

Effect of Some Surfactants on the Expired Bioformulation Belthirul® and Evaluation of its Efficacy Against Fig Moth Larvae, *Ephestia cautella* (Walker)

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Abstract

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A laboratory study was conducted to evaluate the effect of two surfactants Neutrafol-pH and Inex-A compared to the expired Belthirul® formulation (*Bacillus thuringiensis* var *kurstaki*) on the larvae of the fig moth *Ephestia cautella* (Walker). Tests were conducted to determine the time required for wetting, stability of the suspension under normal conditions and compatibility between the entomopathogen and surfactants. Expired formulation, fresh formulation and treated expired formulation were bio-assayed on third instar larvae of the fig moth *E. cautella* to evaluate its efficacy. The results obtained showed that the two surfactants Neutrafol-PH and Inex-A were highly efficient in reforming the expired bioformulation Belthirul®, improved its physical specifications and increased its biological activity on fig moth larvae, and Neutrafol-PH was more effective in increasing the activity and vitality of *B. thuringiensis* var. *kurstaki* in the expired bioformulation. The number of bacterial colonies was 17.3×10^{10} CFU/g, whereas it was 8.0×10^{10} colonies/g in the expired bioformulation treated with Inex-A surfactant, compared with non-treated expired bioformulation which reached 3.9×10^{10} CFU/g. The treated expired bioformulation with Neutrafol-PH and Inex-A increased its efficacy by increasing mortality rate 82.08 and 74.38%, respectively, on the third instar larvae of the fig moth at the concentration of 1 g/L.

Keywords: Efficacy, Belthirul®, surfactants, fig moth, *Ephestia cautella*.

Introduction

The wide pesticides importing by both of public and the private sectors without a good estimate of its actual need led to stocks accumulation all over the world. It is estimated that more than half a million tons of banned and obsolete pesticides are due to pass their expiry date or as a result of poor storage, as temperatures inside non-typical stores during the summer reach 50°C or more, which accelerates the deterioration of pesticides to a state of loss of validity before the expiry of its shelf life (Abd Alrahman, 2022; Al-Mallah & Mustafa, 2010; Al-Tikriti, 1998; Hala *et al.*, 2016), and such stocks are considered as a source for environment pollution (Lohaa *et al.*, 2008).

Bioformulations in the form of wettable powders (WP), are among the most widely used preparations, which are characterized by their wetting, dispersal, suspension, and physical and chemical stability during storage (Teera-Arunsiiri *et al.*, 2003). One of the most imported formulations of entomopathogenic bacteria is *B. thuringiensis* var *kurstaki* formulations used for the control of Lepidoptera larvae (Ali, 2017). Temperature is one of the most important factors affecting the deterioration of the active ingredient of *Bacillus thuringiensis* formulations during storage above 25°C, which negatively affects the stability of the formulations depending on the storage period (Dunkle & Shasha, 1988; Ignoffo, 1992; Khorramvatan *et al.*, 2014).

Previous studies indicated that the toxicity of wettable formulations of *Bacillus thuringiensis* var *kurstaki* was reduced by 60% when exposed to high temperatures during

storage (Muostafa *et al.*, 2018). Zafar *et al.* (2000) found that *B. thuringiensis* formulations were stable and effective for 36 months when stored at laboratory temperature and the toxicity was reduced by 6-10% against cotton bollworm *Helicoverpa armigera*, whereas Brar *et al.* (2005) reported that temperature above 50°C led to a decrease in the vitality of the spores and protein crystals of *Bacillus thuringiensis* formulations.

Published work indicated that products, which are still possible to use in principle, but are not usable because their physical specifications are not suitable for the recommended field use, and thus classified among the undesirable pesticides whose specifications can be relatively improved by adding surfactants, repackaged and then distributed for use on the assumption that they do not harm crops, environment and human health (Al-Tikriti *et al.*, 2001; Hala *et al.*, 2016). This study aimed to test the possibility of reforming the obsolete bioformulation Belthirul® using some surfactants and evaluating its effectiveness on controlling the third instar larvae of the fig moth *Ephestia cautella*.

Materials and Methods

Two packages of the bioformulation Belthirul® (*Bacillus thuringiensis* var *kurstaki*) were brought in the form of WP produced by the Spanish company Probelte fito (pb) in a package weighing 500 g containing powdered spores and toxic secretions of these bacteria prepared in a biocidal way, the concentration of these bacteria was 3200 IU/gm and the use rate was 25-50 g/100 liters of water. One of the two packages was fresh with a production date of 2020, and the

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second package was expired with a production date of 2017, was expired since two years and three months and was collected from the stores of the Ministry of Agriculture. The two surfactants used were Neutrafol-pH (3% N + P₂O₅ 17% + Spreading and adhesive agents) and Inex-A (Ethoxylated Fatty Alcohol 20.2% + Polydimethylsiloxane 1.0%) to reform the expired pesticide.

Rearing of the fig moth *E. cautella*

The insect was reared on artificial nutrient medium consisted of 81% crushed wheat (semolina), 12% glycerine, 6% molasses, and 1% dry yeast at a temperature of 2±27°C and a relative humidity of 50-60% inside the incubator according to the method of (Tarik & Al-Hadethy, 2015)

Laboratory tests

For the purpose of assessing the activity of bacteria in the fresh and expired formulations, *B. thuringiensis* var *kurstaki* from both formulations was grown at 10⁻⁹ dilution in nutrient agar (NA) (Biomedica, India), in four replicates. 24 hours after incubation at 37 °C, the number of bacterial colonies was counted directly on the culture media plates. Standard hard water was prepared by dissolving well 0.304 g of CaCl₂ and 0.139 g of MgCl₂.6H₂O in one liter of distilled water. The hard water solution was kept in 1000 ml glass beakers for use in special physical tests to determining the effect of additives in the expired bioformulation Belthirul® through the following tests: (1) Wetting time test was conducted to determine the effectiveness of the additives to the bioformulation powders (the effectiveness of the disperser and diffuser materials) that were subjected to certain changes that led to powder agglomeration and becoming insoluble in the spray solution, and the time required for getting wet should not exceed one minute (Shaaban & Al-Mallah, 1993), (2) Suspension stability test under normal conditions was conducted to determine the effectiveness of the dispersal and diffusion of materials in the wettable powder formulation, the suspension percent should not be less than 50% (Shaaban & Al-Mallah, 1993). Suspension rate was calculated using the following equation:

$$\text{Suspension rate} = 9/10 \times 100 - (w1-w2)/w1$$

whereas: w1= weight of wettable powder, w2= weight of residue of pesticide powder.

Another study was conducted to study the possibility of enhancing efficacy of the expired Beltirul® by using two surfactants Neutrafol-PH at a concentration of 1.2 ml/L and Inex at a concentration of 0.75 ml/L of the pesticide solution prepared at concentrations 0.063, 0.125, 0.25, 0.5 and 1.0 g/l. The reformed samples were then subjected to bio-assay test on the third instar larvae of the fig moth *E. cautella*. The Belthirul® concentrations were prepared using distilled water that included 0.063, 0.125, 0.25, 0.5 and 1 g of the product/liter of distilled water, in addition to the control treatment (water only), with three replicates in Petri dishes. The exposure method used was by mixing the Belthirul® concentration with diet (wheat semolina) (Al-Mallah & Al-Jubouri, 2016), then sprayed at a rate of 10 ml of

Belthirul solution/15 g wheat semolina, for all the concentrations mentioned above, by a small atomizer and left to dry before distributing to replicates. Each Petri dish contained around 5 grams of treated wheat semolina. In addition, 10 larvae were added to each Petri plate, then placed in the incubator at a temperature of 27 ± 2°C and relative humidity of 65±2%. After 3, 7 and 9 days, the number of dead larvae in each replicate was recorded. Corrected mortality rate was calculated according to Abbott's equation (1925):

$$\text{Corrected mortality rate (MR) (\%)} = \frac{\text{MR Treatment} - \text{MR control}}{100 - \text{MR control}} \times 100$$

Statistical analysis

The laboratory experiments design was a completely randomized design (CRD). The analysis of variance was conducted by SPSS program, and the comparison of means was carried out according to Duncan's multiple range test at a probability level of 0.05 (Al-Sahoki & Waheeb, 1990).

Results and Discussion

Viability of *B. thuringiensis* var. *kurstaki* in the fresh and expired Belthirul® bioformulation

The bacterial viability test of *B. thuringiensis* var. *kurstaki* in the fresh and expired Beltirul® product (Figure 1) showed that the number of bacteria colonies in the fresh bioformulation reached 11.8×10¹⁰ CFU/g, whereas the number of colonies in the expired bioformulation was 3.9×10¹⁰ CFU/g. These results indicated that the bacterial spores have lost their vitality in addition to the decomposition of toxins of the protein crystal in the bioformulation Belthirul® as a result of unsuitable storage conditions, especially in locations where temperatures reach up to 50 °C or more in the summer. Moustafa *et al.* (2018) found that the loss rate (%) in the active ingredient of the two formulations Dipel 2 × 6.4% WP and Protecto 9.4% WP for *Bacillus thuringiensis* subsp. *Kurstaki* when stored at temperature of 35±2 °C after 12 weeks of storage reached up to 5.82 and 12.52%, respectively, which led to a decrease in the toxicity of these two formulations against the second instar larvae of the cotton leaf worm *Spodoptera littoralis* by 60%, whereas the loss rate in the active ingredient were 3.41 and 8.03%, respectively, under a storage temperature of 54±2°C for 14 days.

Stability of adjuvants in the bioformulation Belthirul®

Results obtained (Table 1) showed that the required time to wet the expired bioformulation using hard and normal water exceeded the permissible limits (more than one minute), reaching 1.5 and 1.3 minutes, respectively, compared to the time needed to wet the fresh bioformulation, which amounted to 0.18 and 1.3 minutes, respectively. It was clear that the additives (dispersing and spreading agents) of the expired Belthirul® were exposed to physical and chemical changes that made the powder become agglomerated and less soluble in the spray solution.

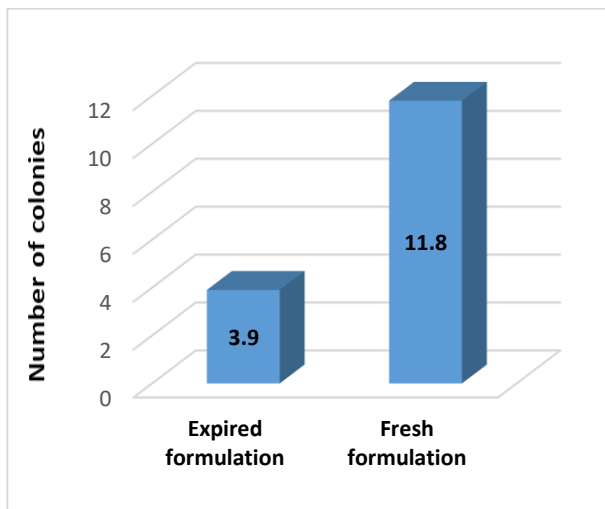


Figure 1. The number of *B. thuringiensis* var. *kurstaki* colonies ($\times 10^{10}$ CFU/g) in the fresh and expired Belthirul[®] formulation.

Table 1. Determining the required Time required for wetting of the fresh and expired Belthirul[®] using hard water and normal water.

Items	Wetting time (min.)	
	Standard hard water	Distilled water
Fresh formulation	0.18 b	0.13 b
Expired formulation	1.50 a	1.30 a

Means followed by the same letters in the same column are not statistically different according to Duncan's multiple range test at $P=0.05$.

Results obtained (Figure 2) also showed that the suspension rate (%) in the expired Belthirul[®] amounted to 22.5%, whereas it reached 79.6% in the fresh product, and it decreased to below the permissible limits (less 50%). This indicates that the adjuvants (dispersing and spreading agents) due to exposure to certain physical and chemical changes that led to the agglomeration of the powder and become less soluble in the spray solution (Al-Tikriti, 1998; Shaaban & Al-Mallah, 1993).

Compatibility of the expired Beltirul[®] formulation with the two surfactants Neutrafol – PH and Inex-A

The compatibility test for the expired Beltirul[®] with the two surfactants Neutrafol -PH and Inex-A (Figure 3) showed that the surfactant Neutrafol-PH was more compatible in increasing the activity and vitality of *B. thuringiensis* var. *kurstaki*, and the number of bacteria colonies reached 17.3×10^{10} CFU/g, followed by the surfactant where the number of bacterial colonies reached 8.0×10^{10} CFU/g compared with the control treatment (without surfactant) which reached 3.9×10^{10} CFU/g, keeping in mind that the number of colonies in the fresh preparation was 11.8×10^{10} CFU/g (Figure 1). The results showed that the expired Belthirul[®] was compatible with the two surfactants of Neutrafol-PH and Inex-A, and that the surfactant Neutrafol-

PH increased the number of bacteria colonies in the expired bioformulation by 4.4 times compared to the surfactant Inex-A which increased the number of bacteria colonies 2-fold, and this may be due to the increase of nitrogen content of the surfactant Neutrafol-PH by 3%, which increases the activity, growth and reproduction of *B. thuringiensis* var. *kurstaki*, as mentioned by Sarrafzadeh (2012). In addition, it was found that urea increases the effectiveness of *B. thuringiensis* bacteria by 2.7 times (Salama *et al.*, 1985; Zhang *et al.*, 2013). Prabakaran & Hoti (2008) found that high levels of amino nitrogen in the culture media (fermenters) increased the biomass of *Bacillus thuringiensis* var. *israelensis* (dry weight 4.78 gL^{-1} and the number of spores 3.24×10^{11} CFU/mL).

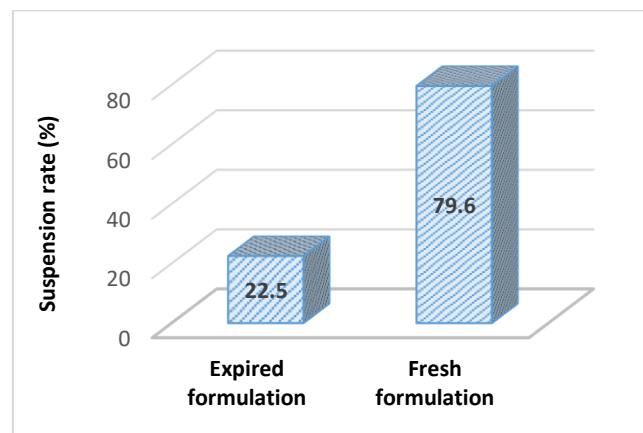


Figure 2. Suspension rate of the fresh and expired Belthirul[®] formulation.

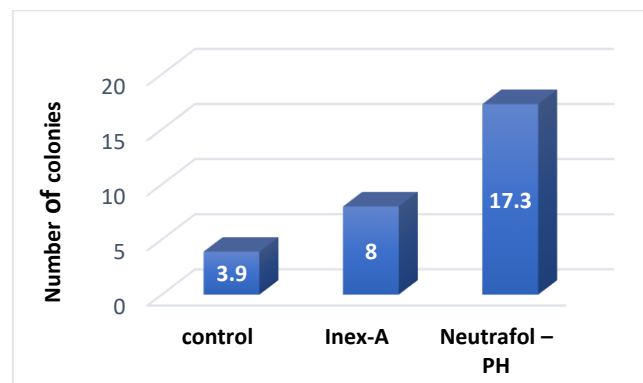


Figure 3. Number of *B. thuringiensis* var. *kurstaki* colonies ($\times 10^{10}$ CFU/g) of the expired Beltirul[®] formulation treated with the two surfactants of Neutrafol-PH and Inex-A.

Ismail (2017) found that the inorganic salts MgSO_4 , CaCO_3 , and boric acid had a stimulating effect on the growth and reproduction of bacteria, as they caused an increase in the number of *B. thuringiensis* var. *kurstak* colonies of 103.95-439.47%, whereas the emulsifier agent Tween 20 caused an increase in the number of colonies reached 2% and this increase may be due to the increase in the permeability of the cell membrane and the increase in the absorption of nutrients. These inorganic salts may increase the absorption

of some dissolved substances in the culture medium such as glycine, proline, betaine, trehalose, which protect the internal components of the bacterial cell (Rudolph *et al.*, 1986).

Additives are compounds that improve the effectiveness of the bioformulation and subsequently reduce its used dose (Rodham *et al.*, 1999; Santos *et al.*, 2019). Several reports indicated that when *B. thuringiensis* was used as a biological control agent, effectiveness was increased by mixing appropriate amounts of acids, salts, oils and adjuvants (Ahmed *et al.*, 1998; Chenzhu *et al.*, 1997; Liu & Tabashnik, 1997; Welland *et al.*, 1997). The activity of *B. thuringiensis* var. *kurstaki* was increased in vitro by adding emulsified lipids, soluble proteins and several types of non-toxic nitrogen compounds, inorganic salts, tannin, organic acids and carbohydrates (El-Moursy *et al.*, 1993; Morris *et al.*, 1995; Salama *et al.*, 1985). Tamai *et al.* (2002) confirmed what previous researchers have stated that the quantities and components of synthetic insecticide formulations, including adjuvants, have an effect on the secondary work sites of microorganisms as well as their ability to change the pH of the medium.

Effectiveness of the fresh Belthirul® formulation on the third instar larvae of the fig moth (*E.cautella*)

The results obtained (Table 2) indicated that there are significant differences in the mortality rate (%) of the third instar larvae of the fig moth *E.cautella* by the influence of different concentrations of the fresh Beltirul® biocide. The interaction between concentrations and storage periods showed that the highest mortality rate was for the concentration 1 g/L 9 days after treatment which reached 96.40%, whereas the lowest mortality rate was for the concentration 0.063 g/L and reached 6.67%, 3 days after treatment.

The average mortality rate due to the effect of exposure periods showed an increase in the mortality rate over time, and reached 24% three days after treatment and up to 59.26% 9 days after treatment. In addition, the results showed that the mortality rate for the concentration 0.063 g/L was 15.09%, then it increased to reach 76.34% for the concentration 1 g/L. Tarik & Al-Hadethy (2015) found the highest mortality rate of first instar larvae of *E. cautella* was 96.9% at the concentration of 1.2×10^6 spores/ml for *Bacillus thuringiensis* eight days after treatment. Whereas, Yıldız & Sezen (2017) reported that *Bacillus thuringiensis* at a dilution of 1.8×10^9 CFU/mL achieved a high mortality rate of the third instar larvae of *E. cautella*.

Effectiveness of the expired Belthirul® formulation on the third instar larvae of the fig moth (*E. cautella*)

Results obtained (Table 3) indicated that interaction between expired Beltirul® concentrations and storage periods gave highest mortality rate of the third instar larvae of fig moth was for the concentrations of 1 g/L 9 days after treatment, and reached 27.63%, whereas no mortality was observed during the first three days at concentrations 0.063, 0.125 and 0.25 g /L. There was an increase in mortality rate over time, as the average mortality rate three days after treatment was 2.00%, and reached 16.81% 9 days after treatment. The results showed that the mortality rate increased with increasing concentration. The lowest average

mortality rate was at the concentration 0.063 g/L and reached 1.53%, then it increased to 19.21% at the concentration of 1 g/L. Results also showed that the expired bioformulation had a decreased effectiveness by more than 60% compared to the fresh bioformulation because of poor storage conditions (Sorokulova *et al.*, 2008).

Table 2. The corrected mortality rate (%) of the third instar larvae of the fig moth *E.cautella* when using the fresh Belthirul® formulation.

Concentration (g/L)	Exposure time (day)			Average per concentration
	3	7	9	
0.063	6.67 i	17.20 gh	21.40 g	15.09 E
0.125	10.00 ih	24.13 gf	32.13 ef	22.08 D
0.25	16.67 gh	37.93 d	64.23 c	39.61 C
0.5	33.33 ef	62.07 cd	82.13 b	59.18 B
1.0	53.33 d	79.30 b	96.40 a	76.34 A
Average of Exposure time	24.00 C	44.13 B	59.26 A	

Values followed by the same lowercase letters in the same row or the same upper-case letters in the same column or row are not significantly different according to Duncan's multiple range test at P=0.05.

Table 3. The corrected mortality rate (%) of the third instar larvae of the fig moth *E. cautella* treated with the expired Belthirul® formulation.

Concentration (g/L)	Exposure time (day)			Average per concentration
	3	7	9	
0.063	0.00 c	0.00 c	4.60 bc	1.53 C
0.125	0.00 c	3.33 bc	6.90 bc	3.41 C
0.25	0.00 c	6.67 bc	20.73 a	9.13 B
0.5	3.33 bc	10.00 b	24.17 a	12.50 B
1.0	6.67 bc	23.33 a	27.63 a	19.21 A
Average of Exposure time	2.00 C	8.67 B	16.81 A	

Values followed by the same lowercase letters in the same row or the same upper-case letters in the same column or row are not significantly different according to Duncan's multiple range test at P=0.05.

The high temperature inside the warehouse also leads to damage and destruction of the additives, especially the adjuvants in the Belthirul® formulations, which results to agglomeration of the powder and becomes less soluble in the spray solution, and the product loses its viscosity properties and absorption on the surfaces of the particles and consequently the merging of solid particles of the active ingredient and their falling off, sedimentation and separation from the spray solution. Al-Tikriti (1998) indicated that there were changes in the chemical and physical properties of the adjuvants that added in pesticide formulations due to heat, which leads to the irregular distribution of the active

ingredient within the solution of the formulation, in addition to the poor spread of the pesticide and its weak ability to wet and penetrate, as mentioned by Moustafa *et al.* (2018), who indicated that the type of formulation and its additives played an important role in the stability of *B. thuringiensis* sub sp *Kurstaki* formulations. Ignoffo *et al.* (1982 & 1983) reported a significant decrease in the efficacy of aqueous suspensions and wettable powders for *B. thuringiensis* var. *israelensis* when stored at 50°C for 28 days. Ignoffo (1992) reported that the half-life of the toxin (Cry and Cyt) of *B. thuringiensis* was less than 10 days at a temperature above 50 °C and the half-life of the dry spores of bacteria at 50 °C was more than 100 days.

Effectiveness of the bioformulation Beltirul® treated with surfactant Neutrafol-PH on the third instar larvae of the fig moth (*E. cautella*)

The results obtained (Table 4) indicated that there are significant differences in the mortality rates of the third instar larvae of the fig moth *E. cautella* when sprayed with different concentrations of the bioformulation Beltirul® treated with Neutrafol-PH surfactant. The interaction between concentrations and storage periods showed that the highest mortality rate (100%) was achieved at the concentrations of 1 g/L 9 days after treatment, whereas the lowest mortality rate was obtained at concentration of 0.063 g/L and reached 10.0% three days after treatment. There was an increase in mortality rates with the increase in exposure period. The average mortality rate three days after treatment was 28.0%, and reached 63.8% nine days after treatment. The results also showed that the mortality rate increased with the concentration increase, as the highest average mortality rate was 82.1% at concentration of 1 g / L and the lowest rate was 16.3% at concentration of 0.063 g/L.

Table 4. The corrected mortality rate (%) of the third instar larvae of the fig moth *E. cautella* by the effect of bioformulation Belthirul® treated with the surfactant Neutrafol-PH.

Concentration (g/L)	Exposure time (day)			Average per concentration
	3	7	9	
0.063	10.00 j	13.83 ij	25.00 gh	16.28 E
0.125	13.33 ij	31.07fg	39.27 ef	27.89 D
0.25	20.00 ih	41.43 e	71.40 c	44.28 C
0.5	36.67 ef	65.53 cd	83.60 b	61.93 B
1.0	60.00 d	86.23 b	100.00 a	82.08 A
Average of Exposure time	28.00 C	47.62 B	63.85 A	

Values followed by the same lowercase letters in the same row or the same upper-case letters in the same column or row are not significantly different according to Duncan's multiple range test at P=0.05.

Efficacy of the bioformulation Belthirul® treated with surfactant Inex on the third instar larvae of the fig moth (*E. cautella*)

Results obtained (Table 5) indicated that there were significant differences in the mortality rate of the third instar larvae of the fig moth *E. cautella* by the influence of different concentrations of the bioformulation Belthirul® treated with the surfactant Inex. The interaction between concentrations and storage periods showed that the highest mortality rate of 93.1% was obtained for the concentrations of 1 g/L nine days after treatment, whereas the lowest mortality rate of 6.7% was obtained for the concentration 0.063 g/L three days after treatment.

The average mortality rate three days after treatment reached 23.3%, whereas nine days after treatment it reached 57.3%. As for the averages of mortality percentages by the effect of concentrations. The results also showed that the average mortality rate increased by increasing the concentration, and reached 74.4% at concentration 1 g/L, whereas the average lowest rate at 0.063 g/L concentration was 14.7%.

Surfactants, including adjuvants, increased the effectiveness of pesticides. Shaaban and Al-Mallah (1993) indicated that the adjuvants improved the characteristics of the pesticide and increased its efficiency and effectiveness against pests by 50%. These include emulsifier agents, wetting and spreading agents, dispersing agents and sticking agents. The adjuvants help to break the surface tension between the pesticide solution and the surfaces of plants and insects and increase the wettability, spread and penetration, as well as their role in increasing the growth and reproduction of bacteria *B. thuringiensis* var. *kurstaki* (Ismail, 2017; Sarrafzadeh, 2012; Zhang *et al.*, 2013).

Table 5. The corrected mortality rate (%) of the third instar larvae of the fig moth *E. cautella* sprayed with biocide Belthirul® treated with the surfactant Inex.

Concentration (g/L)	Exposure time (day)			Average per concentration
	3	7	9	
0.063	6.67 f	13.33 ef	24.17 de	14.72 E
0.125	10.00 f	26.66 d	34.53 d	23.73 D
0.25	13.33 ef	30.00 d	55.17 c	32.83 C
0.5	33.33 d	63.33 c	79.33 b	58.67 B
1.0	53.33 c	76.67 b	93.13 a	74.38 A
Average of Exposure time	23.33 C	42.00 B	57.27 A	

Values followed by the same lowercase letters in the same row or the same upper-case letters in the same column or row are not significantly different according to Duncan's multiple range test at P=0.05.

الملخص

بكر، صفاء زكريا، صالح محمد إسماعيل و خلدون فارس سعيد. 2023. تأثير استخدام مواد خافضة للتوتر السطحي على المركب الحيوي Belthirul[®] منتهي الصلاحية وتقييم كفاءته لمكافحة يرقات عثة التين (*Ephestia cautella* Walker). مجلة وقاية النبات العربية، 41(2): 119-126. <https://doi.org/10.22268/AJPP-041.2.119126>

تم إجراء دراسة مختبرية لتقييم تأثير مادتي تخفيض التوتر السطحي Inex-A و Neutrafol-pH في إعادة فاعلية المستحضر الحيوي منتهي الصلاحية تحت الظروف الاعتيادية، واختبار التوافقية والاختبار الحيوي على يرقات عثة التين (*Ephestia cautella* Walker). تم تحديد الوقت اللازم للابتلال، واختبار ثبات المعلق الحديث والمستحضر منتهي الصلاحية بعد معالجته. أظهرت النتائج أن مادتي تخفيض التوتر السطحي Inex-A و Neutrafol-PH ذات كفاءة عالية في معالجة المستحضر منتهي الصلاحية Belthirul[®] وتحسين مواصفاته الفيزيائية وزيادة فاعليته الحيوية على يرقات عثة التين، وكانت المادة Neutrafol-PH أكثر فاعلية في زيادة نشاط وحيوية البكتريا *B. thuringiensis* var. *kurstaki* في المستحضر الكاسد حيث بلغ عدد مستعمرات البكتريا $10^{10} \times 17.3$ مستعمرة/غ، بينما بلغ $10^{10} \times 8.0$ مستعمرة/غ في المستحضر منتهي الصلاحية والمعالج بمادة تخفيض التوتر السطحي Inex-A مقارنة مع المستحضر منتهي الصلاحية (بدون استخدام مادة تخفيض التوتر السطحي) التي بلغت $10^{10} \times 3.9$ مستعمرة/غ. وقد ازدادت فاعلية المستحضر منتهي الصلاحية و المعالج بمادتي تخفيض التوتر السطحي Neutrafol-PH و Inex-A على يرقات عثة التين بنسبة 82.08 و 74.38%، على التوالي، عند استعمال التركيز 1 غ/ليتر.

كلمات مفتاحية: كفاءة، بلثيرول، مخفضات التوتر السطحي، عثة التين، *Ephestia cautella*

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References

- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18:265-267. <https://doi.org/10.1093/jee/18.2.265a>
- Abd Alrahman, S.S. 2022. Effect of storage on the stability and efficacy of wettable powder formulations of Bicont-T and Antario KAB against fig moth larvae *Ephestia cautella* (Lepidoptera: pyralidae). MSc thesis, College of Agriculture, University of Tikrit, Iraq. 106 pp.
- Ahmed, K., F. Khaliq and B.A. Malik. 1998. Evaluation of synergistic interactions between *Bacillus thuringiensis* and malic acid against chickpea pod borer, *Helicoverpa armigera* (Hbn.) (Lepidoptera: Noctuidae). Pakistan Journal of Biological Sciences, 1:105-108. <https://doi.org/10.3923/pjbs.1998.105.108>
- Al-Sahoki, M. and K.M. Waheeb. 1990. Applications in the design and analysis of experiments. House of Wisdom for Printing and Publishing, Baghdad University, Iraq. 488 pp.
- Ali, O.H. 2017. Use of some vegetable oils, organic nicotinic pesticides and beltherol bio-bacterial formulation and their integration in controlling corn stalk borer *Sesamia cretica* Led. (Lepidoptera: Phalaenidae) on yellow corn. MSc thesis, College of Agriculture, University of Tikrit, Iraq. 86pp.
- Al-Mallah, N.M. and A.A. Mustafa. 2010. Effect of the cold storage period on the acidity percentage of some insecticides. Tikrit University Journal of Pure Sciences, 11(3):255-260.
- Al-Mallah, N.M. and A.R.Y. Al-Jubouri. 2016. Practical applications in pesticides. Part 1. Yazouri Group for Publication and Distribution, University of Mosul, Iraq. 352 pp.
- Al-Tikriti, S.Z.B. 1998. Effect of storage on emulsified commercial formulations of DDVAP and Diazinon. M. Sc. thesis, College of Agriculture, University of Baghdad, Iraq. 47 pp.
- Al-Tikriti, S.Z.B., A.I. Al-Samarraia and S.H. Samir. 2001. Treatment of obsolete phosphorous pesticides in Iraq by using some emulsifying agents. Tikrit University Journal of Agricultural Sciences, 1(2):8-85.
- Brar, S.K., M. Verma, R.D. Tyagi, J.R. Valéro and R.Y. Surampalli. 2005. Starch industry wastewater-based stable *Bacillus thuringiensis* liquid formulations. Journal of Economic Entomology, 98(6):1890-1898. <https://doi.org/10.1093/jee/98.6.1890>
- Chenzhu, W., Z. Shufng, X. Xiufeng, C.Z. Wang, S.F. Zhang, J.H. Zhang and X.F. Xiang. 1997. Effect of tannic acid on the effectiveness of *Bacillus thuringiensis* Var. *Kurstaki* against *Helicoverpa armigera* (Hubn.). Entomologia Sinica, 4:74-81.
- Dunkle, R.L. and B.S. Shasha. 1988. Starch-encapsulated *Bacillus thuringiensis*: a potential new method for increasing environmental stability of entomopathogens. Environmental Entomology, 17(1):120-126. <https://doi.org/10.1093/ee/17.1.120>
- El-Moursy, A.A., A. Sharaby and H.H. Awad. 1993. Some chemical additives to increase the activity spectrum of *Bacillus thuringiensis* var. *kurstaki* (Dipel 2X) against the rice moth *Corcyra cephalonica*. Journal of Islamic Academic Sciences, 6(2):149-154.

- Hala, M.I., S.T. Hanan and K.Y. Naglaa.** 2016. Effect of storage on the stability and biological effectiveness of some insecticides. *Journal of Biological Chemistry and Environmental Science*, 11(2):265-282.
- Ignoffo, C.M.** 1992. Environmental factors affecting persistence of entomopathogens. *Florida Entomology*, 75:516-525.
- Ignoffo, C.M., C. Garcia, M. Kroha and L.T. Couch.** 1982. High-temperature sensitivity of formulations of *Bacillus thuringiensis* var. *israelensis*. *Environmental Entomology*, 11(2):409-411.
- Ignoffo, C.M., C. Garcia, M. Kroha and L.T. Couch.** 1983. The effects of temperature and water on the insecticidal activity and spore viability of a wettable powder foundation of *Bacillus thuringiensis* var. *israelensis*. *Journal of Kansas Entomological Society*, 56(1):88-92.
- Ismail, Z.K.** 2017. The effect of some chemicals and plant extracts on the effectiveness of the biocides Belitherol and Spintor on *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). MSc thesis, College of Agriculture, Tikrit University, Iraq. 118 pp.
- Khorravatan, S., R. Marzban, M. Ardjmand, A. Safekordi and H. Askary.** 2014. The effect of polymers on the stability of microencapsulated formulations of *Bacillus thuringiensis* subsp. *kurstaki* (Bt-KD2) after exposure to ultraviolet radiation. *Biocontrol Science and Technology*, 24(4):462-472. <https://doi.org/10.1080/09583157.2013.871503>
- Liu, Y.B. and B.E. Tabashnik.** 1997. Synergism of *Bacillus thuringiensis* by entylenediamine tetra-acetate in susceptible and resistant larvae of diamond back moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 90(2):287-292. <https://doi.org/10.1093/jee/90.2.287>
- Lohaa, K.M., M. Lamoreea, J.M. Weissb and J. Boer.** 2018. Import, disposal, and health impacts of pesticides in the East Africa Rift (EAR) zone: A review on management and policy analysis. *Crop Protection*, 112:322-331. <https://doi.org/10.1016/j.cropro.2018.06.014>
- Morris, O.N., V. Converse and P. Kanagaratnam.** 1995. Chemical additive effects on the efficacy of *Bacillus thuringiensis* Berliner subsp. *kurstaki* against *Mamestra configurata* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 88(4):815-824. <https://doi.org/10.1093/jee/88.4.815>
- Moustafa, M.A.M., M.A. Saleh, R. Izat, I.R. Ateya and M.A. Kandill.** 2018. Influence of some environmental conditions on stability and activity of *Bacillus thuringiensis* formulations against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 28(1):61-67. <https://doi.org/10.1186/s41938-018-0064-x>
- Prabakaran, G. and S.L. Hoti.** 2008. Influence of amino nitrogen in the culture medium enhances the production of delta-endotoxin and biomass of *Bacillus thuringiensis* var. *israelensis* for the large-scale production of the mosquito control agent. *Journal of Indian Microbiology and Biotechnology*, 35(9):961-965. <https://doi.org/10.1007/s10295-008-0370-5>
- Rodham, D.K., Y. Wang, J.B. Cantwell, P.D. Winn and J. Foundling.** 1999. Formulating microbial biocontrol agents. *Pest Science*, 55(3):340-342. [https://doi.org/10.1002/\(SICI\)1096-9063\(199903\)55:3%3C340::AID-PS894%3E3.0.CO;2-D](https://doi.org/10.1002/(SICI)1096-9063(199903)55:3%3C340::AID-PS894%3E3.0.CO;2-D)
- Rudolph, A., J. Crowe and L. Cowe.** 1986. Effects of three stabilizing agents—proline, betaine and trehalose on the membrane phospholipids. *Archives of Biochemistry and Biophysics*, 245(1):134-143. [https://doi.org/10.1016/0003-9861\(86\)90197-9](https://doi.org/10.1016/0003-9861(86)90197-9)
- Salama, H.S., M.S. Foda and A. Sharaby.** 1985. Potential of some chemicals to increase the effectiveness of *Bacillus thuringiensis* Berl. against *Spodoptera littoralis* (Boisd). *Journal of Applied Entomology*, 100(1-5):425-433. <https://doi.org/10.1111/j.1439-0418.1985.tb02801.x>
- Santos, C.A.M, R.T.S. Santos, J.F. Della'Vechia, F. Griesang, R.A. Polanczyk and C. Ferreira.** 2019. Effect of addition of adjuvants on physical and chemical characteristics of Bt bioinsecticide mixture. *Scientific Reports*, 9(1):1-8. <https://doi.org/10.1038/s41598-019-48939-y>.
- Sarrafzadeh, M.H.** 2012. Nutritional Requirements of *Bacillus thuringiensis* During Different Phases of Growth, Sporulation and Germination Evaluated by Plackett-Burman Method. *Iranian Journal of Chemistry and Chemical Engineering*, 31(4):131-136. <https://doi.org/10.30492/ijcce.2012.5936>
- Shaaban, A. and N.M. Al-Mallah.** 1993. Pesticides. Ministry of Higher Education and Scientific Research, Dar al-Kutub for Printing and Publishing, University of Mosul, Iraq. 520 pp.
- Sorokulova, I.B., A.A. Kumnov, S. Pathiranaa, A.J. Mandell and V. Vodyanoy.** 2008. Novel methods for storage stability and release of *Bacillus* spores. *Biotechnology Progress*, 24(5):1147-1153. <https://doi.org/10.1002/btpr.22>
- Tamai, M.A., S.B. Alves, R.B. Lopes, M. Faion and L.F.L. Padulla.** 2002. Toxicity of pesticides against *Beauveria bassiana* (Bals. Vuill). *Archives of the Biology Institute*, 69(3):89-96.
- Tarik, A. M. and J.M.J. Al-Hadethy.** 2015. Testing of some local isolates of *Bacillus thuringiensis* on the fig moth *Ephesia cautella* (Lepidoptera: Pyralidae) that it was rearing on artificial food in the laboratory. *Diyala Journal of Agricultural Sciences*, 7(1):7-16.
- Teera-Arunsi, A., M. Suphantharika and U. Ketunuti.** 2003. Preparation of spray-dried wettable powder formulations of *Bacillus thuringiensis*-based biopesticides. *Journal of Economic Entomology*, 96(2):292-299. <https://doi.org/10.1093/jee/96.2.292>
- Welland, R.T., P.T. McDonald and M.K. Kish.** 1997. Efficacy of Dimlin R (diflubenzuron) and transgenic Bt cotton on several Lepidopteran species. Pages 1095-1099. In: Proceedings of Beltwide Cotton Conference. January 6-10, 1997, New Orleans, Louisiana, USA.

Yıldız, I. and K. Sezen. 2017. Microbial control using bacteria of the almond moth, *Cadra (Ephestia)cautella* Walker (Lepidoptera: Pyralidae). Journal of Stored Products Research, 74:98-105.

<https://doi.org/10.1016/j.jspr.2017.10.007>

Zafar, A.U., S. Karim, I.A. Nasir and S. Riazuddin. 2000. Shelf life and field evaluation of CAMB *Bacillus thuringiensis* biopesticide against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on tomato. Pakistan Journal of Biological Sciences, 3:804-807. <https://doi.org/10.3923/pjbs.2000.804.807>

Zhang, L., S. Qiu, T. Huang, Z. Huang, L. Xu, C. Wu, I. Gelbič and X. Guan. 2013. Effect of chemical additives on *Bacillus thuringiensis* (Bacillales: Bacillaceae) against *Plutella xylostella* (Lepidoptera: Pyralidae). Journal of Economic Entomology, 106(3):1075-1080.

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