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Bioactivity of Plant Extracts against Cowpea Bruchid *Callosobruchus maculatus* (Fab): A Review

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ABSTRACT

The objective of the present review is to know the bioactive compounds of the plant extract against *Callosobruchus maculatus*. Plants are able to produce a large number of bioactive compounds. Plant extract was found to have a wide range of bioactive compounds like Alkaloids, Carbohydrates, Starch, Glycosides, Flavonoids, Triterpenoids, Resins, Saponins, Steroid, Proteins and Tannins. The high concentration of phytochemicals protects against the *Callosobruchus maculatus*. Recent revelations have shown that synthetic insecticides were found to penetrate into grains and may be toxic. Natural products such as botanical insecticides may provide suitable alternatives. The review obviously designated that plant products have potentials of controlling *Callosobruchus maculatus* in stored cowpea as they are safe, free of residue and strong biological activities that are eco-friendly and biodegradable.

Key words: *Callosobruchus maculatus*, Cowpea, Pest management, Plant products.

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Cowpea [*Vigna unguiculata* (L.) Walp.] is an important grain legume in the tropics and subtropics. It is a native to central Africa and belongs to the family Fabaceae (Cobley, 1956) and is eaten in the form of grain, green pods and leaves (Adejumo, 2012). Cowpea is known as vegetable meat due to high amount of protein in the grain with better biological value on dry weight basis. The grain contains 26.61% protein, 3.99 % lipid, 56.8% carbohydrates, 8.60% moisture, 3.84% ash, 1.38% crude fibre, 1.51% gross energy and 54.85% nitrogen free extract (Owolabi, 2012). It is mostly grown as an intercrop with sorghum, maize and millet (Asiwe, 2006). Cowpea is generally preferred by farmers because of its role in increasing soil fertility through nitrogen- fixation (Blade *et al*, 1997) and production of nutritious fodder for livestock. Insect pests are considered to be largely responsible for this, as their attack can result in 90-100% yield reduction (Jackai and Daoust, 1986). Cowpea (Oyewale and Bamaiyi, 2013) is mostly grown in tropical and sub-tropical regions in the world for vegetable and grains and to lesser extent as a fodder crop. It is a most versatile pulse crop because of its smoothening nature, drought tolerant characters, soil restoring properties and multi-purpose uses. Cowpea seeds are damaged during storage by the cowpea seed bruchid (*Callosobruchus maculatus*) (Fatope *et al*., 1995). The genus Callosobruchus includes at least 20 species, originated mostly from Asia and Africa and occurring mainly in tropical and subtropical regions of the world (Tuda and Chou, 2005). Some of the most common species include *Callosobruchus maculatus* (Fab.), *C. chinensis* (L.), *C. subinnotatus* (Pic.), *C. analis* (F.) and *C. rhodesianus* (Pic.) (Southgate, 1978). The host they infest are a variety of beans such as Vigna, (*Vigna unguiculata* L. Walpers, cowpea; *V. radiata* L. Wilczek, mungbean; *V. subterranea* L. Verdcourt, bambara groundnuts) and other leguminous seeds *viz* chickpea (*Cicer arietinum* L.), green gram (*Phaseolus aureus* Roxb.), black gram (*Phaseolus mungo* Roxb.), red gram (*Cajanus cajan* L.), lentil (*Lens culinaris* Medik.), soyabean (*Glycine max* Mer.), pea (*Pisum sativum* L.), peanut (*Arachis hypogaea* L.) and haricot beans (*Phaseolus vulgaris* L.) (Tuda and Chou, 2005). Callosobruchus maculatus, the cowpea weevil is the most important pest of cowpea (*Vigna unguiculata* L.) during storage (Edde and Amatobi, 2003). Callosobruchus spp. can cause damage of legume seeds up to 100% during storage (Gbaye *et al*. 2011). On an average, damage of pulses caused by these bruchid insects during storage may count to 5-10% in the temperate and 20-30% in the tropical countries (Kiradoo and Srivastava 2010). The adult female lays eggs on the seed surface and the hatching larvae burrow into the seed. The whole development takes place inside a single seed and the adults emerge out by leaving behind holed seed (Messina and Jones 2009). More than one larva can develop within a single grain. Damaged legume seeds have thus reduced weight, become unsuitable for human or animal consumption and have poor germinating ability (Elhag, 2000). Heavy infestation can lead tomouldiness and reduction in commercial value of the seeds (Kiradoo and Srivastava, 2010). Crop losses due to such pests are direct, as well as indirect. Apart from

their direct damage to the stored legume grains they also create conditions that bring secondary infestation by rot organisms mainly fungi and subsequent mycotoxin contamination.

Generally, the infestation starts in the field (Nahdy, 1995) where the adults lay eggs on green or drying pods. Eggs adhere on the surface of pods from where the first instar larvae bore into the seeds through the pod cover. During threshing, no clear-cut evidence or symptoms of damage is visualized (Nahdy *et al.,* 1999). Infestation in the field has no serious implications as the damage in the field is low. However, once infested seeds are stored, huge damage occurs due to rapid multiplication of insects in a very short time (Taylor 1981). Although beginning of infestation occurs from field, no one denies from the cross infestation which also occur in food commodities during storage (Nahdy *et al.*, 1999).

Reports show the polyphenism i.e. the production of above one adult morphs in the life cycle of Callosobruchus spp. in response to environmental variations. They have two distinct adult morphs; a nonflying, inactive, normal or sedentary morph and a flight or active morph (Nahdy *et al.,* 1999; Zannou *et al.,* 2003). This polymorphism of bruchids get up from their different ecological niches (Messina 1990). The adults of flight morph are less fecund, adapted to field infestation during the rainy season and lay eggs on the maturing pods (Huignard *et al,* 1985). After 1 month, when the infested seeds are harvested and stored, adults of the flightless morph emerge. In *Callosobruchus maculatus* conditions like low larval densities, abundant food, high moisture content, intermediate photoperiods and lower temperatures promote the inactive form to develop (Utida, 1972). Being sexually active, they multiply rapidly up to 4-5 generations and after higher larval densities or due to genetic predispositions, adults of the flight morph begin to emerge (Arnold *et al.,* 2012). Study shows that this type of polymorphism is brought by the increase in temperature, seed water content, larval density and post embryonic development (Zannou *et al.*, 2003). The flight morphs are equipped survive until the next rainy season for repeating the cycle (Monge and Huignard, 1991). This polyphenism in the life cycle of bruchids augments their capacity for infestation of agricultural commodities. In this present study, we explain to expression in to the accomplishments of the use of plant products in the managing and control of *Callosobruchus maculatus* (Fab.).

Importance and uses of cowpea

Cowpea [*Vigna unguiculata* (L.) Walp] is an important grain legume in the diet of many people in the third world countries as it provides not only high-quality protein (26.61%) but also constitute the cheapest source of dietary protein for low income sectors of the population (Rachie and Singh, 1985). It is also a good source of carbohydrate (56.8%) calcium, iron, vitamin B and carotene. Cowpea is mainly grown in tropical and subtropical regions in the world for vegetable and grain and to lesser extent as a fodder crop. Although cultivated primarily for its edible seeds, direct consumption of cowpea leaves is also widespread in Africa (Rachie, 1975). In fresh form, the young leaves, immature pods and beans are used as vegetables, while snacks and main meal dishes are prepared from the dried grain (Nelson *et al.*, 1997). In India both green and dried cowpea seeds are suitable for canning and boiling as well.

Pests of cowpea

Plant insect pests, diseases and weeds carry out a serious threat to crop production. Population of weeds, insect pests and diseases have increased over the years especially by the introduction of monoculture farming in the country (Jackai and Adalla, 1997). Cowpea is a crop that is widely reported to be attacked by an array of insect pests and diseases. Adebiyi and Tedela, (2012) reported that the cowpea bruchid is the major threat to this plant. Over 130 species of insects have been reported in cowpea cultivation (Ahmed *et al*., 2009). Hamman *et al.*, (2012) identified the following as some of the insects: Cowpea aphids, *Aphis craccivora* (Koch), Giant coreid bug, *Anoplocnemis curvipes* Stol (Hemiptera: Coreidae); Thrips, *Megalurothrips sjostedti* Tryb. (Thysanoptera: Thripidae); Flower or pollen beetle, *Mylabris spp.* Fab. (Coleoptera: Meloidae); Spiny Brown bug, *Clavigralla tomentosicollis* Germ. (Hemiptera: Coreidae); Green stink bugs, *Nezara viridula* Linn. (Hemiptera: Pentatomidae), thrips (*Megalurothrips sjostedti*), *Ootheca sp.*, *Clavigralla sp.,* and Cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). There are numerous important insect pests of cowpeas worldwide and most locations have 2-4 species being key pests. The maximum damaging pests are flower bud thrips, Megalurothrips sjostedti Tryb. (Thysanoptera : Thripidae), the legume pod borer, Maruca vitrata Fab. (Lepidoptera : Pyralidae) and the pod sucking bug (PSB) compound of which Clavigralla spp. Stal. (Hemiptera : Coreidae), Anoplocnemis curvipes Fab. (Hemiptera : Coreidae), Riptortus dentipes Fab. (Hemiptera: Alydidae) and Aspavia armigera are the maximum damaging.

Damage caused by the pests

The cowpea weevil [*Callosobruchus maculatus* (Fabricius)] is the greatest important postharvest storage pest of cowpea. The weevils occur wherever the cowpea is grown. The adult beetle is minor (3 mm long) and orange-brown with dark markings. The adult lays eggs on the pods that are at maturity stage in the field and on hatching the larvae bore the pod wall and seed coat and enter the seed. Messina (1984) reported high mortality of larvae in the field due to failure of larvae to penetrate the seed after drilling through the pod wall. The adult emergence occurs after harvest (Booker, 1967) in the store where real destruction happens due to re-infestations and easiness of larvae penetration into the seed because usually the seeds are stored after shelling. Re-infestation occurs repeatedly during storage period. In store, each female lays 40-60 white flat eggs and

glues it on the seeds surface; on hatching the larva bore into the seed, where it feed, grow and pupate before developing as adult out of the seed after about 3-4 weeks. A single seed can be infested with multiple larvae (Giga and Smith, 1983; Messina, 1993). It is reported that about 8-10 or more larvae can be found in a single seed. Thus, heavily damaged seeds show numerous exit holes (Ofuya and Agele, 1990). Both sexes can mate soon after appearance and they require neither food nor water to reproduce and can mate several times during their life time. The beetle permanency is slightly affected by relative humidity (Giga and Smith, 1983). Both sexes live an average of 7 days (Messina, 1993). The complete life cycle takes about five weeks; this means that a new generation rises every month during storage. An infestation of up to 100% of the stored seeds has been reported within 3 to 5 months under farmer's storage conditions (Redden *et al.,* 1984; Singh, 1980). The reduction in seed weight is directly proportional to the number of exit holes on the seeds, thus the yield losses can be easily estimated for different accession (Singh *et al.,* 1983). A single beetle is talented to cause a weight loss of grain of up to 3.5% (Booker, 1967).

Survival, oviposition and progeny development of *C. maculatus* in stored cowpea

Cowpea bruchids, *Callosobruchus maculatus* are among the major insect pests of legumes which cause high infestation in cowpea both in the field and in storage (Kang *et al.,* 2013). Cowpea grain stored after harvest is the favorite food of the bruchids (Baributsa, 2010). Initial infestation of cowpea starts in the field just before harvest and the insects are carried into the store where the population builds up rapidly (Deshpande *et al.*, 2011). They lay eggs on the pods of legume hosts as they approach maturity in the field but emergence usually occurs after harvest. In storage, *Callosobruchus maculatus* lay eggs on the seeds and larval development and pupation are completed entirely within a single seed. Although the larvae are the major destructive stage because adult cowpea bruchid do not feed.

The female lays her eggs on the cowpea seed (Fig 1) which hatch in about a week and each tiny grub-like larva bores through the bottom of its egg and into the seed where it feeds, grows and develops, passing through four larval and one pupal stage. This takes about four weeks and once an adult female emerges from the seed, finds a male and mates then begins to lay eggs on other cowpeas. Bruchid, *Callosobruchus maculatus* passes through four main stages in their life cycle which are egg, larva, pupa and adult as shown below in Fig 2.

Management of Cowpea Pests

Due to the devastating effect of insect pests of Cowpea at almost every stage of its development, several approaches have been adopted to control the cowpea pest *C. maculatus*. Research into the control of these pest has centred primarily on the use of synthetic insecticides (Echendu, 1991).



Fig 1: Cowpea seeds after being infected by bruchids, *C. maculatus*.

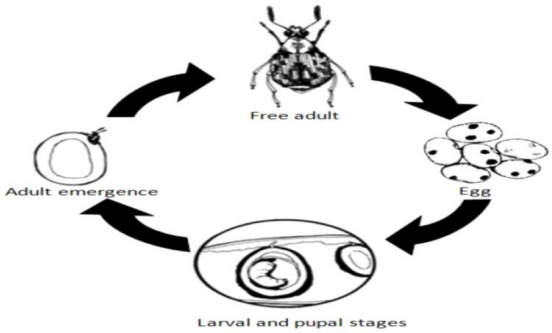


Fig 2: The life cycle of *C. maculatus*.

Dursban and Dimecron, which have been found to be effective against the pests. Over the years, chemical pesticides had made a great contribution to the fight against pests and diseases (Echendu, 1991). However, their widespread and long-term use resulted in insecticide resistance and biomagnifications of insecticides, which in turn resulted in restrictions on their export problems like soil and water contamination and dramatic increase of the damaging residues in many primary and derived agricultural products arose, which endangered both the general environment and human health. Previously insect pests of cowpea have mainly been controlled with synthetic insecticides (Shan, 1997). The use of synthetic organic insecticides in crop pest control programs around the world had caused marvellous damage to the environment, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms. Several management strategies are available such as use of botanical insecticides are naturally occurring chemical extracted from plants which break down readily in the soil and are not stored in plant or animal tissue, insect resistance cowpea variety.

Bio pesticides for *Callosobruchus maculatus*

Abdullahi (2011), Ratnaskera and Rajapkse (2012), Yusuf *et al*. (2011), Ekeh *et al*. (2013) and Adebiyi and Tedela, (2012) independently reported the use of plant materials as biopesticides as alternative to use of chemicals. The use of plant derived biopesticides has the following advantages: plant products are more readily available, biodegradable and less toxic to non-target organisms, selective in action and capable of retarding development of resistance

(Rahman and Talukder, 2006). Abdullahi, (2011) stated that plant materials are safe to biocontrol agents. As a result of the problems of pesticide resistance and negative effects on non-target organisms including man and the environment, chemical pesticides have been reportedly banned in developed countries (Ebenezer, 2010). These resuscitated the idea of botanical insecticides as a promising alternative to pest control. Botanical insecticides are naturally occurring chemical extracted from plants which failure readily in the soil and are not stored in plant or animal tissue. Frequently their effect is not long lasting as those of synthetic pesticides. Botanical insecticides are generally pest-specific and are relatively meaningless to non-target organisms. They are biodegradable and harmless to the environment. Also, the possibility of insect developing resistance to botanical insecticide is less possibility (Isman, 2010). Over 2000 species of plants are known to possessed insecticidal activities.

Anbu Radhika and Sahayaraj (2016) observed the insecticidal activity of pungam oil, they conclude pungam derivatives leads to death of sucking and defoliating insects. The grain safety activity of neem seeds and tobacco extracts in practice for more than 300 year in Indian and Europe (Jotwani and Srikar, 1965, Kulkarni and Joshi, 1998). The formers in Tamil Nadu and Karnadaka use Vitex negundo and Karanja as grain protectants (Ahmed *et al*., 1997). Biocidal activities of some other plants *viz*. *Amaranthus spinosus*, *Apios Americana*, *Brassica nigra*, *Carica papaya andrographis paniculata, Datura metel, Madhuca indica, Moringe oeifera, Ocimum basillicum, Piper nigram, Trigonella foenumgraecum etc.*, have also been detail reviewed by Khatri and Meshram, 2014.

Despite this only a few have been scientifically evaluated (Schmutterer, 1990), *Petiveria alliacea* which is commonly known as Anamu belongs to the family phtolaccacea (Ojo, 1996) reported several biological compounds in the root of *P. alliacea* which comprise: benzalhyde, dibenzyltrisulfide, cis and trans-stibene *e.t.c* of which dibenzyltrisulfide is insecticidal compound. The use of Cashew Nut Shell Liquid (CNSL) has been gaining more attention due to its possession of the active Phenolic compounds, Anacardic acid and Cardol, which also have corrosive and abrasive properties (Lale, 1995). It was demonstrated that low concentration of CNSL could be effective in the management of *C. maculatus*. Similar work was also reported in preventing oviposition in *C.maculatus* (Olotuah and Ofuya , 2010). Research in current years has been revolving more towards selective botanical pesticides, usually apparent to be safer than the synthetic (Arnason *et al*, 1995), while, extensive works on the use of plant extracts in pest management were also documented the use of inexperience and safe protectants of plant origins was extensively reviewed (Mordue, 1993).

Plant extracts used as bio pesticides-powders

Ahmed *et al.,* (2009) reported the parts of the plant used include leaves, stems, seeds or roots. The plant parts would be washed and either air or shade dried, ground and sieved in to powder. The materials are grounded into powder using mortar and pestle or grounded electronically using blender (Ahmed *et al*., 2009; Ogunwolu and Idowu, 1994). It will then be sieved using sieve of appropriate sizes. The resulting fine powder was used in dusting the surface of the cowpea. Application was based on weight/weight (w/w) basis. The powder properly coated the surface of the cowpea seeds before was stored. Adenakan *et al*. (2013), Ekeh *et al*. (2013), Asawalam and Dioka (2012), Yusuf *et al*., (2011) and Ogunwolu and Idowu (1994) reported pesticidal properties of different parts of plant materials such as garlic, Chillies, *Moringa, Dennitia tripetela, Curcuma longa* (Turmeric), Neem and *Clausena* on *Callosobruchus maculatus*. Pirimiphos-methyl caused 100% control (mortality) of *Callosobruchus maculatus* but the performance was not significantly different ($p \geq 0.05$) with the leaf powder of *Clausena anisata* (76.8). The other powders of the plant materials were significantly ($P \leq 0.05$) better than the control (5.0). Similarly, the use of plant materials has significantly reduced the percentage seed perforation. The result showed that there was no significant difference ($p \geq 0.05$) between leaf powder (2.0) and synthetic chemical (0.0). The highest percentage seed perforation was recorded in the control (96.7). Some plant extracts and their parts used by various researchers are listed in Table 1.

Plant materials to be prepared were pulverized and the appropriate quantity soaked in water and the mixture was shaken and stirred thoroughly and applied directly or can be allowed to stand overnight and the mixture is filtered over a muslin cloth (Ahmed *et al*., 2009 and Ahmed *et al*., 2007). The filtrate obtained formed the extract that was diluted with an appropriate quantity of water to form the spray solution (Oparaeke *et al*., 2005; Ahmed *et al*., 2009). Ogunwolu and Idowu, (1994) reported that the crude extract is standardized in methanol: water solvent. The suspension was evaporated, acidified and extracted with chloroform. This was either obtained using water or alcohol as aqueous or ethanol extract. The powder of plant materials was soaked in and left overnight. The mixture was shaken and filtered over clean muslin cloth and the filtrate is now used in biocontrol. Adebiyi and Tedela (2012), Ratnasekera and Rajapkse (2012), Abdullahi (2011), Rahman and Talukder (2006) and Ogunwolu *et al*. (2002) reported different pesticidal properties of some plants such as Moss plant, Neem, Desert date and *Clausena* on *Callosobruchus maculatus*. Adebiyi and Tedela, (2012) showed the effect of extract in the control of *Callosobruchus maculatus*. The result showed that both the water and ethanol extract of *Barbula indica* were able to cause mortality of *Callosobruchus maculatus* significantly ($p \geq 0.05$) better than the control. The highest mortality was achieved with the application of 4 g 100-1mls (63.33 and 83.33) and the lowest mean *Callosobruchus maculatus* mortality was recorded in the control (3.33 and 16.67) for water and ethanol extract, respectively.

Table 1: Some examples of plant materials and the different parts used as.

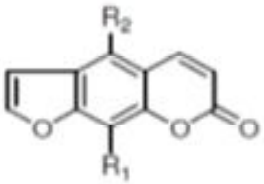
Plant materials	Common name	Family	Parts used	Source
Citrus sinensis O.	Sweet orange	Rutaceae	p	Yusuf *et al.* (2011)
Allium sativum L.	Garlic	Liliaceae	B	Ekeh *et al*. (2013)
Azadirachta indica (A. Juss)	Neem	Meliaceae	S,L	Ogunwolu and Idovru (1994)
Balanites aegyptiaca (L.)	Desert date	Balaniteceae	L	Abdullahi (2011)
Annona spp (L.)	Anona	Annonaceae	L	Ratnasekera and Rajp aske (2012)
Capsicumfructscens	Chilli pepper	Solanaceae	F	Yusuf *et al.* (2011)
Allium cepa (L.)	Onion	Solanaceae	B	Ahmed *et al.* (2009)
Clausena anisata H.		Rutaceae	L, SB, RB	Ogunwolu *et al.* (2002)
l,foringa oleifera Lam	Drum stick	Moringaceae	F, L, S, R	Adenakan *et al.* (2013)
Arachis hypogea L.	Groudnut	Leguminosae	O	Adenakan *et al*. (2013)
Ocimum gratissimum L.	African basil	Lamiaceae	L	Ekeh *et al.* (2013)
Annonasenegalensis	custard-apple	Annonaceae	SB	Fatope *et al.* (1995)
Pers.Balanitesaegyptiaca	soap berry	Zygophyllaceae	R	Fatope *et al.* (1995)
Entada africanaBuill and Perr.	Senegal	Mimosaceae	L	Fatope *et al.* (1995)
Hyptissuaveolens Poit.	Pignut	Labiatae	SH	Fatope *et al.* (1995)
Khayasenegalensis A. Juss.	Gambia mahogany	Meliaceae	SB	Fatope *et al.* (1995)
Mitracarpus Scaber Zucc	button grass	Rubiaceae	SH	Fatope *et al.* (1995)
Parkiaclappertoniana Keay.	African Locust Bean Tree	Mimosaceae	SB	Fatope *et al.* (1995)
Prosoplsafricana Linn	African mesquite,	Mimosaceae	SB	Fatope *et al.* (1995)
*Sclerocaryablrrea (A. Rich).*Höchst.	morula	Anacardiaceae	SB	Fatope *et al.* (1995)
Spenocleazeylanlca Gearth	Goose Weed	Sphenocleceae	SH	Fatope *et al.* (1995)
Allium sativum	*garlic*	Amaryllidaceae	*B*	Edwin, I.E., Jacob, I.E, (2017)
Cordia millenii	manjack	Boraginaceae	S	Edwin, I.E., Jacob, I.E, (2017)
Monodora myristica	seeds of Africa nutmeg	Annonaceae	S	Edwin, I.E., Jacob, I.E, (2017)
Xylopiaaethiopica	negro pepper	Annonaceae	FR	Edwin, I.E., Jacob, I.E, (2017)
Zingiber officinale	ginger	Zingiberaceae	RZ	Edwin, I.E., Jacob, I.E, (2017)
Dysphania ambrosioides	wormseed	Amaranthaceae	L	Mkenda *et al.* (2015)
Tephrosia vogelii	tephrosia	Fabaceae	L	Mkenda *et al.* (2015)
Lippia javanica	fever tea	Verbenaceae	L	Mkenda *et al.* (2015)
Tithonia diversifolia	Mexican sunflower	Asteraceae	L	Mkenda *et al.* (2015)
Murraya koenigii	curry leaf	Rutaceae	L	Mkenda *et al.,* (2015)
Azadirachta indica	nimtree	Meliaceae	L	Mkenda *et al.,* (2015)
Azadirachta indica Juss.	*Neem*	*Meliaceae*	SO	Boeke *et al.* (2004)
Blumea aurita (L.F) DC	*Faux tabac*	*Asteraceae*	LO	Boeke *et al.* (2004)
Capsicum frutescens L.	*Pepper*	*Solanaceae*	Su	Boeke *et al.* (2004)
Carica papaya L.	*Papaya*	*Caricaceae*	Su	Boeke *et al.* (2004)
Cymbopogon citratus (DC. Ex Nees)	*Lemongrass*	*Stapf Poaceae*	L	Boeke *et al.* (2004)
Cymbopogon nardus (L.)	*Citronella grass*	*Rendle Poaceae*	L	Boeke *et al.* (2004)
Cymbopogon schoenanthus (L.)	*Camel grass*	*Sprengel Poaceae*	L	Boeke *et al.* (2004)
Dracaena arborea (Wild.)	*Dragontree*	*Link Liliaceae*	L	Boeke *et al.* (2004)
Helianthus annuus L.	*Sunflower*	*Asteraceae*	S	Boeke *et al.* (2004)
Hyptis spicigera Lam.	*Marubio*	*Lamiaceae*	L	Boeke *et al.* (2004)
Momordica charantia L.	*Bittergourd*	*Cucurbitaceae*	Su	Boeke *et al.* (2004)
Nicotiana tabacum L.	*Tobacco*	*Solanaceae*	L	Boeke *et al.* (2004)
Ocimum basilicum L.	*Sweet basil*	*Lamiaceae*	L	Boeke *et al.* (2004)
Tagetes minuta L.	*Mexican marigold*	*Asteraceae*	L	Boeke *et al.* (2004)

Key: P = peel; B = bulb; S = seed; L = leaves; SB = stem bark; R = root; RB = root bark; F = flower; O = oil, SH = shoot, FR = Fruit, RZ = rhizome, SO = Seed oil, Leaf oil, Su = Slurry.

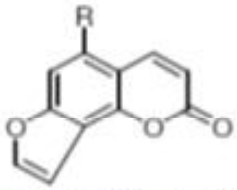
Phytochemical analysis of some plant extracts

Phytochemical constituent in *Citrus sinensis* (Shalaby *et al.*, 2011)

Obtained compounds	Leaves		Peels	
	C. aurantifolia	*C. sinensis*	*C. aurantifolia*	*C. sinensis*
Psoralene (1)	+	+	+	+
Xanthotoxin (2)	-	+	-	+
Bergapten (3)	+	+	+	-
Isopimpinellin (4)	+	-	+	-
Imperatorin (5)	+	+	+	+
Isobergapten (6)	+	-	-	-
Marmesin (7)	-	+	-	-
Umbellifrone (8)	-	+	-	+
Kaempferol (9)	+	-	-	-
Quercetin (10)	-	+	-	+
Myricetin (11)	+	-	+	-
4',5,7- Trihydroxy-3,6- dimethoxy flavone (12)	+	+	-	-
Hyperin (13)	-	+	-	-
Quercetin-3-*O*-robinobioside (14)	-	+	-	-
Rutin (15)	+	+	+	+
Hesperidin (16)	-	-	+	+
Stigmasterol (17)	-	+	-	-
β-Sitosterol (18)	+	+	-	-

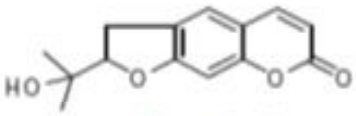


		R_1	R_2
1	Psoralene	H	H
2	Xanthotoxin	OCH_3	H
3	Bergapten	H	OCH_3
4	Isopimpinellin	OCH_3	OCH_3
5	Imperatorin		H

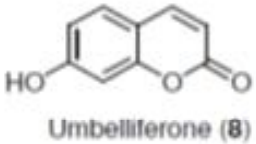


(6_a) Isobergapten R= OCH_3

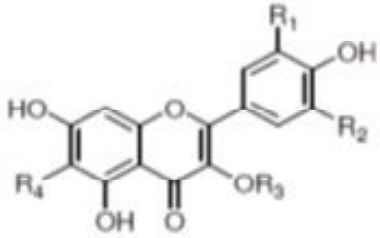
(6_b) Angelicin R=H



Marmesin (7)

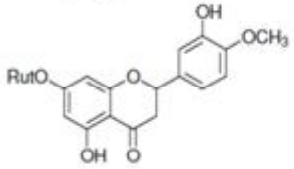


Umbelliferone (8)



		R_1	R_2	R_3	R_4
9	Kaempferol	H	H	H	H
10	Quercetin	OH	H	H	H
11	Myricetin	OH	OH	H	H
12	4',5,7- Trihydroxy-3,6- dimethoxy flavone	H	H	CH_3	OCH_3
13	Hyperin	OH	H	Gal	H
14	Quercetin-3-robinobioside	OH	H	Rob	H
15	Rutin	OH	H	Rut	H

Gal= galactose; Rob=robinobioside (2-*O*-α-rhamnosyl-D-galactose); Rut =rutinose (6-*O*-α-rhamnosyl-D-glucose)

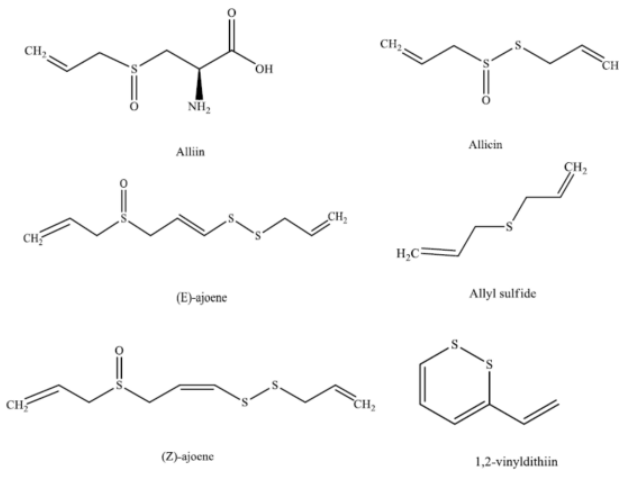


Hesperidin (16)

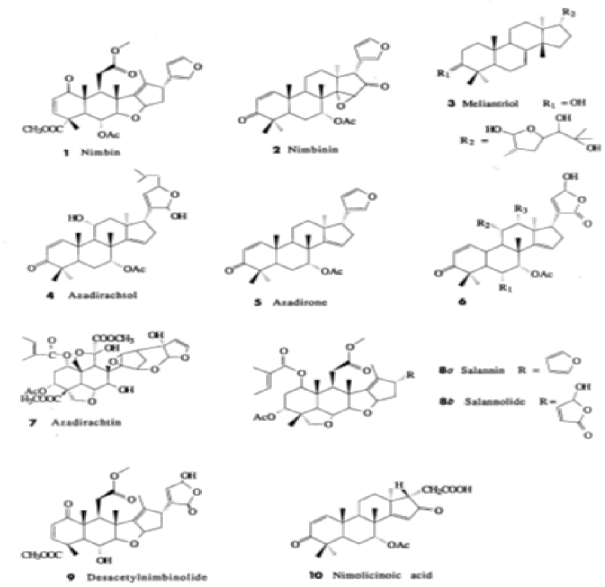
Phytochemical Analysis Some Plant Material.

Plant materials	Alkaloids	Carbo hydrates	Starch	Gly cosides	Flavo noids	Triter penoids	Resins	Saponins	Steroid	Proteins	Tannins
Citrus sinensis O.	+	+	-	-	+	-	+	+	-	+	+
Allium sativum L.	+	+	-	-	+		+	+	+	+	+
Azadirachta indica (A. juss)	+	+	-	-	+		+	+	-	+	+
Balanites aegyptiaca (L.)	+	+	-	-	+		+	+	-	+	+
Annona spp (L.)	+	+	-	-	+		+	+	-	+	+
Capsicumfructscens	+	+	-	-	+		+	+	-	+	+
Allium cepa (L.)	+	+	-	-	+		+	+	-	+	+
Clausena anisata H.	+	+	-	-	+		+	+	-	+	+
I,foringa oleifera Lam	+	+	-	-	+		+	+	-	+	+

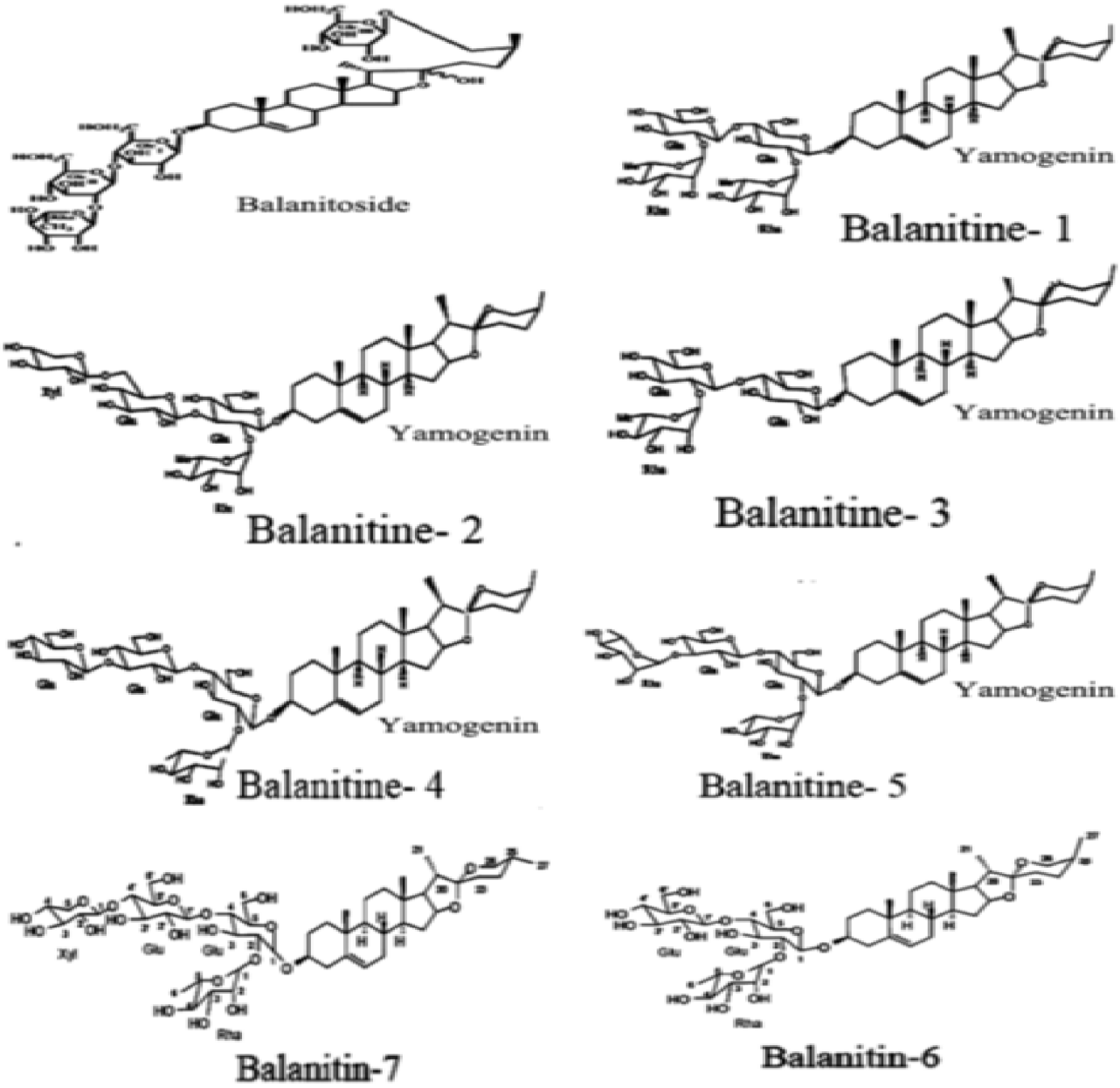
+ = Presence; - = absence.



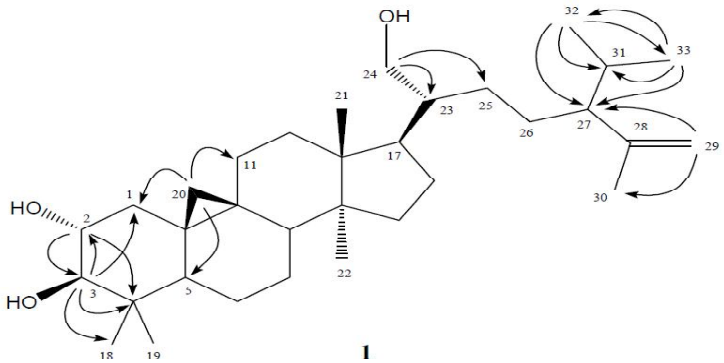
Phytochemical constituent in *Allium sativum* L.
(Martin *et al.*, 2016).



Phytochemical constituent in, *Azadirachta indica*
(Koul *et al.*, 2016).



Phytochemical constituent in *Balanites aegyptiaca* (Chothani, *et al*., 2011).



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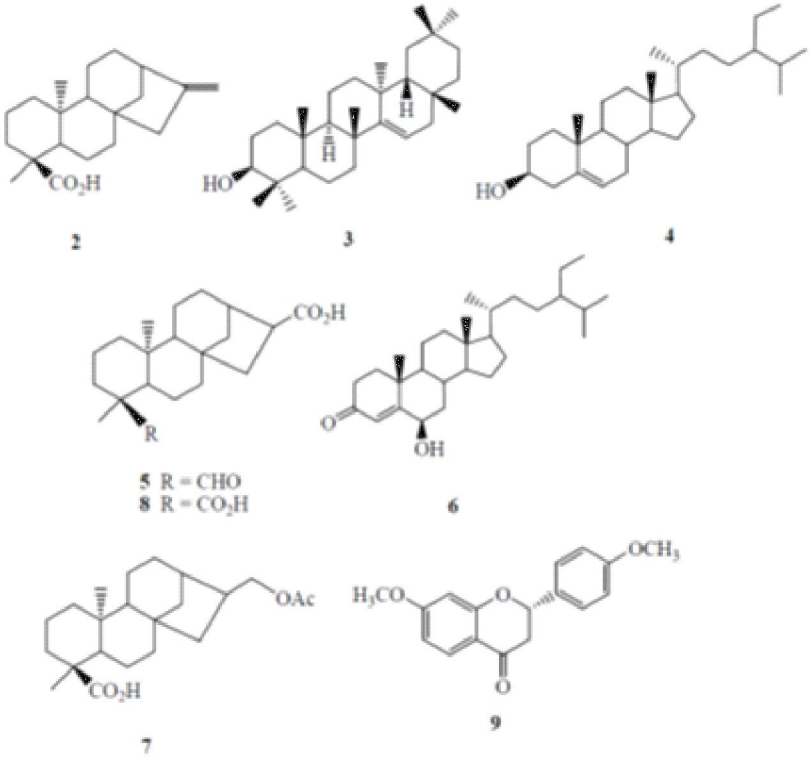
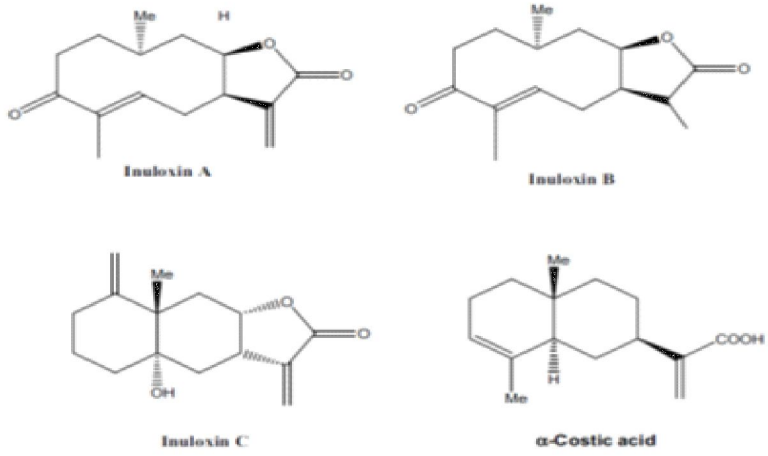


Phytochemical constituent in *Annona* spp (Tran *et al.*, 2013).

Phytochemical constituent in *Dittrichia viscosa* (Faiza *et al.*, 2019)

In recent literature, Faiza *et al* have been reported the efficacy of four bi- and tri-cyclic sesquiterpenes, namely inuloxins A, B and C and α-costic acid, extracted from aerial parts of *Dittrichia viscosa* collected in Algeria was assessed against the cowpea seed beetle *Callosobruchus maculatus*. The compounds were evaluated for their effect on adult mortality, oviposition and adult emergence. Three concentrations (100, 50 and 25 µg/ml) of each compound were tested with chickpea being used as the test plant. Complete adult mortality (100%) was achieved at only 1 day after exposure to inuloxins B and C and á-costic acid with LC50s less than 36 µg/ml. Lethal concentration values (LC50) were determined as 30.4, 35.2, 31.6 and 29.4 µg/ml, respectively, for inuloxins A, B and C and á-costic acid. Oviposition and F1 progeny emergence were significantly reduced (27% and 73%, respectively) after treatment with *D. viscosa* compounds. Our results also revealed that oviposition, adult emergence and sex ratio varied with the sex of the treated mating partner suggesting that the test compounds may have acted as male (or indirect female) chemosterilants resulting in reduced fecundity and fertility of untreated females that mated with treated males.



Bioassay methods

Bioassay methods commonly employed for insecticide toxicity evaluation are topical application, potter's tower method, injection method, dipping method (leaf dip and larvae dip), contact or residual method, film method, etc.

Topical application

A generally employed method is topical application, where the insecticide is dissolved in a relatively nontoxic and volatile solvent such as acetone and small, measured droplets are applied at a chosen location on the body surface on the thorax of individual third stage larvae with an operated micro applicator (Durmusoglu, 2015). A motor driven topical applicator is available with micrometer-driven precision syringe. The advantage of this method is, the high degree of precision and reproducibility that can be attained, large number of tests can be performed in a relatively short time, small number of insects required per replication, simple and inexpensive equipment needed, small amount of toxicant and solvents used.

Potter's tower method

Uniform spraying or dusting on the body of insect can be done by means of potter's tower. Potter (Potter, 1952) designed a spray tower with a twin-fluid nozzle mounted centrally at the top of an open ended metal tube where the sprays falls vertically and deposits on horizontal plane. The topical application on entire insect body can be done by potter's tower by possession the Petri dish containing known number of insects under the bottom part of the tower and spraying inside through nozzle fitted in the lower side by maintaining a particular pressure. This methodology simulates field acquaintance conditions and hence is informative for pest management. The technique has emerged as one of the most convenient methods of dispensing known amount of toxins accurately on insects. The major difficulties of the method are the potter's tower method has a slow execution time and a high initial cost, due to the necessity of purchasing equipment. In this technique the insecticide is directly injected into the insect body (thorax in insects) by hypodermic needle. The method employs a very fine stainless-steel needle (27 or 30 gauge thickness and 0.41 or 0.30 mm in diameter) is used and the quantity of toxicant required is measured by micrometer. Small glass needles of 0.1-0.16 mm in diameter are used for injection in small insects. The insecticide is commonly dissolved in propylene glycol or peanut oil and injected intraperitoneally (into the body cavity). Maintenance should be taken to avoid bleeding by the insect. This method is specifically employed to know the exact amount of toxicant needed inside the body of the insect (Sun, 1963).

Dipping method

This method is active when topical application or injection methods are unreasonable, for example: with small plant-feeding insects, stored-product insects, housefly larvae, insect eggs, red spiders, etc. The insects are picked up with a pair of forceps and dipped into an aqueous solution of the chemical for short periods of time, which is either a suspension or an emulsion (Simon, 2008). Insect absorption methods are convenient field-based bioassays useful for extension and field workers. The methods are simple and are somewhat closer to field application of insecticides. In another dipping method the leaves are dipped in aqueous solution of insecticides for fixed period in serial dilution of different concentrations. The leaves were carefully drained of excess solutions and air dried for an hr before being used (Immaraju *et al*., 1990). After few minutes the known numbers of insects watch feed on the leaves in question are released and the mortality counts are done after a definite period. This method can also be employed against sucking insects which are not easily removed from the leaf surface. The leaves containing insects are dipped in insecticidal solution for a definite period and the population counts before and after definite time interval of dipping are made. This technique allows the product to be evenly distributed on the leaf surface and makes it possible to check whether or not the field doses are effective for the pest control (Siqueira, 2000).

Contact or Residual method

In this method, the formulated insecticide is diluted in a volatile solvent (acetone) and the insecticide solution is coated inside a glass vial. The solvent is allowed to evaporate by rotating the container so that the insecticide is spread evenly over the entire surface leaving a residual film. The dose is varied by the concentration f insecticide solution added to the vials (Simon, 2008). Insects are released on to the treated surface and thus get exposed to residual film. Alternatively, the insecticide is applied evenly on to leaf, glass, filter paper, wood panel or other types of building materials and allowed to dry before exposing the insects to the residual deposits. For uniform application, equipment called Potter's tower is commonly used. The deposits are expressed as milligrams or grams of active ingredient per square meter (mg or g a.i./m2). These techniques do not represent field situations and do not allow us to verify whether doses are being applied efficiently (Bacci, 2009).

Film method

The technique includes insecticide solution is usually deposited on glass surface such as Petri dish, flask, vial, wide mouth jar etc. Petri dishs are most commonly used for evaluating insecticide efficacy. In this approach Petri dishes (5 cm diameter) are coated with one milliliter solution on their inner sides and the solution is allowed for uniform spreading in the Petri dish by swirling it gently and then permitting it dry up at room temperature. The target test insects are then released onto the film of the toxicant in the container. Thereafter; the known numbers of insects are exposed on for a period of 18-24 hours depending upon the

recalculation (Birah, 2008).

Fumigation method

The fumigation method is suitable for stored-product pests. The fumigation would be performed in a closed chamber at 30 ± 2°C and 60 ± 5 % relative humidity. The insecticide is announced into a sealed container along with the insects. Each fumigation test is replicated thrice or more along with control and after exposure the insects would be provided with small quantity of culture medium for a week and moved to recovery room. Adult mortality is recorded at different time intervals from the end of the exposure period (Simon, 2008).

Aqueous solution method

The principle of this method is to disperse the insecticide residue in a small amount of water miscible solvent (acetone or alcohol) into water as a solution or suspension. Then a small number of searching aquatic organisms such as mosquito larvae, micro crustaceans or fish are exposed to the solution. The amount of residue in a treated sample is obtained by comparing its toxicity to that of the standard. The amount of solvent in water should be as little as possible and its toxicity, if any, can be detected by control tests. This method has the advantage of having the whole organism constantly in contact with the medium. Some have claimed uniform management of the toxicant, but settling of the suspended material may occur. High sensitivity is thought to be the result of circulation and absorption of the toxicant through the gills or equivalent organs (Dewey, 1958).

Photomigration method

Photomigration method is another aqueous solution method which was devised by Burchfield *et al.* using negative phototaxic response to larvae of *Aedes aegypti* (Burchfield 1952). The insecticidal solution is carefully evaporated under moderate stream of air to dryness and the residue is dissolved in small amount of acetone and then 50 mL water is added. One to two hundred larvae are then confined behind a porus barrier in a glass trough comprising above solution. After varying time period, the light is turned on and barrier is removed. Viable larvae rapidly migrate away from the light. After one-minute exposure, a second barrier is kept in the trough and the larvae left behind are considered as dead. From a series of dilution T_{50} (The time required to inactivate 50% of the test population) and LC_{50} are worked out. The per cent mortality corresponding to each dose calculated from the number of insects released and the mortality experiential after exposure period. The mortality in control if any will affect the accuracy of the result. In order to over this error, a correction is regularly applied by using the Abott's formula.

The susceptibility of any population to a poison is assessed by constructing a dosage-mortality curve in which the dosage is plotted against the percentage mortality at a given period of time. Such a plot produces a sigmoid curve whose asymptotic methods (infinite ends) at the regions of zero and 100% mortality are difficult to define without extensive testing. In profit transformation, the sigmoid curve is converted to a straight line by plotting the logarithm of the dosage against the profit value of percent mortality. This method of computation yields a straight line, which greatly facilitates the determination of the LC_{50}, LC_{90} or any other lethal concentration/dose.

Utility of bioassay

- Bioassay is assumed to ascertain the potency of the chemicals as insecticides.
- It helps to discovery out the property of synergism,
- potentiation and resentment of a compound when used in a mixture with an insecticide.
- Comparative or relative toxicity of insecticides is worked out on the basis of LC_{50} found as a result of bioassay. This gives an index for selecting promising insecticides for field trial against insect pests.
- Bioassay supports in evaluation of insecticides for their safety to pollinators, predators and pathogens.
- Bioassay can help the formulators in improving the effectiveness for their formulated products, through changes in solvent, spreader, emulsifier, stickers etc.
- The quality of marketed insecticides can be checked through bioassay of samples collected and comparing them with standard.
- The change in values of LC_{50} of an insecticide for an insect with the passage of time indicates variation in susceptibility which helps in detection of resistance if developed in the insect population. Cross resistance to other insecticides, use of synergists and mixed formula--tions to overcome resistance are also estimated through bioassay.
- Formation of toxic metabolites, not quantitatively, due to use of insecticide can be determined by bioassay.
- Through bioassay lethal time LT_{50} required to kill 50% population to test animal. ED_{50} or EC_{50} i.e. the dose or concentration of chemical carries out sterility or other quantitative effects in 50 % population can also be worked out.
- Bioassay can also be applied for estimation of micro quantities *i.e.* residues of insecticides in different commodities in order to alarm the consumers from hazards associated.

Mechanisms of activity of Botanicals Oviposition deterrent

Grownup bruchids do not feed on stored cowpea seeds, but only deposit their eggs. As shown in this study, D. ambrosioides, *T. vogelii* and *L. javanica* have the potential for stored grain legume protection against bruchids by preventing oviposition (Mkenda *et al*, 2015). This is when the plant products prevent the insect from lying eggs on the stored product. This is exhibited by releasing fumes in to the surrounding that prevent mating and subsequent laying of eggs. Rahman and Talukder, (2006) stated that when stored grains mixed with leaf, bark and seed powder of plant material reduced oviposition. Good example of

oviposition deterrent showed that the use of chillies, garlic and peppermint plant parts caused different level of deterrence to *Callosobruchus maculatus*. Chillies and garlic applied at the rate of 50 g per 500 g cowpea seeds greatly reduced oviposition relative to the control. The number of eggs laid on cowpea treated with chillies was not significantly ($p \geq 0.05$) different with garlic but was significantly different with the control. Peppermint however recorded the highest number of eggs laid among the plant treatments but was still better than the control. The efficacy of different plant derivatives against the development of the cowpea weevil, *C. maculatus* fed on cowpea (Radha and Susheela, 2014), *V. unguiculata* seeds. The leaf extracts of romatic plants, *M. koenigii* and *A. indica* were evaluated for their growth, adult mortality and oviposition inhibition of *C. maculatus*. Several compounds have been isolated from the leaves, stem and flowers of *T. diversifolia* (including sesquiterpenes, diterpenes, monoterpenes and alicyclic compounds), some of which have demonstrated biological activities against different species of insects.

Insecticidal properties

Plant materials possessed active ingredients (a.i.) that have insecticidal properties. Neem contained *Azadirachtin, Nimbin, Nimbidin, Selanin*; *Psidium guineense* contained *Guineense*; *Annona* contained *Annonacin*; *Clausena anisata* contained *Clausenol* and *Coumarins*. These materials caused different toxicity properties to insects either by contact, stomach or through respiratory poison to *Callosobruchus maculatus*; Adenakan *et al*., (2013) and Adebiyi and Tedela, (2012) reported toxicity properties of *C. anisata*, Moss plant and *Moringa* plants respectively on *Callosobruchus maculatus*. An example of toxic effect of plant materials was reported by Adenakan *et al*., (2013). The findings showed that *Moringa* flower powder that was applied at the rate of 0.5 g per 30 g cowpea seeds caused insecticidal property in form of mortality. Actellic dust caused highest *Callosobruchus maculatus* mortality 10 hrs after infestation. However, the same control was achieved with the application of leaf powder. All the treatments however caused significant control of *Callosobruchus maculatus* 24 hrs after infestation. The plant extract of *Allium sativum* L. (Garlic), *Cordia millenii* Baker (Manjack), *Monodora myristica* (Gaertn.) (Nutmeg), *Xylopia aethiopica* (Dunal) (Negropepper) and *Zingiber officinale Roscoe* (Ginger) against *Callosobruchus maculatus* (Cowpea weevil) infesting cowpea seeds.

Anti-feeding deterrent

Anti-feeding is sometimes referred to as feeding deterrent. It was defined as the action of a chemical that inhibits feeding although does not kill the insect directly (Manukata, 1977). Saxena *et al*., (1988) defined anti-feedants as chemicals which retard or disrupt insect feeding by rendering the treated materials unattractive or unpalatable. In that situation, the pests get starved to death. This was when plant products prevented insect predation on stored products. The toxicity in effect may be by stomach poison.

Repellency Control

Plant products that were pungent or having irritating odour were used in insect repellency control. Takulder, (2006) defined repellency as a chemical stimulus which causes the insects to make oriented movements away from the source of the stimulus. The use of plants such as Pine tree, *Eucalyptus globules* Labille; Rue, *Ruta graveolens* Linn. and Garlic, *A. sativum* as repellant had been reported (Ahmed *et al.,* 2009). Talukder, (2006) reported the use of essential oil of *Artemisia annua* L. as repellent contrary to storage pests such as *Tribolium castenum* Herbst and *Callosobruchus maculatus* (L.). Some plants materials possessed repulsive odour which drive insect away. This phenomenon occured when fumes were released in to the vicinity, the odour will be perceived by the insect and will drive it away thereby dying due to hunger.

Metamorphosis inhibition

Plant products were used as bio pesticides in arresting insect growth. The effect of growth regulatory plant products can be seen in several ways. There were molecules inhibiting metamorphosis. These compounds preventing completion of life cycle from taking place at the right time or force the insect to go through an early metamorphosis, so that development takes place at a time not favourable for the insect National Research Council (NRC) (1992). Others chemicals have been observed to alter hormones related to this function so that insects suffer malformation. Either, the insects were made sterile or were killed. NRC (1992) reported action of neem extract on some insect pests by way of disrupting their life cycle. The a.i. possessed by plant materials have the ability of inhibiting the life cycle of insects. A good example is *Azadirachtin* found in Neem. The a.i. prevents the insect from completing metamorphosis, or adults were malformed (NRC, 1992). Similarly, Adenakan *et al*., (2013) reported the efficacy of different parts of *Moringa* parts in reducing the development period of *Callosobruchus maculatus*. There was lower mean number of adults emerged at various seed treated with different parts of *Moringa* plant powders. The lowest number of adults that emerged was significantly different from the highest number that emerged in the control treatment.

CONCLUSION

The review of available literature showed that use of different plant materials as powders or in form of extracts significantly reduced seed damage and deter oviposition of *C. maculatus* on cowpea. The use of pirimiphos-methyl was however superior but was not significantly different with the plant materials. Several plants materials were used as the healthier bioactive compounds. The literatures showed that garlic, chilies and peppermint applied at the rate of 0.035–0.55g significantly ($p \leq 0.05$) reduced oviposition, respectively compared to the control. Similarly, powdered flowers of *M. oleifera* applied at the rate of 0.5 g per 30 g of seeds caused

mortality of *C. maculatus* better than the control 8 hours after infestation. The use of *C. anisata* and Permethrin exhibited percentage mortality of cowpea bruchids was high using Permethrin but was not significantly ($p \geq 0.05$) better than *Clausena* leaf powder. Bioactivity of phytochemical plants can be a useful tool for the determination and study of different agricultural pesticides. It can be simple, swift, multipurpose and highly sensitive to a wide range of toxicants. Control of *C. maculatus* on stored cowpea can equally be achieved using plant materials. Adult mortality, oviposition activity repellent activity against *C. maculatus* by bioactive compounds will increase the environmental safety and farmer friendly. The continued use of some plant extracts as grain protectants and bioactive compounds by local farmers should be encouraged through education.

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