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Relative Susceptibility of one *Coleopteran* Stored Product Insects to Permethrin Dust Insecticide and *Chromolaena odorata* Powder Extract

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Abstract

The increased use of pesticides in the control of vector pests has raised some concern over the adverse effect of these chemicals on humans, domestic animals, and their environment. This research was undertaken to harness the beneficial use of dried *Chromolaena odorata* (Siam weed) leaves to control beans weevil (*Acanthoscelides obtectus*) in the storage system. The experimental research method was a complete randomized design with five (5) treatments replicated four times given a total of twenty plates. *Chromolaena odorata* leaves and powders were prepared at a ratio of 1:1. The result of the study revealed higher mortality of bean weevils after seven (7) days in group A with the highest mortality rate obtained from Treatment four (4) with ten (150) grams of dried ground *Chromolaena odorata* leaves and dust insecticide. Based on this study, beans farmers and traders could use *Chromolaena odorata* to control beans weevils in their storage area, instead of synthetic insecticide.

Keywords: Stored Product Insects, Permethrin, Insecticide, *Chromolaena odorata*, Extract

1 Introduction

The use of fumigants and conventional neurotoxic insecticides as grain protectants are unsuccessful in controlling store product pests because of environmental problems such as pollution and mammalian toxicity. Resistance of pests to residual insecticides and the demand for residue-free food have led researchers to evaluation of new-reduced risk insecticides to control stored product pest [1]. One of the most well-studied and most promising alternatives to traditional neurotoxic grain protectants is the use of permethrin [1] Permethrin **i**s a non-systemic insecticide with contact and stomach action, having a slight repellent effect [2]. Permethrin is a contact insecticide effective against a broad range of pests. It controls leaf- and fruit-eating Lepidoptera and Coleoptera in cotton, in fruit, in tobacco, vines and other crops, and in vegetables [3]. It has good residual activity on treated plants. It is effective against a wide range of animal ectoparasites, provides > 60 d residual control of biting flies in animal housing, and is effective as a wool preservative. It provides control of Blattodea, Diptera, Hymenoptera and other crawling insects, also flying insects [4].

The body of adult stored-product insects is made up of an exoskeleton covered with waterproofing waxes and lipids. The wax layer on the insect's epicuticle is damaged and insects lose water through the cuticle. In addition, another mode of action of permethrin is its ability to repel insects [5]. Bean beetles, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), are agricultural pest insects of Africa and Asia that presently range throughout the tropical and subtropical world. This species also is known as the southern cowpea weevil. The larvae of this species feed and develop exclusively on the seed of legumes (Fabaceae) hence the name bean beetle [5]. The adults do not require food or water and spend their limited lifespan (one-two weeks) mating and laying eggs on beans.

The systematic placement of bean beetles is as follows: *Callosobruchus* is one of the genera in the subfamily Bruchinae (seed beetles) that is in the family Chrysomeloidae [6]. This group is part of the order of beetles, Coleoptera (from Greek "sheath-winged" referring to the stiff outer, first pair of wings (elytra that protect the membranous second pair of flight wings). The Coleoptera is the largest of the orders that comprise the class Insecta. Insects are the largest and most diverse (750,000 described species) of all the animal classes that are found in all but marine environments. Insects are protostomous animals and are thus more closely related to mollusks and crustacea than to the deuterstomous vertebrate classes [7]. Spraying harmful insecticides in the *Callosobruchus maculatus* habitat, especially on a bean farm, to reduce the pest population is not practical. The species is an important biological control agent of insect pests in rice fields, thus reducing the population would conflict with agricultural interests. Thus, chemical treatment in infested human settings seems to be the best approach to dealing with rove beetle infestations. In the 1960s, several trials of now-banned pesticides (e.g., DDT, lindane, chlordane, malathion, diazinon, and dieldrin) against P. fuscipes were conducted [8]. However, information about the effectiveness of newer insecticides against bean beetles is lacking.

Pesticides are used widely in agriculture in Nigeria [9]. When effectively applied, pesticides can kill or control pests, including weeds, insects, fungi, bacteria, and rodents. Chemical

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pest control has contributed to dramatic increases in yields for most major fruit and vegetable crops [10]. Its use has led to substantial improvements over the past 40 years in the concept of food security and thus in the health of the public [11]. On the negative side, many pesticides are harmful to the environment and are known or suspected to be toxic to humans [12]. They can produce a wide range of adverse effects on human health that include acute neurologic toxicity, chronic neuro-developmental impairment, cancer, reproductive dysfunction, and possibly dysfunction of the immune and endocrine systems [13]. The diet is an important source of exposure to pesticides. The trace quantities of pesticides and their breakdown products that are present on or in foodstuffs are termed residues. Residue levels reflect the amount of pesticide applied to a crop, the time that has elapsed since application, and the rate of pesticide dissipation and evaporation. Pesticide residues are widespread in the household diet [14]. They are consumed regularly by most individuals, including infants and children. The increased use of pesticides in the control of vector pests and filthy insects has prompted some concern over the adverse effect of these chemical substances on living organisms and their environment [15]. The harmful nature of these synthetic chemical pesticides leads to the inhibition of the actions of enzymes and the blocking of essential processes in organisms.

A pesticide is defined under FIFRA as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insects, rodents, nematodes, fungi, or weeds, or any other forms of life declared to be pests, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant". Pesticides have been used by humankind for centuries. Their use was recorded as early as the eighth century BC when the application of fungicides was documented in Homeric poems [12]. Until the present, numerous mixtures have been developed to control fungi, insects, weeds, and other pests. In the 19th century, sulfur compounds were developed as fungicides, and arsenicals were used to control insects attacking fruits and vegetables. Those compounds were highly toxic and consequently were replaced by chlorinated organic pesticides such as DDT and benzenehexachloride (BHC), which were developed during the 1930s and became widely used in the 1950s and 1960s [10]. Chlorinated hydrocarbon insecticides such as DDT, BHC, dieldrin, aldrin, and toxaphene were enthusiastically adopted by farmers who hoped to control previously uncontrolled insects with what were believed to be relatively safe compounds with long environmental persistence. These chemicals were also used widely in the control pests and insects [16].

The bean weevils or seed beetles are a subfamily (*Bruchinae)* of beetles, now placed in the family *Chrysomelidae*, though they have historically been treated as a separate family. They are Granivores, and typically infest various kinds of seeds or beans, living for most of their lives inside a single seed. The family includes about 1,350 species found worldwide. Bean weevils are generally compact and oval, with small heads somewhat bent under. Sizes range from 1 to 22mm for some tropical species. Colors are usually black or brown, often with mottled patterns. Although their mandibles may be elongated, they do not have the long snouts characteristic of true weevils. Adults deposit eggs on seeds, then the larvae chew their way into the seed. When ready to pupate, the larvae typically cut an exit hole, and then return to their feeding chamber. Adult weevils have a habit of feigning death and dropping from a plant when disturbed [3].

Chromolaena odorata is a perennial shrub forming dense tangled bushes 1.5 - 2.0 m in height, occasionally reaching 6m as a scrambler up trees. *Chromolaena odorata* originated in subtropical and tropical America, had a wide native distribution, from the Southern United States to Northern Argentina, and exhibits considerable variation throughout its distribution. It was argued that the source of the original introduction is likely to have been Jamaica in the West Indies. In the Neotropics, *Chromolaena odorata* is always found in competition or association with a complex of closely related species not present in Asia or Africa. In the new world, *Chromolaena odorata* is common in most habitats except in undisturbed rainforests. It is typically a plant of secondary succession, rapidly invading clearings and persisting until shaded out by the overgrowth of forest trees. The plant can be poisonous to livestock as it has an exceptionally high level of nitrate (5 to 6 times above the toxic level) in the leaves and young shoots; the cattle feeding on these die of tissue anoxia [17]. It is generally regarded as poisonous to animals and thus not recommended as a livestock feed. However, some studies show its benefits in low concentrations, such as up to 5% for egg-laying chickens which also improved yolk color [5]. In Malaysia, where *Chromolaena odorata* is an invasive exotic, plant parts are used by traditional practitioners for the treatment of burns, wound healing, skin infections, post-natal wounds, and as an anti-malarial. It has also been reported to possess anti-inflammatory, astringent, diuretic, and hepatotropic activities [1]. Since the use of chemicals to control bean weevil and other grain pests have been abused causing possible chaos to humans, this study became important for the need for alternative biological pesticides using dried grinded and chopped *Chromolaena odorata* leaves and stems [3].

This study will be helpful to researchers, students, farmers, governmental agencies, etc. It will help in the reduction of the spread of pests and diseases in plants. This work will ensure the appropriate method of reduction of pests without emitting harmful substances into the air. There is increasing use of harmful organophosphates such as Actellic dust and Sniper (2, 2 dichlorovinyl-dimethylphosphate) in the preservation of beans. Ideally, a pesticide must be lethal to the target species, but not to non-target species, including man, unfortunately, this is not the case, therefore the controversy of the use and abuse of pesticides has surfaced. The rampant use of these chemicals, under the adage, “If a little is good, a lot more will be better” has played havoc with humans and other life forms [6].

2 Materials and Methods

2.1 Study Area

The study was carried out in Eziobodo Owerri-west Local Government Area (LGA) of Imo State with headquarters at Umuguma. It is located in Owerri Agricultural Zone, in the rain forest zone about 120km North of the Atlantic coast and lies on latitudes 4^0 14’ North and 6^0 15’ North, longitude 6^0 51’ East and 8^0 09’ East (National Geographical Journal 2004). Owerri West L.G.A has a population of 250,000 people and an estimated area of 295 square kilometers (NPC, 2006). Owerri-West Local Government Area shares boundaries with Ngor-Okpala Local Government Area in the South, Owerri Municipal Council in the East, Mbaitolu Local Government Area in the North, and Ohaji/Egbema Local Government Area in the West. Owerri West L.G.A. has some significant features like the Federal polytechnic which is located at Nekede and the Federal University of Technology (FUTO) which is located at Ihiagwa/Eziobodo/Obinze. The Local Government Area has two dominant seasons: rainy and dry season. Rainfall starts between April and October while the dry season starts from November to early March. The average annual rainfall measures up to 2550 mm, the relative mean temperature ranges annually between 24.50 and 25.50 and the humidity varies according to the time of the year. The area has many secondary schools, and in some communities, two or more secondary schools.

2.2 Data Collection

The *Chromolaena odorata* leaves used for the study were collected in the locality of the Federal University of technology, owerri, Nigeria. The *Chromolaena odorata* was authenticated by Miss Edidiong Uwa. The voucher specimen was deposited in the Herbarium of the Department of Biology. Coleoptera (beans) was gotten from Ihiagwa market, Owerri, Imo state. The weevils used for the study were sourced from beans storage facilities in Ihiagwa market, Owerri, Imo State, Nigeria, while the beans used were obtained from Ihiagwa market, Owerri, Imo state Nigeria. The beans used were free of weevils.

Figure 1: *Chromolaena Odorata* (Source: Eziobodo farm)

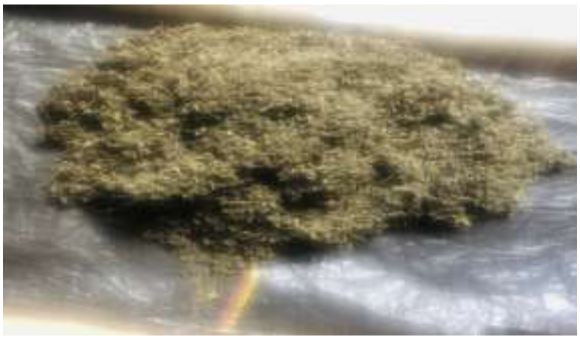

Figure 2: Dried *Chromolaena Odorata*

2.3 Experimental Design and Treatments

Negative control: 0g dried *Chromolaena odorata* leaves were used, 10g of beans grain was weighed and placed in a small container in a cage to simulate buckets of beans, then 10 live weevils were introduced onto the bucket of the beans.

Table 1:

CLP	RI	RII	RIII
T_0(0g)	T_0R_1	T_0R_2	T_0R_3
T_1(50g)	T_1R_1	T_1R_2	T_1R_3
T_2(100g)	T_2R_1	T_2R_2	T_2R_3
T_3(150g)	T_3R_1	T_3R_2	T_3R_3
T_4 50g (chem) insecticide	T_3R_1	$T4R_2$	$T4R_3$

Positive control: 2.5g Actellic Dust (Powder) was weighed and put into the cage that simulates a small container and was covered with a net to allow sufficient air to get in, 10g of beans grain was weighed and placed in a small bucket to simulate bucket of beans in the container, then 10 live weevils were introduced into the bucket of the beans. 2.5g of dust insecticide was introduced into the bucket of beans.

2.4 Treatments with Ground Chromolaena Odorata Mixture

Treatment T0: 0g of a mix of dried ground *Chromolaena odorata* mixture, was weighed and put into the cage that simulates, and was covered with a net to allow sufficient air get in, 10g of beans grain was weighed and placed in small buckets to simulate bucket of beans in the warehouse, then10 live weevils were introduced into the bucket of the cage. Treatment T1:100g of a mix of dried ground *chromolaena odorata* mixture was weighed and put into the cage to simulate and was covered with a net to allow sufficient air to get in, 10g of beans grain was weighed and placed in a small bucket to simulate bucket of beans, then 10 live weevils were introduced into the bucket. Treatment T2:1500g of the dried ground chromolaena odorata, was weighed and put into the cage to simulate a warehouse, and was covered with a net to allow sufficient air get in, 10g of beans grain was weighed and placed in a small bucket to simulate bucket of beans, then 10 live weevils were introduced into the bucket of the cage. Treatment T3:10g of a mix of ground *Chromolaena odorata* leaves was weighed and put into the cage to simulate and was covered with a net to allow sufficient air to get in,10g of beans grain was weighed and placed in a small bucket to simulate bucket of beans, then 10 live weevils were introduced into the bucket. Treatment T4: chem

2.5 Preparation of Chromolaena Odorata

The *Chromolaena odorata* and leaves were washed with distilled water, sliced, and then dried for 30 days at room temperature. After 30 days, the dried *Chromolaena odorata* was ground with a blender. 300g of chopped *Chromolaena odorata* leaves were mixed in each bucket of beans for preparation and also the dust insecticide was mixed with the beans in different containers.

Fig 3: Chromolaena odorata extract

2.6 Number of holes on the bean seeds

Table 2 shows the results on the mean number of holes and weightloss of bean seeds treated with different grams of *Chromolaena odonata* leaf powder (CLP) and permethrin dust insecticide (PDI). CLP (150g) and PDI (50g) gave the lowest mean number of holes (14) and (11) with lower weight loss of 1.15 and 0.9g respectively. The control treatment that was not treated with PDI and CLP gave the highest mean number of holes on bean seeds and the highest weight loss of 48.53.

2.7 Mortality rate of callosobruchus maculatus on permethrin dust insecticide and chromolaena odorata leaf powder

The mortality rate was recorded highest on permethrin dust insecticide (PDI) and 150g *Chromolaena odorata* leaf powder (CLP) having a percentage mortality of 100% respectively and the two treatments had no significance between them in mortality. The results also showed that the control treatment had the highest mean number of bean beetle alive (Table 1). There was no mortality recorded on control treatment.

2.8 Weight loss of bean seeds

Weight loss of bean seeds treated with different grams of *chromolaena odonata* leaf powder (CLP) and permethrin dust insecticide (PDI). CLP (150g) and PDI (50g) gave the lowest mean number of holes (14) and (11) with lower weight loss of 1.15 and 0.9g respectively. The control treatment that was not treated with PDI and CLP had the highest weight loss of 48.53.

2.9 Phytochemical Analysis of chromoleana odorata

The analysis for alkaloids, tannins, flavonoids, saponins, terpenoids, and cardiac glucosides was carried out according to standard methods of [18] and [1].

2.9.1 Alkaloid

A few ml of *Chromolaena odorata* leaf powder was prepared; two drops of Mayer's reagent were added along the side of the tubes. 1.0ml portion was treated similarly with Dragenduff's reagent. The appearance of a creamy precipitate indicates the presence of alkaloids.

2.9.2 Tannins

0.5g of powdered sample of the *Chromolaena odorata* leaf extract was boiled in 20ml of distilled water in the test tube and filtered 0.1% FeCL3 was added to the filtered sample and observed for brownish green or a blue-black coloration which shows the presence of tannins.

2.9.3 Flavonoids

20MG of *Chromolaena odorata* leaf extract was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution was added to it and concentrated sulphuric acid was added later. A yellow color indicates the presence of flavonoids. The yellow color disappeared allowing the solution to stand.

2.9.4 Saponins

2g each of *Chromolaena odorata* leaf powder was boiled together with 20ml of distilled water in a water bath and filtered 10ml of the filtered sample was mixed with 5ml of distilled water in test tubes and shaken vigorously to obtain a stable persistent forth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicated the presence of saponins.

2.9.5 Terpenoids

20mg of *Chromolaena odorata* leaf powder was dissolved in 1ml of chloroform and 1ml of concentrated sulphuric acid was added to it. A reddish-brown discoloration at the interface showed the presence of terpenoids.

2.9.6 Steriods

20ml of *chromolaena odorata* leaf powder was dissolved in 1ml of acetic acid following Liberman Burchard and Salkowkiss procedures. A brownish color and red color at interference indicated the presence of steroids.

2.9.7 Cardiac Glycosides

20ml of *Chromolaena odorata* leaf powder was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution were added. 0.5ml of concentrated sulphuric acid was slowly added along the side of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides or cardiac glycoside constituents.

3 Results

3.1 Mortality rate of callosobruchus maculatus on permethrin dust insecticide and chromolaena odorata leaf powder.

The mortality rate was recorded highest on permethrin dust insecticide (PDI) and 150g *Chromolaena odorata* leaf powder (CLP) having a percentage mortality of 100% respectively and the two treatments had no significance between them in mortality. The results also showed that the control treatment had the highest mean number of bean beetle alive (Table 1). there was no mortality recorded on control treatment.

Table 2: Effect of permethrin dust insecticide and *Chromolaena odorata* leaf powder

Treatments	Mean number of beetle introduced	Mean Number Dead	Mean Number Alive	Mortality (%)
Control(0g)	30 ± 0.1	$0^{d}\pm0.00$	$30^{a}\pm0.10$	0
CLP (50g)	30 ± 0.1	$12^{c}\pm0.17$	$18^{b}\pm0.34$	40
CLP (100g)	30 ± 0.1	$18^{b}\pm0.34$	$12^{c}\pm0.17$	60
CLP (150g)	30 ± 0.1	$30^{a}\pm0.10$	$0^{d}\pm0.00$	100
PDI (50g)	30 ± 0.1	$30^{a}\pm0.10$	$0^{d}\pm0.00$	100

Mean along the column having different superscript of letters differed significantly at P = 0.05
CLP = Chromolaena Leaf Powder
PDI = Permethrin Dust Insecticide

Table 3: Effect of permethrin dust insecticide and *chromolaena odorata* leaf powder on number of holes and weight loss of bean seeds

Treatments	The initial weight of seeds	The final weight of seeds	Mean Number of holes	Weight loss of seeds
Control(0g)	250 ± 1.0	$201.5^{c}\pm12.11$	$198^{a}\pm19.26$	$48.5^{a}\pm6.08$
CLP (50g)	250 ± 1.0	$234.2^{b}\pm18.13$	$76b\pm12.17$	$15.8^{b}\pm2.14$
CLP (100g)	250 ± 1.0	$239.3^{b}\pm20.01$	$59^{c}\pm10.32$	$10.7^{c}\pm1.54$
CLP (150g)	250 ± 1.0	$248.9^{a}\pm1.08$	$14^{d}\pm3.04$	$10.7^{c}\pm1.54$
PDI (50g)	250 ± 1.0	$249.1^{a}\pm1.02$	$11^{d}\pm2.07$	$0.9^{d}\pm0.1$

CLP = Chromolaena Leaf Powder
PDI = Permethrin Dust Insecticide

3.2 Number of holes and weight loss of bean seeds

Table 2 shows the results on the mean number of holes and weightloss of bean seeds treated with different grams of *Chromolaena odonata* leaf powder (CLP) and permethrin dust insecticide (PDI). CLP (150g) and PDI (50g) gave the lowest mean number of holes (14) and (11) with lower weight loss of 1.15 and 0.9g respectively. The control treatment that was not treated with PDI and CLP gave the highest mean number of holes on bean seeds and the highest weight loss of 48.53.

3.3 Phytochemical screening of chromolaena odorate

The results on proximate composition of *C. odorata* leaf powder show a high constituent of carbohydrate (9.873), crude oil (1.883), and ether extracts (1.323). Others were total ash (0.693), crude protein (0.513), and fat (0.293). Table 3, the photochemical analysis of *C. odorate* leaf powder showed that tannins and terpenoids were present in large quantity while alkaloids, flavonoids, saponins, cardiac glycosides and steroids were present in small quantity (Table 4).

Table 4: Proximate composition of *Chromoleana odorata* leaf powder

Constituents	*C. odorata*
Crude fibre (g)	1.88
Crude protein (g)	0.51
Total ash (g)	0.69
Fat (g)	0.29
Carbohydrate (g)	9.87
Ether extract (g)	1.32

3.4 Nutritional Analysis

Standard methods of the Association of Official Analytical Chemists were used to determine the crude protein, crude fat, total ash, and crude fiber contents of the samples *Chromoleana odorata* leaf powder.

Table 4: Phytochemical Analysis of *C. Odonata* Leaf Powder

Parameters	*C. odonata*
Alkaloids	+
Tannins	++
Flavonoids	+
Saponins	+
Terpenoids	++
Cardiac glycosides	+
Steroids	+

Legend:
++ = Present in large quantity
+ = Present

The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogen × 6.25) was also determined by the Kjeldahl method, using 2.0g samples, crude fat was obtained by exhaustively extracting 5.0g of each sample in a soxhlet apparatus using proteleum boiling point range 40-60˚C as the extract. Ash content was determined by the incineration of 10.0g samples placed in a muffle furnace maintained at 550˚C for 5 hours. The crude fiber was obtained by digesting 2.0g of samples with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace at 550˚C for 5 hours. Total carbohydrate was obtained by a different method.

4 Discussion

The pesticide effect of both the ground formulation of *Chromolaena odorata* and synthetic insecticides were evident in this study as shown in the tables 1 and 2. 150grams of *Chromolaena odorata* control killed 100% of the weevils at the end of the study while 50grams of synthetic insecticide killed 100% of the weevils also. Therefore, the pesticidal effect of *Chromolaena odarata* leaf and synthetic insecticides yield the same result. The results of this study are in agreement with the study by [19] where cowpea grains treated with the leaf powder of *Chromolaena odorata* exhibited significant repellent activity against the adults of *Callosobruchus maculatus*.

Other studies also confirmed the pesticidal and insecticidal properties of leaves of *Chromolaena odorata* [1]; [5]; [10]. *Chromolaena odorata* leaf was reported to have insecticidal properties against rice weevil [20]. The insecticidal properties of leaves of *Chromolaena odorata* against the adult stage of Periplaneta Americana (cockroach) were also reported [21-25]. The increase in the mortality rate of bean weevil as observed in this study may be a result of the phytochemical composition of *Chromolaena odorata* plant [26];[27]. The leaf extract of *Chromolaena odorata* has been reported to contain flavonoid, tannin, saponin, and alkaloid [28-33].

The result of the study also showed that the nitrate level of the sample was high at 79.99mg/g. The plant can be poisonous to livestock as it has an exceptionally high level of nitrate (5 to 6 times above the toxic level) in the leaves and young shoots, the cattle feeding on these die of toxic anoxia [34-36]. It is possible that the high nitrate level of the sample contributed to the killing of the weevils through inhalation and oral routes [37-39].

5 Conclusion

The result of the study showed that a mix of *Chromolaena odorata* leaf can be used as an organic pesticide to control pests (bean weevil) in food and food storage facilities. *Chromolaena odorata* leaves and powders were prepared in the ratio of 1:1. The result of the study revealed higher mortality of bean weevils after seven (7) days in the group A with the highest mortality rate obtained from Treatment four (4) with ten (150) grams of dried ground *Chromolaena odorata* leaves and dust insecticide. Based on this study, beans farmers and traders could use *Chromolaena odorata* to control beans weevils in their storage area, instead of synthetic insecticide. The use of this potential organic pesticide especially a grinded form will preclude the use of synthetic pesticides that pose a great threat to human lives. It is worthy of note that the sample (organic pesticides) used for this study was effective in controlling beans weevils in the storage system and it was as effective as a synthetic insecticide.

Recommendation

It is therefore recommended that the department of Agriculture and Health in many countries where *Chromolaena odorata* is largely found, champions the use of the plant (biological/ organic pesticides) nationally and locally as alternative to synthetic pesticides considering its effectiveness and also because it is health and environment friendly.

Ethical issue

Authors are aware of and comply with, best practices in publication ethics specifically about authorship (avoidance of guest authorship), dual submission, and manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that the submitted work is original and has not been published elsewhere in any language. Also, all procedures performed in studies involving human participants were by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures performed in this study involving animals were following the ethical standards of the institution or practice at which the studies were conducted.

Competing interests

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution to data collection, data analyses, and manuscript writing.

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