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Efficiency of Utilizing *Citrullus colocynthis* (L) Essential Oil Alone and When it Mixed with Diatomaceous Earth Against some Stored Products Insect Pests

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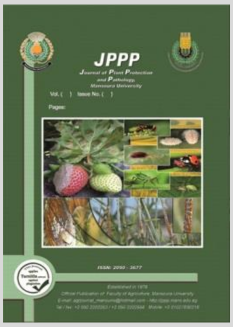

ABSTRACT

Insect pests cause economic damage to cereals in both quality and quantity. The objective of the current study was to assess the contact, fumigant toxicity and repellent activities of *Citrullus colocynthis* L. essential oil (EO) alone, contact activity of DE and the combined effect of EO with various concentrations of Diatomaceous earth DE against the adults of *Sitophilus oryzae L*., *Oryzaephlius surinamensis* (L) *Stegobium paniceum* (L) and *Tribolium castaneum* (Herbst.) under laboratory conditions. The obtained results indicated that mortality rates of insects were positively affected by increasing both of concentration and the exposure time. The fumigant toxicity of *C. colocynthis* was higher than the contact toxicity whereas at 120 h post treatment the LC50 was 2.64, 4.61, 13.4 and 17.32 ml/l for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum* respectively, while in the contact case it was 3.92, 12.6, 36.7 and 48.86 ml/l, respectively. Strong repellent of *C. colocynthis* achived 100, 95.5, 98.8 and 86.6% after 12 hours for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*, respectively at 20 nL/cm^2. Diatomaceous earth achieved strong contact toxicity whereas at 168 h post-treatment LC50 values was 2.287, 2.63, 5.40 and 20.2 % (w/w) for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*, respectively. Based on the obtained results, *S. oryzae* was the most susceptible species, whereas *T. castaneum* was the most tolerant ones. The essential oil *C. colocynthis* has the ability to be utilized as one of the integrated stored product pest management programs.

Keywords: Essential oil, diatomaceous earth, Stored grain insect pests

INTRODUCTION

Food is one of the basic ingredients of life, so food safety and security are main concerns nowadays. One of the most important threats to food safety is the stored grains insect pests which causes uncompensated losses ranged from 11 to 25% with 8% reduction in the world's grain production (Nadeem et al., 2012). Addition, during shipping and transportation insects can infect many stored grain products and producing a large population in a short period resulting in large qualitative and quantitative losses (Korunić et al., 2007; Erika et al., 2019).

For controlling these insect pests, there are many synthetic insecticides used in the developing countries, however it caused several serious problems to the stored food and grain products, human health and the environment (Sun, Y.P. & Johnson, E.R,1960).Thus, we must search for safe alternatives such as Diatomaceous earths (DE) and Essential oils (EO) of aromatic plants instead of toxic chemicals to combat insect pests without causing any damage. Diatomaceous earths (DE) consisted of fossilized bodies of unicellular algae, diatoms that used as inert dust in protecting stored grains against stored products pests with low toxicity to mammals in addition to its efficiency in preventing insect pests, especially in storage(Obeng-Ofori, 1995; Islam et al., 2010; Shah & Khan 2014; Abd El-Aziz & Abd El-Ghany, 2018; Akçali et al., 2018).

The essential oil (EO) of *Citrullus colocynthis*, a perennial plant that resembles the common watermelon, has an insecticidal potential against many harmful insects (Hassan & Omer 2018).The insecticidal activity of this oil has evaluated against numerous insect pests and regarded as a low-toxic, eco-friendly promising substance in pest insects control(Finney,1971; D. Obeng-Ofori et al., 1997; Lorini, I. and A.F. Filho, 2004; Bedini et al., 2015; Panezai et al., 2019).

Nevertheless, the olfactory properties of EO may conflict with the sensory qualities of the foods that have been treated, producing unappealing goods to customers due to their strong smell(Bedini et al., 2018). Also DE must be used at large dosages (0.5–3.5 g/kg of grain) to be effective, which increases the bulk weight of the grain and degrades its quality (Rozman et al., 2015).Thus, essential oils can be administered along with DE to increase their effectiveness against insect pests, allowing the reduction of DE doses (Hasan et al., 2006; Bougherra-Nehaoua et al., 2015).The present study was conducted to evaluate the contact, fumigant toxicity and repellent activities of *C.colocynthis* alone, the contact toxicity of DE and the combination of LC50 of *C.colocynthis* with various concentrations of DE against the adults of *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum* under laboratory conditions.

MATERIALS AND METHODS

This study was conducted in the Stored Grain Laboratory at the Plant Protection Department, Faculty of Agriculture, Moshtohor, Benha University during 2022 to 2023 to determine the efficacy of *C.colocynthis* essential oil

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(EO) and Diatomaceous earth (DE) inert dust and the combined effect of them against the adults of four coleopteran stored grain insect pests: [Rice weevil *Sitophilus oryzae* L. (Curculionidae), Saw toothed grain beetle, *Oryzaephilius surinamensis* (L), Drugstore Beetle, (*Stegobium paniceum* L.) (Anobiidae) and red flour beetle *Tribolium castaneum* Herbst (Tenebrionidae), under the laboratory conditions of 30 ± 1°C and 65 ± 5% R.H.

Rearing cultures:

Fresh washed wheat grains and wheat flour were kept in a freezer at -20°C for seven days or even until used in the experiments to avoid any insect infestation. Adults of *S. oryzae*, were reared on the whole wheat kernels, while *T.*castaneum, *O. surinamensis* and *S. paniceum* were reared on the wheat flour. The insects reared in 1L wide-mouthed glass Mason jars containing 100 g of [(untreated flour mixed with yeast 10:1 (w/w) for *T. castaneum*, *O. surinamensis* and *S. paniceum*) or (untreated whole wheat kernels with moisture content around 14% for *S. oryzae*)]. These jars were covered with muslin cloth and tied with rubber bands to avoid the escape of culture insects and the entry of other insects. About five hundred adults of each insect species (1-2 weeks old) were introduced into the jars for 3days to oviposit in the medium under conditions of 30± 1°C and 65 ± 5% R.H, and 12:12 h. Next, these insects were removed from the food and the jars were kept in the rearing room under these conditions to produce a new generation of adults that will use in the trials.

Experimental Materials:

Citrullus colocynthis, commonly known as Colocynth, a member of Cucurbitaceae is native to Mediterranean region and Asia was used during these investigations. This essential oil was bought from Ragab El-Attar Company, Cairo, Egypt. The essential oil of this plant was kept at 4˚C in a refrigerator until using in the experiments. The diatomaceous earth (Wolf Creek Ranch, California USA) is a DE of freshwater fossil shell containing 89% silicon dioxide which is the tested formulation in the current study that applied as a powder on wheat kernels.

Bioassay Tests:

Contact Toxicity:

The contact toxicity of *C. colocynthis* (EO) and DE against adults of the tested stored-grain insects was assessed as described by (Erika, et al., 2019) Test glass jars (200 ml) with tilted covers were provided with 10 gm of the wheat flour for each of *T. castaneum, O. surinamensis* and *S. Paniceum* or with 10 gm of the whole wheat kernels for *S. oryzae*. This amount of food was hand shacked with either the weighed amount of DE or the concentration of the EO. The EO was mitigated with petroleum ether. In control treatment of EO only food mixed with petroleum ether, whereas in the DE control treatment only untreated grains were used. Thirty adults of each insect species (7 days old) were evaluated in each concentration with three replicates for each treatment. The jars were closed well and incubated at 30±1 °C and 65±5% R.H.. Numbers of dead and alive adults were counted after 1, 2, 3, 5, 7 and 10 days post-treatment and data were subjected to probit analysis to determine LC50 values (Sakuma, 1998).

Repellency Tests:

The repellent activities of *C. colocynthis* essential oil against the adults of *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum.S. oryzea* and *T. castaneum* was tested according to the method described by (Chaubey, 2007). Five concentrations (20, 10, 5, 2.5 and 1.25 nL/cm) and two observation times (12 and 24 h) were considered. The essential oil was mitigated with petroleum ether. Filter paper (9 cm in diameter) was identically cut into two pieces and dilutions of 1 ml of each concentration were dropped evenly on half of the filter paper, while the other half was treated only with 1 ml of petroleum ether (negative control). After a minute of air-dried, both pieces were stuck to the bottom of the Petri dish (11 cm diameter x 3 cm height) with solid glue abreast. Thirty adults (7 days old) were placed at the centre of the dish, then quickly covered with lids. All treatments were replicated three times. The count of insects on both halves of the paper was recorded separately. The following formula used to estimate percentage of repellencey (PR):

$$PR (\%) = [(Nx-Nt) / (Nc+Nt)] \times 100$$

where Nc is the number of insects in the negative control half and Nt is the number of insects in the tested half.

Fumigant Toxicity:

The fumigant activities of *C. colocynthis* essential oil with the same mentioned concentrations in contact bioassay against the adults of *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum* was evaluated. A filter paper was placed on the underside of the cap of a glass jar (2.5 cm diameter, 5.5 cm height and 24 ml. volume). One ml of the essential oil was added to the filter paper, while control treatment using only petroleum ether. Before the cap was tightly placed on the glass jar the solvent was allowed to evaporate for 60 seconds. Thirty mixed sex insects were placed inside each jar with a small amount of diet into cages of wire and gauze (40 mm in diameter and 45mm in height), then incubated jars at 30±1 °C and 65±5% R.H. Results were subjected to probit analysis to determine LC50 values (Sakuma, 1998).

EO combined with DE effects:

The contact toxicity of the *C. colocynthis* EO combined with DE was examined against adults of *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum* (Fields, 2006).

Sub-lethal concentrations (LC50) of the EO was combined with various concentrations of DE (10; 8; 6; 4 and 2 % w/w) to determine if there was an antagonistic or synergistic interaction. LC50 of the EO was mixed with wheat and shacking for 4 min, then the tested concentration of DE was completely mixed with the grains by manually shaking the glass vials for additional 4 min. Subsequently. Thirty insects (7 days old) of each species were introduced separately to each vial, then the caps were tightly screwed and incubated at 30±1 °C and 65±5% R.H. Each treatment repeated three times. The control group was exposed to the same conditions but with untreated wheat. Mortality was recorded after 1, 2, 3, 5 and 7 days from exposure. Then, the time needed to kill 50% of insects (LT50) for each treatment was calculated. To differentiate between synergistic, antagonism, and additive effects, the combination joint action was presented as a Co-toxicity factor (Sun & Johnson, 1960), by applying the below formula:

$$\text{Co-toxicity factor} = \frac{\text{Observed mortality - Expected mortality}}{\text{Expected mortality.}} \times 100$$

This factor was used to categorize the results into three categories as follows: synergistic meant co-toxicity factors ≥ +20; antagonism meant co-toxicity factors ≤−20; and additive meant co-toxicity factors between -20 and+20.

Statistics Analysis:

According to Abbott's formula (Abott, 1925) the data were corrected, and then subjected to probit analyses. Using LDP line software (Finney, 1971) LC50 and LC95 values of the essential oil and the diatomaceous earth against each stored product insect species were estimated. The co-toxicity coefficient was used to evaluate the synergistic effects between the essential oil and the diatomaceous earth by using (CTC) according to (Sun and Johnson, 1960): CTC = LC50 of DE /LC50 of formulation (EO + DE). The effect was considered synergistic if the obtained value was greater than 1, and antagonistic if it was less than 1.

RESULTS AND DISCUSSION

The contact toxicity of *C. colocynthis* essential oil:

Contact toxicity of *C. colocynthis* EO was investigated with four previously stored insect species. Data demonstrate that insect mortality rates affected positively by increased both concentrations and exposure time (Muhammad N. et al., 2012). At the highest concentration of *C. colocynthis* (20 µl/l air), mortalities percentages were 8.8, 10, 6.6 and 3.3% after one day exposure for *S. oryzae, O. surinamensis, S. Paniceum* and *T. castaneum* respectively, these percentages increased with increasing concentration and exposer period to reach 100, 84, 54.4 and 35.5 % after 7 days exposure for the tested insects respectively.

Moreover, at the lowest concentration of *C. colocynthis* (1.25 µl/l air), the essential oil did not show activity to the four tested insect species after 24 h exposure. Otherwise, as the concentration and exposer period increased mortality percentages increased to reach 58.8, 17.7, 12.2 and 3.3 % after 7 days exposure for *S. oryzae, O. surinamensis, S. Paniceum* and *T. castaneum*, respectively (fig1).

In addition, the concentration needed to obtain 50% mortalities for the tested insects were 0.86, 6.87, 15.1 and 42.3 µl/l air against *S. oryzae, Osurinamensis, S. Paniceum* and *T. castaneum*, respectively, while the doses needed to get 95% mortality were 15.3, 102.1, 638.2 and 893.8 % µl/l air, respectively (Table1). At the highest exposure time (168 h) the LC95 values suggested that *S. oryzae* was the most sensitive insect to the EO followed by *O. surinamensis* and *S. Paniceum* respectively, while *T.castaneum* was most tolerant.

Our results consistent with (Muhammad, N. et al., 2012) who evaluated *C. colocynthis* against *T. castaneum* and reported that increasing the chemical doses and exposure times led to an increase in the insect mortality rates. Similar studies have demonstrated that *C. colocynthis* oil showed similar effects like *Azadirachta indica* the essential oil uses in traditional post-harvest practices practices (Tallamy et al., 1997; Madari & Jacobs 2004; Seenivasan et al., 2004; Ahmad, M., 2007; Boateng, & Kusi 2008).

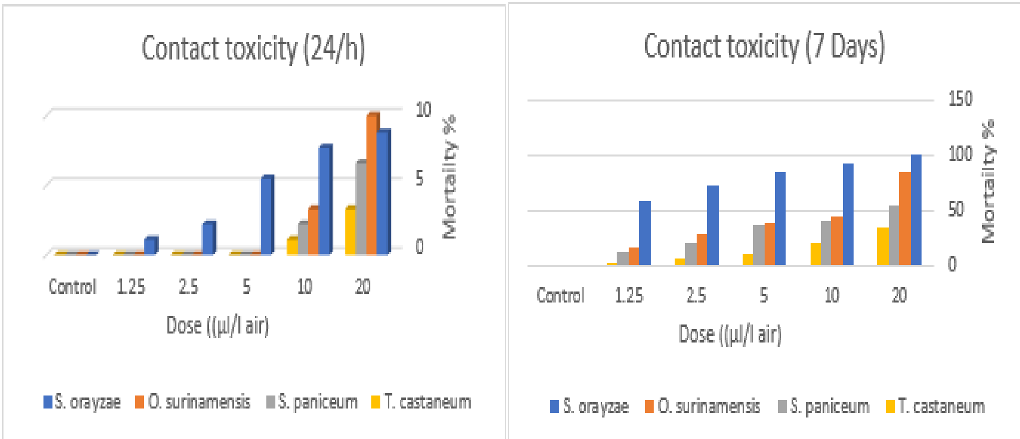

Fig. 1. Contact toxicity % of *C. colothynthus* against adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* after 24 h and 7 days exposure periods.

Table 1. LC_{50} and LC_{95} values of the contact toxicity of *C. colothynthus* essential oil against adults of *S. oryzae O. surinamensis, S .paniceum* and *T. castaneum*

Samples	Insects	Time (h)	LC_{50^a} (µl/l air)	LC_{95^a} (µl/l air)	Slope ± SD	Chi Square (x^2)	p-Value	R.
Citrullus colothynthus	*S.O*	120 h	3.918	577.85	0.76±0.143	15.03	0.002	0.804
		168 h	0.86	15.275	1.32± 0.202	4.997	0.172	0.989
	O.S	120 h	12.6	152.93	1.52± 0.171	23.01	0.00	0.891
		168 h	6.87	102.16	1.403±0.16	15.103	0.002	0.904
	S.P	120 h	36.76	1102.3	1.11± 0.179	0.341	0.952	0.995
		168 h	15.1	638.2	1.01± 0.154	1.646	0.648	0.984
	T.C	120 h	48.86	588.5	1.52±0.25	0.357	0.948	0.995
		168 h	42.3	893.84	1.24± 0.290	0.309	0.951	0.996

Fumigation Toxicity:

Results of fumigation toxicity of *C. colocynthis* EO against *S. oryzae, O. surinamensis, S. Paniceum* and *T. castaneum* are shown in Table (2). It is obviously that, mortality percentages were clearly associated with the applied doses and exposure period.At the highest concentration of EO (20% µl/l air), mortalityies percentages were 20, 12.2, 7.7 and 12.2% after 24 h exposure for *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum,* respectively. Mortality percentages were increased as concentrations and exposure time increased to reach 91, 86.6, 63.3 and 50% after 120 h exposure for, respectively.

While, at the lowest concentration (1.25% µl/l air), mortality percentages were 2.2, 1.1, 0.0 and 1.1% after 24 h exposure for *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum*, respectively. These percentages increased with increasing concentrations and exposure times to reach 38.8, 17.7, 6.6 and 10 % after 120 h exposure, respectively (Fig 2,3 and4).

Probit analysis showed that LC50 values of EO were 2.64, 4.60, 13.4 and 17.3 µl/l air for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*, respectively after 120 h, while the LC95 values were 43.6, 48.5, 155 and 586 µl/l air after 120 h, respectively.

In addition, the times needed to get 50% mortality were 55, 57.8, 96.1 and 114.9 hours against *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*, respectively, while those to obtain 95 % mortality were 240, 244.6, 495 and 1002 hours, respectively (Table 3).

Our results consistent with those of (Delazar et al.,2006; Dehghani et al.,2008; Rajendran & Srianjini, 2008; Regnault-Roger et al.,2012; Abdel-Fattah et al.,2017) who found that essential oil could be used as a safe pesticide for some of stored-product insects either fumigant or repellent. Furthermore, the fumigant toxicity of oils increased by increasing concentrations and exposure duration. In present study the *C. colocynthis* essential oil showed insecticidal activity against *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*. *Sitophilus oryzae* was the most sensitive species for the essential oil *C. colocynthis*, while *T. castaneum* was the highest tolerant as it required much higher doses to be killed .

Table 2. LC_{50} and LC_{95} values of the fumigant toxicity of *C. colothynthus* essential oil against adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum*.

Samples	Time (h)	Insects	LC_{50^a} (µl/l air)	LC_{95^a} (µl/l air)	Slope ± SD	Chi Square(x^2)	p-Value	R.
Citrullus colothynthus	120 h	*S.O*	2.64	43.6	1.35±0.158	9.015	0.029	0.956
		O.S	4.604	48.5	1.61± 0.161	3.686	0.297	0.986
		S.P	13.4	155	1.55±0.174	0.943	0.815	0.995
		T.C	17.32	586	1.08±0.158	1.329	0.722	0.988

Table 3. LT_{50} and LT_{95} of the fumigant toxicity of *Citrullus colothynthus* essential oil against the adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum*

Samples	Conc.	Insects	LT_{50^a} (µl/l air)	LT_{95^a} (µl/l air)	Slope ± SD	Chi Square(x^2)	p-Value	R.
Citrullus colothynthus	20	*S.O*	55.02	240.1	2.57 ± 0.23	13.261	0.004	0.961
		O.S	57.8	244.6	2.62 ± 0.23	34.428	0.00	0.921
		S.P	96.1	494.7	2.31 ± 0.25	2.748	0.432	0.987
		T.C	114.9	1002	1.75 ± 0.22	0.586	0.899	0.995

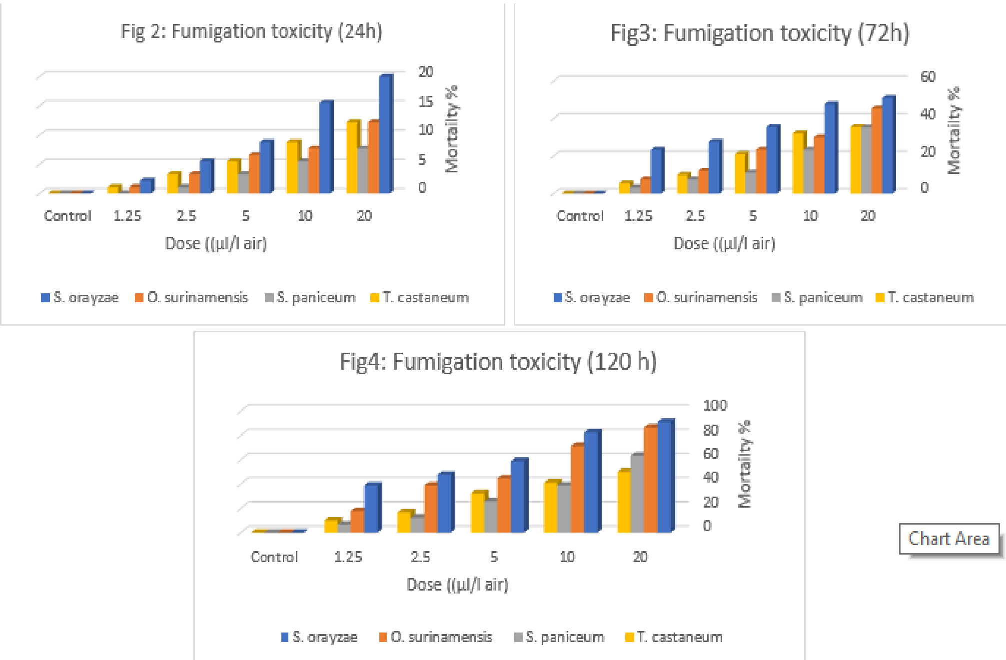

Fig. 2, 3 and 4. Fumigation toxicity % of EO, *C. colothynthus* against the adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* after different exposure times.

Repellent Activity:

Repellent activities of *C. colocynthis* EO against *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* adults evaluated using the area preference method after 12 and 24 h post-treatment (Fig.5). The repellence percentages were increased with increasing concentrations. At the highest concentration of EO (20 nL/cm^2), repellence percentages were 100, 100, 98.8 and 86.6% after 12h exposure for *S.oryzae, O. surinamensis, S. paniceum* and *T. castaneum*, respectively. Repellence percentages were decreased with increasing exposure time to reach 100, 96.6, 83.3 and 53.3 % after 24h exposure, respectively. While, at the lowest concentration (1.25 nL/cm^2) repellence percentages were 65.5, 67.7, 50 and 31.1% after 12h exposure for *S. oryzae, O .surinamensis, S. paniceum* and *T. castaneum*, respectively. These percentages decreased with increasing exposure time to reach 53.3, 47.7, 31.1 and 7.7 % after 24h exposure, respectively (Fig.5).

Our results consistent with (Isman, 2006); Muhammad, I. et al., 2019) who Studied the effects of

Citrullus colocynthis as toxicants against *T. Castaneum* ,the results indicated that the plant extracts evaluated have good insecticidal activity. Other reports evaluated the effects of *C. colocynthis* against stored products insects (Aneeb ali, et al 2019) also evaluated mortality and repellency effect of *C. colocynthis* against *T. castaneum* and revealed that *C. colocynthis* has repellent and toxicant effects against *T. castaneum*. Based on the current study, the *C. colocynthis* essential oil showed high efficiency against all tested insect species and *S. oryzae* was the most sensitive species.

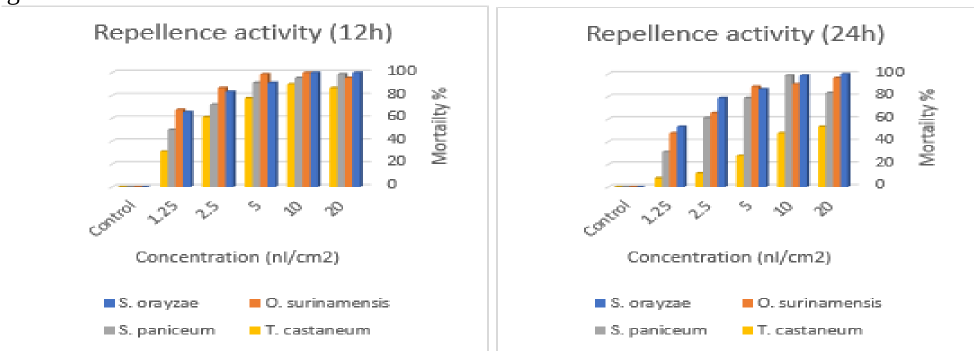

Fig. 5. Repellency Percentages (PR) of *C. colothynthus* against adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* after 12h and 24 h exposure .

The Contact Toxicity of the Diatomaceous Earth:

Contact toxicity of the DE inert dust against adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* was presented in Figure (6). According to (Fields and Korunic 2000),mortality rates were significantly affected by both doses of DE and exposure time, as with increasing doses of DE and exposure time higher mortality rates were gained. At the lowest concentration (2% w/w), mortalityies percentages were 1.1 and 1.1% for *S. oryzae* and *S. paniceum*, respectively, while no mortality recorded for *O. surinamensis* and *T. castaneum* at the same concentration after one day post-treatment. These percentages increased with increasing concentrations and exposer period to reach 54.4, 46.6, 30 and 11.1% after 10 days post-treatment for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*, respectively.

On the other hand, at the highest concentration 10%(w/w), mortalities percentages after one day post-treatment was 12.2, 10, 5.5 and 0.0% for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum*, respectively, since mortality percentages increased with increasing concentrations and exposure time to reach 100, 100, 94.4 and 80 % after 10 days post-treatment, respectively.

Probit analysis showed that, the concentrations needed to achieve 50% mortalities (LC50) for the tested insects were 1.41, 5.53, 16.5 and 30.7 % w/w after 72h post-treatment for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum* respectively, while the concentrations needed to get 95% mortalities (LC95) were 18.5, 14.6, 104 and 190 % w/w, respectively after the same post-treatment time. LC50 values of DE after 168 h post-treatment were 2.287, 2.63%, 5.40 and 20.2 % (w/w) for *S. oryzae*, *O. surinamensis*, *S. paniceum* and *T. castaneum* respectively, while the LC95 values were 7.31, 8.03, 31.2 and 242 % (w/w), respectively(Table 4).

According to the current results, mortality rates after 2 days of exposure ranged from 86.7 to 100% in response to insect species with high efficiency against the tested insect species especially after 10 days of exposure (Ertürk et al., 2017; Korunic et al., 2017).Many studies reported that DE is very safe for ecosystem components including the natural enemies for insects and human beings, so it could be utilized in a storage environment as a natural eco-friendly tool for protecting the stored grain products(Korunic, 1999; Ziaee et al.,2019).

Our results come in the same line with those of (Arthur, 2000; Fields & Korunic 2000; Athanassiou et al., (2004, 2005) who mentioned that adults of *Tribolium* spp. are among the most DE-tolerant species, as *T. castaneum* was the least affected in comparison with the other three species as after the longest exposure time achieved 80% mortality at the highest concentration 10%(w/w), while *S. oryzae* was the most sensitive reached 100% mortality after 7days followed by *O. surinamensis* with 100% mortality after 7days, then *S.paniceum* with 94.4% mortality after 10 days at concentration of 10%(w/w)., so *S. oryzae* considered more susceptible than *Tribolium* spp. (Arthur, 2002; Athanassiou et al., 2003; Kavallieratos et al.,2005; El-Sayed et al., 2010).

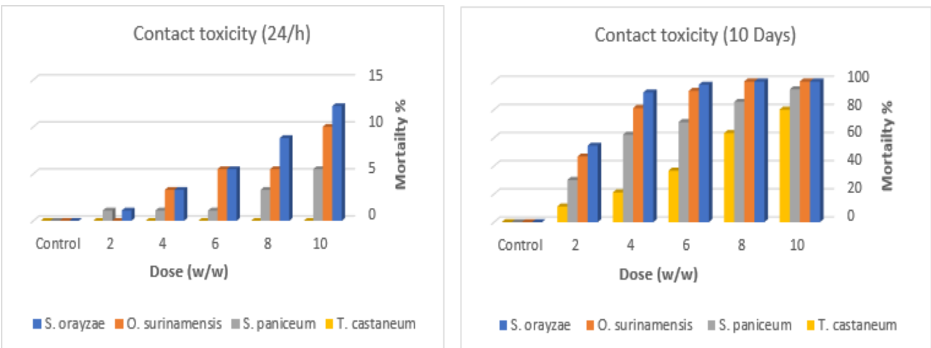

Fig. 6. Contact toxicity % of Diatomaceous earth against adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* after various exposure times.

Table 4. LC_{50} and LC_{95} of Diatomaceous earth against adults of *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* in contact toxicity bioassay.

Samples	Insects	Time (h)	$LC_{50}{}^{a}$ (µl/l air)	$LC_{95}{}^{a}$ (µl/l air)	Slope ± SD	Chi Square (x^2)	p-Value	R.
Diatomaceous earth	*S.O*	72 h	4.414	18.538	2.64± 0.202	31.088	0	0.936
		120 h	3.409	9.252	3.79± 0.318	11.22	0.01	0.969
		168 h	2.283	7.319	3.25± 0.37	6.455	0.09	0.990
	O.S	72 h	5.537	14.693	3.88± 0.34	7.097	0.06	0.981
		120 h	3.945	12.147	3.37± 0.32	14.38	0.002	0.979
		168 h	2.633	8.038	3.39± 0.30	3.279	0.350	0.981
	S.P	72 h	16.510	104.43	2.05± 0.366	8.028	0.045	0.895
		120 h	11.677	105.79	1.72± 0.29	3.039	0.385	0.961
		168 h	5.401	31.276	2.16± 0.267	0.796	0.850	0.995
	T.C	72 h	30.727	190.394	2.08±0.534	1.021	0.796	0.981
		120 h	57.685	1469.02	1.17± 0.357	1.275	0.735	0.948
		168 h	20.267	242.182	1.53± 0.319	2.182	0.535	0.964

Combined toxicity:

In all experiments, the mortality rates of insects treated with Diatomaceous earth (DE) or the essential oil (EO) separately was much lower than those in the combined treatment. When various concentrations of DE [10.0; 8.0; 6.0; 4.0 and 2.0 % (w/w)] combined with LC_{50} of EO, mortalities percentages were 23.3, 17.7, 10 and 5.5 % after one day post-treatment for *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* respectively at the highest concentration. These percentages increased with increasing concentrations and exposure time to reach 100, 100, 96.6 and 93.3% after one-week post-treatment, respectively.

Furthermore, at the lowest concentration of DE, mortalities percentages were 5.5, 5.5, 3.3 and 1.1 % after one day post-treatment for *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* respectively. These percentages were increased with increasing concentrations and exposure time to reach 76.6, 44.4, 40 and 28.8 % after one-week post-treatment for the tested insects, respectively (Table 5).

Also, times needed to achieve 50% mortality in combined treatments of DE with EO was 1.52, 1.75, 3.07 and 4.19 days for *S. oryzae, O. surinamensis, S. paniceum* and *T. castaneum* respectively, while that needed to obtain 95% mortality was 3.23, 4.02, 9.90 and 12.04 days, respectively (Table 6). This result clearly indicated that, the combination of DE with *EO* was most toxic against adults of *S. oryzae*, while it was less affected against *T. castaneum* than other species.

Table 5. Effect of sublethal dose (LD50) of *C. colocynthis* (EO) and its combination with various concentrations of Diatomaceous earth (DE).

		Sitophilus oryzae L.			
Conc.	**Day1**	**Day 2**	**Day 3**	**Day 5**	**Day 7**
10%DE+Lc50 *C. colothynthus*	23.3	56.6	97.7	100	100
8%DE+Lc50 *C. colothynthus*	16.6	32.2	93.3	100	100
6%DE+Lc50 *C. colothynthus*	13.3	23.3	62.2	91.1	100
4%DE+Lc50 *C. colothynthus*	8.8	16.6	47.7	83.3	94.4
2%DE+Lc50 *C. colothynthus*	5.5	12.2	21.1	56.6	76.6
		Oryzaephlius surinamensis L.			
Conc.	Day 1	Day 2	Day 3	Day 5	Day 7
10%DE+Lc50 *C. colothynthus*	17.7	47.7	91.1	100	100
8%DE+Lc50 *C. colothynthus*	12.2	32.2	83.3	95.5	100
6%DE+Lc50 *C. colothynthus*	10	21.1	57.7	85.5	93.3
4%DE+Lc50 *C. colothynthus*	7.7	13.3	31.1	41.1	85.5
2%DE+Lc50 *C. colothynthus*	5.5	10	17.7	32.2	44.4
		Stegobium paniceum L.			
Conc.	Day 1	Day 2	Day 3	Day 5	Day 7
10%DE+Lc50 *C. colothynthus*	10	27.7	41.1	67.7	96.6
8%DE+Lc50 *C. colothynthus*	5.5	21.1	32.2	43.3	91.1
6%DE+Lc50 *C. colothynthus*	5.5	13.3	21.1	38.8	76.6
4%DE+Lc50 *C. colothynthus*	3.3	10	12.2	23.3	58.8
2%DE+Lc50 *C. colothynthus*	3.3	7.7	10	21.1	40
		Tribolium castaneum Herbst			
Conc.	Day 1	Day 2	Day 3	Day 5	Day 7
10%DE+Lc50 *C. colothynthus*	5.5	10	23.3	47.7	93.3
8%DE+Lc50 *C. colothynthus*	3.3	7.7	13.3	32.2	71.1
6%DE+Lc50 *C. colothynthus*	3.3	5.5	10	26.6	63.3
4%DE+Lc50 *C. colothynthus*	1.1	5.5	7.7	17.7	56.6
2%DE+Lc50 *C. colothynthus*	1.1	3.3	7.7	12.2	28.8

Table 6. LT_{50} and LT_{95} of the fumigant toxicity of Diatomaceous earth combined with LC_{50} of *C. colothynthus* essential oil against the adults of *S. oryzae, O. surinamensis, S .paniceum* and *T. castaneum*

Samples	Conc.	Insects	$LT_{50}{}^{a}$ (µl/l air)	$LT_{95}{}^{a}$ (µl/l air)	Slope ± SD	Chi Square(x^2)	p-Value	R.
Citrullus colothynthus	10% DE + LC_{50} of C. colothynthus	S.O	1.52	3.23	5.02±0.04	15.38	0.00	0.93
		O.S	1.75	4.02	4.56±0.40	9.69	0.01	0.98
		S.P	3.07	9.90	3.23±0.27	13.3	0.00	0.95
		T.C	4.19	12.04	3.59±0.31	33.7	0.00	0.92

Although it shown that the combination of DE with *C. colocynthis* EO provided a significant increase in mortality of the tested insects. Additive effect was observed against adults of *S. oryzae* and *O. surinamensis* when DE doses combined with LC50 of *C. colocynthis* (see Table 5). Combinations of DE and EO can be effectively used as an alternative compounds to chemical insecticides against stored product insects since it reduced the time required for complete killing. As presented in other studies *C. colocynthis* EO can be applied as insect repellents with no harmful effects to the environment (Halder et al., 2010).

Our results come in the same trend with those of (Yang, et al., 2010; - Tayeb et al.,2018)who noted that combination of garlic EO with DE decreased the effective dose of both treatments. Similar studies conducted with other essential oils and in many cases the synergic effect between DE and another substance(s) enhanced the effectiveness of a mixture thus allowing the reduction of DE doses to be applied to the stored products (Athanassiou & Korunić 2007; Almaši et al., 2013; Ziaee et al., 2014).

CONCLUSIONS

Laboratory bioassays were carried out to determine the contact, fumigant, and repellent activities of *C. colocynthis* essential oil (EO) which applied alone or in combinationed with diatomaceous earth (DE) against adults of *S. oryzae*, *T. castaneum*, *O. surinamensis* and *S. paniceum*. Overall, our results indicated that *C. colocynthis* EO can be useful in repellent and toxicant formulations. The combination treatment was significantly more effective than single treatment because combination of oil with DE decreased the effective dose of both treatments. Thus, *C. colocynthis* EO combined with DE may have potential role, as an alternative to synthetic insecticides in controlling certain of stored insect species.

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كفاءة استخدام زيت الحنضل النباتي منفردا وعند خلطه مع مسحوق التربه الدياتوميه ضد بعض حشرات المواد المخزونه

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الملخص

أجريت هذه الدراسة لتقييم التأثير الطارد وبالملامسه وبالتبخير لزيت الحنضل النباتي بالاضافه لقييم ناتج الخلط بين الجرعه النصف مميته من زيت الحنضل مع عدة تركيزات من مسحوق التربه الدياتوميه ضد الاطوار الكامله لأربعه من حشرات الحبوب المخزونه هم سوسة الأرز، خنفساء السورينام، خنفساء العقاقير ، خنفساء الدقيق الصدئيه تحت ظروف المعمل. وأظهرت النتائج أن تأثرت معدلات موت الحشرات إيجابيا بزيادة كلا من التركيزات المستخدمه ومدة التعريض. وكان تأثير السميه بالتبخير أكثر فاعليه من تأثيره بالملامسه ، فعند مدة تعريض ١٢٠ ساعة من المعامله بالتبخير كان التركيز المسبب لموت ٥٠% من الحشرات المختبره كان ٢,٦٤، ٤,٦١، ١٣,٤ و١٧,٣٢ مل/لتر بالنسبة لـ سوسة الأرز، خنفساء السورينام، خنفساء العقاقير ، خنفساء الدقيق الصدئيه على التوالي، بينما في حالة المعامله بالملامسه كان ٣,٩٢ ،١٢,٦ ،٣٦,٧ و٤٨,٨٦ مل/لتر لنفس الحشرات على التوالي. كما تم تسجيل تأثير طارد قوي لزيت الحنضل علي الحشرات المختبره فسجل ١٠٠، ٩٥,٥، ٩٨,٨ و ٨٦,٦% لسوسة الأرز، خنفساء السورينام، خنفساء العقاقير ، خنفساء الدقيق الصدئيه على التوالي عند تركيز ٢٠نل/سم٢. كما كان للتربه الدياتوميه تأثير قوي بالملامسه وكان التركيز المسبب لموت ٥٠% من الحشرات المختبره ٢,٢٨٧و ٢,٦٣و ٥,٤٠ و ٢٠,٢ % وزن/وزن % لسوسة الأرز، خنفساء السورينام، خنفساء العقاقير ، خنفساء الدقيق الصدئيه على التوالي.أوضحت النتائج أن سوسة الارز هي الأكثر حساسية بينما كانت خنفساء الدقيق هي الأكثر تحملا لزيت الحنضل النباتي ويمكن الاستفادة من الخلط بين زيت الحنظل والتربه الدياتوميه كأحد برامج المكافحة لآفات المنتجات المخزنة.