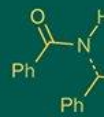


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Development of a clove oil based dust formulation for the management of lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae), in stored paddy

Jayaraj J and Shanthi MDOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5a.8312>**Abstract**

The lesser grain borer, *Rhyzopertha dominica* (Fabricius), is a devastating primary pest of stored cereals, causing substantial quantitative and qualitative losses worldwide. The present investigation was undertaken to develop an eco-friendly clove oil-based dust formulation (CODF) for the management of *R. dominica* in stored paddy. Initial screening of various carrier materials (wood ash, lime, silica, diatomaceous earth, and kaolin) fortified with 0.2% clove oil revealed that diatomaceous earth (DE) exhibited the highest efficacy, completely inhibiting adult emergence (0.00%) and significantly reducing oviposition (0.10 eggs/10 g) compared to untreated control (24.90 eggs/10 g; 94.20% adult emergence). The final formulation (CODF) comprising 0.2% clove oil, 97.8% diatomaceous earth, 1% fumed silica (anti-caking agent), and 1% butylated hydroxytoluene (BHT; stabilizing agent) provided complete protection of paddy grains for up to 120 days, with negligible pest activity observed even after 180 days of storage. GC-MS/MS analysis confirmed the presence of eugenol (36.57%) and caryophyllene (7.02%) as the principal bioactive constituents, while BHT (47.60%) contributed to formulation stability. Thus the prevention of weight loss and preserving geminability of grains effected by admixing CODF with stored paddy is found to be a potential alternative to synthetic insecticides in integrated pest management programs.

Keywords: *Rhyzopertha dominica*, clove oil, diatomaceous earth, dust formulation, stored paddy, botanical insecticide

1. Introduction

Stored grain pests pose a significant threat to global food security, with post-harvest losses estimated to range between 10-40% in developing countries (Phillips and Throne, 2010; Kumar and Kalita, 2017) [29]. Among the diverse array of stored product insects, the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrychidae), is recognized as one of the most destructive primary pests of stored cereals, particularly rice, wheat, and sorghum (Edde, 2012; Hagstrum and Subramanyam, 2009) [12, 16]. Both larvae and adults are internal feeders, boring into sound kernels and reducing them to hollow shells filled with frass, resulting in substantial weight loss, reduced germination capacity, and contamination of grain lots (Arthur *et al.*, 2019; Kavallieratos *et al.*, 2017) [3, 24].

Conventional management of *R. dominica* has relied heavily on synthetic contact insecticides such as malathion, deltamethrin, and pirimiphos-methyl, as well as fumigants like phosphine and methyl bromide (Opit *et al.*, 2012; Nayak *et al.*, 2020) [32, 31]. However, the indiscriminate use of these chemicals has led to several critical concerns including the development of resistant pest populations (Daglish *et al.*, 2014; Afful *et al.*, 2018) [10, 2], harmful residues in food commodities (Bajwa and Kogan, 2004) [6], adverse effects on non-target organisms (Isman, 2006) [19], and environmental contamination (Dubey *et al.*, 2008). Phosphine resistance in *R. dominica* has become particularly alarming, with reports of strongly resistant populations emerging across major grain-producing regions worldwide (Nayak *et al.*, 2020; Sakka *et al.*, 2020) [31, 38]. Furthermore, the phase-out of methyl bromide under the Montreal Protocol has intensified the search for safer and sustainable alternatives (Fields and White, 2002) [14].

Botanical insecticides derived from aromatic plants have garnered considerable attention as promising alternatives to synthetic chemicals for stored product protection (Rajendran and

Sriranjini, 2008; Isman, 2020) [36, 20]. Plant essential oils and their bioactive constituents possess diverse modes of action including repellency, oviposition deterrence, feeding inhibition, and direct toxicity against stored grain pests (Regnault-Roger *et al.*, 2012; Koul *et al.*, 2008) [37, 28]. Clove oil, obtained from the flower buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae), has demonstrated remarkable insecticidal, ovicidal, and larvicidal properties against various stored product insects (Ho *et al.*, 1994; Jairoce *et al.*, 2016; Plata-Rueda *et al.*, 2018) [17, 21, 35]. The principal bioactive constituent of clove oil, eugenol (a phenylpropanoid), has been reported to interfere with octopamine receptors, acetylcholinesterase activity, and detoxification enzymes in insects, leading to neurotoxicity and mortality (Enan, 2001; Regnault-Roger *et al.*, 2012; Jankowska *et al.*, 2018) [13, 37, 22].

Despite the promising bioactivity of essential oils, their practical application in grain storage has been constrained by several limitations including high volatility, rapid degradation, and lack of appropriate delivery systems (Rajendran and Sriranjini, 2008; Isman, 2020) [36, 20]. The development of dust formulations incorporating botanical active ingredients with inert carriers represents a viable strategy to overcome these challenges. Diatomaceous earth (DE), composed of fossilized remains of diatoms, is a well-established grain protectant that causes insect mortality through cuticular abrasion and desiccation (Korunic, 1998; Athanassiou *et al.*, 2011) [25, 4]. The combination of DE with botanical compounds offers potential synergistic effects, enhancing efficacy while reducing the required application rates (Athanassiou *et al.*, 2016; Korunic and Fields, 2020) [5, 27]. Additionally, the inclusion of anti-caking agents and stabilizers can improve the physical properties and shelf-life of such formulations (Vayias and Athanassiou, 2004) [41].

Given this background, the present study was designed with the following objectives: (1) to evaluate various carrier materials (wood ash, lime, silica, diatomaceous earth, and kaolin) fortified with clove oil for their efficacy against *R. dominica* in stored paddy, and (2) to develop an optimized clove oil-based dust formulation (CODF) incorporating anti-caking and stabilizing agents, and to determine its effective dose and residual efficacy over extended storage periods.

2. Materials and Methods

2.1 Insect Culture

A laboratory culture of *Rhyzopertha dominica* was established using adults collected from infested paddy grains obtained from local storage facilities. The insects were reared on clean, uninfested paddy grains (variety: CO 55) with $13 \pm 0.5\%$ moisture content in glass jars (2 L capacity) covered with muslin cloth secured by rubber bands. Cultures were maintained in a BOD incubator at $28 \pm 2^\circ\text{C}$, $65 \pm 5\%$ relative humidity, and continuous darkness. Subculturing was performed at 15-day intervals to ensure a continuous supply of insects of known age. Adult beetles (1-2 weeks old) of mixed sex were used for all experiments.

2.2 Procurement of Materials

Clove bud oil (100% pure essential oil) was procured from a certified commercial supplier (Kazima brand, New Delhi). Diatomaceous earth (DE) of food-grade quality (Jaipur Bio fertilizer, Jaipur) was obtained from a local distributor. Other carrier materials including wood ash (derived from

Prosopis juliflora), hydrated lime (calcium hydroxide), silica powder (precipitated silica), and kaolin clay were obtained from commercial sources. Fumed silica was procured from local market and butylated hydroxytoluene (BHT) was obtained from Purensa global. Technical grade malathion 5% dust was obtained from a local agrochemical supplier for use as a positive control.

2.3 Preparation of Carrier-Based Clove Oil Formulations

For the evaluation of carrier materials, individual dust formulations were prepared by thoroughly mixing 0.2 g of clove oil with 99.8 g of each respective carrier material (wood ash, lime, silica, diatomaceous earth, and kaolin). The mixing was performed using a laboratory-scale mechanical blender operated at 200 rpm for 30 minutes to ensure uniform distribution of the oil throughout the carrier matrix. The resulting formulations were ground using a porcelain mortar and pestle to obtain fine, homogenous powders. All formulations were freshly prepared prior to each experiment and stored in airtight glass containers at 4°C when not in use.

2.4 Evaluation of Carrier Materials against *R. dominica*

The efficacy of different carrier-based formulations was evaluated through bioassays conducted in completely randomized design (CRD) with three replications per treatment. For each replicate, 100 g of clean, uninfested paddy grains (13% moisture content) were placed in a 500 mL glass jar. One gram of the respective formulation (equivalent to 1% w/w application rate) was added to the grains and thoroughly mixed by gentle shaking for 2 minutes to ensure uniform coating. The untreated control consisted of paddy grains without any treatment, while malathion 5% dust applied at the recommended rate of 1 g/100 g grain served as the positive control.

Fifty unsexed adult beetles (1-2 weeks old) were introduced into each jar, which was then covered with muslin cloth secured with a rubber band. The jars were maintained under ambient laboratory conditions ($28 \pm 3^\circ\text{C}$; 65-70% RH). Ten days after insect introduction, the total number of eggs laid was determined by examining the grains under a stereomicroscope at $40\times$ magnification using the egg-plug staining technique described by Milner *et al.* (1983) [30]. Grains were soaked in 1% acid fuchsin solution for 2 minutes, rinsed with distilled water, and the stained egg plugs were counted. Egg hatchability percentage was calculated based on the number of hatched eggs (empty egg plugs) relative to the total number of eggs laid.

Adult emergence was recorded at 25 days after introduction by counting and removing newly emerged adults. Observations were continued until 40 days post-introduction to account for the complete developmental period of the insect. The mean developmental period was calculated as the time from insect introduction until 50% of the total adult emergence was achieved. All observations were recorded and expressed as mean $\pm$ standard error.

2.5 Development of Optimized Clove Oil Dust Formulation (CODF)

Based on the results obtained from the carrier evaluation studies, diatomaceous earth was selected as the carrier material for further formulation development. The optimized Clove Oil Dust Formulation (CODF) was

prepared with the following composition: clove oil (0.2%), diatomaceous earth (97.8%), fumed silica (1.0% as anti-caking agent), and butylated hydroxytoluene (BHT; 1.0% as stabilizing agent). The components were sequentially blended using a mechanical blender. First, clove oil was pre-mixed with fumed silica to facilitate uniform distribution. This mixture was then incorporated into the diatomaceous earth, followed by the addition of BHT. The final blend was mixed at 200 rpm for 45 minutes and passed through a 100-mesh sieve to ensure particle size uniformity.

For comparative purposes, a parallel formulation was prepared without the inclusion of fumed silica and BHT (i.e., only 0.2% clove oil + 99.8% DE) to evaluate the contribution of anti-caking and stabilizing agents to formulation efficacy and longevity.

2.6 Storage Studies with Optimized Formulations

Long-term storage studies were conducted to evaluate the residual efficacy of both the unstabilized formulation (0.2% clove oil + DE) and the final optimized CODF. Paddy grains (100 g per replicate) were treated with the respective formulations at a rate of 1 g/100 g grain (1% w/w) and stored in glass jars under ambient conditions (28±3 °C; 65-70% RH). The treated grains were sampled at intervals of 30, 60, 90, 120, 150, and 180 days post-treatment. At each sampling interval, fifty unsexed adult *R. dominica* were introduced into the jars, and observations on oviposition, egg hatchability, adult emergence, and mean developmental period were recorded as described in section 2.4.

Additionally, at each sampling interval, weight loss percentage and germination loss percentage were determined. Weight loss was calculated using the standard count-and-weigh method (Adams and Schulten, 1978) ^[1] using the formula:

$$\text{Weight loss (\%)} = [(W_u \times N_d) - (W_d \times N_u)] / [W_u \times (N_d + N_u)] \times 100$$

where W_u = weight of undamaged grains, W_d = weight of damaged grains, N_u = number of undamaged grains, and N_d = number of damaged grains.

Germination percentage was determined by placing 100 randomly selected grains from each replicate on moistened filter paper in Petri dishes (9 cm diameter) and incubating at 28±2 °C for 7 days. The number of germinated grains was counted, and germination loss was calculated relative to the initial germination percentage of untreated control grains.

2.7 GC-MS/MS Analysis of CODF

The chemical composition of the optimized CODF was analyzed using Gas Chromatography coupled with Tandem Mass Spectrometry (GC-MS/MS). Analysis was performed on a Shimadzu TQ8040 Triple Quadrupole GC-MS/MS system equipped with a Rxi-5 Sil MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; 5% diphenyl/95% dimethyl polysiloxane). Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. The injector temperature was maintained at 200 °C, and injection was performed in split mode (split ratio 1:10). The oven temperature program was as follows: initial temperature 110 °C (held for 5 min), ramped up to 240 °C at 5 °C/min (held for 5 min). The total run time was 35 minutes.

Mass spectra were acquired in electron ionization (EI) mode at 70 eV with a scan range of m/z 40-600. Identification of compounds was performed by comparing their mass spectra with the National Institute of Standards and Technology (NIST 2023) library and confirmed by comparison of retention indices with literature values. The relative percentage of each compound was calculated from the peak area in the total ion chromatogram.

2.8 Statistical Analysis

All experiments were conducted in a completely randomized design with three replications. Data on oviposition, egg hatchability, adult emergence, weight loss, and germination loss were subjected to square root transformation [$\sqrt{(x + 0.5)}$] to stabilize variance before statistical analysis. Transformed data were analyzed using one-way analysis of variance (ANOVA) using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Treatment means were separated using Duncan's Multiple Range Test (DMRT) at $P = 0.05$ significance level. Untransformed means are presented in tables for clarity, with transformed values and statistical groupings indicated in parentheses.

3. Results

3.1 Evaluation of Carrier Materials Fortified with Clove Oil

The efficacy of different carrier materials fortified with 0.2% clove oil against *R. dominica* biological parameters is presented in Table 1. All carrier-based formulations significantly reduced oviposition, egg hatchability, and adult emergence compared to the untreated control ($P < 0.05$).

Diatomaceous earth (DE) fortified with 0.2% clove oil exhibited the highest level of insect suppression, recording the minimum number of eggs laid (0.10 eggs/10 g), negligible egg hatch (0.01), and complete inhibition of adult emergence (0.00%). This performance was statistically equivalent to the positive control, malathion 5% dust, which also showed complete mortality and prevention of adult emergence. The DE-based treatment resulted in zero adult emergence and, consequently, no measurable developmental period.

Among the other carrier materials evaluated, silica showed the next best performance with oviposition of 0.80 eggs/10 g, egg hatch of 0.08, and adult emergence of 0.68%. Kaolin (0.50 eggs/10 g; 0.35% adult emergence) and lime (0.99 eggs/10 g; 0.83% adult emergence) demonstrated intermediate efficacy, while wood ash was relatively less effective (1.18 eggs/10 g; 1.23% adult emergence). However, all carrier-clove oil combinations were significantly superior to the untreated control, which recorded 24.90 eggs/10 g, 91.60% egg hatchability, and 94.20% adult emergence.

A notable observation was the prolongation of the mean developmental period in all treated grains (ranging from 38.30 to 38.90 days) compared to the untreated control (28.42 days), indicating sub-lethal developmental stress imposed by the treatments on surviving individuals.

3.2 Storage Studies with Unstabilized Formulation

The residual efficacy of the DE + 0.2% clove oil formulation without stabilizer and anti-caking agent over a 180-day storage period is presented in Table 2. The formulation provided excellent protection during the initial

storage period (15-60 days), with negligible oviposition (0.10 eggs/10 g), zero egg hatch, and zero adult emergence. Correspondingly, no weight loss or germination loss was recorded during this period.

At 90 days of storage, a slight increase in pest activity was observed, with oviposition rising to 0.30 eggs/10 g and adult emergence reaching 0.01%. Minimal weight loss (0.30%) and germination loss (0.50%) were recorded. The mean developmental period remained substantially prolonged at 53.61 days.

At 120 and 150 days, oviposition increased to 0.40 and 0.46 eggs/10 g, respectively, with corresponding adult emergence of 0.30% and 0.07%. Weight loss ranged from 0.35% to 0.42%, while germination loss increased to 0.70-0.80%.

By 180 days of storage, the formulation still maintained considerable protective activity, with oviposition at 0.61 eggs/10 g, adult emergence at 0.10%, weight loss at 0.51%, and germination loss at 1.01%. These values remained significantly lower than the untreated control, which exhibited 24.90 eggs/10 g, 94.20% adult emergence, 93.22% weight loss, and 96.43% germination loss ($P < 0.05$).

3.3 Storage Studies with Optimized CODF Formulation

The optimized CODF (0.2% clove oil + DE + 1% fumed silica + 1% BHT) demonstrated superior and prolonged protective efficacy throughout the 180-day storage period (Table 3). Complete protection of paddy grains was maintained for up to 120 days, with zero oviposition, egg hatch, adult emergence, weight loss, and germination loss during this entire period.

At 150 days of storage, only minimal pest activity was detected, with oviposition at 0.20 eggs/10 g, egg hatch of 0.03, and adult emergence of 0.01%. Weight loss and germination loss were 0.22% and 0.50%, respectively. The mean developmental period remained prolonged at 55.67 days.

At 180 days of storage, the CODF continued to provide substantial protection, with oviposition at 0.23 eggs/10 g, egg hatch of 0.05, and adult emergence of 0.03%. Weight loss (0.31%) and germination loss (0.80%) remained negligible compared to the untreated control. The performance of CODF at 180 days was statistically comparable to that of freshly applied malathion 5% dust, which recorded zero oviposition and adult emergence, with weight loss of 0.29% and germination loss of 0.70%.

In contrast, the untreated control exhibited severe infestation, with 24.90 eggs/10 g, 94.20% adult emergence, 93.22% weight loss, and 96.43% germination loss, confirming the vulnerability of unprotected grains to *R. dominica* infestation.

3.4 GC-MS/MS Analysis of CODF

The GC-MS/MS analysis of the optimized CODF formulation revealed a complex mixture of volatile and semi-volatile compounds (Figure 1, Table 4). A total of 16 distinct compounds were identified, with the major constituents being butylated hydroxytoluene (BHT; 47.60%), eugenol (36.57%), and caryophyllene (7.02%). trans-Isoeugenol was present at 6.34%, while the remaining compounds were detected at concentrations below 1%.

The chromatographic profile confirmed that eugenol, the principal phenylpropanoid constituent of clove oil, is well-retained in the formulation along with significant quantities of sesquiterpenes (caryophyllene and its derivatives). The

substantial proportion of BHT reflects its intentional inclusion as a stabilizing antioxidant, while the presence of fumed silica (amorphous silicon dioxide) is not detected by GC-MS/MS due to its non-volatile, inorganic nature.

4. Discussion

The development of effective, safe, and economically viable alternatives to synthetic insecticides for stored grain protection remains a critical research priority in post-harvest entomology (Phillips and Throne, 2010; Isman, 2020) [34, 20]. The present study successfully developed and evaluated a novel clove oil-based dust formulation (CODF) incorporating diatomaceous earth as a carrier, with fumed silica and BHT as functional excipients, for the management of *R. dominica* in stored paddy.

4.1 Carrier Material Selection and Efficacy

The initial screening of various inert carriers fortified with 0.2% clove oil revealed significant differences in their ability to suppress *R. dominica* biological parameters. Diatomaceous earth (DE) emerged as the most effective carrier, completely inhibiting adult emergence and reducing oviposition to near-zero levels. The superior performance of DE can be attributed to its well-established insecticidal mode of action involving the adsorption of cuticular lipids, leading to desiccation and death (Korunic, 1998; Ebeling, 1971) [25, 11]. DE particles adhere to the insect cuticle, abrading the protective wax layer and causing rapid water loss (Athanasios *et al.*, 2011; Korunic and Fields, 2020) [4, 27]. When combined with clove oil, a synergistic effect appears to occur: DE compromises cuticular integrity, facilitating enhanced penetration of bioactive clove oil constituents into the insect hemocoel (Athanasios *et al.*, 2016) [5].

The relatively lower efficacy of other carriers such as wood ash, lime, and kaolin may be due to their inferior adsorptive capacity and lack of intrinsic insecticidal activity. Wood ash has been traditionally used for grain protection in subsistence farming systems (Golob and Webley, 1980) [15], but its efficacy is generally lower compared to DE (Stathers *et al.*, 2008) [39]. Silica, while exhibiting some desiccant properties, lacks the highly porous frustule structure characteristic of DE, which provides superior surface area for cuticular abrasion and oil adsorption (Korunic, 2013) [26]. The prolongation of the mean developmental period observed in all carrier-based treatments (38-39 days compared to 28 days in control) suggests sub-lethal effects on insect physiology. Similar developmental delays have been reported in insects exposed to sub-lethal concentrations of essential oils and their constituents (Jankowska *et al.*, 2018; Koul *et al.*, 2008) [22, 28]. Eugenol, the primary constituent of clove oil, is known to interfere with neuroendocrine regulation of growth and development in insects, potentially through inhibition of acetylcholinesterase and modulation of octopamine receptors (Enan, 2001; Regnault-Roger *et al.*, 2012) [13, 37].

4.2 Efficacy and Longevity of Optimized CODF

The optimized CODF formulation demonstrated exceptional protective efficacy over an extended storage period of 180 days, significantly outperforming the unstabilized formulation. The inclusion of fumed silica (1%) as an anti-caking agent and BHT (1%) as a stabilizing agent markedly enhanced the residual activity of the formulation.

Fumed silica, a synthetic amorphous silicon dioxide with extremely high surface area and purity, serves multiple functions in dust formulations. It acts as a flow aid, preventing caking and ensuring uniform particle distribution (Vayias and Athanassiou, 2004) ^[41]. More importantly, fumed silica possesses intrinsic insecticidal properties similar to DE, enhancing the overall desiccant effect of the formulation (Athanassiou *et al.*, 2016) ^[5]. Its nano-scale particle size (<50 nm) may also facilitate enhanced adhesion to the insect cuticle and grain surface, improving persistence.

Butylated hydroxytoluene (BHT) is a widely used synthetic phenolic antioxidant that functions by donating hydrogen atoms to free radicals, thereby terminating oxidative chain reactions (Yehye *et al.*, 2015) ^[42]. In the context of CODF, BHT likely prevents the oxidative degradation of eugenol and other volatile terpenoids present in clove oil, thereby maintaining the bioactivity of the formulation over extended storage periods. The GC-MS/MS analysis confirmed the substantial retention of eugenol (36.57% of total detected compounds) in the 180-day-old formulation, supporting the stabilizing role of BHT. The decline in efficacy observed in the unstabilized formulation after 60-90 days can be attributed to the gradual volatilization and oxidation of clove oil constituents in the absence of protective antioxidants.

The performance of CODF at 180 days (0.03% adult emergence) was statistically comparable to that of freshly applied malathion 5% dust (0.00% adult emergence), indicating its potential as a viable alternative to synthetic insecticides for medium-term grain storage. This finding aligns with previous studies reporting the efficacy of DE-botanical combinations against stored product pests (Athanassiou *et al.*, 2011; Korunic and Fields, 2020) ^[4, 27]. The minimal weight loss (0.31%) and germination loss (0.80%) recorded in CODF-treated grains after 180 days demonstrate the formulation's ability to preserve both quantitative and qualitative grain parameters, including seed viability.

4.3 Chemical Basis of Insecticidal Activity

The GC-MS/MS analysis of CODF confirmed eugenol (36.57%) as the predominant bioactive constituent, consistent with previous reports on the chemical composition of clove bud oil (Chaieb *et al.*, 2007; Jirovetz *et al.*, 2006). Eugenol, a phenylpropanoid compound, has been extensively documented for its insecticidal, repellent, and antifeedant properties against a wide range of stored product insects (Ho *et al.*, 1994; Jairoce *et al.*, 2016; Plata-Rueda *et al.*, 2018) ^[17, 21, 35]. The insecticidal mode of action of eugenol involves multiple targets including: (1) competitive inhibition of octopamine receptors, leading to disruption of neurotransmission (Enan, 2001) ^[13]; (2) inhibition of acetylcholinesterase activity, causing accumulation of acetylcholine at synapses (Jankowska *et al.*, 2018) ^[22]; (3) interference with cytochrome P450 monooxygenases and glutathione S-transferases, impairing detoxification capacity (Koul *et al.*, 2008) ^[28]; and (4) disruption of ion channel function in neuronal membranes (Regnault-Roger *et al.*, 2012) ^[37].

Caryophyllene (7.02%) and its oxidized derivatives (caryophyllene oxide, caryophyllenyl alcohol, hydroxycaryophyllene) constitute the second major group of bioactive compounds in the formulation. Caryophyllene is a bicyclic sesquiterpene with documented insecticidal and

repellent activities against various arthropod pests (Bettarini *et al.*, 1993; Suthisut *et al.*, 2011) ^[8, 40]. The presence of multiple sesquiterpenoids in the formulation may contribute to synergistic interactions, enhancing overall efficacy and potentially delaying the development of resistance (Huang *et al.*, 2000) ^[18].

The detection of minor constituents including trans-isoeugenol (6.34%), α -calacorene, τ -cadinol, and various humulene derivatives reflects the complex terpenoid profile of clove oil. While present in relatively low concentrations, these compounds may contribute additively or synergistically to the overall bioactivity of the formulation (Bakkali *et al.*, 2008; Pavela, 2015) ^[7, 33].

4.4 Comparison with Previous Studies

The findings of the present investigation are consistent with and extend previous research on botanical-DE combinations for stored product protection. Athanassiou *et al.* (2016) ^[5] reported enhanced mortality of *R. dominica* and *Tribolium confusum* when DE was combined with various essential oils, including thyme and oregano oils. Similarly, Korunic and Fields (2020) ^[27] demonstrated synergistic interactions between DE and plant-derived compounds against stored grain pests. The present study advances this knowledge by developing a fully characterized, stabilized formulation with demonstrated residual efficacy up to 180 days.

The efficacy of clove oil against *R. dominica* observed in this study (complete adult emergence inhibition at 0.2% concentration) corroborates earlier reports by Ho *et al.* (1994) ^[17], who documented strong contact toxicity of clove oil against *Sitophilus zeamais* and *Tribolium castaneum*. Jairoce *et al.* (2016) ^[21] similarly reported significant fumigant and contact toxicity of clove essential oil against *S. zeamais* in stored maize. The present work extends these findings by demonstrating the practical feasibility of delivering clove oil in a dust formulation that provides extended residual protection.

4.5 Limitations and Future Research Directions

While the present study demonstrates the efficacy of CODF under laboratory conditions, several aspects warrant further investigation before widespread field implementation. Large-scale storage trials under varying climatic conditions are necessary to validate the laboratory findings and assess potential interactions with ambient temperature and humidity fluctuations. The efficacy of DE-based formulations is known to be influenced by grain moisture content and relative humidity (Athanassiou *et al.*, 2011) ^[4]; therefore, optimization of application rates under diverse storage environments is warranted.

The spectrum of activity of CODF against other important stored grain pests, including *Sitophilus oryzae* (rice weevil), *Sitotrogacerealella* (Angoumois grain moth), and secondary pests such as *Tribolium castaneum* (red flour beetle) and *Cryptolestes ferrugineus* (rusty grain beetle), requires evaluation. Pest complexes in stored grain typically comprise multiple species with varying susceptibilities to control measures (Hagstrum and Subramanyam, 2009) ^[16].

Long-term toxicological studies assessing the safety of CODF-treated grains for human consumption and livestock feed are necessary to support regulatory approval. While the individual components are generally recognized as safe (GRAS) by regulatory agencies, the potential for interactions or formation of reaction products during extended storage should be investigated.

Finally, the potential for development of insect resistance to botanical formulations, while considered lower than for single-site synthetic insecticides, cannot be discounted

(Isman, 2020) [20]. Rotation strategies and integration with other non-chemical methods within an IPM framework should be developed to ensure long-term sustainability.

Table 1: Evaluation of various carrier materials fortified with 0.2% clove oil against the lesser grain borer, *R. dominica*

S. No.	Treatment	Number of eggs laid/10 gram	Number of eggs hatched	Hatching percentage (%)	Adult Emergence (%)	Mean developmental period (No. of days)
1.	Wood ash	1.18 (1.08) ^b	0.14 (0.37) ^b	0.11 (0.33) ^b	1.23 (1.11) ^b	38.30 (6.19) ^b
2.	Lime	0.99 (0.99) ^{bc}	0.13 (0.36) ^b	0.13 (0.36) ^b	0.83 (0.91) ^c	38.43 (6.20) ^{ab}
3.	Silica	0.8 (0.89) ^{cd}	0.08 (0.28) ^c	0.01 (0.1) ^d	0.68 (0.82) ^{cd}	38.50 (6.20) ^{ab}
4.	Diatomaceous earth	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^d	0 (0) ^e	0 (0) ^d
5.	Kaolin	0.5 (0.71) ^d	0.04 (0.2) ^d	0.08 (0.28) ^c	0.35 (0.59) ^d	38.90 (6.24) ^a
6.	Control	24.90 (4.99) ^a	22.81 (4.78) ^a	91.60 (9.57) ^a	94.20 (9.71) ^a	28.42 (5.33) ^c
7.	CD	0.09	0.1	0.03	0.09	0.01

Figures in parentheses are square root transformed values.

In a column means followed by same alphabets are not significantly different (p=0.05) by DMRT

Table 2: Storage studies without stabilizer and Anticaking agent

Days of Storage	Number of eggs laid/10 gram	Number of eggs hatched	Hatching (%)	Adult Emergence (%)	Mean developmental period (No. of days)	Weight loss (%)	Germination loss (%)
15	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	-	-
30	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^f	0 (0.71) ^f
60	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^f	0 (0.71) ^f
90	0.3 (0.32) ^d	0.02 (0.1) ^d	0.02 (0.1) ^d	0.01 (0.1) ^b	53.61 (7.32) ^a	0.3 (0.55) ^b	0.5 (0.71) ^d
120	0.4 (0.32) ^c	0.03 (0.1) ^c	0.03 (0.1) ^c	0.3 (0.55) ^c	52.11 (7.22) ^b	0.35 (0.59) ^d	0.7 (0.84) ^c
150	0.46 (0.68) ^c	0.05 (0.22) ^c	0.1 (0.32) ^b	0.07 (0.26) ^c	50.36 (7.10) ^c	0.42 (0.65) ^c	0.8 (0.89) ^c
180	0.61 (0.78) ^b	0.08 (0.28) ^b	0.14 (0.37) ^b	0.1 (0.32) ^b	48.32 (6.95) ^d	0.51 (0.71) ^b	1.01 (1.00) ^b
Untreated Control	24.90 (4.99) ^a	22.81 (4.78) ^a	91.60 (9.57) ^a	94.20 (9.71) ^a	28.42 (5.33) ^f	93.22 (9.66) ^a	96.43 (9.82) ^a

Figures in parentheses are square root transformed values.

In a column means followed by same alphabets are not significantly different (p=0.05) by DMRT

Table 3: Final Formulation (CODF) storage studies (0.2% clove oil+ Diatomaceous earth+ Fumed silica (1 %)+ BHT (1%))

Days of Storage	Number of eggs laid/10 gram	Number of eggs hatched	Hatching (%)	Adult Emergence (%)	Mean developmental period (No. of days)	Weight loss (%)	Germination loss (%)
15	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	-	-
30	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e
60	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e
90	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e
120	0.1 (0.32) ^e	0.01 (0.1) ^e	0.01 (0.1) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e
150	0.2 (0.45) ^d	0.03 (0.17) ^d	0.02 (0.14) ^d	0.01 (0.1) ^d	55.67 (7.46) ^a	0.22 (0.47) ^c	0.5 (0.71) ^c
180	0.23 (0.47) ^c	0.05 (0.22) ^c	0.02 (0.14) ^d	0.03 (0.17) ^c	51.34 (7.16) ^b	0.31 (0.56) ^b	0.8 (0.89) ^b
Malathion 5D 180 Days	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0 (0.71) ^e	0.29 (0.54) ^b	0.7 (0.83) ^b
Untreated Control	24.90 (4.99) ^a	22.81 (4.78) ^a	91.60 (9.57) ^a	94.20 (9.71) ^a	28.42 (5.33) ^f	93.22 (9.65) ^a	96.43 (9.82) ^a

Figures in parentheses are square root transformed values.

In a column means followed by same alphabets are not significantly different (p=0.05) by DMRT

5. Conclusion

The present study successfully developed and validated an optimized clove oil-based dust formulation (CODF) comprising 0.2% clove oil, 97.8% diatomaceous earth, 1% fumed silica, and 1% BHT for the management of *Rhyzopertha dominica* in stored paddy. The formulation provided complete protection of paddy grains for 120 days and maintained excellent residual efficacy (adult emergence <0.1%) up to 180 days of storage under ambient conditions. GC-MS/MS analysis confirmed the presence of eugenol and caryophyllene as the principal bioactive constituents, while BHT contributed to formulation stability and prolonged shelf-life. This botanical formulation represents a promising, eco-friendly alternative to synthetic insecticides and can serve as an effective component of integrated pest management programs for stored grains, contributing to reduced post-harvest losses and enhanced food security.

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