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Insecticidal efficacy of *Adenocalymma alliaceum* extracts against cowpea weevils *callosobruchus maculatus* (F) stored *Vigna unguiculata* (L.)

Kavitha GC[a] and Poornima D[b*]

[a]Department of studies in Biotechnology, Davangere University, Davangere-577007, Karnataka, India.

[b*]Department of Studies and Research in Biotechnology, Tumkur University, Tumkuru-572103, Karnataka, India.

Abstract: Insecticidal activities of dried leaf powder and extracts (aqueous and methanol) of *Adenocalymma alliaceum* Miers, were assessed against *Callosobruchus maculatus* with a traditional grain storage insecticide Actellic 2% dust. For each sample, 100 g of cowpea seeds were mixed with respective plant preparation and about 25 adults of *Callosobruchus maculatus* were placed out in a randomized design with three replicates. All three test samples include powder, aqueous and methanolic extracts with irrespective concentration, providing a significant ($P<0.05$) increase in mortality of adult insects. High concentration aqueous extract exhibited a high reduction of bruchid survival and showed a maximum mortality rate of 90.67% followed by leaf powder (85.33%), and methanol extract (78.67%). The aqueous extract was found to be more effective than the methanol extract and plant powder, though it was not as effective as Actellic 2% dust. Further the LC_{10}, LC_{50} and LC_{95} values for the aqueous extract against *C. Maculatus* unveiled through Probit analysis was 0.101g/100g,1.23g/100g and 30.4g/100g respectively followed by plant powder (LC_{10}=0.199g/100g, LC_{50}= 5.37g/100g and LC_{95}=48.97g/100g) and methanol extract (LC_{10}=0.986g/100g, LC_{50}=2.34g/100g and LC_{95}=56.23g/100g). From the results it was evident that the aqueous extracts of *A. alliaceum* exhibit highly toxic effects on *C. maculatus* than that of plant leaf powder and methanol extracts of the A. alliaceum. Consequently, from the study it was obvious that all the tested materials (plant leaf powder, aqueous and methanol extract) of *A. alliaceum* evaluated were found to have potential insecticidal properties and are capable of protecting stored cowpea against *C. maculatus*. Thus, this medicinal plant can be used as an environment- friendly product for inhibition of bruchid pests during the storage of cowpeas.

Keywords: Cowpea weevil, Actellic 2% dust, Storage of grains, *A. alliaceum* extracts, insecticidal.

***Corresponding Author**

Poornima D , Department of Studies and Research in Biotechnology, Tumkur University, Tumkuru-572103, Karnataka, India.

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1. INTRODUCTION

Cowpea is a widely adapted, stress tolerant, annual herbaceous legume from the genus *Vigna unguiculata* (L.) Walp. This pulse is commonly found in tropical and subtropical regions. Thus, it is considered as an important vegetable protein for millions of peoples[1]. Approximately 23 - 25% of cowpea seed proteins are considered as a major source of other nutrition[2] and the stored grains are frequently results in loss of quantitative as well qualitative excellence attributable to microbial deterioration, insect damage and other factors such as chemical and physical are of the order of about 10 - 25%.[3,4,5]. However, this microbial destruction not only causes direct damage but also causes secondary infection from the rot organisms like fungus. Subsequently upon primary and secondary infestation these insects gradually decrease and also cause severe damage on Cowpea which leads to reduction of nutritional content, aesthetic quality of stored grains, organoleptic and reduces weight. In India about 12 million legumes are grown every year but unfortunately, 18.6% of cowpeas alone get infected by *Callosobruchus maculatus* on storage. In Osuji (1985) scrutinize 40 pest species which affect stored food grains. Among which cowpea weevil, *Callosobruchus maculatus* is the most common pest which damages the stored food grains. It is a cosmopolitan polyphagous insect, which are commonly found in tropical and subtropical region, and these insects are considered as the most destructive pest of legume seeds and their grubs are involved in affecting various grains such as cowpea, green pea, broad bean chickpea, lentil and green gram.[6,7,8]. Various synthetic insecticidal techniques are adapted in order to protect the stored grains from this insect infestation. Even though their insecticidal techniques are capable of protecting stored gains, they also exhibit some global problems like mutagenic effects on non-target species, ozone depletion, development of multiple resistance among various insect populations, teratogenicity and carcinogenicity.[9] Consequently, the public issues like human health and environmental damage have diverted attention of everyone towards other alternatives, especially the safer options like exploitation of active constituents from plant sources. Several plants are known to possess bioactive metabolites revealing antifeedant, repellent and toxic effects on a wide range of insect pests.[10,11,12] In addition, many plants are known to protect themselves against insects by producing their own chemical defences like secondary metabolites that are toxic or repellent.[13] Therefore, the use of extracts of plant origin was considered a more effective substitute for synthetic ones as they are easily biodegradable, effective on a number of pests and considered safe in pest control operations as they minimize pesticide residues as well ensure the safety of consumers of the treated grains and the environment.[14,15,16] *Adenocalymma alliaceum* Miers. commonly- known as garlic vine belongs to the family Bignoniaceae. It is a native of South America and has spread to Central America and Brazil. Later it is exported overseas and also grown in favourable climates of India and South Africa.[17] The decoction or infusions of leaves are used for cold, flu, and fever. Infusion of the bark or leaf is used as a remedy for rheumatism, arthritis, uterine disorders, inflammation, and epilepsy. Roots are used in the preparation of cold maceration and tincture and generally taken as a whole-body tonic.[18,19] Leaves are characterized by a pungent garlic-like smell when Crushed, hence used as a substitute for garlic in food. The plant is also used as a mosquito repellent and an antiseptic. [20,21] Rao *et al.* analysed volatile compounds from dried leaves of *A. alliaceum* finding diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide and 1-octen-3-ol as major compounds, organosulfur compounds derived from allicin; the strong garlic aroma present in this plant species is due to naphthoquinones derived from lapachol.[22] The methanol extract of the stem of Adenocalymma has shown cytotoxic activity against colon cancer cells.[23] Chirunthorn *et al.* reported antioxidant and antimicrobial activity with petroleum ether and ethanol extracts. The present study was therefore designed to study the efficacy of *A. alliaceum* (aqueous and methanol) leaves extracts in their ability to control *Callosobruchus maculatus* in stored cowpea grains.

2. METHODOLOGY

2.1 *Plant Materials and Extracts preparation*

The leafy branches of *A. alliaceum* were collected from a nursery in Chitradurga district, Karnataka, India. The plant materials were used against test insect species in three formulations, *viz.* leaf powder, aqueous and methanol leaf extracts prepared as described below. To prepare the powder from the collected plant sample, leaves are separated from twigs, carefully cleaned, shade dried, mechanically powdered and the powders were passed through a sieve of 0.1mm mesh size to standardize particle size. Aqueous and methanol extracts were prepared from the powder wherein 250 g of plant leaf powder was steeped in 500 ml of water and methanol separately that served as the solvent, for 24hrs. The mixture was then passed through Whatman No. 1 filter paper and the filtrates collected were dried over a water bath at 50 ^{0}C.[24] The residue thus obtained (extracts) were stored in a refrigerator maintained at 5-10 ^{0}C until ready for use as a crude active ingredient for bioassays. From each aqueous and methanol extract 0.1, 1, 5 and 10 g were weighed and dissolved separately in 30 ml of water and methanol respectively and further used for the bioassays.[25]

2.2 *Rearing of test insect cultures of Callosobruchus maculatus*

Cowpea weevils (*callosobruchus maculatus*) were isolated from naturally infested seeds collected from the local markets of Chitradurga district, Karnataka, India. The fresh experimental cultures were prepared from the original stocks, the collected insects were placed into a bottle containing undamaged cowpea seeds, and the bottle was covered with muslin cloth fitted with rubber bands (Rup et al. 1984). The contents were kept in an incubator maintained at 30±1 ^{0}C temperature and 70±4% relative humidity as described by Denloye *et al*. Four weeks later the culture was sieved. Sieving was done 24 hrs prior to the test. The old adults were removed and newly emerged adults (0-4days) were collected for bioassay.

2.3 *Media Preparation*

The cowpea seeds were sterilized at 120 ^{0}C for fifteen minutes in an oven to eradicate any infestation present.[26] The sterilized cowpea seeds were kept at room temperature for bioassay.

2.4 *Toxicity Bioassay of Plant Leaf Powder*

The powdered leaf material was weighed out at four rates of 2.5, 5, 10 and 20 g, added initially to 100 g of cowpea seeds, free from any weevils, in 500 ml plastic containers. A conventional insecticide (Actellic 2% dust) was also separately added to 100 g of cowpea seeds in a plastic container, it was used in comparison with an untreated control.[27] Perforated muslin cloth, held tightly in place with several rubber bands, was used to cover each container to ensure adequate ventilation. The seeds and pesticides were shaken thoroughly in the container until the pulverized leaf materials were evenly distributed among the seeds.[28] The content of the plastic containers was allowed to settle down for two hours before the introduction of the insects to each treatment. Twenty- five newly emerged adults (0 to 4 days old) of *C. maculatus* were introduced into each plastic container. The control treatment consisted of cowpea seeds and insects with no plant powder.[29] Each of the treatments was replicated three times and thereafter, the number of adult *Callosobruchus maculatus* that survived in each container was recorded after 30 days of infestation. The percentage mortality of adult weevils was calculated by using the Abbott formula (correcting efficacy % for the natural mortality in the untreated control plots).

2.5 *Toxicity Bioassay of aqueous and methanol extracts*

The plant extracts were subjected for testing insecticidal activity using the method described by Dharmasena *et al.*(2001). 0.1, 1, 5 and 10g of aqueous and methanol extracts were weighed and dissolved in 30 ml water and methanol, respectively were used for the bioassays. Cowpea seeds previously disinfested were divided into eight lots of 100g each and replicated thrice. Each set of seeds were placed in a plastic container and treated with the aqueous and methanol extracts of different concentrations (0.1, 1.0, 5 .0 and 10.0 g /100 g of seeds).[30] The plastic containers were manually rocked for two minutes to ensure that the seeds were coated with the extracts after which they were removed from the plastic container and placed on filter papers for 24 h to allow the solvent to evaporate. Then each lot of seeds were placed in separate fresh plastic containers and twenty-five adult insects were introduced into the plastic container and closed with plastic stoppers bearing gauze windows for ventilation. The control group was constituted with identical amounts of cowpea seeds and number of weevils but without the plant extracts also replicated thrice. The mortality count of the insect pest was taken after the exposure period of 96 h.[31] The insect which did not respond when touched with a fine brush bristle was considered dead and removed. Mortality rates of adult insects were calculated

3. STATISTICAL ANALYSIS

Mortality data recorded, were corrected for natural mortality of the insect pests in the control treatment using the formula proposed by Abbott (1925) and then subjected to Analysis of Variance (ANOVA) and where significant distinction present treatment means were compared at 0.05 significant level using the New Duncan's Multiple Range Test (DMRT) (Zar 1984). Further Mortality of *C. maculatus* was subjected to Probit analysis and $LC_{10,}$ LC_{50} and LC_{95} values of test plant powder and extracts were computed using regression analysis model.[32].

4. RESULTS AND DISCUSSION

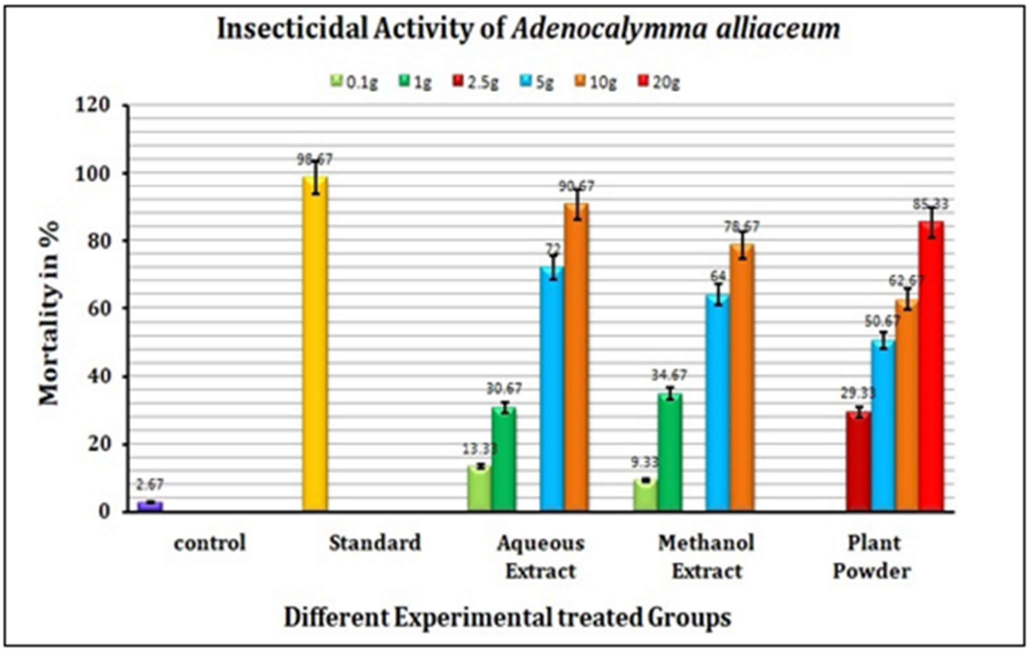
4.1 *Effect of plant leaf powder and extracts on the performance of C. maculatus*

The results of the effects of different concentrations of the leaf powder (botanical pesticide) and leaf extracts (aqueous and methanol) of *A. alliaceum* on adult mortality of *C. maculatus* on stored cowpea seeds are represented in Table 1. From the results, it was evident that all the three plant materials (plant powder, aqueous and methanol extract) revealed concentration-dependent mortality with an exponential increase in the rate of mortality with exposure time albeit vary significantly in their degrees of efficiency (Figure 1). Among the different concentrations tested, the highest adult insect mortality of 85.33% was recorded in the treatment with the highest concentration of (20g/100g seeds) dried and pulverized leaves of *A. alliaceum*, followed by 62.67%, 50.67% and 29.33% at 10g, 5g and 2.5g/100g of seeds, respectively. While Mortality rates of adult weevils taken after 30 days of infestation showed that Actellic 2% dust was the most effective, showing 98.67% mortality of the adult insects, perhaps on account of the fact that this product is a conventional synthetic insecticide specifically formulated with high insecticidal activities on stored product pests (Anon 1993). The mortality rates of the test extracts of *A. alliaceum* at varying concentrations revealed that the least percentage mortality of 13.33% and 9.33% over the 96 h exposure period and the yield of seeds was noticed in aqueous and methanol extracts respectively. A prominent increase in mortality rate of 34.67% and 30.67% was observed at 1g/100g of seeds of aqueous and methanol extracts respectively at 96 h post-treatment, while 72.0% and 64.0% mortality were exhibited by aqueous and methanol extracts respectively at 5g/100g of seeds over 96 h treatment. The highest mortality of 90.67% was observed in 10g/100g of seeds of aqueous extract in 96 h exposure, followed by methanol extract showing 78.67% at same concentration. Differences between the botanical pesticides and plant extracts were significant ($P < 0.05$) at all concentration ranges, against control. In this study the botanical pesticides and plant extracts were obviously -less effective than Actellic 2% dust applied to cowpea seeds.

Table 1: Graph of Plant extract preparation on % mortality of *C. maculatus*

Formulations/Treatment	Concentration (g/100g of seeds)	% Mortality ± SE
Control (Seeds + weevils)	0.0	2.67±1.24
Standard (Seeds + Actellic dust + weevils)	2.0	98.67±1.24
Plant powder (seeds + powder + weevils)	2.5	29.33±1.46
	5.0	50.67±1.24
	10.0	62.67±1.24
	20.0	85.33±1.46

Methanol extract (seeds + Methanol extract + weevils)	0.1	9.33±0.77
	1.0	34.67±0.77
	5.0	64±2.04
	10.0	78.67±1.54
Aqueous extract (seeds + Aqueous extract + weevils)	0.1	13.33±1.02
	1.0	30.67±1.02
	5.0	72±2.14
	10.0	90.67±1.68



The values are mean of three replicates ± SE. SE: Standard Error, Data represents the mean ±SD. All the determinations were performed in triplicates.

Fig 1: Cumulative mean mortality of *C. maculatus* exposed to various concentrations of plant extracts and plant leaf powder.

Further the LC_{10} values of aqueous extract, methanol extract and plant powder against *C. maculates* unveiled through probit analysis were 0.101g/100g, 0.199g/100g and 0.986g/100g of seeds respectively and their corresponding LC_{50} values were 1.23g/100g, 2.34g/100g and 5.37g/100g respectively. LC_{95} values of the *A. alliaceum* aqueous extract was 30.4g/100g, a value lower than that plant powder 48.97g/100g and methanol extract 56.23g/100g are shown in Table 2. Thus, from the results, it was apparent that, aqueous extract of *A. alliaceum* was considerably more toxic to the test insect species (*C. maculatus*) than the plant powder and methanol extracts.

Table 2: Acute toxicity of test plant materials against *C. maculatus*

Formulations	LC_{10}	LC_{50}	LC_{95}	Regression Equation	DF	Slope (±SE)
Plant leaf Powder (g/100g)	0.986	5.37	48.97	Y=-1.43+1.72x	3	1.72±0.2
Methanol Extract (g/100g)	0.199	2.34	56.23	y=0.98+1.19x	3	1.19±0.19
Aqueous Extract (g/100g)	0.101	1.23	30.4	y=1.35+1.18x	3	1.18±0.02

LC_{10} ≡ Lethal concentration that induces 10% mortality in a test population, LC_{50} ≡ Lethal concentration that induces 50% mortality in a test population, LC_{95}≡Lethal concentration that induces 90% mortality in a test population, DF ≡ Degree of freedom, SE: Standard Error

Weevil infestations cause weight loss, quality deterioration resulting in overall unacceptability in markets and impaired germinability of grains and render them unfit for consumption and sale.[32] For so many years the destructive activities and menace of storage pests have been successfully suppressed with the synthetic organochlorine and organophosphate compounds like carbon disulphide, phosphine, malathion, carbaryl, pirimiphos methyl and permethrin.[33] The exploitation of synthetic chemicals to control pests should be avoided due to their carcinogenicity, residual toxicity, hormonal imbalance, long degradation period, environmental pollution and their adverse effects on humans.[34] The mounting problems of pesticides especially those related to large-scale use of broad-spectrum synthetic insecticides have an essential need for effective, biodegradable pesticides with greater selectivity and have created a worldwide interest in the development of alternative strategies, including the discovery of new types of insecticides.[35] However, new insecticides will have to meet entirely different standards; they must be pest specific, non-toxic to mammals, biodegradable, less prone to pest resistance and relatively less expensive.[36] This has led to a reassessment of the century-old practices of protecting stored products using plant derivatives, which have been known to resist insect attacks.[37] The use of plants as insecticides not only ensures safety of the environment and consumption of the treated makes it reliable, readily available for production by the farmer. As a whole, plants with insecticidal potential are a compelling alternative to synthetic ones.[38,39,40] A number of plant products, e.g., oils, powders, ashes, and others, are commonly used by traditional farmers in villages to protect cowpeas from damage in storage.[41,42,43]In addition, many organic compounds of plant origin have been identified to affect pest population in different ways. They include; garlic oils, black pepper, lemon oil, palm oil, soybean oil, citrus peels, and activated kaolin. Various other studies suggest that neem extract was reported has aeffective against beetles.[44,45,46,47]In the present research dried leaf powder, aqueous and methanol extracts of *A. alliaceum showed* their ability to control cowpea weevil *Callosobruchus maculatus* in stored cowpea grains was evaluated.[48] From the study it was apparent that the tested pulverised leaves material and plant extracts (aqueous and methanol extract) were toxic and could be used as a protectant against *C. maculatus*.[49] However, the range of toxicity of aqueous extract of *A. alliaceum* was LC_{10}=0.101g/100g, LC_{50}=1.23g/100g and LC_{95}=30.4g/100g followed by methanol extract: LC_{10}=0.199g/100g, LC_{50}=2.34g/100g and LC_{95}=56.23g/100g and botanical pesticide LC_{10}=0.986g/100g, LC_{50} =5.37g/100g and LC_{95}=48.97g/100g against the target pest. The highest mortality of 90.67% was observed in aqueous extract followed by botanicals (85.33%) and methanol extract (78.67%) of *A. alliaceum*.[50] The results of the present work are corroborated with the report of several workers, like Opareke and Dike (2005), Mukanga et al. (2010), Adedire et al. (2011), Ileke and Oni (2011), who observed that a number of botanicals such as *Stelechocarpus cauliflorus, Azadirachta indica, Ocimum basilicum* are few effectively toxic against storage insect pests including *C. maculatus*.[51,52] *A. alliaceum* contains several of the main sulfur compounds- Allyl methyl disulfide, 1.5-Dithiocane, Dipropyl disulfide are fewgarlic does.[53,54,55]It is these compounds which are responsible for the garlic-like odour and taste of *A. alliaceum*. The leaves and/or flowers contain the known anti-inflammatory and antibacterial plant steroids beta sitosterol, stigmasterol, daucosterol, and fucosterol and various active ingredients which make them useful.[56,57,58,59]Harnafi and Amrani (2007) reported that organic extracts of *A. alliaceum* contain alkanes, alkanols, triterpenes, flavonoids, polyphenols and derivatives of lapachol. Plant materials including garlic disrupt major metabolic pathways leading to rapid death, attractance, deterrence, phagostimulants, and antifeedant or oviposition modifier effects. Also, they may retard or accelerate development or interfere with the life cycle of insects.[60] Garlic plant (*Allium sativum*) holds particular promise as insecticides of natural origin.[61] Pedro (1996) reported that terpenoids are the main chemical components in *A. sativum* that act as fumigant causing insect death owing to anorexia arising from drastic reduction in insect respiratory activities . Allitin, another chemical in garlic, inhibits cholinesterase activity in insects there by insecticidal in nature.[52] Nowadays, researchers are in quest of new classes of naturally occurring pesticides that might be compatible with newer pest control approaches.[63,64] Talukder and Howse (1995) reported that potential use of bioactive plant materials in storage pest management systems might be economical and environmentally friendly. It is obvious from the results of present research work that aqueous extract, methanol extract and dried leaf powderof the *A. aliaceum* can be used as inexpensive and very effective biological pesticides for the control of bruchid pests in stored cowpeas.[65]

5. CONCLUSION

From the results of the current research program, it could be concluded that the tested extracts (aqueous and methanol) and plant leaf powder of *A. alliaceum* had insecticidal properties against cowpea weevils. The broad range of activity of the extracts suggests that multiple mechanisms mediated by the phytoconstituents are responsible for their potent insecticidal property. The tested plant materials could be considered as easy to prepare, cheap, safe and eco-friendly and it may be a possible replacement of chemical insecticides for controlling *C. maculatus* in stored grains, particularly at the household level in technology poor surroundings. Further research is underway to purify and characterize the active principles and to evaluate these phytochemicals against insect pests.

6. AUTHORS CONTRIBUTION STATEMENT

Ms. Kavitha GC conceptualized and gathered the data with regard to this work. Dr. Poornima D gave the necessary inputs towards the designing of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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