GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Effect of *Vernonia amygdalina, Ocimum gratissimum* and their combination on *Callosobruchus maculatus* in cowpea varieties

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GSC Biological and Pharmaceutical Sciences, 2025, 33(03), 212-222

Publication history: Received 27 October 2025; revised on 16 December 2025; accepted on 18 December 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.3.0492

Abstract

This research emphasizes the possible benefits of powdered *Vernonia amygdalina* and *Ocimum gratissimum* as environmentally friendly substitutes for chemical pesticides in the post-harvest control of *Callosobruchus maculatus*; as their availability, affordability, and effectiveness make them promising options for resource-poor farmers in reducing storage losses of Cowpeas. The experiment was conducted in the laboratory of Crop Production Department, Faculty of Agriculture, Prince Abubakar Audu University Anyigba, Kogi State, Nigeria. The location is on Latitude $7^{0}6^{1}$N and Longitude $7^{0}43^{1}$E, which falls within the southern Guinea savannah zone of Nigeria. The daily temperature range is between 25^{0}C - 35^{0}C, relative humidity is moderately high varying between 65 – 80 percent. The experiment consisted of four pest control measures: Bitter leaf powder (3g), Scent leaf powder (3g), 1.5g Bitter leaf powder + 1.5g Scent leaf powder mixture, in addition to control treatment apportioned to three varieties of Cowpea grains (brown, black and black eyed), replicated three times resulting in a total of thirty-six (36) experimental units. Twenty (20) apparently healthy seeds of these three Cowpea varieties and five (5) life adults *Callosobruchus maculatus* (2 males, 3 females) were introduced into each of the plastic containers, measuring 7cm in depth and 4.5cm in diameter with each plastic container covered with white mesh sieve and tied with rubber band. The trial was a 4 x 3 Factorial Experiment with three replications. Generally, the application of *Vernonia amygdalina*, *Ocimum gratissimum* powders and their combination had significant effect on egg laid, weevil multiplication, live weevils, weevil mortality as well as Cowpea damage, with the best controls obtained in the mixture of *Vernonia amygdalina* and *Ocimum gratissimum*, thus recommended for the control of Cowpea weevils, *Callosobruchus maculatus*. However, better performance was observed in grains treated with *Ocimum gratissimum* over those treated with V*ernonia amygdalina*. Brown cowpea variety suffered the most damage to grains compared with other varieties, while the least damage was in black-eyed cowpea variety; an indication that Brown cowpea grains need the most protection, while Black-eyed required the least protection.

Keywords: Egg Laid; Weevil Mortality; Life Weevils; Dead Weevil; Cowpea Damage

1. Introduction

A large number of pests and diseases affect Cowpea during storage. Losses of the grain during post-harvest storage period are very high, leading to serious financial and nutritional losses. *Callosobruchus maculatus* constitute without doubt, the most limiting factor affecting intensive Cowpea production causing extensive damage to stored grains as much as 60 percent grain loss. Cowpea grains offered for sale are usually damaged and when the damage exceeds one or two holes per seed, the price is usually lower than the grain without holes. The use of chemical control methods is prone to several risks such as pesticides poisoning, phytotoxicity, high cost, hazard to handlers and environmental unfriendliness, thus, an insect control method that is inexpensive, environmentally and ecologically friendly which is not detrimental to man will be beneficial to resource-poor farmers in Nigeria.

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Cowpea, *Vigna unguiculata* (L. Walp) is a very important grain legume in West Africa [1], with Nigeria as one of the world's largest producers of the crop averaging 2.92 million tons followed by Niger with 1.10 million tons [1, 2]. The most important producing areas in Nigeria being located in the Savannah regions [3]. Cowpea is however, cultivated in a large range of ecologies in all the continents of the world [4]. It is an extremely valuable crop both as a source of cheap dietary protein for the third world where meat is expensive [5]. The high protein and lysine contents of the seeds make them a natural supplement to single diets of cereals, roots, tubers and fruits [5].

Large harvests obtained by the rural poor farmers in Nigeria are lost in storage majorly due to insect attacks [6]. In general, losses due to pest attack or diseases can be as high as 90 percent in Cowpea [6, 7]. The primary insect causing losses to stored Cowpeas in West Africa is the Cowpea weevil, *Callosobruchus maculatus* with infestation beginning in the field at low level [6, 7, 8, 9]. After the crop is placed in storage, the insect population continues to grow until the Cowpea is completely damaged, while *Bruchidius atrolineatus* causes losses primarily around harvest times but does not reproduce in storage [6, 7, 8, 9].

Biam and Okorie [10] and Ajayi and Lale, [11] reported that Cowpea seed bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a cosmopolitan harvest pest of Cowpea in the tropics. It causes substantial quantitative and qualitative losses manifested by seed perforation and reduction in weight, market value and percentage germination in seeds [12]; observing that infestation can affect 100 percent of the stored Cowpea resulting in up to 60 percent grain loss within a few months [13]. Apart from detrimental economic impact, these losses pose a major threat to food security [14] of rural farmers.

Infestation of Cowpea by this insect starts on the field when the pods are mature at which time the pest lays their eggs on them. The larvae burrow their way into the seed, feeding on the endosperm. The weevil develops into a sexually mature adult within the seeds [15, 16]. An individual bruchid can lay 20 – 40 eggs and in optional conditions with each egg developing into productively active adult in 3 weeks [16, 17].

The use of synthetic pesticides has been the major means through which Cowpea seeds are protected during storage [6]. The use of synthetic pesticides over the years has revealed the nuisance they constitute to the ecosystem such as their undesirable side effects on non-target organisms including man and the fact that they are environmentally disruptive [6, 18, 19].

Resource-poor farmers in Africa employ a range of traditional methods such as use of ash, sand, dry whole pepper, ground pepper, cement dust or other botanical extracts; as aromatic plants have both medicinal and aromatic properties and contain a variety of volatile oils which have insecticidal, anti-feeding and repellent effects on insect pests [6, 18, 19].

The chemical repellency hypothesis states that non-host plant odors repel herbivores by disrupting their ability to locate or feed on the host plant [6, 20]. Consequently, botanical insecticides are becoming more and more well-liked as safer and less expensive alternatives. Several plants-based powders and extracts, including *Vernonia amygdlina* (Bitter leaf) and *Ocimum gratissimum* (Scent leaf), have shown pesticide properties [21, 22, 23, 24, 25].

West Africa is known to be the place of origin of Cowpea weevil, and it is believed to have spread across the globe via the trading of legumes alongside other crops. Also, apart from its global spread through trading, this weevil has prevailed as an agricultural pest due to its tolerance for high degree of inbreeding [26]. The Cowpea weevil is sexually dimorphic; the females are easily distinguished from males. The sexes of the weevils can be visually determined by examining the anatomical (elytra, shape of the abdomen) pattern. The female of this species is dark-coloured with four elytral spots, while the male is pale brown and less distinctly spotted. Also, the plate that covers the end of the abdomen in females is large and dark areas in males [27].

In store, each female lays 40-60 white flat eggs and glues it on the seeds surface; on hatching the larva bore into the seed, where it feeds, grow and pupate before emerging as adult out of the seed after about 3 - 4 weeks. A single seed can be infested with multiple larvae [28. 29]. It is reported that about 8 - 10 or more larvae can be found in a single seed. Thus, heavily damaged seeds show many exit holes [30]. Both sexes can mate soon after emergence and they require neither food nor water to reproduce and can mate several times during their life time. The beetle longevity is slightly affected by relative humidity [31]. Both sexes live an average of 7 days [32]. The complete life cycle takes about five weeks; this means that a new generation rises every month during storage. An infestation of up to 100 percent of the stored seeds has been reported within 3 to 5 months under farmers' storage conditions [33]. The reduction in seed weight is directly proportional to the number of exit holes [33]. This pest poses serious threat to the cultivation of cowpea among resource poor farmers in Nigeria.

Pesticide use has increased significantly and dominates pest management practices worldwide [34]; principally due to changes in farming practice related to increased agricultural activity and the challenges of pest resistance [35, 36]. However, farmers often mishandle and misapply these chemicals [37, 38, 39]. Consequently, there is an urgent need for a safer, more effective, and sustainable approach to control these insect pests. Different kalternative pest management approaches including cultural and biological measures have been implemented [6, 20, 33, 34, 35, 36, 37, 38].

Several plant extracts show insecticidal effects against wide range of insect pests in crops: the use of nicotine, an extract from tobacco against piercing and sucking insects [40]; Retenone to control aphids, thrips and beetle [41]; while neem has been reported to act as an anti-feedant, a repellent and as an insect growth inhibitor due to the presence of *salannin* and *azadirachtins*, the key active ingredients in the plant [6, 42]. Other plant products have also been used on stored grains and field pests: root extracts of *Jatropha caucas* against *Callosobruchus maculatus* [43, 44], seed extracts of *Azadirachta indica* on *Callosobruchus maculatus* [6, 45, 46], *Moringa oleifera* seeds and root extracts in controlling *Callosobruchus maculatus* [6, 47], Bitter leaf [48, 49, 50, 51-55] and Scent leaf (*Ocimum gratissimum* are also known for their pesticide properties.

This study aims at evaluating insecticidal effectiveness of powdered *Ocimum gratissimum* and *Vernonia amygdalina* separately and in combination in the management of *Callosobruchus maculatus* infestations in stored Cowpea. The Specific objectives are:

- To assess Bitter leaf powder for control of *Callosobruchus maculatus* in stored Cowpea grains.
- To evaluate the effect of Scent leaf powder in controlling *Callosobruchus maculatus* in stored Cowpea grains.
- To assess a mixture of Bitter leaf and Scent leaf powders in controlling *Callosobruchus maculatus* in storage.

2. Materials and Methods

The experiment was conducted in the laboratory at the Crop Production Department, Faculty of Agriculture, Prince Abubakar Audu University Anyigba, Kogi State. The location is at (Latitude $7^{0}6^{1}$N and Longitude $7^{0}43^{1}$E) which falls within the southern Guinea savannah zone of Nigeria. The daily temperature range is about 25^{0}C - 35^{0}C. Relative humidity is moderately high and varies from an average of 65-80 percent. The experiment consisted of four pest control measures: Bitter leaf powder (3g), Scent leaf powder (3g), 1.5g Bitter leaf powder + 1.5g Scent leaf powder mixture, in addition to Control treatment apportioned to three varieties of Cowpea grains (brown, black and black eyed), replicated three times resulting in a total of thirty-six (36) experimental units. Twenty (20) apparently healthy seeds of these three Cowpea varieties and five (5) life adults *Callosobruchus maculatus* (2 males, 3 females) were introduced into each of the plastic containers, measuring 7cm in depth and 4.5cm in diameter with each plastic container covered with white mesh sieve and tied with rubber band. The trial was a 4 x 3 Factorial Experiment with three replications.

The containers used in the study were purchased from the open market; thirty-six (36) plastic containers measuring 7cm in depth and 4.5cm in diameter. The Cowpea grains (Brown, Black and Black eyed Cowpea) varieties used in the experiment was sourced from the Anyigba open market and were free from insect infestation and insecticide. Bitter leaf and Scent leaf used for the experiment were harvested from available Bitter leaf and Scent leaf plants. The Bitter leaf and Scent leaf were Sun dried for 7 days to reduce its moisture content and was grounded into smaller particles. Thereafter, 1mm sieve was used to filter the Bitter leaf and Scent leaf powders into a fine powder. The plastic containers were appropriately labeled. The trial was a 4 x 3 Factorial Experiment with three replications.

3. Results and Discussion

Botanicals investigated significantly ($p \leq 0.05$) influenced total numbers of eggs laid at week 2, 4 and 6 after treatment application (Table 1). The control had the highest significant numbers of eggs laid, while the lowest numbers of eggs laid were observed in the combination of *Vernonia amygdalina* and *Ocimum gratissimum* (Bitter leaf + Scent leaf) powders. Within the individual botanicals, *Ocimum gratissimum* (Scent leaf) consistently performed better than *Vernonia amygdalina* (Bitter leaf) in controlling the numbers of eggs laid among the weevils.

Variety investigated also significantly ($p \leq 0.05$) influenced numbers of eggs laid at 2, 4 and 6 weeks after treatment application. Among the cowpea varieties, black-eyed grains consistently witnessed the lowest numbers of eggs laid at 2, 4 and 6 weeks after treatment application, while except at 6 weeks after treatment application, this was followed by the black cowpea grains giving better performance compared to brown cowpea grains.

There were significant interaction effects between botanical and cowpea variety ($p \leq 0.05$) on numbers of eggs laid at 2, 4 and 6 weeks after treatment application. Bitter leaf + Scent leaf powder consistently gave better performances compared with other treatment, while Scent leaf powder did better than Bitter leaf. The highest number of eggs laid (87.33) at the termination of the trial, was in the control black cowpea variety, while treating same black eyed cowpea variety with a combination of *Vernonia amygdalina* and *Ocimum gratissimum* (Bitter leaf + Scent leaf) powders gave the least number of eggs laid (23.67).

Table 1 Effect of *Vernonia amygdalina* and *Ocimum gratissimum* and their combination on eggs laid

Botanical	**Cowpea variety**	**Average number of eggs laid**		
		Week 2	Week 4	Week 6
Control		4.00	36.56	76.67
Bitter leaf		2.89	24.55	45.78
Scent leaf		2.67	16.33	34.33
Bitter leaf + Scent leaf		2.56	7.78	26.67
F-LSD (0.05)		0.014	1.042	1.011
	Brown	3.25	22.17	44.33
	Black	3.00	15.75	54.25
	Black eyed	2.83	26.00	39.00
F-LSD (0.05)		0.011	0.021	0.063
Control	Brown	4.00	45.33	78.67
Control	Black	4.00	26.33	87.33
Control	Black eyed	4.00	38.00	64.00
Bitter leaf	Brown	3.00	23.33	36.33
Bitter leaf	Black	2.67	20.33	61.67
Bitter leaf	Black eyed	3.00	30.00	39.33
Scent leaf	Brown	3.00	15.67	33.33
Scent leaf	Black	2.67	11.00	40.67
Scent leaf	Black eyed	2.33	22.33	29.00
Bitter leaf + Scent leaf	Brown	3.00	4.33	29.00
Bitter leaf + Scent leaf	Black	2.67	5.33	27.33
Bitter leaf + Scent leaf	Black eyed	2.00	13.67	23.67
F-LSD (0.05)		0.564	4.909	5.633

The significant effects of botanical on number of eggs laid was in line with previous observations [56, 57-60], where Scent leaf (*Ocimum gratissimum*) and *Vernonia amygdalina* (Bitter leaf) were reported to have a strong aromatic profile that can mess with a weevil's sense of smell; with extracts or whole leaves placed near grain or legume stores tending to reduce the number of eggs weevils lay - probably because the volatile compounds act as a repellent or disrupt their mating cues. Bitter-leaf powder was also reported to be rich in *sesquiterpene* lactones and flavonoids that act as feeding deterrents and oviposition inhibitors for several stored-product weevils. When incorporated into grain or presented as a treated substrate, the powder typically reduces the number of eggs laid and can lower subsequent adult emergence.

In the same vein botanicals investigated significantly ($p \leq 0.05$) influenced total numbers of live weevil at week 4 and 6 after treatment application (Table 2). The control had the highest significant numbers of live weevils, while the lowest

numbers of live weevils were observed in the combination of *Vernonia amygdalina* and *Ocimum gratissimum* (Bitter leaf + Scent leaf) powders. Within the individual botanicals, *Ocimum gratissimum* (Scent leaf) consistently performed better than *Vernonia amygdalina* (Bitter leaf) in controlling the numbers of live weevils similar to the observation made on eggs laid among the weevils.

The results obtained on live weevils showed significant ($p \leq 0.05$) influence of the treatment imposed on the numbers of live weevils throughout the period of data collection (Table 2). The controls had significantly the highest numbers of live weevils compared to grains treated to *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (Scent leaf), while the lowest numbers of live weevils were observed in grains treated to combination of *Vernonia amygdalina* and *Ocimum gratissimum* (Bitter leaf + Scent leaf) powders. At week 6, treating the Cowpea with mixture of *Vernonia amygdalina* and *Ocimum gratissimum* (Bitter leaf + Scent leaf) completely eliminated live weevils in the lots.

In a similar manner, variety investigated also significantly ($p \leq 0.05$) influenced numbers of live weevils at 4 and 6 weeks after treatment application. Among the cowpea varieties, black-eyed grains consistently witnessed the lowest numbers of live weevils at 4 and 6 weeks after treatment application, followed by the black cowpea grains giving better performance compared to brown cowpea grains. There were significant interaction effects between botanical and cowpea variety ($p \leq 0.05$) on numbers of live weevils at 4 and 6 weeks after treatment application. Bitter leaf + Scent leaf powder consistently gave better performances compared with other treatment, while Scent leaf powder did better than Bitter leaf. These observations were similar to were seen in total numbers of eggs laid.

Treating Cowpea grains to *Vernonia amygdalina* (Bitter leaf), or *Ocimum gratissimum* (Scent leaf) powders or to a combination of *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* significantly influenced Cowpea damage caused by *Callosobruchus maculatus* (Table 3), with the lowest number of seed damage observed in the combination of *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (Scent leaf).

Generally, the outcome of this research demonstrated that combining Bitter leaf (*Vernonia amygdalina*) and Scent leaf (*Ocimum gratissimum*) powders exhibited significant insecticidal effects against the Cowpea weevil (*Callosobruchus maculatus*). The combination of *Vernonia amygdalina* and *Ocimum gratissimum* powders was found to have the highest insecticidal efficacy compared to the individual powders. An indication that combining Bitter leaf (*Vernonia amygdalina*) and Scent leaf (*Ocimum gratissimum*) powder showed synergistic effects, producing significant weevil mortality.

Table 2 Effect of *Vernonia amygdalina* and *Ocimum gratissimum* and their combination on number of live weevils

Botanical	**Cowpea variety**	**Average number of live weevils**	
		Week 4	Week 6
Control		65.30	69.92
Bitter leaf		38.33	42.12
Scent leaf		27.82	21.32
Bitter leaf + Scent leaf		20.00	0.00
F-LSD (0.05)		2.761	3.702
	Brown	36.14	31.47
	Black	46.70	39.96
	Black eyed	31.13	28.59
F-LSD (0.05)		2.334	3.041
Control	Brown	66.33	71.22
Control	Black	74.87	78.00
Control	Black eyed	56.21	60.53
Bitter leaf	Brown	30.12	34.52
Bitter leaf	Black	54.56	50.62

Bitter leaf	Black eyed	30.30	41.22
Scent leaf	Brown	28.12	20.12
Scent leaf	Black	35.22	31.22
Scent leaf	Black eyed	20.12	12.61
Bitter leaf + Scent leaf	Brown	20.00	0.00
Bitter leaf + Scent leaf	Black	22.13	0.00
Bitter leaf + Scent leaf	Black eyed	17.88	0.00
F-LSD (0.05)		5.336	4.925

Table 3 Effect of *Vernonia amygdalina* and *Ocimum gratissimum* and their combinations on the control of Cowpea weevils

Botanical	**Cowpea variety**	**Average number of bore holes / grain**
		Week 6
Control		5.78
Bitter leaf		4.22
Scent leaf		2.34
Bitter leaf + Scent leaf		1.45
F-LSD (0.05)		0.231
	Brown	4.33
	Black	3.75
	Black eyed	3.00
F-LSD (0.05)		0.412
Control	Brown	7.33
Control	Black	6.00
Control	Black eyed	4.00
Bitter leaf	Brown	5.33
Bitter leaf	Black	4.67
Bitter leaf	Black eyed	2.67
Scent leaf	Brown	2.67
Scent leaf	Black	2.67
Scent leaf	Black eyed	1.67
Bitter leaf + Scent leaf	Brown	2.00
Bitter leaf + Scent leaf	Black	1.67
Bitter leaf + Scent leaf	Black eyed	0.67
F-LSD (0.05)		0.048

Scent leaf powder exhibited a better individual efficacy, compared to Bitter leaf powder. In a similar report, Keita et al. [55] reported that powders of Scent leaf provide effective protection against *C. maculatus* without negatively impacting seed germination. While Adeniyi et al. [54] reported that the insecticidal effects of these botanicals are influenced by

exposure time, with extended durations leading to a more pronounced lethal impact on weevils. it has been observed that, the effects of botanicals are not 100 % foolproof and at higher weevil densities the leaf's deterrent can wear off, and also some weevil species seem less bothered [57-60].

4. Conclusion

This research emphasizes the possible benefits of powdered *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (Scent leaf) as environmentally friendly substitutes for chemical pesticides in the post-harvest control of *Callosobruchus maculatus* in storage. Their availability, affordability, and effectiveness make them promising options for resource-poor farmers in reducing storage losses of Cowpeas. However, their usage may be limited where large quantities of grains are involved. Considering the synergistic effects observed in the combination of *Vernonia amygdalina* (Bitter leaf) and *Ocimum gratissimum* (Scent leaf) powders, it may be advantageous to seek combinations of these products for effective control of *Callosobruchus maculatus* in storage grains. Where individual product is to be used, Scent leaf (*Ocimum gratissimum*) should be preferred to Bitter leaf (*Vernonia amygdalina*) as this gave better results on *Callosobruchus maculatus* activities. From the experiment, Brown cowpea grains require the most protection against cowpea weevils, while the Black-eyed cowpea grains require the least protection against cowpea weevils.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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Appendices

		
Black eyed; Black; Brown	Control Brown Cowpea	Brown Cowpea treated with Scent leaf powder
		
Packaged specimen	Control Black Cowpea	Black Cowpea treated with Scent leaf powder
		
Black Cowpea with bored holes	Control Black eyed Cowpea	Black eyed Cowpea treated with Scent leaf powder
		
White eyed cowpea with bore holes	Black Cowpea with bore holes	Brown Cowpea treated with Bitter leaf powder