Plant Archives Vol. 25, No. 2, 2025 pp. 322-326 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.2.045

BOTANICAL PROTECTANTS FOR SUSTAINABLE PULSE STORAGE: DUAL EFFICACY OF PLANT POWDERS AND VEGETABLE OILS AGAINST *CALLOSOBRUCHUS CHINENSIS* OVIPOSITION AND ADULT EMERGENCE IN CHICKPEA (*CICER ARIETINUM*)

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(Date of Receiving-23-05-2025; Date of Acceptance-31-07-2025)

ABSTRACT

The present study evaluated the oviposition deterrent and progeny suppression activities of botanical powders (5% w/w) and edible vegetable oils (1% v/v) against *Callosobruchus chinensis* infesting stored chickpea. Treatments included *Syzygium aromaticum*, *Ocimum sanctum*, *Pongamia pinnata*, *Murraya koenigii* powders, and five oils (sesame, sunflower, groundnut, mustard, castor) compared to Malathion 5% dust (1% w/w) and untreated controls under controlled conditions (25.8 ± 1.2°C; 68 ± 5% RH). All vegetable oils achieved complete oviposition deterrence (100%) and 100% suppression of adult emergence through physical suffocation and chemoreceptor disruption. Among botanicals, *S. aromaticum* showed highest efficacy with 52.55% oviposition deterrence and 92.31% adult emergence suppression, attributed to eugenol-mediated neurotoxicity. *O. sanctum* caused 25.35% oviposition reduction and 91.14% emergence suppression. Notably, oils and *S. aromaticum* outperformed Malathion (22.12% oviposition deterrence; 19.83% emergence suppression), demonstrating their potential as eco-toxicologically safe alternatives for integrated bruchid management.

***Key words*:** *Callosobruchus chinensis*, Oviposition deterrence, adult emergence suppression, Botanical protectants, Stored grain pests

Introduction

Chickpea (*Cicer arietinum* L.) is a vital legume crop cultivated extensively across arid and semi-arid regions due to its high protein content and nutritional value (FAO, 2020). However, post-harvest storage losses pose a serious threat to its productivity, primarily caused by the pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae), a destructive seed-boring pest (Ahmed *et al.*, 2015). Chickpea suffers up to 60% post-harvest losses from *Callosobruchus chinensis* infestations, causing $170 million annual economic damage in India alone (Tripathi *et al.*, 2021). Conventional use of synthetic insecticides and fumigants such as Malathion and phosphine has led to resistance development, environmental persistence, and toxicity to non-target organisms (Regmi *et al.*, 2012; Zettler & Cuperus, 1990). As a sustainable alternative, botanicals offer eco-friendly, biodegradable, and target-specific pest control with minimal residual toxicity (Isman, 2006). Numerous plant-derived powders and oils exhibit ovicidal, larvicidal, and insect growth regulatory (IGR) properties effective against bruchids (Tapondjou *et al.*, 2002). The integration of botanicals into storage pest management could significantly mitigate chemical dependence and reduce post-harvest losses. Synthetic insecticides like Malathion face accelerating resistance (Yadav & Patel, 2019) and environmental hazards. Botanical alternatives offer target-specific action with lower mammalian toxicity (LD >2000 mg/kg) and biodegradability (Isman, 2020). While plant powders disrupt insect development through antifeedant and IGR activities, vegetable oils provide dual physical-chemical protection by coating seeds (Rajendran, 2002). This study addresses a critical gap: comparative efficacy evaluation of botanicals for dual oviposition-progeny suppression in chickpea storage systems an understudied area with high farmer impact potential.

Materials and Methods

Study Site: Post-Harvest Entomology Laboratory, SAS Nagaland University (25º452 453 N, 93º532 043 E; 310m ASL). Ambient conditions: 25.8 ± 1.2°C, 68 ± 5% RH (monitored using calibrated thermohygrometers).

Materials

- Chickpea seeds: Desi variety, disinfested at 60°C for 1h, equilibrated to 12% moisture content
- Insect culture: C. chinensis colony maintained on untreated chickpea, 2-3 day-old adults used for bioassays
- Botanical powders: *S. aromaticum* (clove buds), *O. sanctum* (leaves*)*, *P. pinnata* (leaves), *M. koenigii* (leaves); shade-dried, milled to 150-200 ìm particles, applied at 5% w/w
- Vegetable oils: Cold-pressed sesame, sunflower, groundnut, mustard, castor; emulsified with 0.1% Tween-20, applied at 1% v/w
- Controls: Untreated seeds; Solvent control (0.1% Tween-20); Malathion 5% dust (1% w/w)

Bioassay Design

- Oviposition assay: 25 seeds + 4 beetle pairs per replicate (5 replicates/treatment) in ventilated containers. Eggs counted after 10 days using 40× magnification after methylene blue staining.
- Adult emergence: Monitored daily until 35 days post-oviposition.

Test Insect and Host Grain Preparation

Desi chickpea (*Cicer arietinum* L.) seeds were sourced locally, sieved, sun-dried and disinfested by thermal exposure at 60°C for one hour to eliminate any latent infestations by hidden stages of storage pests. After 10 days of conditioning at room temperature for moisture equilibration, the seeds were used for all bioassays. A pure laboratory colony of *Callosobruchus chinensis* (Coleoptera: Bruchidae) was maintained on untreated chickpea seeds in aerated polypropylene containers. The culture was sustained under ambient photoperiod and temperature regimes. For experimentation, synchronized cohorts of 2–3-day-old adult beetles were utilized to ensure uniformity in reproductive potential and physiological status.

Plant-Derived Treatments and Oil Formulations

Botanical samples comprising foliage of *Pongamia pinnata* (karanja), *Murraya koenigii* (curry leaf), *Ocimum sanctum* (tulsi) and floral buds of *Syzygium aromaticum* (clove) were shade-dried and pulverized using an electric mill to obtain fine powder. Extracts were standardized to 5% w/w (5 g per 100 g seed). Cold-pressed vegetable oils—mustard, castor, sesame, sunflower and groundnut were applied at 1% v/w (1 ml per 100 g seed). All treatments were compared against a positive chemical control (Malathion 5% dust @ 1% w/w) and an untreated control. Formulations were thoroughly homogenized with chickpea seeds via manual mixing and stored in insect-proof, aerated containers (250 mL) labeled accordingly.

Bioassay for Oviposition Suppression and F Emergence Inhibition

For assessment of oviposition deterrence, 25 treated seeds per replicate were exposed to four pairs of *C. chinensis* with three replications. After a 10-day oviposition window, eggs were quantified using a stereomicroscope. Oviposition suppression efficacy was computed as:

$$\text{Oviposition (\%)} = \frac{\text{No. of eggs laid in control - No. of eggs laid in treated seeds}}{\text{No. of eggs laid in control}} \times 100$$

Following egg counting, containers were incubated undisturbed for progeny development. Emerged adults were collected daily and adult emergence suppression was quantified as:

$$\text{Percent adult emergence} = \frac{\text{Number of adult emerged}}{\text{Number of eggs laid}} \times 100$$

Data Processing and Statistical Design

All percentage data (oviposition and adult emergence) were subjected to angular transformation prior to statistical analysis. Data were analyzed using analysis of variance (ANOVA) under CRD. Treatment means were compared using the Critical Difference (CD) at a 5% level of significance ($P \leq 0.05$) to discern statistically significant toxicological effects across treatments. Data arcsine-transformed, analyzed by one-way ANOVA ($P<0.05$) with SAS 16.0 software. Reported with F-statistics.

Results and Discussion

Oviposition Deterrence

Initially for the evaluation of oviposition deterrent effects, chick pea seeds were treated with the plant powders and vegetable oils in 5 replications after that, a sample of 100g of seeds from each treatment were taken from each replication and 4pairs of the *Callosobruchus chinensis* were released. The data of oviposition was calculated once the pests started laying eggs (approximately 10-15 days). The number of eggs were

Table 1: Effect of different plant powders and vegetable oils on reduction in oviposition of C. chinensis on stored chick pea.

Treatments	Reduction in oviposition (%)
Pongamia pinnata @ 5% w/w: (T_1)	23.64 (26.97)
Murraya koenigii @ 5% w/w:(T_2)	8.26 (12.48)
Ocimum sanctum @ 5% w/w:(T_3)	25.35 (30.05)
Syzygium aromaticum@ 5% w/w:(T_4)	52.55 (46.47)
Sesame oil @ 1% v/w: (T_5)	100.00 (0.05)
Sunflower oil @ 1% v/w: (T_6)	100.00 (0.01)
Groundnut oil @ 1% v/w: (T_7)	100.00 (0.05)
Mustard oil @ 1% v/w: (T_8)	100.00 (0.04)
Castor oil @ 1% v/w: (T_9)	100.00 (0.02)
Malathion 5% dust @ 1% w/w: (T_{10})	22.12 (25.97)
Untreated control: (T_0)	0.00 (0.00)
Sem±	***4.59***
CD (P=0.05)	***13.47***

counted using the magnifying glass. As per the data in Table 1 and Fig. 1, highest percentage of reduction in oviposition was observed in the seeds treated with vegetable oils (100.00). The seeds treated with Murraya koenigii showed the least percentage reduction of oviposition (8.26) amongst all the treatments.

The evaluation of various botanical powders and vegetable oils revealed significant interference with the reproductive cycle of *Callosobruchus chinensis*, as evident from their oviposition deterrent activity and inhibition of adult emergence. The results clearly demonstrated that both oviposition and post-embryonic development were disrupted to varying extents depending on the treatment. forming impermeable barriers that disrupted egg adhesion. Botanicals varied significantly ($P<0.001$): *S. aromaticum* (52.55%) > *O. sanctum* (25.35%) > *P. pinnata* (23.64%) > *M. koenigii* (8.26%). Malathion showed only 22.12% deterrence confirming field resistance issues (Yadav & Patel, 2019). Similar oviposition-suppressive and ovicidal effects of oils were previously reported by Verma and Pandey (1978) and

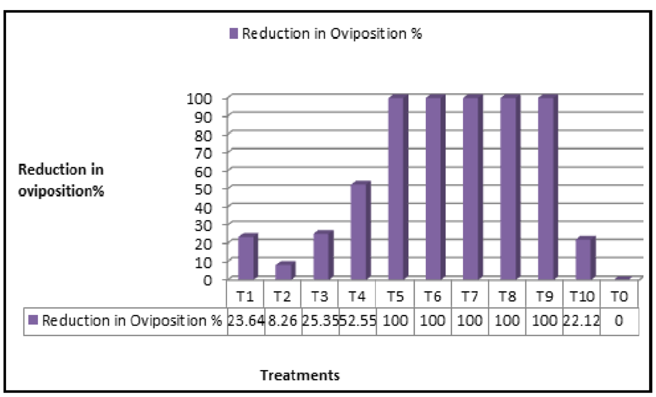

Fig. 1: Reduction in oviposition%.

Ali *et al.*, (1983), confirming the broad-spectrum efficacy of lipid-based grain protectants.

Adult Emergence Suppression

The adult emergence was calculated for the 100g of seeds taken for the oviposition immediately once the adult emerges from the eggs. The adult emergence was counted at regular interval of 24hrs for 35 days until there is no further emergence of adult.

Among the most efficacious interventions, vegetable oils namely sesame, sunflower, groundnut, mustard and castor oils at 1% v/w achieved complete inhibition of oviposition (100%), resulting in zero adult emergence (100% inhibition). Oils caused 100% suppression (no progeny; Fig. 2) via ovicidal suffocation. *S. aromaticum* achieved 92.31% suppression (2.25% emergence vs. control's 29.5%), while *O. sanctum* reached 91.14% (Table 1). *M. koenigii* (7.58%) and Malathion (19.83%) were least effective. These findings suggest a robust dual mode of action, where the oils likely act as physical barriers forming hydrophobic films over the seed coat, preventing egg adhesion and suffocating eggs by obstructing gas exchange. These findings coincided with Pathania, M and Thakur, A. K (2020) they observed that number of adult emerged (30.67%) was least in the seeds treated with curry leaf powders than other treatments. Percentage of adult emergence was 2.00% in the seeds treated with Syzygium aromaticum. This treatment was found to be best treatment next to treatment by vegetable oils. These findings were on par with the findings of Gautam *et al.*, (2000); Aslam *et al.*, (2002) and Ahmed Zia (2004) they evaluated some edible plant products against pulse beetle where clove powder was effective in reducing the percentage of adult emergence.

Among the botanicals, *Syzygium aromaticum* (clove) powder at 5% w/w exhibited the highest efficacy, reducing

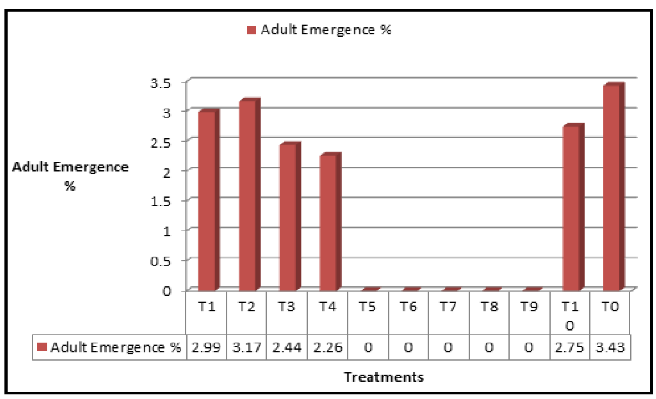

Fig. 2: Percentage of adult emergence. (T1- *Pongamia pinnata*; T2- *Murraya koenigii*; T3- *Ocimum sanctum*; T4- *Syzygium aromaticum*; T5- Sesame oil; T6- Sunflower oil; T7- Groundnut oil; T8- Mustard oil; T9- Castor oil; T10- Malathion 5% dust; T0- Untreated control)

oviposition by 52.55% and limiting adult emergence to 2.25%, corresponding to an adult emergence inhibition of 34.10%. The phytochemical eugenol, a phenolic monoterpene predominant in clove, is well known for its neurotoxic, antifeedant, and insect growth regulatory (IGR) properties. The disruption of hormonal homeostasis during larval development, possibly via ecdysteroid antagonism or juvenile hormone mimicry, likely contributed to the high IAE values. This aligns with the findings of Gautam *et al.*, (2000), Aslam *et al.*, (2002), and Ahmed Zia (2004), who reported substantial reproductive suppression and postembryonic toxicity in bruchids treated with clove derivatives.

Ocimum sanctum (tulsi), another potent botanical, resulted in a 25.35% reduction in oviposition and 2.44% adult emergence, yielding an adult emergence inhibition percentage of 28.86. The phytoconstituents such as thymol, methyl eugenol, and linalool are likely responsible for neuroendocrine disruption, leading to incomplete metamorphosis or failed adult eclosion. These results are in agreement with Ratnasekera and Rajapakse (2010) and Singh (2011), who attributed the reduced emergence and fecundity of *C. chinensis* to the antigonadotropic and IGR effects of *O. sanctum*-based treatments.

Treatment with *Pongamia pinnata (*karanja) powder at 5% w/w led to 23.64% oviposition deterrence and 2.99% adult emergence, equating to only 12.83% inhibition of adult emergence. Although furanoflavonoids such as karanjin and pongamol are known for their larvicidal and antifeedant activities, the comparatively lower IAE percentage suggests limited impact on post-ovipositional development, possibly due to inadequate penetration or metabolic detoxification by the insect.

Murraya koenigii (curry leaf) was the least effective among botanicals, showing only 8.26% oviposition deterrence and 3.17% adult emergence, translating to a modest inhibition of adult emergence of 7.58%. This suggests the active constituents, likely carbazole alkaloids such as mahanimbine and girinimbine, may not be present in concentrations sufficient to exert significant IGR activity under the tested conditions.

Interestingly, the chemical control, Malathion 5% dust at 1% w/w, achieved only 22.12% oviposition reduction and 2.75% adult emergence, resulting in aninhibition of adult emergence of 19.83%. While Malathion, an organophosphate insecticide, acts as a cholinesterase inhibitor and is effective on contact, its lack of hormonal interference may limit its efficacy on developing immature stages post-oviposition. Moreover, declining performance may also be attributed to resistance development due to extensive use, as noted in various postharvest pest populations. The untreated control exhibited the highest reproductive success, with no oviposition deterrence and 3.43% adult emergence, serving as a baseline for efficacy comparison.

Collectively, these findings indicate that certain botanicals—particularly *S. aromaticum* and *O. sanctum* and vegetable oils possess strong oviposition deterrent and IGR-like properties, comparable or superior to synthetic standards. Their mechanisms include behavioral deterrence, neuroendocrine disruption, and physical inhibition of embryogenesis, making them suitable candidates for incorporation into eco-compatible integrated pest management (IPM) programs for stored grain ecosystems.

Conclusions

Syzygium aromaticum (5% w/w) and edible oils (1% v/w) provide high-efficacy, low-risk protection against *C. chinensis*, outperforming Malathion. Clove's neuroendocrine disruption and oils' physical barrier mechanisms achieved >90% progeny suppression—critical for breaking pest cycles in storage.

Future Research Priorities

1. Phytotoxicity screening: Germination impact of oils under long-term storage
2. Synergistic formulations: *S. aromaticum* + mustard oil nanoemulsions for enhanced persistence
3. Farmer adoption studies: Cost-benefit analysis of botanical treatments ($/kg seed)
4. Resistance management: Rotation schemes integrating botanicals with reduced-risk synthetics

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