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Efficacy of olive mill wastewater for protecting *Bacillus thuringiensis* formulation from UV radiations

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ABSTRACT

The effectiveness of 10 low-cost UV-absorbers in protecting *Bacillus thuringiensis* subsp. *kurstaki* BLB1 toxins against inactivation by UV-A and UV-B irradiation was evaluated in this study. Among them, two by-products, molasses and olive mill wastewater (OMW) were selected for further studies. They were tested at different concentrations of 0.05, 0.1, 0.15 and 0.2% using the para-aminobenzoic acid (PABA) as a common UV protectant. Interestingly, addition of PABA and OMW to BLB1 formulations was found to be most effective in protecting BLB1 spores at 90.8 and 76.4% respectively and in preserving delta-endotoxin concentration at a level of 81.7 and 72.2%, respectively when used at a concentration of 0.2%. The lowest preserved spores (46.3%) and delta-endotoxin level (12.4%) was found using molasses. In contrast, spore count and delta-endotoxin concentration were completely reduced after an exposure of unprotected *Bt* strain BLB1 to UV radiations up to 96 h. SDS-PAGE analysis of protected and unprotected samples revealed that delta-endotoxin bands (130, 65–70 kDa) were conserved until 96 h of UV exposure in presence of PABA or OMW compared with their disappearance in presence of molasses after 72 h of exposure and their dramatically decline from 8 h of exposure in unprotected mixture. A complete loss of larvicidal toxicity against *Ephestia kuehniella* was found after 24 h of exposure in absence of any UV-absorber. Addition of OMW or PABA offered the highest levels of insecticidal activity with 63.2 and 74.7% of residual toxicity, respectively. Whereas, molasses addition, as UV protectant retained only 26.3% of residual activity after 96 h of exposure. Therefore, addition of OMW by-product to *Bt* formulation may be a suitable alternative to others synthetic chemical compounds. OMW may also provided added value, be environmentally friendly and less hazardous, when used at low concentration.

1. Introduction

Application of microorganisms for biological control is drawing increasing attention to environmentally hazardous chemical pesticides. *Bacillus thuringiensis* (*Bt*) serotypes are the most studied and markedly used bacterium as bioinsecticide (Schnepf et al., 1998). They are Gram positive soil bacteria characterized by their ability to produce crystalline inclusions containing delta-endotoxin proteins such as *Bt kurstaki* which express the 27–130 kDa proteins (Kim et al., 2013). Many formulations of *Bt kurstaki* are available and applied under field conditions, however the major drawback of using *Bt* protoxins and spores as active ingredients in biopesticides against Lepidopteran pest species is their lack of persistence due to UV radiations. Generation of free radicals following oxidation of amino acids and destruction of tryptophan and histidine residues were the major mechanisms that have been suggested for the inactivation of *Bt* protoxins (Ignoffo and Garcia, 1978; Pozsgay et al., 1987; Becker et al., 1992). This disadvantage led to the search for protective measures to reduce the damaging effect of UV radiations on *Bt* toxins. Photostability of *Bt* biopesticide, could be achieved by encapsulation with sodium alginate, gelatin, starch, carboxymethylcellulose and carrageenan (Kuppusamy et al., 1989; Elcin, 1995; Elcin et al., 1995; Prabakaran and Hoti, 2008). It could be accomplished also by using different synthetic organic compounds such as congo red, uric acid, para-aminobenzoic acid (PABA), benzaldehyde, malachite green and melanin which have been evaluated as suitable UV protectants for various entomopathogens (Krieg et al., 1980; Dunkle and Shasha, 1989; Cokmus et al., 2000; Maldonado-Blanco et al., 2002; Lee et al., 2006; Arthurs et al., 2006). However, these compounds were considered as expensive products and are not recommended to be disseminated in the environment. Therefore, in our laboratory, attempts have been made to look for cheaper alternatives such as the use of molasses and olive mill waste water (OMW).

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Application of such products will contribute not only to the valorization of industrial by-products, but also to the production of low-cost *Bt* formulation. In the present study, we report screening of various low-cost UV protectants on the basis of their effects on delta-endotoxin concentration, spore count and toxicity against *Ephestia kuehniella* larvae after UV-A and UV-B exposure. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was also done to study the correlation between delta-endotoxin degradation (130 kDa, 65–70 kDa), toxicity loss and contribution of UV protectants in photoprotection.

2. Materials and methods

2.1. Bacterial strains

Bt subsp. *kurstaki* strains BLB1 and BNS3 Cry$^-$ were used in the present work. The BLB1 strain was used for large-scale bioinsecticides production and formulation studies because of its high toxicity to a wide range of Lepidopterean insect larvae including *E. kuehniella* (Saadaoui et al., 2009).

2.2. Inocula preparation

Inocula were prepared by transferring cells from nutrient agar slants into 3 ml of Luria broth (LB) medium (Sambrook et al., 1989) and incubated overnight at 30 °C. Aliquots (0.2 ml) were used to inoculate 250 ml Erlenmeyer flasks containing 50 ml LB medium. After 6 h incubation at 30 °C in a shaker set at 200 rpm, the OD_{600} was determined. The culture broth was used to inoculate the complex medium to start with an initial OD_{600} of 0.15 corresponding to almost 2×10^8 CFU/ml and 0.05 g/l dry biomass (Ghribi et al., 2007).

2.3. Bioinsecticide production, in shake flask, using complex medium

The *Bt* strain BLB1 was grown in a complex medium at the optimized conditions for delta-endotoxin production (Ghribi et al., 2007). This medium is composed of (g/l): starch, 30; soya bean, 25; KH_2PO_4, 1; K_2HPO_4, 1; $MgSO_4$, 0.3; $MnSO_4$, 0.01; and $FeSO_4$, 0.01. The pH was adjusted to 7.0 before sterilization at 121 °C for 20 min. In the shake flask, $CaCO_3$ was sterilized separately and added at a concentration of 20 g/l for the improvement of pH stability. The 1000 ml flasks with four baffles containing 50 ml of culture medium were incubated for 72 h at 30 °C in a rotary shaker set at 200 rpm.

2.4. Recovery of active components from Bt fermented broth

Bt fermented broth was aseptically centrifuged at 9000 rpm for 30 min at 4 °C to collect spores and delta-endotoxins in the pellet (centrifugate). The active compounds lost in the supernatant (enzymes, vegetative insecticidal proteins (Vips)) during centrifugation were concentrated by ultrafiltration process. The sample to be formulated was prepared by mixing the centrifugate and the retentate of ultrafiltration in the optimal proportion of 25% w/v.

2.5. Spore count and delta-endotoxin determination

Spore count was estimated by counting colony forming units (CFU) after 72 h of growth. Hence, culture samples were heated at 80 °C for 10 min, appropriate dilutions were plated on solid LB medium and incubated at 30 °C overnight.

Delta-endotoxin concentration was determined in the solubilized crystal preparation from culture medium as described by Zouari et al. (1998). In summary, 1 ml of culture medium was centrifuged for 10 min at 9000 rpm and the pellet was washed twice with 1 M NaCl and twice with distilled water. The pellet was suspended in 1 ml of 50 mM NaOH (pH 12.5) in order to solubilize delta-endotoxin crystals. After 2 h of incubation at 30 °C, total solubilized proteins in the supernatant were measured by using Bio-Rad reagent (Bio-Rad Protein assay, cat. 500-0006, München, Germany) according to the method of Bradford (Bradford, 1976). Toxin contents were calculated as the result of subtracting the total proteins measured with BNS3 Cry$^-$ strain from the total proteins measured with the toxin producing strains.

2.6. Bioassays

Bioassays were carried out using third instars larvae of *E. kuehniella*, as described by Tounsi et al. (2005). Each test was repeated in triplicate. Fifty percent lethal concentration (LC_{50}) was calculated from pooled raw data by probit analysis using programs written in the R. language (Venables and Smith, 2004). Bioassay controls were performed using the strain, BNS3 Cry$^-$.

Toxicity against *E. kuehniella* larvae was expressed in percent of residual toxicity remained after exposure to UV-A and UV-B radiations. It was calculated as follows:

$$\text{Residual toxicity} = \left(\frac{\text{toxicity of exposed mixture containing UV protectant}}{\text{toxicity of the mixture without exposure}}\right) \times 100$$

2.7. Screening of UV protectants

Ten low-cost compounds were screened for their UV protectant properties. Molasses, OMW, starch, olive oil, mineral oil, corn oil, casein, gelatin, talc and coal.

The mixture centrifugate/retentate without UV protection additive and the mixture centrifugate + retentate + para-aminobenzoic acid (PABA) were used as negative and positive controls, respectively.

The mixtures thus, formulated were well mixed and stored in dark for 24 h to allow dissolution of the additives. A dilution of 1/10 (final volume: 5 ml) of each mixture was performed, in order to obtain a sample with delta-endotoxin concentration almost equal to concentration of *Bt* biopesticides used in field application. The diluted mixtures were exposed to UV-A and UV-B radiations in an open Petri dish on the same height at 12 cm distance from the lamps. To compensate the loss of water due to evaporation, the volume of each sample was adjusted with sterile distilled water before determination of spore count, toxicity and delta-endotoxin concentration. For stabilizing the UV output, the lamps were started 15 min before the beginning of irradiation experiments (6 h of exposure).

Based on the initial screening, two promising UV protectants, molasses and OMW were selected for further studies with PABA as positive control. These UV protectants were incorporated with mixtures at different concentrations of 0.05, 0.1, 0.15 and 0.2% and then subjected to UV-A and UV-B treatment up to 96 h. Spore count, delta-endotoxin concentration, residual toxicity and SDS-PAGE analysis were determined at 24 h intervals. Whereas, the mixture without UV protectant was exposed to UV-A and UV-B for 24 h and SDS-PAGE analysis was studied at 8 h intervals. All these experiments were carried out with three replications and repeated on three different days.

2.8. Source of UV-A and UV-B radiations

UV-A and UV-B radiations were emitted simultaneously and uninterrupted by lamps, L1 and L2, respectively. These lamps were a UV source from UV transilluminator (Desaga, Heidelberg,

Table 1
Effect of UV radiations on spore count, delta-endotoxin concentration and residual toxicity of *Bt* mixture containing different UV protectants after 6 h exposure.

UV protectants	CFU (10^7 spores/ml)	Delta-endotoxins (mg/l)	Residual toxicity (%)	Protection grade
Positive control	352 ± 13	3183.1 ± 63	97.5 ± 7.8	A
OMW	356 ± 12.5	3198.6 ± 64	96.8 ± 6	A
Molasses	350 ± 14	3166.6 ± 62	97 ± 5.8	A
Mineral oil	316 ± 13	2203.3 ± 55	82 ± 5	B
Coal	309 ± 11	2163.3 ± 53	75.7 ± 5	B
Gelatin	306 ± 10.5	2110 ± 48	65 ± 5	B
Starch	300 ± 12	2093.7 ± 58	60 ± 5.3	B
Casein	306 ± 9	2083.3 ± 54	60 ± 4	B
Olive oil	274 ± 11	1680 ± 34	49 ± 4	C
Corn oil	271 ± 8	1679.6 ± 41	48.4 ± 4	C
Talc	265 ± 8	1661.9 ± 36	45 ± 2.7	C
Negative control	262 ± 7	1700 ± 46	45 ± 2	C

All compounds were tested at 0.1% concentration.
A: >90% protection; B: 50–90% protection; C: <50% protection.
Positive control: mixture + PABA; Negative control: mixture without UV protectant.

Germany), (220 V, 30 W) which emitted UV-A and UV-B radiation with a wavelength of 366 and 306 nm respectively.

2.9. SDS-PAGE analysis

Integrity of delta-endotoxins of all the mixtures with or without UV protectants was examined by using 12% SDS-PAGE. Aliquots (50 μl) were taken from the samples exposed or not to UV radiations, centrifuged, washed twice with 1 M NaCl and twice with distilled water, suspended in Laemmli sample buffer, boiled for 5 min, analyzed by SDS-PAGE and finally stained using Coomassie blue (Laemmli, 1970).

2.10. Contamination

Growth of pathogenic microorganisms was tested as per the protocol reported by Lisansky et al. (1993) in three mixtures: (centrifugate + retentate + molasses); (centrifugate + rententate + OMW) and finally (centrifugate + retentate + propionic acid). The latter formulation was used as positive control in this study since propionic acid was known to exhibit antimicrobial activities. Molasses, OMW were used at the same concentration of 0.2%, whereas, propionic acid was used at a concentration of 0.3%.

2.11. Statistical analysis

All results related to determination of bioassays, CFU counts and delta-endotoxin concentration were the average of three replicates of three separate experiments. They were statistically analyzed by SAS software (Version 6) using Duncan test performed after analysis of variance (ANOVA).

3. Results

3.1. Screening of UV protectants

Evaluation of low-cost UV protectants effectiveness revealed that molasses and OMW at 0.1% concentration (Table 1), offered a significant *Bt* bioactive components photoprotection, since spore count, residual toxicities, and delta-endotoxin concentration not differ significantly to the positive control (PABA). A moderate protection has been shown by using mineral oil, coal, gelatin, starch and casein since delta-endotoxin concentration and spore count were of 2203.3, 2163.3, 2110, 2093.7, 2083.3 mg/l and 316, 309, 306, 300, 306×10^7 spores/ml, respectively. However, no protection was observed in the case of olive oil, corn oil and talc since delta-endotoxin concentration, spore count and residual toxicities were not significantly different when compared to the negative control (without UV protectant) exposed to UV radiations. Thus, among the different low-cost UV protectants tested, molasses and OMW were the most effective in protecting *Bt* bioactive components and were selected for further studies."

3.2. Spectroscopic properties of UV protectants

The spectroscopic absorbance of UV protectants (molasses, OMW and PABA), used at the same concentration of 0.2% was presented in Fig. 1. Compared to PABA, which showed the higher absorbance in UV-B region (280–315 nm) and in a small part of UV-A region (315–400 nm), molasses and OMW can absorb in the entire UV-A and UV-B radiation range with higher absorbance detected for OMW.

3.3. Effect of UV radiations on spore count

Determination of spore count after exposure to UV radiations showed that spore photoprotection was linearly related to the concentration of the used UV protectants (Fig. 2). Without UV protectants, spore count declined to 28×10^7 after 24 h of exposure, whereas, it remained at a concentration of 340×10^7, 324×10^7 and 276×10^7 spores/ml corresponding to 97.1, 92.5 and 78.8% of the existing spores in the mixture, when using PABA, OMW and molasses, respectively at a concentration of 0.2%. After 48 h of exposure to UV radiations, spore count decreased continuously

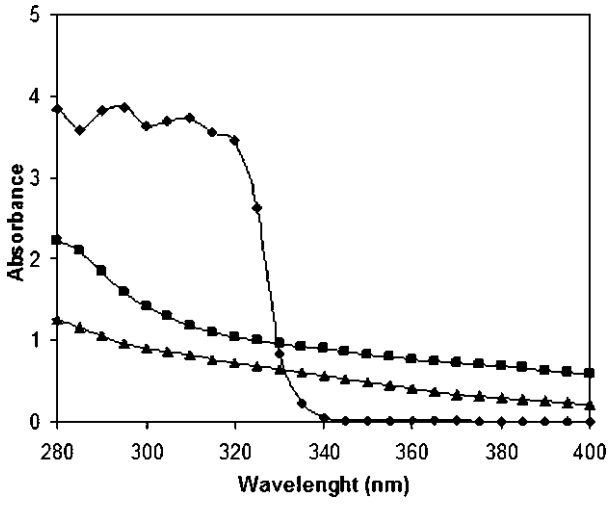

Fig. 1. UV absorbance profiles of PABA, molasses and OMW. (♦) PABA; (■) OMW; (▲) molasses.

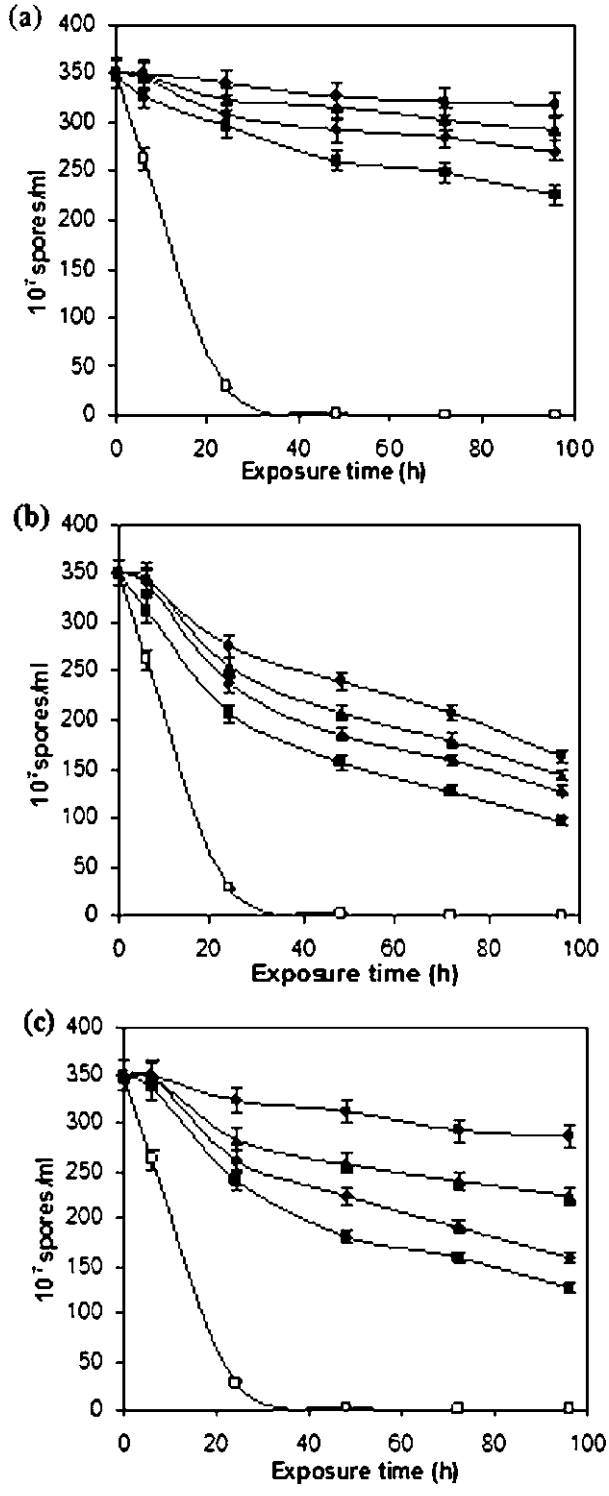

Fig. 2. Effect of UV-A and UV-B irradiation on spore count of *Bt* mixture with different concentrations of PABA (a); molasses (b); OMW (c). (□) negative control; (■) 0.05%; (♦) 0.1%; (▲) 0.15% (●) 0.2%.

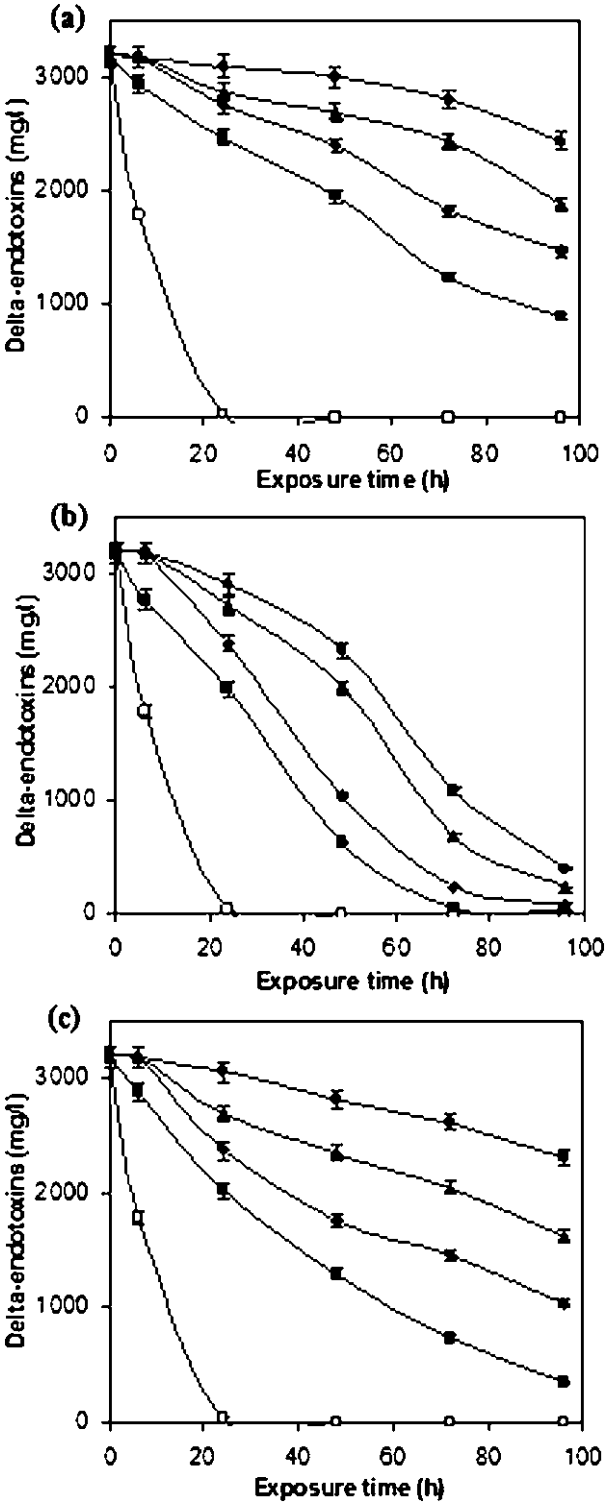

Fig. 3. Effect of UV-A and UV-B irradiation on delta-endotoxin concentration of *Bt* mixture with different concentrations of PABA (a); molasses (b); OMW (c). (□) negative control; (■) 0.05%; (♦) 0.1%; (▲) 0.15% (●) 0.2%.

when using molasses at all the tested concentrations. While using OMW or PABA at 0.2% can maintain 89.1 and 93.6% of the existing spores, respectively, at the same exposure time.

Extension of mixture exposure to UV radiations up to 96 h revealed that spore count could be maintained at 286 and 318×10^7 spores/ml corresponding to 81.7 and 90.8% when using OMW or PABA, respectively at a concentration of 0.2%, while molasses offered the least protection since spore count declined to 162×10^7 spores/ml corresponding to 46.3% of the existing spores.

3.4. Effect of UV radiations on delta-endotoxin concentration

The assessment of delta-endotoxin concentration during UV exposure showed that without UV additives, delta-endotoxin

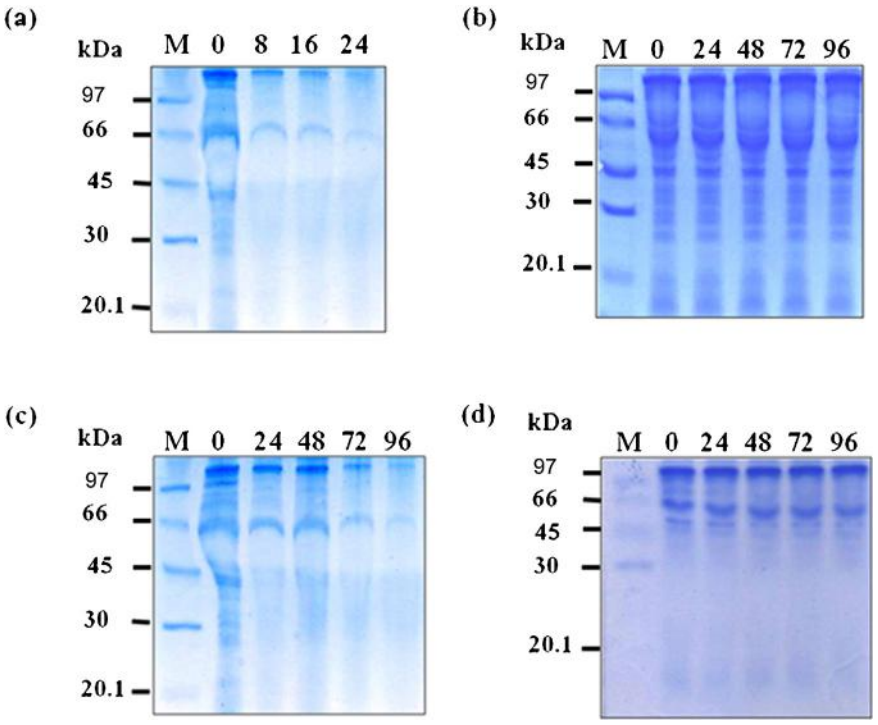

Fig. 4. SDS-PAGE analysis of delta-endotoxins from negative control (a) and *Bt* mixture with selected UV protectants after UV-A and UV-B irradiation; (b) PABA; (c) molasses; (d) OMW: M: Low Molecular Weight, lane 1: before irradiation, lane 2: 24 h, lane 3: 48 h, lane 4: 72 h, lane 5: 96 h. (a): M: low molecular weight, lane 1: before irradiation, lane 2: 8 h, lane 3: 16 h, lane 4: 24 h.

concentration declined dramatically starting from 6 h of exposure (Fig. 3). Whereas, a significant *Bt* toxins photoprotection was observed using molasses, OMW or PABA until 24 h of exposure since delta-endotoxin concentration remained stable at 2900, 3058, and 3099 mg/l, respectively. After prolonged exposure to UV radiations, toxins photoprotection was observed only when using OMW or PABA at a concentration of 0.2%. While, at lowest concentration, delta-endotoxin level continued to decrease reaching 1881, 1465 mg/l and 1632, 1039 mg/l by using PABA and OMW at a concentration of 0.15 and 0.1%, respectively.

3.5. SDS-PAGE analysis

As shown in Fig. 4, photoprotection of *Bt* bioactive components by using a by-product (OMW) or a synthetic component like the PABA, preserved intact *Bt* delta-endotoxins until 96 h of exposure. In contrast, a progressive degradation of 130 and 65 kDa toxin bands from 72 h of exposure was observed by using molasses as an UV protectant confirming toxicity decrease after such time of exposure. SDS-PAGE analysis of the mixture without UV protection additives showed a total disappearance of *Bt* toxin bands after 24 h of exposure to UV radiations.

3.6. Effect of UV radiations on toxicity against E. kuehniella larvae

Residual toxicity evaluation against *E. kuehniella* larvae after 6 h of exposure to UV radiations, showed that incorporation of OMW or molasses in *Bt* formulation maintained insecticidal activity at 96.8 and 97%, respectively, similarly to the synthetic compound (PABA) (97.5%) (Fig. 5). In contrast, after 24 h of exposure, residual toxicity decreased to 18.5% in the negative control and continuous exposure up to 96 h resulted in complete loss of insecticidal activity. At such exposure time, a significant reduction in toxicity was observed when using molasses as UV protectant (26.3%). Whereas, addition of OMW or PABA as an UV protectants offered the highest photoprotection since 74.7 and 63.2% of residual toxicity could be maintained, respectively.

3.7. Contamination

In order to determine eventual microbial contamination, growth of pathogenic microorganisms (*Salmonella*, *Staphylococcus*, yeast, total and fecal coliforms) was assessed in three formulations for a period of 1 year: (centrifugate + retentate + molasses),

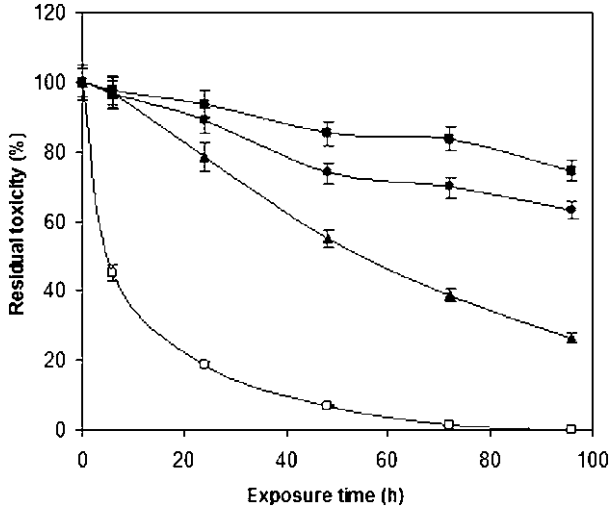

Fig. 5. Effect of UV-A and UV-B irradiation of *Bt* mixture with selected UV protectants on toxicity against *E. kuehniella* (□) negative control; (▲) molasses; (●) OMW; (■) PABA.

(centrifugate + rententate + OMW) and finally (centrifugate + retentate + propionic acid). The obtained results showed the absence of pathogenic microorganism in the three tested formulations (result not shown).

4. Discussion

Sunlight is known to inactivate the biopesticide preparations based on *Bt kurstaki* owing to UV radiations. Among the UV components, UV-C (254 nm) is absorbed by the ozone layer and only a negligible amount reaches the Earth's surface. Hence, UV-A (320–400 nm) and UV-B (280–320 nm) are considered to be the major components responsible for the photodegradation and inactivation of various biopesticides under field conditions. To prolong the survival and efficacy of *Bt* based biopesticides in the environment, various techniques were applied, such as the encapsulation (Cohen et al., 1991), granular formulations (Ahmed et al., 1973) and addition of photostabilization additives (Dunkle and Shasha, 1989). The latter was based on the realization of a specific interaction between the chromophores or additives and the active site of *Bt* containing the photosensitive amino acids.

In this study, effects of UV-A and UV-B radiations on *Bt* strain BLB1 unprotected mixture (retentate + centrifugate) were studied. It was demonstrated that the exposure for 6 h caused a 53.4% loss of delta-endotoxin concentration, 74.4% reduction in spore count and 55% decrease in insecticidal toxicity toward *E. kuehniella*. Prolonged exposure up to 96 h resulted in complete loss of spore count, severely decline of delta-endotoxin level illustrated by a total degradation of 130 and 65 kDa delta-endotoxin bands and consequently a dramatically fall of larvicidal toxicity against *E. kuehniella*. Hadapad et al. (2009) also observed a 50% reduction in larvicidal mortality toward *Culex quinquefasciatus* and a complete loss of *B. sphaericus* spore viability after the exposure of spore suspensions to UV-B for 6 h. Total *B. sphaericus* larvicidal mortality reduction was reached after 24 h continuous UV-B irradiation. Thus, sensitivity of *Bt* bioactive components to UV radiations limits its persistence in field conditions and justifies the addition of solar protectants in formulations for prolonging the survival and efficacy in the environment.

In this report, we have screened various cheaper organic compounds for the UV property, among them, OMW and molasses were found to be promising solar protectants that have offered good protection against UV-A and UV-B radiations.

Due to its good *Bt* sunlight protection (Maldonado-Blanco et al., 2002), the PABA was used as positive control in this study. Indeed, PABA scavenges reactive oxygen species and protects DNA against UV and free radical damage (Miao et al., 1995) and is used as a sunscreen because of its UV absorbing property especially against UV-B (Hu et al., 1995). While, considering the high cost and the disadvantages of its dissemination in the environment, low-cost and eco-friendly products were recommended for UV-protection. For this reason, we focused our research on the use of two by-products namely OMW and molasses. In fact, the olive-fruit processes produce in addition of oil, a large quantity of a characteristic effluent (OMW) causing a major environmental problem because of the difficulty of treatment and disposal. Owing to its composition on various phenolic compounds, OMW was used as a good fertilizer in agricultural soils, had an antimicrobial effect and showed strong insecticidal activity toward the olive psyllid, *Euphyllura olivina* (Magdich et al., 2012; Debo et al., 2011). We studied during this report its ability as a photoprotectant using very small concentrations (0.05, 0.1, 0.15 and 0.2%) comparing with higher ones (2.5 g/l) responsible for OMW phytotoxicity (Hachicha et al., 2009; Debo et al., 2011). On the other hand, molasses, a by-product of the manufacture or refining of sucrose from sugar cane, was used mainly as a supplement for livestock feed or as a substrate for ethanol production (Guimaraes et al., 2007) and was also incorporated in many *Bt* formulation (Adjalle et al., 2009). It offered a significant photoprotection of *Bt* bioactive compounds until 6 h of exposure to UV radiations. But prolonged exposure of *Bt* mixture with molasses up to 96 h was never studied nor the effect on delta-endotoxin concentration and their integrity.

The insecticidal activity of BLB1 *Bt* is mainly due to the presence of delta-endotoxin proteins. SDS-PAGE analysis showed that BLB1 protein profile was composed of two major bands, one of 130 kDa corresponding to Cry1 protoxins and the other of 65–70 kDa corresponding to Cry2 and/or Cry1 toxins after protoxins proteolysis. A dramatically decline (from 8 h of exposure) was observed in delta-endotoxin bands in absence of any UV-additive, showing the necessity of UV absorber to avoid the degradation of toxins bands. Indeed, UV radiations provoked the formation of peroxide radicals causing the instability of the 130 kDa and 65–70 kDa delta-endotoxins, this effect was also found by Ignoffo and Garcia (1978). Using molasses, the persistence of delta-endotoxin bands still to 48 h and then disappeared during prolonged exposure. The obtained result joined the lower levels of delta-endotoxin as well as spore count and confirmed the little potential of the UV-absorbance by molasses. This was explained by the weak content of molasses in aromatic organic compounds and unsaturated groupings in their structures responsible of the higher absorbance of UV comparing with PABA and OMW (Adjalle et al., 2009). Interestingly, SDS-PAGE analysis revealed the persistence of intact 130 kDa and 65–70 kDa delta-endotoxin bands along the UV exposures (96 h) when adding the PABA or OMW to *Bt* mixtures. This result proved the preservation of delta-endotoxin and spore count at 2438, 1863.3 mg/l and 318, 286×10^7 spores/ml in the presence of PABA or OMW, respectively at a concentration of 0.2% after a prolonged UV exposure (96 h). It was clear that OMW absorb in the entire UV-A and UV-B radiation range, hence it offered a double protection comparing with the PABA absorbing highly in UV-B. The absorbance of molasses was the lowest one but in the entire UV-A and UV-B radiations.

Furthermore, the impact of spores and delta-endotoxin photoprotection, were traduced by the evaluation of the residual toxicity against *E. kuehniella* larvae. The exposure of unprotected *Bt* mixture to UV radiations resulted in a fall of toxicity at 24 h, this effect was the consequence of the lower spore and toxin concentration, the two major factors contributing to larvicidal toxicity. In contrast, the addition of OMW or PABA to the samples maintained the toxicity at high levels (63.2% and 74.7% of residual toxicity, respectively) after 96 h of UV exposure. Once more, the molasses provided the lowest UV-protection since it gives only 26.3% of residual toxicity, after 96 h of exposure. In addition, the use of OMW is being explored to reduce the utilization of propionic acid as anti-microbial agent which will reduce the overall coast of *Bt* formulation.

Our study showed the importance of UV-protectants addition in *Bt* formulations for prolonging the survival and efficacy of biopesticide in fields. Regarding the limits of PABA product use, we found that OMW could be a promising solar protectant that provides a good protection against the UV radiations.

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