Musa balbisiana* and an East African.

Highland Banana cv Mbwazirime respectively resistant and susceptible to artificial inoculation with *Xcm* in the evaluation by Ssekiwoko et al. (2006) were included to act as reference checks for the current trial. In all, a total of 1000 plants that were regenerated from corms of suckers obtained from symptomless mother plants were established in plastic pots containing steam sterilized top loam soil. Emerging plants from corms were monitored through establishment for a period of at least 21-30 days to confirm their disease-free status. The experiment was laid down in a Completely Randomized Design (CRD) arrangement with four replications each comprising of ten plants per genotype in the trial.

Xcm strain used for artificial inoculation of banana in pot trial

Xcm strain used for inoculation of banana test plants in the pot trial was isolated from infected banana cv Kayinja (Mudonyi et al., 2017). Then cultured and maintained by regularly sub-culturing on Yeast colonies characteristic of *Xanthomonas* were resuspended in sterile water in a bottle. The turbidity of the inoculum suspension of *Xcm* was adjusted using a spectrophotometer at an optical density of 600nm that corresponds to a concentration of $1x10^8$ CFU/ml.

Artificial inoculation of banana plants in pot trial

Initially before inoculation of experimental plants, the pathogenicity of *Xcm* isolate from Kayinja was confirmed by infection of susceptible banana cultivar Kisansa (AAA) following procedure described by Ssekiwoko et al. (2006) and Mudonyi et al. (2017). These Kisanasa plants had been micropropagated in tissue culture, weaned in poly pots containing steam sterilized top loam soil and allowed to go through an acclimatization period of one month in the screen house. Infection of these potted plants were via injection into meristematic tissue within the petiole of topmost expanded youngest leaf, with 0.5ml of (10^8CFU) utilizing a hypodermic syringe and needle. The negative control plants were injected similarly with an equal volume of sterile water. After this initial pathogenicity test confirmation, similar approach was used to inoculate all pot trial test plants by injection with 0.5ml of the bacterial suspension. Artificial inoculation was carried out in the evening when ambient relative

humidity conditions were rising and temperature decline from an average day maximum of 30°C to avoid wound drying before bacterial multiplication and colonization of plant tissue (Karamura et al., 2008).

Monitoring disease progress in pot trial

Monitoring BXW progress on artificially inoculated pot trial plants maintained under screen house conditions lasted 60 days post inoculation. The screen house temperature conditions were at an average maximum of 28-30°C and minimum of 18-21°C. Monitoring was made twice a week and data was recorded on disease latency (incubation) period, disease incidence and disease severity. Disease severity was semi quantitatively estimated using a scale of 0-5 (where 0=no symptoms on all leaves, 1=one inoculated leaf wilted, 2=two to three leaves wilted, 3=four leaves wilted, 4=all leaves wilted and 5=plants dead; Winstead et al. 1952). Disease index for each genotype was determined using following formula;

$$\text{Disease index} = [(0xa) + (1xb) + (2xc) + (3xd) + (4xe) + (5xf)/ (nx5)] 100$$

Where, 0, 1, 2, 3, 4, 5 is disease severity scale for each respective plant a, b……f and n = total number of plants inoculated (25) for each genotype. This disease index was expressed as a percentage and therefore a very high index meant a highly susceptible banana genotype and low index meant resistant genotype (0% means resistant, 1- 40% moderately resistant, 41-59% susceptible, 60-100% highly susceptible).

Field evaluation of selected *Musa* germplasm accessions

Banana genotypes evaluated in the field

10 promising genotype selections from among those tested in the pot trial (Table 2) were advanced and further evaluated under natural field conditions. These plants were raised from corms and planted in holes measuring 45 x 45 cm wide and 60 cm deep and at a spacing of 3 m x 3 m. The field trial layout was a Randomized Complete Block Design (RCBD) with four blocks each comprising of 16 plants per genotype. A total of 640 test plants were planted in the trial. It was then surrounded with spreader row of plants of banana cultivar Kayinja that is highly susceptible to BXW.

Field trial inoculation and management

The spreader row plants that comprised of BXW- susceptible banana cv Kayinja were inoculated by spraying their male bud flowers and cushions with 0.5 ml of suspension *Xcm* strain. This strain was also the one that was used for inoculation of plants in pot trial evaluation. This *Xcm* strain was previously isolated from banana cv Kayinja. Field test plants were kept weed free by regular herbicide spray with Roundup and hand pulling around the plants. Tools for weeding, pruning and de-suckering were not used to avoid possible damage to suckers which would have potentially exposed plants to some other infections and to avoid spread of *Xcm* infection in the field trial via contaminated tools. Removal of male buds was also avoided to promote natural vector transmission of *Xcm* when moving from various male flowers of banana plants while foraging for nectar.

Monitoring BXW progress in field trial

Field trial plants were observed monthly for a period of one year and data collected on latency period, disease incidence and severity. Disease incidence was computed as a percentage of plants showing BXW characteristic symptoms for each cultivar per block. Disease severity was estimated utilizing modified 0-5 scale of Winstead et al. (1952a), (where 0=no symptoms, 1=inflorescence symptoms on the male bud, rachis and the bunch, 2=mother plant wilted, 3=mother plant dead, 4=one or more daughter plant(s) wilted, and 5=entire mat dead. Disease severity data was then used to calculate disease severity index (DI) for each genotype in the block utilizing formula described in the pot trial above.

Statistical analysis of data collected from pot and field trials

For both pot and field trials, disease indices were subjected to analysis of variance (ANOVA) using GenStat 12th Edition (VSN International Ltd, 2009). Means were separated using Fisher Least Significance difference at 5%.

RESULTS

Banana resistance to meristematic tissue injection with *Xcm* under screen house conditions

Identity of Xcm for evaluation of banana resistance confirmed

The strain of *Xcm* utilized in evaluation of banana resistance (Figure 2a) was found to induce wilt symptoms in all eight inoculated plants (100% incidence) of banana "cv" Kisansa within 14 days of pathogenicity confirmation test trial (Figure 2a). These infected plants wilted and eventually died by end of 32 days post-inoculation. Negative control reference test plants that were inoculated with sterile distilled water did not develop wilt symptoms (Figure 2b) during the same observation period. Cultural characteristics were established by re-isolation of causative bacteria onto YPGA (Figure 1). This pathogenic bacteria formed shiny, yellow, mucoid and smooth colonies on YPGA (Figure 2c). These colony characteristics on YPGA are typical of Xcm. After conducting these in vitro and pathogercity confirmatory tests, this Xcm strain was used for infection of all test plants in subsequent pot and field evaluation trials.

Banana is susceptible to Xcm by artificial inoculation

All the banana genotypes that were evaluated under screen house conditions became infected by Xcm via artificial inoculation but the response to infection over time was highly variable (Table 3). For example, 12 of the genotypes evaluated exhibited the shortest latency period of 12 days. Also latency period was 14-16 days for 14 genotypes and 20 days for one genotype PV.03.44. M. balbisiana which is a diploid with B genome (Table 1) exhibited the longest latency period of 32 days. Further, whereas about 29% of inoculated plants of M. balbisiana

Table 2. List of selected banana genotypes evaluated for resistance to BXW in field trial.

Genome group	Name of genotype	Genotype ploidy level
A	M. ornata	Diploid (AA)
	M. zebrina	Diploid (AA)
	M2	Triploid (AAA)
	M9	Triploid (AAA)
	KM5	Triploid (AAA)
	Mbwazirume	Triploid (AAA)
B	*M. balbsiana*	Diploid (BB)
AB	BurroCEMSA	Triploid (ABB)
	Saba	Triploid (ABB)
	FHIA3	Tetraploid (AABB)

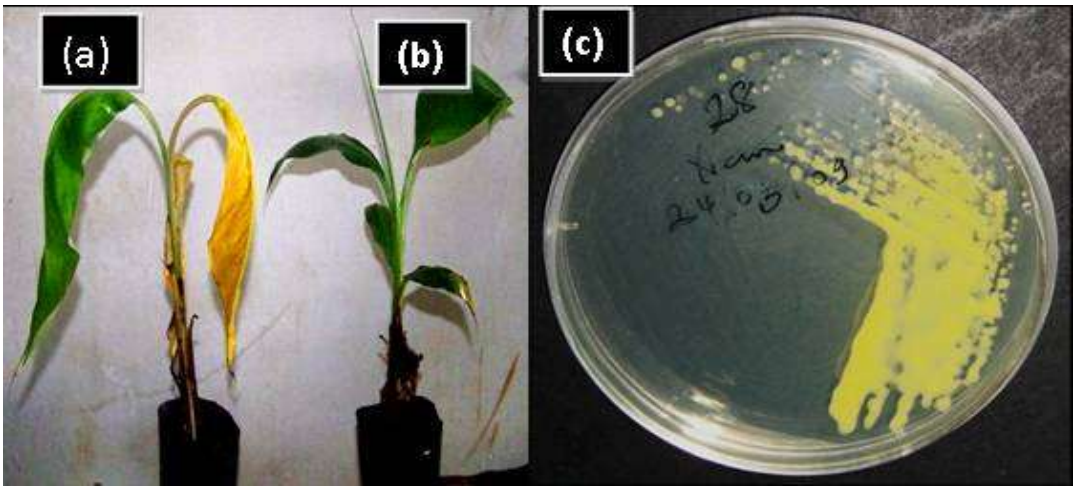

Figure 1. *Xcm* infected banana and colony characteristics on YPGA. Potted Kisansa banana plants inoculated with *Xcm* (a) and sterile water (b). Cultural appearance of pathogenic bacteria on YPGA (c) that was re-isolated from the symptomatic plant (a) was typical for *Xcm*.

Figure 2. Appearance of bacterial wilt symptoms on inflorescence of genotype Saba in the field trial. Symptom appearance on healthy-looking inflorescence (A) gradually progressed from wilting of male bud and rachis (B) to premature fruit ripening (C) and finally rotting of the entire.

showed bacterial wilt symptoms, all the plants of the other genotypes evaluated by artificial inoculation developed wilt symptoms both on inoculated and non inoculated leaves within 44 days after inoculation (Table 3). Wilt symptom manifestation by these other genotypes was neither linked to their ploidy level nor to their genome group (A, B or AB) as described in Table 1. Interestingly, wilt symptoms on the infected plants of M. balbisiana that is a diploid with BB genome were restricted to the inoculated leaf only and also these plants eventually outgrew such symptoms of infection 44 days after inoculation. On the other hand no such symptom recovery was observed for all the inoculated plants of the other genotypes evaluated by artificial inoculation. Also with exception of M. balbisiana, disease index at 26 days after inoculation of the different genotypes was highly variable and ranged from 40-83%. However at 44 days after inoculation the disease index for all these genotypes was not significantly different and ranged from 93-100% (Table 3).

Artificial inoculation of potted plants via injection with 0.5 ml (10^8CFU) of bacteria suspension into meristematic tissue within the petiole of youngest fully expanded leaf of each plant utilizing a hypodermic syringe and needle was performed in the evening when ambient temperature was declining to below 25˚C and relative humidity rising to above 80%.

Persistent bract trait is associated with field resistance of banana to *Xcm*

Infection of banana genotype plants by *Xcm* in the field trial was achieved by spraying flowers of spreader plants of susceptible banana cultivar Pisang awak with bacterial suspension. Then infection by *Xcm* was transmitted naturally by foraging insect vectors from these inoculated spreader plants to the healthy test plants. Under these field trial conditions, initial manifestation of bacterial wilt symptoms varied significantly among the banana genotypes evaluated. For example, banana genotypes Saba (BBB) and BurroCEMSA (ABB) were the first to show symptoms of infection (Figure 2). Later four additional genotypes also showed wilt symptoms while another four genotypes did remain symptomless (Table 4). For those plants of genotypes that became infected by *Xcm* under field trial conditions, initial symptoms appeared on the healthy looking inflorescence (Figure 2A). Symptoms on the inflorescence began with wilting of the male bud followed by that of the rachis (Figure 2B), then premature ripening and rotting of fruits (Figure 2C, D), and finally death of the entire plant.

The infection by *Xcm* was transmitted naturally by foraging insect vectors from the inoculated spreader plants of susceptible cultivar Pisang awak to the healthy looking test plants of genotypes evaluated in the field trial.

Six genotypes that were evaluated in the field trial developed wilt symptoms and four did remain symptomless (Table 4). Notably, these six genotypes that wilted under field trial conditions of BXW spread have dehiscent male bud bracts and the four that remained symptomless have persistent male bud bracts (Figure 3).

This persistent bract trait on the inflorescence was apparently associated with banana field resistance to infection by *Xcm* via insect transmission.

The disease incidence and severity index for the six banana genotypes that became infected by *Xcm* under field conditions of disease spread varied significantly (Table 4, $p < 0.001$). The highest disease incidence of 65% and severity index of 63.5% was recorded for genotype Saba (ABB). In addition, disease incidence and severity index that varied from 14.6-55% and 13.7-44% respectively was recorded for the remaining five genotypes that became infected by *Xcm*. Finally, disease incidence and severity index of 0% (Table 4) was recorded for four genotypes inclusive of two cooking varieties, Mbwazirume (AAA) and recently released matooke hybrid M9 (AAA). These four genotypes that did not show symptoms of infection in the field trial had developed wilt symptoms within 12-32 days after artificial inoculation with *Xcm* under screen house trial conditions (Table 3). Additionally, it was only *Xcm*-infected *M. balbisiana* potted plants that were also able to recover fully from bacterial wilt symptoms developed 32 days after artificial inoculation.

Unlike for artificial inoculation in pot trial (Table 3) where all genotypes tested became infected by *Xcm*, four of the ten banana genotype selections that were advanced and evaluated under field conditions of bacterial wilt spread were not infected by *Xcm*. These interesting genotypes have persistent male bud bracts and include two important cooking banana varieties; recently released banana hybrid M9 and popular variety Mbwazirume.

DISCUSSION

Evaluation of banana for resistance to Xcm in the screen house

This study evaluated 4 indeginous banana cultivars, 2 banana hybrids, 3 wild relatives of banana and 17 introduced banana germplasm accessions for resistance towards Xcm infection by injection of inoculum into meristematic tissue of the leaf petiole. The technique was very effective since 100% of inoculated plants became infected (Table 3). Apparently, the inoculum was directly deposited in the main transport system enhancing inoculum delivery to all parts of the plant to cause infection. On the other hand, inoculum for the field trial plants was placed onto the flowers of spreader plants to enable easy access by foraging insects for transmission to healthy plants. All the genotypes evaluated in the screen house except M. balbisiana that is diploid with BB genotype succumbed to BXW via artificial inoculation.

Table 3. Variation in response of banana genotypes to infection by *Xcm* via artificial inoculation under screen house conditions at National Agricultural Research Laboratories, Kawanda.

Genotype	Latency period	Mean incidence (%)		Disease index (%)	
		26 dai	44 dai	26 dai	44 dai
Pisang nangk	12-22	100^{a}	100^{a}	83^{i}	100^{a}
Red bogoya	16-20	100^{a}	100^{a}	82^{hi}	100^{a}
Dumingi	16-22	100^{a}	100^{a}	82.5^{i}	100^{a}
GCTC.V.215	12-22	100^{a}	100^{a}	75^{gh}	100^{a}
Nakasabira	16-22	97^{b}	100^{a}	73^{fgh}	100^{a}
ITC	12-20	100^{a}	100^{a}	69^{efg}	100^{a}
Williams	12-22	100^{a}	100^{a}	69^{efg}	100^{a}
Pisang rajabul	12-22	100^{a}	100^{a}	69^{efg}	100^{a}
Pitu	12-22	100^{a}	100^{a}	68^{defg}	100^{a}
IC2	12-22	100^{a}	100^{a}	64^{cdef}	100^{a}
Cavendish	14-22	100^{a}	100^{a}	63^{cde}	100^{a}
Long tavoy	12-20	100^{a}	100^{a}	62^{c}	100^{a}
Amou	16-22	100^{a}	100^{a}	60^{cde}	100^{a}
M2	14-22	100^{a}	100^{a}	60^{cde}	100^{a}
M9	16-20	100^{a}	100^{a}	60^{cde}	100^{a}
Saba	16-22	100^{a}	100^{a}	59^{cd}	100^{a}
Mbwazirume#	16-22	100^{a}	100^{a}	59^{cd}	100^{a}
KM5	12-22	100^{a}	100^{a}	58^{c}	100^{a}
Kafunze	12-22	100^{a}	100^{a}	58^{c}	100^{a}
FHIA3	12-22	100^{a}	100^{a}	53^{bc}	100^{a}
M. ornate	12-26	100^{a}	100^{a}	53^{bc}	93^{b}
burroCEMSA	16-22	100^{a}	100^{a}	51^{b}	100^{a}
M. zebrina	16-22	91^{e}	100^{a}	51^{b}	100^{a}
PV.03.44	20-22	100^{a}	100^{a}	47^{ab}	100^{a}
Pisang mas	14-26	94^{c}	100^{a}	47^{ab}	100^{a}
P.A. 03.22	16-22	92^{d}	100^{a}	40^{a}	100^{a}
M. balbsiana	32	0^{f}	28.6^{b}	0j	0.0^{c}
LSD(5%)		0.0464***	0.02575***	9.1***	0.932***
CV%		10	5.4	30.1	2

Presence of B genotype in other bananas did not confer resistance to BXW. This result is in agreement with previous evaluation where all indigenous edible banana cultivars in East and Central Africa succumbed to the disease (Ssekiwoko et al., 2006; Michael et al., 2006). In this study, two East African highland banana cultivars Nakasabira and Kafunze that were not previously screened were evaluated and equally found susceptible, suggesting there is limited possibility that there is resistance among the East African highland bananas to BXW infection. Most of M. balbisiana plants consistently did not develop symptoms in this study. About one third of M. balbisiana plants developed symptoms that were restricted to only the inoculated leaf but these plants overgrew these symptoms by 32 days after inoculation. The result suggests that M. balbisiana is resistant to Xcm which also is in agreement with findings reported by Ssekiwoko et al. (2006) and Tripathi et al. (2008). M. balbisiana is thus so far, the only possible source of resistance to Xcm and could be used for breeding improvement of banana for resistance to Xcm following both conventional and genetic engineering approaches.

Evaluation of banana for resistance to natural transmission of Xcm by insects in the field

Unlike for the result of artificial inoculation technique of injecting inoculum into meristematic tissue in the leaf petiole under screen house trial conditions additional banana genotypes to M. balbisiana showed field resistance to Xcm by insect transmission. Field resistant geneotypes included popular banana cultivar Mbwazirume, recently released banana hybrid M9, and genotype M.

Table 4. Variation in response of banana genotypes to infection by *Xcm* under field conditions for bacterial wilt spread. Wilt incidence and severity data was recorded until 360 days after inoculation of flowers of spreader-plants of susceptible banana cultivar Pisang awak (ABB) surrounding each experimental block that had been sprayed with bacterial suspension.

Genotype	Disease incidence (%)	Disease severity index (%)
Saba (BBB)	65.0^{g}	63.5^{e}
BurroCEMSA (ABB)	55.0^{f}	44.0^{d}
M2 (AAA)	40.0^{e}	38.5^{cd}
Km5 (AAA)	35.0^{d}	27.0^{bc}
M. ornata (AA)	32.5^{c}	30.5^{cd}
FHIA3 (AABB)	14.6^{b}	13.7^{ab}
M. balbsiana (BB)	0^{a}	0^{a}
M9 (AAB)	0^{a}	0^{a}
Mbwazirume (AAA)	0^{a}	0^{a}
M. zebrina (AA)	0^{a}	0^{a}
LSD (5%)	0.1555***	14.46***
CV%	150.5	155.5

Figure 3. Persistent (Top panel: A and B; Bottom panel: A, B and C) and dehiscent (Top panel: C) male bud bract traits of banana inflorescence. Banana hybrid M9 (Bottom panel: A), popular cultivar Mbwazirume (Bottom panel: B) and wild relative of banana *M. balbisiana* (Bottom panel: C) that have persistent male bud bracts also remained wilt symptomless throughout the period of observation in the field trial.

zebrina. These field resistant banana genotypes were observed to have persistent male bud bracts. In contrast, the other cultivars that succumbed to infection by Xcm in the field had dehiscent male bud bracts. The wet wounds left behind after dehiscence of male bud bracts observed on inflorescence of the banana genotypes that succumbed to Xcm infection in the field may have acted as entry points for Xcm carried around as body contaminate of insects foraging for pollen and nectar. Inflorescence of M9 and M. zebrina on the contrary were left with a dry wound after dehiscence of male bud bracts that may not be suitable entry points for spread of Xcm by foraging insects. Apparently, persistent bract trait is a preformed structure that may enable banana resist ingress of pathogenic bacteria as implied by description of Lucas (1998). Karamura et al. (2008) reported that certain bananas had persistent bracts and or dry wounds left after abscission of bracts and male flowers that enabled them resist natural infection by insects. Since these field resistant bananas with persistent bracts include popular cultivar Mbwazurime and recently released banana hybrid M9 as well as other edible banana cultivars grown by the farmers that are highly preferred by the consumers they may be planted by farmers and integrated in already recommended cultural practices being promoted for control of BXW. Finally, gene(s) controlling persistent bract trait in banana should be identified, isolated and utilized for genetic engineering improvement of farmer preferred Xcm-susceptible banana cultivars.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of sugarcane hybrid clones for cane and sugar yield in Nigeria

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Field experiment was conducted in year 2015/2016 at the National Cereals Research Institute Badeggi, Niger State (sugarcane research field) to evaluate the performance of sixteen sugarcane genotypes. The clones were planted in a Randomized Complete Block Design (RCBD) and replicated three times. Analysis of variance showed significant differentiation among studied genotypes. The results revealed that among the evaluated genotypes ILS 708-05 was characterized by highest potential cane yield (105.54 t/ha). BD 1576-14 significantly had highest brix (24.90%) among the tested clones. Genotypes that performed better than the Check ([Standard] B 47419) in terms of cane yield, less flowers and tolerance to smut should be advance to multi-location trials.

Key words: *Saccharum officinarum*, hybrid clones, brix and morpho-agronomic traits.

INTRODUCTION

Sugarcane (*Saccharum* spp.) is one of the most important species cultivated in the tropics and subtropics. It belongs to the genus *Saccharum* of the family *Poaceae*. The genus comprises six species that include *Saccharum spontaneum, Saccharum officinarum, Saccharum robustum, Saccharum edule, Saccharum barberi* and *Saccharum sinensis* (D' Hont et al., 1998). Cox et al. (2000) reported that modern sugarcane varieties that are cultivated for sugar production are found among interspecific hybrids between *S. spontaneum* and *S. officinarum.* In Nigeria sugarcane is an important industrial cash crop (Olaoye, 2006). Nigeria is the largest consumer of sugar in Africa after South Africa. However, the country only produces 2% of its requirement, estimated as 1.7 million tons while imports up to 98% of the commodity. Statistics have shown that Nigeria spends ₦200 billion on sugar importation and consumes 1.43 trillion metric tonnes of sugar yearly.

The goal of sugarcane breeding programme is to increase sugar yield by increasing sugar production per unit area. According to Glaz and Gilbert (2000), sugarcane production can only be improved through the adoption of promising varieties and technologies. Increased cane yield is a function of higher genetic potential of the variety (Nazir et al., 1997). According to Olaoye (2005), production of sugarcane seedlings from genetically diverse parents or breeding clones is essential for developing high yielding, disease and insect resistant cultivars for industrial production or by local chewing cane farmers. Increasing sugar content in sugarcane crop is closely associated with height, diameter and number of the stalks, along with sugar

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accumulation in the stalk as reported by Katia et al. (2012). Sugar yields have been generally improved by increased total biomass rather than directly by increasing sugar concentration in stalks (Jackson, 2005). Aitken et al. (2008) reported that important traits to be considered in increasing sugar yield are crop vigor and productivity of the ratoon crop. Oni (2016) reported that sugar industries in Nigeria rely more on cultivars brought from overseas rather than those developed in Nigerian Research Institutes, due to inadequate information about the performance of the cultivars that were bred in the country. Assessment of performance, adaptation of different sugarcane genotypes to different environments and evaluation of their traits are necessary before a variety is released for commercial cultivation. Clone selection at pre-commercial stage can help in the identification of improved genotypes for commercial production of sugarcane (Glaz and Gilbert, 2000). This study was undertaken to evaluate some exotic hybrid clones to reveal their yield and industrial potentials over the existing varieties.

MATERIALS AND METHODS

The field evaluation study of fifteen promising sugarcane hybrid clones was conducted at sugarcane research field of the National Cereals Research Institute (N 09° 04' 14.0, E 006° 05' 26.9, Elevation: 103 m), Badeggi, Niger State, Nigeria. The fifteen clones (ILS_1576-02, ILS_1576-20, BD_1576-31, BD_1576-07, BD_1354-17, BD_1576-14, ILS_708-05, 1LS_169-06, BD_1354-20, ILS708-02, BD_1388-23, ILS_1260-03, BD_1388-31, BD_1388-33, BD_1388-43) were hybrids of crosses originated from Mauritius that were raised and evaluated through series of selection process at National Cereals Research Institute Badeggi and University of Ilorin Sugar Research Institute. The fifteen clones were promising genotypes selected to advance yield trial in the year 2016. The clones were planted in a Randomized complete block design (RCBD) and B-47419 (a prominent commercial variety) was used as a Check (Standard). Each clone was planted on 5 m x 5 m plot and replicated three times. Each plot was made up of 6 rows with inter row distance of 1 m. Ten setts were planted per row and each sett comprise of three buds. Agricultural practices for sugarcane production were adopted from the recommendations of NCRI Badeggi. Data were collected on germination and establishment of leaves at 21 and 42 days after planting respectively, tiller count at 4 months after planting; stalk girth, stalk length, number of stools per plot, number of millabe canes per plot and cane yield per plot at maturity before harvest. Percentage germination was assessed at 21 days after planting. The total number of emerged buds per net plot was counted.

$$\text{Germination (\%)} = \frac{\text{No of sprouted buds per net plot}}{\text{Total number of buds on the setts planted per net plot.}} \times 100$$

Tiller count: It is the number of the secondary growth taken from the gross plots at 3 month after planting.
Plant height: 3 and 6 months after planting plant height was recorded using a graduated meter rule from the base (ground level) of the plant to the tip of the last unfolded leaf of selected tagged sugarcane plants within the net plots.
Stalk length: this was measured from the base of the stalk to the last node with the aid of a graduated meter rule.
Number of millabe cane: Sugarcane stalk with internodes that can be milled were counted from the net plot at 12 months after planting.
Stalk girth: (cm) a veneer caliper was used for measuring the stalk girth.

Brix (sugar content) was measured by refractometer at 8th and 12th months after planting. Brix is the percentage of weight of soluble solids in juice measured by a hand refractometer (Payne, 1968). Hand refractometer is graduated in percent (1 to 30) and used to determine the level of soluble sugar in the juice squeezed out of crushed sugarcane stalk. Other parameters include flowering behavior, and smut incidence per plot. The extent of flowering was recorded on a rating scale of 1 to 3 (3 = Profuse flowering, 2 = Shy flowering and 1= No flowering). Observation of flowering starts from August to December since, that is the period sugarcane flowers in our environment (Nigeria). Smut incidence was checked every month (starting from germination to harvesting) and the numbers of smutted stalks are taken.

$$\text{Smut per plot (\%)} = \frac{\text{Number of smutted stalk per plot}}{\text{Total number of stalk per plot}} \times 100$$

The collected data were used for analysis of variance (ANOVA) using Crop Stat (version 7.2) package. LSD (least significant difference at 5% level of probability) used for means was significant.

RESULTS AND DISCUSSION

The results showed significant ($P \leq 5\%$) differences among the tested genotypes for most of the studied traits except germination and establishment count, tiller number, plant height (six months after planting), millable canes/plot and number of stalks/stool (Tables 1 and 2). B 47419 variety was characterized by the tallest plants (161.27 cm) and was significantly different from the other genotypes except BD 1354-17, ILS 169-06, BD 1354-20, ILS 708-02, BD 1388-23, ILS 1260-03, BD 1388-31and BD 1388-43. BD 1576-14 had the highest percentage germination (52.8). BD 1354-17 showed higher establishment number (144.3), which was significantly the same with other genotypes. The significant differences noted among the studied genotypes was in accordance with the findings of Arain et al. (2011), who recorded significant differences for plant height while evaluating 11 sugarcane clones for qualitative and quantitative traits under the agro-climatic condition of Thatta.

Table 2 shows the mean values of yield parameters for the hybrid clones at maturity (12 months after planting MAP). The highest number of cane stools per plot was presented in ILS 708-02 genotype which was significantly the same with B47419 and the least cane stools/plot was recorded in BD 1576-31 genotype. The significant variation in stalk length and number of millable canes per plot in this study is in accordance with the result of Muhammad et al. (2014), who reported assessment of sixteen sugarcane varieties in Pakistan. Maximum stalk girth (2.90 cm) was recorded for ILS 708-05 genotype, which is comparatively better than the stalk girth (1.93

Table 1. Mean values of hybrid sugarcane clones at vegetative stage.

GENOTYPE	GERM	EST	TILLER	PLH3	PLH6
B 47419	40.9	128.7	173.3	161.3	287.5
ILS 1576-02	36.1	48.7	61.7	96.5	271.4
ILS 1576-20	40.5	114.3	95.3	88.3	285.9
BD 1576-31	19.5	67.3	67.0	91.5	274.4
BD 1576-07	39.6	125.0	99.3	102.5	293.4
BD 1354-17	36.9	144.3	96.0	126.2	360.8
BD 1576-14	52.8	124.7	158.0	105.5	289.5
ILS 708-05	25.9	76.7	87.7	114.6	264.3
ILS 169-06	35.7	47.3	25.7	124.6	276.4
BD 1354-20	44.9	102.3	114.7	140.3	290.2
ILS 708-02	37.6	117.3	146.7	142.0	291.4
BD 1388-23	33.2	138.3	86.7	144.1	286.7
ILS 1260-03	44.5	73.3	53.7	125.3	285.3
BD 1388-31	51.3	88.3	54.3	141.6	303.3
BD 1388-33	32.1	54.7	33.0	112.9	277.5
BD 1388-43	46.3	69.00	98.0	131.8	288.5
Significances	ns	ns	ns	*	Ns
LSD (P<5%)	30.5	68.5	97.8	42.2	68.9
CV (%)	47.4	43.2	64.7	20.8	14.3

GERM= Germination (%), EST= Establishment Number, Tiller= Number of Tillers, PLH3= Plant height (cm) at 3 months after planting, PLH6= Plant height (cm) at 6 months after planting, ns= not significant, *, **= significant, CV= coefficient of variation. Data represents Means with LSD at P≤5%.

Table 2. Mean values of the yield parameters among hybrid sugarcane clones at maturity.

Varieties	MILLAB	STALKS	STOOLP	STALKG	STALK LNT	SINGLES	CANEY
B47419	196.3	8.7	28.7	1.9	187.2	0.6	89.2
ILS 1576-02	138.3	9.6	20.0	2.2	152.9	0.5	60.2
ILS 1576-20	165.7	7.5	26.0	2.2	186.1	0.8	64.2
BD 1576-31	111.3	7.8	15.0	2.3	174.4	0.9	46.3
BD 1576-07	152.3	8.6	31.0	2.1	170.4	0.5	84.3
BD 1354-17	167.3	9.1	32.7	2.2	206.1	0.8	103.4
BD 1576-14	140.0	7.5	26.0	2.2	179.9	0.7	74.3
ILS 708-05	153.0	7.5	21.3	2.9	202.7	0.9	105.5
ILS 169-06	134.7	9.7	27.7	2.1	213.3	0.8	70.1
BD 1354-20	151.3	8.6	29.7	2.4	211.1	1.0	89.4
ILS 708-02	155.7	5.3	35.0	2.7	214.9	1.1	80.5
BD 1388-23	179.0	7.4	28.3	2.3	203.1	0.8	85.6
ILS 1260-03	103.0	6.0	29.0	2.3	206.5	0.8	77.4
BD 1388-31	124.7	7.7	32.3	2.4	199.8	1.0	102.5
BD 1388-33	76.7	7.9	21.7	2.4	212.7	0.9	65.7
BD 1388-43	141.0	9.5	27.3	2.3	204.1	0.7	83.4
Significances	Ns	ns	*	**	**	*	**
LSD (P<5%)	70.9	2.8	9.0	0.3	13.5	0.3	23.4
CV (%)	29.7	20.9	20.0	7.9	9.6	21.6	17.5

Data represents Means with LSD at P≤5%. Millab= Millable canes/plot, STALKS= Stalks/stool, STOOLP= Stools/plot, STALKG= Stalk girth (cm), STALK LNT= Stalk length (cm), SINGLES= Single stalk weight (kg), CANEY= Cane yield (t/ha), ns= not significant, *, **= significant, CV= coefficient of variation.

Table 3. Mean values of the sugar content (Brix) of the hybrid clones at 10 and 12 months after planting.

Varieties	Brix at 10 months	Brix at 12 months
B47419	19.3	20.7
ILS 1576-02	20.9	24.07
ILS 1576-20	18.0	21.3
BD 1576-31	20.6	23.9
BD 1576-07	22.0	23.8
BD 1354-17	17.9	19.9
BD 1576-14	23.7	24.9
ILS 708-05	18.7	20.9
ILS 169-06	19.7	22.5
BD 1354-20	17.7	20.9
ILS 708-02	21.2	23.7
BD 1388-23	20.5	20.7
ILS 1260-03	19.9	20.2
BD 1388-31	17.6	19.7
BD 1388-33	17.7	21.0
BD 1388-43	19.2	19.9
Significances	**	**
LSD (P<5%)	2.2	1.6
CV (%)	6.8	4.3

BRIX 10= Brix at 10 months (%), BRIX 12= Brix at 12 months (%), ns= not significant, *, **= significant, CV= coefficient of variation
Data represents Means with LSD at P≤5%.

cm) of the commercial Check variety (B47419). ILS 708-02 had the heavier single stalk weight which was significantly not differrent from BD 1576-31, ILS 708-05, BD 1354-20, BD 1388-23, BD 1388-31 and BD 1388-33. The highest cane yield (105.54 t/ha) was recorded in ILS 708-05 genotype, while ILS 1576-02 genotype gave the lowest cane yield (60.22 t/ha) among the other genotypes. The high cane yield expressed by some genotypes in this study can be attributed to diverse genetic composition of the clones which arose from the wide genetic differences of their parents. Maqbool et al. (2001) reported that higher cane yield is the function of higher genetic potential of a variety. The result of cane yield in this study was similar to the work of other researchers that reveal significant differences in cane yield among some sugarcane genotypes. Khan et al. (2003, 2002), Junejo et al. (2002) and Muhammad et al. (2014) reported high cane yield of different sugarcane genotypes in their respective studies.

At ten twelve months after planting, the highest brix (23.70 and 24%, respectively) indicating the sugar content was recorded in BD 1576-14 hybrid clone. This was significantly at par with BD1576-07 and greater than those recorded for other genotypes (Table 3). The results also revealed that sugar content of some genotypes improves with sugarcane crop age between ten to twelve months. This can sometimes be used to identify maturity period of the sugarcane variety. The variation among the studied clones in brix percentage agrees with the report of a study carried out in Nigeria by Kwajaffa and Olaoye (2014); this showed significant variation among 20 genotypes (from 17.8 to 25.0%). Mohammed et al. (2014) reported non-significant differences among some sugarcane varieties for brix percentage and stated that it may be due to the uniform expression of genes for these attributes.

The results in Table 4 showed smut incidence of the studied hybrid clones. It revealed that not all genotypes were affected by the smut disease in the first plant crop including the Check (B47419) when smut incidence was scored at 3, 6 and 10 months after planting. However, eight hybrid clones were infected with smut whip at one time or more during this study. Similar trend had also been reported by Hafiz et al. (2009) that there exist differences in reaction to smut disease among tested cultivars/lines. It was confirmed that the source of resistance against smut exist among the genotypes studied and can be utilized to evolve new high yielding sugarcane varieties.

Table 5 showed differences observed among the studied genotypes for flowering attribute. It was observed that four genotypes (ILS 1576-20, BD 1576-07, BD 1388-23 and BD 1388-33) flower less and three genotypes (B47419, ILS 1576-02 and BD 1388-33) do not flower in

Table 4. Smut incidence observed in the study genotypes at 3, 6 and 10 months after planting.

Varieties	SMUT 3	SMUT 6	SMUT 10
B47419	N	N	N
ILS 1576-02	N	N	N
ILS 1576-20	N	N	1
BD 1576-31	N	N	N
BD 1576-07	N	N	N
BD 1354-17	N	N	N
BD 1576-14	N	N	N
ILS 708-05	N	N	1
ILS 169-06	N	N	1
BD 1354-20	N	N	N
ILS 708-02	N	1	N
BD 1388-23	N	2	1
ILS 1260-03	N	2	1
BD 1388-31	N	N	N
BD 1388-33	1	2	1
BD 1388-43	1	1	2

Note: N= no smut observed, 1-3= smutted stalks/plot (%).

Table 5. Flowering behavior of the hybrid sugarcane clones at 12 months after planting.

Genotypes	Flowering observation at 12 Months
B47419	Nil
ILS 1576-02	Nil
ILS 1576-20	Shy
BD 1576-31	Profuse
BD 1576-07	Shy
BD 1354-17	Profuse
BD 1576-14	Profuse
ILS 708-05	Profuse
ILS 169-06	Profuse
BD 1354-20	Profuse
ILS 708-02	Profuse
BD 1388-23	Shy
ILS 1260-03	Profuse
BD 1388-31	Shy
BD 1388-33	Nil
BD 1388-43	Profuse

this environment (NCRI sugarcane field). The genotypes that flowered profusely were not advanced to multi-locational trial, because flowering is an undesirable attribute under commercial sugarcane production. Flowering results in progressive reduction in cane and sucrose yield if harvesting is delayed in such varieties (Fadayomi et al., 1995).

Conclusion

The genotypes (BD_1354-17, BD_1354-20 and BD_1388-31) that showed tolerance to diseases/pests, high yielding and flower less can be selected for more evaluation under different ecologies. Those genotypes (BD_1576-31, BD_1576-07, BD_1576-14, and ILS_708-

02) that have better sucrose/cane yield but susceptible to diseases/pest and flower profusely can be maintained and used as parents in germplasm for further improvements.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Status and distribution of soil available micronutrients along a hillslope at Ekpri Ibami in Akamkpa Local Government Area of Cross River State, Nigeria

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A field study of the status and distribution of available soil micronutrients along a hillslope of Ekpri Ibami was carried out on a 50 ha land. The aim of the study is to evaluate the micronutrient status and distribution and their relationship with some selected soil properties. A total of 16 soil samples were collected from each pedogenic horizons of four profile pits dug along a hillslope classified as upper slope, middle slope, lower slope and valley bottom. The micronutrients determined in the laboratory were iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) while the soil properties determined were particle size distribution (sand, silt, and clay), pH and organic carbon. The result obtained showed that the soils of Ekpri Ibami are characterized by sandy loam texture with sand= 706.9 g/kg, silt = 93.6 g/kg and clay = 199.5 g/kg. Soil reaction showed that the soils are strongly acidic (5.1) with low organic content (0.73 g/kg). The result of the soil micronutrients indicates that Fe content was high (5 mg/kg) in all the slope positions; copper was rated medium (0.2-2.0 mg/kg); zinc was rated low (0.8 mg/kg) at upper slope and lower slope while the middle slope and valley bottom were rated medium (0.81-2.0 mg/kg); manganese was rated medium (1.1-5.0 mg/kg) in all the slope positions. The results indicate that the sand particles, pH, and organic are the main soil properties which influences availability of micronutrients in the soil due to their significant relationships. The significant correlation among the studied available micronutrients points to the fact that, their abundance and release to plant is controlled by similar factors. The soils will not require supplementary application of Fe rich fertilizer since they are above critical limits of arable production but a complementary supply of copper, zinc, and manganese fertilizers are strongly recommended to enhancing the soil fertility status of the area.

Key words: Micronutrients, hillslope, soil productivity, food production, sustainability.

INTRODUDTION

Micronutrients are important elements that are assimilated by plants in trace amount. In as much as the element are required by the plant in small quantities, vital plant metabolism are impaired or limited if the elements are unavailable thus leading to plant growth dysfunctionality and reduced yield. This also could lead to poor soil fertility status that wholly determines crop productivity level. Furthermore, iron (Fe), manganese (MN)

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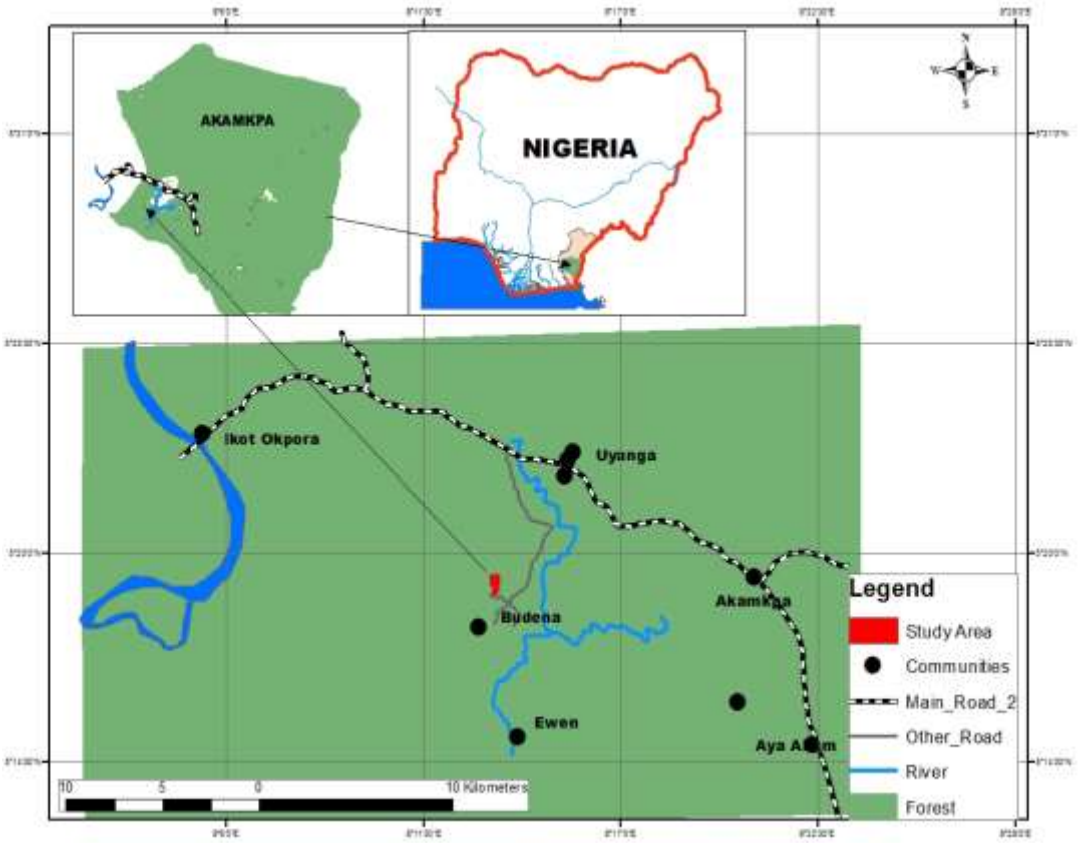

Figure 1. Location map of the study area.

manganese (Mn), copper (Cu) and zinc (Zn) are positively charged micronutrients which form complexes with different enzymes and other organic compound functional groups; Zn cofactor more than 100 enzymes (Shenkin, 2006). These elements are also important in gene display, breakdown of proteins, nuclear acids, growth substance, chlorophyll and secondary metabolites, metabolism of carbohydrates and lipids, stress tolerance and so on (Rengel, 2003; Gao et al., 2008).

The focus on food security and sustainable crop production by the government of Federal Republic of Nigeria through the Ministry of Agriculture and Rural Development by the use of up to date information of nutrient status of soils has become of utmost concern. The status and distribution of available micronutrients within a soil profile system along a land scape has been considered essential for the understanding of the soil inherent ability to sustain an adequate amount or supply of these elements to crop so as to meet the demand of zero hunger by 2030 through sustainable food production (United Nation, 2012).

Several researchers have championed the importance of assessing the soil micronutrient status (Chaudar et al., 2012; Ibrahim et al., 2011; Mustapha et al., 2011) and also its quantities through geostatistical calculations (Garima et al., 2015). This study therefore aimed to evaluate the status and distribution of available Fe, Mn, Cu and Zn in soil profiles along a hillslope at Akamkpa Local Government Area of Cross River State.

MATERIALS AND METHODS

Study location

Cross River State is on a latitude of 6°10'2" N and longitude of 8°39'36" E of the Greenwich meridian. The study was at Ekpri Ibami area which lies between latitudes 05°18'53" and longitude 08°13'25" at an elevation of 82 to 111 m above sea level located in the tropical sub-humid environment, within Akamkpa Local Government Area of southern Cross River State (Figure 1).

Climatic condition of the study area

The study area experiences humid tropical climate with distinct wet and dry seasons. Rainfall ranges between 1500 and 3500 mm per annum while the relative humidity was between 80 and 90%. The mean annual temperature is between 25.4 and 27.5°C (NIMET, 2015). Rainfall, relative humidity, temperature and sunshine data for the study area were adapted from Calabar weather station of the Nigerian Meteorological Agency since the study area (Akamkpa)

Table 1. Physicochemical of properties of Agricultural land of Ekpri Ibami.

Soil parameter	Range	Mean
Sand (g/kg)	496.4-875.2	706.9
Silt (g/kg)	29.6-143.6	93.6
Clay (g/kg)	80-380	199.5
Textural class	Sandy loam	-
pH (H_2O)	3.9-5.1	5.1
OC gkg^{-1}	0.04-2.59	0.73

falls within 100 km range of this synoptic station as recommended by Afangide et al. (2010).

Geologic formation

The basement complex rocks occupy about 10,000 km^2 in Southeast Nigeria (Ekwueme et al., 1990), out of which about 40% is found in Cross River State which makes up the Oban-Obudu massif and, a continuation of the African-pan Basement Complex of the Cameroun highlands. The characteristics of the material reflect the processes that form the underlying and the influence of the environment where they occurred.

Vegetation and land use

Southern Cross River State falls within the tropical rainforest climatic zone. According to FDALR (1990), the vegetation of the study area is predominantly secondary forest re-growth. Annual crops identified consist of *Zea mays*, *Manihot* species, *Oryza sativa*, *Musa* species, *Dioscorea* species, and perennial crops such as *Carica papaya*, *Elaeis guineensis*, *Hevea brasiliensis* and *Irvingia gabonensis*. Dominant trees, climbers, and shrubs such as *Daniella oliveri, Ficus* species*, Khaya senegalensis, Laxifora* species*, Combretum* species*, Alcohornea* species*, Andropogon* species*,* and *Digitaria* species, are scattered almost evenly while African bamboo trees grow wildly near the streams and lowland areas.

Sampling method

The study was carried out on a 50 ha (500,000 m^2) land. Four (4) profile pits were dug along the hillslope of Ekpri Ibami representing upper slope, middle slope, lower slope and valley bottom geomorphic positions labeled as I, II, III and IV, respectively. Soil samples were collected from each identified and described pedogenic horizons of the four profile pits making a total of sixteen samples and transported to the laboratory for analysis.

Laboratory analysis

Particle size distribution was determined using the hydrometer method as described by Bouyoucos (1957). Soil pH was determined in 1:1 soil water suspension with a glass electrode pH meter. Organic carbon was determined by the wet oxidation method of Walkley and Black (1934). The extractable micronutrients: Zn, Cu, Fe and Mn, were extracted using 0.1 M HCl solution (Osiname et al., 1973) and determined on an atomic absorption spectrophotometer at a specific wavelength.

Statistical analysis

The result was subjected to discrete statistics and a Pearson correlation coefficient was used to show the relationships between micronutrients and some selected soil properties.

RESULTS AND DISCUSSION

The results of the laboratory analysis are shown in Tables 1 and 2.

Physicochemical properties of soils of Ekpri Ibami hillslope

The particle size distribution of soils of Ekpri Ibami hillslope showed that sand content ranged from 496.4 to 875.2 g/kg, silt from 29.6 to 143.6 g/kg and clay from 80 to 380 g/kg with mean values of 706.9, 93.6 and 199.5 g/kg, respectively. This suggests that the soils are coarsed-textured, covering 70.6% of the agricultural land and may be faced with the problem of water and nutrient adsorption for plants. This could be attributed to the parent material from which the soils originate from (Akamigbo, 1984). The soil pH ranged from 3.9 to 5.1 with a mean value of 5.1 and rated as strongly acidic (<5.5) based on the rating suggested by Karltun et al. (2013). This pH value of the soils has been established not to support some plant nutrients, thus not optimally available to plants for uptake, plus this range of pH is not compatible to plant root growth (Tisdale et al., 2003). Accordingly, the organic carbon content of the soils ranged from 0.04 to 2.59 gkg^{-1} with a mean value of 0.73 gkg^{-1}. Status of the organic carbon was totally low when compared with the rating provided by Adaikwu et al. (2013).

Status and distributions of micronutrients

Available iron (Fe)

The DTPA extractable iron content in the soils ranged from 69.5 to 109 $mgkg^{-1}$ (mean 92.8 $mgkg^{-1}$), 84.7 to 180.9 $mgkg^{-1}$ (128.1 $mgkg^{-1}$), 42.57 to 111.16 $mgkg^{-1}$ (mean 87.75 $mgkg^{-1}$) and 103.71 to 142.32 $mgkg^{-1}$ (116.69 $mgkg^{-1}$) for upper slope, middle slope, lower slope and valley bottom, respectively (Table 2). The result obtained showed that the iron content of the soils

Table 2. Profile distribution of extractable micronutrient.

PEDON No.	Depth (cm)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)
UPPER SLOPE PEDON I N05° 19' 16.84" ; E008° 13' 36.1" ; 108 m ASL					
Ap	0-17	94.5	0.32	0.30	2.38
Bt1	17-62	98.2	0.22	0.38	3.16
BC	62-122	69.5	0.29	0.21	2.04
Crt	122-200	109	0.36	1.47	2.23
	Mean	92.8	0.30	0.59	2.45
	CV	18.0	19.90	100.13	20.05
MIDDLE SLOPE PEDON II N05° 19' 25.3" ; E008° 13' 35.7" ; 108 m ASL					
Ap	0-16	180.9	0.23	1.03	2.02
Bt1	16-65	145.5	0.27	1.10	2.37
Bt2	65-112	84.7	0.43	0.69	2.70
Cr	112-200	101.3	0.37	0.46	3.47
	Mean	128.1	0.33	0.82	2.64
	CV	34.0	28.14	36.52	23.45
LOWER SLOPE PEDON III N05° 19' 11.3" ; E008° 13' 36.8" ; 102 m ASL					
Ap	0-8	99.03	0.32	0.42	1.08
Bt1	0-53	42.57	0.45	1.07	2.04
Bt2	53-114	98.25	0.26	0.63	1.06
Crt	114-200	111.16	0.34	0.66	2.67
	Mean	87.75	0.34	0.70	1.71
	CV	34.98	23.16	39.11	45.85
VALLEY BOTTOM PEDON IV N05° 19' 23.3" ; E008° 13' 33.8" ; 102 m ASL					
Ap	0-19	103.71	0.36	0.57	3.06
Bt1	19-60	106.08	0.23	0.72	3.94
BC	60-124	142.32	0.20	1.06	4.37
Cr	124-200	114.63	0.58	1.26	3.64
	Mean	116.69	0.34	0.90	3.75
	CV	15.19	50.48	34.83	14.67

of Ekpri Ibami hillslope was high (>5.0 $mgkg^{-1}$) when compared with the rating by Esu (1991). The high iron (Fe) content obtained in the soils may be attributed largely to weathering of biotite ($Al_{1.24}Fe_{1.4}H_{1.64}K_{0.98}Mg_{0.71}Na_{0.02}O_{12}Si_{1.36}Ti_{0.16}$) as a result of the geological formation of the study area been basement complex origin. Similarly, it is known that soil parent material has an influence on chemical compositions of soil (Irmak et al., 2007). The result of iron content obtained in soils of Ekpri Ibami under a humid rainforest condition was similar to that of Gubi Bauchi North (44.75 $mgkg^{-1}$) under a Sahel Savannah condition.

The high Fe content in the soil (above the limiting value of 2.5 mg/kg for crop production) connotes that Fe deficiency is not likely for crops grown on these soils. However, the presence of Fe in high concentrations in soils could lead to its precipitation and accumulation and upon complex chemical reactions leading to the formation of phlintite (laterite). This upon alternate wetting and drying could irreversibly yield hard consolidated material (petrophlintite or ironstone) which could restrict root penetration and drainage. This observation is similar to that of Ephraim (2012).

Available copper (Cu)

Table 2 shows that copper (Cu) in the soils ranged from 0.22 to 0.36 mg/kg (mean = 0.30 mg/kg), 0.23 to 0.43 mg/kg (mean = 0.33 mg/kg), 0.26 to 0.45 mg/kg (mean = 0.34 mg/kg), and 0.20 to 0.58 mg/kg (mean = 0.34 mg/kg) for upper slope, middle slope, lower slope and valley bottom, respectively. The result obtained shows that the soils falls within the medium (0.2-2.0 mg/kg) micronutrient fertility rating for copper content as given by Esu (1991). Generally, copper shows an irregular

Table 3. Pearson correlation coefficient (r) between soil properties and micronutrients.

Correlation	Fe	Cu	Zn	Mn	Sand	Silt	Clay	pH	OC
Fe	1								
Cu	-0.410	1							
Zn	0.378**	0.281**	1						
Mn	0.203**	0.026**	0.169**	1					
Sand	0.314**	0.085**	0.018**	0.508**	1				
Silt	-0.345	-0.141	-0.549	-0.156	-0.505	1			
Clay	-0.224	-0.042*	0.190**	-0.518	-0.944	0.192**	1		
pH	0.232**	-0.298	0.015**	0.121**	0.463**	-0.291	-0.415	1	
OC	0.175**	-0.146	-0.020*	-0.012*	0.282**	-0.168	-0.256	0.236**	1

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level. OC: Organic carbon.

distribution down the profile in all the distinct landscape positions while the highest been obtained at the lower slope and valley bottom; this could be attributed to contribution of surface washed materials deposited.

Available zinc (Zn)

Table 2 shows the result of zinc (Zn) content in the soils. Extractable zinc was rated low based (<0.8 mg/kg) on the critical values of Esu (1991) at the upper slope and valley bottom while the middle slope and valley bottom were rated medium (0.81-2.0 mg/kg). The “low status” soils would require Zn fertilization for a better arable crop production. In general, Zn was observed to follow an irregular increase and decrease pattern with depth in all the landscape positions. Singh and Shukla (1985) and Bassirani et al. (2011) reported similar results, this also conforms to the findings of Ephraim (2012) in soils in Gubi, Bauchi North, Nigeria.

Available manganese (Mn)

Manganese (Mn) content in the soils of Ekpri Ibami ranged from 2.04 to 3.16 mg/kg (mean = 2.45 mg/kg), 2.02 to 3.47 mg/kg (mean = 2.64 mg/kg), 1.06 to 2.67 mg/kg (mean = 1.71 mg/kg) and 3.06 to 4.37 mg/kg (mean = 3.75 mg/kg) at the upper slope, middle slope, lower slope and valley bottom, respectively. The manganese content showed an irregular increase and decrease with depth in all the profiles which is similar with the result obtained by Onyekwere et al. (2017) and Ephraim (2012). According to the rating of Esu (1991), it is rated ‘medium’ in all the horizons of the profile. This suggests that the soils Mn content is above the critical available range of <3 mg/kg reported by Lindsday and Norveil (1978) and >1.0 mg/kg reported by Esu (1991). This finding is in contrast with the report by Haque et al. (2000), Beyene (1982), Dibabe et al. (2007) and Tena and Beyene (2011) who reported that amount of extractable Mn is generally high in the tropical soils and Mn toxicity is even more common than deficiency.

Relationship of available micronutrients with soil properties

Presented in Table 3 is the coefficient of correlation ($p<0.05$ and $p<0.01$) between available micronutrients and some soil physicochemical properties at Ekpri Ibami hillslope. This affirms the study by Brady (1995) who highlighted the significant influence of micronutrients and soil physicochemical properties. As shown in Table 3, iron (Fe) gave a significant correlation with Zn (r = 0.378**), Mn (r = 0.203**), Sand (r = 0.314**), pH (r =0.232**) and Organic carbon (r = 0.175**) at 5% level of significance indicating that as one parameter increases there is also a corresponding increase with the other. Copper (Cu) showed a positive correlation with Zn (r =0.281**), Mn (r = 0.026**) and Sand (r = 0.085**) but gave a negative but significant correlation with clay (r = -0.042*). Zinc (Zn) gave a positive and significant correlation with Mn, sand and clay but a negative significant correlation with organic carbon. While manganese (Mn) gave a positive correlation with sand (r = 0.508**) and pH (r = 0.121**) but a negative significant correlation with organic carbon (r = -0.012*).

Sand gave a positive correlation with pH and organic carbon; silt a positive and significant correlation with clay while pH gave a positive and significant correlation with organic carbon (r = 0.236**). This contradicts the results of Sidhu and Sharma (2010) and Kumar and Babel (2011) who reported that the available micronutrients increased with increase in organic carbon but corresponds with decreased with increase in pH. Tisdale et al. (2003) stated that micronutrients react with soil organic matter to form stable complexes; these micronutrient cations which are bound by organic matter are more available to plants than the inorganic forms. The findings of this study affirms the report of Brady and Weil (2002) and Esu (2010) who stated that the availability of most micronutrients in soils depends on soil organic carbon content.

Conclusion

The status and distributions of available Fe, Cu, Zn and Mn at the hillslope of Ekpri Ibami proposes a complementary supply of copper, zinc and manganese fertilizers and organic manure to sustain food crop production in the studied soil, while iron is not likely to be limited in such soils. The results indicate that the sand particles, pH and organic are the main soil properties which influences availability of micronutrients in the soil due to their significant relationships. The significant correlation among the studied available micronutrients points to the fact that, their abundance and release to plant is controlled by similar factors. So it is recommended that for sustainable food production and availability continuous use of organic materials: farm yard manure and crop residue. Also, the farmers should embrace management practices such as minimal tillage which will in turn improve soil fertility and maintain the availability of micronutrients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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