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**Research paper**

# Edible plant rhizome-based biocide for management of *Callsobruchus chinensis* (Coleoptera: Bruchidae)

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## ABSTRACT

**Investigation on bio-efficacy of edible rhizome powder of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa)* against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) undertaken at Department of Entomology, Assam Agricultural University, Jorhat at different dosage admixed with green gram seed revealed that the turmeric rhizome powder recorded the highest adult mortality (97.00%) at 10 g/100 g (w/w) green gram seed at 72 hours after treatment (HAT) as against 31.07% in the case of ginger rhizome powder. The highest oviposition inhibition (100%) was seen in the case of turmeric against 63.23% in ginger at 10 g/100 g gram seed after 120 HAT. The LC50 values of turmeric (1.2 g/100 g seeds) were lower than ginger (11.87 g/100 g seeds) for 96 HAT. Turmeric rhizome powder showed significant adult toxicity, oviposition deterrence and ovicidal properties suggesting its possible incorporation in the IPM practices against storage insect pests of pulses.**

***Key words***: Anti-ovipositional, *Callosobruchus chinensis*, Ginger, Ovicidal, Toxicity, Turmeric

## INTRODUCTION

Agriculture is one mainstay of livelihood, civilization, culture, and heritage of India (Pathak *et al*., 2022) as 2/3$^{rd}$ of the Indian population primarily dependent on this sector. The Gross Value Added (GVA) in agriculture estimated to be 18.80% during 2021-22, suggesting its inevitable importance in Indian Economy (Economic Survey of India, 2022). With the advent of industrialization along with the profuse application of agricultural technologies, newer challenges including attack of pest resurgence, environmental pollution, biodiversity imbalance, etc. were commonly encountered in Indian agriculture. Although India could be able to become self-sufficient in several areas, but improper handling and storage of the agricultural produce is a cause of concern. About 14.0 mt of foodgrains get lost during storage in India annually (Banga *et al*., 2020) of which insect pests alone cause nearly 20-25% storage loss (Rajashekar *et al*., 2012) damaging and deteriorating the primary production resources affecting the sustainable crop production system on many occasions. More than 600 species of coleopterans, 70 species of lepidopterans and 355 species of mites cause quantitative and qualitative losses in stored products (Guru *et al*., 2022; Rajendran, 2002; Tyagi *et al*., 2019), including deterioration of grain chemical composition in the form of starch and proteins of the grains (Grish *et al*., 1975; Pushpamma and Reddy, 1979). Amongst the biotic stresses, the pulse beetle *Callosobruchus chinensis* L. (Coleoptera : Bruchidae) is one of the cosmopolitan and notorious insect species (Srivastava and Dhaliwal, 2010) causing up to 100% loss of stored pulses in tropical countries including India (Dubey *et al.,* 2010), as it multiplies at a faster rate in high temperature and humidity conditions (Appleby and Credland, 2004). Empirical approaches including use of chemicals for management of stored pulses have produced enormous successes in recent past but showed several negative consequences (Ayman *et al.,* 2023), compelling stakeholders to search for green alternative or the natural products in recent times (Pirali-Kheirabadi and da Silva, 2010). Several researchers have been often claiming suitability use of plant origin materials including spices and other plant parts (Akinneye *et al.,* 2006) showing biological toxicity and reproduction inhibition (Emeasor *et al.,* 2005; Nadra, 2006). Our present investigation aimed at screening and use of edible rhizome powder of edible spice crop for management of *C. chinensis* in stored green gram to figure out suitable alternative safer to humans, natural enemies, and the environment.

## MATERIALS AND METHODS

The experiment was conducted at the Post Graduate Research Laboratory, Department of Entomology, Assam Agricultural University, Jorhat (Longitude: 94°22′E, Latitude: 26°75′N, Altitude: 91 meters above MSL) during 2018-19. Ginger, *Zingiber officinale* (Family: Zingiberaceae) and turmeric, *Curcuma longa* (Family: Zingiberaceae) are the herbaceous plants, whose rhizome is commonly used as spice in Indian cuisine. The mature ginger rhizomes are fibrous in nature and hold biologically active compounds like gingerol, shogoal and zingiberene showing antifeedant and growth inhibition properties against several storage pests (Pino *et al.,* 2004). The turmeric powder had also showed pesticidal properties against *Tribolium castaneum*, *Sitophilus granarius*, *Rhyzopertha dominica* (Jilani and Su, 1983), *S. oryzae* (Chander *et al.*, 1991), and *S. zeamais* (Tavares *et al.*, 2013). The rhizomes of ginger and turmeric (Var. Local) were collected from local market, washed thrice with double distilled water for removal of all foreign materials, dried to reduce the moisture level up to 7-8% under shade to avoid photo-degradation, ground to finer particles, and sieved with 150 $\mu$m mesh size to obtain a fine powder. Powdered plant products were then stored in airtight glass containers under refrigeration until their use in experiments.

Mass culture and maintenance of *C. chinensis* was conducted during 2018-19 on green gram seeds, *Vigna radiata* L. (Family: Fabaceae, Variety: Local). Gram seeds were sorted to remove all foreign materials and broken pieces followed by their disinfection in refrigerator at -20ºC for 24 hours to kill all hidden infestation of storage insect pests. One kilogram of green gram was put into insect rearing plastic containers (Make: General, Capacity: 5 litre), on which 10 pairs of adult males and females in a ratio of 1:1 was released for egg-laying and removed after 48 hours and containers were put into the BOD incubator at 30ºC temperature and 80-85% relative humidity.

To evaluate the bio-efficacy of ginger and turmeric rhizome powder against adult *C. chinensis*, 100g un-infested green gram seeds were put into a small plastic container (Make: General, Capacity: 200 ml) replacing the top cover with muslin cloth for aeration. Afterwards, selected edible rhizome powders were admixed @ 1.0, 2.0, 3.0, 4.0, 5.0, 7.5 and 10.0 g per 100g of seed (w/w basis) along with a control for comparison. A total of 20 naive adults (1:1 sex ratio) were released into each of the plastic containers and the data on percent adult mortality was recorded at 6, 12, 24, 48, 72, and 96 hours after treatment (HAT). Each of the treatments, including control was replicated thrice and the data on adult mortality (%) was calculated with the formula:

Mortality (%) = (Nos. of dead insects/ Nos. of insects released) x 100

Following the same procedure, a separate experiment was also conducted to record the rate of oviposition as affected by the rhizome powders at different time intervals. The data on oviposition deterrence (%) was calculated with the formula:

Percent oviposition deterrence (POD) = (Ec-Et)/Ec X 100

Where, Ec = No. of eggs laid on control seeds and Et = No. of eggs laid on treated seeds.

To find out the ovicidal properties, twenty (20) naive adults at 1:1 sex ratio was released into plastic containers having 100 g of green gram seed for egg laying and removed after 12 hours of introduction. Later, 10 numbers of seeds bearing at least one egg each were marked with a permanent marker, put back into the container and treated with the edible rhizome powders @ 1, 5, and 10 g per 100 g seed. The total number of adults that emerged out of the marked seeds was counted when more than 90% adult emergence was seen in the control and the percent (%) adult emergence computed with the formula:

Adult emergence (%) = (Nos. of adult emerged / Nos. of eggs laid) x 100.

The data on adult mortality (%) were subjected to Abbott's correction (Abbott, 1925) on obtaining mortality in the control, angular transformed and analysis of variance (ANOVA) with Fisher Test at 5% level of significance. The data on adult mortality were subjected to probit analysis to calculate $LC_{50}$ values using SPSS computer software (Version 12.0). The data on anti-ovipositional and ovicidal properties were also subjected to ANOVA following completely randomized block design.

## RESULTS AND DISCUSSION

### *Adult mortality*

Table 1 represents a significant dose and time dependent bio-efficacy of turmeric powders in terms of adult mortality of *C. chinensis* at different time interval. The highest adult mortality (100%) was recorded at a dosage of 10 g/100 g seeds after 120 and 96 HAT followed by 97%, 85%, 60%, 33.3%

**Table 1.** Effect of turmeric rhizome powder on adult mortality of *C. chinensis*

| Dosage (g/ 100 g seeds) | Adult mortality (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **6 HAT** | **12 HAT** | **24 HAT** | **48 HAT** | **72 HAT** | **96 HAT** | **120 HAT** |
| 10 | 10.00 (21.00) | 33.33 (40.65) | 60.00 (54.05) | 85.00 (71.68) | 97.00 (85.48) | 100 (90.99) | 100 (90.99) |
| 7.5 | 8.33 (20.23) | 25.00 (33.76) | 53.33 (49.34) | 70.00 (59.86) | 93.33 (81.51) | 100 (90.99) | 100 (90.99) |
| 5.0 | 6.67 (18.76) | 23.33 (33.60) | 46.67 (47.94) | 70.00 (39.75) | 91.67 (78.73) | 98.33 (89.40) | 100 (90.99) |
| 4.0 | 5.00 (14.91) | 21.67 (32.77) | 38.33 (43.18) | 66.67 (43.18) | 86.67 (73.46) | 90.00 (77.06) | 100 (90.99) |
| 3.0 | 3.33 (20.23) | 18.33 (29.70) | 26.67 (33.92) | 41.67 (43.29) | 58.33 (51.81) | 70.00 (39.75) | 85.00 (71.96) |
| 2.0 | 1.67 (10.35) | 8.33 (20.23) | 26.67 (33.92) | 38.33 (43.18) | 53.33 (49.60) | 70.00 (59.86) | 81.67 (68.20) |
| 1.0 | 0.00 (0.327) | 6.67 (18.76) | 16.67 (23.85) | 28.33 (34.81) | 41.67 (43.29) | 53.33 (49.60) | 61.67 (54.89) |
| Control | 0.00 (0.327) | 0.00 (0.33) | 5.00 (0.33) | 5.00 (0.33) | 5.00 (0.33) | 5.00 (0.33) | 8.33 (0.33) |
| SEd± | 1.02* | 1.25** | 1.31** | 1.07** | 1.31** | 0.76** | 0.72** |
| CD (P=0.05) | 2.16 | 2.65 | 2.79 | 2.28 | 2.79 | 1.61 | 1.53 |
| CD (P=0.01) | 2.98 | 3.65 | 3.84 | 3.14 | 3.84 | 2.22 | 2.10 |

Data presented are the mean of three replications with 20 samples each; Data within parentheses are the mean of the Abott's corrected angular transformed values; *-Significant at p=0.05, **-significant at p=0.01; HAT- Hours after treatment

and 10% at 72, 48, 24, 12 and 6 HAT, respectively, as compared to the control causing 0-8.33% mortality. Turmeric powder at 1.0 g/100 g seed did not cause any adult mortality after 6 hours of treatment, which got increased up to 61.67% at 120 HAT (Table 1). Application turmeric powder caused adult mortality ranging between 61.67-100% at 120 HAT as compared to 8.33% in the control. Chander *et al.* (2003) reported that admixing turmeric powder cause 63.20% suppression of progeny of lesser grain borer (*Rhyzopertha dominica*) at 0.5% concentration level, which might be due the strong repellency of *ar*-turmerone and turmerone as reported by Su *et al.* (1982) against *Tribolium castaneum*. *Ar*-turmerone was found to elicit repellency ranging between 43.10-62.90% against *Tribolium castaneum* after 8 weeks of treatment against *Tribolium castaneum* (Su *et al.*, 1982). The $LC_{50}$ values of turmeric rhizome powder against adults of *C. chinensis* was recorded to be 1.20 g, 3.06 g and 6.90 g per 100 g green gram seeds at 96, 72 and 48 hours of treatment, respectively (Table 3). Subahar *et al.* (2022) had reported that the turmeric rhizome extract decreases the immunoreactivity of octopamine (OA) and tyramine (TA) in the midgut of *Aedes aegypti* larvae along with damage to the epithelial cells microvilli, cell membranes, nucleus, mitochondria, and other cell organelles, which might be true in the case of higher bioactivity against *C. chinensis*. *Curcuma longa* shows inhibitory effect on Glutathione S-transferase (GST) and Acetylcholinesterase (AChE) enzymatic activity of the 3rd instar *G. intestinalis* larvae, causing death of the insect (Attia *et al.*, 2022).

Application of ginger rhizome powder also revealed a dose and time dependent mortality of *C. chinensis* over the exposure period with the highest mortality of 68.33% at a dosage of 10 g/100 g seeds after 120 HAT as compared to 0-6.67% mortality in the control. The adult mortality ranged from 25.00-68.33% at different dosage of treatments as compared to 6.67% in the control at 120 HAT, which was found to ranging between 13.33- 5.00% after 6 hours of treatment (Table 2). Similar kinds of observation was also reported by Longe (2016), recording less than 50.0% adult mortality of *C. maculatus* in stored cowpea on application of ginger rhizome powder. The $LC_{50}$ values of ginger rhizome powder against adults of *C. chinensis* was recorded to be 11.87 g, 68.78 g and 89.53 g per 100 g green gram seeds at 96, 72 and 48 hours of treatment, respectively (Table 3), which is larger as compared to turmeric rhizome powder suggesting lower insecticidal properties. The essential bioactive compound of ginger includes zingiberene, zingiberol and kaemferol, which serve as a receptor that damage the digestive tract, and respiratory system apart from serving as feeding deterrent against insect (Yahya Syukur *et al.*, 2018), which might be the possible reason for obtaining mortality against *C. chinensis*.

**Table 2.** Effect of ginger rhizome powder on adult mortality of *C. chinensis*

| Dosage (g/ 100 g seeds) | Adult mortality (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6 HAT | 12 HAT | 24 HAT | 48 HAT | 72 HAT | 96 HAT | 120 HAT |
| 10 | 13.33 (25.36) | 20.00 (29.51) | 25.00 (31.47) | 28.33 (32.36) | 31.67 (35.06) | 60.00 (52.45) | 68.33 (59.14) |
| 7.5 | 11.67 (24.30) | 16.67 (26.33) | 21.67 (28.47) | 25.00 (31.47) | 28.33 (32.43) | 40.00 (42.76) | 46.67 (47.54) |
| 5.0 | 10.00 (21.00) | 15.00 (23.92) | 20.00 (26.18) | 23.33 (29.38) | 26.67 (32.33) | 31.67 (35.63) | 40.00 (42.76) |
| 4.0 | 8.33 (20.23) | 13.33 (22.76) | 18.33 (25.10) | 20.00 (26.18) | 23.33 (29.46) | 30.00 (35.11) | 36.67 (40.35) |
| 3.0 | 8.33 (20.23) | 13.33 (22.73) | 18.33 (25.20) | 20.00 (26.18) | 23.33 (29.46) | 25.00 (31.47) | 33.33 (37.82) |
| 2.0 | 8.33 (20.23) | 13.33 (22.76) | 16.67 (22.52) | 18.33 (25.10) | 21.67 (28.38) | 23.33 (29.46) | 30.00 (35.11) |
| 1.0 | 5.00 (14.91) | 10.00 (18.60) | 10.00 (13.18) | 11.67 (16.75) | 13.33 (18.60) | 18.33 (25.20) | 25.00 (31.47) |
| Control | 0 (0.33) | 3.33 (0.33) | 6.67 (0.33) | 6.67 (0.33) | 6.67 (0.33) | 6.67 (0.33) | 6.67 (0.33) |
| SEd± | 1.14* | 1.75* | 1.70* | 2.15* | 2.25* | 1.32** | 1.29** |
| CD (p=0.05) | 2.42 | 3.70 | 3.61 | 4.55 | 4.78 | 2.81 | 2.73 |
| CD (p=0.01) | 3.33 | 5.10 | 4.97 | 6.27 | 6.58 | 3.87 | |

Note: Data presented are the mean of three replications with 20 samples each; Data within parentheses are the mean of the Abott's corrected angular transformed values; *-Significant at p=0.05, **-significant at p=0.01; HAT- Hours after treatment

### *Anti-ovipositional properties*

The male and female *C. chinensis* started mating at once after emergence and continued throughout its entire life while the oviposition behavior of the female involved distinct phases of patrol, stop over site and egg deposition onto the green gram seeds. The data on antiovipositional properties of turmeric rhizome powder against *C. chinensis* was found to gradually decline from 12 HAT up to 24 HAT at 1.0 g/100 g seeds (Figure 1). The highest of 100% ovipositional inhibition was recorded with a dosage of 10.0 g/100 g seeds at 120 HAT as compared to 90.32%, 88.89%, 79.13%, 72.17%, 71.52% and 52.62% inhibition at 96, 72, 48, 12, 6 and 24 HAT, respectively. The lowest oviposition inhibition (14.35%) was recorded at the least dosage of the treatment of 1.0 g/100 g of grain at 6 HAT. The oviposition inhibition was ranged between 66.67-100% at 120 HAT (Figure 1). The females preferred to lay eggs on clean seed surface (Wilson, 1988), which might be the case for recording lowest numbers of eggs on seeds admixed with turmeric powder. Amongst the bioactive compounds isolated from turmeric powder, *viz*., *ar*-turmerone and turmerone showing strong repellency against *Tribolium castaneum*. Turmerone was found unstable thermally, which get converted to more stable *ar*-turmerone under ambient temperature and air (Su *et al*., 1982). These bioactive compounds might have played a role

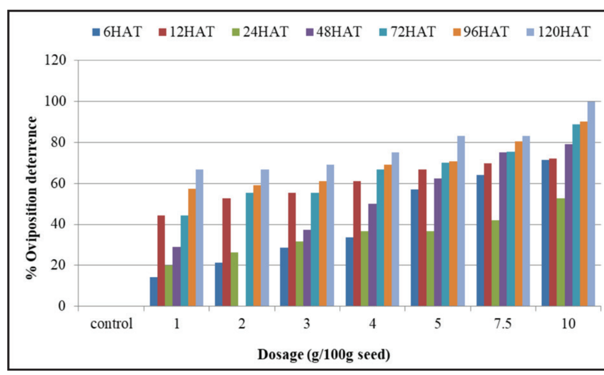


**Fig. 1.** Anti-ovipositional properties of turmeric powder against *C. chinensis*

against *C. chinensis* causing low oviposition and high mortality. In the present investigation, it was observed that the patrol phase got stopped due to application of edible plant powders resulting in low oviposition over the treated seeds, which is in close conformity with the works of Arora and Singh (1970) and Ofuya and Agele (1989).

The data on the effect of ginger rhizome powder on oviposition is presented in Figure 2 and the data revealed the highest oviposition inhibition of *C. chinensis* (76.67%) at 6 HAT @ 10 g/100 g seed, followed by 68.07% at 48 HAT. The lowest inhibition of 23.33% was recorded at the least dosage of the treatment (1.0 g/100 g of seed) at 6 HAT (Figure 2). Since host selection for oviposition follows a series of

**Table 3.** $LC_{50}$ values of edible rhizome powder against *C. chinensis*

| Name of the plant product | Hours after treatment (HAT) | $LC_{50}$ values (g/100 g seeds) | Regression equation (y=a + bx) | Df | $X^2$ | Slope SEm± | 95% Fiducial limit | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | upper |
| Turmeric | 48 | 6.90 | 1.851+2.400x | 19 | 45.085 | 2.400±0.114 | 8.38 | 11.99 |
| | 72 | 3.06 | 0.863+1.678x | 19 | 37.969 | 1.678±0.096 | 2.69 | 3.44 |
| | 96 | 1.20 | 2.269+2.466x | 19 | 46.666 | 2.466±0.130 | 0.91 | 1.46 |
| Ginger | 48 | 89.53 | -0.725+0.762x | 19 | 30.805 | 0.762±0.114 | 36.39 | 698.03 |
| | 72 | 68.78 | -0.604+0.721x | 19 | 26.609 | 0.721±0.107 | 31.28 | 365.37 |
| | 96 | 11.87 | -0.093+1.248x | 19 | 35.080 | 1.248±0.101 | 9.83 | 17.02 |

Note: y= probit kill, x=log dose; Mortality based on 3 replications each with 20 individuals; Df= degrees of freedom

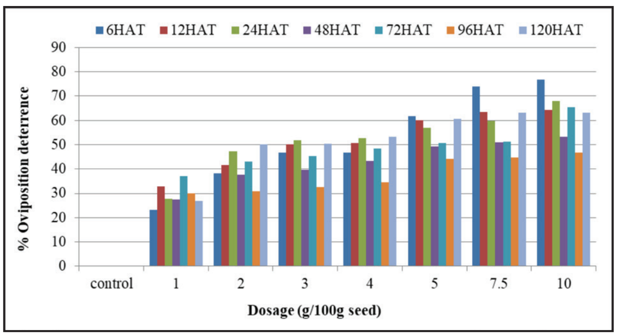


**Fig. 2.** Anti ovipositional properties of ginger powder against *C. chinensis*

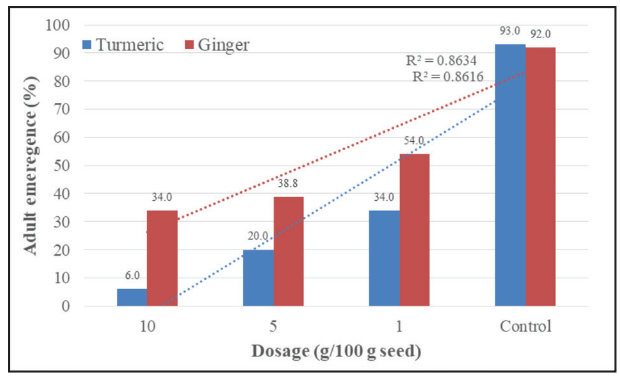


**Fig. 3.** Ovicidal effect of ginger and turmeric powder against *C. chinensis*

chronological events like (host finding, recognition, acceptance, or rejection), visual and chemical cues play a vital role in this process. Treatment of green gram with the edible plant powders may alter its color, taste and odour which are perceived by the insect's eyes, gustatory and olfactory receptors, respectively and may deter the selection of such treated grain as a suitable host for oviposition as obvious in our present investigation. Suthisut *et al*. (2011) reported that the major bioactive compounds present on ginger was found to be camphene, camphor, 1,8-cineole, alpha-humulene, isoborneol, alpha-pinene, beta-pinene and terpinen-4-ol, which were found eliciting contact toxicity, feeding retardancy and ovipositional repellency properties against the adults of *Sitophilus zeamais* and *Tribolium castaneum*, which might true in the present study.

### *Ovicidal properties*

The data on ovicidal activity of rhizome powders (Figure 3) revealed that the turmeric powder has produced significant effect in reducing the adult emergence of *C. chinensis.* The lowest adult emergence (6.00%) was observed @ 10 g/100 g seed dosage, while the highest (34.00%) was observed at the least dosage of 1.0 g/100 g seeds as compared to the 93.00% hatching in the control. On the other hand, the ginger rhizome powder recorded the lowest adult emergence (34%) @ 10 g/100 g seed dosage as against 92% adult emergence in the control (Figure 3). The toxicity might probably be due to accumulation of higher quantities of the bio-active compounds on the treated seed, leading to low adult emergence with slight deviation due to temperature and relative humidity.

## CONCLUSION

Although, both the tested edible plant rhizome powders showed significant effect in terms of adult mortality, oviposition deterrence and ovicidal properties, but bioactivity of the turmeric rhizome powder outperformed against *C. chinensis* revealing its possible incorporation in integrated storage insect pest management strategy in the near future.

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## CONFLICT OF INTEREST

The author declares no conflict of interest in publishing the manuscript.

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