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Carrier selection and dose optimization of Himalayan cypress (*Cupressus torulosa*) needle essential oil for stored grain pest control

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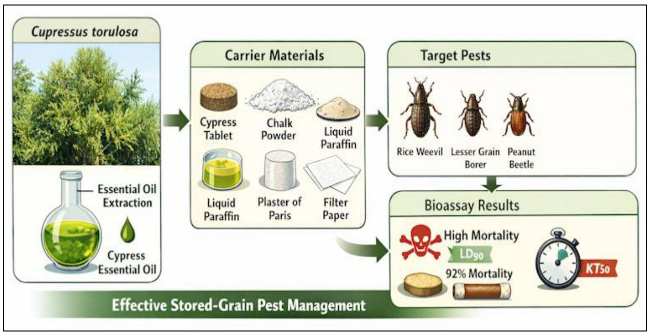
Abstract
Storage pests are a major nuisance and pose a serious threat to a country's food security, causing substantial post-harvest losses every year. In this context, essential oil-based pest management approaches are considered effective and environmentally safe alternatives to synthetic pesticides. Therefore, the present study was conducted to evaluate the efficacy of *Cupressus torulosa* (Cupressaceae) essential oil formulations obtained from two different locations i.e. Ogla (CTO) and Munsyari (CTM) in Uttarakhand, India against three noxious stored-grain pests, namely *Sitophilus oryzae* (rice), *Rhyzopertha dominica* (dal), and *Carpophilus dimidiatus* (peanut).Six different doses of essential oil (EO), ranging from 5 to 30 µL/58 cm², were administered with and without carrier materials to determine their efficacy and identify the most suitable carrier at a constant concentration of 1000 ppm. The knockdown time (KT_{50}) values and mortality rates for each pest at each dose were recorded. The CTM formulation exhibited a highly effective dose (LD_{90}) at 0.515 µL/cm², achieving 92% mortality within 48 hours, whereas the CTO formulation reached LD_{90} at 0.7 µL/cm² within 74 hours. Furthermore, when effective doses of both CTM and CTO essential oils were tested with various carrier materials including *C. torulosa* tablets, chalk powder, liquid paraffin, plaster of Paris (PoP) cylinders, filter paper, and cigar filters, the cigar filter emerged as the most effective carrier for EO delivery.

Keywords: *Sitophilus oryzae, Rhyzopertha Dominica, Carpophilus dimidiatus*, essential oil, stored pest management

Introduction
From agricultural fields to post-harvest storage systems, insect pests cause devastating damage across the world. These pests belong predominantly to two diverse insect orders *viz*. Lepidoptera and Coleoptera which include weevils, borers, and moths. Globally, the number of stored-grain insect pest species is estimated to be as high as 1,663 (Hagstrum *et al*., 2013) [11]. In India alone, these pests cause an annual loss of 25-30% of stored food grains (Singh *et al*., 2021; Kumar & Kalita, 2017) [14, 22]. Worldwide, damage may reach 40% in cereals and up to 73% in legumes (Shaaya *et al*., 1997; Mesele *et al*., 2019; Endshaw *et al*., 2020) [8, 18, 21]. Beyond quantitative losses, these pests also inflict qualitative damage, including depletion of essential nutrients in cereals, legumes, spices, and other commodities. Contamination through insect excreta further raises serious public-health concerns. The economic implications of such losses are substantial and pose a threat to national food security. Numerous research studies published each year explore different strategies for managing these destructive pests in households and warehouses. Control measures reported in the literature include chemical pesticides, chemosterilants, botanicals, insect growth regulators (IGRs), oviposition deterrents, antifeedants, hormones, long-lasting insecticide-impregnated nets (LLINs), and essential oils (Chidege *et al*.,2024; Bordoloi *et al*,2024; Ahmad *et al*., 2022) [2, 5, 6]. The National Food Security Mission (NFSM) guidelines also emphasize the importance of Integrated Pest Management (IPM) for sustainable stored-grain protection. Essential oils are widely recognized for their bioactive properties; however, their efficacy often depends on the selection of an appropriate carrier material, as the volatile constituents need stabilization for optimal performance. In present investigation *C. torulosa* commonly known as Himalayan cypress belongs to conifer family Cupressaceae, distributed in western and central Himalayan region of India have been selected as test material as this family are mainly known for their oil-bearing nature, medicinal and insecticidal properties (Almadiy *et al*.,2023; Liu *et al*.,2022) [3, 16]. Therefore, the present study aims at standardizing the effective dose of *C. torulosa* essential oil and to identify the most effective carrier material for delivering an environmentally safe essential oil for the management of major stored-grain insect pests.



Materials and Methods
For the present study three robust storage insect pests were selected as model insects of bioassay experiments each one from cereals, oilseed legumes, and pulses. Namely, *Sitophilus oryzae* from Rice, *Rhyzopertha Dominica* from Lentils (dal) and *Carpophilus dimidiatus* from Peanuts. The chosen pests were collected and mass multiplied by rearing with food grains in laboratory and finally used for multiple bioassay experiments. Storage pest bioassay was carried out

in multiple trials for a year from January to December 2024 in phytochemistry laboratory of Chemistry and Bioprospecting Division at ICFRE-Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, TamilNadu, India.

Essential oil of *C. torulosa* needle from two population's *viz*. CTO and CTM (located at Ogla and Munsyari respectively in Uttarakhand, India) were used for the present study. The purified essential oil was considered as stock.These two stocks with 1000 ppm concentrations were tested against targeted pests along with different carriers such as tablet, chalk powder, Plaster of paris cylinder (1x1 cm), liquid Paraffin, Whatman's filter paper and cigar filter.The tablet was formulated using coarse powder of the shade dried needles of the *C. torulosa* along with excipients such as cellulose and magnesium salts.

Petri plates (58 cm^2) and small plastic containers of same size were used as test chamber based on requirement. Insects were released with food grains inside the chamber. To create a controlled environment and ensure that only test insects are present inside the chamber thePetriplates and containers were covered properly with another Petriplates and gada cloths respectively. 10 insects were introduced in each replication and in control. Three replications of each experiment were taken where dose ranged between 5 - 30 µL (i.e.5 µl 10 µl, 15 µl, 20 µl, 25µl and 30 µl) for both CTO and CTM stocks were tested against all three pests. The experiment was conducted at room temperature (25 ± 2°C). Equal quantity of carrier materials was used for each replication during the experiments.

Each test plate was monitored at a regular interval up tothe mortality of last individual after the treatment and knockdown time of 50%population (KT_{50}) was documented. Further, the mortality data was analysed by statistical method to find LD_{90}value.Relative Efficacy (Percent Reduction in KT_{50}) were also calculated for both treatments CTO and CTM. Using the following formula.

$$\text{Relative Efficacy (\%)} = \frac{KT_{50}\text{ of control/ less effective pesticide} - KT_{50}\text{ of more effective pesticide}}{KT_{50}\text{ of control/less effective pesticide}} \times 100$$

Table 1: Experiment details

Test Insects	Treatment formulations	Replications	Insects/ replication	Dose(µL)/58cm^2	Carrier materials evaluated
1. *Sitophilus oryzae* 2. *Rhyzopertha dominica* 3. *Carpophilus dimidiatus*	CTO & CTM	3 (R1, R2, R3)	10 nos. of healthy adults	5,10,15,20,25,30	Tablet, Chalk powder, Liq. Paraffin, Plaster of Paris, Cigar filter, Filter paper

Sitophilus oryzae *Rhyzopertha Dominica* *Carpophilus dimidiatus*

Fig 1: Test Insects of the storage grain pest experiment

Fig 2: a). Laboratory multiplication of test insects for bioassay trials; Storage pest bioassay with different carriers b). Tablet, c). Plaster of paris, d). Chalk powder, e). Cigar filter

Observations and Results

The knockdown time required to affect 50% of the exposed insect population (KT_{50}) was assessed in multiple experimental treatments, and only statistically significant outcomes were included in the analysis. The evaluation of various carrier materials namely paraffin, chalk powder, tablet formulations, plaster of Paris (PoP) cylinders and filter papers revealed that none were able to induce KT_{50} within the initial 96hours exposure period. Even at the highest tested concentrations, these carriers required an extended duration of approximately 15-20 days to achieve KT_{50}, indicating limited efficacy for rapid insecticidal action and untreated control showed more than two months of survivals of pests.

In contrast, formulations utilizing purified crude oil and cigar filter as carriers demonstrated significantly reduced KT_{50} values along with enhanced mortality rates, as presented in Figures 3 and 4. These results suggest improved bioavailability and release efficiency of the active compounds when delivered through this carrier system. Moreover, direct application of crude oils derived from CTM and CTO via spray administration resulted in rapid and complete insecticidal activity, achieving KT_{50} within 24-48 hours of exposure.

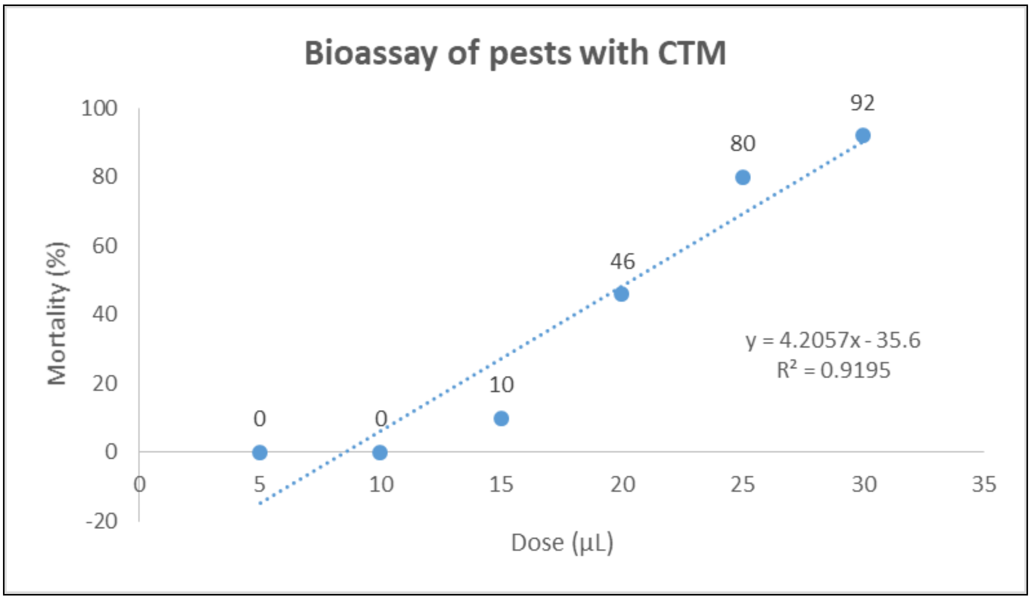


Fig 3: The LD50 bioassay with CTM formulation against storage pests using cigar filter as carrier

The bioassay of CTM with cigar filter against the test insects revealed (figure 3) a clear dose-dependent increase in mortality. Probit analysis of the dose-mortality data showed a strong linear relationship between dose and percent mortality, described by the regression equation $y = 4.2057x - 35.6$, with a high coefficient of determination ($R^2 = 0.9195$). The median lethal dose (LD_{50}) was estimated to be approximately 20.3 µL, while the lethal dose causing 90% mortality (LD_{90}) was approximately 29.9 $\mu L/58cm^2$ (i.e. $0.515\mu L/cm^2$)**.** These results indicate that CTM exhibits high toxicity against the test insects, achieving substantial mortality at relatively moderate doses.

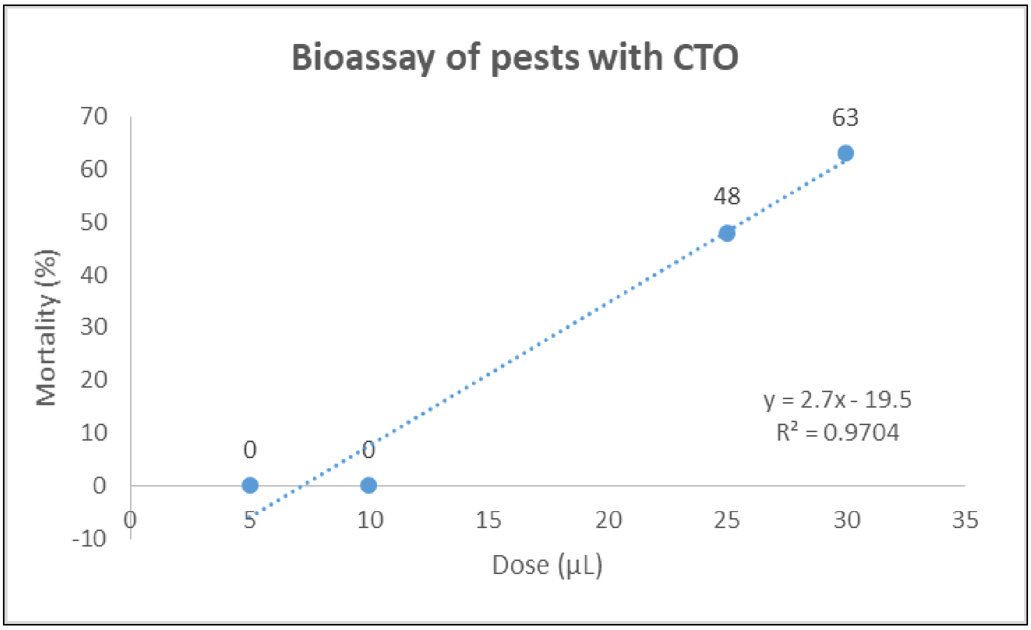


Fig 4: The LD50 bioassay with CTO formulation against storage pests using cigar filter as carrier

In other hand, the bioassay of CTO with cigar filter against the test insects also showed (figure 4) increase in mortality with increasing dose. Probit analysis revealed a strong dose-mortality relationship, described by the regression equation $y = 2.7x - 19.5$, with a high coefficient of determination ($R^2 = 0.9704$). The median lethal dose (LD_{50}) of CTO was estimated to be approximately 25.7 µL, while the dose required to cause 90% mortality (LD_{90}) was approximately 40.6 µL/ 58 cm^2 (i.e. 0.7µL/cm^2). These findings indicate that CTO is toxic to the test insects, although higher doses are required to achieve comparable mortality levels when compared with CTM.

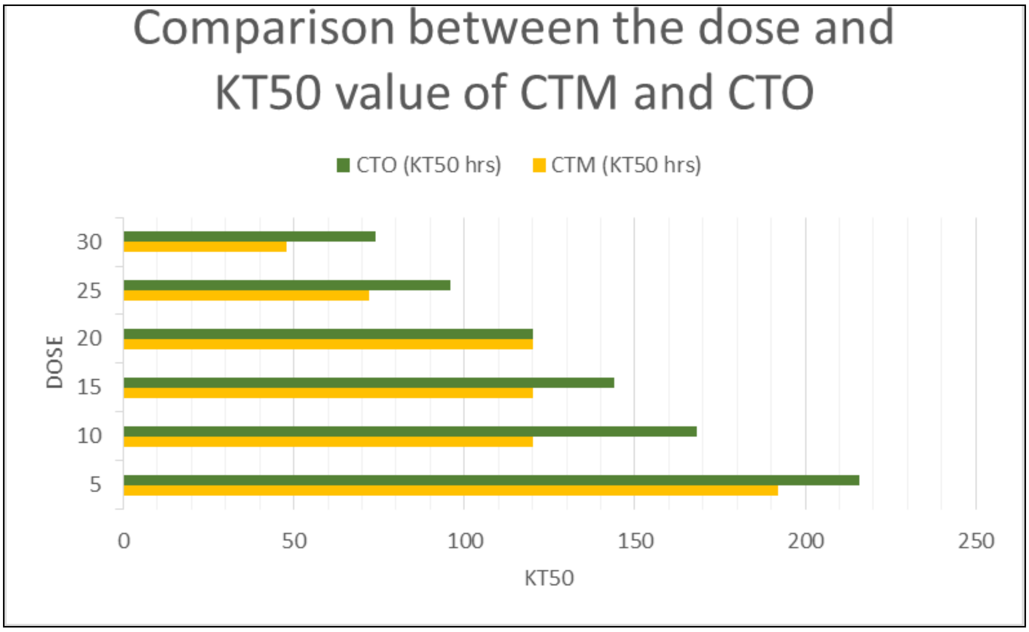


Fig 5: Comparison between the dose and KT_{50} of CTO and CTM

Table 2: Rapid reduction (%) of target pests of CTM over CTO.

Dose (µL)	Relative Efficacy (%)
5	11.11
10	28.57
15	16.67
20	0.00
25	25.00
30	35.14

When both treatments were compared (figure 5) in terms of speed of action, CTM consistently produced faster knockdown effects than CTO across all tested doses (table-1). The KT_{50} values of CTM decreased sharply with increasing dose, reaching 48 h at 30 µL, whereas CTO recorded a comparatively higher KT_{50} of 72 h at the same dose. At lower doses (5-15 µL), CTM also demonstrated shorter KT_{50} values (120-192 h) than CTO (144-216 h), indicating quicker toxic action even at sublethal concentrations.

Overall, the combined evidence from dose-mortality and knockdown-time analyses demonstrate that CTM is more efficacious than CTO, requiring lower doses to achieve equivalent mortality and producing faster knockdown of the target pests. These results suggest that CTM may be a more effective candidate for pest management applications. During the investigation Rice weevil *Sitophilus oryzae* came out as the sturdiest pest among three. Dal pest *Rhyzopertha Dominica* and peanut beetle *Carpophilus dimidiatus* controlled in almost similar time frame.

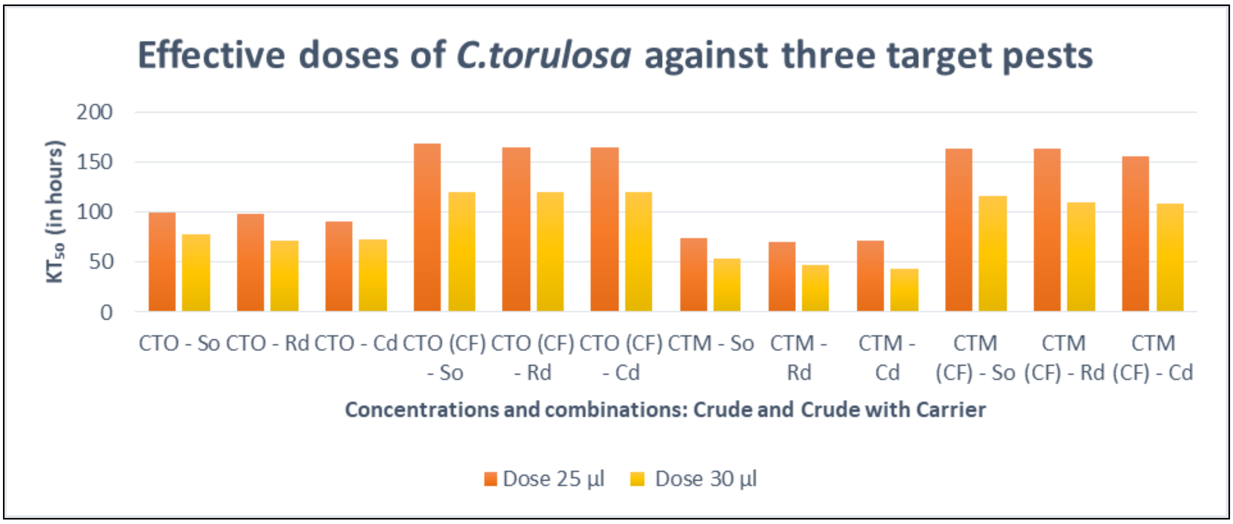


Fig 6: Comparison of effective doses of *C. torulosa* crude oil (CTO&CTM) and EO with carrier Cigar filter against three target pests.

Effective doses of *C. torulosa* crude oil (CTO and CTM), with and without cigar filter (CF) as a carrier, against three target storage pests*S. oryzae* (So), *R. dominica* (Rd), and *C. dimidiatus* (Cd) have been evaluated during the study revealed (Figure 6) higher dose (30 µl) consistently resulting in lower KT_{50} values compared to 25 µl across all test insects, indicating faster knockdown at increased concentration. Among crude oil formulations without carrier, CTO exhibited higher KT_{50} values than CTM for all three pests, suggesting comparatively lower toxicity of CTO. In contrast, CTM demonstrated greater efficacy, particularly against *C. dimidiatus*, where the lowest KT_{50} values were recorded at both doses. Across pest species, *C. dimidiatus* showed the highest susceptibility, followed by *R. Dominica*, while *S. oryzae* was relatively more tolerant.

The KT_{50} values of *C. torulosa* formulations delivered through a cigar filter carrier (CF) varied distinctly among the three target pests (*Sitophilus oryzae*, *Rhyzopertha Dominica*, and *Carpophilus dimidiatus*), indicating differential susceptibility to carrier-mediated exposure.

For both CTO (CF) and CTM (CF) treatments, *S. oryzae* consistently exhibited the highest KT_{50} values at both 25 µl and 30 µl doses, reflecting comparatively lower susceptibility under CF-based delivery. In contrast, *C. dimidiatus* showed the lowest KT_{50} values, suggesting greater sensitivity to the slow-release formulation, while *R. Dominica* demonstrated an intermediate response.

At 25 µl dose, CTO (CF) resulted in KT_{50} values of approximately 168 h for *S. oryzae*, 165 h for *R. Dominica*, and 165 h for *C. dimidiatus*. Increasing the dose to 30 µl reduced KT_{50} uniformly to about 120 h across all three pests, indicating that dose elevation partially overcomes species-specific tolerance under carrier-based delivery.

A similar trend was observed for CTM (CF), where *S. oryzae* again showed the highest KT_{50} (≈164 h at 25 µl and 116 h at 30 µl), followed by *R. Dominica* (≈164 h and 110 h), and *C. dimidiatus* (≈156 h and 108 h). Notably, CTM (CF) produced slightly lower KT_{50} values than CTO (CF), particularly at the higher dose, indicating marginally enhanced efficacy of CTM when formulated with a cigar filter carrier.

Discussion

Botanicals have long been prioritized by researchers for pest management because of their environmental safety and biodegradability. Consequently, numerous investigations have been conducted to control insect pests and weevils using plant-derived products. Stored-grain weevils, which cause severe post-harvest losses, have been effectively managed in earlier studies using essential oils extracted from spices, citrus, and other aromatic plants (Bakkali *et al*., 2008; Owolabi *et al*., 2009; Devi and Devi, 2013; Sagheer *et al*., 2013; Gonzales *et al*., 2015; Ghosh *et al*., 2023; Hamza *et al*., 2024; Kavallieratos *et al*., 2024) [1, 4, 7, 10, 12, 13, 20].

Essential oils derived from *Hesperocyparis arizonica*, *Hesperotropsis leylandii*, *Juniperus* and *Pfitzeriana* belonging to the family Cupressaceae, when applied at a concentration of 1000 ppm, were previously reported to cause 87-100% mortality of major stored-product pests such as *Trogoderma granarium*, *Oryzaephilus surinamensis*, *Alphitobius diaperinus*, and *Tenebrio molitor* at both adult and larval stages (Kavallieratos *et al*., 2024) [13].

Similarly, Almadiy *et al*. (2023) [3] demonstrated that nano-emulsified essential oil of *Cupressus sempervirens*, another species of *Cupressus* achieved complete (100%) control of *S. oryzae* at a dose of 40 µL/cm². Hamza *et al*. (2024) [12] reported 100% larval repellency of the noxious storage pest *T. granarium* using gamma-irradiated oil of *Thuja orientalis*, another species belongs to Cupressaceae family, in a fumigant assay at a dose of 0.126 µL/cm². In the present study, crude essential oil of *Cupressus torulosa*, another member of the same family, applied at 1000ppm concentration, resulted in 50% knockdown of *S. oryzae*, *R. Dominica*, and *C. dimidiatus* within 48 - 72 hours in an average with the concentration0.517µL/cm^2.*S. Oryzae* had been found the most robust among all three selected target pests in present study quite similar with the study of Kumar *et al*. (2020) [15] where in same dose *R. Dominica* showed higher mortality compared to *S. oryzae*. Phytochemical screening of *C. torulosa* essential oil (Lohani *et al*.,2012) [17] revealed the presence of several terpenoid compounds in sufficient quantities, including α-pinene, β-pinene, limonene, p-cymene, γ-terpinene, 4-terpineol, and α-terpineol. These compounds are known to act as neurotoxic agents, repellents, and fumigants by disrupting insect nervous systems, thereby explaining the high efficacy observed in both contact and fumigant bioassays against stored-grain insect pests.

Conclusion

From the present investigation, it is concluded that *C. torulosa* formulations in both CTM and CTO forms exhibited promising results against the storage pests tested. However, the former formulation was found to be slightly more toxic and efficient and had a quick mode of action. From the carrier materials tested, cigar filter material was found to be most appropriate for safe and efficient delivery of the active principles. Thus, *C. torulosa*-based lures in CTM form also hold a good prospect for further development and testing for practical use against several stored-grain insect pests.

Conflict of Interest

The authors declare no conflict of interest.

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