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Pesticidal Efficacy of Four Botanical Pesticides on Survival, Oviposition and Progeny Development of Bruchid, *Callosobruchus maculatus* in Stored Cowpea, *Vigna unguiculata*

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Authors' contributions

This work was carried out in collaboration between both authors. Author PAM reviewed the literature and wrote the first draft of the manuscript. Author PAN edited the manuscript and the final manuscript was read and approved by both authors.

Review Article

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ABSTRACT

Cowpea (*Vigna unguiculata*) production is limited by various insect pests that attack and damage the crops both in the field and during storage. Cowpea bruchids, *Callosobruchus maculatus* are the major insect pests of cowpea which infest the cowpea grains in the field, and then carried into the store where the population builds up rapidly. Chemical insecticide application is one of the management options that has been used for many years to control the insect pests. However, due to the side effects associated with those insecticides, there has been a resurgence need of using botanical pesticides to control insect pests in the field as well as in storage. This review aims to increase an awareness of using selected botanicals (*Tephrosia vogelii*, *Chenopodium ambrosioides*, *Tithonia diersifolia*, *Lippia javanica* and *Vernonia amygdalina*) as the cheap, effective and environmental friendly insect pest management strategy against bruchids in stored cowpea. The bioactive compounds from these plants offer great potential of developing botanical pesticides against postharvest insects in stores.

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1. INTRODUCTION

Cowpea, *Vigna unguiculata* L. Walp is a legume widely cultivated in tropical and subtropical countries which belongs to the family Leguminosae, subfamily Papilionaceae and Tribe Phaseolae [1]. In Tanzania, cowpea is grown mostly in semi-arid areas such as Shinyanga, Mtwara, and Kigoma because of its ability to tolerate moisture stress conditions [2]. Cowpea has unusual health benefits, with protein content of about 23%-25%, making it suitable in the diet of many people in third world countries who in most cases can't afford to buy other protein sources such as meat and fish [3-7]. Cowpea is mostly cultivated as food crop and many people in Africa and other developing countries highly appreciate leafy vegetable species [6,8]. Cowpea as a legume is also efficient in fixing nitrogen and consequently enriches the soil for growth of other crops [4]. However, cowpea production in Tanzania is low and does not meet the increasing demand as important food crop due to high infestation by insect pest in field as well as during storage which causes loss of weight, nutritional value and viability of stored grains [9]. The most important insect pest in stored cowpea is the bruchid, *Callosobruchus maculatus*. In Africa, it has been found that about 30% to 80% of the total cowpea production valued at over US 300 million dollars is either lost or suffers damage annually as a result of bruchid infestation [4]. Losses attributed to bruchids are estimated to be at about 87 to 100% within storage duration of 3 to 6 months that results in both quantitative and qualitative reduction, manifested by food and nutrition insecurity, and low incomes to small scale farmers [6,10] Consequently, there is a need for new approaches to control insect pests in stored cowpea in order to enhance food security among the people.

2. SURVIVAL, OVIPOSITION AND PROGENY DEVELOPMENT OF BRUCHIDS (*Callosobruchus maculatus*) IN STORED COWPEA

Cowpea bruchids, *C. maculatus* are among the major insect pests of legumes which cause high infestation in cowpea both in the field and in storage [11]. Cowpea grain stored after harvest is the favorite food of the bruchids [12]. 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 [13,14]. They lay eggs on the pods of legume hosts as they approach maturity in the field but emergence usually occurs after harvest [9]. In storage, *C. maculatus* lay eggs on the seeds and larval development and pupation are completed entirely within a single seed. Although infestation of cowpea grain starts in the field, larvae are the major destructive stage because adult cowpea bruchid do not feed [1].

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 as shown in Fig. 2. 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 [12].

Bruchid, *C. maculatus* passes through four main stages in their life cycle which are egg, larva, pupa and adult as shown below in Fig. 2.



Fig. 1. Cowpea seeds after being infected by bruchids, *C. maculatus*
Source: [12]

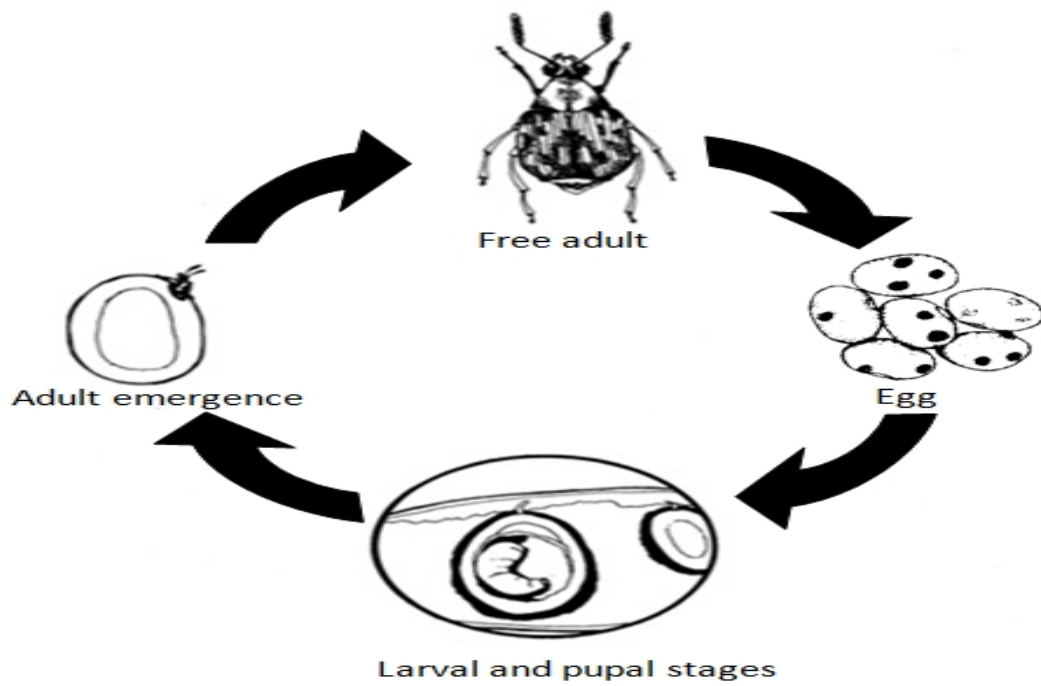


Fig. 2. The life cycle of *C. maculatus*

3. COMMON METHODS USED TO CONTROL BRUCHIDS IN STORED COWPEA

3.1 Synthetic Chemical Pesticides

Chemical insecticides have been employed in the control of insect pests and often proved to be very effective. They may be applied as a powder formulation i.e Actellic super dust (Pirimiphos-methyl), liquid formulation i.e karate or fumigant/ gas formulation depending on the intended use of the pesticide [15] as shown in Table 1.

Table 1. Different Chemical formulations used in insect pest management

Solids	Liquids	Gases
Dust or powders, Granules, Pellets, Tablets Particulates or Baits, Dry flowables, Wettable powders, Ear tag/ Vapour strips, Seed treatment WDGs	Suspensions (Flowables), Emulsifiable Gels, Aerosol, volume Microemulsions	Concentrate Solutions, concentrates, Ultralow concentrates, Fumigants sold as liquids or solids

Source: [15]

When some of these formulations are sprayed in the field to control the insect pests, they end up in the soil and other parts of the environment such as air and water [16,17]. Consequently, the use of synthetic insecticides in crop protection programs around the world has resulted in disturbances of the environment, pest resurgences, pest resistance to pesticides and lethal effect to non-target organisms in the agro-ecosystems in addition to direct toxicity to users [18]. Furthermore, these control measures have been difficult to be employed by most of small holder farmers who have always insufficient income to enable them to buy the synthetic pesticides, thus they rely on a variety of pesticidal plants to control the insect pests [19]. Even where synthetic insecticides are affordable to growers (e.g through government subsidies), they are often applied at inappropriate application rates due to illiteracy, poor labeling or use of old, expired products, leading to very low yield of agricultural products [20]. In addition, pesticides of chemical origin can affect human health directly or indirectly by disrupting ecological systems that exist in rivers, lakes, oceans, streams, wetlands, forests and fields [21]. Consequently, there is an increasing interest in the use of plant origin bio-pesticides in order to reduce the problems of environmental pollution, killing of non-target species and health risks to humans, as well as reducing the cost of purchasing synthetic chemical pesticides.

3.2 Biological Control

Biological control can be defined as “the intentional introduction of an exotic, usually co-evolved, biological control agent also referred as natural enemy for permanent establishment and long-term pest control” [22]. There is a great potential of using biological control method to control insect pests in stored products which help to reduce the use of pesticides on food and provide for high quality food products. Among the important natural enemies in stored products are the parasitoid wasps in the families Braconidae, Ichneumonidae, Pteromalidae and Bethyridae [23-25]. These natural enemies have shown to be very effective in controlling a number of insect pests in stored products. For example, *Dinarmus basalis* is a solitary parasitoid that attacks larval, pre-pupal and pupal stages of *C. maculatus* [26,27]. The

hymenopteran *D. basalis* is an ectoparasitoid larvophagous species, that represents 80% to 90% of the bruchid larvophagous parasitoid in the cowpea fields and storage [14,28]. It has been reported that, *D. basalis* adults were more susceptible to the essential oils of different plants introduced into granaries at harvest time in an attempt to control bruchids, and potentially reduced density of parasitoid populations and increase seed losses due to bruchids infestation [28,29]. When *D. basalis* adults were put into the storage systems in the absence of any treatment, successive generations of the parasitoids maintained the *C. maculatus* population at a low density [29,30]. The parasitoid *D. basalis* showed to suppress the bruchid population and without parasitoids the seeds were completely damaged in control treatment. It is obvious that, the use of biological control method in crop protection is promising and it is very important to ensure that the use of pesticides do not harm the natural enemies useful in biological control.

3.3 Botanical Pesticides

Botanical pesticides are attractive alternatives to synthetic chemical insecticides for pest management derived from plants with insecticidal properties. They have several advantages over the synthetic pesticides due to fact that they are cheap, easy to prepare, non-poisonous to human due to short life span and in most cases readily available and have more than one active ingredient which work synergistically making it difficult for pests to develop resistance [21]. Consequently, they offer a greater role in the production and postharvest protection of food in most developing countries.

Recently, a number of botanical pesticides have been found to be effective against short lived insect pests in the field as well as during storage of grains [31-35]. Some of these botanical pesticides that have been explored and found to be sustainable in control of the insect pests in stored cowpea include powders from *Artemisia annua*, *Azadirachta indica* and *Ocimum gratissimum* [4]. Others such as leaf powders of tobacco (*Nicotiana tabacum*), Mexican marigold (*Tagetes minuta*), and *Tephrosia vogellii*, mixed in different combinations and proportions, were also found to be effective against bruchids (*Callosobruchus maculatus*) on cowpea [36]. *Nicotiana tabacum* was also found to be the best botanical pesticide followed by *T. vogellii* and then *T. minuta* in reducing pod and seed damage by bruchid in stored cowpea [37]. Oils extracted from *Balanites aegyptiaca* seeds and Cashew nut have been found to be effective against the survival and development of cowpea weevil *Callosobruchus maculatus* in cowpea seeds during storage leading to 100% mortality [38]. *Capsicum frutescens* seed powder and *Capsicum annum* seed powder dust have toxicity effect to *C. maculatus* and *S. zeamais* [39]. Bio pesticides, including botanicals, can offer a safe and effective alternative to conventional insecticides for controlling major insect pests within an integrated pest management program. Based on socio-economic status of the small scale farmers in Africa, there is a need for continuous research towards replacement of the hazardous chemical insecticides by cheap, effective, easily available and eco-friendly natural plant products with active safe components.

4. BIOACTIVE COMPOUNDS FOUND IN THE FOUR BOTANICAL PESTICIDES (*Tephrosia vogellii*, *Tithonia diersifolia*, *Lippia javanica* AND *Vernonia amygdalina*) FOR PESTICIDAL ACTIVITY

4.1 *T. vogellii*

This is a nitrogen-fixing species, cultivated in many parts of Africa as a fish poison, a pesticide and for improving soil fertility [40,41]. Two distinct chemotypes in *T. vogellii* has

been identified, among them, is Chemotype 1 (Fig. 3b) which contains rotenoids, including deguelin, rotenone, sarcolobine, tephrosin and α -toxicarol, required for pest control efficacy [42]. Rotenone and deguelin are the major rotenoid compounds in *T. vogelii*. All rotenoid compounds have the same A, B, C, D ring systems as rotenone, the variations being in the E ring, as exemplified by deguelin and elliptone in the Fig. 3a below.

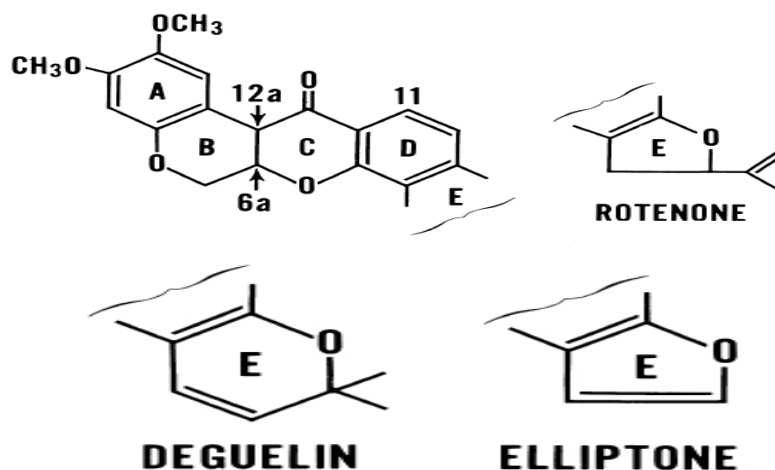


Fig. 3a. Structural formulas of rotenone, dequelin and elliptone

Source: [20]

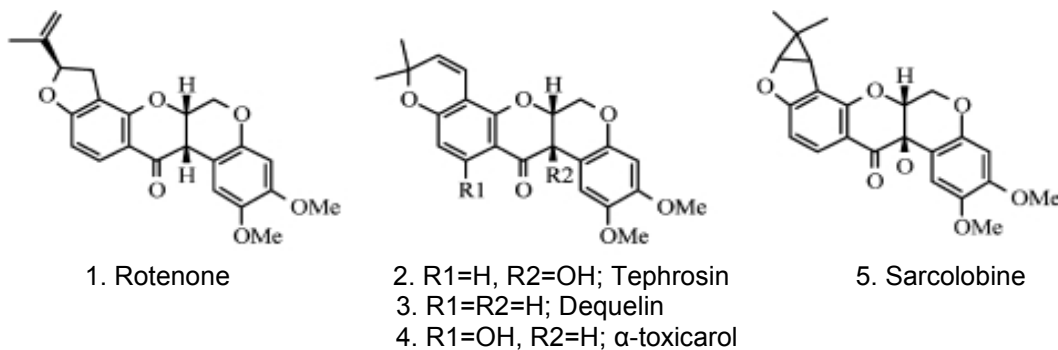
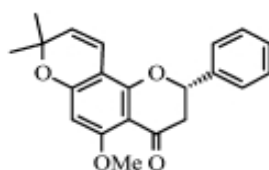


Fig. 3b. Chemo-type 1 compounds of *T.vogelii*

Source: [43]

Chemotype 2 (C2) contains no rotenoids, but only prenylated flavanones including, obovatin 5-methylether (Fig. 3c.) and therefore, no pesticidal activity [42].



Obovatin-5-O-methylether

Fig. 3c. Chemo-type 2 compound of *T. vogelii*

Source: [43]

Tephrosia has been largely used to control field pests rather than storage pests, and has been found to be very effective in control of a number of hard-to-kill field insects including cucumber beetle, leafhoppers, squash bugs, flea beetles, harlequin bug, spittle bugs, thrips, scales, mites, and some fruit worms [44]. Therefore, there is a need to search for further information on how efficacy is *T. vogelii* in the control of bruchids in stored cowpea.

4.2 *T. diversifolia*

T. diversifolia, commonly known as Mexican sunflower or tree marigold, is a common shrub in field boundaries, grasslands and disturbed lands in East Africa which was introduced into West Africa as an ornamental plant [45]. It has been used traditionally in different purposes. As a medicine, it is suitable for constipation, stomach pains, indigestion, sore throat, liver pains and to treat malaria [46,47]. It has been also reported to have anti-inflammatory, analgesic, antimalarial, antiviral, antidiabetic, anti-diarrhoeal, antimicrobial, antispasmodic, vasorelaxant and cancer-chemopreventive activities [46,48]. The leaves of *T. diversifolia* are effective in the treatment of sprains, bone fractures, bruises and contusions, stomach pains, indigestion, sore throat, hepatitis, cystitis and jaundice [48]. *Tithonia* species also have pesticidal activity. They are well known for sesquiterpenes, diterpenes, monoterpenes (Fig. 4) and alicyclic compounds isolated from the leaves, stem and flowers, some of which have biological activities against insects [47]. The insecticidal properties and other health benefits found in *T. diversifolia* call for further research in the utilization of this plant in the storage of grain legumes such as cowpea.

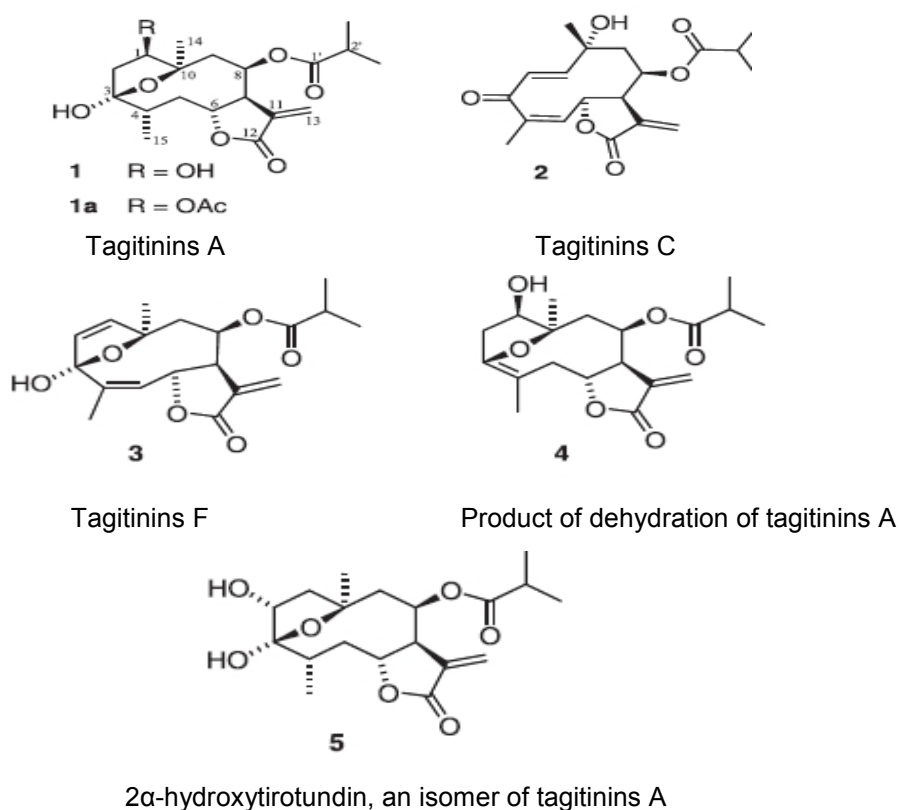


Fig. 4. Sesquiterpene lactones from *Tithonia diversifolia* and derivative 4

Source: [46]

4.3 *L. javanica*

Lippia species are members of the Verbenaceae family represented by herbs, shrubs and small trees [49]. *L. javanica* also known as fever tea or lemon bush, is an erect, small, woody annual shrub that grows to approximately 2 m high, commonly found in grassland on hillsides and stream banks and as a constituent of the scrub on the fringes of forests that are often adjacent to farmland [50,51]. It is of an aromatic in nature, used extensively as a traditional medicine to treat minor ailments and other microbial infections such as coughs, colds, candida albicans, candida krusei, and Cryptococcus neoformans and other respiratory diseases and skin infection [52]. It is reported to be effective in controlling aphids and red spider mites in cabbage, rapes and tomatoes [53,54]. Polar extracts of *Lippia* species have potential as environmentally friendly alternatives for the control of various insect pests [55]. Few chemotypes (Fig. 5.a-d) have been identified in this medicinal plant which includes; myrcenone, carvone, piperitenone, an ipsenone and linalool and the major one being myrcenone [49]. This calls for further studies on how this plant can be used effectively in controlling cowpea bruchids in stored products due to the occurrence of chemotypes in natural plant populations. Below are the structures and the chemical formula of the four chemotypes found in *L. javanica*.

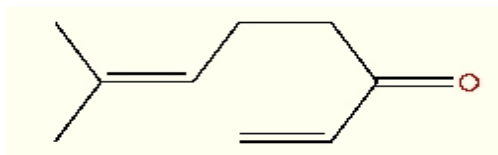
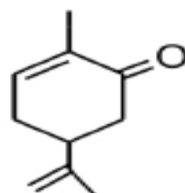


Fig. 5a. Myrcenone structure with chemical formula C₉H₁₄O

Source: <http://www.pherobase.com/database/compound/compounds-detail-myrcenone.php>

Accessed on 04/01/2014 at 22.30hrs



Carvone

Fig. 5b. Carvone with chemical formula C₁₀H₁₄O

Source: [56]

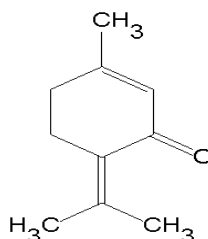


Fig. 5c. Piperitenone structure with chemical formula C₁₀H₁₄O

Source: <http://www.chemsynthesis.com/base/chemical-structure-23046.html>

Accessed on 04/01/2014 at 23.15hrs

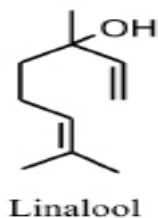


Fig. 5d. Linalool structure with chemical formula $C_{10}H_{18}O$

Source: [56]

4.4 *V. amygdalina*

V. amygdalina is a well known African medicinal plant that provides anticancer agents such as vernodaline, vernolide and vernomygdine [57]. It is the most known medicinal plant in the genus *Vernonia* [58]. The plant also consists of some anti oxidant compounds, and some peptides [59]. The aqueous extract of this plant have been found to have cell growth inhibitory effects in prostate cancer cell line [57,59]. The plant has antihelminthic, antitumorigenic, hypoglycaemic and hypolipidaemic activity and both the leaves and the roots are used traditionally in phytomedicine to treat fever, hiccups, kidney disease and stomach discomfort [58]. It is also found to be useful to nursing mother as it improves lactation, sugar level control in diabetes patients and the leaves or other parts of the plant can be used solely or mixed with other plants in the treatment of various suspected illnesses [60]. In addition, *V. amygdalina* has been reported to contain large quantity of Thiamine, Pyridoxine, Ascorbic acid, Glycine, Cysteine and Casein hydrolysate significantly more than other botanicals such as *Bryophyllum pinnatum*, *Eucalyptus globules* and *Ocimum gratissimum* [61]. Phytochemical screening of ethanol extracts of *V. amygdalina* leaf samples showed the presence of alkaloids, favonoids, saponins, tannins, phlobatannins, terpenoids and cardiac glycosides [62]. The most well known compounds in *V. amygdalina* include vernolide and vernodaline with the chemical structures as shown in Fig.6 (a-b) below. The presence of various bioactive compounds used in different purposes in *V. amygdalina* encourages scientists and researchers for further studies on the efficacy of this plant in controlling insect pests. Hence there is a need to search for effectiveness of this botanical in controlling bruchids in stored cowpea.

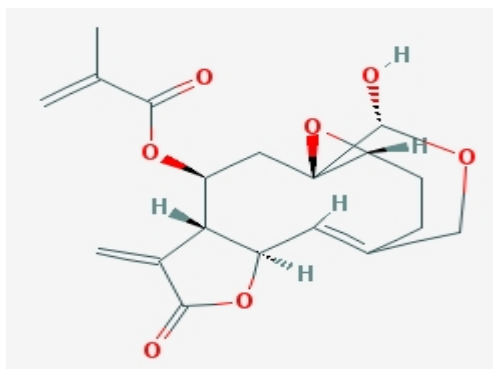


Fig. 6a. Chemical structure of vernolide

Source: <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=11774&viewopt=PubChem>

Accessed on 20/03/2014 at 2215 hrs

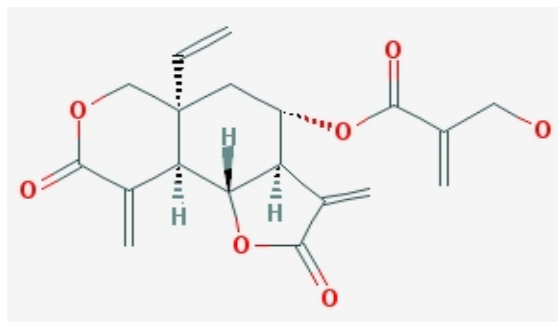


Fig. 6b. Chemical structure of vernodaline

Source: <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=11767>

Accessed on 20/03/2014 at 2228 hrs

4.5 *C. ambrosioides*

C. ambrosioides is a perennial plant that is used as a traditional insecticide to preserve post harvest grains from weevil attack, and for medicinal purposes to treat intestinal parasites, nervous infections, cough, pulmonary obstruction, typhoid, influenza, skin and kidney infection [63-66]. The plant has also been reported to exhibit antipyretic, antifungal, antiviral, antibacterial, sedative, analgesic, antioxidant and insecticidal activities [67-72]. It has been reported that *C. ambrosioides* consists of ascaridole, sabinene, β -pinene, α -terpinene, p-cymene, limonene, (E)- β -ocimene, γ -terpinene, 1, 4-epoxy-p-menth-2-ene, 1, 2, 3, 4-diepoxy-p-menthane and phytol as important medicinal and insecticidal compounds [63,73,74]. Fig. 7 below shows the major structures and the mechanism of formation of ascaridole and other monoterpenoid constituents in the genus *Chenopodium*.

The composition of the genus *Chenopodium* varies quantitatively and qualitatively within and between natural populations and no correlation to the geographical distribution. Ascaridole which is a bicyclic monoterpene with unusual bridging peroxide functional group is the major constituent (40 – 70%) in *C. ambrosioides* [63]. Other reports also show that, α -terpinene and p-cymene are also present in large amount [68,75,76]. *C. ambrosioides* is an important plant species with both repellent and insecticidal potential for controlling stored grain pests of different families such as bruchidae [77]. Further researches on the ways to increase agriculture production through the control of insect pests using botanicals with potential insecticidal activity will be of much value to the present and future generation.

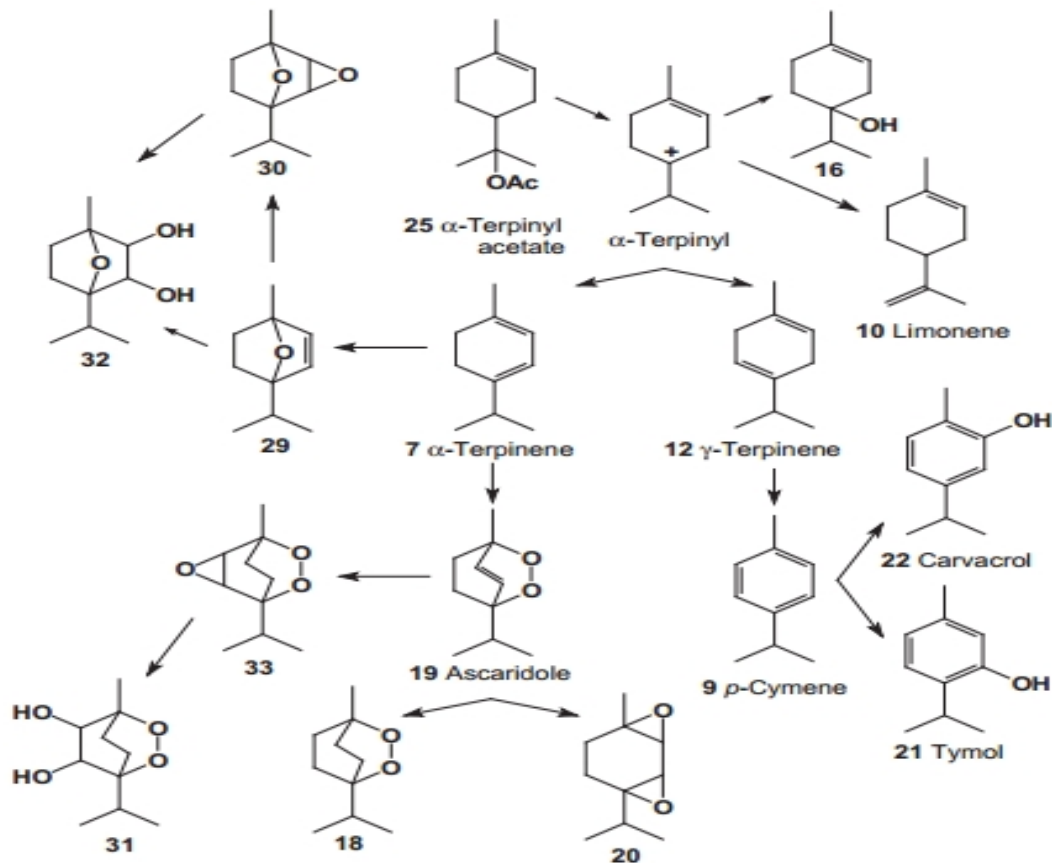


Fig. 7. Mechanism of formation of ascaridole and other monoterpenoid constituents in the genus chenopodium

Source: [63]

5. RESPONSE OF INSECT PESTS TO BOTANICAL PESTICIDES

Various insect pests can respond to different botanical pesticides in different ways depending on the physiological characteristics of the insect species as well as the type of the pesticidal plant. The components of various pesticidal plant can be classified into six groups namely; repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants, and attractants [78].

5.1 Repellency

An insect repellent is a substance which once applied on the surface of anything discourages insects from landing or climbing on that surface. A botanical pesticide with a repellent property keeps away the insect pest, and it is intuitively attractive in protecting the crops [20]. They offer protection with minimal impact on the ecosystem, as they drive away the insect pest from the treated materials by stimulating olfactory or other receptors [79,80]. Repellents from plant origins are considered safe in pest control because they have low or none pesticide residue making them safe to the people, environment and ecosystem in

general [80]. The plant extracts, powders, and essential oil from the different bioactive plants have been reported as important repellent against stored grain insect pests [78]. The aqueous extracts of *T. vogelii* was found to have repellent activity against golden flea beetle in *Jatropha* and *Sitophilus zeamais* in stored maize grain [81,82]. In addition, the leaves of *L. javanica* were found to have mosquito repellent activity [50]. This justifies the insecticidal properties of these pesticidal plants as repellents and as a result, there is a need to search for their repellent activity against bruchids in stored cowpea.

5.2 Feeding Deterrents/Antifeedants

The term antifeedant, or feeding deterrents are chemicals that inhibit feeding or disrupt insect feeding by rendering the treated materials unattractive or unpalatable [78,79]. The insects remain on the treated material indefinitely and eventually starve to death. They are of great value in protecting the stored products from insects. Bisabolangelone, a sesquiterpene, podophyllotoxin, a lignan, and aginosid, a saponin, were found to be strong deterrent to *P. saucia* larvae in a feeding choice test [83]. Other naturally occurring antifeedants include; glycosides of steroidal alkaloids, aromatic steroids, hydroxylated steroid meliantriol and triterpene hemiacetal [84]. Other feeding deterrents include thymol, citronellal and α -terpineolare which are effective against tobacco cutworm, and *Spodoptera litura* larvae [85]. Also azadirachtin from *Azadirachta indica* is a potent antifeedant to many insects though it is considered non toxic to mammals [20]. It was also found that there is a significant antifeedant, toxic and repellent effect of aqueous extract of *T. vogelii* against golden flea beetle in *Jatropha* [81]. This awareness has created an interest in the search of efficacy of this pesticidal plant in the control of cowpea bruchids in storage.

5.3 Toxicity

Toxic substance refers to any poisonous substance of plant or animal origin which can cause death of an organism [21]. Various researches worldwide show that many plant products are toxic to stored product insects and listed the use of forty three plant species expressing toxicant effects of different species of stored-products insects [86]. Rotenone is considered as a toxic compound since it is a mitochondrial poison which blocks the electron transport chain and prevents energy production [87]. As an insecticide, it is a stomach poison because it must be ingested to be effective [20]. The essential oil vapours distilled from anise, cumin, eucalyptus, oregano, and rosemary were also reported as fumigants and caused 100% mortality of the eggs of *Tribolium confusum* and *Ephestia kuehniella* [88]. Many botanicals including *T. diversifolia*, *T. vogelii*, *V. amygdalina* and *L. javanica* have been found to have toxic effect against many microbial and insect pest [43,47,48,51,54,57,61,89,90,91]. Further investigation on how the toxicity effect of these botanicals can be enhanced by checking the frequency of their application and optimum rates to control cowpea bruchids is important.

5.4 Growth Retadants

Plant extracts showed deleterious effects on the growth and development of insects, reducing the weight of larva, pupa and adult stages and lengthening the development stages [79]. Plant derivatives also reduce the survival rates of larvae and pupae as well as adult emergence [56]. It has been reported that both azadirachtin and neem seed oil increased aphid nymphal mortality significantly at 80% and 77% respectively, and at the same time increasing development time of those surviving to adulthood [92]. Many botanicals have

been reported to have a pronounced effect on the developmental period, growth and adult emergence [93]. It is also known that, the extract of *V. amygdalina* possessed the active principles such as terpenoids, tannins, alkaloids, saponins and glycosides which inhibited growth of *Streptococcus mutans* and *Staphylococcus aureus* [60]. Sesquiterpene lactones tagitinin A, tagitinin C and a flavonoid hispidulin isolated from *Tithonia diversifolia* were also found to have inhibitory effects on germination of radish, cucumber and onion seeds [94]. The essential oil of *Lippia* have been reported to have contact and fumigant toxicity as well as repellent and oviposition-deterrent activities towards four pests of stored grain [95]. Consequently, the possibility of employing these pesticidal plants to control bruchids in stored cowpea may be worthy of further investigation.

5.5 Sterility

Sterility can be induced by Sterile Insect Technique (SIT) or a chemosterilant, a chemical compound that interferes with the reproductive potential of sexually reproducing organism [96]. Chemosterilants are used to control economically destructive or disease-causing pests (usually insects) by causing temporary or permanent sterility of one or both of the sexes or preventing maturation of the young to a sexually functional adult stage [97,98]. It has been reported that plant parts, oil, extracts, and powder mixed with grain reduced insect oviposition, egg hatchability, postembryonic and progeny development [93,99]. Hexane extracts of *Andrographis lineat*, *A. paniculata* and *Tagetes erecta* showed 100% ovicidal activity against *Anopheles subpictus* [100]. Some botanical insecticides are used as chemosterilants, for example, at the physiological level azadirachtin blocks the synthesis and release of molting hormones from the prothoracic gland, leading to incomplete ecdysis in immature insects and in adult insects it leads to sterility [20]. From very early times, plant materials have been used traditionally as natural protectants of stored grains. The utilization of botanical insecticides in stored grains pest control seems to be very promising; as a result, there is a need for further research on various botanical pesticides with an intention to improve food security.

6. CONCLUSION

The role of botanical pesticides in insect pest management and crop protection play a minor role due to limited scientific information and continued use of effective but 'toxic' commercial pesticides. The regulatory environment and public health needs should create opportunities for the use of botanicals which are environmentally benign, safe to human and animal health and more cost effective than synthetic pesticides

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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