The Synergism and Larvicidal efficacy of microalgae Fischerella muscicola extracts and Nestor against meal moth Plodia interpunctella larvae

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Abstract

To develop bioinsecticides from environmentally friendly natural products, the present study was conducted to assess the toxicity of ethanol and aqueous extracts of the microalga *Fischerella muscicola* and the pesticide Nesto each separately, based on the LC50 values of concentrations (10, 25, 50, 75 and 100 µg/ml), in addition to Estimation of the synergy ratios of these extracts with Nestor at a ratio of 1:1 and 1:5 (pesticide:extract) against third and fifth instar larvae of Indian meal moth *Plodia interpunctella*, after 24, 48 and 72 hours of surface treatment. The results proved that the ethanolic extract had a very toxic effect on larvae, as it recorded a significant superiority in toxicity to the pesticide Nestor, as the LC50 values against third instar larvae were 0.018, 0.028 and 0.042 µl/larva, and LC50 values for fifth instar larvae were 0.113, 0.074 and 0.047 µl/l 24, 48, and 72 hours of working time, respectively. The values of the synergy ratios indicated that the extracts of the alga *Fischerella* had a synergistic effect with the insecticide Nestor clearly in increasing the toxicity against the larvae when they were mixed with the insecticide in both proportions, as the killing rates increased with the increase in the mixing ratio, which was manifested by a significant decrease in the LC50 values, especially in the aqueous extracts. It alone recorded any killing rates after 72 hours of treatment, while it gave high killing rates when mixed with the pesticide, especially at a ratio of 1:1. Accordingly, a number of active phenolic compounds present in algae extracts were identified, qualitatively and quantitatively, using High Performance Liquid Chromatography (HPLC) technology. The crude ethanolic extract (40.19%) and Kaempferol in the crude aqueous extract (44.1%).

1. Introduction

The economic importance of the Indian meal moth *Plodia interpunctella* was first recognized by Chittenden in 1894 (Williams, 1964) and is known by common names including Dried fruit moth, Horn compressed vegetable moth, (Hagstrum et al., 2013). Today it is the number one pest of stored products; They infect many grains and processed stored products, but also attack ground grain products, nuts, spices, peas, beans, lentils, chocolate and other commodities (Kumar, 2017). Indian flour is more prevalent than meal moth, as it is considered the first pest in flour mills (Subramanyam, 1996), it feeds on a wide range of grains, including barley, buckwheat, corn, oats, rice and wheat, or on dried fruit meals such as raisins, apricots, figs , peaches and plums, it also feeds on nuts such as almonds, peanuts, hazelnuts and walnuts, as well as peas, beans, lentils and various stored commodities (Williams, 1964) .

Adults are distinguished by wings of yellowish-white, brown and reddish-brown colors, while the larvae are white or yellowish-white. The insect begins its life from the eggs that it lays in the crevices of the food, then the larvae that feed on the food itself up to the pupal stage, and finally the short-lived flying adults that Do not feed (Rees, 2007). After the eggs hatch, the new larvae disperse in search of food, and their small size helps them enter airtight containers. (Subramanyam, 1996). Full-grown larvae are yellowish-white in color. The larvae pass through several larval stages and molts and eventually turn into a pupae inside a cocoon. The larval stages in total take 55 days at 20°C and 65%RH (Kumar, 2017; Subramanyam, 1996; Bell, 1975)On average, the female lays 200 eggs either singly or in groups on infected stored commodities (Kumar, 2017; Rees, 2007; Gorham, 1999).

Micro-bacteria (cyanobacteria)

Algae and bacteria are essential organisms of surface waters, and their presence is of ecological importance, and sometimes raises public health concerns. Various control measures are taken, as the interactions of microalgae, macroalgae, submerged aquatic plants, and bacteria appear to be important phenomena that require deeper understanding. by those involved in research and management of microbial water quality (Cho et al., 2022). The term cyanobacteria is used to describe a class of microorganisms that use photosynthesis to obtain the energy they need to live and reproduce. Bacteria are considered to be prokaryotes as they do not contain a nucleus or any other internal structures that have lipid membranes around them. Its oldest name is blue-green algae because its color is usually bluish-green, and it often grows in water (Cameán and Jos, 2020; Nienaber and Steinitz-Kannan, 2018). They can be found almost anywhere on Earth, producing a variety of toxic compounds (cyanotoxin), some of which are toxic to animals, but these toxins rarely raise public health concerns under most circumstances, as due to their low and normal numbers they usually grow unnoticed. One, however; under appropriate conditions; These organisms can be a real problem and have a significant impact on public health (Nienaber and Steinitz-Kannan, 2018).

Fischerella muscicola . moss

The prokaryotic alga was previously classified under different morphological types, but the modern

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classification included it in the order Nostocales, as it was found that the alga shares the same ancestry with members of the order (Rastogi, 2021). It is one of the autotrophic genera capable of fixing atmospheric nitrogen, the moss has a filamentous, cylindrical, branched, heterogeneous shape, its apical chiles are circular in shape (Al-Qassimi, 2020), and it divides in more than one plane (Sarma, 2013), single-row lateral branches partially or completely emerge from the sheaths , and can grow into a new algal filament (Esser, 1986) Microalgae can be grown easily compared to plants due to the limited requirements of the algae, they require only a small area for cultivation and contain either marine or brackish water, and wastewater can be used (Mol and Thirumurthy, 2020), as Chlorella sp showed a mechanism A defense against many pathogens by absorbing nitrogen and phosphorous from wastewater, algae cultivation also needs alternative nutrients such as industrial waste, which can be added to the agricultural environment in order to reduce production costs without affecting sustainability (Renuka et al., 2018). , and there are many studies in which microalgae were used as pesticides against various pests, including a study in which the extract of Nostoc carneum was used against the larvae of the cotton leaf worm Spodoptera littoralis (Saber et al., 2018), and a second study in which the extract of Spirulina platensis was used against the larvae of The second and fourth instars of the cotton leaf worm (Rashwan and Hammad, 2020).

Phenolic compounds isolated from algae

Phenolic compounds are found in a huge variety of marine terrestrial plants due to their importance and contribution to the growth and survival of living organisms, as they help in defense against bacteria and predators, (Jimenez-Lopez et al., 2021). Among the compounds discovered in algae are: :

Phenolic Acids Bioactive compounds involved in many functions, including nutrient absorption, protein synthesis, enzymatic activity, photosynthesis, and biochemical or bioantagonism. These molecules regularly bind to other molecules such as simple and/or complex carbohydrates, organic acids, and other bioactive molecules such as flavonoids or terpenoids, and are usually classified according to the number of carbons in the chain, which are attached to the phenolic ring (Luna-Guevara et al. , 2018; Liwa et al., 2017).

Flavonoids Phenolic compounds are structurally characterized by a heterogeneous cyclooxygen bonded to two aromatic rings, which can then vary according to the degree of hydrogenation. There are many studies on the content of flavonols in terrestrial plants, but studies of flavonoid content in algae are rare, but some indicate that marine algae are a rich source of antioxidants and other flavonoids (Cotas et al., 2020; Bilal Hussain et al., 2019; Mukherjee et al., 2019).

Cytochrome P450 enzymes

They are also known as Mixed Function Oxidases, Monooxygenases, and Multi Function Oxidases. They are enzymes specialized in decomposing toxic substances and extraneous substances in the cell. They are found in most living organisms, either in a soluble form, as in microorganisms, or associated with cellular membranes, especially the endoplasmic reticulum and mitochondrial membranes in higher organisms (Lee et al., 1995). It can be found in most parts of the body, but the liver is the main center of its existence because most of the foreign substances and drugs that enter the body are metabolized with the help of enzymes in the liver. These enzymes play an important role in the metabolism and detoxification of a large number of endogenous compounds, as well as their metabolism of a large number of exogenous compounds known as xenobiotics, including insecticides and phytotoxins (Feyeresien, 1999).

The presence of these enzymes in insects is concentrated in the midgut, Malpighian tubes and fat bodies (Feyeresien, 1999) and the concentration of any of these enzymes varies in different tissues (Scott et al., 1998), as it is now certain that the activities of these enzymes as well as their concentrations (levels) In insects, they vary greatly during evolution, and the patterns of activity of each of these enzymes vary between one developmental stage and another, and even between one age and another within the same evolutionary stage (Abbas, 2013). These enzymes play an important and essential role in the metabolism of chemicals and the detoxification of drugs, natural toxins and exotic biomaterials (Egbichi, 2009; Al-anizy, 2005).

Insecticide synergists

Synergists, whether chemical manufactured from them or extracted from natural compounds, are by nature non-toxic or low-toxic compounds if used alone, while using them in low concentrations with pesticides causes an increase in the efficiency and toxicity of insecticides as they increase the speed of pesticide penetration through the insect's cuticle, as well as discourage it. CYP450 enzymes, thus increasing the toxicity of pesticides in the least amount and least harm to the environment, in addition to delaying the ability of insects to develop resistance against pesticides. Non-toxic (or less toxic if used alone) and when mixed with insecticide increases the toxicity of the pesticide more than if it was alone (O'Brien, 1967). Synergists have also been defined as non-toxic substances in themselves or they are almost toxic, adding them in small quantities leads to an increase in the toxicity of pesticides by inhibiting the work of enzymes that metabolize pesticides, and this depends on the method of the synergist attack or its effect on the active sites, as the effectiveness of the action is high. With metabolic inhibitors (Abbas, 2013). There is also the term quasisynergism given by (Sun and Johnson, 1972) to substances that want the penetration, transport or speed of access of the pesticide to the target sites, regardless of its mechanism of action.

Insecticides have been used for about a century in the control of agricultural pests. It was found (Weed, 1938) that the compound N-isobutylinducyleneamid led to an increase in the efficacy (toxicity) of the pyrethrum pesticide, and then (Eaglson, 1940) discovered that the oil of Sesame seeds led to an increase in the toxicity of the pyrethroid insecticide. After that, Haller et al., 1942 confirmed that sesame seed oil led to an increase in the toxicity of pyrethroid pesticides, and they attributed the reason for this to the fact that the oil contained compounds from the Methylene dioxyphenyl group. Compounds (MOP), or the so-called (1,3-benzodioxole) such as sesamin and sesamolin, derivatives of these compounds have been used for several years to increase the effectiveness of insecticides, until the first compound of this group was prepared, which is pyronyl butoxide in 1947 by Wachs, which has been shown to increase the effectiveness of pyrethroid insecticides, and later laboratory and field studies confirmed that this compound and a number of its derivatives increase the toxicity of pyrethroid insecticides against many types of insects (Abbas, 2013).

Most of the main insecticides synergists are basically inhibitors of mixed-functional oxidation enzymes, and each group of them is specialized and affects according to a specific mechanism or method, as (Wilkinson, 1976) indicated that there are four main types of interactions of synergists with mixed-functional oxidation enzymes, but two are accepted. Only the first is the non-campetitive inhibition hypothesis (Raffa and Thomas, 1985) which states that synergists (MDP derivatives) when metabolized by mixed-functional oxidative enzymes give rise to carboxolium ions and free radicals and carbanions, respectively. With a number of components of the system of mixed-function oxidative enzymes and their inhibition, which means that the amount of inhibition does not depend on the concentration of the synergist, but rather on the amount of the metabolite. The second is the hypothesis of purely competitive inhibition, which is the most accepted to this day. Its content is that the synergists act merely as basic materials for mixed-functional oxidative enzymes, a substitute for the pesticide, meaning that the amount of enzyme inhibition depends on the concentration (amount) of the synergist (Casida et al. , 1966), and (Franklin, 1972) added that the residual enzyme activity after reaching its maximum inhibition is due to the amount of non-inhibiting enzymes, which in turn are used for synergistic metabolism.

2. Materials and Methods

Insects approved in biological tests, Meal moth Indian *Plodia interpunctella*, Lepidoptera, were bred from colonies prepared for the study. In industrial media consisting of sterilized wheat flour by freezing for a period of three days to ensure that it is free from eggs and second insect larvae, in addition to it dry bread yeast in a ratio of 20:1 (yeast: flour), equal amounts (200 g) of culture medium (food) were placed in Sterile plastic bottles with 70% ethanol, with a capacity of 600 g, 100 adult insects were placed in each bottle, then the bottles were placed in the incubator after covering their nozzles with sterile chiffon pieces and fixing them with rubber bands, and incubated at a temperature of 30 ° C and a relative humidity of 60-70%, taking into account the exchange of breeding media Every 30 days (Saleem et.al., 2013). As for the Indian flour moth, *Plodia interpunctella*, it was bred on industrial media prepared from a mixture of crushed nuts (almonds, walnuts and peanuts). Equal amounts of culture media (250 g) were placed in sterilized plastic bottles with ethanol 70%, each of which capacity was 600. Insects were placed in each bottle of 50 random pupae of the Indian flour moth, and in order to isolate the eggs of the Indian flour moth, 25 pairs of pupae were placed inside a special box made by hand using a sieve with holes diameters of 800 micrometers, the sieve was fixed on a plate or bowl, and its opening was closed After mating, eggs were collected every 24 hours and placed on sterile food media in special boxes. The first hatching of eggs was observed after five days. As for the larval stages, the insect recorded five larval stages, separated by six days. After 8-9 days, the fifth instar larvae turned into pupae, and these pupae needed 6-10 days, depending on the season of the year, to complete the morphological transformation into an adult. They survive for 10-14 days, while the majority of males live for half that time (Curts and Landolt, 1992).

Fischerella muscicola **. moss**

Two species of *Fischerella muscicola* belonging to the Cyanobacteria group, obtained from the algae laboratory of the College of Education for Pure Sciences/University of Mosul, were used in this study.

The pesticide Nestor

In the current study, Nestor 20 sp insecticide was used. This insecticide belongs to the new methylated nicotine-methylated Neonicotinoides group. It has the form of a water-soluble powder Solnble powder. Its active substance is acetamiprid 20% (w/w) Acetamiprid 20% (w/ w), and its manufacturer is Agri scinences, and the following is its most important information as mentioned (Yu, 2015):

(1E)-N-[(6-chloro-3-pyridinyl)methyl]-N'-cyano-N-methylethanimidamide

Agricultural solutions and media

- M HCL (2M) hydrochloric acid solution

Prepared by following the law of molarity on concentrated hydrochloric acid

The law of dilution was then applied to calculate the volume of hydrochloric acid, and the volume was then completed to 1 liter of distilled water.

- Algae Growth Medium Chu 10

Chu 10 liquid medium was used to grow *Fischerella muscicola*, according to the following components:

Materials	Weight (g/l) distilled water
$Ca(NO_3)_2$	0.4
K_2HPO_4	0.1
Na_2CO_3	0.2
$MgSO_4.7H_2O$	0.25
Na_2SiO_3	0.25
Ferric Ammonium Citrate	0.05

The development medium was prepared by dissolving these components with the above-mentioned weights per 1 liter of distilled water. The prepared medium was placed in a 3-liter glass container (bioreactor) and sterilized with a sterile Autoclave (autoclave) at a temperature of 121 °C and a pressure of 1 atmosphere for 20 minutes (Al-Hayali, 2020; AL-Kateb, 2017).

- Algae sample growth

Vicherella alga was cultivated under sterile conditions in liquid cultures (simple hand-made bioreactors) by preparing the liquid Chu 10 medium and placing it in a 3-liter glass bottle whose lid had previously been prepared with two holes for the purpose of inserting rubber tubes through them. The glass bottle, while the second tube heading outside the bottle is connected to a syringe filter (0.45 mm) and connected to a second rubber tube connected to a small pump of the types used in fish tanks in order to introduce air into the reactor, and the second hole passed through a second rubber tube used to empty The excess air inside the bottle, and this reactor was placed in 25±2°C growing chambers equipped with 2500 lux illumination over 24 hours (Al-Hayali, 2020; Falch et. al, 1995).

- Preparation of Extracts

The development reactor was filtered using a 250 ml funnel fitted with filter paper (Whatman No.1), the amount of algae precipitated on the filter paper was collected and left to dry in the shade at room temperature 2±25 for two to three days, then it was ground with a ceramic mortar until it turned into a fine powder, and also kept in airtight containers until use. According to the method (Ale Grand et al., 1988) 2 gm of vicherella alga were weighed and put into a 500 ml beaker, 50 ml petroleum ether for defatted process, 50 ml ethyl alcohol, and 50 ml distilled water were added. Close the flask with aluminum foil tightly and place it on the magnetic stirrer at medium speed for 72 hours, then filter the mixture using Whatman No. 200 ml of 70% ethyl alcohol was added to it, and after closing the mouth of the flask with aluminum foil, it was placed again on the magnetic stirrer for another 72 hours, then filtered with filter paper and the extract was concentrated by a REV Rotary vacuum evaporator at a temperature of 40 ° C. . The remaining precipitate was soaked for the third time with 200 ml of distilled water in a 500 ml beaker, and placed on the magnetic stirrer at a temperature of 60 ° C to obtain the hot water extract, then the crude extracts were placed in opaque glass bottles, closed tightly and kept in the refrigerator until use (Harborne, 1984).

Basic test solutions

Depending on the results of the preliminary tests, stock solutions of the pesticide were prepared by placing the required weight of each Nestor pesticide or the extract in a graduated cylinder of 10 ml and completing the volume with distilled water to 10 ml, from which five concentrations were prepared (10, 25, 50, 75 and 100 µl/ ml), by adding 9 ml of distilled water to 1 ml of the concentration of the basic solution and in the same way the required concentrations (micro g / ml) of the extracts were prepared using the appropriate solvents for each of them. At a temperature of 4°C until use.

Vital tests experiments were conducted on the insect of the current study with 3 replicates for each concentration, and 10 larvae for each replicate, taking into account that the insects are active and homogeneous in size. The third and fifth instar larvae of the Indian meal moth were treated by the surface treatment method to achieve the following:

A- Determination of the toxicity of the pesticide.

b- Determination of the toxicity of aqueous and alcoholic extracts of alga

C- Estimation of the potential synergistic effect of aqueous and alcoholic extracts of Vacherila alga on the toxicity of the pesticide Nestor. The solutions were used in mixing ratios of 1:1 and 1:5 (pesticide:extract).

Surface treatment Topical Application

Initial experiments showed that the appropriate amount of solvent to carry the tested materials (microliter or microgram) for each larva was 10 microliter, so the insects were treated by adding 10 microliter of each of the test solutions to the dorsal side (upper surface) of the insect's chest by means of a microsyringe, 1000µl)). As for the insects of the control group, they were treated with distilled water or ethanol used to dissolve the tested substance. After 15 minutes, the insects were inspected to ensure their safety. These treated insects were placed in Petri dishes, and the dishes were placed in the incubator. Mortality percentages were recorded after each of 24 hours and 48 hours. 72 hours from the start of the test.

statistical analysis

In cases where the homicide rates in the control group exceeded 10%, the homicide percentages in the coefficients were corrected by applying Abbot's equation (Abbot, 1925) to correct the homicide percentages:

- Determination of the lethal concentration value LC50% of the tested insects

The values of LCs for 50% of tested insects, Slope, and Upper and Lower Confidence Limits were determined using MSDOS by Probit software. Exe, Propit program) for the statistical analysis of the results of toxicity tests according to the (Finney, 1952) method.

- Estimation of Synergestic Ratio (SR)

To calculate the values of synergy ratios, the listed equation was used dividing the value of the dose or LC50 of the tested insects from the pesticide alone, by the value of the dose or LC50 of the pesticide with the extract (Brattesten and Metcalf, 1970). It is equal to the number of times the increase in pesticide toxicity caused by the extract.

Diagnosis using acid hydrolysis of raw extracts to detect phenols

There are phenols inside plants linked by a glycosidic bond with sugar, forming glycosides, and for the purpose of purification and identification of phenols, the process of acid hydrolysis is carried out to release phenols from sugar by breaking the glycosidic bonds based on the method of the researcher (Harborne, 1973), when 25 ml of hydrochloric acid (HCL) was added at a concentration of 2 molar extracts to 5 ml of crude ethanol extract of algae, they were put into a glass flask and the mixture was heated in a Reflux Condensor at 70 °C for 1 hour, then the mixture was cooled down and 50 ml of ethyl acetate was added to it in a 250 ml separating funnel over two phases, the first is 25 ml, and the mixture is shaken well, then left on a stand until two layers appear; An organic layer and an aqueous layer, the upper organic layer containing sugar-free free phenolic compounds was separated, and the above steps were repeated a second time with the lower aqueous layer, then concentrated using the rotary evaporator to obtain the phenolic extract, the process was also repeated for the aqueous extracts, and the phenolic extracts were preserved In opaque bottles in the refrigerator until use.

Identification of active phenolic compounds quantitatively and qualitatively using high-performance liquid chromatography technology (HPLC).

A high-performance liquid chromatograph based on polarity and capillary action was used to separate separated phenolic compounds from plants, as most of these compounds are weakly acidic (ionize under basic conditions and dissolve easily in different polar solvents) (Al-Hayali, 2020). Research vehicles in the laboratories of the Ministry of Science and Technology / Department of Environment and Water using a high-performance liquid chromatography (HPLC) type (SYKAM) of German origin, with a separation column C18 with dimensions (4.6 x 250 mm), and a UV/VIS SPD-20A detector at The wavelength is 280 nm, and the carrier phase was used: methanol: distilled water: formic acid 5:25:70 (V:V:V) to separate the phenols, and the flow rate of the carrier phase was 1.3 ml/min flow rate.

3. Results and discussion

The results of biological tests for estimating the toxicity of Nestor and extracts of *Fischerella* alga against Indian meal moth larvae depended on the values of concentrations and lethal doses for fifty percent of the tested insects (LC50 and LD50), and the minimum and maximum confidence limits at the 95% upper and lower confiddence limits 95%. As well as the values of the toxicity regression lines (slope), and the estimates of the synergistic effect of the extracts were based on the values of synergy ratios, confidence limits and slope values. The results showed the toxicity or non-toxicity of the test solutions, and how the toxicity of the poisonous ones varied significantly with the difference in the sex of the insect and its developmental stage, as well as the method of treatment. The results also showed that the highest rates of killing appeared in the method of surface treatment, Topical Application, while the method of exposure to Petri dishes was the Contact treatment. It showed relatively little or no killing rates in low concentrations, especially with the Indian flour moth, which can crawl and hang on the sides and covers of dishes away from the toxic substance on the bottom, and it brushes the inner surface of the entire dish with its silk threads thus reducing the amount of the toxic substance that touches her body.

Toxicity of the insecticide Nestor to Indian meal moth larvae

The results in Table (1) indicate that the pesticide Nestor is toxic to the larvae of the Indian flour moth, and that its toxicity varied clearly with sex, developmental stage and method of treatment. different insects, as (Zhang et al., 2022) confirmed that exposing the soybean aphid Aphis glycines to acetamiprid shortened the life cycles, parturition time and pre-reproductive period for adults, and (Wang et al., 2020) confirmed that acetamiprid is highly toxic against Bombyx mori larvae after continuous exposure to a low dose of the insect, and its residues in the midgut did not degrade until after 96 hours of treatment. The results showed that the toxicity of the pesticide in the surface treatment method of Indian meal moth larvae is more than that of the baffling flour beetle, the Indian meal moth larvae were more sensitive to pesticide concentrations of 1.5, 2.9 and 6.6 times, respectively, during 24, 48 and 72 hours, It was found that the third instar larvae of the Indian meal moth are 1.8, 1.9 and 2.4 times more sensitive to it than the fifth instar larvae, respectively, during 24, 48 and 72 hours, and that it is also more toxic to the adults of the baffled flour beetle than to the larvae of 0.5, 1.1 and 0.2 hours, respectively. 24, 48 and 72, and the results showed that its toxicity in the surface treatment method was double what it was in the exposure method to treated Petri dishes.

Table (1): LC50 values of third and fifth instar larvae after 24, 48 and 72 hours of treatment with Nestor alone

Larval stage	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.169	0.064	0.477	1.040	0.065	0.030	0.142	1.210	0.030	0.015	0.056	1.409
Fifth	0.305	0.118	0.785	1.261	0.126	0.064	0.249	1.603	0.074	0.039	0.139	1.585

Toxicity of ethanolic and aqueous extracts of *Fischerella* seaweed to Indian meal moth larvae.

The results of toxicity tests in Table (2) indicated that the ethanolic extract is very toxic to Indian meal moth larvae and that its toxicity varied significantly with sex and developmental stage. This study agreed with other studies in which extracts of cyanobacteria were used as insecticides. If (Rashwan and Hammad, 2020) confirmed that the ethanolic extract of spirulina algae Spirulina platensis showed high toxicity against second and fourth instar larvae of Spodoptera littoralis, it also agreed with the assertion of (Saber et al., 2018) that the crude ethanolic extract From the alga nostoc carneum showed insecticidal activity against the larvae of the cotton leaf worm Spodoptera littoralis, also confirmed (Al-Dulaimi, 2021) the effect of organic extract, ethanolic extract and acetone extract of Vicherella alga on pathogenic bacterial species S.amirabileusonia, K.p.p.p. , P. aeruginosa and E.coli, if the organic extract recorded the best results in inhibiting growth, while the ethanolic extract recorded relatively lower results than the organic, while the aqueous extract did not record any positive results. It is clear from comparing the results of the toxicity of the ethanolic extract in these tables with the toxicity of the pesticide Nestor in Table (1) that the toxicity of the ethanolic extract to larvae is 7.0 times more than that of the pesticide Nestor, as the results showed that it is more toxic to flour moth larvae. Indians had more pesticides 3.0, 1.8 and 1.6 times, respectively, during 24, 48 and 72 hours.

Table (2): LC50 values of third and fifth instar larvae after 24, 48 and 72 hours of surface treatment with ethanolic extract of the alga *Fischerella muscicola*

Larval stage	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.042	0.024	0.075	1.456	0.028	0.017	0.046	1.697	0.018	0.011	0.028	1.953
Fifth	0.113	0.063	0.190	1.709	0.074	0.044	0.124	1.709	0.047	0.029	0.078	1.717

The results of this study were in agreement with (Abbas, 2013) that the fourth instar larvae of the baffling flour beetle, the rusty red flour beetle and the long-headed flour beetle are more sensitive to the pesticides Actaer and Lannate than their adults. Larval ages are lower than in adults, making them less susceptible to detoxification reactions. They also found (Al-Attar and Abbas, 2003) that the smaller third instar larvae of the red and bewildering flour beetle Coopex, Decis and Vapcocidin than the larger fifth and seventh instar larvae. . The results of the current study agree with (Soummane et al., 2011) that methanolic and dichloromethane extract from the aerial parts of a number of medicinal plants are more toxic to the larvae of the Mediterranean fruit fly Ceratitis capitata than to its adults, and also agree with (Saidana et al., 2007) in The extracts of petroleum ether, methanol, and chloroform for several types of plants were very effective against adults and larvae, but the larvae were more sensitive than adults, and this difference in toxicity can be attributed to the behavior of the larva because its body wall (cuticle layer) is less solid than it is In adults, which makes the larvae more susceptible to pesticides, and this is consistent with what was previously confirmed by (Cercelius and Knowles, 1976) in the larvae of the leaf blight insect. As for the fact that the tested substances are more toxic by the superficial treatment of larvae and adults, this may be due to the speed of penetration, and this is consistent with what was confirmed by (Cercelius and Knowles, 1976) about the presence of high concentrations of the pesticides, chlordymiform and demethylated clodiform inside the bodies of the third instar larvae of the leaf-pest insect after Surface treatment, attributing this to the speed of permeability.

Synergistic effect of aqueous extracts on the toxicity of Nestor to Indian meal moth larvae.

It is evident from the results of the biological tests in the table (3) and (4) that adding the aqueous extract to the pesticide Nestor caused a significant increase in the toxicity of the pesticide against larvae, and this was reflected on the values of LC50, as it decreased significantly and clearly than it was for the pesticide alone, recording killing rates that exceeded 80% within 24 Only an hour, when adding the extract to the pesticide at a ratio of 1:1, the toxicity of the pesticide to Indian meal moth larvae increased by 19.7, 38.2 and 26 times, respectively, and the killing rates were almost similar in the third and fifth instar larvae using both extracts with a slight superiority in the third instar larvae.

Table (3): LC50 values of the pesticide Nestor mixed with the aqueous extract in a ratio of 1:1 after 24, 48 and 72 hours

	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
Larval stage	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.004	0.001	0.010	1.177	0.000	0.000	0.002	1.106	0.000	0.000	0.000	0.000
Fifth	0.021	0.011	0.040	1.507	0.006	0.003	0.013	1.379	0.004	0.002	0.009	1.737

Table (4): LC50 values of the pesticide Nestor mixed with the aqueous extract in a ratio of 5:1 after 24, 48 and 72 hours

	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
Larval stage	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.001	0.000	0.003	1.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fifth	0.015	0.004	0.052	0.742	0.004	0.001	0.016	0.686	0.002	0.001	0.007	0.851

Synergistic effect of the two ethanolic extracts on the toxicity of the pesticide Nestor to Indian meal moth larvae

It is clear from the results of the biological tests in Tables (5) and (6) that adding the ethanolic extract to the pesticide Nestor caused a significant increase in the toxicity of the pesticide against larvae and adults by the surface treatment method, and this was reflected in the LC50 values, which decreased significantly and clearly from what they were for the pesticide alone and for the crude extract alone. When the algal extract was added to the pesticide in a ratio of 1:1, the toxicity of the pesticide to Indian meal moth larvae increased by 39.5 and 47.7, respectively, during the 24 and 48 hours, and the killing rates were almost similar in the third and fifth larval instars with a slight superiority in The effect on third instar larvae. As for the mixture of algae extract Vicherella caused an increase in the rates of killing than the pesticide alone 26.1 and 28.6 times, respectively, during the 24 and 48 hours, but the killing rates decreased than in the extract alone, 1.1 and 1.3 times during the 24 and 48 hours. Conversely, the 5:1 mixing ratio also caused a significant increase in pesticide toxicity, but the killing ratios all appeared slightly lower than the killing ratios when the mixing ratio was 1:1.

Table (5): LC50 values of Nestor mixed with ethanolic extract at a ratio of 1:1 after 24, 48 and 72 hours

	After 24 hour of treatment				After 48 hour of treatment				After 72 hour of treatment			
Larval stage	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.004	0.002	0.009	1.595	0.000	0.000	0.003	1.106	0.000	0.000	0.000	0.000

Fifth	0.009	0.004	0.034	1.007	0.004	0.001	0.010	1.376	0.000	0.000	0.002	0.729

Table (6): LC50 values of Nestor mixed with ethanolic extract at a ratio of 1:1 after 24, 48 and 72 hours												
After 24 hour of treatment					After 48 hour of treatment				After 72 hour of treatment			
Larval stage	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope	LC50	Confidence Limits		Slope
		Lower	Upper			Lower	Upper			Lower	Upper	
Third	0.012	0.004	0.030	1.374	0.004	0.002	0.009	1.613	0.000	0.000	0.000	0.000
Fifth	0.048	0.017	0.135	1.032	0.008	0.003	0.023	1.082	0.001	0.000	0.005	1.044

The fact that the synergistic effect did not increase in the present study at the mixing ratio of 5:1 than it is at the mixing ratio of 1:1 indicates that the synergistic effect of these substances reached its maximum at the ratio 1:1, and the effectiveness of the extracts can be attributed to the synergistic effect of the pesticide. Nestor to its inhibition of the enzymes that metabolize the pesticide, and this conclusion is confirmed by the results of many studies that proved that the various phenolic compounds present in the extracts are strong inhibitors of mixed-functional oxidative enzymes, especially the cytochrome enzyme CYP3A4, which is responsible for metabolizing the vast majority of foreign biological substances, including pesticides, including what was mentioned by (Lakshmi et al., 2021) in their study, the hexane extract of Croton bonplandianum was used with two types of pyrethroids (Cypermethrin and Lambda-cyhalothrin) against the dengue vector Aedes aegypti. Better killing activity for mosquito larvae. This conclusion also confirms what was mentioned (Li et al., 2021) about the effect of the crude extract of Artemisia sieversiana on the locust Oedaleus asiaticus. They confirmed that the extract can be used as a synergist with the entomopathogenic fungus Metarhizium anisopliae, and that this compound mixture can become a control agent. Biological alternative for the control of insect pests. The conclusions in the current study confirm what was stated (Gonçalves Diniz et al., 2020) in their study in which they used combinations of isolates of the fungus Fusarium caatingaense with aqueous and ethanolic extracts of the tobacco plant Nicotiana tabacum against the cochineal insect Dactylopius opuntiae. Watering the tobacco plant with the entomopathogenic Fusarium fungus resulted in a mortality rate of 98.7%.

Quantitative and qualitative diagnosis of reactive phenolic compounds by using high-performance liquid chromatography (HPLC) technique.

A number of phenolic compounds were diagnosed and separated from crude extracts, as well as from phenolic extracts and petroleum ether of algae extract. Table (7) shows each of the standard phenolic compounds, standard retention time (minutes), and area (area) for the standard compounds.

Table (7): Standard phenolic compounds and standard retention time Reten. time (minute)					
Standard phenolic compounds		Reten time	Standard phenolic compounds		Reten time
1	Apigenin	4.78	6	p-Coumaric acid	5.88
2	Caffeic acid	11.80	7	Rutin	8.10
3	Ellagic acid	3.65	8	Sinapic acid	10.12
4	Hydroxybenzoic acid	12.19	9	Vanillic acid	13.88
5	Kaempferol	6.82			

The results and table (8) showed the phenolic compounds in the extracts of alga *Fischerella muscicola*, the compound of Kaempferol, that this compound appeared in the petroleum ether extract of *Fischerella* alga with a percentage of (51.4%) in the phenolic water extract, and (16.1% of the crude extract, in the aqueous extract).) in petroleum ether extract. and the compound Rutin, which appeared in the highest percentage (47.58%) in the phenolic ethanol extract, the arrangement of the compounds was as follows: Rutin > Sinapic acid > p-Coumaric acid > Ellagic acid > Hydroxybenzoic acid in proportions (47.58> 40.15> 39.88> 31.58> 28.99), while the compound Kaempferol appeared in the highest percentage (51.4%) in the phenolic water extract, and the arrangement of the compounds was as follows: Kaempferol > Ellagic acid > Caffeic acid > Apigenin > Sinapic acid > Vanillic acid with percentages (51.4> 40.9> 39.5> 28.9> 22.6> 20.6), while the petroleum ether extract showed the compound Kaempferol with the highest percentage (16.5%), and the arrangement of the compounds was as follows: Kaempferol > Vanillic acid > Sinapic acid (16.5 > 11.0 > 9.5). It is clear from the above that the compound most present and having the highest percentage in algae is Kaempferol, followed by Ellagic acid, then Caffeic acid. Studies on phenolic materials indicated that the phenolic content in algae products depends on the different extraction conditions used, as well as the type of solvent used for extraction (Ethanol, methanol, chloroform, hexane and ethyl acetate) (Machu et al., 2015), and these results are in agreement with the study (Al-Hayali, 2020; El-Harbawi, 2019) that species of cyanobacteria algae contain different types of phenolic compounds such as Keampferol, Rutin, Qurcetine and Catechine. And I also agreed with (Singh et al., 2017) who confirmed that the crude extract of twenty species of Cyanobacteria contains Qurcetin, Keampferol, Galic acid and Rutin.

Table (8): Comparison of the ratios of phenolic compounds in extracts of *Fischerella* alga

	Extract Phenolic compounds	Aqueous extract		Ethanolic extract		Petroleum Ether Extract
		Before acid hydrolysis	After acid hydrolysis	Before acid hydrolysis	After acid hydrolysis	
1	Apigenin	21.4	28.9	-	-	-
2	Caffeic acid	33.6	39.5	-	-	-
3	Ellagic acid	32.59	40.9	20.59	31.58	-
4	Hydroxybenzoic acid	-	-	18.99	28.99	-
5	Kaempferol	44.1	51.9	-	-	16.5
6	p-Coumaric acid	-	-	33.58	39.88	-
7	Rutin	-	-	40.19	47.58	-
8	Sinapic acid	16.98	22.6	32.59	40.15	9.5
9	Vanillic acid	14.5	20.6	-	-	11.0

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