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# *Croton dichogamus* (Pax) leaf powder as a protectant of stored Bambara groundnuts (*Vigna subterranea* (L.) Verdc against *Callosobruchus maculatus,* Fabricius: Coleoptera: Bruchidae) in Tanzania

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## Abstract

The insecticidal efficacy of the dried leaves powder of *Croton dichogamus* was evaluated to determine the effectiveness and optimize their use as a grain protectant against storage insect, *Callosobruchus maculatus*. *Croton dichogamus* at the dosage of 10, 5 and 1% (w/w) was compared with Actellic Super Dust and untreated control. The experiment was laid out in Randomized Complete Block Design (RCBD) with (6) replications. The treatments were evaluated on *C. maculatus* live insects, grain damage, mortality and oviposition. The results revealed that the dry powder from *C. dichogamus* leaves considerably reduced the infestation of *C. maculatus*, seeds damage, oviposition and caused insect mortality. The higher dosage (10%w/w) of *C. dichogamus* powder displayed lower grain damage, fewer live *C. maculatus* and few seeds with eggs and high insect mortality. Therefore, from the findings of this study, it can be concluded that the powders form *C. dichogamus* leaves at the higher concentration protected the Bambara groundnut seeds against infestation by *C. maculatus*. Further research is recommended to determine the bioactive compounds from the leaves of *C. dichogamus*, mode of action and toxicity effects on non-target organisms.

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## Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an under-utilized climate-resilient leguminous crop native in Africa (Atiku *et al.*, 2004; Mayes *et al.*, 2019; Mpotokwane *et al.*, 2008). Bambara groundnut is mainly cultivated in Africa for its highly nutritious grains consumed as fresh or pulses or ground into flour. Despite the fact that the grains are highly nutritious containing approximately 20% protein, 56.3% carbohydrate, 6.0% fat, 5.8% fiber, and 2% mineral and regarded as a complete food it has however remained one of the orphan crop (Halimi *et al.*, 2018; Murevanhema & Jideani, 2013). In Tanzania, Bambara groundnut is ranked the third after peanut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) in terms of production and social-economic importance (Azam-Ali *et al.*, 2001).

The crop is highly suitable for the dry regions and marginal soils where farmers cannot access irrigation and fertilizers resulting to the poor performance of other legumes as compared with Bambara groundnuts (Mayes *et al.*, 2019). It has been reported that Bambara groundnut is highly tolerant to erratic environmental conditions such as drought, temperature and depleting soil fertility a situation aggravated by climate change and continuous cultivation without replenishing soil nutrients (Mayes *et al.*, 2019). In Tanzania, the yield of Bambara groundnut is very low ranging between 500-800kg $ha^{-1}$ as compared with the optimum yield of 1,500-2,000kg $ha^{-1}$ where the crop is well managed (NARI, 2015). The low yield of the crop is partly attributed to pre-harvest and post-harvest insect infestation (DAFF, 2016). The most important post-harvest insect pest affecting Bambara groundnuts is the bruchids (*Callosobruchus maculatus,* and *C. subinnotatus*) (Maina & Lale, 2004). Bambara groundnuts are highly susceptible to infestation by bruchids *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic.) (Maina & Lale, 2004). The infestation of bruchids start from the field to the storage. In storage bruchids reduce the quantity, quality, viability of the seeds and the market value of the grains. In Nigeria, it has been reported that bruchids on stored legume grains can cause up to 99% grain loss if grains of susceptible variety is left unprotected by insecticide (Umar & Turaki, 2014). In an attempt to control bruchids farmers usually apply synthetic insecticides in the first place. When correctly used the synthetic pesticides are effective in controlling insect pests (Cooper & Dobson, 2007). However, the use of synthetic pesticides is associated with negative side effects to consumers, the environment and the non-target animals (Aktar *et al.*, 2009). Various strategies such as cultural control measures, breeding for resistance, and application of pesticidal plants are used to control insects on crops (Ajayi & Lale, 2000). The use of pesticidal plant powders is a promising strategy (Chikukura *et al.*, 2011; Chougourou *et al.*, 2016).

*Croton dichogamus* (Pax*:* Euphorbiaceae) is a naturally growing shrub widely distributed in parts of the Northern Tanzania including districts of Babati and Mbulu where it is traditionally used by agro-pastoralists for the control of storage insects and treatment of urinary tract infection (Qwarse *et al.*, 2018). However, despite being used traditionally for controlling storage insects, there is no scientific evidence for the effectiveness of this plant as a grain protectant. Therefore, the current study has evaluated the effectiveness of this plant as a potential Bambara grain protectant against storage insects.

## Materials and methods

*Collection and preparation of plant materials*

The fresh leaves of *Croton dichogamus* were collected from Babati in Manyara Tanzania (04°18'04.6" S, 35°24'49.7" E, and 1570m above sea level. The pesticidal plant leaves were air-dried under the shade for 14 days at room temperature (22°C–26°C) and relative humidity (RH) of 75±5%. The dry leaves were ground using the electric grinding mill to form the powder, which was stored in 10L plastic containers covered with the airtight-lid in dark condition according to (Anjarwalla *et al.*, 2016; Mkenda *et al.*, 2015).

*Collection and rearing bruchids*

The stock bruchids (*Callosobruchus maculatus*) was obtained from infested Bambara groundnuts collected from the Singida Municipal Market and identified by an entomologist from Tanzania Pesticide Research Institute (TPRI). The insects were reared on untreated Bambara groundnut grains kept in six 10L buckets half-filled with 5kg of untreated Bambara groundnuts grains.

The buckets were covered with a fabric mesh to prevent bruchids from escaping from the container. The containers were kept at room temperature for 21 days at the storage house at the Nelson Mandela Institute of Science and Technology (NM-AIST), Tanzania.

*The experimental setup*

Untreated grains used in this experiment were obtained from the Tanzania Agricultural Research Institute-Naliendele (TARI-Naliendele). The grains were weighed into cotton storage bags and admixed with powders from *Croton dichogamus* leaves at the rate of 1%, 5%, 10% (w/w). The positive control, Actellic Super Dust, Syngenta (Pirimiphos-methyl) was applied as per manufacturer's recommendation while the negative control remained untreated.

The experiment was laid out in a randomized complete block design (RCBD) with (6) replications. The buckets heavily infested with bruchids was placed inside the room and opened for the dispersing bruchids to naturally infest any of the treatments randomly placed inside the room.

*Data collection*

A grain sample of 150gm was drawn from each storage bag using a metal grain sampler. The grain was sieved for easy counting of the number of live bruchids, number of dead bruchids, number of damaged seeds, number of seeds with eggs attached on the surface were counted after 30 days' interval for all treatments for 120 days.

After the assessment, the grains, live insects and pesticidal plant powder were returned into the respective containers. The dead bruchids were removed and discarded after every counted to avoid re-counting the same dead insect.

*Data analysis*

Data collected were subjected to analysis of variance (ANOVA) using the STATISTICA 8 edition. The Fisher's Least Significance Difference (LSD) was used to compare treatment means at p = 0.05 level of significance.

**Results**

*Effectiveness of C. dichogamus leaf powders on live C. maculatus on Bambara groundnuts.*

The present study revealed a significant difference in the number of live bruchids ($p<0.001$) between treatments throughout the storage period (Table 1). For 120 days of the storage, the untreated bags (negative control) generally contained the highest number of live bruchids 3.00 ± 1.15, 13.67 ± 1.67, 25.67 ± 3.53 and 70.00 ± 9.61 followed by *C. dichogamus* (1%w/w) 1.83 ± 0.40, 4.00 ± 0.63, 10.67 ± 0.67 and 26.83 ± 5.33 and *C. dichogamus* (5%w/w) 1.50 ± 0.34, 3.17 ± 0.48, 8.33 ± 1.31 and 17.67 ± 3.20. In the first 30 days, only a few insects (0.33 ± 0.21) were observed in the seeds treated with the high dosage of C. *dichogamus* (10% w/w). There were no insects observed on the bags treated with the positive control, Actellic super dust for 30 and 60 days of the storage. For 60, 90 and 120 days of the storage the positive control and *C. dichogamus* (10% w/w) showed high effectiveness in controlling the bruchids on stored Bambara groundnuts despite few insects observed. Generally, the number of live *C. maculatus* was dependent on *C. dichogamus* application rates (1, 5 and 10 w/w%).

The higher dosage of C. *dichogamus* (10% w/w) displayed the highest effectiveness for the entire storage time.

*Effectiveness of C. dichogamus leaf powders on C. maculatus mortality on Bambara groundnut grain.*

The mortality of *C. maculatus* in Bambara groundnuts seeds treated with different concentrations of *C. dichogamus* powders is significantly different from the bruchids mortality in untreated seeds (Table 2).

The insect mortality increased with the increase of the dosage of the leaf powder and exposure time. Generally, positive control Actellic Super Dust showed the highest bruchids mortality for the entire storage time followed by *C. dichogamus* (10%w/w) for 30, 60, 90 and 120 days respectively. The lowest mortality was observed in bags treated with *C. dichogamus* (1%w/w) throughout the storage time where low insect mortality was observed in untreated bags in the 120 days of the storage.

**Table 1.** Mean number of live insects (*C. maculatus*) in stored Bambara groundnuts.

| Treatment | Mean number of live *C. maculatus* | | | |
|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days |
| *C. dichogamus* (1%w/w) | 1.83 ± 0.40ab | 4.00 ± 0.63b | 10.67 ± 0.67b | 26.83 ± 5.33b |
| *C. dichogamus* (5%w/w) | 1.50 ± 0.34b | 3.17 ± 0.48b | 8.33 ± 1.31b | 17.67 ± 3.20bc |
| *C. dichogamus* (10%w/w) | 0.33 ± 0.21c | 1.17 ± 0.48c | 3.83 ± 0.75c | 9.17± 0.95cd |
| Actellic Super Dust | 0.00 ± 0.00c | 0.00 ± 0.00c | 0.33 ± 0.33c | 1.33± 0.88c |
| Negative Control | 3.00 ± 1.15a | 13.67 ± 1.67a | 25.67 ± 3.53a | 70.00± 9.61a |
| One-way ANOVA F-statistic | 5.72** | 42.60*** | 37.50*** | 25.66*** |

**; *** significant at p ≤ 0.01 and 0.001 respectively. Means within the same column followed by the same letter (s) are not significantly different at (p = 0.05) using Fisher's Least Significant Difference (LSD) test.

**Table 2.** Mean number of dead insects (*C. maculatus*) in stored Bambara groundnuts.

| Treatment | Mean number dead C. maculatus | | | |
|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days |
| *C. dichogamus* (1%w/w) | 0.17 ± 0.17b | 0.67 ± 0.33b | 1.17 ± 0.60c | 5.33 ± 1.26b |
| *C. dichogamus* (5%w/w) | 1.00 ± 0.52b | 2.00 ± 0.68b | 7.17 ± 1.35c | 13.00 ± 2.89b |
| *C. dichogamus* (10%w/w) | 1.83 ± 0.98a | 4.67 ± 0.80a | 15.33 ± 4.19b | 39.00 ± 5.88a |
| Actellic Super Dust | 3.67 ± 0.67a | 6.00 ± 1.00a | 25.67 ± 5.93a | 43.00 ± 12.50a |
| Negative Control | 0.00 ± 0.00b | 0.00 ± 0.00b | 0.00 ± 0.00c | 1.33 ± 0.88b |
| One-way ANOVA F-statistic | 3.72* | 11.71*** | 10.08*** | 13.31*** |

*; *** significant at p ≤ 0.05 and ≤ 0.001 respectively. Means within the same column followed by the same letter (s) are not significantly different at (p = 0.05) using Fisher's Least Significant Difference (LSD) test.

*Effectiveness of C. dichogamus leaf powders on Bambara groundnut grains damage by C. maculatus*

The result of the experiment revealed the significant difference (p≤0.001) in the effect of *C. dichogamus* powders on Bambara groundnut seeds damage. The highest grain damage was observed in untreated seeds for the entire storage time for 30, 60, 90 and 120 days respectively (Table 3). *C. dichogamus* at different rates showed effectiveness in reducing the Bambara groundnut seeds damage. Seeds treated with the high concentration of *C. dichogamus* (10%w/w) highly protected Bambara groundnut seeds in a similar way as Actellic Super Dust. The ability of the *C. dichogamus* leaf powder to protect the damage of the crop by *C. maculatus* is dependent on the dosage of the powder. Few damaged seeds were recorded on bags treated with Actellic Super Dust for 60, 90 and 120 days. Generally, the higher the dosage the lower the damage but the increased with the duration of the storage.

**Table 3.** Mean number of damaged Bambara groundnut seeds by *C. maculatus*.

| Treatment | Mean number of damaged Bambara groundnut | | | |
|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days |
| *C. dichogamus* (1%w/w) | 2.17 ± 0.83b | 4.33 ± 1.48b | 26.83 ± 7.13b | 211.33 ± 39.51a |
| *C. dichogamus* (5%w/w) | 1.67 ± 0.84b | 2.50 ± 1.09b | 25.83 ± 7.50b | 181.83 ± 8.23a |
| *C. dichogamus* (10%w/w) | 0.5 ± 0.34b | 2.17 ± 0.60b | 24.00 ± 4.16bc | 89.67 ± 14.52b |
| Actellic Super Dust | 0.00 ± 0.00b | 1.67 ± 1.20b | 3.33 ±1.76c | 6.67 ± 3.18b |
| Negative Control | 10.00 ± 2.08a | 13.67 ± 3.67a | 69.67 ± 9.74a | 257.33 ± 64.91a |
| One-way ANOVA F-statistic | 14.29*** | 7.81*** | 7.88** | 8.63*** |

**; *** significant at p ≤ 0.01 and 0.001 respectively. Means within the same column followed by the same letter (s) are not significantly different at (p = 0.05) using Fisher's Least Significant Difference (LSD) test.

*Effectiveness of C. dichogamus leaf powders on oviposition of C. maculatus on Bambara groundnut seeds.*

All levels of *C. dichogamus* leaf powder significantly reduced the number of eggs laid by *C. maculatus* on the surface of the seeds (p<0.001) (Table 4). The highest number of Bambara groundnut seeds with eggs on the surface was observed in untreated bags (55.33 ± 26.19, 74.67 ± 29.34, 157.67 ± 37.7 and 160.00 ± 34.04) for 30, 60, 90 and 120 days of the storage respectively.

The least number of Bambara groundnut seeds with eggs was observed in Actellic dust and *C. dichogamus* (10%w/w). generally, the higher dosage of *C. dichogamus* reduced oviposition of *C. maculatus*. However, there was an increase in the number of seeds with eggs on the surface with time. This indicates that the ability of the *C. dichogamus* to deter oviposition of *C. maculatus* decreased with the time from 30, 60, 90 and 120 days respectively.

**Table 4.** Mean number of Bambara groundnut seeds with eggs on the surface.

| Treatment | Mean number of Bambara groundnut seeds with eggs | | | |
|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 120 days |
| *C. dichogamus* (1%w/w) | 8.67 ± 6.18b | 46.33 ± 9.82ab | 60.50 ± 24.15b | 102.17 ± 34.25ab |
| *C. dichogamus* (5%w/w) | 4.83 ± 1.58b | 26.83 ± 6.74bc | 49.17 ± 2 5.04b | 63.17 ± 12.36bc |
| *C. dichogamus* (10%w/w) | 3.33 ± 1.52b | 8.33 ± 3.02c | 45.67 ± 11.68b | 54.00 ± 14.48bc |
| Actellic Super Dust | 0.00 ± 0.00b | 3.33 ± 1.67c | 7.67 ± 3.84b | 8.00 ± 6.11c |
| Negative Control | 55.33 ± 26.19a | 74.67 ± 29.34a | 157.67 ± 37.74a | 160.00 ± 34.04a |
| One-way ANOVA F-statistic | 6.04** | 6.16 ** | 3.76* | 3.84* |

*; **; *** significant at p ≤ 0.05, 0.01 and ≤ 0.001 respectively. Means within the same column followed by the same letter(s) are not significantly different at (p = 0.05) using Fisher's Least Significant Difference (LSD) test.

**Discussion**

The results of the present study revealed varied efficacy of *C. dichogamus* dried leaf powder as protectant of Bambara groundnut protectant against *C. maculatus*. The results showed that the number of damaged Bambara groundnut seeds was dependent on exposure time and dosage. The highest concentration of *C. dichogamus* dry leaf powder effectively protected the Bambara groundnut seeds against *C. maculatus* damage. There was continuous increase of the number of seeds damaged for all treatments from 30-120 days of the storage. The increase of the damaged number of the Bambara groundnut seeds was partly due to increase in the number of live *C. maculatus* which may be due to degradation of the bioactive compounds in *C. dichogamus* over time. The highest concentration (10%w/w) of *C. dichogamus* protected the Bambara groundnut seeds in the similar way as the positive control (Actellic dust).

The results showed the highest number of live *C. maculatus* and oviposition on the untreated bags whereby those treated with different dosages of *C. dichogamus* dried leaf powder displayed less number of live *C. maculatus*. The highest dosage of *C. dichogamus* (10%w/w) effectively prevented the emergence of *C. maculatus* and oviposition as positive control (Actellic super dust) for 120 days of the storage. The difference in the number of live bruchids and oviposition is an indication that *C. dichogamus* dried leaf powder is an insect repellent and an oviposition deterrent. The high mortality of the adult *C. maculatus* was revealed in the Bambara groundnut seeds treated with *C. dichogamus* (10%w/w) similar to the seeds treated with actellic super dust. The killing ability of *C. dichogamus* may be due presence of diverse toxic bioactive compounds such as crotofolane, diterpenoids, crotoxide A and B previously isolated from *C. dichogamus* leaves (Jogia *et al.*, 1989) or due to the action of fine powder blocking the insect spiracles resulting to death due to suffocation (Obembe & Kayode, 2013). Moreover, previous studies have reported that the *croton* species are rich in terpenoids which have insecticidal properties (Castilhos *et al.*, 2018). The findings of the current are supported by the previous studies using croton species where an ethanolic extracts from the leaves and stems of

*Croton rhamnifolius*, *Croton jacobinensis*, *Croton sellowii, and Croton micans* which showed insecticidal property against *Plutella xylostella* L (diamond back moth) on kales. Furthermore, (Qwarse *et al*., 2018) reported that *C. dichogamus* leaves are traditionally used by small holder famers as a protectant against storage insects in Mbulu district, Northern zone of Tanzania. Therefore, the current study has provided justification of the insecticidal potential of the dried powder from *C. dichogamus* leaves.

## Conclusion

In conclusion, the results obtained from this study proved that the powder form the *C. dichogamus* were effective in reducing the infestation of *C. maculatus* on treated seeds. The significant difference in the number of live *C. maculatus*, mortality, seeds damage and oviposition is an indication that *C. dichogamus* dried leaf powder can affect the developmental stages of *C. maculatus* and significantly protect the Bambara groundnuts against damage by *C. maculatus*. The high dosage of *C. dichogamus* (10%w/w) leaf powder was the most effective among all concentrations used in this study. Further research is needed to determine the bioactive compounds found on *C. dichogamus* leaves, mode of action and toxicity effects on non-target organisms such people who consume the seeds which are protected by the powder from these plant leaves for its optimum usage and safety.

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## Conflicts of interest

We declare no conflicts of interest.

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