Effectiveness of the biopesticide Amyloland and the two chemical pesticides in controlling of the red rusty flour beetle *Tribolium castaneum* (Herbst)

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https://doi.org/10.59658/jkas.v11i4.2794

Received:
July 14, 2024

Accepted:
Aug. 15, 2024

Published:
Dec. 15, 2024

Abstract

This study evaluated the efficiency of the bacterial biopesticide Amyloland and the two chemical pesticides Coragen and Conan in controlling adults and the second instar larvae of the red rusty flour beetle *Tribolium castaneum* under laboratory conditions. The results of the study showed that the pesticide Amyloland achieved the highest mortality percentage of second-instar larvae at a concentration of 3.5 g L^{-1}, reaching 100% after 9 days of treatment, compared to the two concentrations of 2.5 and 3 g L^{-1}, which recorded a mortality percentage of 73.33% and 96.67%, respectively, for the same time. In the treatment of adults, the concentration of 3.5 g L^{-1} gave the highest mortality percentage of 90% after 13 days of treatment compared to the two concentrations of 2.5 and 3 g L^{-1}, which achieved a mortality percentage of 50% and 66.67%, respectively, for the same time. As for treating the two chemical pesticides, the Coragen pesticide achieved superiority and significant differences over the Conan pesticide in the adult instar. As for the treatment of the second larval instar, there are no significant differences between the two pesticides in the percentage of mortality during the treatment periods. The results of the Coragen pesticide indicated that the highest percentage of mortality for adults, at a concentration of 0.20 ml L^{-1}, reached 100% after 9 days of treatment. The concentrations of 0.15 and 0.10 ml L^{-1} recorded a mortality percentage of 76.7% and 56.7%, respectively. As for Conan pesticide in the adult instar, it was given at a concentration of 0.6 g L^{-1}, the highest mortality percentage of 63% after 9 days of treatment, while at the two concentrations of 0.5 and 0.4 g L^{-1}, the percentage of mortality 50% and 43.3%, respectively. From the results, we notice that the percentage of mortality increases with increasing concentration and length of time.

Keywords: *Trobilium castaneum*, Amyloland, Coragen, Conan

Introduction

One of the most important future challenges for the agricultural sector is the safety of food, grains and their products to cover the increasing population of the world. Post-harvest losses in agriculture are considered a major threat to people's food security, as

losses caused by post-harvest insects are estimated at approximately 10% in developed countries. In more than 20% of developing countries [1], Stored grains and their products are exposed to many insect pests that belong to different orders, especially Coleoptera. These insects infect stored products to a large extent, as more than 600 species of this order have been diagnosed as storage pests against wheat grains and their products [2,3]. *T. castaneum* is considered one of the most widespread warehouse pests and has a devastating economic impact, infecting many food types [4]. This insect is considered a secondary pest, but at the same time, it feeds on many grains, flour, seeds, nuts, and dried products [5]. Infestation with this insect can lead to the destruction of stored products directly as a result of the larvae and adults feeding on these stored products or indirectly through the female laying her eggs on the infected food as well as her unwanted, toxic and highly volatile secretions such as benzoqunones, as well as the secretion of other chemicals from Glands in the chest and posterior abdominal region [6].

Wheat crop production in Iraq for the 2023 agricultural season reached approximately 424800 tons, and the cultivated area amounted to 8419850 dunums [7]. Despite the great effectiveness of chemical insecticides against most insects, they have many negative effects on human health and the environment [8,9,10]. In addition, these insects have developed resistance to insecticides such as organophosphate pesticides and pyrethroids[11,12,13]. prompted researchers to study new alternatives and strategies leading to radical changes. In pest management, toxic chemical pesticides are replaced with less toxic to humans and less dangerous to the environment [14]. Because of the economic importance of the flour beetle, the red rusty *T. castaneum* and the aim of finding alternative materials that are environmentally friendly and safer for humans and animals to use in combating this insect, the research aimed to evaluate the pesticide of bacterial origin Amyloland biologically and the two chemical pesticides Coragen and Conan in controlling adults and second instar larvae of the flour beetle.

Materials and Methods

A colony flour beetles, the red rusty *T. castaneum*, was obtained by collecting samples of flour infested with insects from local markets in Kerbala Governorate. The insect was diagnosed based on the book Basics of Insect Classification using the taxonomic key of the family (Tenebrialida) [15]. It was taken a group of sterilized glass bottles with dimensions of (10 x 5) cm and placed in each glass bottle 250 g of sterilized flour, which was previously placed in the refrigerator at a temperature of 5-7°C below zero for 24 hours to eliminate all stages of insects in the flour, so that the flour was safe from infection Insecticides [16]. After that, 20 pairs (The gender of the insect is identified by the papillae on the posterior abdominal end of the female, which are more prominent than the male) of adult insects were placed in each glass bottle for mating and laying eggs while covering the bottle nozzles to allow them to ventilate and simultaneously prevent their escape from the glass bottle. Then, these bottles were placed in an incubator at a temperature of 2 ± 28 °C and a humidity of 5 ± 70%, taking into account the continuous renewal of the colony after each generation to obtain the different larval ages to conduct experiments on them.

Obtaining the second larval instar of the insect

To determine the second larval instar of the insect, approximately 50 pairs of adult insects were taken and placed in plastic boxes with a capacity of 10 x 5 cm, each containing 10 g of sterilized flour. 10 pairs were placed in each box; then these boxes were placed in the incubator under a temperature of 2 ± 28 °C, and the relative humidity was 5 ± 70%, therefore for two weeks to lay eggs. After that, the adults were removed, and the plastic boxes were left in the incubator for 5 days to complete the egg hatching, with daily follow-ups of the hatching process. After that, successive moults were observed, and accordingly, the second larval instar was isolated to conduct a pesticide test on those larvae [17].

Bioevaluation of Amyloland pesticide of bacterial origin

Three recommended concentrations (2.5, 3 and 3.5 g L^{-1}) of Amyloland pesticide were prepared, which are (Table 1). Each concentration was placed in a 100 ml sprayer, and calibration was performed until it was ready. As for the comparison treatment, only normal distilled water was used. 12 Petri dishes were used, and three replicates were added for each of the concentrations used in the experiment, in addition to the comparison replicates. Then 5 g of wheat was placed in each dish, and the dishes were sprayed with the previously prepared concentrations. For each concentration, three dishes with the comparison were sprayed with ordinary distilled water and 1 ml for each. Petri dish, then left for 15 min to dry. After that, 10 adult insects were transferred to each dish using small brushes, and the dishes were placed in an incubator at a temperature of 2 ± 28 °C and a humidity of 3 ± 70%. After that, the percentage of mortality was taken after (1, 3, 5, 7, 9, 11 and 13) days of treatment, and the results were corrected according to the equation[18].

$$\text{Corrected mortality percentage} = \frac{\text{Mortality percentage in the treatment} - \text{Mortality percentage in the control}}{100 - \text{Mortality percentage in the control}} x100$$

Then, the same experiment was repeated using the same concentrations and time period as before, while the second instar larvae were used instead of the adults used in the first treatment. The percentage of mortality was taken after the same period had passed, and the results were corrected using the same previous equation.

Biological evaluation of Coragen and Conan pesticides in the percentage of mortality of adults and the second larval instar of the beetle

Three concentrations (0.10, 0.15 and 0.20 ml L^{-1}) of Coragen pesticide and three concentrations (0.4, 0.5 and 0.6 g L^{-1}) of Conan pesticide were prepared separately (Table 1) and according to the concentrations recommended by their manufacturer and mixed. Mix well with water, then put each concentration in a 100 ml sprayer. In addition to the control treatment, the sprayer was calibrated to make it ready for use, which was satisfied with distilled water only. Plastic Petri dishes with a capacity of (9 x 16) cm were taken, with three replicates for each concentration. Then 10 adult insects were placed in each dish (replicate), and these dishes were sprayed with the different concentrations prepared in advance. The control dishes were sprayed with distilled water in 1 ml for each dish; then

left to dry. Then, these insects were transferred to other dishes containing an amount of 5 g of sterile, infested flour. They were placed in the refrigerator at a temperature of 5 - 7 °C for 24 hours to become sterile and ready for use. Then, these insects were transferred to the Petri dishes using A small brush, and these dishes were placed in an incubator at a temperature of 2 ± 28 °C and a relative humidity of 5 ± 70%. The percentage of destruction was taken after (1, 3, 5, 7 and 9) days of treatment, and the results were corrected according to the equation [18]. This process was repeated with the same concentrations and periods, but by taking the second larval instar instead of the adults, then taking the percentage of mortality during the same previous time, the results were corrected according to the same previous equation.

Table (1): Pesticides used in the study

Trade Name	Active Ingredient	Recommendation Dosage
Amyloland	*Bacillus amyloliquefaciens*	2.5 - 3.5 g L^{-1}
Coragen 20 SC	Chlorantraniliprole	0.1 - 0.2 ml L^{-1}
Conan 20 WG	Dinotefuran	0.4 – 0.6 g L^{-1}

Statistical analysis

The results were analyzed using the statistical analysis program Genstat (2009) according to a completely randomized design (CRD). The least significant difference was tested at the 5% probability level to determine the significant differences between the treatments[19].

Results and discussion

Bioevaluation of Amyloland pesticide on percentage of mortality of adults and the second larval instar of the flour beetle

The results of the study showed that using the pesticide Amyloland against adults and second-instar larvae of the flour beetle had the highest mortality percentage of 90%, at a concentration of 3.5 g L^{-1} after 13 days of treatment, compared to the rest of the concentrations used in the treatment, with significant differences, as the mortality percentage for the concentrations reached (2.5 and 3 g L^{-1}) 50% and 66.67%, respectively, after the same previous period has passed (Table 2).

Table (2): Effect of the pesticide Amyloland on the percentage of mortality in adults of the red rusty flour beetle (*T. castaneum*).

Age group	Concentrations	Corrected percentage of mortality over periods (day)							Means ($g\ L^{-1}$)
		1	3	5	7	9	11	13	
Adults	**2.5 g L^{-1}**	3.33	3.33	6.67	10.00	26.67	36.67	50.00	19.52
	3 g L^{-1}	3.33	6.67	16.67	26.67	43.33	53.33	66,67	30.95
	3.5 g L^{-1}	3.33	10.00	16.67	26.67	56.67	80.00	90.00	40.48
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Means (day)		2.50	5.00	10.00	15.83	31.67	42.50	51.67	
L.S.D 0.05	Time periods = 5.851			Concentrations = 4.423			Interaction = 11.703		

The results also showed (Table 3) that using the bacterial pesticide (Amyloland) on the second larval instar at a concentration of (3.5 g L^{-1}) achieved the highest mortality percentage of 100% after 9 days of treatment, compared to other concentrations (2.5 and 3 g L^{-1}), in which the percentage of mortality of 73.33% and 96.97%, respectively, and for the same time.

Table (3): Effect of the pesticide Amyloland on the mortality percentage of the second larval instar of the red rusty flour beetle (*T. castaneum*)

Age group	Concentrations	Corrected percentage of mortality over periods (day)							Means ($g\ L^{-1}$)
		1	3	5	7	9	11	13	
Second larval instar	**2.5 g L^{-1}**	0.0	13.33	33.33	60.00	73.33	86.67	90.00	50.95
	3 g L^{-1}	0.0	30.00	43.33	80.00	96.67	100	100	64.29
	3.5 g L^{-1}	0.0	33.33	50.00	86.67	100	100	100	67.14
	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Means (day)		0.0	19.17	31.67	56.67	67.50	71.67	72.50	
L.S.D 0.05	Time periods = 7.358			Concentrations = 5.562			Interaction = 14.716		

The results we have achieved when treating the bacterial pesticide Amyloland on adults and second-instar larvae show that the mortality percentage increases with the increase in the pesticide concentration and the length of time. We note that the second-instar larvae were more sensitive to the pesticide than adults, as the mortality of larvae

was faster than that of adults and for all The concentrations used are because the adult insect has a hard structure covering its body that resists the effect of the pesticide, or the reason may be attributed to the slow feeding of the adult insect and its tolerance of hunger, which protects it from the effect of the pesticide. As for the larvae, they are more greedy in their feeding, and these results are consistent with the findings of [20]. It was found that the alcoholic extract of black pepper fruits had a greater effect on the larvae than the adults, who were less affected. The Amylolifaciens bacteria that make up the Amyloland pesticide can form toxin crystals inside the insect's body [21], when the insect is swallowed by the insect while feeding on grains. In wheat treated with the pesticide, these substances dissolve in the basal medium of the digestive juice using a certain type of enzyme. The bacteria are characterized by the ability to be active and grow inside the insect's digestive tract because they are not affected by the secretions of the digestive tract. They have a high ability to penetrate the wall of the digestive canal and the external body wall because of their ability to secrete enzymes and break down the protective tissue, which then reaches the blood tissue and thus leads to the mortality of the insect [22].

Biological evaluation of the pesticides Coragen and Conan on the percentage of mortality of adults and the second larval instar of the red rusty flour beetle

The results (Table 4) showed that Coragen pesticide had a clear effect on the second larval instar at the concentrations (0.20 ml L^{-1}), as it achieved the highest mortality percentage of 100% after 7 days of treatment, while it achieved the two concentrations (0.10 and 0.15 ml L^{-1}), where the mortality percentage of 76.7% and 80.0%, respectively.

Table (4): Effect of the pesticide Coragen on the mortality percentage of the second larval instar of the red rusty flour beetle (*T. castaneum*)

Age group	Concentrations	Corrected percentage of mortality over periods (day)					Means (ml L^{-1})
		1	3	5	7	9	
Second larval instar	**0.10 ml L^{-1}**	26.7	50.0	76.7	76.7	90.0	64.0
	0.15 ml L^{-1}	40.0	50.0	83.3	80.0	96.7	70.0
	0.20 ml L^{-1}	43.3	66.7	90.0	100	100	80.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0
Means (day)		27.5	41.7	62.5	64.2	71.7	
L.S.D 0.05	Time periods = 6.74		Concentrations = 6.03			Interaction = 13.47	

The results also showed that when adults were treated with the pesticide Coragen, it was observed that the concentration (0.20 ml L^{-1}) was superior with significant differences over the rest of the concentrations used, as the mortality percentage of 100% after

9 days of treatment, while the concentrations (0.10 and 0.15 ml L^{-1}) gave a mortality percentage of 56.7% and 76.7%, respectively, according to the same period (Table 5).

Table (5): Effect of the pesticide Coragen on the mortality percentage in adults of the red rusty flour beetle (*T. castaneum*)

Age group	Concentrations	Corrected percentage of mortality over periods (day)					Means (ml L^{-1})
		1	3	5	7	9	
Adults	**0.10 ml L^{-1}**	26.7	36.7	43.3	46.7	56.7	42.0
	0.15 ml L^{-1}	26.7	36.7	53.3	56.7	76.7	50.0
	0.20 ml L^{-1}	33.3	50.0	63.3	76.7	100	64.7
	Control	0.0	0.0	0.0	0.0	0.0	0.0
Means (day)		21.7	30.8	40.0	45.0	58.3	
L.S.D 0.05	Time periods = 9.82		Concentrations = 8.78			Interaction = 19.64	

Through these results extracted from the experiment, we find that the percentage of insect mortality increases with increasing pesticide concentration and time and that the second instar larvae were more sensitive and affected by the pesticide than the adults. For the same reason mentioned when treated with Amyloland pesticide, Coragen pesticide works through its direct effect on the muscular system and on the ryanodine receptors in The insect, which is responsible for the contraction of muscle cells, by controlling the amount of calcium ions transferred from its intracellular reserve to the cytoplasm of the cells [23]. The pesticide binds to the ryanodine receptors in the muscle cells, which leads to the opening of ion channels and the flow of calcium ions from its internal store of cells to the cytoplasm in a large way and when Depletion of calcium reserves in muscle cells leads to weak muscle contraction and the insect stops feeding, as well as rapid paralysis that leads to death [24].

The results (Table 6) showed that Conan pesticide had a significant effect on the mortality percentage of adults, as the concentration (0.6 g L^{-1}) exceeded by 63% after 9 days of treatment, compared to the concentrations (0.4 and 0.5 g L^{-1}), which reached the mortality percentage 43.3% and 50%, respectively, for the same period.

Table (6): Effect of the pesticide Conan on the mortality percentage in adults of the red rusty flour beetle (*T. castaneum*)

Age group	Concentrations	Corrected percentage of mortality over periods (day)					Means (g L^{-1})
		1	3	5	7	9	
Adults	**0.4 g L^{-1}**	0.0	6.7	20.0	26.7	43.3	19.3
	0.5 g L^{-1}	0.0	10.0	23.3	30.0	50.0	22.7
	0.6 g L^{-1}	0.0	10.0	26.7	36.7	63.0	27.3
	Control	0.0	0.0	0.0	0.0	0.0	0.0
Means (day)		0.0	6.7	17.5	23.3	39.2	
L.S.D 0.05	Time periods = 7.22		Concentrations = 6.46			Interaction = 14.45	

The results presented (Table 7) indicated that treatment with Conan pesticide significantly affected the mortality percentage of the second larval instar of the beetle, as the two concentrations achieved (0.6 and 0.5 g L^{-1}) by giving them the highest mortality percentage of 100%, after 9 days of treatment, while the concentration achieved (0.4 g L^{-1}) mortality percentage reached 80% after the same period.

Table (7): Effect Conan pesticide in the second larval instar mortality percentage of the red rusty flour beetle (*T. castaneum*)

Age group	Concentrations	Corrected percentage of mortality over periods (day)					Means (g L^{-1})
		1	3	5	7	9	
Second larval instar	**0.4 g L^{-1}**	0.0	13.3	50.0	73.3	80.0	43.3
	0.5 g L^{-1}	0.0	33.3	56.7	70.0	100	52.0
	0.6 g L^{-1}	0.0	36.7	63.3	80.0	100	56.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0
Means (day)		0.0	20.8	42.5	55.8	70.0	
L.S.D 0.05	Time periods = 8.25		Concentrations = 7.38			Interaction = 16.50	

We note from the study results that larvae are more affected than adults and that the time period also increases the mortality percentage [25]. The active substance of Conan pesticide, the compound dinotefuran, is considered a part of the group of neonicotinoid

pesticides. The compound dinotefuran's mechanism of action includes disrupting the insect's central nervous system by inhibiting the nicotinic acetylcholine receptors, which are responsible for transmitting nerve impulses chemically in the sites of synapses [26]. These compounds bind irreversibly with the receptors. Acetylcholin, which is found in nerve synapses as a result of the similarity of its stereoscopic structure to the compounds of this group, leads to the accumulation of Acetylcholin and the continued stimulation of the nervous system due to the inability of the Acetyl Cholinesterase enzyme to break down the compounds of this group because they are not Acetylcholin, which leads to a malfunction in the functioning of the nervous system and the death of the insect [27, 28].

From the above, we can conclude that the Amyloland pesticide has an active and influential role in eliminating adults and second-instar larvae of the beetle, especially the concentration (3.5 g L^{-1}), and this may be due to its efficiency in penetrating the tissues of the insect and thus causing its death. We also conclude that the Coragen and Conan pesticides have played a role this is important in getting rid of beetle colonies, but this is due to the efficiency of the concentrations used (0.20 ml L^{-1} and 0.6 g L^{-1}) on these insects and thus achieving the highest mortality percentage.

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