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Development and evaluation of eco-friendly natural oil-based gel balls for the effective control of household and stored-grain insects

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Abstract
Growing concerns about the toxic residues and ecological damage caused by synthetic pesticides have led to the exploration of safer, natural alternatives. The present work focuses on the development of a natural insecticidal/insect repellent Gel Ball formulation, incorporating selected essential oils (based on their established insect-repellent and insecticidal properties) dispersed in a gel formulation to enhance stability and release properties. Oils such as neem, eucalyptus, rosemary, citronella, clove, lemon grass, tea tree and peppermint were chosen for their known insect-repelling and insecticidal activities. The prepared formulation was evaluated for its physical attributes including appearance, efficacy, stability and screened for general environmental safety. Its bio-efficacy was assessed against commonly encountered house hold insects, including *Tribolium castaneum* (red flour beetle), *Sitophilus oryzae* (rice weevil), and *Musca domestica* (housefly). The results indicated that the formulation produced significant repellency and mortality while showing no harmful effects on stored commodities. The results of environmental safety studies revealed that the formulation provides a safe, biodegradable, and sustainable alternative to conventional synthetic insecticides for the effective management of insects in domestic and storage settings.

Keywords: Natural insect repellent/insecticide, Essential oils, Bio-safety, Gel Balls, *Tribolium castaneum*, *Sitophilus oryzae*, *Musca domestica*

Introduction

Insecticides/Pesticides play a crucial role in protecting food grains and public health by minimizing losses caused by insect infestations. However, the extensive and indiscriminate use of conventional synthetic pesticides/insecticides has led to serious challenges, including toxic residues in food, environmental contamination, pest resistance and adverse health effects on humans, animals and non-target organisms (Damalas CA & Eleftherohorinos IG, 2011, Isman MB, 2020) [4,12]. Pesticides such as organophosphates and carbamates, although effective, often persist in the environment and disrupt ecological balance (Aktar MW *et al*., 2009, Ioannis G, 2011, Sanchez-Bayo Francisco *et al*., 2019) [1, 11,18]. In recent years, there has been a marked shift toward eco-friendly pests/insects control alternatives, particularly those from plants. Essential oils are one of the alternatives which are extensively used with significant efficacy against large number of insects or pests. Essential oils are natural, volatile compounds extracted from aromatic plants that possess insecticidal, repellent and anti-feedant properties. They are biodegradable, non-persistent and relatively safe to humans and animals, making them ideal candidates for domestic pest/insect management and protection of stored food products (Natchiappan S *et al*., 2024, Elbehery HH & Ibrahim S, 2024) [15.5]. Despite their strong bioactivity, the practical application of essential oils is often limited by their volatility, poor solubility and short residual activity. Incorporating these oils into a base oil and converting into specific gel based dosage form enhances stability, allows for gradual release of active constituents, and prolongs its efficacy. The present work aims to develop a natural oil-based gel; incorporating selected essential and fixed oils—neem, eucalyptus, rosemary, citronella, clove, lemongrass, tea tree, and peppermint—each known for their potent insecticidal and repellent properties (Isman MB, 2006) [13].

The present study focuses on the formulation development, study of physical properties of the formulation, evaluation of the formulated dosage form for insecticidal/insect repellent activity against commonly encountered household and storage pests, namely *Tribolium castaneum* (red flour beetle), *Sitophilus oryzae* (rice weevil), and *Musca domestica* (housefly), its stability and basic biosafety studies.

Pictures showing insects; *Tribolium castaneum, Sitophilus oryzae, Musca domestica*

Tribolium castaneum **(Red Flour Beetle)**	***Sitophilus oryzae*** **(Rice Weevil)**	***Musca domestica*** **(Housefly)**
		

Materials and Methods

Materials

The oils; neem, eucalyptus, rosemary, citronella, clove, lemongrass, tea tree, coconut and peppermint—were procured online from Veda Oils and few from local market Nashik. Label claim was considered for purity authentication. The base oil used was cold pressed coconut oil. For test, infested grains (wheat and rice) containing *Tribolium castaneum* and *Sitophilus oryzae*, maintained under laboratory conditions were used. Efficacy against *Musca domestica* was assessed using a direct exposure model wherein freshly cut vegetable samples were placed under controlled conditions and fly/ feeding attempts. Landing and repellency responses were recorded following the gel ball formulation treatment. Suitable optimum temperature and relative humidity were maintained (27 ± 2°C, 65 ± 5% relative humidity) for the effective growth and activity of the insects, Sodium alginate, guar gum and calcium chloride (AR grade) were used for the preparation of Gel Balls.

Table 1: Table showing the ingredients and their composition of optimized batch exhibiting highest efficacy, stability and safety under tested conditions

Sl No.	Ingredients	Composition
Gel Base		
1	Sodium alginate	2.5 g
2	Guar gum	0.5 g
3	Purified water	QS-100 ml
Oil Phase		
1	Neem oil	0.5 mL
2	Eucalyptus oil	1.0 mL
3	Coconut oil	10.0 mL
4	Citronella oil	0.5 mL
5	Lemongrass oil	0.5 mL
6	Peppermint oil	0.5 mL
7	Tea tree oil	1.0 mL
8	Clove oil	0.5 mL
9	Rosemary oil	0.5 mL
10	Gum acacia (emulsifier)	0.3 g
11	Calcium chloride (Cross linking)	3% w/v in water

Methodology

A. Formulation Development

a. Preparation of Gel/Gum Solution: Purified water (80 mL) was heated to 50–60°C, and the weighed quantity of sodium alginate was gradually dispersed with continuous stirring. Guar gum was then added slowly with constant stirring to obtain a homogeneous mixture. The volume was adjusted to 100 mL with warm purified water and stirred until a uniform viscous solution was formed. The resulting solution was allowed to stand for 1 hour to ensure stabilization.

b. Preparation of Oil Phase: All the essential oils (listed in Table 1) were mixed with coconut oil (carrier oil). Weighed quantity of gum acacia was added and stirred gently until uniform consistency.

c. Gel Mixture solution: Slowly oil phase was added to gum solution by stirring with a moderate speed to get homogenous gel dispersion.

d. Preparation Gel Balls: A 3% w/v calcium chloride solution was prepared. The gel mixture was dropped into the solution using a dropper to form gel balls, which were allowed to cure for 30 minutes. The formed balls were separated by filtration using a conventional strainer, rinsed once with purified water, and air-dried at room temperature for 5 hours (Smrdel P. *et al*., 2008) [19]. The dried balls were elastic and firm in consistency.

e. Packing and Storage of Gel Balls: Balls were packed in wax-coated Kraft paper containing 15 manually perforated vents (primary packaging), then sealed in aluminium foil–laminated pouches and subsequently placed in rigid corrugated cartons to prevent mechanical damage. The packets were stored at room temperature.

B. Evaluation of Formulation for insecticidal activity (Bio efficacy) and eco safety:

Evaluation was carried out under the following heads (parameters)

a. Physical evaluation
b. Insecticidal bio efficacy (Insecticidal/repellent property)
c. Safety assessment
d. Stability tests
e. Shelf life assessment

a. Physical Evaluation;

- **Appearance**: Dosage forms were tested for Shape, Surface texture, colour and presence of cracks (Results: Table: 4)

- **Diameter & Weight Uniformity:** Measured diameter using vernier caliper. Weight uniformity was calculated by weighing 20 balls together and then individually. Mean weight was calculated and compared with individual ball weight by taking standard deviation. Percentage weight variation was determined and compared with standard acceptance values (Hari PR *et al*., 1996) [8] (Results: Table: 4)

- **Mechanical strength**: The crushing strength of the gel balls was determined using a manual compression method. A single gel ball was placed on a rigid, flat glass plate and covered with a flat metal plate to ensure uniform load distribution. Incremental weights were applied vertically, starting from 10 g and increasing in increments of 20 g, until permanent rupture of the gel ball occurred. The minimum weight required to cause irreversible structural failure was recorded as the crushing weight. The test was performed on 20 individual gel balls, and the results were expressed as mean crushing strength ± standard deviation (SD). *(*George M., & Abraham TE, 2006, Anal AK & Stevens WF 2005) [7,2]. (Results: Table:4)

b. Insecticidal Bio efficacy studies:

- **Mortality Test**: Bio-efficacy was assessed using a laboratory-maintained infested grain insect model. Approximately 100 g of wheat grains (for *Tribolium castaneum*) and 100 grams of rice grains (for *Sitophilus oryzae*) were evenly spread, placed separately in experimental chambers (eight centimetre diameter), each containing approximately 25 healthy adult insects. Mortality was recorded at time intervals of 1, 2, 4, 8, 24, and 48 hours. Percentage mortality was calculated using the standard formula (Brari, J, 2020[3]

% Mortality=Dead insects / Total insects x100.

Results were expressed as mean percentage mortality for each group. For each species, three experimental groups were maintained; GroupI-Control group (without gel balls), Group II-Blank group (plain gel balls without active ingredient) and Group III Treatment group (insecticidal gel balls). Two gel balls maintained at equal distance, were used for the study. (Results: Table: 5)

c. Repellence Test: Approximately 100 g of wheat grains (for *Tribolium castaneum*) and 100 grams of rice grains (for *Sitophilus oryzae*) were placed separately in experimental chambers, each containing approximately 25 healthy adult insects. The set up was kept under standard laboratory conditions (27 ± 2°C, and 65 ± 5% RH). Number of insects were counted at 1st, 2nd and 3rd hour, after placing the gel balls at the centre of the insects infested grains. Two gel balls maintained at equal distance, were used for the study. Percentage repellency was calculated after observing the insects which are moving away from the gel balls to the corner of the chamber using the formula given below (Khalil, M., *et al*., 2024)[14]. (Results Table: 6)

- **Repellency against *Musca domestica*** was assessed through a direct exposure model using freshly cut vegetables to evaluate fly landing and contact behaviour in the treated environment. Test was carried out by placing the cut vegetable (tomato and onion) in large open petriplates. One gel ball was placed on each cut surface (Tian, Y., *et al*., 2024) [20, 21]. (Results Table: 6)

Table 3: Interpretation of data for Repellency

Percent Repellency (PR) (%)	Interpretation
0-20	Very low / negligible repellency
21-40	Moderate repellency
41-60	Good repellency
61-80	High repellency
81-100	Very high / complete repellency

d. Stability Studies: According to the standard protocol, stability studies were carried out at 25°C ± 2°C for six months and 40°C ± 2°C for two months. The samples were tested periodically for physical changes like surface cracking, changes in bio-efficacy and oil seepage. (ICH guidelines, 2003)[10] (Results: Table: 7)

e. Shelf- Life assessment: Shelf life assessment was carried out by evaluating the stability, efficacy and physical integrity, by adopting ICH (Q1A) guidelines for pesticides/Insecticides (with slight modification). Test gel balls were stored at different temperature as shown in table below. After the study period, physical evaluation for appearance, diameter, weight variation, texture and biological efficacy studies were carried out and the variations were calculated. In this study, shelf life (t_{90}) was considered as the time required for the formulation to retain not less than 90% of its initial bio-efficacy against stored-grain insect species (ICH guidelines, 2003) [10] (Results: Table: 7)

Table 2: Various physical conditions applied for the shelf life assessment of insecticidal gel balls

Study Type	Temperature	RH	Duration
Long-term	25°C ± 2°C	60% ± 5% RH	10 months
Accelerated	40°C ± 2°C	75% ± 5% RH	6 months
Refrigerated	5°C	—	3 months

f. Environmental Safety assessment: As per OECD guidelines basic safety assessment evaluation was carried out. Evaluation procedure is described below.

g. Acute Earthworm Toxicity Test (OECD 207) [16]: The acute toxicity of the insecticidal gel balls towards non-target soil organisms was evaluated in accordance with OECD Guideline 207 (Earthworm, Acute Toxicity Tests). Adult Indian earthworms (*Pheretima posthuma*), were selected for the study. Garden soil was used for screening. Test gel balls (Five) were crushed and incorporated uniformly into the soil, while control groups received untreated soil and blank gel -treated soil. Twenty earthworms were introduced into each test container, and the experiment was conducted under controlled laboratory conditions (25 ± 2 °C, 12;12 h light: dark cycles). Mortality, behavioural abnormalities like reduced mobility and morphological changes were recorded after 7 and 14 days of exposure. (García-Delgado Carmen *et al*., 2020, Hlaskova Lenka *et al*., 2018) [6,9], (Results: Table: 8)

h. Ready Biodegradability Test (OECD 301 Series) [17]: Biodegradability of the gel ball formulation was assessed following OECD Guideline 301 (Ready Biodegradability Tests), using the closed bottle (CO_2 evolution and O_2 consumption) method. The test substance (100mg of gel) was exposed to aerobic microbial inoculum (10ml of surface water sample from

natural pond) under controlled conditions for 28 days. Biodegradation was determined by measuring oxygen consumption and carbon dioxide evolution and comparing it to theoretical oxygen demand (TO_2D) and theoretical CO_2 production (TCO_2P). (Results: Table: 7)

Statistical Analysis: All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical significance between treated and control groups was analyzed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Results and Discussion

Table 4: Results of Physical Evaluation Studies

Parameter	Initial	3 Months	6 Months	Acceptance Criteria
Appearance	Smooth, spherical, yellowish brown, no cracks	No change	Yellowish, slightly dark brown	Yellowish, slightly dark brown, no cracking or shape change
Diameter (mm)	8.08 ± 0.05	8.06 ± 0.02	8.06 ± 0.02	±5% variation
Mean Weight (g)	1.50 ± 0.03	1.48 ± 0.04	1.48 ± 0.05	±7.5% variation
Mechanical Strength	Unbroken, Intact	Unbroken Intact	Unbroken Intact	No fragmentation or change texture

Table 5: Percentage mortality (Mean ± SD) of stored grain insects *T. castaneum* and *S. oryzae* following exposure to the test formulation at various time intervals

Insects	Group	1 hr (%)	2 hr (%)	4 hr (%)	8 hr (%)	24 hr (%)	48 hr (%)
T. castaneum	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	4 ± 0.8	12 ± 1.6
	Blank	0 ± 0	0 ± 0	0 ± 0	0 ± 0	8 ± 5.22	9 ± 3.24
	Treatment	24 ± 0.82	36 ± 5.26	48 ± 5.62	56 ± 1.24	80 ± 0.86	92 ± 0.88
S. oryzae	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	8 ± 0.88	14 ± 1.68
	Blank	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16 ± 5.22	20 ± 3.22
	Treatment	32 ± 0.84	56 ± 1.20	72 ± 2.68	84 ± 1.20	88 ± 2.42	96 ± 1.62

Values are presented as Mean ± SD (n = 25 per group). Percentage mortality was calculated relative to the total insects exposed. Data were analysed using two-way ANOVA followed by Tukey's post-hoc test, with statistical significance set at $p < 0.05$.

Table 6: Repellence Activity of Gel Ball Formulation against test Insects

Insect Species	1 hr (%)	2 hr (%)	3 hr (%)	Interpretation
Tribolium castaneum	86 ± 0.22	88 ± 4.02	98 ± 3.26	Very Strong repellency
Sitophilus oryzae	82± 4.4	88 ± 0.6	98 ± 2.22	Very Strong repellency
Musca domestica	88 ± 5.2	90 ± 4.0	99 ± 3.42	Very strong repellency

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

Table 7: Results of stability and shelf life studies of insecticidal gel balls

Parameter	Initial	25°C ± 2°C (3 Months)	25°C ± 2°C (6 Months)	40°C ± 2°C (1 Month)	40°C ± 2°C (2 Months)	Acceptance Criteria
Appearance	Smooth, spherical	No change	No change	No change	Slight softening	No cracking/discoloration
Surface Texture	Uniform	Uniform	Uniform	Slight soft	Soft but intact	No fragmentation
Diameter (mm)	8.02 ± 0.05	8.01 ± 0.04	7.99 ± 0.06	7.95 ± 0.05	7.92 ± 0.07	±5% variation
Mean Weight (g)	1.50 ± 0.03	1.49 ± 0.04	1.47 ± 0.05	1.45 ± 0.04	1.42 ± 0.06	±7.5% variation
Oil seepage	Absent	Absent	Absent	Absent	Absent	No seepage
Bio-efficacy	Average 94% for the insects tested					≥90% mortality
Bio-efficacy	Average 98% for the insects tested					≥90% Repellency

Data were expressed as mean ± SD (n = 3). Statistical comparison between initial and stability samples was performed using one-way ANOVA, and differences were considered significant at $p < 0.05$.

Table 8: Environmental Safety Assessment of Gel Ball Formulation as per OECD 207 guidelines for mortality studies and OECD 301 for biodegradability test

Test Model	Parameter Evaluated	Control	Blank	Treatment	Interpretation
Acute Earthworm Toxicity	Mortality (%) – Day 7	0 ± 0	0 ± 0	0 ± 0	No acute toxicity
	Mortality (%) – Day 14	0 ± 0	0 ± 0	1 ± 1.2	Practically non-toxic
	Behavioural changes(14 days)	None	None	Mild, reversible hypoactivity (Day14)	No significant adverse effects
	Morphological abnormalities	None	None	None	Safe to soil target organism
Biodegradability Test	(Day 7)-% Biodegradation	—	—	48 ± 2.42	Moderate degradation
	(Day 14)-% Biodegradation	—	—	72 ± 3.12	Substantial degradation
	(Day 28)-% Biodegradation	—	—	98 ± 2.84	Readily biodegradable
	Oxygen Consumption (mg O_2/L)	—	—	Increased progressively	Active microbial degradation
	CO_2 Evolution (%)	—	—	Corresponding to 80–85% of theoretical CO_2	Confirmed mineralization

Values are expressed as Mean ± SD (n = 20 for earthworm study). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

A biodegradation level ≥60% within 28 days was considered indicative of ready biodegradability as per OECD criteria

Results and Discussion

Physical Parameters: The natural oil-based gel balls were smooth, spherical, elastic, and crack-free, confirming successful ionic cross-linking of sodium alginate with calcium chloride. The optimized formulation showed uniform diameter (8.08 ± 0.05 mm), acceptable weight variation (±7.5%), and adequate mechanical strength, indicating suitability for handling and storage (Table 4).

Insecticidal Bio-efficacy: Significant time-dependent mortality was observed against *Tribolium castaneum*, *Sitophilus oryzae*, and *Musca domestica*. Mortality reached 92% (*T. castaneum*) and 96% (*S. oryzae*) within 48 h, while control groups showed negligible mortality. Repellency exceeded 80% within 1 h and reached 98–99% at 3 h, indicating very strong repellency (Tablle 5). The enhanced efficacy may be attributed to synergistic action of essential oils affecting octopaminergic and GABA-mediated neurotransmission.

Environmental Safety: As per OECD 207, 0% mortality was observed in *Pheretima posthuma* at Day 7 and only 1% at Day 14, with no morphological abnormalities, indicating practical non-toxicity. Biodegradability testing (OECD 301) showed 98% degradation at 28 days, confirming ready biodegradability.

Stability and Shelf-Life: No significant changes in physical parameters or bio-efficacy (≥90%) were observed under long-term (25°C) and accelerated (40°C) conditions. Slight softening at 40°C did not affect integrity. Encapsulation within the cross-linked matrix likely minimized volatilization, supporting an estimated shelf-life of around 12 months for optimal efficacy and stability.

Conclusion

The study successfully developed eco-friendly natural oil-based gel balls exhibiting >90% insecticidal activity within 48 h and strong immediate repellency against *T. castaneum*, *S. oryzae*, and *M. domestica*.. The formulation demonstrated environmental safety, negligible non-target toxicity, and ready biodegradability as per OECD guidelines, along with satisfactory stability under varied storage conditions. Overall, the developed gel-ball dosage form represents a effective, sustainable, biodegradable, and safer alternative to conventional synthetic insecticides for household and stored-grain pest management.

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