The Effectiv of the Testing Chemical Pesticide Nishan 20sc and the Biopesticide Spinosad on the Larvae and Pupae of the *Culex Theileri Theobald* Mosquitoes of the Genus *Culex* (Diptera: Culicidea) in Samarra

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Abstract:

The study was conducted in the Department of Life Sciences – College of Education – University of Samarra, which aims to combat mosquito larvae and pupae using the chemical pesticide 20 Sc Nishan and knowing the percentage of its effect on the larvae and pupae of the *Culex thieleri* mosquito along with the use of the biological pesticide known as Spinosad and knowing which of the two is more effective in eliminating mosquito larvae and pupae in particular. *Culex* genus, and three concentrations of the chemical pesticide were used (0.25, 0.5, 1) ml/L.

The effect of different concentrations of Nishan 20 Sc pesticide on the death of *Culex* mosquito larvae indicated that there were significant differences in the killing rates due to the interaction between the concentrations and the duration of exposure, as the highest killing rate was 100 mosquito larvae. *Culex* % at a concentration of 1.0 ml pesticide/liter after 72 hours of treatment, while the lowest killing rate was 30.0% at a concentration of 0.25 ml pesticide/liter after24hours of treatment. Only distilled water was used in the control plants and for a period of 72 hours,24 and 48 hours for each concentration. Three concentrations of the biocide known as Spinosad, 12% (0.50, 0.25, 0.125) ml/L, were used for each Spinnosad pesticide to kill *Culex* mosquito larvae. There were significant differences in the killing rates due to the interaction between the concentrations and the duration. Exposure: The highest killing rate of *Culex* mosquito larvae was 100% at a concentration of 500 ml pesticide/liter after 72 hours of treatment, while the lowest killing rate was 23.3% at a concentration of 0.125 ml pesticide/liter after 24 hours of treatment. It was used in control laboratories.

Only distilled water, with a time period of 72, 48, and 24 hours for each concentration. For each concentration, three replicates were used for both larvae and pupae, and 10 larvae were placed in each replicate, Also, 10 pupae were placed in each replicate for each replicate of the experiment. The results of both pesticides gave high killing results when The concentration was 1 ml/liter after 72 hours for the chemical pesticide 20 Sc Nishan, while the biopesticide Spinosad was more effective at a concentration of 0.5 ml/liter after 72 hours.

The results of the study confirmed that there is a direct relationship between increasing the concentration, the time period of exposure, and the percentage kill rates, and that mosquito larvae were more sensitive than pupae in being affected by all the treatments under study.

keywords: *Culex*, , Spinosad, IPM, Nishan, DDT

Introduction:

Mosquitoes are medically important insects as they transmit many dangerous and deadly pathogens to humans and animals. Mosquitoes remain one of the most important major threats to public health and economic growth in many parts of the world Mosquitoes are Diptera insects that act as biological and mechanical vectors for many diseases.

Parasites and mosquitoes can transmit endemic and even epidemic diseases and other diseases[1].

Mosquitoes are small insects belonging to the Diptera order of the Culicidea family, Mosquitoes can be identified by their small size, which is approximately 3-9 mm. They have one pair of wings covered by scales, long legs, and parts The mouth of female mosquitoes contains a long proboscis and its mouthparts are piercing and absorbent to feed on blood to mature the eggs, while male mosquitoes have mouthparts that are only absorbent to feed on plant juices [2].

The mosquito control program relies heavily and extensively on chemical pesticides, as is known in the field. Integrated pest control (IPM) has many benefits, including that mosquitoes are unable to develop resistance to them, unlike chemical pesticides, and it also contributes to combating all immature stages of mosquitoes [3].

Control using chemical pesticides has a rapid and significant effect on insects, but they cause great damage to humans, animals, plants, soil, and the environment. Chemical pesticides affect targeted and non-targeted insects, and their cost is high, in addition to their persistence in animal and plant tissues, which leads to the emergence of resistance in vectors [4]. The insecticide (Diphenyl-Dichloro-Trichloroethane) DDT

was among the first pesticides used to control arthropods that transmit diseases. It was originally sprayed to combat malaria and typhus during World War II , With the end of the war, DDT became used to control mosquitoes that transmit malaria after it was proven Its effectiveness in Europe. Through the application of DDT, malaria infection rates decreased significantly throughout the world. In 1947, the first resistance to DDT appeared in Anopheles taeniorhynchus mosquitoes. *Culex* mosquitoes in Baghdad became resistant and tolerant to high concentrations of DDT, despite widespread resistance, For both organophosphorus pesticides and brothroids, large numbers of their pesticides are still used to control mosquitoes, largely because there are very few effective alternatives [5].

They are among the most important types of insect control because they quickly kill insects, are easy to use, and give quick results, Chemical pesticides have been used to completely eliminate insects of all kinds. The disadvantages of this control include the occurrence of environmental disturbances, as well as the deposition of chemical components in food chains, the emergence of insects that have resistance to the pesticide, and the indistinguishability of toxic and dangerous chemical pesticides between beneficial insects, as well as against predators and natural enemies of the targeted insect, as well as those with it. A toxic effect on humans, animals, plants, soil and air, which leads to an imbalance in environmental systems and the economic and expensive cost of the pesticide.

Nishan 20sc is a non-systemic pesticide used to combat all motile and immobile insect stages. The pesticide category works to eliminate a wide spectrum of insects, especially some Diptera families. Its toxicity is by contact. The molecular formula of the pesticide Nishan is C12H4CL2F6N4, Nishan 20 Sc can be sprayed comprehensively and has the ability to be mixed with other pesticides except alkaline ones.

Biopesticide: Spinosad is one of the pesticides that has been recently used in pest control and is a natural metabolic product produced by Actinomycetes bacteria, For the spinosa species, Saccharopolyspor Spinosad is a mixture of two types of metabolites, Spinosy A and Spinosyn B, which are deadly infectious toxins when taken orally, as it has a direct effect on the nervous system of the insect, as it works in binding to the Nicotinic Acetyl cline receptor of the insect and thus causes involuntary movement of the body's muscles that It results in tremors and paralysis of the insect's body, all chemical pesticides, regardless of their method of action, will lead to the emergence of resistance in the target species, and thus the necessity of finding new pesticides to replace them, In addition to that, the unintended harm to non-target species that results from the application of pesticides determines their benefit [6].

Aim of the Study:

Testing the effectiveness of the chemical pesticide Nishan 20sc , the biocide Spinosad on the larvae and pupae of *Culex theileri theobald* mosquitoes.

Materials and Methods:

Areas of Salah al-Din Governorate/ Samarra were chosen to collect the larvae and pupae of the *Culex* ***theiler*** *theobald* mosquito, especially the areas where there are many ponds and swamps in which mosquitoes breed.

The larvae and pupae were collected during the months of November and December and in separate areas of Samarra, which is the Qasr al-Ashiq area, the southeastern coast of the city. Al-Qalaa, Al-Abbasiya area, Al-Qatul area pool.

The biopesticide Spinosad was obtained from in Baghdad on the recommendation, The chemical pesticide Nishan 20sc was obtained from a pesticide seller in the city of Samarra.

Laboratory breeding of mosquitoes *Culex theiler theobald* :

A new strain was used in the study in Samarra, which was recorded for the first time in the city of Samarra by the researcher *Culex theileri theobald*, belonging to the genus *Culex* , It was obtained in different areas of the Samarra district, which are the Qasr al-Ashiq area, the southeastern coast of the city of the Citadel, the Abbasiya area, and the pool of the Qatul area, in which it is found. Ponds, swamps, and stagnant water, where Culex mosquitoes abound due to the presence of organic materials, vegetation, wild animals, and birds. Larvae and pupae of *Culex* mosquitoes were collected during the months of November and December, and samples of larvae and pupae of *Culex theileri theobald* mosquitoes were classified at the University of Baghdad Research Center and Natural History Museum in their book No. 3/ 7/1287 on 12/12/2023 and the diagnosis was made by.

A medium-handled scoop was used for the purpose of collecting mosquito larvae and pupae, then they were placed in plastic containers prepared for this purpose with a capacity of 2 liters and containing a liter of water similar to water in the wild environment and free of chlorine. It was transferred to the incubator under laboratory conditions at a temperature of 2 ± 26 °C and a relative humidity of 5. ± 65% and a lighting period of 10-14 hours.

The experiments were conducted in sterile plastic petri dishes, and tap water was placed in it exposed to the sun for three days to get rid of the chlorine present in the water.

The experiments were conducted with three replicates for each concentration, in addition to using a control factor for each of the three concentrations and using 10 larvae as well as 10 pupa, the concentrations of the biocide were (0.125, 0.250, 0.5) Spinosad ml/L, the concentrations of the Nishan pesticide were (0. 25, 0.5, 1) ml/L for each replicate. The test was done after 24, 48, and 72 hours for the chemical pesticide and the biocide known as Spinosad. When the dead larvae and pupae were counted, a medium-sized scoop was used to collect mosquito larvae and pupae, then they were placed in plastic containers prepared for this purpose, with a capacity of 2 liters and containing a liter of water similar to water in the wild environment and free of chlorine. They were transferred to the incubator under laboratory conditions at 100 °C. The temperature was 26±2°C, the relative humidity was 65±5%, and the lighting period was 10-12 hours. After that, they were placed in the rearing cage, whose dimensions were length 85 cm x width 50 cm x height 85 cm, which was designed by the researcher. The larvae were fed by adding ground and prepared mouse diet. Of wheat, yellow corn, and protein (soybeans) in a ratio of 1:1:1, 2 grams per tank [7].

Biscuits were also used to feed the larvae, and the water was replaced every 7 days to avoid rotting. Females need proteins and blood for the purpose of fertilization and egg production, so they must be fed. On a blood meal by placing the feathered pigeons from the chest and back areas in the breeding cage for the purpose of absorbing the blood. After that, plastic boxes were placed inside the breeding cage containing water to lay the eggs. The eggs are then transferred to pots with dimensions of 25 x 25 cm to raise the larvae.

Figure(1) Mosom breeding cage is designed by the researcher

Prepare concentrations of Nishan 20 Sc pesticide to eliminate *Culex theileri theobald* larvae and pupae

Different concentrations of Nishan pesticide were prepared, namely, (1,0.50,0.25) ml/liter, according to the recommendations of the pesticide manufacturer, and 1000 ml of distilled water was added to each concentration. As for the control, only distilled water was used without adding the pesticide, and three concentrations were used for each concentration.

Replicates of mosquito larvae were obtained after 24, 48 and 72 hours. The results were recorded and it was found that the highest percentage of killing larvae was after 72 hours at a concentration of 1 ml/l. The same previous steps were performed on the Virgin Mary. Trade name: Nishan 20 sc. Store at room temperature.

Active ingredient: Aziloctin

Dosage recommended by the manufacturer: 1 ml/l

Producing company: HOUSE AGRICULTVRE

Production date: 03/2022

Effective date: 03/2025

Figure (2) Explains the chemical pesticide Nishan 20 SC

Preparing spinosad pesticide concentrations to eliminate *Culex theileri theobald* larvae and pupae

Different concentrations of spinosad pesticide were prepared, namely (0.125, 0.250, 0.5) ml/liter, and 1000 ml of distilled water was added to each concentration. As for the control, only distilled water was used without adding the pesticide for a period of (24, 48, 72) hours.

Trade name: Mozkill

Active ingredient: spinosad 12%

Dosage recommended by the manufacturer: 1 ml/L

the Producing company:

Production date: 2023/6

Effective date: 6/2025

For each concentration, three replicates of mosquito larvae were used after 24, 48 and 72 hours. The results were recorded and it was found that the highest percentage of killing larvae was after 72 hours at a concentration of 0.5 ml/l The same previous steps were performed on the pupal stage.

Statistical Analysis:

The results analyzed statistically by applying the statistical program (MINITAB VER.17) according to the Anova analysis test (Anova), the mathematical averages were

compared according to the Duncuns Multiple Range test and at a possibility of $0.05 \geq$ p [8].

Results:

Three Chemical pesticide concentrations were used in this experiment Nishan 20 SC $(0.250, 0.500, 1.0)$ milligrams / L by each concentration of three repeat, for a period of $(24, 48, 72)$ hours, the control coefficient of the experiment was used in the distilled water to know the killing rate And put in each bis 10 larva of larvae and pupal of 10 mosquitoes. And one put one tired of each concentration in all the repeated, knowing that the concentration recommended by the company was 1 ml of the pesticide, but it was very toxic, as the larvae were eliminated by 100% in less than 24 hours that led to the use of the above -mentioned concentrations.

The results of **Table** (1) showed the effect of the different concentrations of Nishan 20 SC in the destruction of larvae with mosquito Concentration of 1.0 ml pesticide / L after 72 hours of transaction, while it was the lowest killing rate of 30.0 % at the concentration of 0. 25 ml / L after 24 hours of treatment, while the average killing rate showed the effect of the concentrations that the highest killing rate was when concentrated 1.0 ml / L. It was killed after 24 hours 34.2 %.

The results of the current studies have interviewed with what it reached [9], as she showed through her study that there are moral differences in the virgin killing rates for home flies 24 and 48 hours after her treatment of Mid Al -Mallathion .

The results of the current study match with what was reached [10] in his study in which the diclorfos was used in the anti -household adults, as the pesticide was given the highest killing rate of 46.77 - 50.55% .

The results of the current study agreed with the results of the study [11]. conducted in Sudan to know the sensitivity of the phlebotomus papatasi insect that was collected from three regions of Sudan for many pesticides such as (Permethrin, DDT, Malathion and Proboxur Provoxur. Which showed the inability of the insect to resist the toxic influence of the triumphic pesticide in two regions, and this insect showed its resistance to the pesticide in the last or third region .

The results of the current study agreed with the findings of the [12] when using the diclorovus pesticide in the fight against the mosquitoes that the highest killing of the pesticide in the larvae life is 100 % in the PPM100 concentration and the lowest killing rate of 66. 16 % in the PPM10 concentration.

Table (1) The effect of Nishan's pesticide on larvae *culex* mosquito

Concentration ml / L	24 Hours	48 Hours	72 Hours	Concentration Average
0.25	3.00 g	5.33 e	7.67 c	5.33 C
0.50	4.33 f	6.33 d	8.67 b	6.44 B
1.0	6.33 d	8.67 b	10.00 a	8.33 A
Control	0 h	0 h	0 h	0.00 D
Time average	3.42 c	5.08 b	6.58 a	

*Similar lowercase letters horizontally mean there is no statistical difference between them.

*Capital letters that are vertically similar mean there is no statistical difference between them.

When increased the recesses used and this is due to the NISHAN 20 SC, the toxic effect increases with increased concentrations and exposure duration, and that this toxic effect inhibits the cholel cholin ethrase in the nervous system of the insect and thus leads to an increase in the secretion of the Astyle Colin that has a role in transferring the instrument ,the nervous so that this substance accumulates at the end of the nerves, and paralysis of the insect occurs and then its death.

The results of the Table (2) showed the effect of the different concentrations of Nishan 20 SC in the death of pupal in mosquito Concentration of 1.0 ml pesticide / L after 72 hours of treatment, while the lowest killing rate was 13.3 % at the concentration of 0. 25 ml / L after 24 hours of treatment, while the average killing rate showed the effect of the concentrations that the highest killing rate was when concentrated 1.0 ml / L. It was killed 24 hours later 22.5.%.

The results of the current studies were interviewed with the findings of [9], as she showed, through her study, that there are moral differences in the pupal killing rates for home flies 24 and 48 hours of treatment with Mid Al -Mallathion.

The results of the current study match with what was reached [10]. in his study in which the diclorfos was used in the anti -household anti On the watermelon fly B. Diversa, where it was noticed that the killing rate increases by increasing the period of exposure to the toxic substance and the killing rate reached 50 % when the period increases to 24 hours.

Table (2) The effect of Nishan's pesticide on pupal *culex* mosquito

Concentration ml / L	Hours 24	Hours 48	Hours 72	Concentration Average
0.25	1.33 h	3.33 f	6.00 d	3.56 C
0.50	2.33 g	5.00 e	7.33 c	4.89 B
1.00	5.33 de	8.33 b	10.00 a	7.89 A
Control	0	0	0	0.00
Time average	2.25 c	4.17 b	5.83 a	

*Similar lowercase letters horizontally mean there is no statistical difference between them.

*Capital letters that are vertically similar mean there is no statistical difference between them.

When increased the recesses used and this is due to the NISHAN 20 SC, the toxic effect increases with increased concentrations and exposure duration, and that this toxic effect inhibits the cholel cholin ethrase in the nervous system of the insect and thus leads to an increase in the secretion of the Astyle colin that has a role in transferring the instrument The nervous so that this substance accumulates at the end of the nerves, and paralysis of the insect occurs and then its death.

Discussions:

1- When the concentrations used increase, this is due to the toxic effect that Nishan 20 Sc pesticide has, which increases with increasing concentrations and duration of exposure. This toxic effect inhibits the enzyme acetyl cholin esterase in the nervous system of the insect and thus leads to an increase in the secretion of acetylcholine, which has a role in transmitting instructions. Nerve, such that this substance accumulates at the end of the nerves, causing paralysis and then death of the insect.

2- This pesticide works to affect mosquitoes as a result of the binding of the compounds of this pesticide, Spinosyn A and Spinosyn D, to the nerve receptors of the nervous system, thus inhibiting the enzyme Acetylcholin esterase, which decomposes the substance acetylcholin that transmits nerve impulses, thus preventing its connection with nerve connections, leading to the occurrence of involuntary movements that result in body tremors and paralysis. And then death,

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اختبار فعالية المبيد الكيميائي Nishan 20sc والمبيد الحيوي Spinosad على يرقات وعذارى بعوض *Culex theileri theobald* التابعة لجنس ***Culex*** (Diptera: Culicidea) في سامراء

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المستخلص:

أجريت الدراسة في قسم علوم الحياة – كلية التربية – جامعة سامراء التي تهدف الى مكافحة يرقات وعذارى البعوض بأستعمال المبيد الكيميائي Nishan 20 Sc ومعرفة نسبة تأثيره على يرقات وعذارى بعوض *Culex thieleri* مع استعمال المبيد الحيوي المعرف باسم Spinosad ومعرفة الاكفأ بينهما في القضاء على

يرقات وعذارى البعوض وخاصة جنس *Culex* وتم استعمال ثلاث تراكيز للمبيد الكيميائي (0.25, 0.5 ,1) مل / لتر, تأثير التراكيز المختلفة من مبيد Nishan 20 Sc في هلاك يرقات *بعوض Culex* الى وجود فروق معنوية في نسب القتل للتداخل بين التراكيز ومدة التعريض اذ كانت اعلى نسبة قتل يرقات بعوضة *Culex* 100 % عند تركيز 1.0 مل مبيد/ لتر بعد مرور 72 ساعة من المعاملة , بينما كانت اقل نسبة قتل 30.0 % عند تركيز 0. 25 مل مبيد / لتر بعد مرور 24 ساعة من المعاملة, استعمل في معامل السيطرة فقط الماء المقطر وبفترة زمنية (24, 48 ,72) ساعة لكل تركي, تم استعمال ثلاث تراكيز للمبيد الحيوي المعرف ب Spinosad 12% (0.25,0.5 , 0.125) مل / لتر, ولكل مبيد Spinnosad في هلاك يرقات بعوض *Culex* الى وجود فروق معنوية في نسب القتل للتداخل بين التراكيز ومدة التعريض اذ كانت اعلى نسبة قتل يرقات بعوضة *Culex* 100 % عند تركيز 500 مل مبيد/ لتر بعد مرور 72 ساعة من المعاملة , بينما كانت اقل نسبة قتل 23.3% عند تركيز 0.125 مل مبيد / لتر بعد مرور 24 ساعة من المعاملة ,استعمل في معامل السيطرة فقط الماء المقطر وبفترة زمنية (24, 48 , 72) ساعة لكل تركيز تم استعمال ثلاث مكررات لكلا من اليرقة والعذراء ووضع في كل مكرر 10 يرقات وكذلك وضع في كل مكرر 10 عذارى لكل مكرر من التجربة واعطت نتائج كلا المبيدان نتائج قتل عالية عند تركيز 1 مل / لتر بعد مرور 72 ساعة بالنسبة للمبيد الكيميائي Nishan 20 Sc اما المبيد الحيوي Spinosad كان اكثر فعالية عند تركيز 0.5 مل / لتر بعد مرور 72 ساعة.

اكدت نتائج الدراسة ان هناك علاقة طردية بين زيادة التركيز والفترة الزمنية للتعريض ونسب القتل المئوية وكانت ليرقات للبعوض اكثر حساسية من العذارى في تأثرها بجميع المعاملات قيد الدراسة .

الكلمات المفتاحيه:

DDT, Nishan, IPM , Spinosad , *Culex*