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Evaluation of toxicity of neem essential oil and its nano-emulsion against adult *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

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Abstract: The saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) is a widespread warehouse pest that attacks a range of stored food products. Although chemical pesticides are effective to control this beetle, however, these agents are associated with harmful effects on humans and to the environment, indicating the need for safer and ecofriendly alternatives. In the present study, we aimed to compare the efficacy of neem (Azadirachta indica) essential oil and its nano-emulsion in the control of O. surinamensis adults. Neem essential oil was prepared using a steam distillation method, whereas the nano-emulsion was prepared using a non-ionic surfactant. Adults of O. surinamensis were exposed to both preparations, and mortalities were recorded after 24 h. The mean nano-emulsion droplet size was found to be 63.94 nm, and electron microscopy revealed that the nanoparticles occur either as scattered particles or agglomerated in small groups, with size ranging from 6.94 to 13.9 nm. The lethal concentrations of the neem essential oil and its nano-emulsion that cause 50% mortality (LC50) among O. surinamensis adults were 18.2 and 15.7 µL/L of air, respectively, indicating that the LC50 of the nano-emulsion against these beetles was 13.7% lower ($p < 0.05$) than that of the parent essential oil. Although, beetles were found to be susceptible to both preparations, the higher efficacy of the nano-emulsion as compared to the essential oil indicates that it would be a more promising option for the control of O. surinamensis.

Keywords: Nanocomposites, O. *surinamensis*, neem essential oil, food

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تقييم سمية الزيت العطري للنيم ومستحلب النانو له ضد الطور الكامل لخنفساء الحبوب المنشارية (غمدية الأجنحة: الخنفساء ذات الصدر المنشاري)

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مستخلص البحث: خنفساء الحبوب المنشارية تعتبر من آفات المخازن عالمية الانتشار والتي تهاجم مدى واسع من المنتجات والأغذية المخزونة. وبالرغم من فعالية المبيدات الحشرية الكيميائية في مكافحتها إلا أنها ارتبطت بالعديد من الأضرار على الإنسان وبيئته الطبيعية. لذا دعت الحاجة إلى البحث عن بدائل للمبيدات الكيميائية تكون أكثر أماناً وفي ذات الوقت صديقة للبيئة. وتهدف دراستنا الحالية إلى مقارنة فعالية الزيت العطري لنبات النيم مع مستحلب النانو لزيت النيم لمكافحة خنفساء الحبوب المنشارية. وقد تم معاملة الطور البالغ للحشرة وحساب نسب الإماتة بعد 24 ساعة. وكان متوسط حجم قطرات النانو التي تم تحضيرها 63.94 نانومتر، كما تبين من خلال الفحص بالمجهر الإلكتروني أن جسيمات النانو تظهر إما كجسيمات متناثرة أو مجتمعة في مجموعات صغيرة، وبأحجام تتراوح ما بين 6.94 إلى 13.9 نانومتر. دلت النتائج أن تركيز الزيت العطري للنانو ومستحلب النانو له والذي أحدث 50% من الإماتة للطور الكامل للحشرة هو 18.2 و15.7 ميكرو لتر/لتر هواء على التوالي، وهذا يظهر بأن التركيز المميت لخمسين بالمئة من الحشرات لمستحلب النانو قد كان أقل بنسبة 13.7% عن الزيت العطري للنيم مما يدل على أنه الأكثر فعالية. وبشكل عام أظهرت نتائج المقارنة بين الزيت العطري للنيم و مستحلب النانو للنيم قد يكون له مستقبلاً واعداً في مكافحة خنفساء الحبوب المنشارية.

كلمات مفتاحية: تركيبات نانونية، زيت النيم العطري، مكافحة آفات المنتجات المخزونة.

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1. Introduction

Stored grains and other products often become infested with pests that inflict varying degrees of damage, which accordingly reduce product quantity and quality, thereby resulting in considerable economic loss. According to the Food and Agricultural Organization (FAO), approximately 17% of food produced annually is lost, of which 10% is attributable to insect infestation (Stathas et al. 2023). Among pests that affect foodstuffs, the saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) causes severe damage to stored food (Gharsan 2022; Kousar et al. 2021), and thus it would be highly desirable to develop effective strategies for controlling and eliminating the damage caused by this beetle. Although in this regard, chemical insecticides are effective to control of *O. surinamensis*, they are associated with a number of potentially harmful effects with respect to human health and the environment (Kim et al. 2017). Consequently, studies are necessary to identify safer and effective alternatives, such as plant extracts, for the control of stored product insects (Ahmed et al. 2021; Alhaithloul et al. 2023; Garay et al. 2020). For example, Gharsan et al. (2018) have reported that onion and lavender oils are particularly effective in combating grain beetles, resulting in mortality rates reaching 100% for onion oil at all concentrations and 90% for lavender oil at a concentration of 4 µL/mL. Additionally, Al Qahtani et al. (2012) established that among the three types of plant powder they assessed, the ginger powder was the most effective in combating grain beetles, with a lethal concentration that causes 50% mortality (LC_{50}) as low as 0.14 mg/g. Neem extracts are among those plant extracts that have widely been used and proven to be effective in the control of insect pests of stored products (Saxena et al, 2018). For example, a study of the efficacy of different neem products (those of leaves, seeds, and bark) against cowpea beetle (*Callosobruchus maculatus*) infestation in bean seeds revealed that powdered seed had the highest protective effect, resulting in the lowest weight loss percentage of bean seeds (0.09%) compared to that in control group (1.16%) (Rajab & Abdullahi 2020). Neem contains a range compound with favorable bioactive properties, among which terpenoids are the most important and can be extracted from different parts of the plant. Additionally, azadirachtin has been identified as one of the most active biological components in neem (da Costa et al. 2014). Moreover, neem contains more than 20 sulfur compounds, which are responsible for its distinctive odor (Lokanadhan et al. 2012). Recently, the efficacy of plant extracts in the control of insects has attracted considerable research attention, notably in the context of the emergence and development of nanotechnology, which has contributed to enhancing the stability of active compounds (Hashem et al. 2020; Melanie et al. 2022; Palermo et al. 2021). In this regard, the toxicities of citronella essential oil and its nano-emulsion against *O. surinamensis* have previously been evaluated, however, to date, there have been no similar studies that have examined the toxicity of neem oil against this beetle. Consequently, in this study, we aimed to evaluate the efficacy of neem essential oil and its nano-emulsion against the adults of *O. surinamensis*.

2. Materials and Methods

2.1 Insect rearing

Saw-toothed grain beetles (*O. surinamensis*) were collected from infected oats and dates from the market of Al-Baha City, Saudi Arabia, and then transferred to the laboratory of the Biology Department, College of Science, University of Al-Baha. The insect was identified by microscopic examination and using identification keys (Vendl et al. 2019). The insects were reared on dry dates within plastic containers (1 L) in an incubator at 27 ± 2°C and 65% ± 5% relative humidity and maintained for several generations to obtain a sensitive population.

2.2 Extraction of neem essential oil

Neem essential oil was extracted from the fresh leaves of neem plants (*Azadirachta indica*, A. Juss, 1830) collected during July 2019 from the Department of Medicinal and Aromatic Plants Research, Institute of Horticulture Research (El-Kanater El-Khairia farm), Egypt. The extracted oil was purified using a steam distillation method. Fresh neem leaves were dried, ground, and 100 gm of powder was placed in 300 ml of water and then distilled using a Clevenger apparatus for 3 hours. (Wu et al. 2019).

2.3 Preparation of a neem nano-emulsion

A nano-emulsion of the neem extract was prepared by mixing 10 mL of neem essential oil with 5 mL of a non-ionic surfactant (Tween 80), followed by slow stirring until a homogeneous mixture had formed. Thereafter, the mixture was diluted with 85 mL of water to obtain final volume of 100 mL, followed by continuous stirring for 30 min using a magnetic stirrer to ensure adequate dispersion and complete incorporation. The mixture thus obtained was divided into two portions, both of which were sonicated at 700 W using a SONOPULS HD 2200 ultrasonicator (Bandelin, Germany): one portion for 15 min and the other for 1.5 h. The size of the particles in each portion of the 10% neem nano-emulsion were determined using a hydrodynamic light scattering analyzer (DLS) following storage for 90 d at 27°C (Hassanin et al. 2017).

2.4 Determination of nano-emulsion droplet size

The size of the neem nano-emulsion droplets was measured at 27 °C using a Zetasizer Nano ZS particle sizer (Malvern Instruments, UK). Briefly, prior to measurement, 30 µL of the nano-emulsion was diluted with 3 mL of water at 25 °C. Molecule size information is presented as the mean Z-normal of three independent batches of nano-emulsions (Hassanin et al. 2017).

2.5 Transmission electron microscopy

The samples for transmission electron *m*icroscopy observation were prepared initially by pipetting a 20-µL diluted sample onto a 200-mesh copper specimen grid coated in film, followed by incubation for 10 min, with subsequent blotting of excess liquid. Thereafter, the grid was stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. Finally, the dried coated grid was observed using a Tecnai G20 Super TWIN double-tilt transmission electron microscope (FEI, Netherlands) operated at 200 kV (Hassanin et al. 2017)

2.6 Fumigant toxicity

To assess the toxicity of neem essential oil and its nano-emulsion against *O. surinamensis*, 10 adult insects (2 weeks old) were placed in 1-L plastic containers, in which were suspended filter papers (1 cm × 1 cm) soaked with graded concentrations of neem essential oil as well as its nano-emulsion (10, 15, 20, and 25 µL/L). Control group insects were maintained in containers in which untreated filter papers were suspended. The insects were exposed to the test materials for 24 h, after which the number of dead insects was counted under a microscope. The experiments were performed under laboratory conditions at 27 ± 2°C with 65% ± 5% relative humidity (Gharsan et al. 2022; Malacrinò et al. 2016).

2.7 Statistical Analysis

The percentage mortality was calculated and corrected according to the formula proposed by Abbott (1925), as follow:

Percentage mortality of treated insects = (percentage of dead insects in the control/total number of tested insects) × 100

After calculation of the percentage mortalities, the toxicity data were analyzed to determine the lethal concentration to 50% of the insects (LC_{50}; Ldp line) using probit analysis (Finney 1952). Significant differences between the groups were determined by one-way ANOVA, followed by Tukey's honest significant difference (HSD) tests, and statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS (USA-based IBM Corp.).

3. Results

3.1 Droplet size and polydispersity of the neem nano-emulsion

The mean droplet diameter of the prepared neem nano-emulsion was 63.94 nm (Fig. 1), and the value of the polydispersity index, an indicator of polydispersity and a measure of the regularity and stability of droplet size, of this preparation was found to be 0.364.

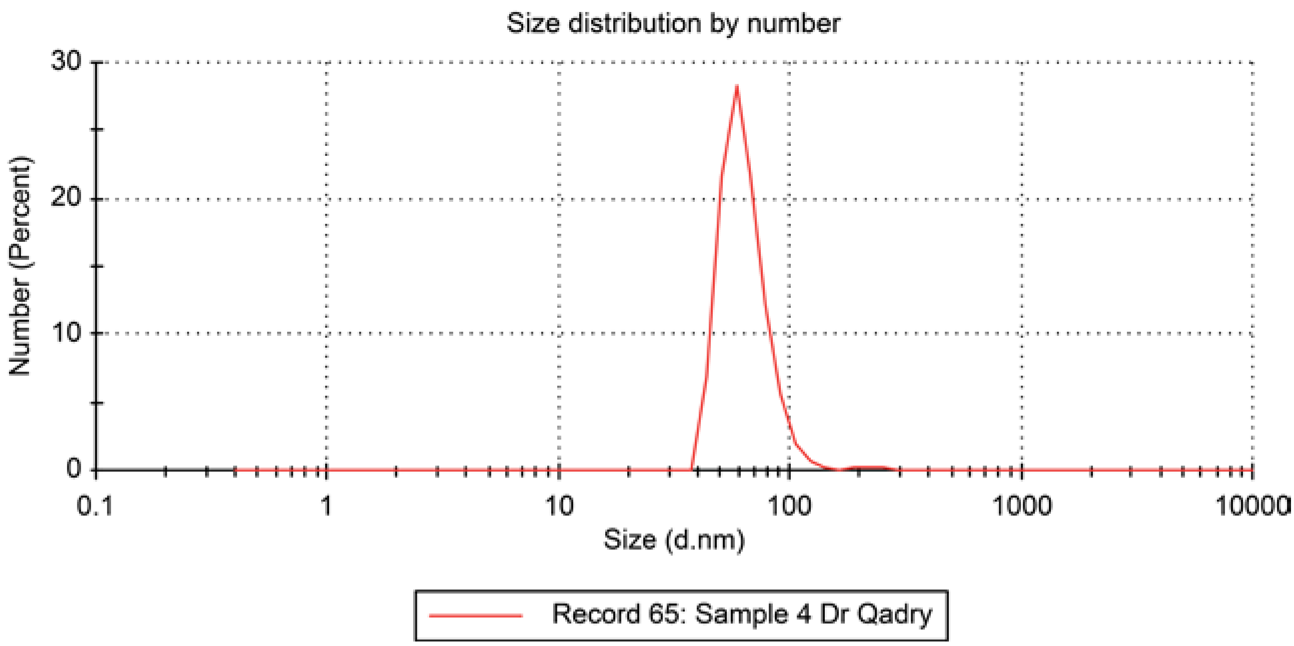

Figure 1. Mean droplet size (nm) of neem nano-emulsion.

3.2 Characteristics of neem nano-emulsions

The shape and size of the nanoparticles, which were determined from transmission electron micrographs, revealed that the particles are dark and spherical, and they appeared either as single scattered particles or in small clusters ranging in size from 6.94 to 13.9 nm (Fig. 2).

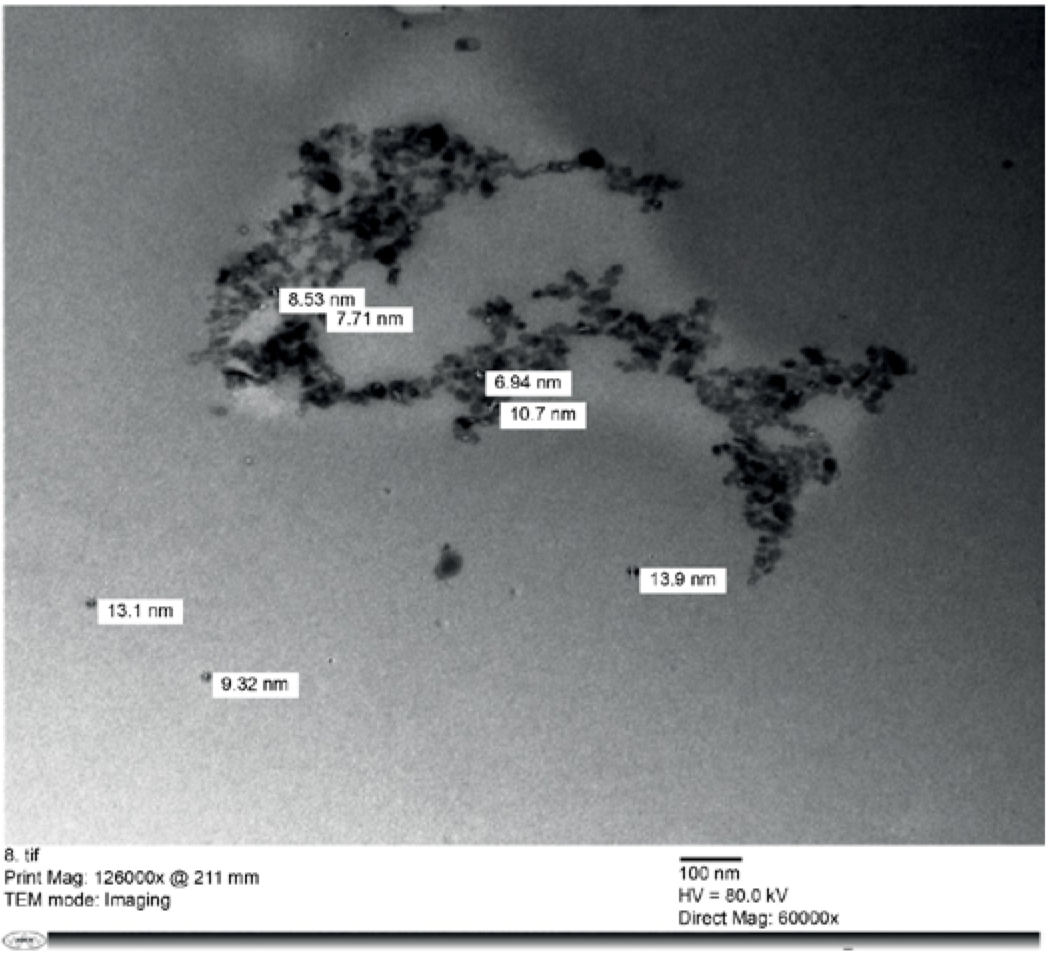

Figure 2. Representative transmission election microscopy image of neem nano-emulsion

3.3 Toxicity of neem essential oil against *O. surinamensis* adults

The essential oil of neem showed a concentration-dependent increase in toxicity against *O. surinamensis* adults, with mean mortality rates of 8%, 28%, 57%, and 81% , being recorded at concentrations of 10, 15, 20, and 25 µL/L, respectively (Table 1 and Fig. 3). On the basis of these findings, the LC_{50} and LC_{95} values of neem essential oil against *O. surinamensis* adults were calculated as 18.2 and 35.5 µL/L, respectively.

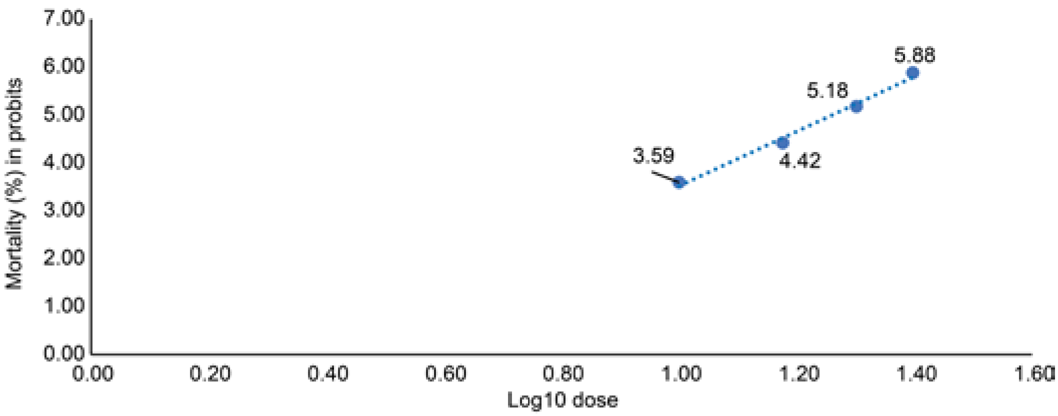

Figure 3. Regression line of Log concentration-mortality of neem essential oil on *Oryzaephilus surinamensis* adults.

Table 1 Toxicity of neem essential oil and neem nano-emulsion against *Oryzaephilus surinamensis* adults

	Conc. µL/L of air	Mean toxicity (percentage)	Sig.	LC50	LC95	Slope	Sig.
Essential oil of neem	10	8	0.782	18.2a*	35.5	5.7	0.02
	15	28					
	20	57					
	25	81					
	Control	0					
Neem nano-emulsion	10	3.3	0.031	15.7b	22.6	10.6	
	15	25					
	20	91.6					
	25	100					
	Control	0					

* Different letters following values in the same column indicate statistically significant differences at the $p < 0.05$ level.

3.4 Toxicity of neem nano-emulsion against *O. surinamensis* adults

Consistent with the parent oil, the neem nano-emulsion was found to show a concentration-dependent increase in toxicity against the insects, with mean mortalities ranging from 3.3% to 100% at concentrations from 10 to 25 µL/L of air (Table 1 and Fig. 4), and an LC_{50} value of 15.7 µl/L.

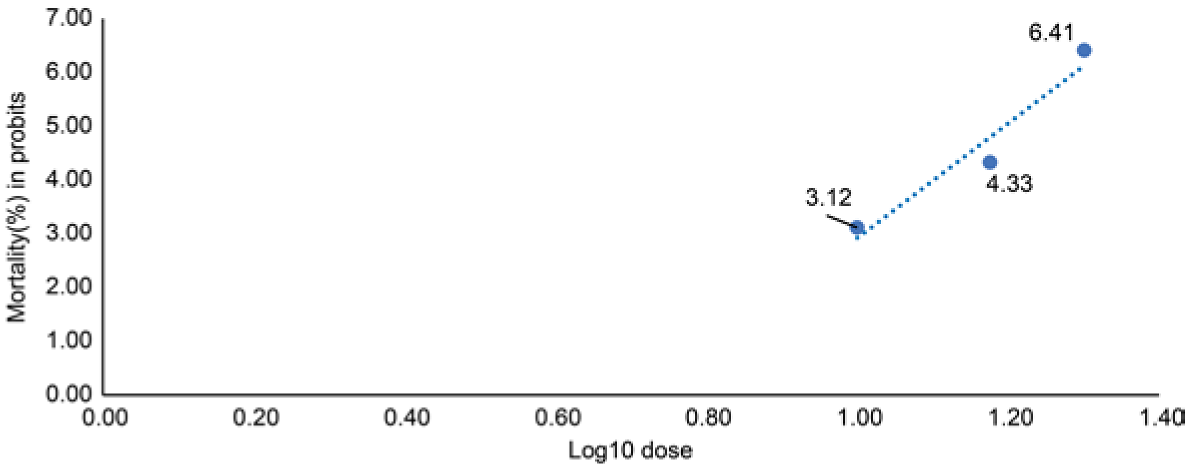

Figure 4. Regression line of Log concentration-mortality of neem nano-emulsion on *Oryzaephilus surinamensis* adults.

4. Discussion

In this study, it has been observed that the neem essential oil was effective in eliminating *O. surinamensis*, which is consistent with the findings of previous studies that has evaluated the exterminatory effects of neem against insect pests of stored products (Mantzoukas et al. 2020; Muhammad & Kashere 2020; Onu et al. 2015). Neem contains range of active substances, which are presumed to contribute to the efficacy of neem extracts in controlling insects, among which, the limonoids present in neem are believed to be responsible for its insecticidal properties. Although azadirachtin has been identified as the most active compound in neem, other limonoids may also contribute in enhancing its activity and efficacy, as well as to preventing insect resistance (Iqbal et al. 2021). Compared with that of the neem oil, we noted a 13.7% reduction in the LC_{50} of the nano-emulsion against *O. surinamensis* adults, thereby indicating that the nano-emulsion was more effective than its parent essential oil in controlling these beetles. These results are consistent with previous findings indicating that the conversion of essential oils to nano-emulsions enhances their efficacy in eliminating insects (Giunti et al. 2019; Gharsan et al. 2022; Manjesh et al. 2022; Palermo et al. 2021; Sabbour 2020). For example, da Costa et al. (2014) found that the mortality of *Zabrotes subfasciatus* increased when exposed to nanocomposites of neem extract, and also noted that the compounds were more stable than the parent extract.

In the present study, we obtained a value of 63.94 nm for the mean droplet size of the prepared neem nano-emulsion, which is within the 20–200 nm droplet size range, considered to be suitable for a good nano-emulsion (Ibrahim 2020; Ostertag et al. 2012). The neem nano-emulsion was prepared using a surfactant (Tween 80), which has been reported to ensure the stability of emulsions of essential oils (Mansouri et al. 2021). For example, Ali et al. (2017) have confirmed that the use of Tween 80 in combination with high-energy sonication promoted the formation of a stable and durable nano-emulsion. In addition, to enhance the stability of plant extracts, there are certain other advantages also associated with the use of nano-emulsions in the field of pest control, including an increased solubility of the active constituents and enhanced wettability during application (Mustafa & Hussein 2020). Moreover, the small size of the nanoparticles comprising nano-emulsions facilitates active constituent penetration through the insect cuticle, thereby enhancing their ability to eliminate insects (Margulis-Goshen & Magdassi 2013). However, one of the limitations of the present study is that all experiments were conducted under laboratory conditions, and consequently, it is necessary to further validate our findings under actual storage conditions.

Conclusively, the findings of this study will make a valuable contribution to the field of pest control, and in particular control of the pests of stored products, as plant extract nanoparticles have shown to possess a number of important advantages. Moreover, the continued application of nanotechnology will no doubt contribute to the discovery of other effective compounds for control of the insect pests of stored products.

5. Conclusions

In this study, we compared the pesticidal activities of a neem essential oil and its nano-emulsion against the adults of *O. surinamensis*. The conversion of neem essential oil to a nano-emulsion was found to significantly enhance its toxic effects against *O. surinamensis*. Although the efficacy of natural plant products in controlling insects is often attributable to the action of their active compounds, these plants products and their bioactive compounds tend to be unstable and are susceptible to deactivation or degradation by multiple factors. However, converting compounds of plant origin to a corresponding nano form can enhance their stability, thereby making them more promising candidates for the control of insect pests. Moreover, nano-emulsions can be incorporated as a complementary component of integrated pest management programs.

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7. References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. *econ. Entomol*, 18(2), 265-267.

Ahmed, N., Alam, M., Saeed, M., Ullah, H., Iqbal, T., Al-Mutairi, K. A., ... & Salman, M. (2021). Botanical insecticides are a non-toxic alternative to conventional pesticides in the control of insects and pests. *Global decline of insects*, 1-19.

Al Qahtani, A. M., Al-Dhafar, Z. M., & Rady, M. H. (2012). Insecticidal and biochemical effect of some dried plants against *Oryzaephilus surinamensis* (Coleoptera-Silvanidae). *The Journal of Basic & Applied Zoology*, 65(1), 88-93.

Alhaithloul, H. A., Alqahtani, M. M., Abdein, M. A., Ahmed, M. A., Hesham, A. E. L., Aljameeli, M. M., ... & El-Amier, Y. A. (2023). Rosemary and neem methanolic extract: antioxidant, cytotoxic, and larvicidal activities supported by chemical composition and *molecular docking simulations. Frontiers in Plant Science*, 14, 1155698.

Ali, E. O. M., Shakil, N. A., Rana, V. S., Sarkar, D. J., Majumder, S., Kaushik, P., ... & Kumar, J. (2017). Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, Rhizoctonia solani and Sclerotium rolfsii. *Industrial crops and products*, 108, 379-387.

da Costa, J. T., Forim, M. R., Costa, E. S., De Souza, J. R., Mondego, J. M., & Junior, A. L. B. (2014). Effects of different formulations of neem oil-based products on control Zabrotes subfasciatus (Boheman, 1833) (Coleoptera: Bruchidae) on beans. *Journal of Stored Products Research*, 56, 49-53.

Finney, D.J. (1952). Probit Analysis (2nd Ed), *Cambridge University Press*, New York, 388-390.

Garay, J., Brennan, T., and Bon, D, (2020). Review: Essential oils a viable pest control alternative. International Journal of *Ecotoxicology and Ecobiology*, 5, 13-22.

Gharsan, F., Jubara, N., Alghamdi, L., Almakady, Z., & Basndwh, E. (2018). Toxicity of five plant oils to adult Tribolium castaneum (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Florida Entomologist*, 592-596.

Gharsan, F. N., Kamel, W. M., Alghamdi, T. S., Alghamdi, A. A., Althagafi, A. O., Aljassim, F. J., & Al-Ghamdi, S. N. (2022). Toxicity of citronella essential oil and its nanoemulsion against the saw-toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Industrial crops and products*, 184, 115024.

Gharsan, F. N. (2022). Estimating the Susceptibility of Four Wheat Cultivars to the Saw-Toothed Grain Beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). *International Journal of Food Science*, 2022.

Giunti, G., Palermo, D., Laudani, F., Algeri, G. M., Campolo, O., & Palmeri, V. (2019). Repellence and acute toxicity of a nano-emulsion of sweet orange essential oil toward two major stored grain insect pests. *Industrial Crops and Products*, 142, 111869.

Hashem, A. S., Ramadan, M. M., Abdel-Hady, A. A., Sut, S., Maggi, F., & Dall'Acqua, S. (2020). Pimpinella anisum essential oil nanoemulsion toxicity against Tribolium castaneum? Shedding light on its interactions with aspartate aminotransferase and alanine aminotransferase by molecular docking. *Molecules*, 25(20), 4841.

Hassanin, M. M., Halawa, A. E., & Ali, A. A. A. (2017). Evaluation of the activity of thyme essential oil nanoemulsion against Sclerotinia rot of fennel. *Egyptian Journal of Agricultural Research*, 95(3), 1037-1050.

Ibrahim, S. S. (2020). Essential oil nanoformulations as a novel method for insect pest control in horticulture. *Horticultural crops*, 195-209.

Iqbal, T., Ahmed, N., Shahjeer, K., Ahmed, S., Al-Mutairi, K. A., Khater, H. F., & Ali, R. F. (2021). Botanical insecticides and their potential as anti-insect/pests: Are they successful against insects and pests?

Kim, K. H., Kabir, E., & Jahan, S. A. (2017). Exposure to pesticides and the associated human health effects. *Science of the total environment*, 575, 525-535.

Kousar, T., Zaib-un-Nisa Memon, H. A., Sahito, W. M. M., Jatoi, F. A., & Shah, Z. H. (2021). 1. Biology, morphology, and varietal distribution of Saw-toothed grain beetle, *Oryzaephilus surinamensis* (L) on date palm dry and semi-dry dates at district: Khairpur, Sindh-Pakistan. *Pure and Applied Biology* (PAB), 10(3), 539-548.

Lokanadhan, S., Muthukrishnan, P., & Jeyaraman, S, (2012). Neem products and their agricultural applications. *Journal of Biopesticides* 5: 72.

Malacrinò, A., Campolo, O., Laudani, F., & Palmeri, V. (2016). Fumigant and repellent activity of limonene enantiomers against Tribolium confusum du Val. *Neotropical entomology*, 45(5), 597-603.

Manjesh, K., Kundu, A., Dutta, A., Saha, S., & Neelakanthaiah, B. S. (2022). Bio-insecticidal nanoemulsions of essential oil and lipid-soluble fractions of Pogostemon cablin. *Frontiers in Plant Science*, 13, 874221.

Mansouri, S., Pajohi-Alamoti, M., Aghajani, N., Bazargani-Gilani, B., & Nourian, A. (2021). Stability and antibacterial activity of Thymus daenensis L. essential oil nanoemulsion in mayonnaise. *Journal of the Science of Food and Agriculture*, 101(9), 3880-3888.

Mantzoukas, S., Ntoukas, A., Lagogiannis, I., Kalyvas, N., Eliopoulos, P., & Poulas, K. (2020). Larvicidal action of cannabidiol oil and neem oil against three stored product insect pests: effect on survival time and in progeny. *Biology*, 9(10), 321.

Margulis-Goshen, K., & Magdassi, S. (2013). Nanotechnology: an advanced approach to the development of potent insecticides. *Advanced technologies for managing insect pests*, 295-314.

Melanie, M., Miranti, M., Kasmara, H., Malini, D. M., Husodo, T., Panatarani, C., ... & Hermawan, W. (2022). Nanotechnology-based bioactive antifeedant for plant protection. *Nanomaterials*, 12(4), 630.

Muhammad, A., & Kashere, M. A. (2020). Neem, Azadirachta indica L. (A. Juss): An eco-friendly botanical insecticide for managing farmers insect pest problems-a review. *FUDMA Journal of Sciences*, 4(4), 484-491.

Mustafa, I. F., & Hussein, M. Z. (2020). Synthesis and technology of nanoemulsion-based pesticide formulation. *Nanomaterials*, 10(8), 1608.

Onu, F. M., Ogu, E., & Ikehi, M. E. (2015). Use of Neem and Garlic Dried Plant Powders for Controlling some Stored Grains Pests. *Egyptian Journal of Biological Pest Control*, 25(2).

Ostertag, F., Weiss, J., & McClements, D. J. (2012). Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. *Journal of colloid and interface science*, 388(1), 95-102.

Palermo, D., Giunti, G., Laudani, F., Palmeri, V., & Campolo, O. (2021). Essential oil-based nano-biopesticides: Formulation and bioactivity against the confused flour beetle Tribolium confusum. *Sustainability*, 13(17), 9746.

Rajab, Y. S., & Abdullahi, U. (2020). Efficacy of Various Neem Products in Control of Storage Pests (Callosobruchus maculatus) of Cowpea (Vigna unguiculata). *IOSR Journal of Agriculture and Veterinary Science* (IOSR-JAVS), 13(3), 23-26.

Sabbour, M. M. A. (2020). Efficacy of nano-formulated certain essential oils on the red flour beetle Tribolium castaneum and confused flour beetle, Tribolium confusum (Coleoptera: Tenebrionidae) under laboratory and storage conditions. *Bulletin of the National Research Centre*, 44, 1-7.

Saxena, R. C., Jilani, G., & Kareem, A. A. (2018). Effects of neem on stored grain insects. *In Focus on phytochemical pesticides* (pp. 97-112). CRC Press.

Stathas, I. G., Sakellaridis, A. C., Papadelli, M., Kapolos, J., Papadimitriou, K., & Stathas, G. J. (2023). The Effects of Insect Infestation on Stored Agricultural Products and the Quality of Food. *Foods*, 12(10), 2046.

Vendl, T., Stejskal, V., & Aulicky, R. (2019). Comparative tarsal morphology of two secondary stored product beetle pests, *Oryzaephilus surinamensis* (L.) and *Cryptolestes ferrugineus* (Stephens), that vary in their climbing ability on smooth surfaces. *Journal of stored products research*, 82, 116-122.

Wu, H., Li, J., Jia, Y., Xiao, Z., Li, P., Xie, Y., ... & Li, C. (2019). Essential oil extracted from Cymbopogon citronella leaves by supercritical carbon dioxide: antioxidant and antimicrobial activities. *Journal of Analytical Methods in Chemistry*, 2019.