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Insecticidal Impact of some Natural Oils and their Nano -Emulsions on Sitophileus *oryzae*

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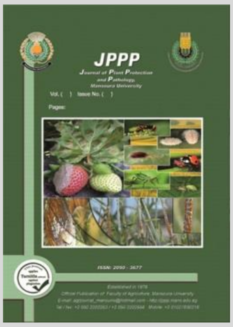

ABSTRACT

In vitro studies were conducted to evaluate the effects of essential oils (EOs), Cymbopogon citratus, Syzygium aromaticum and their nano-emulsion (NEs) by mixing with grains against Sitophilus oryzae (L.). Malathion was used as a standard reference and had the highest adverse effect among those followed by NEs and EOs, since % mortality, % reduction of progeny S. oryzae adults raised,and % loss of wheat grains decreased with high concentration and time. Malathion has a highly toxic effect, followed by NEs and EOs, where the LC50 values of malathion, S. aromaticum nano-emulsion, C. citratus nano-emulsion, S. aromaticum oil and C. citratus oil were (835.6 & 277.0), (15166.4 & 9516.3), (18817.0 & 13333.4), (17232.7 & 10371.5) and (45342.6 & 15706.1) after one and two weeks, respectively. The mortality rate for malathion was 95%, followed by the nano-emulsions S. aromaticum and C. citratus (83.3 and 70.0%) and EOs (76.7 and 66.0%), respectively. While the percentage of reduction in progeny at the high concentration of malathion was 93.5, and was 94.6 and 76.6% for NEs, and 91.8 and 83.7% for EOs, respectively. Moreover, Malathion had the lowest percentage of wheat grain weight loss, followed by NEs and EOs, compared to the untreated control (29%). Furthermore, NEs have a lower percentage of germination wheat grains. Finally, the use of NEs is a promising eco-friendly tool for the control of S. oryzae .

Keywords: *S.oryzae* , natural oils, nano-emulsion

INTRODUCTION

Rice is a principal diet for up to two billion people, it has minerals, vitamins, and carbohydrates (Akhtar et al., 2015). Rice grains could be attacked by Sitophilus oryzae (L.) (Coleoptera: Curculionidae), causing losses to the stored rice,and adults and larvae feed on grains (Baloch, 1992) and many other cereals (Batta, 2004). Under normal storage, the grain deteriorates by 80% due to insect feeding (Palipane, 2001), which leads to a decrease in the quality and quantity of rice grains. (Tabassum et al., 2012).

Implications of toxic residues insecticides and fumigants are big problems due to environmental hazards,high mammalian toxicity (Isman, 2000), and the development of resistance to insecticides (Dubey et al., 2008). The natural oils (EOs) are important source of pests control (González et al., 2019).

Natural oils are considered safer and more eco-friendly to the environment than synthetic insecticides due to their natural characteristics (Campolo, 2018), have lower toxicity for mammals, and have a complex of chemical compounds with multiple modes of action to overcome resistance (Isman, 2006; Correa et al., 2015 and Mossa, 2016).

Syzygium aromaticum is a plant widely used in tropical and subtropical countries. It has phenolic compounds, several biological activities, and insecticidal effects (Bandara and Senevirathne, 2021).

Cymbopogon citratus has been used in medicine. It has been confirmed in several previous studies to have also shown insecticidal potential (Sonja et al., 2021).

Nanoformulation of the EOs is more effective than that of substances (Choupanian and Omar 2018). Furthermore, they can affect target sites (Pavoni et al., 2019). Nanoproducts are technologies.

to produce pesticides and increase the insecticidal activity of oil nano-emulsions (NEs) against stored grain insects (Adak et al., 2020).

This study aims to reduce the negatives associated with the use of chemical insecticides and replace them with natural oils, as well as increase the effect of essential oils, C. citratus and S. aromaticum (EOs) on insects by oil nanoemulsion on adults of S. oryzae and their F1progeny, and to study the effect of natural oils and their nanoemulsions on wheat grain weight loss and germination compared to the recommended insecticide, malathion.

MATERIAL AND METHOD

***Sitophilus oryzae* tested**:

Sitophilus oryzae (L.) (curculionidae Coleoptera) was used in wheat grains under *in vitro* conditions of 28+2ºC and 70±5 R.H.% in the laboratory of Egyptian Agricultural Research center.

***Sitophilus oryzae* culture**

S. oryzae cultures were kept under the same conditions in glass jars containing 400 g each. Wheat kernels were previously sterilized to kill any stage of insects, by heating at a temperature of 70°C for one hour. The glass jars were covered with muslin and held in place with rubber bands. Unsexed adults (300-400) (1-2 weeks old) were introduced into jars to lay eggs under controlled conditions.

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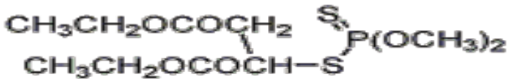
After three days, all insects were separated from the food and the grain containers were kept in the rearing room. Fresh adults (0–2 days old) of *S. oryzae* were used in the experiments (Egbon *et al*., 2012).

Tested Compounds:

Insecticide: Malathion:



IUPAC name: diethyl (dimethoxythiophosphorylthio) succinate; S-1,2-bis (ethoxycarbonyl) ethyl O,Odimethyl Phosphorodithioate (Martin, 1971). Formulation of malathion (1% WP). Source: Kafr El-Zayat Company, Egypt.

Plant Oils:

The oils of *Cymbopogon citratus* (Poaceae) and *Eugenia aromatic* (Myrtaceae) *a*; were provided by Hashem company for natural oils, Egypt

Perepation of nanoemulsion:

Nanoemulsions (NEs) of the oils *S.aromaticum* and *C. citratus* were prepared according to (Hamouda *et al*., 1999 and Ozogul *et al*., 2017). O/W thick oil in aqueous nanoemulsion was prepared using the tested oils (14% v/v of the total coarse emulsion), ethanol (3% v/v), and Tween 80 (3% v/v), accounting for 20 %. (v/v) of total emulsion. The oil phase components were mixed and kept for 1 hour at 86°C. Then mix with water (80%) and leave for 3 minutes. Finally, centrifuge at 10,000 mg. The nanoemulsion of the tested oils was stored in dark bottles at ambient temperature (Hashim *et al*., 2018).

Contact toxicity assay (mixed with medium):

All experiments were carried out at 28+2ºC, 70±5 R.H. %; and 12:12 (L: D) h. Healthy wheat grains were used and processed at a temperature of 40 oC for 30 minutes. The contact test by mixing with feeding medium technique was used to evaluate the insecticidal effect of the *C. citratus* and *S. aromaticum* oil nano-emulsions against *S. oryzae*. Serial concentrations were 5000, 10000, 15000, and 20000 (ppm) for the two essential oils and oil nano-emulsions prepared in acetone. The considerable concentrations used were 400, 600, 800 and 1000 ppm for malathion insecticide. One ml of each concentration separately was applied onto the surface of 20 g of wheat grains in 50 ml plastic bottles using a fine pipette. The jars were shaken by hand to mix the grains with each concentration. Each treatment and untreated control were replicated three times. The treated wheat was left in jars for a convenient time until the solvent evaporated. Twenty newly emerged adults of *S. oryzae* (one -two weeks old) were added to each replicate, then covered with muslin cloth and kept under *in vitro* conditions. Mortality counts were recorded after one and two weeks. All results were corrected with Abbott's formula (1925). The toxicity index of the tested compounds was determined according to (Sun, 1950) as follows:

$$\text{Toxicity index} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the tested compound}} \times 100$$

Progeny test:

The insects were removed from the grains and left inside the jars until the emergence of F 1 progeny according to the method described by (El-Lakwah *et al*.,1992) to determine the reduction percentages in adults progeny by the following equation:

$$\textbf{\% Reduction percentage} = \frac{C-T}{C} x100$$

C= number of adults in untreated control
T= number of adults in treatment

Losses in wheat grains weight:

The decrease in wheat grain weight resulting from infection with S. oryzae was estimated after three months of treatment after screening the insects from the wheat grains, and the percentage of weight loss was calculated as dry weight loss with the equation (Harris and Lindbland, 1978):

$$\%\text{weight loss} = \frac{\text{Initial dry weight of seeds} - \text{dry seeds weight after 3 months}}{\text{Initial dry weight of seeds}} \times 100$$

Test of germination :

Test of germination were accomplished on wheat grains of each treatment according to Qi and Burkholder (1981) and germination percentages were recorded four days after treatment after three months post-treatment by equation:

$$\%\ germination = \frac{No.of\ seeds\ germination}{Total\ No.of\ seeds\ used\ (60)} \times 100$$

Characterization of essential oils and their nano formulations:

individual compounds in both samples, (*Cymbopogon citratus* and *Eugenia aromatic*) were determined by gas chromatography mass (GC- MS) as in method (Adams, 2017).

Statistical analysis:

statistical analysis of data according to Duncan's test (Duncan, 1955). LC_{50} and LC_{90} calculated using LdP Line (http://embakr.tripod.com/ldpline/ldpline.htm),(Abbott, 1925).

RESULTS AND DISCUSSION

Contact toxicity assay:

The results obtained in Table (1) and Figures (1, 2, 3, 4, and 5) showed that malathion achieved the highest mortality percentages against *S. oryzae* (60.0 and 95.0 %) after one and two weeks, respectively, and the percent reduction was 93.5 % in the higher concentration. Also, data in tables (1 and 2) showed that nanoemulsion oils (NEs) for *S. aromaticum* and *C. citratus* had high mortality percentages when used with the concentration after one and two weeks (66.7 and 83.3 %) and (58.4 and 70.0 %) and percent of reduction was 94.6 and 76.6 %, respectively. The oils *S. aromaticum* and *C. citratus* had high mortality when it used with the highest concentration after one and two weeks (63.0 &76.7) and (43.0& 66.0) the percentages of reduction were 91.8 and 83.7%, respectively. The mortality percentage of *S. oryzae* increased with concentration and time after *S. aromaticum* applied due to secondary metabolites and strong toxic effects on insects (Forim *et al.*, 2012), also,eugenol has grain protectant action against *C. maculatus* (Mann, 2012). Studying thetoxicity of malathion and *S. aromaticum* and *C. citratus* oils against *S.oryzae* showed that LC_{50} were (835.6 & 277.0 ppm) for malathion, (17232.7& 10371.5 ppm) for *S. aromaticum* oil and (45342.6 & 15706.1ppm) for *C. citratus* oil after first and second week, respectively, also, acquired toxicity of nano emulsion (NEs) of the oils *S. aromaticum* and *C. citratus*. LC_{50} were (15166.4& 9516.3ppm) and (18817.0& 13333.4ppm) after one and two weeks, respectively.

Table 1. Toxicity of malathion and oils, S. aromaticum and C. citratus against S. oryzae

Treatments	Concentration (ppm)	One week						Two weeks					
		Mortality (%)	*LC_{50}	Confidence limits		Toxicity index	Slop value (S.V.)	Mortality (%)	LC_{50}	Confidence limits		Toxicity index	Slop value (S.V.)
				Lower	Upper					Lower	Upper		
Malathion	400	20.7	835.6	745.8	984.4	100	2.7	66.7	277.0	172.3	350.0	100	2.8
	600	33.3						83.3					
	800	46.7						90.0					
	1000	60.0						95.0					
S. aromaticum nano-emulsion	5000	23.0	15166.4	12972.0	18099.4	5.5	1.7	27.0	9516.3	8113.0	10831.2	3.0	2.2
	10000	36.0						53.4					
	15000	46.0						65.3					
	20000	59.0						74.3					
	25000	66.7						83.3					
C.citratus nano-emulsion	5000	20.2	18817.0	15689.8	24328.1	4.4	1.5	25.0	13333.4	11336.7	15684.1	2.1	1.7
	10000	33.0						40.0					
	15000	45.0						53.0					
	20000	50.2						60.0					
	25000	58.4						70.0					
S. aromaticum oil	5000	22.0	17232.7	14529.5	21501.2	4.8	1.5	30.0	10371.5	8653.9	12028.0	2.7	1.8
	10000	34.3						46.7					
	15000	43.8						60.0					
	20000	53.0						70.0					
	25000	63.0						76.7					
C. citratus oil	5000	20.0	45342.6	28134.8	184574.3	1.8	1.0	21.0	15706.1	13447.7	18825.0	1.8	1.7
	10000	26.3						37.0					
	15000	31.0						46.0					
	20000	36.3						56.0					
	25000	43.0						66.0					

- **LC_{50} was Lethal concentration for half the insects.**

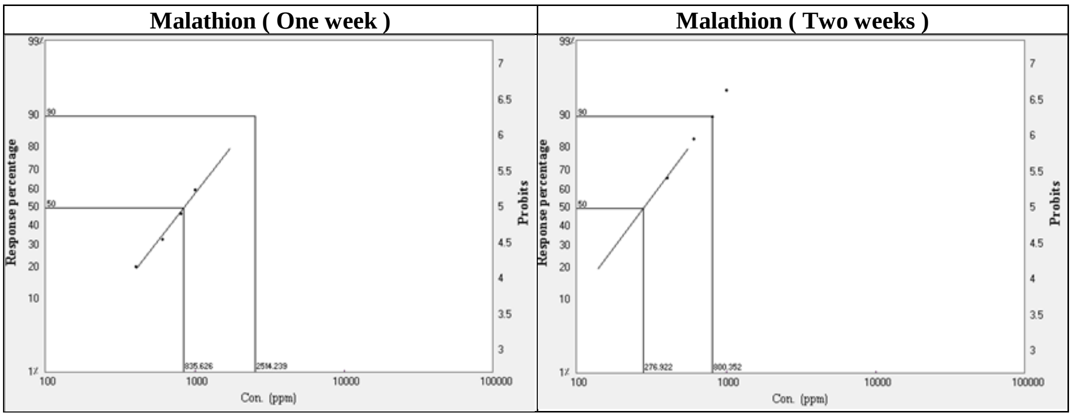


Fig. 1. Frequency of malathion sensitivity against of *Sitophileus oryzaet*; LC_{50} and LC_{90} after one and two weeks, respectively.

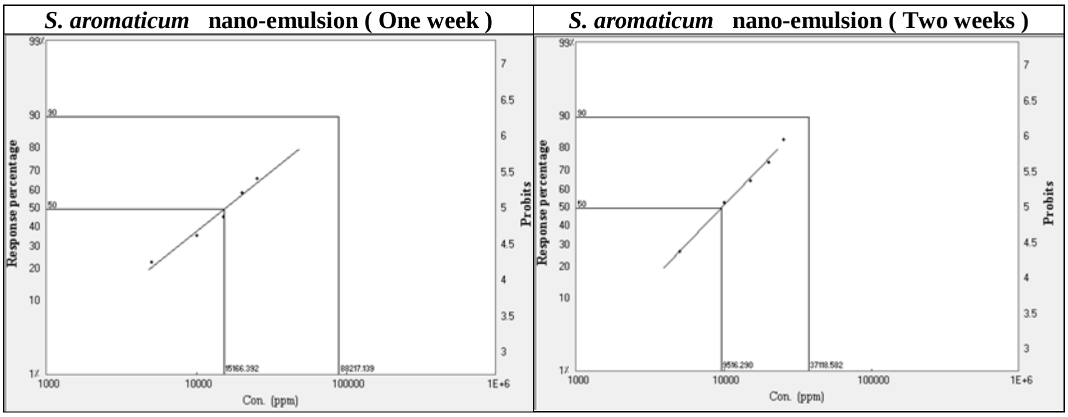


Fig. 2. Frequency of S. aromaticum nano-emulsion sensitivity against of Sitophileus oryzaet; LC50 and LC90 after one and two weeks, respectively.

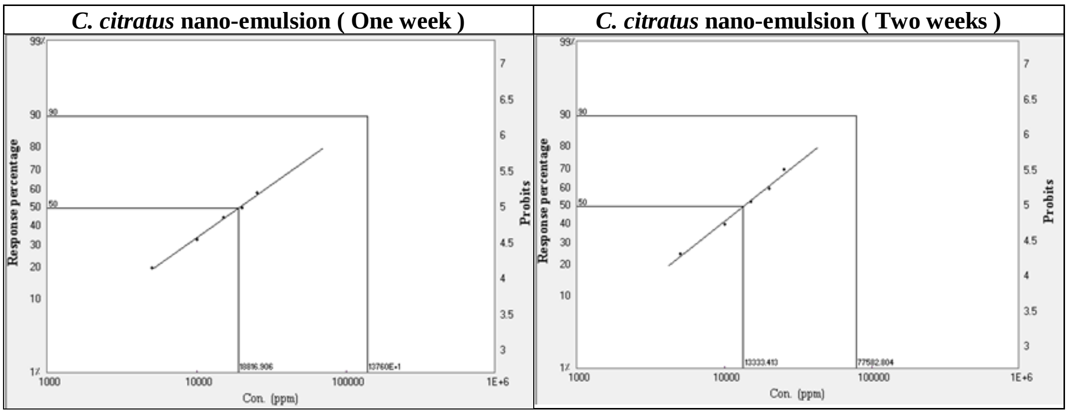


Fig. 3. Frequency of *C. citratus nano-emulsion* sensitivity against of *Sitophileus oryzaet*; LC_{50} and LC_{90} after one and two weeks, respectively

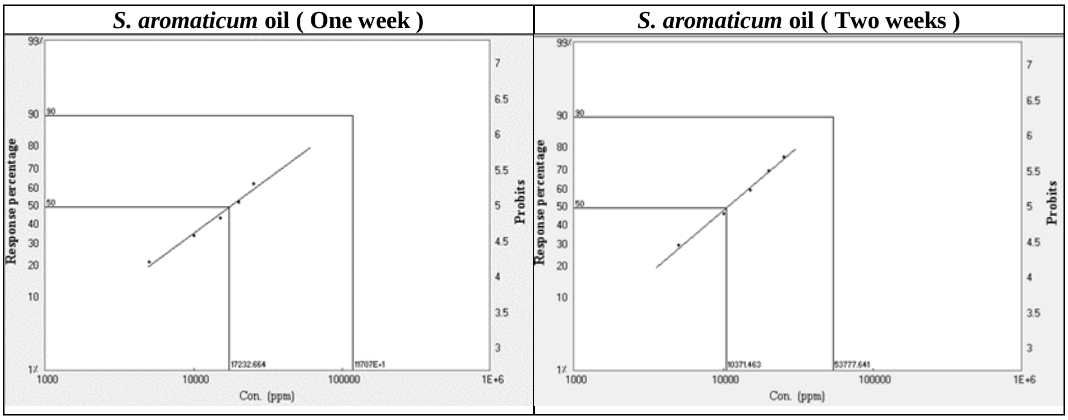


Fig. 4. Frequency of *S. aromaticum oil* sensitivity against of *Sitophileus oryzaet*; LC_{50} and LC_{90} after one and two weeks, respectively.

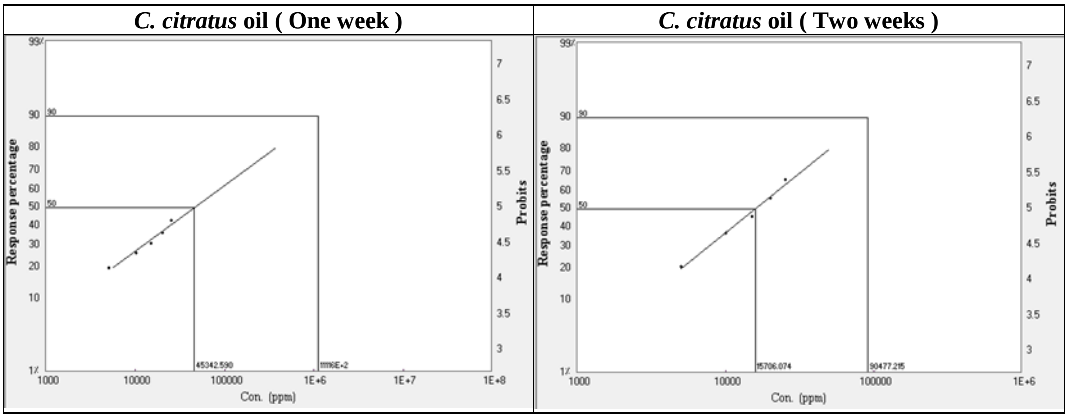


Fig. 5. Frequency of *C. citratus oil* sensitivity against of *Sitophileus oryzaet*; LC_{50} and LC_{90} after one and two weeks, respectively.

LC_{50} values of the tested materials were positively correlated with exposure time in all treatments, where LC_{50} values after one week were higher than those after two weeks in all treatments. Nanoemulsions help improve the natural oil's physical stability, water diffusion, and bioavailability, as tiny oil droplets cover the targeted surface area with limited non-target toxicity and irritation. (Nenaah *et al.*, 2015) and interact with the target sites (Pavoni *et al.*, 2019, Turek and Stintzing, 2013). The insects that died after the application of *S. aromaticum* were unable to cast off their exoskeleton, therefore, the effect of *S. aromaticum* is due to their ability to penetrate the chitin of insects

(Ntonifor *et al.*, 2010 and Oigiangbe *et al.*, 2010). Toxicity index of malathion was highly effective in *S. oryzae*, followed by, Nano emulsion oils (NEs) and the EOs alone. In addition, *S. aromaticum* oil and its Nano emulsion (NE) were more effective than *C. citratus* oil and its Nano emulsion (NE).

Reduction of progeny:

The data in Table (2) revealed that when the concentration of all treatments increased, the emergent progeny decreased and the percentage reduction increased. Malathion had the strongest adverse action on adult progeny among the used compounds since it reduced the emerging progeny of *S. oryzae*, followed by nanoemulsion oils (NEs) and the oils.

Wheat grains weight loss:

All treatments significantly reduced the percentage loss of grain weight with increasing concentrations. Results in Table (2) showed that malathion was the main agent for reducing weight loss (1.2 %), followed by nanoemulsion oils (NEs) for *S. aromaticum* (1.6%) and *C. citratus* (2.1 %) and (3.3 &2.3 %) %) for the oils *S. aromaticum* and *C. citratus* , respectively, compared to untreated control 29 %.

Germination tested

Table 2. Effect of treatments on % mortality, % reduction, % losses and % germination and wheat grains in S. oryzae

Treatments	Concentration (ppm)	Mean no. of adult emergence	Reduction (%)	Losses (%)	Germination (%)
Malathion	400	50o	72.3j	6.2i	98a
	600	25s	86.4f	4.3j	99a
	800	17u	90.8d	2.8l	98a
	1000	12w	93.5b	1.2o	99a
S. aromaticum nano-emulsion	5000	135e	26.6u	11.3e	93cd
	10000	105i	42.9q	10.1f	92de
	15000	65m	68.7k	9.3g	90f
	20000	20t	89.1e	7.3h	92de
	25000	10x	94.6a	1.6n	94c
C. citratus nano-emulsion	5000	158c	14.1w	14.3c	90f
	10000	120f	34.8t	10.3f	91ef
	15000	80k	56.5o	7.3h	90f
	20000	62l	61.4m	4.2j	92de
	25000	43p	76.6i	2.1m	87g
S. aromaticum oil	5000	150d	18.5v	7.2h	90f
	10000	110h	40.2r	4.6j	90f
	15000	70l	61.0n	3.6k	96b
	20000	33q	82.1h	2.1m	94c
	25000	15v	91.8c	3.3k	92de
C. citratus oil	5000	165b	10.3x	16b	84h
	10000	115g	37.5s	12d	91ef
	15000	82j	55.4p	7.3h	90f
	20000	60n	67.4l	4.5j	92de
	25000	30r	83.7g	2.3m	87g
Control	-	184a	-	29a	71i
LSD(0.01)	-	2.385	0.300	0.539	1.644
LSD(0.05)	-	1.789	0.225	0.404	1.233
P_{Value}	-	00.0**	00.0**	00.0**	00.0**

****, * indicated that P < 0.01 and P < 0.05 respectively, Means followed by same letter do not significantly. Means were compared by the least significant difference (LSD) test, differences being considered significant and highly significant at P = 0.05 at P = 0.01 respectively.**

In Table (3) the results showed that malathion exhibited non-significant activity on germination, while the oils of *S. aromaticum* and *C. citratus* and their nanoemulsions had a slight impact on germination. Active compounds of natural oils have modes of action that prevent the development of insect-resistant populations, and are non-toxic to mammals. (Isman, 2000)

Chemical Components of natural oils

Data in Table (3) revealed that the main compounds (%) of *S. aromaticum* are: eugenol (67.30), 1-propanol, 2-(2-hydroxypropoxy) - (18.94%), trans-13-Octadecenoic acid, methyl ester (9.16).

Table 3. EO Main Compounds (%) Syzygium aromaticum and Cymbopogon citratus

Oils	Compound Name	RT	Area %	Molecular Formula
Syzygium aromaticum	Eugenol	12.68	67.30	C10H12O2
	1-Propanol, 2-(2-hydroxypropoxy)-	5.47	18.94	C6H14O3
	trans-13-Octadecenoic acid, methyl ester	28.85	9.16	C19H36O2
Cymbopogon citratus	Citral	10.54	38.57	C10H16O
	2,6-Octadienal, 3,7-dimethyl-, (Z)-	9.78	25.79	C10H16O
	Estragole	8.78	7.04	C10H12O

EO Main Compounds (%) of *C. citratus* estimated by GC-MS are trans-citral (38.57%), 2, 6-Octadienal, 3, 7-dimethyl-, (Z)- (25.79%) and Estragole (9.16)

The different effects between the two oils may be due to the type and the amount of chemical compositions (Boughendjioua, 2018 and Sawadogo *et al*., 2022). EOs and their bioactive compounds affect stored grain insects by inhibiting the acetylcholinesterase (Isman 2000). Eugenol was effective on a wide range of insects (Dohi *et al.,* 2009), and eugenol's activity is due to the octpaminergic system (Enan, 2001). Mechanisms of insecticidal action through the bioactive compounds of *S. aromaticum* also apply to *S. oryzae*. (Dohi *et al.,* 2009). EOs penetrated the waxy layer of insects were quite lipophilic, they could quickly enter and interfere with physiological functions (Bachrouch *et al*., 2015). Eugenol was one of the chemical compounds monoterpenes, it has volatile and lipophilic characteristics, penetrates inside insects, and has an effect on physiological functions (Saad *et al.*, 2018). Eugenol impacted adenosine triphosphatase (ATPase) activities in *S. oryzae* (Saad *et al.,* 2018). EOs exhibited anti-AChE activity against adults of *S. oryzae* (Bhavya *et al*., 2018). *C. citratus* had worldwide and it had toxicity of terpenoid constituents (Kobenan *et al.,* 2021).

CONCLUSION

The current study demonstrated that nanoemulsions of *S. aromaticum* and *C. citratus* essential oils had significant potential to control *S. oryzae*. *S. aromaticum* (NEs) oils had distinctive effect on parameters, mortality, and reduction of progeny compared to untreated control *S. aromaticum* exhibited insecticidal activity , followed by *C. citratus* (NEs) , which delayed the biological growth and reduced the weight grain losses caused by S. *oryzae.* Malathion has many serious hazards on human and environment. Natural oils (*S. aromaticum and C. citratus)* helped reduce the negative effects of insecticides such as malathion to have less toxicity, overcome insect resistance, and ensure the safety of the environment and non-toxic untargeted bioorganisms.

The major bioactive compounds for *S. aromaticum* and *C. citratus* had insecticidal activity in *S. oryzae*. So, the application of nanoemulsions of *S. aromaticum and C. citratus n*atural oils can be an alternative to insecticides and control *S. oryzae*.

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تأثير بعض الزيوت الطبيعية ومستحلباتها النانوية في سوسة الارز

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الملخص

أجريت الدراسة الحالية في المعمل لتقييم فعالية كلا من زيت القرنفل وزيت حشيشة الليمون والمستحلب النانوى (NEs) لهما ضد سوسة الأرز عن طريق الخلط مع الحبوب بالمقارنة مع المبيد الحشري الملاثيون الموصى به. وأشارت النتائج أن الملاثيون كان له التأثير الأعلى عن مستحلبات النانو يليه الزيوت ، حيث زاد كلا من نسبة الموت لسوسة الأرز البالغة والخفض في الذرية كما انخفضت نسبة الفقد في الوزن من حبوب القمح مع زيادة التركيز وفترة التعرض. ومن ثم فقد بلغت LC_{50} (835.6 & 277.0) و (15166.4 & 9516.3) و (18817.0 & 13333.4) (17232.7 & 10371.5) و (45342.6 & 15706.1) ppm بعد أسبوع وأسبوعين على التوالي ، وبلغ معدل الوفيات 95.0٪ للملاثيون يليه مستحلب النانو لزيت القرنفل ثم مستحلب النانو لزيت حشيشة الليمون (83.3 & 70.0٪) والزيوت (76.7 & 66.0٪) على التوالي. بينما كانت نسبة الانخفاض في الذرية عند أعلى التركيز 93.5 % بسبب الملاثيون و 94.6 & 76.6٪ لمستحلبين النانو (NEs)، 91.8 & 83.7٪ للزيوت الإثنين على التوالي. بالإضافة إلى ذلك زيت القرنفل ومستحلبه النانوي أعلى تأثيرًا بالمقارنةً بزيت حشيشة الليمون ومستحلبه النانوي على التوالي. كان الملاثيون أقل نسبة فقد في وزن حبوب القمح تليها مستحلب النانو (NEs) والزيوت مقارنة بالحبوب الغير معالجة بنسبة 29.0٪. وأدى مستحلب النانو إلى تثبيط نسبة إنبات حبوب القمح بشكل طفيف متبوعًا بـ EO مقارنة بالكنترول، بعد ثلاثة أشهر بعد المعاملة. أخيرًا، يعد استخدام مستحلب النانوى لكلا من زيت القرنفل وزيت حشيشة الليمون من الأدوات الواعدة الصديقة للبيئة وتعتبر بديل للمبيدات الحشرية لحماية الحبوب المخزنة ضد سوسة الأرز في برنامج مكافحة الآفات المتكامل.