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Research Article

Insecticidal Activity of Polyethylene Glycol Nanocapsules of Clove Essential Oil against *Sitophilus oryzae* and *Tribolium castaneum*

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Abstract

The present study was aimed to prepare clove oil nanocapsules (PEG-ClO NCs) and to investigate their insecticidal potential against stored grain pests including *Sitophilus oryzae* and *Tribolium castaneum.* Nanocapsules were synthesized by the melt dispersion method. The 10% PEG-ClO NCs formed the most stable nanocapsules with 270.30 nm size, 0.25 PDI, and 90.03% encapsulation efficiency. Scanning electron microscopy images showed slightly irregularly shaped nanocapsules in a good dispersion. The major phytochemicals identified in GC-MS analyses of pure clove oil were eugenol, phenol, and caryophyllene. Contact toxicity bioassay revealed that clove essential oil nanocapsules showed 100% mortality against *Tribolium castaneum* and *Sitophilus oryzae* after 3 and 7 days of exposure respectively. The LC50 values for nanocapsules were 2260.89 and 10498 mg/kg against *S. oryzae* and *T. castaneum.* The residual contact toxicity of pure oil was reduced gradually and after 4 weeks it showed 61.67 % and 58.36 % mortality upon exposure as compared to PEG-ClO NCs that showed 95.67 % and 93.33% mortality against *Sitophilus oryzae* and *Tribolium castaneum* respectively. The results of this study suggested that PEG-based nanocapsules prevented the clove essential oil evaporation and rapid degradation while allowing a persistent and slow release. It is concluded that PEG nanocapsules loaded with clove essential oil are feasible to be used as bioinsecticide against stored grain pests for a long time.

Keywords: *Syzgium aromaticum*; Polyethylene glycol (PEG); Essential oil; *Sitophilus oryzae*; *Tribolium castaneum*

1. Introduction

Stored grains such as rice, maize, barley, and wheat are the primary sources of proteins, dietary fibers, and carbohydrates. Unfortunately, 20-50% of stored grains get destroyed annually in developing countries and 10-20% in developed countries due to insect infestation [1]. Along with direct consumption of kernels, the destruction inflicted by insects to stored grains also includes the aggregation of insect cadavers, webbing, frass, and exuviae. These make cereal grains unhealthy for consumption, resulting in monetary losses [2]. Moreover, changes induced by insect infestation in the storage environment mostly led to the formation of moist and warm “hotspots” suitable for the invasive development of fungi which further produces harmful mycotoxins [3].

Stored grain pests, based on their feeding habits, are classified into primary and secondary pests [4]. *Tribolium castaneum,* commonly known as (Red flour beetle), is considered a secondary pest. It is notoriously destructive with a reported potential to destroy 246 commodities, making it the most resilient polyphagous insect pest

globally [5]. It usually feeds over cereals, pulses, seeds, flour, spices, nuts, rice, starchy material, fruits, and bakery items [6]. *Sitophilus oryzae* (Coleoptera: Curculionidae), commonly known as rice weevil is a primary pest responsible for deteriorating 10-65% of grains in normal storage conditions and could probably cause 80% damage in prolonged storage conditions [7]. *S. oryzae* can induce serious infestation in paddy, maize, sorghum, wheat, and other cereals [8, 9]. Both red flour beetle and rice weevil are major destructors and are responsible for serious financial detriment globally [4, 10].

There are various methods for stored grain pest control including mechanical routes (using different storage structures) chemical, biological, and physical practices. The most practical and convenient approach is fumigation, considering its quick and effortless implementation over various storage locations specifically silos, bunkers, jute sacks, warehouses, and bulkers [11, 12]. However, utilizing synthetic insecticides is the most stereotypical approach to control these insect pests, either by direct application over grains or simply by gas fumigation [13]. Nowadays, methyl bromide and phosphine are being used extensively for managing pests intruding on stored commodities like grains and dried fruits worldwide [14, 15].

Although methyl bromide is a very quick and effective disinfectant, it is one of the major ozone-depleting substances. Therefore, obeying 'Montreal Protocol' it has been phased out and ceased globally [11, 16]. In developing countries, the disinfestation of stored commodities generally hinges on phosphine gas [17]. Serious upsurge in phosphine resistance at different levels in *T. castaneum* [18, 17]. and *S. oryzae* has also been reported [17, 19, 20].

Besides resistance development, the excess usage of these insecticides induces environmental toxicity [21, 22]. Pesticides also exert carcinogenic effects such as mammary tumors, hematopoietic, uterine, and lymphatic malignancies and disturb immune responses [23, 24]. All these issues have intensified the need to find efficient alternatives that must be safe, biodegradable, cost effective [25]. This obligation for secure control agents has promoted the development of bioinsecticides [26]. From this perspective bioinsecticides, based on essential oils (EOs) are believed to be a better substitute for conventional insecticides [27, 28].

Essential oils are natural, aromatic, lipophilic and volatile compounds that are composed of complex secondary metabolites such as terpenes and terpenoids [29]. Phytochemicals that are naturally present in plants EOs act as a neurotoxicant. EOs also invade insect's respiratory systems by obstructing their spiracle pleading them to suffocate and die [29]. EOs can stimulate biochemical dysfunction by entering the insect body through inhalation, skin absorption, and ingestion simply because of their lipophilic nature [30]. EOs also exhibit sub-lethal toxicity and repellency towards insects. The EOs can restrict the oviposition and feeding habits of insects [31]. Clove (*Syzygium aromaticum*) EO having eugenol and caryophyllene as major phytonutrients, hinders eggs and grubs' developmental stages inside grains thoroughly [13, 32].

Regardless of EOs striking pesticidal potential, their implementation at commercial level and over large-scale is deferred because of their high volatility, chemical instability, poor solubility as well as sensitivity towards temperature, moister, and PH [33, 34]. To overcome these problems nanoformulations of essential oils can be utilized. Nanoformulations can increase physical stability, facilitate controlled release, and protect the deterioration of bioactive constituents. Furthermore, nanoencapsulation of EO provides higher mobility, increasing water solubility and permeability into the insect's cuticle [35, 36].

A broad range of agents like synthetic polymers, phospholipids, natural polymers, inorganic material, and lipids are available that can be used for encapsulation [37]. The most commonly used polymers include chitosan, chitin, alginates, cyclodextrin, and polyester like poly-ε-

caprolactone and polyethylene glycol (PEG) [38]. PEG 6000 is preferred over other available options because it improves the solubility of EOs and is intoxicant itself with no immunotoxicity or antigenicity. PEG does not intrude into any enzymatic activity and gets easily defecated after being consumed by a human being [39]. The present study was planned to prepare PEG-based clove essential oil nanocapsules and to examine their insecticidal potential against stored grain pests.

2. Materials and Methods

2.1. Extraction of essential oil

Dry clove buds (*Syzygium aromaticum*) were obtained from the local market of Lahore. After removing the unnecessary dust particles, the grinding of clove buds was carried out using an electric blender. Clove oil was extracted by using a traditional hydro distillation plant at 100 ^{o}C. The clove powder (50g) was mixed with 300 ml distilled water in a 500 ml round bottom flask. The extraction time was approximately 6 hours. After the hydro-distillation process, the obtained solution contained a mixture of water and oil. Oil was separated from the obtained solution by using anhydrous magnesium sulphate followed by filtration. Collected essential oil was stored in a cool and dark place before any further testing [40].

2.2. Rearing of Insects

Adults of *Sitophilus oryzae* and *Tribolium castaneum* were collected from infested rice and wheat grains obtained from the local market of Lahore, Pakistan. Both insect species were identified by using a stereomicroscope. *T. castaneum* adults were reared in a clean plastic jar (2L capacity) and the medium for this culture was a mixture of 5% instant yeast and 95% sterilized wheat flour. The culture was maintained at an optimal temperature 26±2^{o}C, 65±5 RH, and a photoperiod of 12;12h (L:D). Moreover, *S. oryzae* were also reared on sterilized rice grains, in the laboratory at 26±2^{o}C with 65±5% RH and photoperiod of 12h;12h (L:D). Jars were covered by muslin cloth to prevent insects from escaping and ensure proper ventilation [41, 42]. Cultures of both species were maintained for 7 months in the Applied Entomology and Toxicology Laboratory, Department of Zoology GC University, Lahore

2.3. Preparation of PEG-based clove oil nanocapsules (PEG-CIO NCs)

The melt dispersion technique (with a few modifications) was used for the preparation of PEG-coated nanocapsules. Several parts of PEG 6000 (from Sigma-Aldrich) 100g per part were separately heated using a hotplate stirrer at 65°C. After PEG is melted completely, 8, 10, 12, and 15ml of clove oil were added separately to prepare different concentrations (8,10,12 and 15ml/100g PEG) of PEG-CIO NCs, namely P1, P2, P3, and P4 respectively. Equal distribution of clove oil within melted PEG was ensured by mixing it continuously with a glass rod for at least 30min. The mixture was then cooled immediately at -4^{o}C for 45 mins and then ground in a cold mortar. Finally, the powder was sieved through stainless steel 230 mesh sieve. The powder was stored in a zipper bag at 27±2°C in a desiccator containing calcium chloride to prevent moisture absorption before further testing [39].

2.4. Size and distribution of clove oil nanocapsules

The polydispersity index (PDI) and size of PEG-CIO NCs were assessed a day after the preparation of nanocapsules by using the Dynamic light scattering (DLS) technique [42]. From each concentration of PEG-CIO NPs powder (0.2g) was taken and suspended in 10 ml distilled water for about 30min. After equilibrating the sample for 2 minutes measurements were performed at 25^{o}C. The procedure was replicated thrice [43, 44].

2.5. Encapsulation Efficiency (%EE)

The percentage encapsulation efficiency (%EE) was evaluated by forming a standard concentration curve. After 2 days of storage, 0.1g powder from each sample of PEG-CIO NCs formulations was dissolved in 2ml of absolute ethanol. A series of different concentrations were prepared for each mixture by mixing pure clove oil with absolute ethanol. A colorimetric assay was carried out to determine the absorbance of these samples by using UV-

visible spectrophotometry (Agilent Technologies Cary 60 UV-Vis). Absorbance was determined at 290nm and the values obtained were then compared to the standard curve. The percentage encapsulation efficiency (% EE) was calculated by comparing these values with the original amount of EO incorporated [45].

2.6. Clove oil composition pre-/post-Nano formulation

Gas chromatography-mass spectrometry (GC MS model:7890B, 5977A, Agilent USA) was used for the phytochemical analysis of pre-/post-nanoformulations. 0.5g of 10% PEG-CIO NPs formulation was dissolved in 5ml of distilled water and then heated for 50^{o}C for about 30min followed by the addition of 4ml absolute ether to recollect the EO extracted [46].

2.7. Contact toxicity

Pure clove oil as well as selected PEG-CIO NCs with highest %EE were used to treat 20g of milled rice obtained from the local market of Lahore. The experiment was performed in an air-tight glass container. In the case of S. *oryzae*, rice grains were treated with doses of 180, 360, 540, 720g, and 900 mg/kg of pure clove essential oil and nanoformulations (containing equal concentration of pure oil mentioned above taking account the encapsulation efficiency). In the case of *T. castaneum*, the concentration for pure clove oil was 900, 1125, 1350, 1575, and nano-formulations containing equal concentration of pure oil. Pure oil solutions were prepared by using acetone as a solvent. The rice samples were treated with different concentrations followed by drying them for 1h to evaporate the solvent before releasing insects. Nano formulation powder and rice samples were mixed vigorously to spread the formulation evenly.

Twenty adults were released into each glass jar with different concentrations of pure oil and nanoformulations and were stored at 28±2^{o}C, 75±5% RH. Rice samples treated with PEG 6000 and acetone were used as a control. A Glass jar with untreated rice was established as a negative control. All treatments to evaluate contact toxicity were replicated 3 times and mortality was recorded after 3 and 7 days. The insects showing no mobility of legs and antennas even after disturbing them with pins were considered dead [32].

2.8. Residual toxicity

Residual bioassays were conducted periodically after 1 day of storage as well as for 1-4 weeks of storage. Pure clove oil as well as nanoemulsions were applied once to establish treatment groups. The treated rice was stored and assessed for residual toxicity periodically. The experimental model was the same as described above. This experiment was performed on a regular basis for 4 weeks [47].

3. Results

3.1. GC-MS analysis

Screening of pure clove oil phytochemicals was performed by using GC-MS analysis. The eugenol, phenol, and caryophyllene were identified as major constituents. Analysis of results from GC-MS showed that there were slight chemical variations between the pure clove oil and oil released by nanocapsules. The major components before the nanoformulations were eugenol, phenol, and caryophyllene. Eugenol was maintained throughout the storage period even after formulation but there was a reduction in phenol and caryophyllene content after encapsulation (Table 1).

3.2. Characterization of PEG-CIO NCs

The average size of PEG-CIO NCs was about 200, 220,270, and 305 nm for P1, P2, P3, and P4 respectively. The size of PEG-CIO NPs P4, containing higher concentration is noticeably larger compared to the one with lower concentrations. The prepared nanoformulations had low PDI values ranging from 0.19 to 0.59. The P3 (10ml/100g PEG) showed the best results for all 3 variables that were analyzed *i.e.,* P3 showed the highest %EE of 90±1.25, had low PDI (<0.3), and was relatively smaller in size (Table 2). Therefore, P3 (10ml/100g PEG) nanoemulsion was selected for further experiments to assess the insecticidal potential against *T.*

Castaneum and *S. oryzae* adults.

3.3. Morphology of PEG-CIO NCs

The PEG-CIO NCs were formed in the form of dry solid powder. The morphology of the optimized formulation (P3) was visualized by scanning electron microscope (SEM). The obtained nanocapsules appear slightly irregular and were in good dispersion with a size range of 200nm (Figure 1).

3.4. Insecticidal activities

The results of contact toxicity of PEG-CIO NCs toward *Sitophilus oryzae* and *Tribolium castaneum* adults showed that after 3 days of exposure, PEG-CIO NCs did not show any significant mortality. In contrast clove oil showed 100% mortality even after 24 hours of exposure in both insects but after 7 days of exposure, nanoparticles showed 100% mortality at the highest concentrations of 1000mg/kg and 20000 mg/kg in both *S. oryzae and T. castaneum* respectively (Figure 2 and Figure 3).

The LC_{50} and LC_{90} values obtained after treating *S. oryzae* and *T. castaneum* with PEG-CIO NPs for 3 days were higher as compared to the values obtained after 7 days of exposure (Table 3). The LC_{50} values were 7580.67 and 13200.5 mg/kg and LC_{90} values were 17358.1 and 30969.1 mg/kg for *S. oryzae* and *T. castaneum* respectively after 3 days of exposure. Moreover, the LC_{50} values for clove oil cannot be measured as it showed 100% mortality after 24 hours of exposure so it LC_{50} was much smaller as compared to nanoformulations.

However, no mortality was recorded in the negative control group. The LC50 (7580.67 and 13200.5 mg/kg) and LC_{90} (17358.1 and 30969.1) values for *S. oryzae* and *T. castaneum* respectively after 7 days of exposure were smaller.

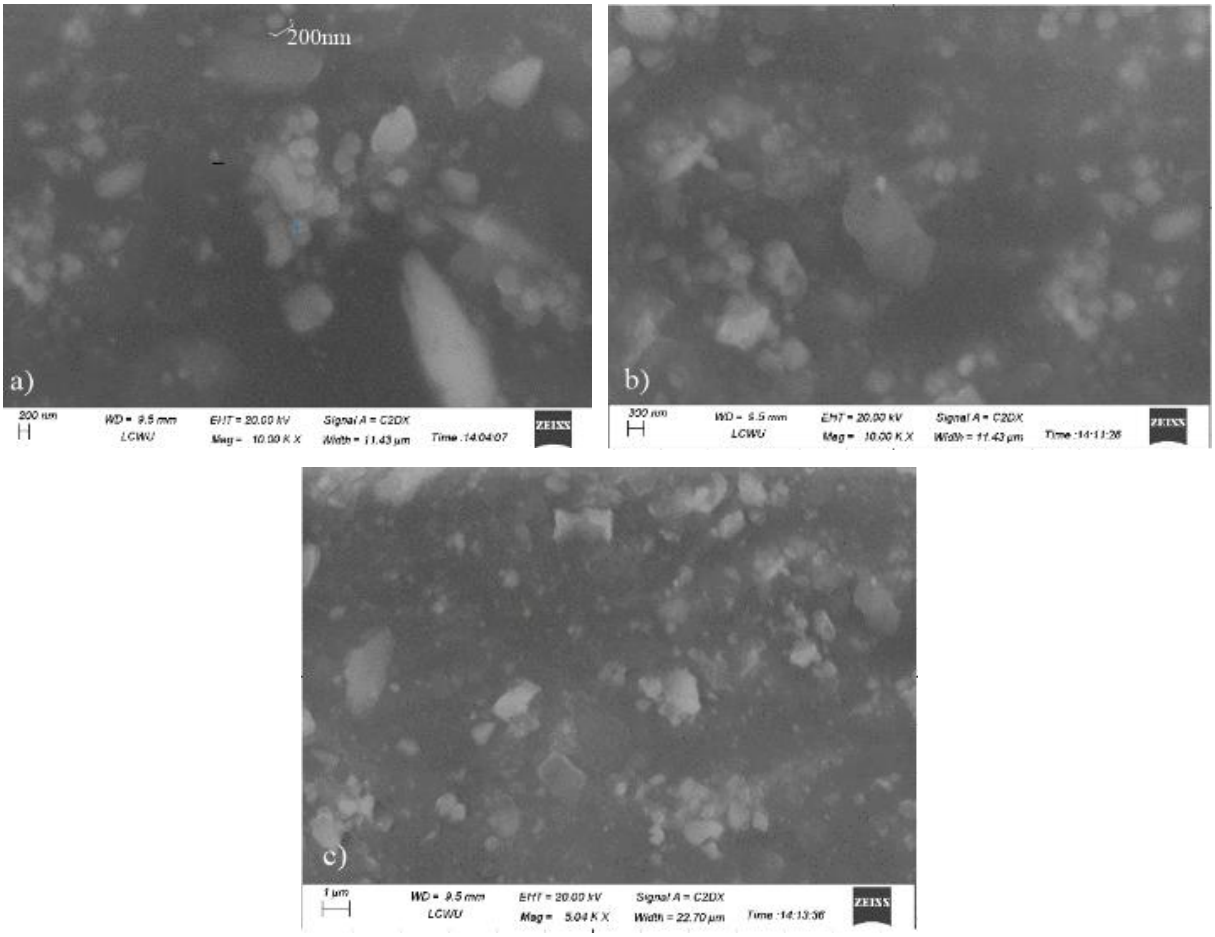
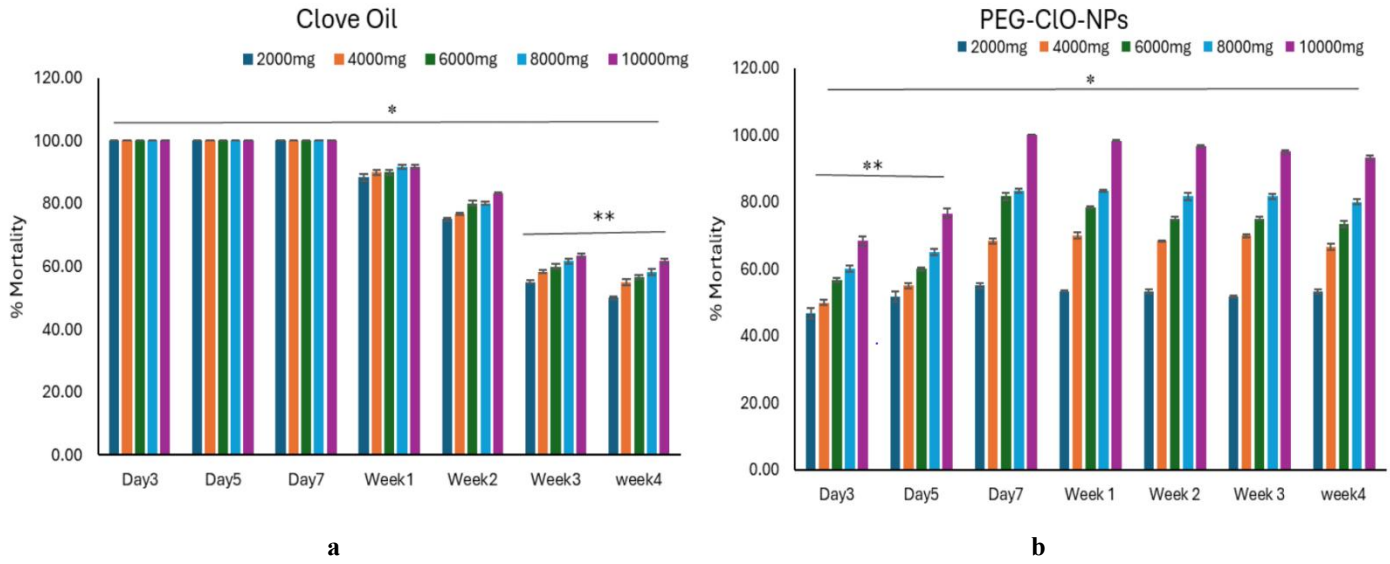


Figure 1. SEM micrograph of PEG-ClO NCs (10ml:100g) of clove oil to PEG a) at 200nm b) at 300nm c) at 1µm.

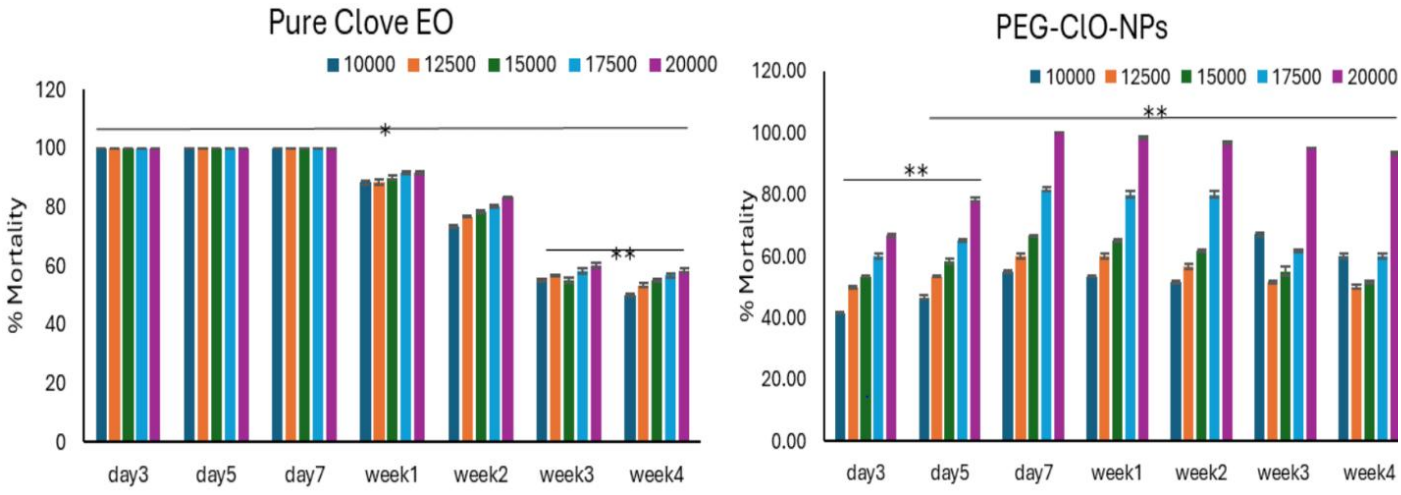
Table 1. Component analysis of major constituents of pure clove oil and post-encapsulated clove oil.

Sr. No.	Components	Pre-encapsulation %	Post encapsulation%
1	Phenol	87	4.59
2	Eugenol	98	85
3	Caryophyllene	99	91



a **b**

Figure 2. The % mortality of *Sitophilus oryzae* after exposure with (a) clove essential oil and (b) polyethylene glycol-based clove oil nanocapsules (PEG-ClO NCs) treated rice that were stored for various durations.



a **b**

Figure 3. The % mortality of *Tribolium castaneum* after exposure with (a) clove essential oil and (b) polyethylene glycol-based clove oil nanocapsules (PEG-ClO NCs) treated rice that were stored for various durations.

Table 2. The average size, polydispersity index (PDI), and percentage encapsulation efficiency of polyethylene glycol-based clove oil nanocapsules.

Groups	P1	P2	P3	P4
Size (nm)	200 ±2.19	220 ±1.64	270.30 ±1.29	305 ±3.06
PDI	0.19 ±0.007	0.22 ±0.004	0.25 ±0.006	0.59 ±0.012
Encapsulation efficiency (%)	78 2.21	84 ±1.95	90.03 ±1.25	86 ±2.78

Table 3. Toxicity of polyethylene glycol-based clove oil nanocapsules (PEG-CIO NCs) against *S. oryzae* and *T. castaneum* after 3 and 7 days of exposure.

Insect	Exposure	Dose (mg/kg)	LC50/LC90	Lower Limit	Upper Limit	ANOVA
S. oryzae	3 Days	2000 4000 6000 8000 1000 10000	7580.67/17358.1	6457.29 9257.30	14001.0 25077.5	X^2= 1.402 $F_{5,12}$= 14.577 P>0.05
T. castaneum		12500 15000 17500 20000	13200.5/30969.1	118662 22618.5	17490.2 1233739	X^2=0.0925 $F_{5,12}$=26.961 P>0.05
S. oryzae	7 Days	2000 4000 6000 8000 1000 10000	2260.89/7569.99s	929.048 6685.34	3125.57 8946.81	X^2= 3.592 $F_{5,12}$14.577 P>0.05
T. castaneum		12500 15000 17500 20000	10498.2/19123.2	4999.40 16724.8	12547.0 26414.1	X^2= 3.608 $F_{5,12}$=103.342 P>0.05

3.5. Residual toxicity

To investigate the residual toxicity of PEG-CIO NCs (P3) and clove oil, mortality of insects was observed at intervals of 3, 5, 7 days and 1-4 weeks. The contact toxicity of PEG-CIO NPs toward *Sitophilus oryzae* and *Tribolium castaneum* adults was increased by increasing the dosage and exposure time. However, the contact toxicity of pure clove oil decreased gradually after storage. After 4 weeks of storage, PEG-CIO NCs still caused 95% and 93% mortality whereas clove oil contact toxicity was reduced significantly to 62.12 and 59.01% towards *S. oryzae* and *T. castaneum.*

4. Discussion

Stored grains and a few other stored commodities are highly prone to insect infestations during their storage period [48]. Insect pest infestations of grains are absurdly controlled by different synthetic insecticides [49]. Essential oils along with their phytochemicals are grabbing much attention nowadays. Therefore, in this research study, clove essential oil was selected to develop insecticides against stored grain pests. Plant EOs have some limitations associated with their use. These limitations can be subdued by encapsulating EOs to enhance their efficacy and stability [34, 50]. Therefore, the present study aimed to synthesize polyethylene glycol (PEG) based insecticidal nanocapsules containing clove essential oil .

The results of GC-MS analysis suggest that Eugenol, phenol, and caryophyllene were three phytochemicals that were present as major constituents. Ibrahim [32] also mentioned the eugenol as major components of clove oil.

However, post-encapsulation results showed a slight difference in the content ratio of these phytochemicals before and after encapsulation. These results were similar to another study conducted by Ikawati et al. [39]. The minor differences observed in GC-MS analysis before and after encapsulation might be due to the diffusion and volatility of core material right through the wall material which was PEG in this study.

Different ratios (oil: PEG) showed variations in the Polydispersity index (PDI) and average size values of nanocapsules. The smallest and largest size noticed in our study was 200 and 305nm along with PDI values of 0.24-0.59 in P1 and P4 respectively. A lower PDI value usually indicates homogenous dispersion and stability of nanoformulations. Ideally, the PDI value should be > 0.30 [51]. The 10% PEG-based nanocapsules of clove oil (P3) showed 270.3 nm of average size and 0.25 PDI, these results are in accordance with Ibrahim [32]. Ikawati et al. [39] also reported an average size of 179 nm along with 0.24 PDI of the clove oil nanocapsules. Gonzalez et al. [52] reported that PEG-based nanocapsules containing bergamot and geranium EOs show an average size of < 255nm and their PDI values are <0.270.

Nanoformulations with higher percentage encapsulation efficiency (%EE) indicate maximum entrapment of active ingredients within the encapsulated system. The highest %EE noticed in this study was 90% for 10% PEG-ClO (P3) indicating that it provides maximum entrapment of clove essential oil. These results were similar to some previous studies showing 89.54% of % EE for clove EO nanocapsules prepared by the melt dispersion method [32]. However, Ikawati et al. [39] reported 77% encapsulation efficiency although the method and oil used for the preparation of nanocapsules were similar for all these studies. The reason behind the variation observed in % Encapsulation efficiency of nanocapsules prepared with a similar technique might be due to the amount of essential oil [53]. Based on these results, the PEG-CIO nanocapsules of 10% ratio were selected to analyze their toxicity against *Tribolium castaneum* and *Sitophilus oryzae*.

The polyethylene glycol-based clove essential oil nanocapsules (PEG-ClO NCs) showed remarkable toxicity against *S. oryzae* and *T. castaneum*. Toxicity increased after increasing the dosage. It was noticed *that S. oryzae* showed 100% mortality at 10000 mg/kg while *T. castaneum* showed 55% mortality at the same concentration on day 7. The LC_{50} values were 7580.67 and 13200.5 mg/kg against *S. oryzae* and *T. castaneum* respectively after 3 days of exposure. The LC_{50} values decreased to 2260.89 and 10498.2 mg/kg respectively after 7 days of exposure. The *S. oryzae* was more sensitive as compared to *T. castaneum* and showed more mortality even at low concentrations. Yang et al. [54] concluded that garlic EO nanocapsules showed 80% mortality against *T. castaneum* while garlic oil showed 11% mortality even after 6 months of exposure Hemalatha and Kumar [55] observed that cardamom EO-NPs caused 85% mortality against *Oryzaephilus surinamensis*.

The results of residual contact toxicity showed that the insecticidal potential of PEG-ClO nanocapsules and pure oil varied depending on the exposure period and dose. The toxicity of nanoformulations reduces gradually after a long storage period because phytochemical release also becomes slow over time. The reduction in insecticidal potential in this result was like that of Ikawati et al. [39] but in contrast, Ibrahim [32] concluded that ClO-PEG NPs were more toxic than pure oil even at their lowest concentration and toxicity of nanoformulations increased over time. Results might be different because the insect tested was *Rhyzopertha dominica* which could be more sensitive toward these formulations just like *S. oryzae* in our study.

In the present study contact toxicity of clove oil was initially more than nanocapsules containing clove oil which might be due to the adsorption route involving direct contact with the cuticles or interrupting the respiratory route of the insect. But toxicity for pure oil

eventually dropped immediately after a few weeks of application while the toxicity of nanocapsules was time dependent showing slow but persistent toxicity. Therefore, it can be used as an effective method to control stored grain pests for a long period of time. The nanocapsules can be easily removed from grains by simply washing them off as PEG makes them soluble in water. Thus, PEG-based clove essential oil holds potent application potential to be used as a protectant for stored grain against insect pests.

5. Conclusion

The insecticidal potential of clove EOs was improved by encapsulation using the melt dispersion method. This approach increased the stability of oils and facilitated a slow and persistent release of active phytochemicals responsible for insecticidal activity. S. *oryzae* was more sensitive towards clove essential nanocapsules as compared to *T. castaneum*. This study suggested that for long-term and effective control of stored grain insect pests, PEG-based clove essential oil nanocapsules could be an effective method.

However, it is suggested to unfold the exact mode of action of these nanocapsules. The long-term stability of these nanocapsules should further be explored.

Authors Contribution

Kunza Abdul Qayyum: Methodology, Project administration and Writing – original draft; Hafiz Muhammad Tahir and Aamir Ali: Conceptualization, Supervision and Writing – original draft; Ayesha Muzamil and Muniba Tariq: Project administration and Writing – review & editing; Bushra Mushtaq: Methodology and Writing – review & editing; Fatima Ijaz: Data curation, Formal Analyses; Asjid Ghaffar: Investigation, Project administration, Visualization ad Validation.

Conflicts of Interest

There are **no** conflicts of interest reported by the writers.

Ethical Statement

All the procedures were performed as per guidelines and approval of the Bioethical Committee, Government College University Lahore, Punjab, Pakistan

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Data Availability statement

The data presented in this study are available on request from the corresponding author.

REFERENCES

1. Suleiman, R. A., & Rosentrater, K. A. Grain storage in developing countries. In Storage of cereal grains and their products, 2022, 113-133. Woodhead Publishing.
2. Wang, Y., Yuan, Z., & Tang, Y. Enhancing food security and environmental sustainability: A critical review of food loss and waste management. Resources, Environment and Sustainability, 2021. 4(1), 100023.
3. Fones, H. N., Bebber, D. P., Chaloner, T. M., Kay, W. T., Steinberg, G., & Gurr, S. J. Threats to global food security from emerging fungal and oomycete crop pathogens. Nature Food, 2020. 1(6), 332-342.
4. Banga, K. S., Kumar, S., Kotwaliwale, N., & Mohapatra, D. Major insects of stored food grains. International Journal of Chemical Studies, 2020. 8(1), 2380-2384.
5. Gowda, G. B., Patil, N. B., Sahu, M., Prabhukarthikeyan, S. R., Raghu, S., Pandi, G. P., Adak, T., Swain, C. K., Pokhare, S., Mohapatra, S. D. & Rath, P. C. Differential gut bacteria in phosphine resistant and susceptible population of *Tribolium castaneum* (Herbst) and their biochemical and molecular characterization. Pakistan Journal of Zoology, 2022. 54(3), 1331-1338.

6. Ileke, K. D., & Olaposi, A. E. Application of Two Ocimum Species Powders in the Control of Red Flour Beetles, *Tribolium castaneum* Herbst (Coleoptera: Tenebronidae), during Wheat Grain Storage. Food Science and Engineering, 2024. 5(1), 112-122.
7. Demis, E. Assessment and Efficacy of Botanicals Against Rice Weevil *(Sitophilus Oryzae L.)* on Rice (Oryza Sativa L.) Under Storage Condition in Fogera District, North-Western Ethiopia (Doctoral dissertation), 2022.
8. Ferrero, A., Fogliatto, S., Barberi, A., & Vidotto, F. Relationship between weedy rice (*Oryza sativa*) infestation level and agronomic practices in Italian rice farms. Weed Science, 2021. 69(5), 565-574.
9. Kavallieratos, N. G., Boukouvala, M. C., Nika, E. P., Eleftheriadou, N., & Avtzis, D. N. Immediate and delayed mortality of four stored-product pests on concrete surfaces treated with chlorantraniliprole. Insects, 2021. 12(12), 1088.
10. Elnabawy, E. S. M., Hassan, S., & Taha, E. K. A. Repellent and toxicant effects of eight essential oils against the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Biology, 2021. 11(1), 3.
11. Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annual Review of Entomology, 2020. 65(1), 333-350.
12. Guru, P. N., Mridula, D., Dukare, A. S., Ghodki, B. M., Paschapur, A. U., Samal, I., Nikhil Raj, M., Padala, V. K., Rajashekhar, M., & Subbanna, A. R. A comprehensive review on advances in storage pest management: Current scenario and future prospects. Frontiers in Sustainable Food Systems, 2022. 6(2022), 993341.
13. Ikawati, S., Himawan, T., Abadi, A. L., & Tarno, H. Toxicity nanoinsecticide based on clove essential oil against Tribolium castaneum (Herbst). Journal of pesticide science, 2021. 46(2), 222-228.
14. Hasan, M. M., Aikins, M. J., Schilling, M. W., & Phillips, T. W. Comparison of methyl bromide and phosphine for fumigation of *Necrobia rufipes* (Coleoptera: Cleridae) and *Tyrophagus putrescentiae* (Sarcoptiformes: Acaridae), pests of high-value stored products. Journal of Economic Entomology, 2020. 113(2), 1008-1014.
15. Shan, C., Li, B., Li, L., Du, X., Ren, Y., McKirdy, S. J., & Liu, T. Comparison of fumigation efficacy of methyl bromide alone and phosphine applied either alone or simultaneously or sequentially against *Bactrocera correcta* in *Selenicereus undatus* (red pitaya) fruit. Pest Management Science, 2023. 79(12), 4942-4951.
16. Arora, S., Stanley, J., & Srivastava, C. Temporal dynamics of phosphine fumigation against insect pests in wheat storage. Crop Protection, 2021. 144(1), 105602.
17. Wakil, W., Kavallieratos, N. G., Usman, M., Gulzar, S., & El-Shafie, H. A. Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. Insects, 2021. 12(4), 288.
18. Sakka, M. K., & Athanassiou, C. G. Evaluation of phosphine resistance in three *Sitophilus* species of different geographical origins using two diagnostic protocols. Agriculture, 2023. 13(5), 1068.
19. Behera, S. K., Shaw, S. S., Rath, P. C., Adak, T., Basana, G. G., Guru, P. P. G., Annamalai, M., Pati, P., Mandol, L., & Patil, N. B. Toxicity and resistance levels of phosphine against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.) populations, CABI Database, 2023. 60 (2), 287-296.
20. Tanda, A. S., Kumar, M., Tamta, A. K., & Deeksha, M. G. Advances in Integrated Management Technology of Insect Pests of Stored Grain. In Advances in Integrated Pest Management Technology, 2022. 157-196. Springer, Cham.

21. Araújo, M. F., Castanheira, E. M., & Sousa, S. F. The buzz on insecticides: a review of uses, molecular structures, targets, adverse effects, and alternatives. Molecules, 2023. 28(8), 3641.
22. Singh, K. D., Mobolade, A. J., Bharali, R., Sahoo, D., & Rajashekar, Y. Main plant volatiles as stored grain pest management approach: A review. Journal of Agriculture and Food Research, 2021. 4(1), 100127.
23. Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., Singh, G., Barnwal, R. P., & Singla, N. Global trends in pesticides: A looming threat and viable alternatives. Ecotoxicology and Environmental Safety, 2020. 201(1), 110812.
24. Turner, M. C., Cogliano, V., Guyton, K., Madia, F., Straif, K., Ward, E. M., & Schubauer-Berigan, M. K. Research recommendations for selected IARC-classified agents: impact and lessons learned. Environmental Health Perspectives, 2023. 131(10), 105001.
25. Rajmohan, K. S., Chandrasekaran, R., & Varjani, S. A review on occurrence of pesticides in environment and current technologies for their remediation and management. Indian Journal of Microbiology, 2020. 60(2), 125-138.
26. Ashaolu, C. A., Okonkwo, C. O., Njuguna, E., & Ndolo, D. Recommendations for effective and sustainable regulation of biopesticides in Nigeria. Sustainability, 2022. 14(5), 2846.
27. Khursheed, A., Rather, M. A., Jain, V., Rasool, S., Nazir, R., Malik, N. A., & Majid, S. A. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. Microbial Pathogenesis, 2022. 173(1), 105854.
28. Assadpour, E., Can Karaça, A., Fasamanesh, M., Mahdavi, S. A., Shariat-Alavi, M., Feng, J., Kharazmi, M. S., Rehman, A., & Jafari, S. M. Application of essential oils as natural biopesticides; recent advances. Critical Reviews in Food Science and Nutrition, 2024. 64(19), 6477-6497.
29. Gadge, A. S., Pushpalatha, M., Narwade, D. K., & Dhananjay, V. Nanoformulated Essential Oils for Enhanced Pest Management. Biotica Research Today, 2022. 4 (11), 764-767.
30. Cimino, C., Maurel, O. M., Musumeci, T., Bonaccorso, A., Drago, F., Souto, E. M. B., Pignatello, R., & Carbone, C. Essential oils: Pharmaceutical applications and encapsulation strategies into lipid-based delivery systems. Pharmaceutics, 2021. 13(3), 327.
31. Isman, M. B. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. Phytochemistry Reviews, 2020. 19, 235-241.
32. Ibrahim, S. S. Polyethylene Glycol Nanocapsules Containing *Syzygium aromaticum* Essential Oil for the Management of Lesser Grain Borer, *Rhyzopertha dominica*. Food Biophysics, 2022. 17(4), 523-534.
33. Jesser, E., Lorenzetti, A. S., Yeguerman, C., Murray, A. P., Domini, C., & Werdin-González, J. O. Ultrasound assisted formation of essential oil nanoemulsions: Emerging alternative for *Culex pipiens* pipiens Say (Diptera: Culicidae) and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) management. Ultrasonics sonochemistry, 2020. 61(1), 104832.
34. Chaudhari, A. K., Singh, V. K., Kedia, A., Das, S., & Dubey, N. K. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: Prospects and retrospects. Environmental Science and Pollution Research, 2021. 28(1), 18918-18940.
35. Subramani, T., & Ganapathyswamy, H. An overview of liposomal nano-encapsulation techniques and its applications in food and nutraceutical. Journal of Food Science and Technology, 2020. 57(10), 3545-3555.
36. Ngwuluka, N. C., Abu-Thabit, N. Y., Uwaezuoke, O. J., Erebor, J. O., Ilomuanya, M. O., Mohamed, R. R., Soliman, S. M., Elella, M. H., & Ebrahim, N. A.

Natural polymers in micro-and nanoencapsulation for therapeutic and diagnostic applications: part I: lipids and fabrication techniques. Nano Microencapsulation Technology and Applications, 2021. 3-54.

37. Sivadasan, D., Sultan, M. H., Madkhali, O., Almoshari, Y., & Thangavel, N. Polymeric lipid hybrid nanoparticles (plns) as emerging drug delivery platform—A comprehensive review of their properties, preparation methods, and therapeutic applications. Pharmaceutics, 2021. 13(8), 1291.
38. Sun, C., Zeng, Z., Cui, H., & Verheggen, F. Polymer-based nanoinsecticides: current developments, environmental risks and future challenges-a review. Biotechnologie, Agronomie, Société et Environnement, 2020. 24(2), 59-69.
39. Ikawati, S., Himawan, T., Abadi, A. L., & Tarno, H. Thermostability, photostability, and toxicity of clove oil nanoparticles against *Cryptolestes ferrugineus* (Stephens)(Coleoptera:Laemophloeidae). Biodiversitas Journal of Biological Diversity, 2020. 21(10), 1-8.
40. Draz, K. A., Tabikha, R. M., Eldosouky, M. I., Darwish, A. A., & Abdelnasser, M. Biotoxicity of essential oils and their nano-emulsions against the coleopteran stored product insect pests *Sitophilus oryzae* L. and *Tribolium castaneum* herbst. International Journal of Pest Management, 2024. 70(4), 729-743.
41. Abdel-Hady, A. A., Ramadan, M. M., Lü, J., & Hashem, A. S. High-temperature shock consequences on the red flour beetle (*Tribolium castaneum*) and the rice weevil (*Sitophilus oryzae*). Journal of Thermal Biology, 2021. 100(1), 103062.
42. Lima, L. A., Ferreira-Sá, P. S., Garcia Jr, M. D., Pereira, V. L. P., Carvalho, J. C. T., Rocha, L., Fernandes, C. P., Souto, R. N. P., Araújo, R. S., Botas, G., & Cruz, R. A. (2021). Nano-emulsions of the essential oil of *Baccharis reticularia* and its constituents as eco-friendly repellents against *Tribolium castaneum*. Industrial crops and products, 2021. 162(1), 113282.
43. Lino, R. C., Carvalho, S. M. D., Noronha, C. M., Sganzerla, W. G., Rosa, C. G. D., Nunes, M. R., & Barreto, P. L. M. Development and characterization of poly-ε-caprolactone nanocapsules containing β-carotene using the nanoprecipitation method and optimized by response surface methodology. Brazilian Archives of Biology and Technology, 2020. 63(1), e20190184.
44. Abadi, A. V. M., Karimi, E., Oskoueian, E., Mohammad, G. R. K. S., & Shafaei, N. Chemical investigation and screening of anti-cancer potential of *Syzygium aromaticum* L. bud (clove) essential oil nanoemulsion. 3 Biotech, 2022. 12(2), 49.
45. Yang, K., Liu, A., Hu, A., Li, J., Zen, Z., Liu, Y., Tang, S., & Li, C. Preparation and characterization of cinnamon essential oil nanocapsules and comparison of volatile components and antibacterial ability of cinnamon essential oil before and after encapsulation. Food Control, 2021. 123(1), 107783.
46. Mostafa, A. A. F., Yassin, M. T., Al–Askar, A. A., & Al-Otibi, F. O. Phytochemical analysis, antiproliferative and antifungal activities of different *Syzygium aromaticum* solvent extracts. Journal of King Saud University-Science, 2023. 35(1), 102362.
47. Brabec, D., Lanka, S., Campbell, J. F., Arthur, F. H., Scheff, D. S., & Yan-Zhu, K. Aerosolized Insecticide Spray Distributions and Relationships to Efficacy against Stored Product Pests. Insects, 2023. 14(12), 914.
48. Demis, E., & Yenewa, W. Review on major storage insect pests of cereals and pulses. Asian Journal of Advances in Research, 2022. 5(1), 41-56.
49. Hung, N. H., Quan, P. M., Dai, D. N., Satyal, P., Huong, L. T., Giang, L. D., Hung, L. T., & Setzer, W. N. Environmentally-friendly pesticidal activities of Callicarpa and Karomia essential oils from Vietnam

and their microemulsions. Chemistry & Biodiversity, 2023. 20(3), e202200210.

50. Hazafa, A., Murad, M., Masood, M. U., Bilal, S., Khan, M. N., Farooq, Q., Iqbal, M. O., Shakir, M., & Naeem, M. Nano-biopesticides as an emerging technology for pest management. In Insecticides-Impact and Benefits of Its Use for Humanity. 2021. 101285 IntechOpen.
51. Che Marzuki, N. H., Wahab, R. A., & Abdul Hamid, M. An overview of nanoemulsion: concepts of development and cosmeceutical applications. Biotechnology & Biotechnological Equipment, 2019. 33(1), 779-797.
52. González, J. O. W., Gutiérrez, M. M., Ferrero, A. A., & Band, B. F. Essential oils nanoformulations for stored-product pest control–Characterization and biological properties. Chemosphere, 2014. 100, 130-138.
53. Solomon, B., Gebre-Mariam, T., & Asres, K. Mosquito repellent actions of the essential oils of *Cymbopogon citratus, Cymbopogon nardus* and *Eucalyptus citriodora*: evaluation and formulation studies. Journal of Essential Oil Bearing Plants, 2012. 15(5), 766-773.
54. Yang, F. L., Li, X. G., Zhu, F., & Lei, C. L. Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae). Journal of Agricultural and Food Chemistry, 2009. 57(21), 10156-10162.
55. Hemalatha, N., & Kumar, K. R. Synthesis and characterization of PEG-cinnamon essential oil nanoparticles and their application as an insecticidal agent. Journal of Advanced Scientific Research, 2021. 12(03 Suppl 1), 142-148.

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