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ORIGINAL ARTICLE

Evaluation of citronella oil nanoemulsion formulation against the insect-stored pest *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae)

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Abstract

Citronella (*Cymbopogon nardus*) produces essential oil and has the potential to be developed as a botanical insecticide. However, in its development, botanical insecticides encountered several obstacles. Utilizing nanotechnology in nanoemulsion preparations is one method to overcome these challenges. This research aimed to determine the contents of the citronella oil nanoemulsion (CiONano) and citronella oil non-nano emulsion (CiONonNano) formulations and evaluate the toxicity, repellency, and prevention of oviposition against female adults of *Callosobruchus maculatus*. This was the first work to evaluate the nanoemulsion of citronella oil prepared from spontaneous emulsification against *C. maculatus*. Chemical content testing used the GCMS method. A toxicity test using the contact method (LC_{50}), used a probit program, while testing for repellency and oviposition deterrence was carried out using the no-choice method. The highest chemical component of CiONonNano and CiONano was citronella (37.56 and 38.97%, respectively), followed by citronellol (17.71 and 18.99%, consecutively) and geraniol (14.78 and 15.38%, respectively). In general, the CiONano formulation showed higher repellency and toxicity than CiONonNano. The LC_{50} values of CiONano were 10.03%. These values were 4.49 times lower than the LC_{50} of CiONonNano. However, the results of the oviposition inhibition test showed different results, the CiONonNano formulation had a higher oviposition inhibition capacity for adult female *C. maculatus* than CiONano. As a result, it is necessary to optimize the CiONano formulation to obtain consistent results in controlling *C. maculatus.*

Keywords: *Cymbopogon nardus*, essential oil, oviposition deterrent, repellency, toxicity

Introduction

The *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) insect is a highly destructive pest that causes significant damage to various types of beans, including green beans, kidney beans, lentils, black lentils, soybeans, and others (Nisar *et al.* 2021; Mansouri *et al.* 2022). These insects, which threaten grains, often enter and establish themselves in storage facilities due to local microclimates and inadequate retention measures during processing and storage (Hagstrum and Phillips 2017). This insect can damage cowpea seeds up to 100% in 60 days (depending on the variety) (Nwosu and Ikodie 2021). Historically, controlling storage pests has involved the use of chemical fumigation methods, such as phosphine or other fumigants like carbonyl sulfide (Daglish *et al.* 2018), as well as synthetic insecticides, such as organophosphates, organochlorines, carbamates, malathion, and others. These methods offer faster and more practical results. However, their excessive and improper use can lead to insect resistance, health issues, and environmental problems (Daglish *et al.* 2018; Kalpna *et al.* 2022). Phosphine resistance in insects has become a global concern, as reported in several countries (Wakil *et al.* 2021). To overcome these various problems, utilizing botanical insecticides can be an alternative method of controlling *C. maculatus* (Rohimatun *et al.* 2023).

Indonesia has a variety of spice and medicinal plants that can be used as botanical insecticides, such as citronella [*Cymbopogon nardus* (L.) Rendl. (Poaceae)]. Citronella produces essential oils as botanical insecticides (Kaur *et al.* 2021). Citronella oil has several chemical compounds, including citronellal, geraniol, citronellol, germacrene-D, elemol, limonene, linalool, and others (Caballero-Gallardo *et al.* 2021; Kaur *et al.* 2021). The chemical compounds can affect pests through insecticidal and repellency activities. Repellent activity and toxicity also work on several storage pests, such as *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Hernandez-Lambraño *et al.* 2015), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae) (Loko *et al.* 2021) and *Callosobruchus maculatus* (Hassan *et al.* 2018).

Increasing food demand and the requisiting of safe control for storage pests have led to a scientific focus on developing environmentally friendly pesticides, one of them being the development of nanotechnology (Jasrotia *et al.* 2022). Nanotechnology has the potential to improve the effectiveness and longevity of active compounds, and reduce agricultural inputs while overcoming the drawbacks of conventional pesticides (Jasrotia *et al.* 2022; Kumar *et al.* 2019). The development of the nanotechnology process can improve its absorptivity, clarity, stability, and activity due to the small particle size, which makes the particle`s surface wider (McClements 2012). The application of nano-biopesticides for storage pest management is still limited, and information regarding their synthesis, variations, effectiveness, and mode of action is still lacking (Singh *et al.* 2021). The development of nano-particles derived from active substances in plants as insecticides is substantial due to its various advantages (Baliyarsingh and Chandan 2023). Therefore, optimizing the role of citronella as a botanical insecticide against storage pests can be done through technological development into nanoemulsion form.

The essential oil-based nanoemulsions have advantages in contemporary agriculture and environmentally friendly pest control (Du *et al.* 2016; Mossa *et al.* 2019). Nanoemulsions are water-based, and their production requires significantly fewer organic solvents than conventional emulsion concentrates (Du *et al.* 2016; Kaur *et al.* 2021). Additionally, Kaur *et al.* (2021) stated that nanoemulsions are an efficient strategy to increase the stability of the characteristics of bioactive materials, reduce volatility, and prevent environmental impacts. In this work we evaluated nanoemulsion of citronella oil prepared from spontaneous emulsification against *C. maculatus*. Thus, this research aimed to identify the chemical components and evaluate the toxicity, repellency, and oviposition deterrence of citronella nanoemulsion formulations against the storage pest *C. maculatus* in the laboratory. In the future, the results of this research can be considered for application in the field or storage.

Materials and Methods

Preparation of citronella oil nanoemulsion formulation

Citronella oil was purchased from the Ministry of Agriculture's experimental garden in Manoko, West Bandung. Citronella oil was obtained by distilling the leaves and stems of the citronella plant using the steaming method. Then it was formulated as CioNonNano. CioNano nanoemulsion was prepared using a low--energy technique by spontaneous emulsification. The Tween 80 used in this research was the analytic reagent CAS 9005-65-6. This technique consists of two mixing phases, namely the organic phase (10% citronella oil, surfactant and cosurfactant at a specific concentration) and the water phase (distilled water and cosurfactant) to produce a nano-sized formulation. The particle size of the formulation was obtained by using a Particle Analyzer Machine. The droplet sizes were around 90–160 nm with PDI of 0.2–0.4 and zeta potential of –28 to –8.8 mV (Yuliani and Noveriza 2019).

Insect mass rearing

The insect of *C. maculatus* was acquired from Pasar Anyar in Bogor. *C. maculatus* was reared in an air-conditioned room with at 27 ± 2°C, relative humidity of 75 ± 2%, and a photoperiodism of 12 h. Some *C. maculatus* insects were placed in jars (16 cm in diameter, 16.3 cm in height) containing mung beans as feed and insect breeding medium. The jars were covered with gauze for air circulation. *C. maculatus* adults were transferred to a new jar containing fresh mung beans every 14 days. Female adults of *C. maculatus* aged 2–3 days were used for treatment.

GC-MS analysis

Identification of the active compounds of CiONonNano and CiONano formulation using GC-MS (Gas Chromatography-Mass Spectrometry) analysis

(Agilent Technologies 7890 Gas Chromatography with auto sampler and 5975 Mass Selective Detector and Chemstation data system). The conditions used were an HP Ultra 2 column with a capillary length of 30 m × 0.20 mmI.D; injection volume 5 ml, temperature 250°C with program temperature 80–280°C (26 min) and constant gas flow mode with Helium carrier gas. The data obtained was compared to 5795 Mess Selective Detector and Chemstation data systems) in the Regional Health Laboratory of DKI Jakarta Province.

Toxicity test

The CiONonNano and CiONano preparations were tested with concentrations of 0.05, 1.00, 2.00, 4.00, 8.00 and 16.00% (v/v) and the control (acetone) that were repeated five times by using the fumigation method modified from da Silva Moura *et al.* (2019) and Ismail (2022). Each treatment was dissolved with acetone. The study used filter paper glued to a Petri dish (diameter 8 cm). A total of 0.5 ml of CiONonNano and CiONano solutions according to the treatment concentration was dropped onto filter paper in a spiral using a micropipette from inside to outside. The filter paper was left until there was no smell of acetone for 1–2 min. A total of 10 female adults of *C. maculatus* were put into a Petri dish and were given 5 g of green beans as feed. Then, the Petri dish was closed quickly and sealed with food-grade plastic to prevent oil evaporation during exposure. Mortality observation was conducted at 72 days after treatment (DAT). The data of C. *maculatus* mortality was analyzed by the probit method to determine lethal concentration (LC) values using the PoloPlus Ver 2.0 Program (Rohimatun *et al.* 2020).

Repellency test

The repellency test was carried out using the filter paper method (Boodram and Khan 2019) with five replications. Whatman paper (diameter 8 cm) was cut into two parts and glued to a Petri dish (diameter 9 cm). The filter paper was divided into two parts, one for citronella oil treatment and the other for control. As much as 0.50 ml of CiONano and CiONonNano solution treatment (concentrations of 0.25, 0.50, 1.00, and 2.00%) and control (acetone) was dripped onto the filter paper until the acetone evaporated. A total of 10 female adults of *C. maculatus* were put in the middle of the Petri dish. Observations were made 15, 30, and 45 min after treatment (MAT) and 1, 3, 6, 24, 48, and 72 h after treatment (HAT) by calculating the number of *C. maculatus* in the control and treatment sides. The inhibition percentage was calculated using the formula:

$$\text{PR (\%)} = \frac{(\text{NC} - \text{NT})}{(\text{NC} + \text{NT})} \times 100\%,$$

where: PR – repellency (%); NC – number of insects on control paper; NT – number of insects on treated paper.

The repellency (%) was categorized into six classes, namely 0 with 0.00–0.10% repellency, I with 0.11–20.00% repellency, II with 20.10–40.00% repellency, III with 40.10–60.00% repellency, IV with 60.10–80.00% repellency, and V with 80.10–100% repellency. The repellency index (RI) was calculated with the formula:

$$\text{RI} = \frac{2\text{G}}{(\text{G} + \text{P})} \times 100\%,$$

where: G – insects attracted to the treatment (%); P – insects attracted to the control (%) (Jilani and Su 1983). The RI values ranged between zero and two. The RI > 1 means indicated the treatment's attractiveness and lower repellency than the control. RI = 1 indicates neutral treatment, which means similar repellency between the treatment and the control. RI < 1 shows repellent treatment (a more outstanding repellency of the treatment than the control) (Padín *et al.* 2013).

Oviposition deterrent test

The oviposition deterrent test also used the filter paper method (Boodram and Khan 2019). Five concentrations (2.00, 1.00, 0.50, 0.25%, and the control) were used as the treatment with five replications. The 0.50 ml preparation of the treatment and control were dripped circularly and evenly on the surface of the filter paper and then left until the acetone evaporated. Furthermore, 5 g mung beans were placed on the filter paper, followed by 10 female adults of *C. maculatus*. The observations included calculating the number of eggs oviposited on the mung bean from 1 day after treatment until no eggs were laid (day 10). The oviposition deterrent was calculated based on the formula:

$$\text{OD} = \frac{\text{EC - ET}}{\text{EC + ET}} \times 100\%,$$

where: OD – oviposition deterrent (%); ET – number of eggs laid on the treatment; and EC – number of eggs laid on control. The percentage of repellency and oviposition deterrents were variance analyzed using the IBM SPSS Statistics 26.0 program (IBM SPSS Statistic Version 26.0 2019). If there was a significant difference, it was continued with the Tukey test with a 5% confidence interval.

Results

Chemical composition of citronella oil formulation

The highest component of both CiONonNano and CiONano was citronellal (37.56 and 38.97%), followed by citronellol (17.71 and 18.99%, respectively) and geraniol (14.78 and 15.39%, respectively). Detailed CiONonNano and CiONano GCMC analysis results are presented in Table 1.

Toxicity of CiONano and CiONonNano

The treatment of citronella oil can lead to the mortality of *C. maculatus* inline with an increase in treatment concentration and duration of exposure. Figures 1 and 2 showed the correlation between concentration and mortality of *C. macullatus* treated with CiONano and CiONonNano. In this study, the CiONano formulation caused higher mortality to *C. maculatus* than CiONonNano. CiONano was more toxic to female adults of *C. maculatus*, as indicated by lower LC_{50} values than CiONonNano (10.03% versus 45.00%) (Table 2). The LC_{50} values shown by CiONano 10.03% were 4.49 times higher than CiONonNano.

Repellency and Repellent Index of CiONano and CiONonNano

All treatment concentrations, both CiONonNano and CiONano, showed repellency against female adults of *C. maculatus*. In general, it can be seen that CiONano showed higher repellency than CiONonNano. Furthermore, the percentage of repellency of CiONano seemed to be more stable, up to 6 HAT, while in CiONonNano, it was only up to 45 MAT (Table 3).

In the CiONonNano treatment, all treatment concentrations showed a repellency >80% up to 45 MAT. In 1 HAT, the CiONonNano treatment also showed > 80% repellency, except in the 1.00% treatment. At 3 HAT, the repellency of CiONonNano decreased, except for the 2.00% treatment. At 24 HAT, treatments 0.25 and 0.50% showed 68.00% repulsion (Class IV), while 1.00 and 2.00% treatments showed more reduction, with 60.00 and 56.00% (Class III) repellency, respectively. At 48 and 72 HAT, 0.25% of the treatment

Table 1. Chemical composition of CiONonNano and CiONano based on GC-MS analysis

No.	Chemical constituents	Molecule	Retention Time [minutes]		Concentration [%]	
			CiONonNano	CiONano	CiONonNano	CiONano
1.	D-Limonene	$C_{10}H_{16}$	9.833	11.508		3.77
2.	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$		14.424		1.04
3.	Citronellal	$C_{10}H_{20}O$	24.284	25.238	37.56	38.97
4.	Linalool	$C_{10}H_{18}O_2$	26.041		1.19	
5.	(1R,2R,5S)-5-Methyl-2-(pro-l-e-2-yl) cyclohexanol	$C_{10}H_{18}O$	26.518		2.18	
6.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-, [1S-(1. α., 2. α., 4. β.)]- / Elemene	$C_{15}H_{24}$	27.346	1.89		
7.	dl-Isopulegol	$C_{10}H_{18}O$		29.985		1.26
8.	Caryophyllene	$C_{15}H_{24}$	27.505	32.066	2.72	2.61
9.	2,6-Octadiene,2,6-dimethyl-	$C_{10}H_{18}$	29.147	35.319	2.17	1.86
10.	Germacrene D	$C_{15}H_{24}$	30.342	38.002	1.12	1.32
11.	Geranyl acetate	$C_{12}H_{20}O_2$		40.238		1.10
12.	Citronellol	$C_{10}H_{20}O$	31.685	40.529	17.71	18.99
13.	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$	31.748		1.06	
14.	Geraniol	$C_{10}H_{18}O$	33.452	44.576	14.78	15.39
15.	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$		48.337		1.04
16.	Benzene, (1-butyloctyl)-	$C_{18}H_{30}$		48.529		1.92
17.	Benzene, (1-ethyldecyl)-	$C_{18}H_{30}$		49.277		1.89
18.	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$		50.039		1.75
19.	Cyclohexanemethanol, 4-ethenyl-.α., .α., 4-trimethyl-3-(1-methylethrnyl)-,[1R-(.α.,3.α.,4.β.)-	$C_{15}H_{26}O$	38.109	50.463	1.81	1.36
20.	Glycerin	$C_3H_8O_3$		53.806		1.96

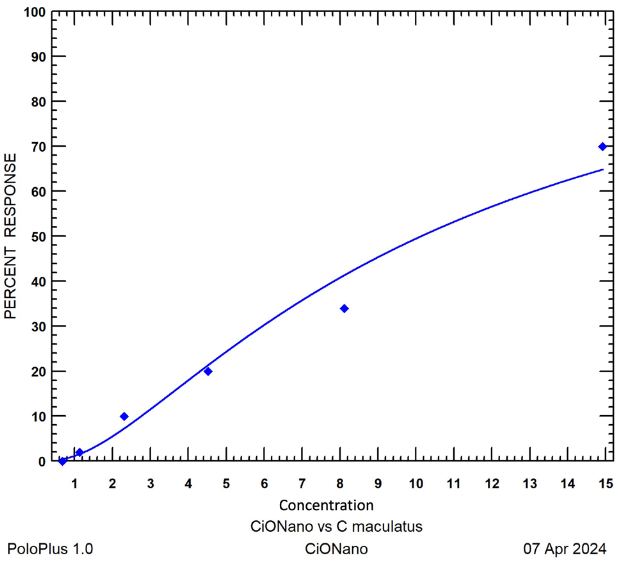


Fig. 1. The correlation between concentration and mortality of *Callosobruchus macullatus* treaten with CiONano

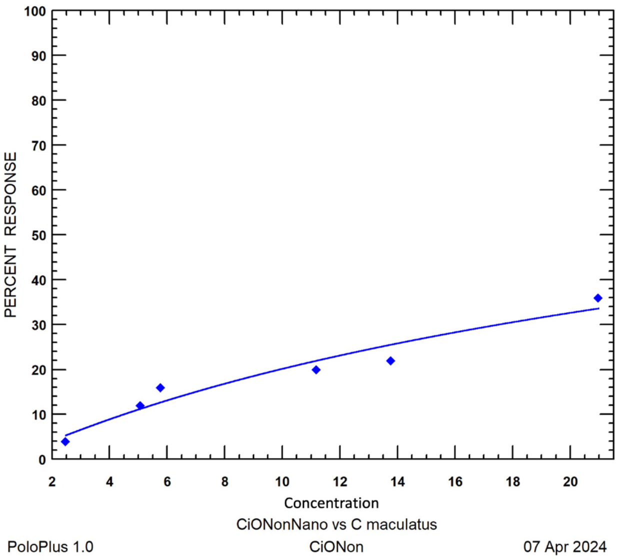


Fig. 2. The correlation between concentration and mortality of *Callosobruchus macullatus* treaten with CiONonNano

was seen to be more stable against female imago of *C. maculatus*, which was indicated by the same repellency class. There was a 4% decrease in repellency in the CiONonNano 0.50%. The CiONonNano 1.00 and 2.00% showed an increase in the repellency of 24 and 20.00%, respectively. This CiONonNano formulation tended to be unstable after 1 HAT.

The CiONano treatment in Table 3 showed repellency > 80.10% (Class V) at all concentration levels until 3 HAT, except at a concentration of 0.25%. In the 0.25% CiONano treatment at 6 HAT, there was a 20% decrease in repellency; at 24 HAT, there was a very sharp decrease, namely 44%. At 48 and 72 HAT, the durable CiONano percentage repellency was in class IV.

The repellency in class V was stable in the CiONano at 0.50%. However, the CiONano 0.50% were in class III at 6 and 24 HAT. The CiONano 1.00% also showed a long-lasting result until 3 HAT, but it decreased at 6 HAT, being in class IV. The percentage repellency in the CiONano 1.00% treatment at 24 HAT increased again and was stable at 48 and 72 HAT. The treatment of 2.00% CiONano was more stable than the other treatments. From 15 MAT to 3 HAT, the repellency was stable in class V, decreased at 6 HAT, and stabilized at 24, 48, and 72 HAT in class IV. From Table 3, all treatments showed repellency to *C. maculatus*. However, when the repellency index (IR) was calculated, the CiONonNano treatment was in the attractive category starting from 3 HAT. In contrast, the CiONano treatment at all exposure times showed the repellency category at all observation times (Table 4).

Oviposition deterrent

The results of the oviposition deterrent test showed that CiONano and CiONonNano could inhibit the oviposition of female adults of *C. maculatus*. However, from the first day until the last observation, the female adults of *C. maculatus* laid fewer eggs in the CiONonNano treatment than CiONano (Figure 5 and Table 5).

Oviposition deterrent CiONonNano at all concentrations was also higher than CioNano treatment and statistically significant. The difference in oviposition deterrent of the two at concentrations of 0.25, 0.50, 1.00, and 2.00% were 27.07, 26.90, 27.22 and 31.28%,

Table 2. Toxicity of CiONonNano and CiONano against adult females of *Callosobruchus maculatus*

Citronella oil formula	a ± SE	b ± SE	LC_{50} (CI 95%) [%]	Chi-square	Hetero-geneity
CiONano	2.56 ± 0.27	2.44 ± 0.31	10.03 (8.28–12.88)	3.44	0.86
CiONonNano	2.88 ± 0.313	1.28 ± 0.31	45.00 (25.743–189.871)	1.28	0.32

a – intercept, b – slope, CI – confidence interval

Table 3. Repellency (%) of CiONonNano and CiONano against adult females of *Callosobruchus maculatus*

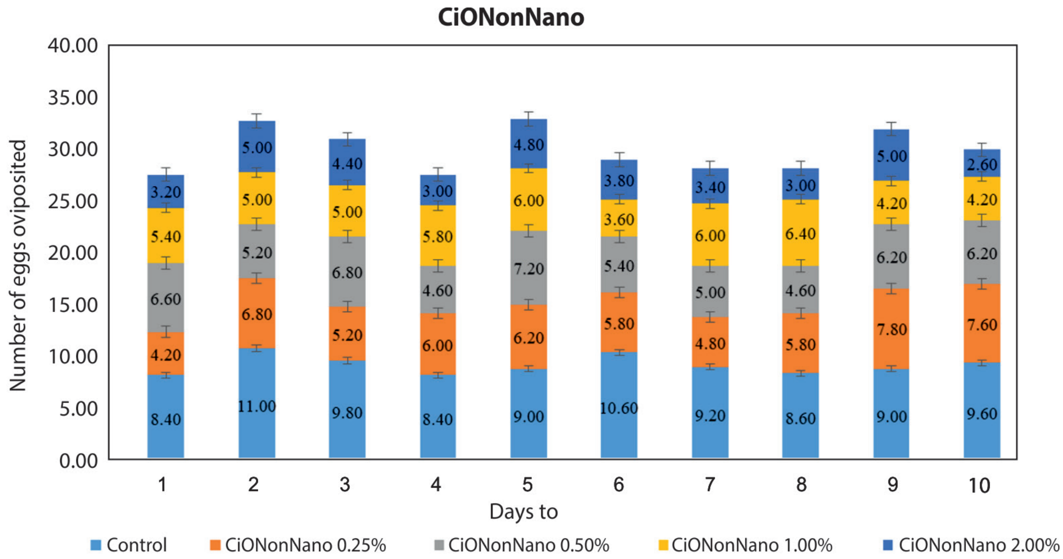
Time	Repellency percentage							
	CiONonNano concentration							
	0.25% ± SEM	class	0.50 % ± SEM	class	1.00 % ± SEM	class	2.00 % ± SEM	class
15 MAT	92.00 ± 4.90 a	V	96.00 ± 4.00 a	V	92.00 ± 8.00 a	V	100.00 ± 0.00 a	V
30 MAT	84.00 ± 7.48 a	V	96.00 ± 4.00 a	V	92.00 ± 4.90 a	V	100.00 ± 0.00 a	V
45 MAT	84.00 ± 4.00 a	V	100.00 ± 0.00 a	V	84.00 ± 4.00 ab	V	96.00 ± 4.00 a	V
1 HAT	96.00 ± 4.00 a	V	84.00 ± 4.00 ab	V	68.00 ± 10.20 abc	IV	96.00 ± 4.00 a	V
3 HAT	68.00 ± 18.55 a	IV	64.00 ± 17.20 abc	IV	32.00 ± 20.60 bc	II	88.00 ± 4.90 ab	V
6 HAT	48.00 ± 17.44 ab	III	40.00 ± 15.49 c	II	12.00 ± 27.28 c	I	28.00 ± 8.00 c	II
24 HAT	68.00 ± 8.00 a	IV	68.00 ± 4.90 abc	IV	60.00 ± 14.14 abc	III	56.00 ± 11.66 bc	III
48 HAT	65.33 ± 7.42 a	IV	76.00 ± 11.66 abc	IV	60.00 ± 16.73 abc	III	64.00 ± 17.20 abc	IV
72 HAT	64.00 ± 9.80 ab	IV	60.00 ± 15.49 abc	III	84.00 ± 4.00 ab	V	84.00 ± 7.48 ab	V
	CiONano concentration							
15 MAT	100.00 ± 0.00 a	V	96.00 ± 4.00 a	V	100.00 ± 0.00 a	V	100.00 ± 0.00 a	V
30 MAT	100.00 ± 0.00 a	V	96.00 ± 4.00 a	V	100.00 ± 0.00 a	V	96.00 ± 4.00 a	V
45 MAT	92.00 ± 4.90 a	V	100.00 ± 0.00 a	V	100.00 ± 0.00 a	V	96.00 ± 4.00 a	V
1 HAT	96.00 ± 4.00 a	V	100.00 ± 0.00 a	V	100.00 ± 0.00 a	V	92.00 ± 4.90 ab	V
3 HAT	72.00 ± 13.56 a	IV	100.00 ± 0.00 a	V	88.00 ± 8.00 ab	V	84.00 ± 7.48 ab	V
6 HAT	52.00 ± 22.45 ab	III	48.00 ± 8.00 bc	III	72.00 ± 4.90 ab	IV	72.00 ± 4.90 ab	IV
24 HAT	8.00 ± 22.45 b	I	60.00 ± 12.65 abc	III	80.00 ± 8.94 ab	V	80.00 ± 8.94 ab	IV
48 HAT	80.00 ± 6.32 a	IV	88.00 ± 4.90 ab	V	68.00 ± 14.97 abc	IV	80.00 ± 6.32 ab	IV
72 HAT	80.00 ± 0.00 a	IV	84.00 ± 7.48 ab	V	80.00 ± 6.32 ab	IV	68.00 ± 13.56 ab	IV
f	4.24		5.25		4.51		6.18	
Sig.	0.00		0.00		0.00		0.00	

SEM – standard error of means; MAT – minutes after treatment; HAT – hours after treatment. Numbers followed by the same letters in the same column are not significantly different based on Tukey 5% ($p < 0.05$)

Table 4. Repellent Index (RI) of CiONonNano and CiONano against the female adult of *Callosobruchus maculatus*

Time	0.25%		0.50%		1.00%		2.00%	
	RI ± SEM	classification	RI ± SEM	classification	RI ± SEM	classification	RI ± SEM	classification
CiONonNano concentration								
15 MAT	0.36 ± 0.22	repellent	0.18 ± 0.18	repellent	0.36 ± 0.36	repellent	0.00 ± 0.00	repellent
30 MAT	0.72 ± 0.34	repellent	0.18 ± 0.18	repellent	0.36 ± 0.22	repellent	0.00 ± 0.00	repellent
45 MAT	0.72 ± 0.18	repellent	0.00 ± 0.00	repellent	0.72 ± 0.18	repellent	0.18 ± 0.18	repellent
1 HAT	0.18 ± 0.18	repellent	0.72 ± 0.18	repellent	1.44 ± 0.46	attractant	0.18 ± 0.18	repellent
3 HAT	1.44 ± 0.83	attractant	1.62 ± 0.77	attractant	3.06 ± 0.93	attractant	0.54 ± 0.22	repellent
6 HAT	2.34 ± 0.78	attractant	2.70 ± 0.70	attractant	3.96 ± 1.23	attractant	3.24 ± 0.36	attractant
24 HAT	1.44 ± 0.36	attractant	1.44 ± 0.22	attractant	1.80 ± 0.64	attractant	1.98 ± 0.52	attractant
48 HAT	1.56 ± 0.33	attractant	1.08 ± 0.53	attractant	1.80 ± 0.75	attractant	1.62 ± 0.77	attractant
72 HAT	1.62 ± 0.44	attractant	1.80 ± 0.75	attractant	0.72 ± 0.18	repellent	0.72 ± 0.34	repellent
CiONano concentration								
15 MAT	0.00 ± 0.00	repellent	0.04 ± 0.04	repellent	0.00 ± 0.00	repellent	0.00 ± 0.00	repellent
30 MAT	0.00 ± 0.00	repellent	0.04 ± 0.04	repellent	0.00 ± 0.00	repellent	0.04 ± 0.04	repellent
45 MAT	0.08 ± 0.05	repellent	0.00 ± 0.00	repellent	0.00 ± 0.00	repellent	0.04 ± 0.04	repellent
1 HAT	0.04 ± 0.04	repellent	0.00 ± 0.00	repellent	0.00 ± 0.00	repellent	0.08 ± 0.05	repellent
3 HAT	0.28 ± 1.36	repellent	0.00 ± 0.00	repellent	0.12 ± 0.08	repellent	0.16 ± 0.07	repellent
6 HAT	0.48 ± 0.22	repellent	0.52 ± 0.08	repellent	0.28 ± 0.05	repellent	0.28 ± 0.05	repellent
24 HAT	0.22 ± 0.06	repellent	0.92 ± 0.22	repellent	0.40 ± 0.13	repellent	0.20 ± 0.09	repellent
48 HAT	0.20 ± 0.00	repellent	0.12 ± 0.05	repellent	0.32 ± 0.15	repellent	0.20 ± 0.06	repellent
72 HAT	0.20 ±	repellent	0.16 ± 0.07	repellent	0.20 ± 0.06	repellent	0.32 ± 0.14	repellent
f	4.20		5.61		6.10		10.71	
Sig.	0.00		0.00		0.00		0.00	

SEM – standard error of means



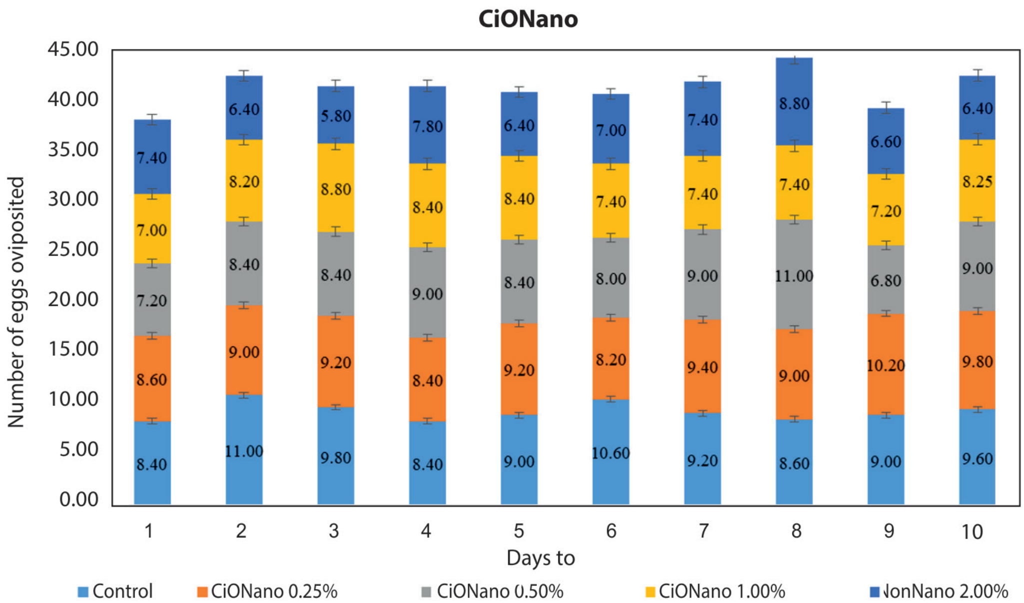


Fig. 3. Number of eggs oviposited by adult female *Callosobruchus maculatus* day 1 until 10 on mung bean placed on paper that had been treated with CiONano and CiONonNano

Table 5. Oviposition deterrent of CiONonNano and CiONano against female adult of *Callosobruchus maculatus*

Treatment	Concentration	Oviposition deterrent ± SEM
CiONonNano	0.25%	51.22 ± 3.84 abc
CiONano		24.15 ± 1.80 d
CiONonNano	0.50%	53.16 ± 6.60 abc
CiONano		26.26 ± 4.43 d
CiONonNano	1.00%	58.18 ± 11.57 ab
CiONano		30.96 ± 4.95 cd
CiONonNano	2.00%	69.04 ± 8.82 a
CiONano		37.76 ± 0.75 bcd

SEM – standart error; $p < 0.05$

respectively (Table 5).

Discussion

Optimizing formulation through nanotechnology can increase the solubility, absorbency, efficiency, and effectiveness of the absorption of active ingredients. The release of active ingredients is more controlled and can protect from critical and other factors (Mossa *et al.* 2019). Some of the chemical contents in CioNano, which function as insecticides, are higher than in CioNonNano. Apart from that, some chemical ingredients also only appear in CioNano. It shows that nanoemulsions have the potential to be developed because of their various advantages. Droplet size was one factor that influenced the difference in the test results of CiONonNano and CiONano toxicity against *C. maculatus*. Because of the smaller droplets, more particles can enter the insect's body through the spiracle and cuticle (Saed *et al.* 2022) because they are tiny (< 200 nm) and kinetically more stable (Aswathanarayan and Vittal 2019). The CioNano in this research was more toxic than CiONonNano. The LC_{50} and LC_{95} values of CiONano were 4.49 and 121.12 times lower than CiONonNano. It could be caused by CiONano droplet size being between 70–140 nm (average 114.50 nm), while in CiONonNano, it was around 1740–5262 nm. In addition, the CiONonNano is more heterogeneous than CioNano (Yuliani *et al.* 2023).

Besides having toxicity effects on *C. maculatus*, citronella oil can repel insects (dos Santos *et al.* 2022). The CiONano generally has a higher RI than CiONonNano, with the percentage repellency >90% up to 3 HAT. However, along with the exposure time, the repellency percentage fluctuated. However, the CiONano treatment appeared to be more stable than CiONonNano. Decreasing repellence value over time is common in tests using essential oils. The reduction in repellency can be affected by a decline in active components during the treatment process and exposure time. The use of kaffir lime leaf essential oil at 24 h also decreased the repellency index (Fajarwati *et al.* 2015), although, in the middle of the observation, there was an increase.

The essential oils in plants also produce odors and have different effects depending on the organ of the plant that produces them. Essential oils in flowers can be used to help pollinate, while in fruit they can be used for distribution media to seeds, and in leaves and stems they function as an insect repellent. It is evident from our study that they operate similarly for the beetle species under consideration. *T. castaneum* can also be repelled by essential oils from other Poaceae genera, specifically *Cymbopogon martinii* (Roxb) Wats. and *Cymbopogon flexuosus* (Nees ex Steud.) Watson (Caballero-Gallardo *et al.* 2021), which is related to *C. nardus*. Active ingredient molecules CioNano make it easier to enter the insect's body extracellularly and then be captured by chemoreceptors on the cilia located in the antennae. The presence of these foreign materials can cause nerve depolarization, which will trigger the transmission of electrical impulses to the antennae lobes of insects to elicit a rejection response or block the sense of smell, which in turn acts as a barrier for insects to recognize their hosts (Brito *et al.* 2020). As a result, the CioNano is better than CioNonNano in repelling female adults of *C. maculatus.*

Citronella oil has proven capable of deterring insect oviposition (Caballero-Gallardo *et al.* 2021). Citronella oil has been shown to reduce the number of eggs produced by *C. maculatus* (de Souza Alves *et al.* 2019). Citronellol, geraniol, and citronellal contained in citronella oil are monoterpenes (Kaur *et al.* 2021). They promise alternative fumigants and effects on biological parameters, such as growth rate, life span, and reproduction of insects. The other chemical compound, namely d-limonene in CiONano of 3.77%, plays an important role as an insecticide, which has mortality, ovicidal and repellent effects (de Andrade Rodrigues *et al.* 2022).

Citronella oil is toxic and has repellent activity on *C. maculatus*. It also significantly decreased the oviposition rate and emergence of adults (de Souza Alves *et al.* 2019). CioNonNano and CiONano treatments could deter egg laying by the adult female of *C. maculatus*. However, at the same concentration, CiONonNano was more effective in preventing the egg laying of female imago than CiONano and was significantly different. Factors that affect the results of the oviposition inhibition test include insects, method, concentration, and duration of exposure. In this treatment, the adult female population in CiONano had higher fecundity than CiONonNano. The higher the concentration of treatment, the higher the inhibition of oviposition of female *C. maculatus*. In this treatment, the egg number was observed until the last imago laid its eggs (day 10). This long exposure time causes the active ingredient CiONano, which has a smaller particle size than CiONonNano, to evaporate faster. It is required to optimize nanoemulsion, in order for it to produce consistent performance for controlling storage pests.

Conclusion

The essential oil from citronella had functions as a botanical insecticide and had a toxic effect, repellent activity, and oviposition deterrent against female adults of *C. maculatus*. The citronella oil formulated in nanoemulsion (CiONano) was more toxic and more repellant than non-nanoformulation (CiONonNano), yet at the same concentration CiONonNano had higher deterrence levels of adult female oviposition of *C. maculatus*. Consequently, to consistently achieve *C. maculatus* management control, the CiONano formulation needs to be optimized. Finally, this experimental work was not in-depth enough and deserves to be developed with more attention to the related mechanisms as to how essential oil of citronella and emulsions works on *C. maculatus.*

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