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Activity of nanoemulsion botanical insecticides from *Myristica fragrans* and *Jatropha curcas* essential oil against *Sitophilus zeamais*

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Abstract. *Nuryanti NSP, Budiarti L, Dulbari, Sutrisno H, Sudrajat D, Yuriansyah, Priyadi, Rahmadi R, Rochman F, Sari EY, Maharani JS. 2023. Activity of nanoemulsion botanical insecticides from* Myristica fragrans *and* Jatropha curcas *essential oil against* Sitophilus zeamais. *Biodiversitas 24: 5610-5617.* Maize weevils also known as *Sitophilus zeamais*, are primary pests that decrease in the quality and quantity of grain products. This study aimed to examine the impact of nanoemulsion formulas of *Myristica fragrans* and *Jatropha curcas* essential oils on *S. zeamais* adults. The investigation focused on the toxicity, ability to inhibit population growth, intensity of damage caused, and effect on seed weight loss. The essential were extracted using the distillation method, and the toxicity was tested through contact, fumigant, and seed dressing. A probit program was used to analyze the toxicity level (LC_{50} and LC_{95}). The results showed that *M. fragrans* essential oil had the highest toxicity in contact treatment and the lowest when applied via fumigation. Furthermore, this method shows an LC_{50} value for the contact method of 0.53% and fumigation of 1.01%, while the LC_{95} value is 4.38% and 10.60% respectively. Based on the LC_{95} botanical insecticide from *J. curcas*, all three methods were toxic to *S. zeamais* adults, with contact being the highest, followed by seed dressing, and fumigant, at 5.23, 6.44, and 19.94%, respectively. The botanical insecticides derived from *M. fragrans* essential oil through contact provided the best inhibitory activity on population development, intensity of damage, and seed weight loss, with values of 18.00, 16.81, and 13.96%. Plant-based pesticides not only cause pest mortality but also maintain seed quality by reducing weight loss, intensity of pest attacks, and inhibiting the development of pest populations in storage.

Keywords: Contact toxicity, fumigation, pest control, population inhibition

INTRODUCTION

Weevil known as *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is one of the primary pests that infest stored grain products such as rice, corn, and sorghum in both tropical and subtropical regions (Gałęcki et al. 2019). The pest is responsible for causing both qualitative and quantitative damage which depends on factors such as storage structure, as well as physical and chemical properties of the corn. *Sitophilus zeamais* thrived in hot and humid areas and attacked various types of cereals, with corn being its main target (Gowda et al. 2019). Currently, the dominant pest control methods in storage rely on synthetic insecticides and fumigants due to their quick and reliable efficacy (Stejskal et al. 2021). However, the persistent use of these chemicals gives rise to some issues, including unintended harm to non-targeted insects (Haddi et al. 2020), decreased biodiversity of natural predators (Janssen and van Rijn 2021), and disturbance to the natural balance of the ecosystem (Alyokhin et al. 2020). For a significant period, botanical insecticides have been considered an attractive for managing insect pests (Hikal et al. 2017). The majority of botanical insecticides with insecticidal activity are biodegradable and pose less harm to mammals (Riyaz et al. 2022). This suggests the potential to replace potent plant-based botanical insecticides with their synthetic counterparts.

Botanical insecticides have shown their effectiveness, safety, affordability, and ease of processing and application, ensuring a practical choice for farmers in developing nations (Regnault-Roger et al. 2012). Improving the availability and efficacy of new pesticides necessitates rigorous testing of the bioactivity of plant secondary metabolites and their formulation techniques. Extensive evaluation and documentation have been conducted on more than 75 plant species from diverse families to determine their effectiveness as insecticides against stored-grain pests (Sarma et al. 2019). Notably, essential oils derived from *M. fragrans* have shown promise in exhibiting insecticidal properties. The viability of these botanicals in managing a wide range of insect pests was explored (Damalas and Koutroubas 2020). Consequently, ongoing research seeks to explore the viability of these botanicals in managing a wide range of insect pests. Faheem and Abduraheem (2019) reported that the essential oil content of *M. fragrans* was able to suppress the population of *A. verbasci*. Additionally, *M. fragrans* had insecticidal activity against *S. oryzae* (Demeter et al. 2021) and *C. sativum* at a concentration of 1.0 g/10 g seed had significantly negative effects on *C. maculatus* (Juma'a et al. 2022).

To offer eco-friendly pest control alternatives, it is crucial to conduct bioactivity testing and create insecticide formulations from various essential oils derived from botanicals. The main advantages of formulating insecticides are to enhance the durability of the active ingredients during storage and distribution, simplify the handling and application of the product, safeguard the active compounds against unfavorable environmental factors, and improve the effectiveness of the active ingredients by promoting contact and interaction with the intended pests (Li et al. 2021).

Nanoemulsions are emulsions made up of an oil phase, an aqueous phase, and surfactant molecules that do not mix together (Gupta 2020). Due to their small droplet size, they prevent the coalescence of particles, which ensures an even distribution of the dose and system dispersion (Aswathanarayan and Vittal 2019). Nanoemulsion showed potential as a suitable formulation for botanical insecticides. Pavoni et al. (2019) reported that nanoemulsions of *P. anisum* and *M. piperita* essential oils, as well as geraniol, cis-jasmone, and farnesol, were more repellent. The level of repellence activity increased with the size of the oil particles. For instance, a citral-based nanoemulsion with particles that were 99 nm in size at 2% concentration showed a repellence index of 66, while the same formulation with 816 nm particle size had minimal activity (Singh and Pulikkal 2022). Nuryanti et al. (2018) achieved success in creating a nanoemulsion formulation by blending extracts of *P. retrofractum* and *T. erecta*. They incorporated Polysorbate and Triton x-100 emulsifiers using the inversion phase technique, resulting in particle sizes ranging from 80.41 to 143.81 nm. However, the creation of nanoemulsion formulations for insecticides derived from *M. fragrans* essential oil and *J. curcas* seed extracts, intended for controlling pests in warehouses through contact, fumigation, and seed dressing methods, remains an area that has not yet been explored. Such nanoemulsion formulations have the potential to enhance the effectiveness of active ingredients, improve spreadability, and mitigate seed weight loss. This study aimed to evaluate the effects of nanoemulsion formulations containing essential oils from *M. fragrans* and *J. curcas* on adult *S. zeamais* insects. The investigation focused on analyzing the toxicity of the formulations, the potential to impede population growth, the degree of damage caused, and impact on seed weight loss.

MATERIALS AND METHODS

Mass rearing of *Sitophilus zeamais*

The insects used in this study were obtained from the food crop production laboratory at Politeknik Negeri Lampung and identified as *Sitophilus zeamais* species. Furthermore, they were mass-reared by adopting the methodology stated by (Babarinde et al. 2021). Corn seeds that had been thoroughly cleaned using water and sieved were acquired from the Politeknik Negeri Lampung farm. Sterile polyethylene insect cages with a capacity of 25 cm × 10 cm × 15 cm were used to accommodate 500 g of corn seeds. Approximately 20 male and female adult beetles were introduced into each cage, covered with Tricho cloth, and secured using rubber bands which prevented the weevils from escaping or entering. The cages, containing the corn seeds and adult *S. zeamais*, were kept at a temperature of 30ºC ± 2.0 and a relative humidity of 75-90%. After a week, insects that have died and have not infected the corn seeds are removed by sieving them and then discarding them. Meanwhile, the corn seeds containing the beetle eggs were kept for about 4 weeks until the emergence of adult insects. For the experiments, only adult insects between 2 to 4 days old were utilized.

Extraction of essential oil

The *M. fragrans* seeds were obtained from traditional markets located in the province of Bandar Lampung, while the *J. curcas* seeds were collected from Rama Murti Village, Seputih Raman District, Lampung Tengah Regency (4°52'16.7"S 105°22'46.5"E). Both seeds were dried at room temperature around 25-27°C for 2 days until a moisture content of 20-30%, and ground with a grinder type-FFC23 with a speed of 5800 rpm until they become powder. To extract the essential oil from the *M. fragrans* plants, a Clevenger-type water steam distillation apparatus was utilized, Steam distillation is basically the process of distilling plant material with steam in a boiler. In this method, the material is placed on a perforated plate above the steam inlet. It is easy to control how much steam is generated in the steam-generating mechanism. In addition, since the steam generator is outside the distillation unit, the ambient temperature of the place where the material to be distilled is kept below 100°C, and the occurrence of damage due to heat effects can be prevented or reduced (Akdağ and Öztürk 2019). On the other hand, the oil from *J. curcas* was extracted using a manual press. The *J. curcas* seed powder was steamed for 30 minutes before the oil was obtained by pressing the *curcas* seeds manually. After distillation, the essential oils were kept in a refrigerator at 4°C until ready to be used in the treatments.

Nanoemulsion method

Both *J. curcas* and *M. fragrans* essential oil were transformed into a nanoemulsion formula using a modified low-energy emulsification technique through the phase inversion method, as described by Nuryanti et al. (2020). The emulsification process involved the gradual addition of distilled water phase drop by drop into the essentioal oil + Polysorbate phase at a controlled rate of 4 ml per minute. The formulation which consisted of *M. fragrans* or *J. curcas* seed oil (separately) added with polysorbate emulsifier (1:1; v:v) was then homogenized using a magnetic stirrer while slowly adding distilled water. Furthermore, stirring was continuously maintained at 750 rpm for a duration of 30 minutes starting from the beginning of the material blending process. The process of creating an emulsion occurred under conditions that did not exceed 29°C, specifically at room temperature.

Contact toxicity assays

The essential oil formulas were assessed for their toxicity to adult *S. zeamais*, and a preliminary test was performed to establish a range of concentrations that resulted in mortality rates of 5-99%. The formula was prepared in five different concentrations of 0.125%, 0.25%, 0.5%, 1.0%, and 2.0% using distilled water and polysorbate as emulsifier. Meanwhile, the control group included distilled water without the essential oil formula. The experiment was conducted using the methodology described by Prijono (2011). To create each formula, a 1:1 mixture of the oil and polysorbate (v/v) was first prepared, diluted with distilled water to reach a total volume of 100 ml, and then stirred at 750 rpm for 30 minutes.

A plastic cylinder cage measuring 8 cm in diameter and 10 cm in height was used to house ten adult weevils. Each tested formula solution, totaling 0.4 ml, was sprayed onto the weevils using a small hand sprayer. After being treated with the solution, the beetles were moved into plastic jars containing corn seeds. This process was repeated 5 times, and their mortality was then recorded at 24, 48, 72, and 96 hours after treatment. Probit analysis was utilized to determine the LC values of the essential oil formula (Enan 2001). Furthermore, additional bioassays were conducted employing the solutions that corresponded to the LC_{15}, LC_{35}, LC_{55}, LC_{75}, and LC_{95} values.

Fumigant activity

The fumigant activity of the essential oil formula was evaluated using 70 mL glass vials, each containing ten 3-5 days-old adult beetles. To create the fumigation setup, 8 cm diameter pieces of Whatman (No. 1) filter papers were cut and infused with the essential oil concentrations. These pieces were then fixed to the screw-cap lids of the glass vials. Based on the results of preliminary tests, a range of concentrations from 15% to 95% was selected for the experiment. This was conducted to establish a concentration range for exact treatment. Each procedure was repeated 5 times to ensure accuracy. The control treatment consisted of filter paper pieces infused with an extraction solution without the essential oil formula. After 48 and 72 hours of exposure, the mortality of the beetles was meticulously recorded and analyzed as part of the evaluation process.

Seed dressing activity

The toxicity test using the seed dressing method involved selecting white corn kernels that were free from infestation and visibly healthy. This was based on the study conducted by (Soe et al. 2020) and entailed soaking 4.5 g of white corn kernels in nanoemulsion preparations of botanical insecticides derived from *M. fragrans* and *J. curcas*. The concentrations used were 0.125%, 0.25%, 0.5%, 1%, and a control. The soaking process lasted for 5 minutes, allowing the insecticide to be absorbed into the seeds. Subsequently, the corn kernels were drained, air-dried, and put in a plastic cup with a diameter of 10 cm and a height of 5 cm. Each treatment was infested with 10 imago *S. zeamais* (4 days old). Finally, the experiment was repeated five times, and the mortality of insects was observed and recorded at 24, 48, 72, and 96 hours after treatment.

Population deterrence test

The population deterrence experiment employed nanoemulsion formulas and followed seed dressing, fumigation, and contact toxicity methods. The concentrations tested were equivalent to the LC_{75} value obtained in the previous tests. Furthermore, the process of preparing the formula solution for the experiment was similar to the mortality test. The formula was diluted in polysorbate (1:1, v:v), and then distilled water was added to make a 100 ml volume. A total of 10 grams of corn seeds were dipped in the formula for 1 minute, with the control treatment using only the polysorbate solution. After treatment, the seeds were drained, put in a 250 ml plastic cup, and covered. About 5 pairs of adult-aged *S. zeamais* between 3 to 5 days were released into the plastic cage and allowed to lay eggs on the corn seeds for 35 days. The number of adult beetles present after this period, was recorded. The calculation of the population deterrence percentage was conducted using the formula provided by (Harikarnpakdee and Chuchote 2018) as follows:

$$PA = \frac{NC - NT}{NC} x100\%$$

Where:
PA : percentage of population deterrence (%),
NC : The count of grown-up individuals on the control,
NT : The number of adult individuals that appeared in the treatment

Seed weight loss percentage

Seed weight loss resulting from infestation by *S. zeamais* was addressed through the application of a nanoemulsion formula derived from *M. fragrans* and *J. curcas*, employing contact, fumigation, and seed dressing techniques. The concentration tested matched the LC_{75} value determined in the toxicity assessment. The procedure for preparing the formula solution for the weight loss test closely resembled that of the toxicity test. To quantify the decrease in seed weight due to pest attacks by *S. zeamais*, the initial weight of corn seeds was measured before any treatment, and their final weight was recorded 35 days after treatment. Subsequently, the percentage of corn seed weight loss caused by the infestation was determined by applying the formula established (Odjo et al. 2022). The weight loss percentage is calculated using the formula:

$$WL = \frac{a - b}{b} x100\%$$

Where:
WL : percentage of seed weight loss (%),
a : initial seed weight (g),
b : final seed weight (g) (Odjo et al. 2022)

Intensity of seed damage

The intensity of seed damage was assessed by computing the percentage of seeds infested by *S. zeamais* within 100 seed samples that had been stored for 35 days. Damage severity represents the ratio of damaged seeds to the total number of seed samples in each plastic cup

container. Following the 35-day treatment period, the corn seeds were extracted from the plastic cups and sorted into two categories: those that remained intact or uninfested by *S. zeamais* and those that exhibited damage from *S. zeamais* infestation. The calculation of damaged seeds followed the formula provided by (Hendrival et al. 2019):

$$IS = \frac{a}{a+b} x100\%$$

Where:
IS : Intensity of seed damage (%),
a : number of damaged seeds by *S. zeamais*,
b : number of seeds that were not attacked by *S. zeamais*

The LC_{50} and LC_{95} values were calculated using the probit analysis software POLO-PC to assess the relationship between concentration and insect mortality, while analysis on inhibition of population growth, intensity of damage, and seed weight loss was conducted using fully random methodology. To assess the homogeneity of data variance, we conducted Bartlett's test and tested for data additivity using Tukey's test. If the assumptions were met, variance analysis was employed to analyze the data, and the middle value of the treatment was tested for differences using the Least Significant Difference (LSD) test at a 5% significance level.

The droplet size analysis

The analysis of droplet size in the nanoemulsion was conducted using a Particle Size Analyzer (PSA) following the method described by Nuryanti et al. (2018). To perform this analysis, three droplets from the sample were first diluted with 20 mL of distilled water (aquadest). Subsequently, the prepared sample was transferred into disposable cuvettes, which were then positioned at a 90° angle from a detector. The instrument housing the prepared sample was closed, and measurements were conducted using Zetasizer software, with the solvent's refractive index as input and laser intensity adjustment. The resulting measurement provided the average droplet size.

RESULTS AND DISCUSSION

The *Myristica fragrans* and the *Jatropha curcas* toxicity

The nanoemulsion formulation of *M. fragrans* showed the highest level of toxicity when applied through direct contact after 72 and 96 hours of exposure. The concentration required to cause a 50% mortality rate (LC_{50}) was determined to be 0.60%, while that of 95% was 7.38% (Table 1). In contrast, the fumigation method showed the lowest toxicity to *S. zeamais*. Table 1 showed that the LC_{50} and LC_{95} values for fumigation were 1.51% and 14.99%, respectively. The consistent toxicity trend of the *M. fragrans* essential oil nanoemulsion formulation observed 72 hours after application remained unchanged even after 96 hours of treatment. The contact method continued to exhibit the highest level of toxicity, followed by the seed dressing method, while the fumigation method displayed the lowest showed that the LC_{95} values for these methods were 4.38%, 6.24%, and 10.60%, respectively.

Among the different application techniques, the contact method exhibited the highest toxicity for the nanoemulsion formula derived from *J. curcas*. This was followed by the seed dressing, while fumigation showed the lowest. After a 72-hour application, the respective LC_{95} values for *S. zeamais* mortality according to Table 1 were 9.02%, 11.81%, and 26.34%. After subjecting the maize weevils to a 96-hour exposure, similar results were observed. The contact method continued to exhibit the highest level of toxicity, while seed dressing showed the lowest, as presented in Table 1.

Population growth inhibition, intensity of damage, and weight loss activity of *M. fragrans* and *J. curcas*

The utilization of *M. fragrans* seed essential oil *M. fragrans* as a botanical insecticide through the contact method showed superior inhibitory activity in terms of population growth, intensity of damage, and weight loss. The corresponding values were discovered to be 18.00%, 16.81%, and 13.96%, respectively.

Table 1. Toxicity of nanoemulsion formulas from *Myristica fragrans* and *Jatropha curcas* essential oil to the maize weevil (*Sitophilus zeamais*) adults after 72 and 96 h exposure

Types of essential oils	Methods	Intercept[a]± SE[c]	Slope[b]± SE[c]	LC50[d] (CI[e] 50%)(%)	LC95[d] (CI[e] 95%) (%)
M. fragrans (72 h)	Contact	0.33±0.11	1.51±0.28	0.60 (0.17-1.14)	7.38 (2.73-32.99)
	Fumigation	-0.30±0.12	1.65±0.28	1.51 (0.84-2.82)	14.99 (5.95-24.63)
	Seed dressing	0.20±0.11	1.59±0.29	0.74 (0.49-1.03)	8.02 (4.24-28.88)
M. fragrans (96 h)	Contact	0.48±0.12	1.80±0.30	0.53 (0.37-0.72)	4.38 (2.56-12.02)
	Fumigation	0.10±0.11	1.16±0.28	1.01 (0.50-1.66)	10.60 (4.63-29.30)
	Seed dressing	0.21±0.11	1.79±0.29	0.70 (0.52-1.00)	6.24 (3.74-16.04)
J. curcas (72 h)	Contact	0.23±0.11	1.47±0.28	0.69 (0.46-0.99)	9.02 (4.29-43.54)
	Fumigation	0.52±0.11	1.12±0.18	0.89 (0.18-3.37)	26.34 (5.53-47.16)
	Seed dressing	0.13±0.11	1.40±0.28	0.80 (0.51-1.16)	11.81 (5.47-63.46)
J. curcas (96 h)	Contact	0.14±0.11	1.59±0.29	0.67 (0.27-1.19)	5.23 (2.32-10.21)
	Fumigation	0.14±0.12	1.15±0.18	0.74 (0.29-1.63)	19.94 (4.64-48.85)
	Seed dressing	0.31±0.11	1.63±0.29	0.63 (0.42-0.88)	6.44 (3.56-20.77)

Note: a[a]: intercept of probit regression line, b[b]: probit regression slope, [c]SE: Standard Error. [d]LC: Lethal Concentration, [e]CI: confidence interval

In contrast, the fumigation method displayed lower activity, with inhibition values of population growth, intensity of damage, and weight loss at 12.33%, 49.63%, and 54.83%, respectively. Nanoemulsion from *J. curcas* also showed the same activity results as *M. fragrans*. The utilization of *J. curcas* as a botanical insecticide in the contact method exhibited outstanding effectiveness in terms of suppressing population growth, reducing damage intensity, and minimizing weight loss. The inhibition values were 22.00%, 23.69%, and 22.03% respectively. On the other hand, fumigation exhibited lower activity, with inhibition values of 12.67%, 54.50%, and 60.86%, respectively (Table 2).

Particle size

Nanoemulsion Droplet Size Analysis was carried out using a particle size analyzer (Nuryanti et al. 2018; Horison et al. 2019). Particle size is the average diameter of a particle, which is an important parameter in nanoemulsions (Bourbon et al. 2018). The particle size test results of the two formulas tested were included in the nanoemulsion (Figure 1 and 2). Adak et al. (2020) stated that nanoemulsion is an emulsion that has a particle diameter of 0.1 to 200 nm. Small nanoemulsion particle sizes are desired to achieve optimal efficiency. The smaller particle size of the nanoemulsion can increase the penetration and absorption of secondary metabolites into the insect's body (Pascual-Villalobos et al. 2019).

Figure 1 illustrates the particle size distribution of *M. fragrans* essential oil when combined with a 1:1 (v:v) polysorbate emulsifier, using the phase inversion emulsification technique, the particle sizes initially fall within the range of 100 to 600. The nanoemulsion's particle size distribution reaches a peak at approximately 17.5%, with particles measuring around 76 nm on average. The average particle size is 144.1 nm.

Figure 2 depicts the particle size distribution of *J. curcas* essential oil when combined with a 1:1 (v:v) polysorbate emulsifier. Employing the phase inversion emulsification technique results in particle sizes that initially fall within the range of 90 to 600. The nanoemulsion's particle size distribution reaches a peak at approximately 18.5%, with particles measuring around 82 nm on average. The average particle size is 150.2 nm.

Table 2. *Myristica fragrans* and *Jatropha curcas* activity on inhibition of population growth, intensity of damage, and seed weight loss

Kind of essential oils	Methods	Population growth inhibition (%) ± SD	Damage intensity (%) ± SD	Weight loss (%) ± SD
M. fragrans	Contact	18.00 ± 1.00 b	16.81 ± 1.32 a	13.96 ± 2.33 a
	Fumigation	12,33 ± 2,52 a	49.63 ± 3.23 c	54.83 ± 4.92 c
	Seed dressing	14.33 ± 3.23 ab	21.39 ± 2.10 b	22.11 ± 6.41 b
J. curcas	Contact	22.00 ± 3.61 b	23.69 ± 2.80 a	22.03 ± 5.62 a
	Fumigation	12.67 ± 1.53 a	54.50 ± 5.60 c	60.86 ± 5.36 b
	Seed dressing	16.00 ± 1.00 a	28.09 ± 1.10 b	26.83 ± 1.68 ab

Note: The percentage value of population growth inhibition, damage intensity, and weight loss followed by the same letter in the same column was not significantly different from the 5% LSD test

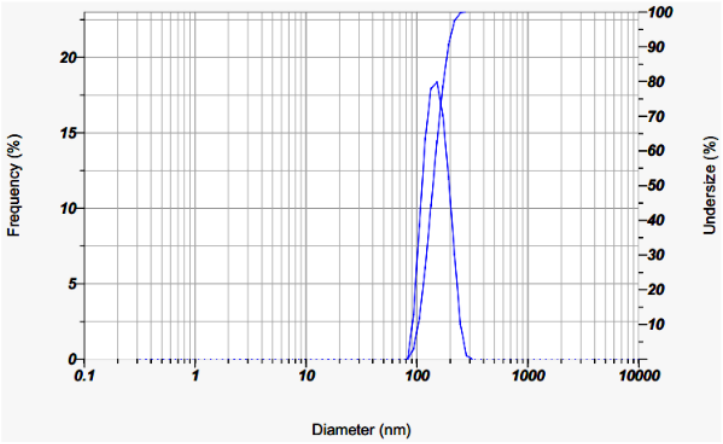

Figure 1. *Myristica fragrans* nanoemulsion particle size using inversion phase

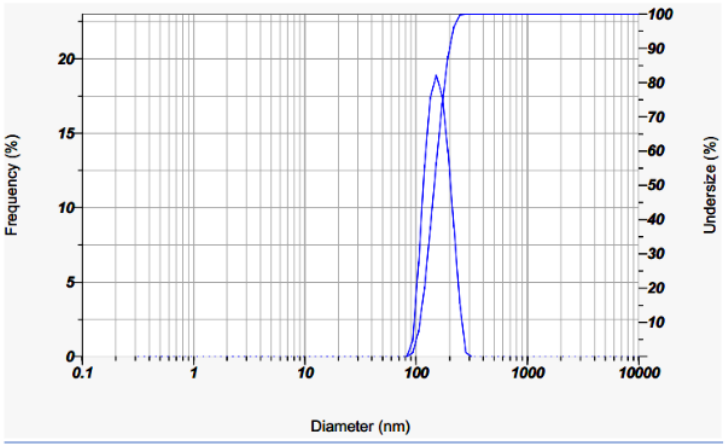

Figure 2. *Jatropha curcas* nanoemulsion particle size using inversion phase

Discussion

The study examined the effectiveness of botanical insecticides, such as *M. fragrans* and *J. curcas* essential oil formulations, on adult *S. zeamais* beetles using contact, seed dressing, and fumigation methods. This assessment was conducted 96 hours after exposing the *S. zeamais* insects to these two types of botanical insecticides. An application of *M. fragrans* nanoemulsion at a concentration of 0.67% resulted in a 50% mortality rate among the *S. zeamais* pests after 96 hours of exposure. Furthermore, to achieve a 95% toxicity level of *M. fragrans* towards the tested insect population, a concentration of 4.38% was found to be necessary. It's worth noting that the toxicity of *J. curcas* was found to be lower compared to that of *M. fragrans*. The toxic activity against 50% and 95% of the *S. zeamais* population was observed at concentrations of 0.67% and 5.23%, respectively, as detailed in Table 1.

The toxic effect of *M. fragrans* essential oil as a natural insecticide have been documented in previous studies (Abdullah et al. 2021; Mssillou et al. 2022). These studies have highlighted its efficacy against various pests, including adult female German cockroach *(B. germanica)*, larvae, and adult Red flour beetle (*T. castaneum)*, as well as *C. maculatus*. Abou-Elnaga (2014) demonstrated that *M. fragrans* essential oil exhibits larvicidal activity against third instar larvae of *C. pipiens* and *A. aegypti*, both of which belong to the Culicidae (Diptera) family.

Ashokkumar et al. (2022) reported that the essential oil of *M. fragrans*, extracted using various methods, contains major chemical components such as sabinene, eugenol, myristicin, caryophyllene, β-myrcene, and α-pinene. Furthermore, it has been confirmed that *M. fragrans* essential oil exhibits antimicrobial, anti-inflammatory, anticancer, antimalarial, hepatoprotective, antiparasitic, insecticidal, and nematicidal activities. The main phytochemical compound discovered in *M. fragrans* essential oil were predominantly terpene hydrocarbons, including sabinene and pinenes, along with camphene, pcymene, phellandrene, terpinene, limonene and myrcene. Terpene derivatives contributed to about 60 to 80% of the composition with linalool, geraniol, and terpineol, making up around 5 to 15%. Additionally, phenylpropanoids such as myristicin, elemicin, safrole, eugenol, eugenol, and their derivatives, constituted approximately 15 to 20% of the oil. Myristicin, a form of methoxy-safrole, typically accounts for around 4% of the total composition. In vivo and in vitro sublethal treatments of trimyristin and myristicin caused a significant inhibition of acetylcholinesterase (AChE), acid phosphatase (ACP), and alkaline phosphatase (ALP) activities in the nervous tissue of *L. acuminate* (Jaiswal et al. 2009).

The results showed that the nanoemulsion of *M. fragrans* and *J. curcas* seeds also had activity in inhibiting population development, intensity of damage, and weight loss. The contact method gave the highest activity for all observed parameters compared to the fumigation and seed dressing methods for both botanical insecticides from essential oils of *M. fragrans* and *J. curcas* seeds. The toxicity of *J. curcas* is associated with several secondary metabolite components it contains, such as saponins, lectins (curcins), phytates, protease inhibitors, curcalonic acid, and phorbol esters (Choudhary et al. 2021). According to (Choi 2019; Li et al. 2022), phorbol ester is a tetracyclic diterpenoid that is generally known for its tumor-inducing activity. Phorbol ester mimics the action of diacyl glycerol (DAG), an activator of protein kinase C, which regulates different signal transduction pathways and other cellular metabolic activities. They occur naturally in many plants of the Euphorbiacaeae and Thymelaeaceae families. The biological activity of phorbol ester is very structurally specific. Forbol ester, even at very low concentrations, shows toxicological manifestations in animals fed diets containing phorbol ester. Detoxification ability has also been reported in several mollusks and rat liver homogenates. Besides having antinutritional and toxic effects, some phorbol ester derivatives are also known for

their antimicrobial and antitumor activities. The molluscicide and insecticidal properties of phorbol ester indicate its potential to be used as an effective biopesticide and insecticide.

Creating a nanoemulsion formula from *M. fragrans* and *J. curcas* using the inversion phase emulsification method, along with the incorporation of polysorbate as an emulsifier, is capable of generating particle sizes below 200 nm. The average particle size of the nanoemulsion derived from *M. fragrans* is smaller than that of *J. curcas*, yet both qualify as nanoemulsions. The inversion phase emulsification method effectively reduces particle sizes to the nanometer scale, as documented by Asadinezhad et al. (2019). This process involves transferring the oil phase into the liquid phase as droplets, thereby increasing liquid pressure, leading to the fragmentation of oil droplets into smaller sizes and their dispersion within the liquid phase, as explained by Lu and Wang (2023).

The emulsification technique plays a pivotal role in determining the size of the nanoemulsion. Nuryanti et al. (2018), reported the usage of polysorbate as an emulsifier also contributes to nanoemulsion size. A higher concentration of emulsifier results in the production of smaller nanoemulsion particles. According to Choupanian et al. (2017), the incorporation of more than 1.5 times polysorbate and alkyl polyglucoside as an emulsifier in a neem oil formulation can reduce particle size to less than 100 nm. Smaller particle sizes enhance the toxicity of the nanoemulsion due to the increased dispersion of particles that target insects.

This present study suggested that the contact method using *M. fragrans* and *J. curcas* essential oil formulation had the most promising potential to be developed as a botanical pesticide for *S. zeamais*. However, further study is required to validate these reports.

In conclusion, the nanoemulsion formulations of *M. fragrans* and *J. curcas* displayed toxicity to adult *S. zeamais* by contact, seed dressing, and fumigation activities. The stable pattern of toxicity observed in the *M. fragrans* nanoemulsion formulation remained unchanged after 72 and 96 hours. Among the three methods, the contact method displayed the highest level of toxicity, followed by the seed dressing method, and the fumigation method showed the lowest level of toxicity consistently. The LC_{95} values for these methods were recorded at 4.38%, 6.24%, and 10.60%, respectively. Furthermore, the use of botanical insecticides derived from *M. fragrans* through contact, fumigation, and seed dressing methods exhibited greater efficacy in population growth inhibitation (18.00%), damage intensity (16.81%), and seed weight loss (13.96%). The *M. fragrans* nanoemulsion demonstrated a particle size distribution of approximately 17.5% with an average particle size of 76 nm. The particle size distribution of *J. curcas* nanoemulsion, in contrast, peaked at approximately 18.5%, with an average particle size of roughly 82 nm. Consequently, there is potential to progress the expansion of nanoemulsion formulations sourced from *M. fragrans* for the management of *S. zeamais*.

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