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Insect repellent nettings: An application of plant-based Insecticides: Preventing the dispersal of the red flour beetle

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Abstract
Plant-based insecticides serve as an alternative safer approach to chemical insecticides. The applicability of nano-emulsion in post-harvest management of stored food commodities rises rapidly since its novel formulation improves the time of efficacy of plant-based insecticides. This study used thyme essential oil and neem oil as core materials to form nano-emulsions with whey protein isolate. The efficacy of formulated nano-emulsion was determined against *Tribolium castaneum.* During the filter paper assay, it was found that the repellent activity ranged from 73 to 90%. The mortality of *T. castaneum* adults was also observed with the nano-emulsion. Hence, a toxicity screening of individual oils was carried out, wherein, thyme essential oil was responsible for the cent percent mortality in 24 hours. Insect repellent Nettings (IRNs) were also fabricated by treating the nets with the developed nano-emulsion. The results showed ineffectiveness in repelling *T. castaneum* as the repellent activity was below 70% and the repellent action of IRNs reduced drastically after 24 hours of treatment. It was found that the size of *T. castaneum* adults was small enough to easily pass through the holes of IRNs. However, IRNs act as a primary barrier as their active components induced the mortality of the model insect studied.

Keywords: Botanical insecticides, nano-emulsion, repellent activity, mortality, nettings

Introduction
The vital area for the management of insects in a storage facility is prevention (Wilkins *et al*., 2021) [21]. Prevention is better than cure (disinfestation). This also applies to the stored grains as disinfestation costs, resistance development, chemical residue, and manpower are a major concern. Hence, prevention is a must strategy to be overlooked. There had been the use of prevention techniques like filling cracks and sealing other entries to the food storage house, with screenings (Stejskal, Vendl, Aulicky, & Athanassiou, 2021) [19]. However, the screens often get clogged with dust, and the passage of air is limited. The maintenance of airflow inside the storage house is a complication in such a case.
Insects fly around the storage houses. The flight activity of these stored product insects may lead to cross-infestation of the fresh produce from the infested facility (Jian, 2019) [6]. This may be contributed by air movement in and around the facility. The transportation of infested grains to a storage facility may complicate things further (Gerken & Campbell, 2022) [3]. The presence of even a small number of insects in any stage will multiply manifold. There have been complaints of insects invading the space of surrounding localities. Prevention of cross-infestation is the need of the generation with reduced usage of chemical insecticides for the management of stored product insects.
Botanical insecticides were employed successfully for field crops and are widely available in the market. Botanical insecticides have the property to repel insect pests (Mangang, Tiwari, Rajamani, & Manickam, 2020; Manickam, Mangang, & Rajamani, 2021) [11, 12]. These have not been used in stored product insect management due to limited controlled release technologies. Recently many studies were performed to achieve controlled release of the active components using nano-emulsion formulations (Kavallieratos, Nika, Skourti, Boukouvala, *et al*., 2022; Kavallieratos, Nika, Skourti, Xefteri, *et al*., 2022; Sharma, Loach, Gupta, & Mohan, 2020) [7-8, 18]. Botanical insecticides act as the potential alternative to the use of chemical insecticides. However, in addition to screening of the botanical, researchers need to focus on the development and fabrication of applicable tools using known botanical insecticides (Isman, 2020; Mangang & Manickam, 2022) [10].

One such tool to prevent infestation of grains is the use of long-lasting insecticide incorporated nettings. These fine meshes were used to prevent malaria outbreaks from mosquito bites (Okumu *et al*., 2012) [15]. The recent studies focused on the use of insecticide nettings to protect stored product insects (Agrafioti, Arvanitakis, Rumbos, & Athanassiou, 2021; Gerken & Campbell, 2022; Scheff *et al*., 2021) [1, 3, 17]. The nets were incorporated with chemical insecticides, however, over the years, people tend to shift towards a healthier lifestyle avoiding chemicals. The use of chemical insecticides was limited and botanical insecticides seem to be a promising alternative method. In the present study, the repellent effect of nettings treated with a nano-emulsion of oils (botanical insecticides) was determined against *T. castaneum.*

Materials and methods

Culturing of red flour beetle

The populations of *T. castaneum* were acquired from the stock culture of the storage entomology laboratory, NIFTEM-T, Tamil Nadu, and reared to get uniform aged insects continuously throughout the experiments. The plastic jar was sterilized properly using ethanol to prevent microbial contamination. The mixed-age of *T. castaneum* was released at a rate of 50 insects per plastic jar which is half-filled with wheat grain powder. The mouth of the plastic jar was covered with muslin cloth and it was fastened with a rubber band to prevent escape. This insect was reared at a temperature of 32-37 °C and relative humidity of 70-80%. The adults of *T. castaneum* were sieved out the following day and the flour was kept idle separately to check the emergence of F1 progeny. The emerged adults were used for the studies.

Preparation of Nano emulsion formulation

The Neem kernel and thyme leaves were procured from the local farmers of Thanjavur. Neem kernel oil was obtained by solvent extraction using hexane. Thyme essential oil was obtained by hydro-distillation using Clevenger apparatus. Tween 20, an emulsifier was used to mix the above solutions. Based on preliminary data, the two extracts were mixed at a 1:1 ratio (1.5ml each), with 2.5ml of Tween 20. Whey protein isolate (4g in 100ml distilled water) was used to encapsulate the emulsion (Fig.1) and provide a controlled release of the active components present in the mixture.

Fig 1: Nano emulsion of botanicals

Filter paper assay

The Whatman filter paper no.1 (9cm diameter) was cut into halves. Based on method followed by Mangang *et al*. (2020) [11], the micro pipette was used to treat 0.6ml of nano emulsion to one half of the filter paper. The other half of the filter paper was treated with a mixture of tween 20 and protein solution. The two halves were reattached and kept inside the petri-plate. The adults of *T. castaneum* (20 in number) was released inside the petri-plate in the middle of the two halves of filter paper. The number of insects that move away from the treated side to the untreated side were considered as repelled and calculated using equation 1. The observations were taken for 30 days to observe the effectiveness of the nano-emulsion. The adult insects were released on the 1st day and on the 16th day of the 30-day long study to determine the effectiveness of nano emulsion as a repellent. The experiments were conducted in triplicates (Fig.2).

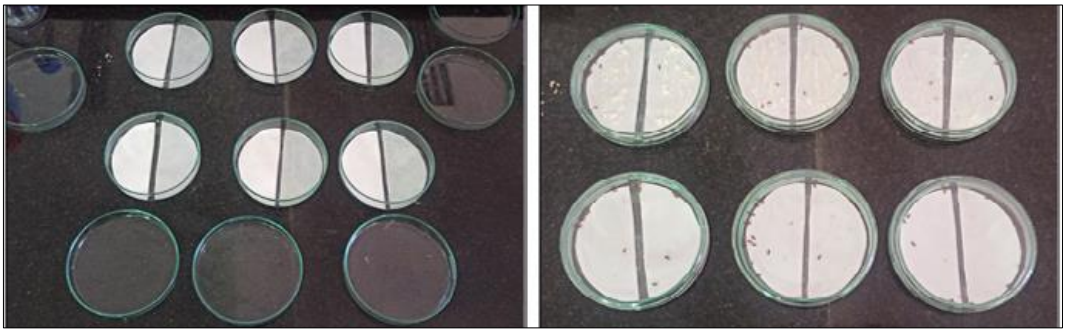

Fig 2: Filter paper assay

Screening of individual botanicals for its toxicity

The neem kernel oil and thyme essential oil were screened for its toxicity. Whatman filter papers (9cm diameter) was treated with each extract (100 µL) and dried for 5 minutes. The *T. castaneum* adults (120 numbers) used in the study was kept devoid of food for a day. The treated filter paper was kept inside the closed petri plate (0.79 µL.cm^{-3}) along with the adults of *T. castaneum* (20 numbers) and grains (2g). Each study was triplicated.

Insect Repellent Nettings (IRNs)

The nettings (made from cloth) were purchased from the local market of Thanjavur, Tamil Nadu, India. The net was treated with emulsified neem oil using tween 20 and protein solution

to form nano encapsulated powder when dried in the nets. The solution (100ml) was poured in a non-sticky tray. The nets were treated by dipping in the emulsified solution, followed by drying before application (Fig.3). Based on preliminary studies, it was found that 4.6 ml of emulsified solution was absorbed in the nettings ($35\times13cm^2$) which was tabulated by measuring the amount of emulsified solution remained in the tray after a dip.

Fig 3: Treatment of nettings with the emulsified botanicals

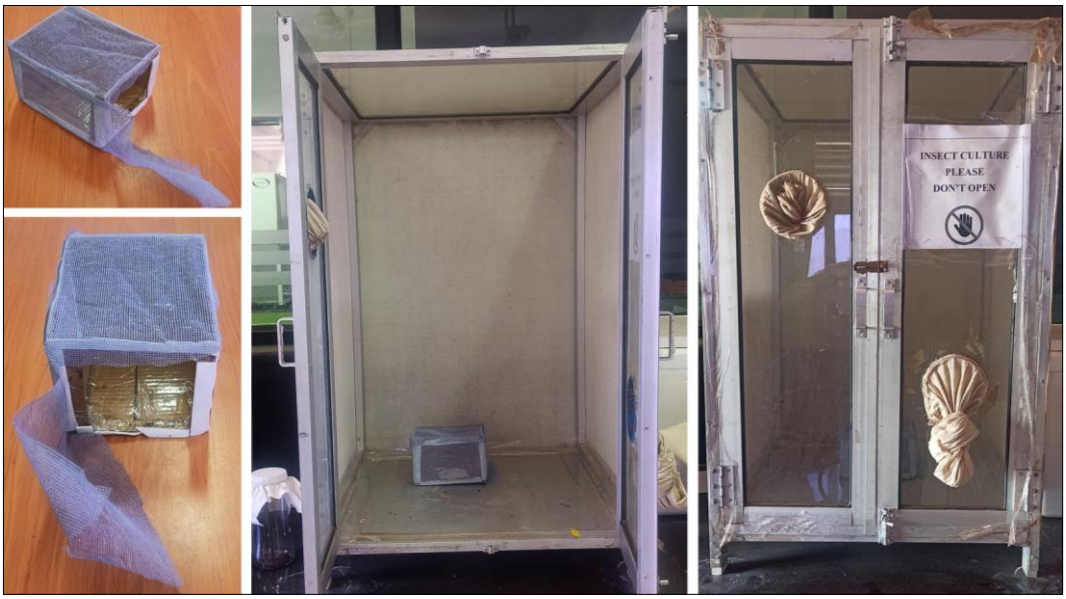

Fig 4: Test arena for checking the efficacy of Insect Repellent Nettings (IRNs)

The dried nets were used to cover the box containing the foods of *T. castaneum* (Fig.4) and kept inside the cage. The adults of *T. castaneum* (30 numbers) were released into the insect cage and checked for the repellent activity of the nets used. The insect cage was covered with muslin cloth on the top, sides and back to allow passage of air flow. The bottom of the cage was metallic and doors were made of glass to make the insects visible from the outside. The number of adults that were unable to enter through the treated nets was considered repelled. The repellent activity (R) percentage was calculated using equation 1.

$$R\ (\%) = \frac{\text{Number of insects repelled}}{\text{Total number of insects used}} \times 100 \quad (1)$$

Statistical analysis

The repellent activity (%) of emulsified botanicals obtained was calculated and given in terms of Mean ± SD. The mortality of *T. castaneum* adults during filter paper assay was also tabulated and grouped using Turkey test. The amount of significance was determined using one way ANOVA at 99% level of significance ($p<0.01$).

Results and discussion

Effect of nano-emulsions as a repellent against *T. castaneum* in Filter paper assay

The results of nano emulsions of botanical extracts showed 73 to 90 per cent repellency but there was no significant difference ($p<0.05$) observed in the nano-emulsion treated filter paper (Table 1) up to 4th day. The repellent activity against *T. castaneum* was effective for 4 days. The mortality of *T. castaneum* adults was observed during the repellency test of nano-emulsion from the 5th day of storage. In the control treatment, it was found that the repellent activity up to the 10th day was ranging from 40 to 60 per cent (Table 1). However, the adults were found dead on the 11th day of storage study in the case of control treatment.

Another set of *T. castaneum* adults, on the 16th day of storage showed repellent activity of 90%. The repellent activity was not significantly different up to 22nd day. However, the mortality of the insects was observed from the 23th day. Similarly, the mortality was observed on the 26th day of the storage study in the control treatments (Table 2).

Table 1: Percentage repellent activity of nano-emulsions obtained in filter paper bioassay for the first 15 days

Time		Repellent activity (%)	
		Nano-emulsion treated Half	Control treatment without nano-emulsion
Day 1	30minute	80.0±10.00^{a}	53.3±11.55^{bc}
	60minute	80.0±13.23^{a}	56.7±5.77^{bc}
	2h	86.7±7.64^{a}	56.7±5.77^{bc}
	4h	90.0±5.00^{a}	60.0±0.00^{a}
	8h	91.7±2.89^{a}	56.7±5.77^{bc}
	16h	90.0±5.00^{a}	53.3±5.77^{bc}
	24h	83.3±2.89^{a}	46.7±5.77^{bc}
Day 2		73.3±2.89^{a}	46.7±5.77^{bc}
Day 3		75.0±13.23^{a}	50.0±0.00^{bc}
Day 4		83.3±5.77^{a}	50.0±10.0^{bc}
Day 5		All dead	43.3±5.77^{bc}
Day 6		All dead	40.0±0.00^{b}
Day 7		All dead	46.7±5.77^{bc}
Day 8		All dead	40.0±0.00^{b}
Day 9		All dead	50.0±0.00^{bc}
Day 10		All dead	43.3±5.77^{bc}
Day 11		All dead	All dead
Day 12		All dead	All dead
Day 13		All dead	All dead
Day 14		All dead	All dead
Day 15		All dead	All dead

Table 2: Percentage repellent activity of nano-emulsions obtained in filter paper bioassay for 16th to 25th day with new set of adult *Tribolium castaneum*

Time		Repellent activity (%)	
		Nano-emulsion treated Half	Control treatment without nano-emulsion
Day 16	30minute	90.0±0.00^{a}	53.3±11.55^{a}
	60minute	90.0±0.00^{a}	56.7±5.77^{a}
	2h	90.0±0.00^{a}	56.7±5.77^{a}
	4h	90.0±10.0^{a}	60.0±0.00^{a}
	8h	90.0±0.00^{a}	56.7±5.77^{a}
	16h	90.0±0.00^{a}	53.3±5.77^{a}
	24h	76.7±5.77^{a}	53.3±5.77^{a}
Day 17		73.3±5.77^{a}	43.3±5.77^{a}
Day 18		75.7±5.77^{a}	50.0±0.00^{a}
Day 19		80.0±10.00^{a}	60.0±10.0^{a}
Day 20		80.0±10.00^{a}	46.7±5.77^{a}
Day 21		83.3±11.55^{a}	46.7±0.00^{a}
Day 22		80.0±10.00^{a}	46.7±5.77^{a}
Day 23		All dead	43.3±5.77^{a}
Day 24		All dead	50.0±0.00^{a}
Day 25		All dead	43.3±5.77^{a}
Day 26		All dead	All dead
Day 27		All dead	All dead
Day 28		All dead	All dead
Day 29		All dead	All dead
Day 30		All dead	All dead

Table 3: Toxicity of individual plant-based insecticides used in the study

Plant-based insecticides (Without dilution)	Mortality (%) in 24 hours
Neem oil	0±0.00
Thyme essential oil	100±0.00

The results of the toxicity study showed that thyme essential oil was responsible for the mortality of *T. castaneum* adults (Table 3). Neem is an excellent source of insect growth regulator as it contains Azadirachtin (Benelli *et al*., 2017) [2]. It has been used traditionally to keep the insects away from the treated grains (Tripathi, Gupta, & Gupta, 2020) [2]. In this study, the results showed that neem oil were unable to cause the mortality of *T. castaneum* adults. Thyme essential oil, which have excellent potential for the mortality of the insect, have several medicinal values. The studies on thyme oil suggested that they are excellent antibacterial, antifungal, antifungal agents (Kowalczyk, Przychodna, Sopata, Bodalska, & Fecka, 2020) [9]. These properties may be beneficial if used along with grains, since, grains were prone to fungal infection when there is short increase in humidity. There have been studies on the use of thyme essential oil as a preservative in bakery products (Gonçalves *et al*., 2017) [4] and also possess therapeutic properties (Salehi *et al*., 2018) [16].

When repellent assay was studied in the petri plate, it was found that the nano-emulsion was significantly repellent against the adults of *T. castaneum* up to the 4th day. Further, the repellent activity of nano-emulsion dropped (51.7-53.3%). Upon closure examination, it was found that the adults were

dead. This may be due the toxicity of neem and thyme against *T. castaneum.* The repellent effect of the nano-emulsion reduced as the time increased during the filter paper assay.

The same filter paper was tested on the 16th day with new sets of *T. castaneum* adults. It was observed that the repellent activity was present in the nano-emulsion treated filter paper. However, the mortality was caused after 7 days with the new sets of *T. castaneum* adults (Table 2), wherein, the life span was increased by 3 more days when compared to the first triplicates studied (Table 1). Observing the successful repellent behaviour of the nano-emulsion against *T. castaneum* adults in the filter paper assay, further analysis was subjected.

Repellent activity of treated nettings

The effect of nettings treated with nano-emulsion showed repellent activity for a day (Fig 5). The mortality of *T. castaneum* adults were also observed during the first day. The use of Thyme essential oil and neem oil as a repellent had been proven previously by many research studies. However, the repellent effect of nettings treated with these botanical insecticides reduced drastically after a period of 24 hours.

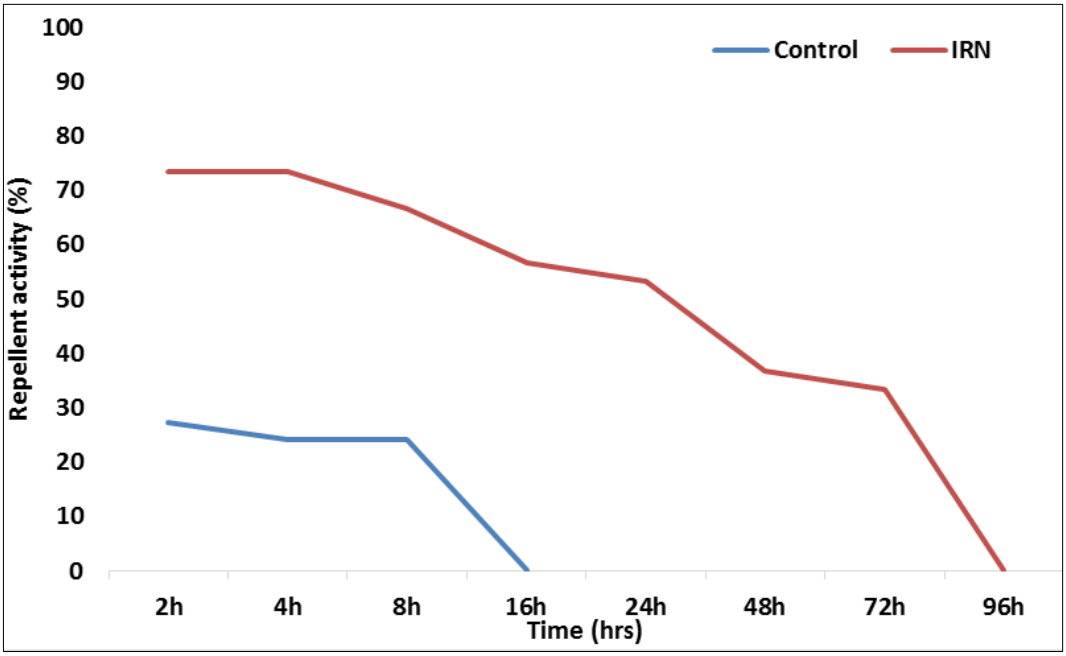

Fig 5: Repellent activity of treated and untreated insect repellent nettings (IRN) against the adults of *T. castaneum*

The attempt to increase the efficacy of botanicals was successful. The developed nano-emulsion was used in treating the nettings to form IRNs. However, the use of nettings as an application for the management of *T. castaneum* seemed unsuitable. There are two possible ways for the inefficient repellent behaviour of nettings observed in the study (Fig.5). The first reason may be that the efficacy of nano emulsion is less against

T. castaneum. Another reason may be that the holes of nettings were large enough for easy passage of *T. castaneum* adults. However, the first possible reason can be neglected as the results from filter paper assay showed effective repellent activity up to a period of 30 days. This proved that the use of nettings for management of stored product insect may not be an effective method. The nettings may work against mosquito or used against field pest. However, its possible use for prevention against stored product insect will be inadequate.

Earlier, Msango and Longwe (2013) [14] worked on developing an effective Long Lasting Insecticide Nettings (LLIN) to cover the phosphine fumigated area of a ware house in Malwi for controlling tobacco beetle, *L. serricorne*. Morrison Iii and Wilkins (2018) [13] studied the effect of LLIN on the behaviour of *R. dominica* and *T. castaneum*. The mobility and dispersal of *T. castaneum* and *Trogoderma variabile* was also determined when exposed to LLIN (Wilkins, Zhu, Campbell, & Morrison III, 2020) [22]. Wilkins *et al*. (2021) [21] conducted another research to prove that LLIN are effective for the management of insects. They reported that LLIN can be used for intercepting immigration of insects and also be used to cover gaps of the storage house (like windows, vents). Another possibility is to attract and kill using LLIN along with traps. Since the use of LLIN in stored product insects are elevating in the few years, Scheff *et al*. (2021) [17] incorporated deltamethrin in LLIN. The effect of the developed LLIN was observed for its effectiveness against three stored product insect's *viz. T. castaneum, R. Dominica, S. oryzae* and the results showed ineffectiveness against each species. The present study showed similar findings about its inadequacy in repelling the adults of *T. castaneum.* However, these nettings seem to provide mortality effect up to an extent. Hence, the individual botanical insecticides used in the study was screened to find out the insecticide responsible for the mortality of *T. castaneum* adults. The results showed that thyme essential oils were responsible for the mortality of the model insect studied.

Conclusion

The use of LLIN in controlling malaria outbreak brought the idea of using nettings for the management of stored product insects. Along with this ideology, nano-emulsions were also formulated which showed excellent repellent effect against the adults of *T. castaneum.* As an application to the use of plant based insecticides for controlled release and longer efficacy for a set period, the developed nano-emulsion were used to treat the nettings. During the research, it was found that the nettings were unable to repel the adults of *T.*

castaneum. However, the nettings showed mortality towards the adults and thyme essential oil was responsible for it. Even though, the nettings were not repelling, it has the potential to provide mortality against the stored product insects. This research recommends the use of nettings as an integral part of integrated pest management program.

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