Research Article

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Ability of *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powder to protect bean against infestation of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) in Tubah, North-West Cameroon

Capacité des poudres de feuilles de *Chenopodium ambrosoides* et *Tephrosia vogelii* dans la protection du haricot contre l'infestation d'*Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) à Tubah, Nord-Ouest Cameroun

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Running title: Plant powder efficacy on Acanthoscelides obtectus

Abstract
Acanthoscelides obtectus, bean weevil is one of the most detrimental insect pests on stored bean. The infestation starts from the field and continues during storage. Many control methods are used to reduce the damage caused by this pest. The most used is synthetic residual chemical insecticides, however effective it causes many environmental problems. The search for environmental friendly control methods becomes an imperative. In this issue *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powders were tested on *A. obtectus* regarding adult toxicity, F_1 progeny production, and reduction of grain damage and suppression of population growth. Four contents (4; 8; 16 and 32 g/kg) of each plant powder were mixed with bean grain (*Phaseolus vulgaris*). The mortality was recorded within 1, 4, 7 and 10 days post exposure. The two leaf powders induced significant mortality of adults. Total mortality (100%) was recorded by *C. ambrosoides* leaf at 16 g/kg from 4 days post exposure. The same performance was observed for *T. vogelii* at its highest content (32 g/kg) within 10 days post-exposure. The LC_{50} values within 10 days were 1.48 g/kg and 2.05g/kg respectively for *C. ambrosoides* and *T. vogelii*. The F_1 progeny production was considerably inhibited by the two plants. The inhibition was complete with *C. ambrosoides* leaf powder at its highest content (32 g/kg). *T. vogelii* in the same condition induced 86.47% progeny reduction. *Tephrosia vogelii* and *C. ambrosoides* leaf powder effectively suppressed population growth and almost completely diminished the grain damage in more than eight months storage. Considering these results the two plants can successful protect stored bean against damage induced by *A. obtectus* in Tubah (North-West Cameroon). These plant products could supersede the synthetic insecticides used by smallholders in grain storage.
Keywords: *Acanthoscelides obtectus*, stored bean, infestation, *Chenopodium ambrosoides, Tephrosia vogelii*

Résumé
Acanthoscelides obtectus est l'un des insectes ravageurs les plus destructeurs du haricot durant le stockage. L'infestation par ce ravageur débute dans les plantations et se poursuit lors du stockage. Plusieurs méthodes sont utilisées pour réduire les dégâts causés par ce ravageur ; les insecticides chimiques sont les plus utilisés, bien qu'efficaces, ceux-ci sont la cause de plusieurs problèmes environnementaux. Ainsi la recherche des méthodes de protection plus écologiques est plus que nécessaire. Dans cette optique, les poudres des feuilles de *Chenopodium ambrosoides* et *Tephrosia vogelii* ont été testées sur *A. obtectus* concernant la toxicité sur les adultes, la production de la progéniture F_1, la réduction des dégâts et la suppression de la croissance de population. Quatre concentrations (4 ; 8 ; 16 et 32 g/kg) de chaque poudre ont été mélangées séparément au grain de haricot, *Phaseolus vulgaris*. La mortalité des adultes a été enregistrée après 1, 4, 7 et 10 jours post traitement. Les deux poudres ont induit une mortalité significative. *C. ambrosoides* a induit une mortalité totale à 16 g/kg en quatre jours. La même performance a été réalisée par *T. vogelii* à la dose maximale (32 g/kg) en 10 jours d'exposition. Les concentrations létales induisant 50% de mortalité (LC_{50}) en 10 jours étaient de 1,48 et 2,05

g/kg respectivement pour *C. ambrosoides* et *T. vogelii*. Les deux plantes ont considérablement inhibé la production de la progéniture F_1. L'inhibition complète (100%) a été atteinte par *C. ambrosoides* à la dose maximale (32 g/kg). Dans les mêmes conditions *T. vogelii* a induit une inhibition de 86,47% de production F_1. Les poudres de feuilles de *C. ambrosoides* et T. *vogelii* ont inhibé la croissance de la population et complètement réduit les dégâts sur les grains en huit mois de stockage. Considérant ces résultats, ces plantes pourraient avantageusement remplacer les insecticides de synthèse utilisés par les paysans dans la protection des grains stockés.

Mots clés : *Acanthoscelides obtectus*, haricot en stockage, infestation, *Chenopodium ambrosoides*, *Tephrosia vogelii*

1. Introduction

Cereals and pulses constitute the major staple food grains around the world precisely in Africa, especially in Sub-Saharan African. Many cereals and pulses crops are widely cultivated around this part of the world. The common bean, *Phaseolus vulgaris* belonging to the pulses is one of the most cultivated and used pulses in Africa. According to FAO (2019), the world bean production was 31405912 tonnes on an area of 36458894 ha with a yield 8614 hg/ha in 2017 (FAO 2019). African continent in the same year produced 6851757 tonnes on 7266713 ha for a yield of 9429 hg/ha. Whereas Cameroonian bean production in 2017 was 413072 tonnes for a cultivated area of 310650 ha with the yield of 13297 hg/ha (FAOSTAT 2019), which was low compared to the world and even African production. Common bean is a legume commonly grown in sub-Saharan Africa for food, cash, animals' food, and as soil improver (Masangwa et al. 2013). The haricot bean is the second most important grain crops in next to maize in terms of production and consumption in Africa as a whole (Broughton 2003) and in Cameroon especially in the Western Highlands.

Beans are often considered as the "poor man's meat" and consumed as seeds (mature or immature) as well as a vegetable (both leaves and pods) (Høgh-Jensen et al. 2013). The common bean is an important source of proteins, amino acids, carbohydrates and vitamins. Then it is significant as source for food security and nutrition. This bean covers considerably the needs of rural populations in terms of nutrients. The cultivation of this legume allow to the smallholders to generate income that can be used for financial issues. *Phaseolus vulgaris* crop by fixing nitrogen in soil enriches it and increases at the same time its fertility. This soil fertility improvement enhances culture yield cultivated on these farms, and all this in a biological manner. In tropical or subtropical areas especially in the North-West region of Cameroon, the cultivation of this legume is mostly done by women. This culture enables the women to generate income which empowers them.

The world demand for common bean is highly increasing because of its significance to human nutrition as a source of proteins, complex carbohydrates, vitamins, and minerals (Bennink 2005). Its importance in reducing blood cholesterol level and combating chronic heart diseases, cancers and diabetes is also gaining recognition from human health point of view (Singh 1999; Bennink 2005).

The important uses of common bean make its storage imperative in order to ensure availability along the year. Its cultivation is done once the year whereas its uses and marketing are carried out through the year. Several factors affect this legume availability, those are biotic and abiotic in addition to poor agricultural practises and technology, which seriously reduce yield in sub-Saharan Africa. Stored beans suffer heavy losses in terms of both quality and quantity, and these losses are mostly caused by bean bruchids (Coleoptera: Bruchidae). Beans in Africa fall under attack by some species of bruchids: the bean bruchid or common bean weevil, *Acanthoscelides obtectus*; the cowpea bruchids *Callosobruchus chinensis* and *C. maculatus*, Rhodesian bean weevil, *C. Rhodesianus*; spotted bean weevil, *Zabrotes subfasciatus* and even some unidentified Bruchidius species (Giga et al. 1992).

Acanthoscelides obtectus is the most detrimental stored common bean pest. The storage pest is widely distributed in Africa, Central and South America, New Zealand, USA and Southern Europe (Mason & McDonough 2012). It develops primarily on common bean but has been found on other beans (Mason & McDonough 2012; Vilca Mallqui et al. 2013). *Acanthoscelides obtectus* exhibits high tolerance to varied degrees of temperature, thus, it is found in cool highland areas as well as the warmer parts of the tropics (Wendt 1992). These beetles are small (3 to 5 mm (1/6 inch)), adults are grey and oblong in shape, with the body covered by yellowish green hairs (Mason & McDonough 2012). Infestation starts in the field when females lay eggs on the mature beans in plant pods. Multiple whitish eggs are laid loosely on a single bean pod or in pod cracks and multiple larvae may emerge from a single bean, unlike many storage insects where just one insect emerges per seed

(Godrey & Long 2008). Bean weevils develop on the mature bean pods in the field but will also infest beans in storage facilities (Baier & Webster 1992).

The common bean availability can be increased by reducing storage losses. Then the control of storage pest such as *A. obtectus* appears very important. Damage caused by *A. obtectus* on bean could be reduced through chemical, biological, physical control and host plant resistance, which are important components of integrated pest management strategies. However, the use of synthetic residual chemicals dominates in Cameroon and North-West region in particular. These chemicals, although effective, cause many environmental problems such as pollution, diseases and resistance in pests among others (Subramanyam & Hagstrum 1995; Park et al. 2003). Furthermore, the majority of farmers in Africa are resource-poor and have neither the means nor the skills to obtain and handle pesticides appropriately. Therefore, an environmentally safe and economically feasible pest control practice needs to be available. Botanicals are relatively environmentally safe. They are generally assumed to be more biodegradable leading to less environmental problems (Nukenine et al. 2010; Regnault-Roger et al. 2012; Goudoungou et al. 2018). Some studies reported the use of products derived from *Chenopodium ambrosoides* (Denloye et al. 2010; Mkenda & Ndakidemi 2014) and *Tephrosia vogelii* (Belmain *etal*., 2012; Stevenson *etal*., 2017, Dougoud etal., 2019) in insect pest control. Some interesting findings were carried out concerning the storage insects as *Sitophilus zeamais* and *Callosobruchus maculatus* (Denloye et al. 2010; Mkenda & Ndakidemi 2014; Ogendo et al. 2004; Mkenda et al. 2015; Kawuki et al. 2005). Essential oils, leaf powder, and different plant formulations have been mixed with stored grain; the effectiveness of this method of protection depends not only on the type of the plant and quantity used but also on the circumstances under which the plant is cultivated and the time at which it is harvested (Dougoud et al. 2019; Stevenson et al. 2012). Temperature, humidity, harvest site affect the insecticidal properties of plant used as insecticide (Figueiredo et al. 2008; Gahukar et al. 2014; Bohinc & Trdan 2017). Also, the species of pest insect affects the efficiency of plant used to protect stored grain (Nukenine et al. 2010). Taking into consideration all these remarks, the study of the ability of *C. ambrosoides* and *T. vogelii* to protect stored common bean in North-West region of Cameroon revealed useful especially for the smallholders of this locality. Therefore, the objective of this research work was to determine the ability of *C. ambrosoides* and *T. vogelii* to protect stored common bean against *A. obtectus* attack in ambient conditions of Tubah locality, North-West region of Cameroon.

2. Materials and methods

2.1- Insect rearing

The strain of *Acanthoscelides obtectus* used in this study was collected on stored common bean by smallholders in Bambui, North-West Cameroon. The bruchids were reared on disinfected common bean (*Phaseolus vulgaris*) in 900 mL glass jars and kept under fluctuating laboratory conditions (T = 23.03±1.87°C; hr = 75.01±7.5%). Temperature and humidity during the study were recorded by data logger (Data logger Model EL-USB-2, LASCAR, China). The insects used in the experiment were those obtained from the fourth generation in order to allow adaptation to their new environment. Since the life duration of *A. obtectus* adult is short, the insects used were aged ≤ 2 days to allow a better assessment of the plant powder effectiveness.

2.2- Commodity used: common bean, *Phaseolus vulgaris*

The variety of common bean, *Phaseolus vulgaris* used during experimentation was obtained from a smallholder stock around Bambui, North-West region, Cameroon. Before experimentation, broken grains, pieces of stone, sand and other foreign materials were removed from the stock. Then, the bean was kept in the freezer at −20 °C for 14 days for disinfestation. After disinfestations from all types of living organisms, the bean was kept in ambient conditions of laboratory for 14 days to allow its acclimatisation. Then the moisture content of bean was determined before experiment and it was 11.95±0.36 %. After all these steps, the bean was ready for use as substrate for insect rearing and bioassays.

2.3- Plant products

Leaves of *C. ambrosoides* and *T. vogelii* were collected in May 2018 in Tubah, a subdivision of Mezam division, North-West region, Cameroon. Tubah is located between latitude 4° 50′ to 5°20′ North and longitude 10°35′ to 11°59′ East of Green Wish Meridian (Ndenecho 2009). The identity of the plant was confirmed by an Ethno-botanist of the University of Bamenda who accompanied the researchers during plant harvest. The leaves were dried at room temperature for 14 days and then crushed. The crushed leaves were ground until the powder passed through a 0.20-mm sieve. Then, the powder was stored in a freezer at −20 °C until needed for bioassays.

2.4- Mortality bioassay

Different quantities of leaf powder of *C. ambrosoides* and *T. vogelii* (0.2; 0.4; 0.8 and 1.6 g) were added to 50 g of common bean contained in glass jars covered with perforated lids to allow aeration. The mix up of bean and plant powder was manually shaken during five minutes to allow uniform coating of plant powder on grain. Then 20 *A. obtectus* adults of non determined sex were added in each class jar. The control was constituted by infesting the bean with the same number (20 insects) of bruchid as previously but without plant powder. After adding insects, the jars were covered and displayed on shelves in ambient laboratory conditions (T = 23.03±1.87°C; hr = 75.01±7.5%). In order to determine mortality, the observations were carried out within 1, 4, 7 and 10 days. During observations the dead and alive *A. obtectus* were counted. The insect was considered dead after many delicate contacts with entomological forceps without any reaction.

2.5- Progeny production

After recording mortality within 10 days of exposure (setion 2.4), the plant powder and insects were discarded, and infested beans were maintained in the same glass jars for further observations. The experiment was displayed on the shelf in the fluctuating laboratory conditions (T = 23.03±1.87°C; hr = 75.01±7.5%). The observations were carried out every starting from the fifth week after removing insect and powder from the jars. From this period, progeny counting was done once a week every week up to the last progeny emergence

2.6- Damage and population growth bioassays

The quantities of 0.8; 1.6; 3.2; and 6.4 g of *C. ambrosoides* or *T. vogelii* leaf powder were added to 200 g of common bean separately to constitute the contents of 4; 8; 16 and 32 g/kg. The jars containing bean and plant powder were hand shaken to permit uniform distribution of powder on grain surface. Then 20 adult bruchids of non determined sex were in the glass jars. The control was constituted by the same quantity of bean and same number of bruchids but without plant powder. The jars containing infested and treated bean were displayed on the shelves. The storage was carried out during eight months, from June 2018 to January 2019. After this period the number of insects in each dosage was recorded in order to determine the population growth. Damage assessment was performed by measuring and counting the number of damaged and undamaged grains using the method of Adams & Schulten (1978).

2.7- Data analysis

Abbott‘s formula (Zar 1999) was used to correct for control mortality before Analysis Of Variance (ANOVA) and probit analysis. Data on cumulative corrected mortality, reduction in F_1 progeny, damage, weight loss and germination percentage were arcsine-transformed [(square root(x/100)] and the number of F_1 progeny was log-transformed (x + 1). The transformed data were subjected to the ANOVA procedure using SPSS package Version 20.0 (IBM Corporation 2011; Finney 1971). Tukey’s test (P = 0.05) was applied for mean separation. The student T test was used to compare *C. ambrosoides* and *T. vogelii* leaf powder concerning the different insecticidal parameters. Probit analysis (Finney 1971; Abbott 1925) was conducted to determine lethal dosages causing 50% (LC_{50}) and 95% (LC_{95}) mortality of *A. obtectus* adult at 1, 4, 7 and 10 days after treatment application. The probit analysis was also used to determine the effective content causing 50% (EC_{50}) reduction of F_1 progeny.

3. Results

3.1- Mortality and toxicity of leaf powder on *Acanthoscelides obtectus*

Chenepodium ambrosoides and *T. vogelii* leaf powder significantly induced mortality of *A. obtectus* (F = 9.45-2937.79; P<0.01) (Table 1). This mortality was increased with increase exposure period and content. Then the efficacy was dose-dependent. The complete mortality of insects was reached by *C. ambrosoides* leaf powder from the contents of 16 g/kg with four days exposure, whereas the same performance was realised by T. vogelii leaf at its highest content (32 g/kg) within 10 days exposure. *C. ambrosoides* and T. vogelii leaf at their lowest content (4g/kg) induced respectively 57.50% and 1.25% within one day exposure, whereas in the same order with the same content these plants caused respectively 93.32% and 76.40% *A. obtectus* mortality within 10 days exposure. *Chenopodium ambrosoides* just within one day exposure caused almost complete mortality (98.75%) at its highest content (32 g/kg). The lethal content 50 (LC_{50}) and 95 (LC_{95}) decreased as the exposure period increased (Table 2). *C. ambrosoides* recorded the lowest LC_{50} and LC_{95} within 10 days, which were respectively 1.475 g/kg and 4.364 g/kg. *Tephrosia vogelii* leaf powder recorded 63.885 g/kg within four days but within 10 days the LC value felt to 10.359 g/kg.

3.2- Progeny inhibition

The production of *Acanthoscelides obtectus* progeny was significantly inhibited by *C. ambrosoides* and *T. vogelii* leaf powder (Table 3). This inhibition was dose-dependent, as the content increased, the production of progeny also decreased. The complete inhibition of F_1 progeny was achieved by *C. ambrosoides* at its highest content (32 g/kg). Bean treated *T. vogelii* leaf powder at 32 g/kg recorded 7.75 insect then an inhibition of 86.47%. The two leaf powders even at their lowest content (4 g/kg) considerably inhibited the production of F_1 progeny. *Chenopodium ambrosoides* and *T. vogelii* reduced *A. obtectus* F_1 progeny by 84.39% and 34.69% respectively at 4 g/kg. *Chenopodium ambrosoides* revealed more effective *T. vogelii*, this was confirmed by the efficacy content 50 (EC_{50}) and 95 (EC_{95}). *Chenopodium ambrosoides* EC_{50} and EC_{95} values were lower than those of *T. vogelii*.

3.3- Suppression of insect population and reduction of grain damage

Chenopodium ambrosoides and *T. vogelii* leaf powder significantly suppressed the population growth of *A. obtectus* (Table 4; Figure 1) and reduced grain damage and weight loss (Table 4; Figure 2). This was dose dependent, as the content increased inversely to the number of emerged bruchids, percentage of damaged bean and weight loss reduced. The bean treated with content of 8 g/kg *C. ambrosoides* recorded almost complete suppression of insects (20.50 bruchids). With the same powder from 16 g/kg, there was not population growth, only 20 insects introduced from the beginning of the experiment were found dead (Figure 1). The reduction of damage in bean treated with *C. ambrosoides* followed the same tendency, from the content of 4 g/kg the weight loss was almost completely suppressed (<1 %). From 8 g/kg, the weight loss was less than 0.1. Bean treated with *T. Vogelii* leaf powder at its lowest content suffered from considerable damage (weight loss: 34.30 %), which linked to the proliferation of bruchids at this content, it was 255.25 insects in this treated bean. However the bean treated with this leaf powder content suffered less damage compared to the untreated or control sample, which recorded 54.36% weight loss and 499 emerging insects. *Tephrosia vogelii* at its highest (32 g/kg) content suppressed almost completely *A. obtectus* emergence (26.75 insects), and reduced almost totally damage (<1 % weight loss).

4. Discussion

Chenopodium ambrosoides and *T. vogelii* leaf powder significantly induced mortality of *A. obtectus* adult, which was dose dependant. In addition this mortality varied according to the plant species; *C. ambrosoides* revealed more effective than *T. vogelii*. The increase in mortality according to the time exposure and leaf powder concentration was due to the increase of quantity of active components contained in plant products then also their toxicity against insect. The difference in term of efficacy concerning plant extracts on an insect species depends on several factors such as extracts types, application methods, and plant species. In this study, there were two species characterized by different chemical compositions. *Tephrosia vogelii* mainly contains rotenoids (deguelin; rotenone; sarcolobine; tephrosin; α-toxicarol) (Stevenson et al. 2012). *Chenopodium 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 (Cavalli et al. 2004). *Tephrosia* contains rotenoids, to which it owes its insecticidal properties (Isman 2008; Stevenson et al. 2012). Rotenone has been used as an insecticide for over 150 years (Isman 2008).The difference in chemical composition gives to the two used plants different effectiveness against *A. obtectus*. That was observed in this experiment by the high efficacy of *C. ambrosoides* leaf powder compared to that of *T. vogelii*.

In addition to the chemical composition the toxicity of a plant can be also attributed to its formulation, which allows acting physically such as powder. Insect mortality may be due to the physical action of the powder formulations, since the particles may block spiracles of the test insects and cause death by asphyxiation (Denloye et al. 2010). Plant powders cause abrasion of insect cuticle and lead to water loss (De Sousa et al. 2005), which may cause stress and eventual death.

Several plants were found to be toxic against *A. obtectus*, for example Waweru et al. (2017) reported that *Jacaranda mimosifolia* and *Bougainvillea spectabilis* powder prepared in concentrations of 2.5g, 5g and 10g showed potential mortality on *A. obtectus*. This mortality was considerably influenced by content and time exposure as in the present study. These authors observed that the insecticidal activity was more evident after 72 and 96 hours where 5g and 10g of the powder showed significant difference with 2.5g and the control.

Some studies highlighted the effectiveness of *C. ambrosoides* against storage insets pests; Guzzo et al. (2006)., reported that the leaf powder of *C. ambrosoides* was more toxic to *S. zeamais* than either *C. maculatus* or *T. castaneum* with 48 h LC_{50} values of 0.46 g/kg, 1.60 g/kg and 2.14 g/kg, respectively. In the present study *C. ambrosoides* leaf recorded LC_{50} value of 2.44 g/kg within 96 h (4 days). This difference was due to the insects' species, commodity used, temperature and relative humidity. A wide range of factors affects secondary metabolite content and concentration in plants and thus the concentration of active ingredients in botanical used as insecticides. Different plant parts are highly variable in active ingredient content and concentration (Dougoud et al. 2019).

As emphasised by Nta et al. (2019) the ability to reduce progeny production should be one of the basic characteristics of an effective grain protectant. *Tephrosia vogelii* and *C. ambrosoides* leaf powder significantly inhibited F_1 progeny production of *A. obtectus*. This inhibition was dose dependent and varied according to the plant species. Several studies revealed the inhibitory effect of plant extracts on stored pest beetles in particular coleopteran pest insects of stored cereals and pulses. Tapondjou et al. (2002) showed that the dry ground leaf of *C. ambrosoides* inhibited F_1 progeny production and adult emergence of the *C. chinensis* and *C. maculatus*. Denloye et al. (2010) reported that *C. ambrosoides* extract had the potential for practical control of insects by acting as oviposition suppressant and ovicide. Leaf powder of several plants has shown their ability to reduce the production of *A. obtectus* F_1 progeny. As presumed by Ntonifor et al. (2011), *C. ambrosoides* leaf powder has larvicidal and/or ovicidal effects in addition to its adulticidal activities. According to its phytochemical composition done by several compounds, that may having synergistic and /or potentiating interactions, are lethal both to the adult and immature stages of pest insect. The mechanism can also be responsible for inhibiting the *A. obtectus* production of F_1 as observed with the same plant concerning *S. zeamais*. The same assumption can be also made for *T. vogelii*, which is an aromatic plant even though these two plants do not belong to the same family. The tendency concerning adult mortality induced by two used plants was maintained for progeny inhibition; *C. ambrosoides* was more effective than *T. vogelii*. The high mortalities of *A. obtectus* induced by *C. ambrosoides* leaf powder just at exposure (one day exposure) seriously reduced the number of insects able to lay egg. That situation, by significantly reducing number of laid eggs, inevitably induced the inhibition of progeny production. At six weeks after treatment, the leaf powder of *Solanum melongena*, *Parkia biglobosa*. *Ipomoea batatas*, *Colocasia esculenta*, *Tridax procumbens* and *Terminalia catappa* individually had significant effect on progeny emergence (Nta et al. 2019). Among the six plants tested by these authors, *T. catappa* and *S. melongena* were very effective in inhibiting the reproduction and progeny emergence of *A. obtectus*.

A good grain protectant must be able to suppress pest population in order to reduce considerably even completely damage. In the present study, *T. vogelii* and *C. ambrosoides* leaf powder significantly reduced *A. obtectus* population growth and considerably lowered damage in treated *P. vulgaris* grain. The used plant powders in this study significantly reduced *A. obtectus* population growth and bean damage at all their dosages. The effectiveness of *C. ambrosoides* leaf powder was high even at the lowest concentration. With the low contents of *T. vogelii* there was recorded considerable population growth. However the reduction of grain loss remained significant. As observed in some studies especially in field trials with homemade botanical insecticides, the lower pest mortality may not always mean lower efficacy (Aziz et al. 2013; Tembo et al. 2018). The lower performance observed concerning the lower dosages especially with *T. vogelii* could be attributed to the reduction of the quantity of active compounds due to their volatility, when the storage period goes on. In this case the treatment needs to be renewed in order to improve effective grain protection. Ntonifor et al. (2011) reported that the maize grain treated with 0.5g/50g of *C. ambrosoides* and stored for 90 days (3 months) suffered about double grain weight loss than that of 45 days (one and half month) of storage. But from 1g/50g of same plant powder, there was not weight loss up to 90 days storage (Ntonifor et al. 2011). Those findings corroborate what has been observed in the present study.

Different plants species have been used in many African countries to protect their stored cereals and legumes grains against storage pests which are mostly insects. During a study carried out in Benin (West African country), Loko et al. (2018) found that the majority of farmers (88.70%) used medicinal plant with insecticidal or repellent effect to conserve their stored grain. They reported that six plant species amongst which *Cinchona officinalis*, *Khaya senegalensis*, *Xylopia aethiopica*, *Capsicum frutescens*, *Azadirachta indica* and *Ocimum gratissimum* were used to prevent infestation of bean seeds. All of the plants species except *X. aethiopica* were used in form of leaf or seed powder (Loko et al. 2018). In addition to different plant species, different plant parts are used by African farmers. Then insecticidal compounds can be found in various plant parts. Leaf, stem and root powder of *Chromolaena odorata* exhibited insecticidal activity against cowpea beetle, *C. maculatus* (Osariyekemwen & Benedicta 2017).

Tephrosia vogelii and *C. ambrosoides* leaf powder considerably suppressed damage. In particular at their highest tested dosage, they almost completely diminished the grain damage and weight loss up to more than eight months storage in Tubah locality. These results showed the insecticidal potential and the good performance of these plants in grain protection in Tubah, and this could be extrapolated to the whole country (Cameroon).

In conclusion, plant used in stored grain protection is a practice commonly used by smallholders to conserve small quantity of grains. In addition to being accessible, this method is less expensive and easily applicable. This method requires less technical equipments compared to the synthetic chemical insecticides. These plants used by smallholders are available and most of them are used in pharmacopoeia and human nutrition. The high biodegradability of plant extracts makes them useful in the framework of ecological protection of agricultural products. Then, it becomes important even imperative to improve the bioactivity and most precisely insecticidal efficacy of these plants by testing on others insect pests and in other stored grains. Studies need to be carried out concerning mammalian toxicity in order to promote these plants use for the protection of grain for human consumption.

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Tables

Table 1: Mortality induced by *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powder on *Sitophilus zeamais* adult within different exposure periods

Contents (g/kg)	% Mortality (Mean ± S.E.)		t value
	C. ambrosoides	*T. vogelii*	
	1d		
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	-----
4	57.50 ± 6.29^{b}	1.25 ± 1.25^{bc}	7.83*
8	66.25 ± 4.27^{b}	1.25 ± 1.25^{bc}	12.04**
16	86.25 ± 4.27^{a}	5.00 ± 0.00^{ab}	41.84***
32	98.75 ± 1.25^{a}	7.50 ± 1.44^{a}	38.12***
$F_{(4; 15)}$	93.76***	9.45**	
	4 d		
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
4	79.67 ± 6.66^{b}	11.51 ± 5.31^{bc}	6.84*
8	97.50 ± 2.50^{a}	24.21 ± 5.07^{ab}	12.70**
16	100.00 ± 0.00^{a}	25.53 ± 3.93^{ab}	18.94***
32	100.00 ± 0.00^{a}	39.74 ± 4.22^{a}	14.27**
$F_{(4; 15)}$	182.93***	13.04***	
	7 d		
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
4	81.94 ± 5.05^{b}	30.19 ± 3.62^{c}	11.68**
8	98.61 ± 1.39^{a}	43.76 ± 4.64^{bc}	7.55*
16	100.00 ± 0.00^{a}	57.74 ± 6.03^{b}	7.01*
32	100.00 ± 0.00^{a}	79.51 ± 6.71^{a}	3.05^{ns}
$F_{(4; 15)}$	340.79***	38.37***	
	10 d		
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
4	93.32 ± 0.18^{b}	76.40 ± 3.73^{b}	4.74*
8	100.00 ± 0.00^{a}	89.98 ± 3.33^{a}	3.01^{ns}
16	100.00 ± 0.00^{a}	98.33 ± 1.67^{a}	1.00^{ns}
32	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	----
$F_{(4; 15)}$	2937.79***	314.87***	

Means ± S.E. followed by the same lower case letter in a column for the same powder at same period do not differ significantly at P< 0.05 (Tukey's test); ns: P > 0.05, *: P < 0.05, **: P < 0.001, ***: P < 0.0001

Table 2: Toxicity parameters of *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powder on *Sitophilus zeamais* adult within 1, 4, 7 and 10 days of exposure period

Periods (days)	Products	Slope±SE	R^2	LC_{50} (95% FL)	LC_{95} (95% FL)
1	*C. ambrosoides*	1.881±0.127	0.755	3.767(2.270 ; 5.044)	28.196(19.380 ; 57.045)
	T. vogelii				–
4	*C. ambrosoides*	3.851±0.450	0.287	2.444(1.074; 3.209)	6.534(5.413; 10.328)
	T. vogelii	0.931±0.16	0.539	63.885(32.563; 548.406)	–
7	*C. ambrosoides*	4.346±0.571	0.299	2.472(1.426; 3.072)	5.908(5.136; 7.905)
	T. vogelii	1.442±0.100	0.770	9.954(7.479; 12.903)	137.537(70.054; 517.882)
10	*C. ambrosoides*	3.492±0.817	0.350	1.475(0.576; 2.109)	4.364(3.816; 4.963)
	T. vogelii	2.336±0216	0.541	2.047(1.038; 2.875)	10.359(8.274; 15.239)

LC: efficacy content; SE: standard error; FL: fuducial limit; –: LC values were out of products contents range used in this experiment.

Table 3: Progeny production of *Acanthoscelides obtectus* in bean treated with *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powder under fluctuating laboratory conditions

Content (g/kg)	Products		t value
	C. ambrosoides	*T. vogelii*	
	Mean number of F_1 progeny (Mean±SE)		
0	54.75±3.99^{a}	54.75±3.99^{a}	
4	8.25±4.39^{b}	36.25±5.54^{ab}	3.15^{ns}
8	1.50±0.65^{b}	28.00±6.54^{bc}	3.45*
16	0.25±0.25^{b}	18.25±5.04^{bc}	3.74*
32	0.00±0.00^{b}	7.75±2.25^{c}	3.44*
$F_{(4; 15)}$	78.27***	13.38***	
	Inhibition of adult emergence relative to control (%)		
0	0.00±0.00^{b}	0.00±0.00^{d}	
4	84.39±8.48^{a}	34.69±6.12^{c}	-3.59*
8	97.26±1.19^{a}	49.69±9.86^{bc}	-4.39*
16	99.26±0.38^{a}	67.99±6.74^{ab}	-4.93*
32	100.00±0.00^{a}	86.47±2.94^{a}	-4.61*
$F_{(4; 15)}$	126.37***	28.90***	
EC_{50}(95% FL) g/kg	1.791(0.277; 2.821)	7.552(5.159; 9.973)	
EC_{95}(95% FL) g/kg	6.596(5.110; 12.047)	77.774(42.732; 273.064)	

Means ± S.E. followed by the same lower case letter in a column do not differ significantly at P< 0.05 (Tukey's test); ns: P > 0.05, *: P < 0.05, ***: P < 0.0001; EC: efficacy content; FL: fudicial limit; SE: standard error.

Table 4: *Acanthoscelides obtectus* population increase and grain weight loss in bean treated with *Chenopodium ambrosoides* and *Tephrosia vogelii* after eight months storage under ambient laboratory conditions

Content (g/kg)	Products		t value
	C. ambrosoides	*T. vogelii*	
	Insect number (Mean±SE)		
0	499.00±36.95^{a}	499.00±36.95^{a}	
4	28.50±5.91^{b}	255.25±53.77^{b}	-4.39*
8	20.50±0.50^{b}	58.25±16.97^{c}	-2.29^{ns}
16	20.00±0.00^{b}	48.25±18.02^{c}	-1.57^{ns}
32	20.00±0.00^{b}	26.75±3.75^{c}	-1.80^{ns}
$F_{(4; 15)}$	162.34***	41.74***	
	Weight loss (%)		
0	54.36±4.79^{a}	54.36±4.79^{a}	
4	0.65±0.06^{b}	34.30±3.38^{b}	-9.85**
8	0.22±0.06^{b}	6.46±0.63^{c}	-10.07**
16	0.08±0.02^{b}	3.70±0.35^{c}	-10.45**
32	0.03±0.01^{b}	0.68±0.15^{c}	-4.05*
$F_{(4; 15)}$	127.52***	78.92***	

Means ± S.E. followed by the same lower case letter in a column do not differ significantly at P< 0.05 (Tukey's test); ns: P > 0.05, *: P < 0.05, ***: P < 0.0001.

Figures

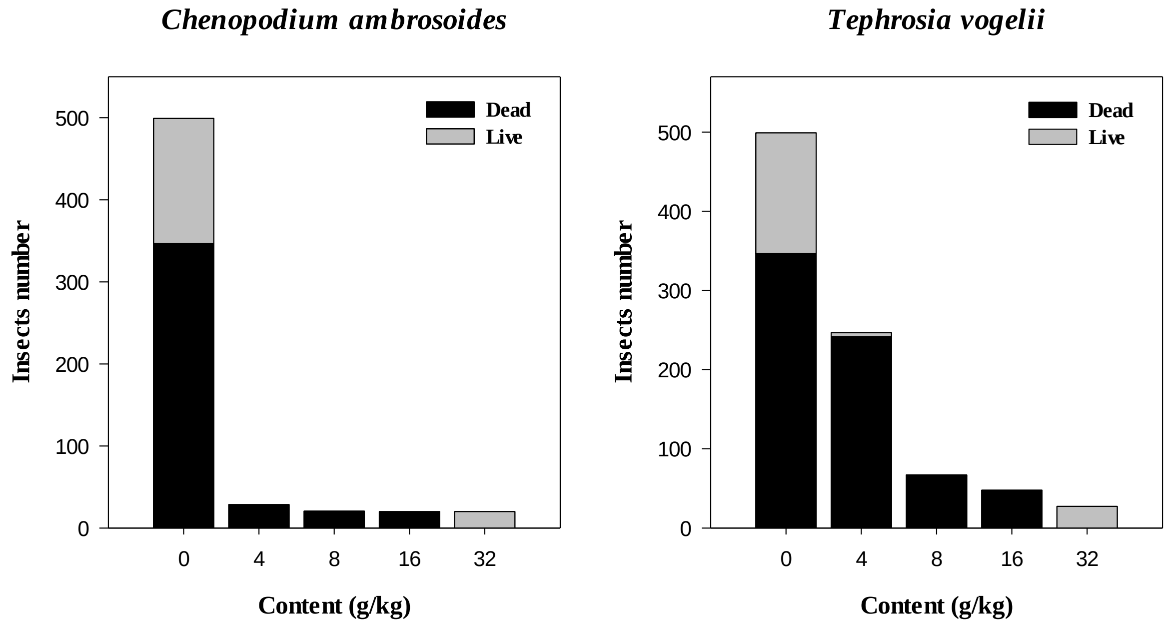
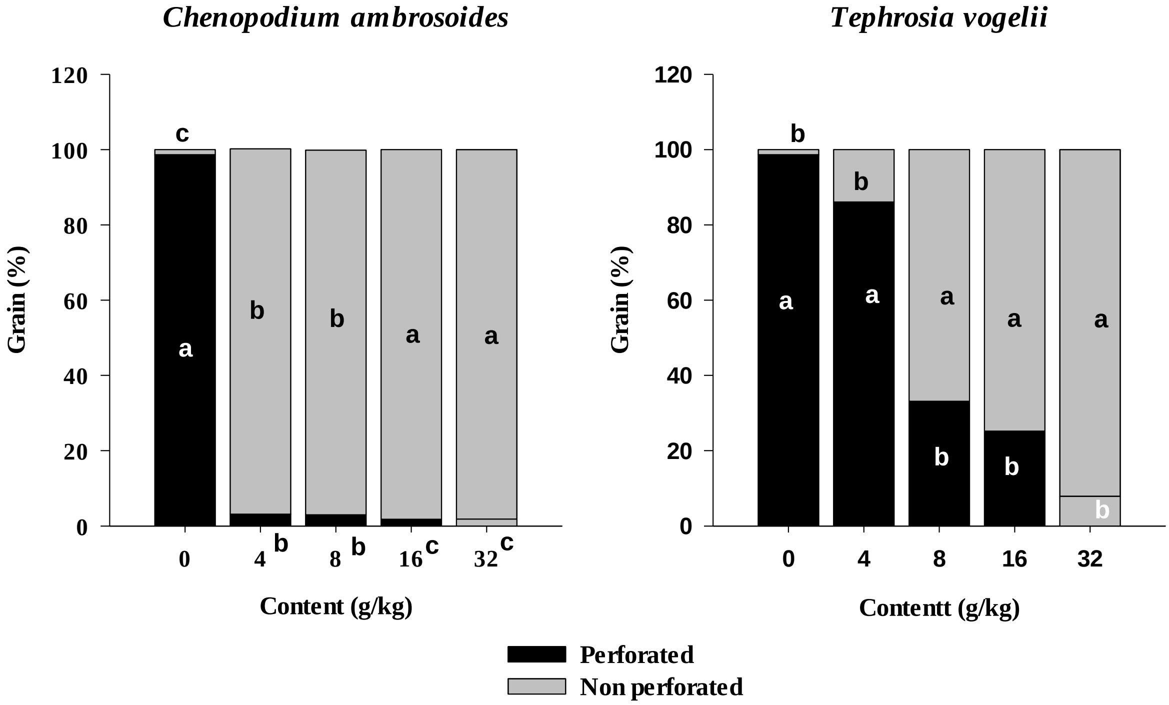


Figure 1: Dead and alive insects recorded after eight months storage of common bean treated with *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powder under ambient laboratory conditions



The bands of the same colour with the same lower case letter do not differ significantly at P< 0.05 (Tukey's test).
Figure 2: Damage grain recorded in beans treated with *Chenopodium ambrosoides* and *Tephrosia vogelii* leaf powder after eight months of storage

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