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Insecticidal efficacy of the combination of diatomaceous earth and essential oil of *Ammoides verticillata* against *Tribolium confusum*.

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Abstract :-In order to evaluate the insecticidal activity of a formulation of the mixture made of *ammoides verticillata* essential oil with diatomaceous earth on *Tribolium confusum*, the *Tribolium* were exposed to diatomaceous earth at different concentrations: 0.0mg, 0.2mg, 0.8mg, 1.4mg, 2mg, and 3mg/cm² in Petri dishes, and their mortality rate was monitored every 24 hours for up to 120 hours, and to different concentrations of the essential oil (00μl, 02μl, 04μl and 8μl). We checked their mortality rate after 24h, 48h, 72h, 96h, and 120h. After having positive results, we tested the concentrations of the combination of diatomaceous earth and essential oil of *Ammoides verticillata* as follows; lethal dose (LD) 10 of diatomaceous earth + LD10 of essential oil, LD30+LD30, LD50+LD50, LD70+LD70 and LD90+LD90 in petri dishes. The mortality of *Tribolium confusum* population treated with diatomaceous earth and essential oil was highly significant (P<0.000), the statistical results gave us a LC50 at 0.60mg/cm² for diatomite and LC50 of 3.04μl for essential oil. The combination of essential oil and diatomite gave us very significant results. We noticed the maximum mortality (100%) with LD90+LD90 after 48 hours of exposure.

Keywords:-Diatomaceous earth, Essential oil, *Ammoides verticillata*, *Tribolium confusum*, Insecticidal efficacy.

1. Introduction

Cereals play an important role in the agricultural system globally. They are a main source of human and animal food (Slama et al., 2005). Cereals provide approximately 50% of the calories eaten by humans and 60% of the raw materials used in making compound feed for animals. In Algeria, the cereal sector is one of the most important segments of agricultural production (Djermoun, 2009). There are 3.5 million hectares of cereal production, of which 1.6 million hectares are durum wheat. The annual average consumption of the cereal products was about 205 kg per person in Algeria (Chehat, 2007). Generally, cereals are stored as food supply, as animal feed, and as seed for the next planting season.

Wheat storage and preservation are designed to maintain the original quality of the grains with minimal change. However, there are significant losses at production, and at central storage facilities like warehouses or silos. Losses are a result of insects, rodents, molds and bacteria (Khayra et al., 2019). After being stored, food products are exposed to insect pests such as *Tribolium confusum*. These pests cause sufficient damage to produce and limit both qualitative and quantitative production issues. Their increase or proliferation is mainly due to favorable environmental conditions for growth and survival. Some pests damage seeds while are still maturing and continue in the store (Pruthi and Singh, 1950). Losses, according to the Algerian Interprofessional Office of Cereals (O.A.I.C.), can exceed 35% in the past few years (Ahmad, 2016). The most frequently utilized means of controlling unwanted insects is to use chemical insecticides (Soejarto and Farnsworth, 1989). Additionally, synthetic insecticides can work their way into stored grains and can ultimately be toxic to consumers (Ogendo et al., 2004). Therefore, integrating pest management methods is crucial, utilizing prevention and biological control solutions at every opportunity (Kebdani, 2017).

The use of diatomaceous earth is among the proposed alternatives; it is non-toxic and environmentally friendly (Ross, 1981). Also, aromatic plants are among the most potent botanical insecticides. Their essential oils are rich in bioactive chemical compounds. Biopesticides based on essential oils could represent a promising alternative for pest resistance management programs (Isman, 2000).

The objective of this study is to evaluate the insecticidal activity of *Ammoides verticillata* essential oil in combination with diatomaceous earth against the grain storage pest *Tribolium*

confusum, in order to provide a reliable alternative method to protect stored food products against this pest.

2. Methods

2.1. Insect collection and laboratory bioassays

To test the insecticidal activity of diatomaceous earth (DE) against *Tribolium confusum*, we took samples of wheat infested with *T. confusum* from the Tissemsilt-Algeria cereals and legumes cooperative (CCLS). The insects were stored in plastic bags containing wheat to ensure food supply.

2.2. Insecticidal activity of diatomaceous earth against *Tribolium confusum*

We concentrated on a local material called diatomaceous earth. Diatomaceous earth is quite common in Algeria, and is also known as kieselguhr or diatomite. Diatomaceous earth is a porous and crumbly siliceous sedimentary rock that is almost entirely composed of the fossilized remains of diatoms. The diatomaceous earth was collected from the Bider area (Tlemcen, Algeria). It contains high-pressure coesite, silicon dioxide (SiO₂) at 77%, magnesium oxide (MgO) at 4.5%, potassium oxide (K₂O) at 1.0%, calcium oxide (CaO) at 6.8%, sulfur (VI) oxide (SO₃) at 0.6%, phosphorus (V) oxide (P₂O₅) at 1.0%, iron (III) oxide, hematite HP (Fe₂O₃) at 4.1%, vanadium phosphide (PV) at 0.4%, phosphorus sulfide (P₄S₇) at 1.0%, titanium oxide (TiO₂) at 0.2%, aluminum oxide (Al₂O₃) at 2%, phosphorus (P) at 1.0%, and sodium oxide (Na₂O) at 0.4%, as determined by Bounouira et al. (2019).

Insects were exposed to diatomaceous earth at different concentrations: 0.0 mg, 0.2 mg, 0.8 mg, 1.4 mg, 2 mg, and 3 mg/cm² in Petri dishes (9 cm diameter) containing wheat to feed the *Tribolium confusum* (Figure 1).

All experimental were kept in the lab at 24° ± 2°C, and their mortality rate was monitored every 24 hours for up to 120 hours.



Figure 1: Bioassays against *Tribolium confusum*.

2.3. Insecticidal activity of *Ammoides verticillata* essential oil on *Tribolium confusum*

In this study, we used essential oil extracted from *Ammoides verticillata*.

The *Tribolium confusum* were introduced into Petri dishes with 10 grains placed on filter paper treated with different concentrations of the essential oil (2 μ L, 4 μ L, 8 μ L). Three repetitions were performed for each dose with a control group (Figure 2). Mortality rate was monitored after 24h, 48h, 72h, 96h, and 120h.

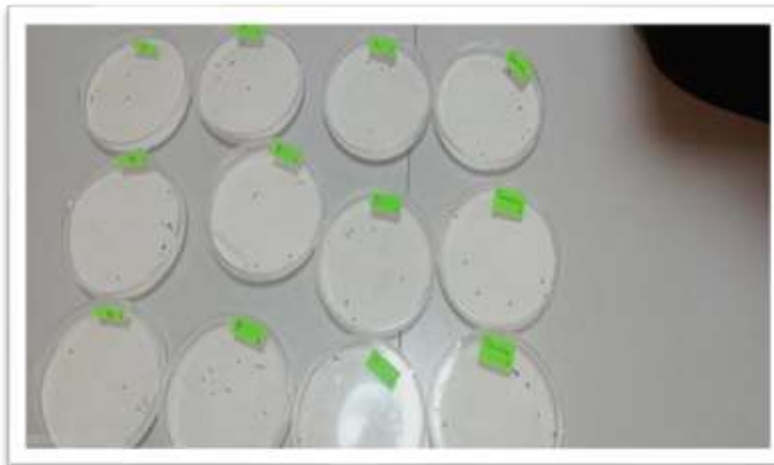


Figure 2: Mortality test of *Ammoides verticillata* essential oil on *Tribolium confusum*.

After having positive results after the use of diatomaceous earth and essential oil and after the estimation of lethal concentrations, we tested the concentrations of the combination of diatomaceous earth and essential oil of *Ammoides verticillata* as follows; LD10 of diatomaceous earth + LD10 of essential oil, LD30+LD30, LD50+LD50, LD70+LD70 and LD90+LD90 in petri dishes, and in boxes which contain 10 corn seeds and 10 individuals of *Tribolium*.

2.4. Methods of analysis and data processing

The methods used in this study are based on analysis of variance (ANOVA), Duncan's test, and lethal concentration calculations. We calculated lethal concentrations to determine the DE and essential oil doses that caused mortality rates from 10% to 90% in the *Tribolium confusum* population. Statistical analyses were carried out using SPSS software.

3. Results

In this study, we focused on evaluating the insecticidal activity of a local material called diatomaceous earth, which is abundant in Algeria, and an essential oil from a local plant (*Ammoides verticillata*) on the wheat storage pest *Tribolium confusum* (Figure 3).

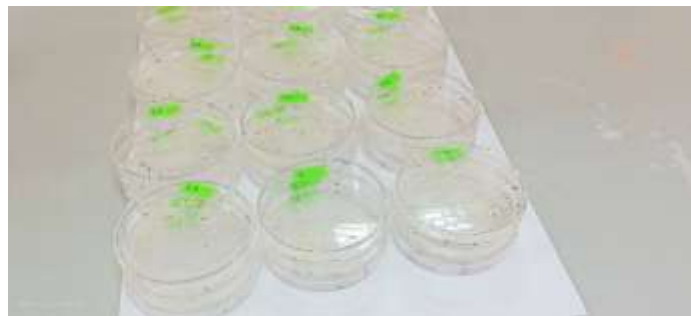


Figure 3: Mortality test of *Tribolium confusum* population treated with diatomaceous earth.

3.1. Effective concentrations of diatomaceous earth

The mortality of *Tribolium confusum* population treated with diatomaceous earth was highly significant depending on the doses and exposure time ($P < 0.000$; $P < 0.000$, respectively) (Table 1).

Table 1: ANOVA test for mortality of *Tribolium confusum* population treated with diatomaceous earth (DE) at 0, 0.2, 0.8, 1.4, 2, and 3 mg/cm².

	ANOVA SS	DDL	MEAN SQUAR	F	Pr > F
DOSE	178,4	17	10,4941	5,083	6,22E-07
TIME	247,622	4	61,9056	29,99	2,21E-14

Previous studies have shown that different strains of the same species exhibit varying susceptibilities to DE (Rigaux et al., 2001), and that the concentration affects mortality and efficacy (Fields and Korunic, 2000).

To compare the effectiveness of different concentrations of our DE, we performed Duncan's test (Table 2). We observed similar effectiveness between concentrations of 0 and 0.2 mg/cm², with less than 30% mortality after 120 hours of exposure.

A similar efficacy was found between concentrations of 0.8 and 1.4 mg/cm², with a mortality rate under 60%. High efficacy was observed at 2 and 3 mg/cm² with over 80% mortality after 120 hours.

Table 2: Susceptibility of *Tribolium confusum* population to different concentrations of diatomaceous earth.

Dose (mg/cm ²)	DUNCAN GROUPING		
	1	2	3
00	1.66A		
00,2	2.66A		
0,8		5.66B	
1,4		6.00B	
2,0			8.66C
3,0			9.00C

In the following table (Tab 3), we compared the sensitivity of the *Tribolium confusum* population treated with diatomite, based on observed mortality. It would be interesting to know the lethal concentrations to control this population.

3.2. Estimation of lethal concentration (LC) values for the *Tribolium confusum* population.

To identify the diatomite concentrations that determine mortality for the *Tribolium confusum* population as a function of time, we calculated the lethal concentrations. The following table shows the lethal concentrations of diatomite.

Table 3: Estimated lethal concentrations (LC) after treatment with diatomaceous earth on *Tribolium confusum* population.

LETHAL DOSES	95% CONFIDENCE LIMITS FOR DOSE		
	Estimate (μL)	Terminal inferior	Terminal superior
10	0.15	0.007	0.285
20	0.202	0.023	0.426
30	0.305	0.054	0.576
40	0.433	0.110	0.761
50	0.600	0.210	1.017
60	0.833	0.378	1.432
70	1.182	0.646	2.267
80	1.780	1.052	4.468
90	3.141	1.758	13.471

The results show a significant toxic effect of DE on the *Tribolium confusum* population with LC50 at 0.600 mg/cm² and LC90 at 3.141 mg/cm².

3.3. Effective concentrations of *Ammoides verticillata* essential oil

The mortality of *Tribolium confusum* population by *Ammoides verticillata* essential oil was also highly significant depending on the doses and exposure time ($P < 0.000$ $P < 0.000$, respectively) (Table 4).

Table 4: ANOVA test for mortality of *Tribolium confusum* population treated with *Ammoides verticillata* essential oil at 0, 2, 4, and 8 μL .

	ANOVA SS	DF	Mean Square	F	Pr < F
μDOSE	614.983	11	55.9076	146.7	6.69E-31
TIME	24.4333	4	6.1083	16.03	3.61E-08

We compared the efficacy of different concentrations of our *Ammoides verticillata* essential oil using Duncan test (Tab 5). The control population showed an average mortality of 2.66 within 120h of treatment with *Ammoid verticillata* essential oil. We observed similar efficacy between concentrations 0mg, and 0.2 mg/cm² with a mortality rate of less than 30% within 120 h of exposure (Figure 4). The 8 μ concentration had an average mortality of 9.33 after 120h of treatment with *Ammoides verticillata* essential oil.



Figure 4: Mortality test of *Tribolium confusum* population treated with *Ammoides verticillata* essential oil.

Table 5: Susceptibility of *Tribolium confusum* population to different concentrations of *Ammoides verticillata* essential oil

DOSE (μL)	Subset for $\alpha=0,05$		
	1	2	3
00	2.66A		
2.00		3.00B	
4.00		6.00B	
8.00			9.33C

3.4. Estimation of lethal concentration (LC) values for the *Tribolium confusum* population

The following table shows the estimated lethal concentrations of *Ammoides verticillata* essential oil, which determine the mortality of the *Tribolium confusum* population as a function of time.

Table 6: Estimated lethal concentrations after treatment with *Ammoides verticillata* essential oil on the *Tribolium confusum* population.

LETHAL DOSES	95% CONFIDENCE LIMITS FOR DOSE		
	Estimate (μL)	Terminal inferior	Terminal superior
10	1,22	0,09	2,06
20	1,67	0,26	2,56
30	2,10	0,53	3,05
40	2,54	0,94	3,65
50	3,04	1,53	4,55
60	3,65	2,26	6,24
70	4,42	3,03	9,87
80	5,54	3,84	18,81
90	7,57	4,92	49,74
99	15,88	8,05	553,28

According to the results in Table 17, the essential oil of *Ammoides verticillata* has a significant toxic effect on *Tribolium confusum* with an LC50 of 3.04 μL and LC90 of 7.57 μL .

3.5. The insecticidal activity of the combination of diatomite and essential oil of *Ammoides verticillata*

The combination of essential oil and diatomite gave us very significant results.

We noticed an increase in the mortality rate after using the combination, after 24 hours we noted a rate of 50% after 120 hours with the LD10+LD10 dose, for the LD50+LD50 dose we noted a mortality rate of more than 80% and We noticed the maximum mortality of 100% with LD90+LD90 after 48 hours of exposure (figure 5).

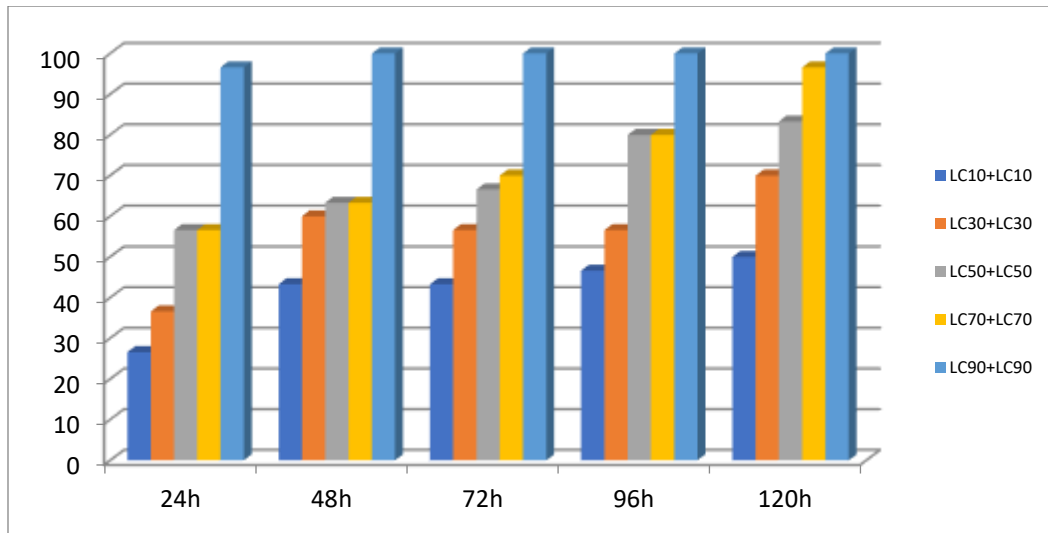


Figure 5: The insecticidal activity of the combination

4. Discussion

In this study, we tested the insecticidal activity of the combination of diatomaceous earth and essential oil of *Ammoides verticillata* against *Tribolium confusum*.

According to Bounouira 2020, the insecticidal activity of DE on *Ceratitis* and *Agriotes* produced highly significant results. Glenn Moore, an entomologist at Northrop King, believes that DE can be used as an insecticide for crops (Decrosta, 1979).

The effect of DE on insects is significant. Because its mode of action is entirely mechanical, it does not release harmful residues into other organisms' digestive systems, and insects cannot develop immunity or resistance to it. Diatomaceous earth is essentially a lethal dust bordered with microscopic sharp edges. Ingestion of this lethal powder by insects causes them to become dehydrated from the inside, as well as destruction of their interior, which happens quickly, usually within minutes. Since it is a mechanical insecticide, insects cannot develop immunity or resistance against DE. Therefore, it can be used to control insects for a long time without the manifestation of insecticide resistance that is often reported for other insecticides (Islam and Rahman, 2016).

A study conducted in 1943 by the USDA demonstrated an 86% kill rate of bean weevils using DE. Another study, uncontrolled, involved cotton fields in California and DE's

effectiveness in comparison to chemical pesticides. Researchers discovered much larger crop yields using DE (Ross, 1981).

Several studies have been conducted on the insecticidal activity of DE, including recent ones against: *Rhizopertha dominica* (Fields and Korunic, 2000 ; Stathers and al., 2002) , *Tribolium castaneum* (Rigaux and al., 2001 ; Reza and al., 2012 ; Kabir, 2013), *Tribolium confusum* (Mewis and Ulrichs, 2001), *several Sitophilus species* (Islam and al., 2010 ; Mewis and Ulrichs, 2001; Bounouira and al., 2022), *Callosobruchus maculatus* (Islam and al., 2010), *Agriotes lineatus* (Bounouira and al., 2019) and *Ceratitis capitata* (Bounouira and al., 2024).

Based on previous studies and our results, DE can be used as a natural pesticide that is safe for humans and the environment.

Medicinal aromatic plants are considered biopesticides due to the composition of their essential oils, which help control insect pests (Ketho, 2004). Botanical pesticides are useful in both developed and developing countries, especially in post-harvest protection. Essential oils and their components are truly toxic to many insects through contact and fumigation (Saheb, 2007).

Essential oil-based biopesticides form a promising class of insecticides as they contain multiple compounds with different modes of action. The insecticidal activity of *Ammoides verticillata* Essential has been confirmed in several studies against various insect species, such as *Sitophilus zeamais* (Bounouira and al., 2022) and *Bactrocera oleae*, a major olive pest in the Mediterranean region (Senouci H., 2020). This activity primarily pertains to the predominant compounds. Limonene has been researched and utilized as a botanical insecticide (Sfara and al., 2009). Additionally, the extent of its insecticidal efficacy may be attributed to the interactions among all the constituents found in the oil (Belabbes et al., 2017). Terpenes has been shown to have significant toxicity against a panel of insect pests (Khanikor and al., 2013), Carvacrol has been shown potent insecticidal and acaricidal activity with a broad spectrum against agricultural, stored-product, and arthropod pests. It showed displaying strong and selective activity against *Alphitobius diaperinus* (Panzer), a cosmopolitan pest insect of stored food grain products such as flour, and of poultry-rearing facilities (Ahn and al., 1998).

Based on previous research and our results, the essential oil of *Ammoides verticillata* is a natural pesticide that is both effective and environmentally safe.

5. Conclusion

Our findings indicate that using a combination of diatomaceous earth and *Ammoides verticillata* essential oil is highly recommended for controlling *Tribolium confusum*. This is because of the dual benefits of the essential oil and diatomaceous earth, which may be used as an alternative to insecticides.

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