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Laboratory evaluation of antioxidants as UV-protectants for *Bacillus thuringiensis* against potato tuber moth larvae

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Abstract: The effectiveness of several antioxidant materials in protecting *Bacillus thuringiensis* var. *kurstaki* (HD-73) against inactivation by solar irradiation was assessed in the laboratory. The addition of antioxidant materials (UV-absorbers) to *B. thuringiensis* var. *kurstaki* (HD-73) formulation prolonged the residual insecticidal activity, resulting in greater effectivness against the potato tuber moth larvae, *Phthorimaea operculella*.

Key words: *Bacillus thuringiensis, UV, photoprotectants, Phthorimaea operculella.*

INTRODUCTION

Bacillus thuringiensis is a Gram-positive soil bacterium, characterized by its ability to produce crystalline inclusions composed of insecticidal crystal proteins (ICPs) during sporulation (Hofte and Whiteley 1989). The worldwide use of *B. thuringiensis* is based on the specific toxicity of ICPs against target pests. However, the use of *B. thuringiensis* as insecticide is limited in field applications because of the rapid inactivation of toxins and spores after exposure to sunlight. Ultraviolet (UV) is mainly responsible for this inactivation because of its impact on cells by direct DNA damage (e.g. pyrimidine dimmers, cross-linking with proteins) or by producing reactive oxygen-derived free radicals (Pusztai *et al.* 1991 and Zhang *et al.* 2007).

Solar inactivation of *B. thuringiensis* and other entomopathogens is a widely recognized phenomenon. Exposure for short periods less than 24 hours to wavelengths below 400 nm inactivates spores, degrades protein structures and may inactivate *B. thuringiensis* crystalline toxins (Dunkle and Shasha 1989). The half-life for *B. thuringiensis* has been estimated at 3.8 hours when exposed to an UV radiation in natural sunlight (Ignoffo *et al.* 1977). Spores of *B. thuringiensis* lost 53% of their toxicity for larvae of *Plodia. interpunctella* after 2 houre of UV-irradiation (Johnson *et al.* 1998). *B. thuringiensis* exposed on grapevine leaves in direct sunlight lost more than 50% activity against the light brown apple moth *(Epiphyas postvittana)* on the first day (Bailey *et al.* 1996).

The response of *B. thuringiensis* to solar radiation is complicated by differing responses of the spores and crystalline endotoxin. A variety of responses to the UV spectrum by *B. thuringiensis* have been observed including absorption by spores at 270 nm and crystals at 273nm (Morris 1983), inactivation of spores at 330 nm (Griego and Spence 1978), and crystal inactivation caused by tryptophan destruction (Pozsgay et al. 1987).

Numerous attempts have been made to develop protective measures against damaging UV radiation under field conditions, but success has been limited. Techniques reported to give short-term protection from solar inactivation of *B. thuringiensis* include encapsulations (Raum and Jackson 1966; Dunkle and Shasha 1989), addition of clay granules (Raum and Jackson 1966; Ahmed et al. 1973), and addition of water – soluble or suspensible UV-absorbing compounds to spray formulations (Jaques 1972; Hostetter et al. 1975). Most of these compounds are impractical for large –scale application of *B. thuringiensis*. There is a need for a highly effective, long-term, protective system which could be formulated in commercial products or easily added to tank mixes.

The studies suggested that, exposure of *B. thuringiensis* to any wavelength or combinations of wavelengths below 500 nm will inactivate it depending on exposure time and intensity levels. Therefore, methods capable of shielding *B. thuringiensis* from the entire UV-energy component in sunlight (200-400 nm) should have the highest potential for extending insecticidal activity.

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To be an effective control agent for the potato tuber moth, *phthorimaea operculella*, *B. thuringiensis* must maintain insecticidal activity long enough to affect the maximum number of early instars in the first generation without repeated applications.

The present series of laboratory tests were designed to determine if the addition of antioxidant materials (UV-absorbing chemicals) or combinations of such materials could significantly prolong the residual insecticidal activity of *B. thuringiensis* and increase the effectiveness of the pathogen against *P. operculella* larvae.

MATERIAL AND METHODS

Pathogen:

B. thuringiensis var. *kurstaki* (HD-73) was used during this study. For production of endotoxin preparation, growing cultures were used to inoculate 500 ml-conical flasks each containing 50 ml of fermentation broth media (M_2 medium). To prepare 1 liter from this medium, Proflo (10g), Peptone (2g), Dextrose (15g), Yeast extract (2g), $MgSO_4.7H_2O$ (0.3g), $FeSO_4.7H_2O$ (0.02g), $ZnSO_4.7H_2O$ (0.02g), $CaCO_3$ (1.0g) and $MnSO_4$ (0.02g) were dissolved in 1 liter distilled water and pH adjusted to 7.0. The flasks were incubated for 48-72 hours in a controlled environment incubator shaker, operated at 300 rpm and 30ºC, depending on time necessary to complete lysis. At the end of incubation period, the culture was centrifuged and the spore-endotoxin complex was precipitated using lactose-acetone procedure as described by Dulmage *et al.* (1970).

Tested Insects:

Standard laboratory colony of the potato tuber moth *P. operculella* was reared on potato tubers *Solanum tuberosum* as a natural host under controlled conditions of about 26±2°C and 70±5% R.H. Eggswere obtained from the stock culture and kept in Petri-dishes till hatching. The rearing technique by EL-Sherif (1966) was adopted. Pupae were individually kept in specimen tubes (1×3 cm^2) till adult emergence. Adult moth were kept in oviposition cages that consist of chimney glass, about 8 cm in diameter and 16 cm high, the lower rim of which rested on the bottom of a Petri-dish lined with a disk of filter paper and the upper rim covered with muslin. Each cage was provided with a small plastic cover containing a piece of cotton soaked in 5% sugar solution. Eggs were obtained from the stock culture and kept in Petri-dishes till hatching. Groups of newly hatched larvae were transferred into Petri-dishes containing fresh pieces of potato. Larval development was allowed to continue until adult emergence.

Photoprotectant Materials:

The following antioxidant materials were selected as experimental UV protectants based on their photoprotective activity which is related to their antioxidant properties, efficiently scavenging singlet molecular oxygen and peroxyl radicals (Sies *et al.,* 1995), vitamin (A): (β-Carotene or Retinol) a yellowish, crystals solid (ScienceLab. Company, 2005), vitamin (E): (alpha-tocopherol) an odorless, tasteless, light yellow, oily liquid or viscous liquid; this fat-soluble vitamin has been used in large doses as an antioxidant (ScienceLab. Company, 2005), vitamin (C): (Ascorbic acid) an odorless, pleasant taste, white to slightly yellowish, powdered solid; soluble in cold water (ScienceLab. Company, 2005) and selenium: an odorless, solid metallic powder, non-toxic material (ScienceLab. Company, 2005). Charcoal: an odorless solid powder (ScienceLab. Company, 2005); was tested as a photoprotectant based its ability to act as an oxygen sink, preventing the formation of free active radicals or by impeding all light including ultraviolet (Burges, 1998 and Ignoffo and Garcia, 1978 and Ragaei, 1990). Titanium dioxide: an odorless, tasteless, powdered solid (ScienceLab. Company, 2005); was also tested as a photoprotectant depending on its ability as UV reflector (Bull *et al.*, 1976).

These materials were tested as photoprotectants or anti-UV to B. thuringiensis (HD-73) alone and in combination with each other such as vt. (A+E) mixture, vt. (A+C) mixture, vt. (E+C) mixture, vt. (A+E+C) mixture, vt. (A+E+C)+slenium mixture) and vt. (A+E+C)+ ttanium dioxide.

Effect of Ultraviolet Irradiation on the Efficacy of B. Thuringiensis Var. Kurstaki (HD-73):

Radiation was provided by UV source (a set of two 275 W, high intensity, mercury sun lamps with built-in aluminium reflectors (Model RS 40, Sylvania Lighting). The distance between the two lamp bulbs was 25 cm (center to center). The test samples were placed at a vertical distance of 47 cm from the front glass surface of the bulbs .This provided essentially the same irradiation conditions as those described by Saxena *et al.*, (2002). The lamps were allowed to burn for 10 minutes before exposing the samples. 50 mg of the biomass of *B. thuringiensis* (HD-73) was suspended in 100 ml distilled water to obtain 500 µg/ml concentration, Tween 80 was added at 0.04% as a wetting agent to ensure good dispersal of the preparation and then placed in Petri-dish (9cm in diameter). The dish containing the suspension was exposed to UV source. The exposure periods were 0, 4, 8,12,16,20 and 24 hours. An exposure time of 4 hours in the solar simulator is equivalent to ≈ 1.5 days in the field (McGuire *et al.*, 1996).

Bioassay was carried out on the first instar larvae of *P.operculella* by dipping potato slices for 5 minuets in 1.5% ripening agar containing *B. thuringiensis* (HD-73) suspensions exposed to the different periods of UV. The slices were picked up and left to dry at room temperature (26±2°C) and each placed in a small plastic cup (4 cm height and 4.5cm diameter), each to confine 10 first instar larvae of *P.operculella*. 5 replicates were made for each exposure period. The same number was used as a control where potato slices were dipped in 1.5% agar suspension without *B. thuringiensis* (HD-73) for 1 minute. The slice-agar coverage technique was used to ensure that the pathogen was well reached to the potato tuber moth larvae. Records on the number of surviving and dead individuals were taken after 7 days. The percentage of observed larval mortality reported and corrected according to Abbott formula (Abbott, 1925):

Corrected percent mortality = [(T-C) / (n-C)] x100

Where:

T =number of dead larvae in treated replicates.

C = number of dead larvae in control replicates.

n = the original number of the larvae used.

Relative Absorbance of B. thuringiensis var. kurstaki (HD-73) and Photoprotectant Materials to Different Light Wavelengths:

The absorbance of suspensions of *B. thuringiensis* (HD-73) and photoprotectant materials (alone and in combinations) were measured using Automatic recording UV–Spectrophotometer (Beckman DU640, Beckman, Coulter, Inc., Fullerton, CA, U.S.A.). The scanning range used was 200-700 nm. The absorbance was recorded on a chart recorder.

Effect of Photoprotectants on Persistence of B. thuringiensis var. kurstaki (HD-73):

The scanned materials with different absorbance ranges in the ultraviolet and visible range were selected for testing individually and in combinations at the concentration of 0.1g and mixed well with 0.05g *B. thuringiensis* (HD-73), then mixed with 1.0g refined Arabic gum as a sticker and left overnight for drying and then scraped in a mortar.

After sticking *B. thuringiensis* (HD-73) with the photoprotectant materials by Arabic gum, they suspended in 100 ml distilled water individually and in combinations, Tween 80 were added at 0.04% as a wetting agent to ensure good dispersal of the preparation and then placed in Petri-dishes (9cm in diameter). The dishes containing the suspensions were exposed to UV source .The exposure periods were 0, 4, 8, 12, 16, 20 and 24 hours. The control was *B. thuringiensis* var. *kurstaki* (HD-73) alone and mixed with only Arabic gum. Bioassays were carried out on the 1st instar larvae of *P. operculella* as previously mentioned.

The percentage of original activity remaining (%OAR) of *B. thuringiensis* var. *kurstaki* (HD-73) after exposing to UV irradiation was computed according to Patel *et al*. (1996) and Ignoffo and Batzer (1971):

$$\%OAR = \frac{\%\text{ cause-specific mortality from exposed } B.\ thuringiensis}{\%\text{ cause-specific mortality from unexposed } B.\ thuringiensis} \times 100$$

RESULTS AND DISCUSSION

Effect of Ultraviolet Irradiation on the Efficiency of B. thuringiensis var. kurstaki (HD-73):

Table (1) and Figure (1) showed that, exposure of *B. thuringiensis* (HD-73) to artificial UV light resulted in a gradual deterioration in virulence; it lost almost $^{1}/_{2}$ of its activity against 1st instar larvae of *P. operculell*a after 4 hours of exposure to UV irradiation. The larval mortalities were 90, 49, 35, 30, 22, 10 and 4.0% after exposing to artificial UV irradiation periods of 0, 4, 8, 12, 16, 20 and 24 hours, respectively. The activity of *B. thuringiensis* (HD-73) decreased sharply as a result of the exposure to the artificial UV light measured by decreasing in the larval mortalities of *P. operculell*a from 90 % before exposing to UV irradiation to 4.0% after 24 hours of exposure.

Relative Absorbance of B. thuringiensis var. kurstaki (HD-73) and Photoprotectant Materials to Different Light Wavelengths:

The UV and visible absorption spectra of *B. thuringiensis* (HD-73) and tested photoprotectants were obtained using spectrophotometer scanning at wavelength range of 200-700nm (Table (2) and Figures (2-4). The tested materials showed a variation with respect to their absorbance values for different wavelengths.

Table 1: % larval mortality of 1st instar larvae of *P.operculella* treated with *B. thuringiensis* var. *kurstaki* (HD-73) at (500 µg/ml) previously exposed to different periods of UV irradiation (artificial UV light).

UV-Irradiation period (in hours)	Mean% corrected larval mortality ± SE	% of Original Activity Remaining (%OAR)*
0	90 ± 1.73	-
4	49 ± 5.20	54.44
8	35 ± 5.77	38.89
12	30 ± 2.89	33.33
16	22 ± 1.15	24.44
20	10 ± 2.31	11.11
24	4.0± 1.15	4.44
Untreated control	2.0 ± 0.58	-

*%OAR = (% cause-specific mortality from exposed *B. thuringiensis* ÷ % cause-specific mortality from unexposed *B. thuringiensis*) ×100

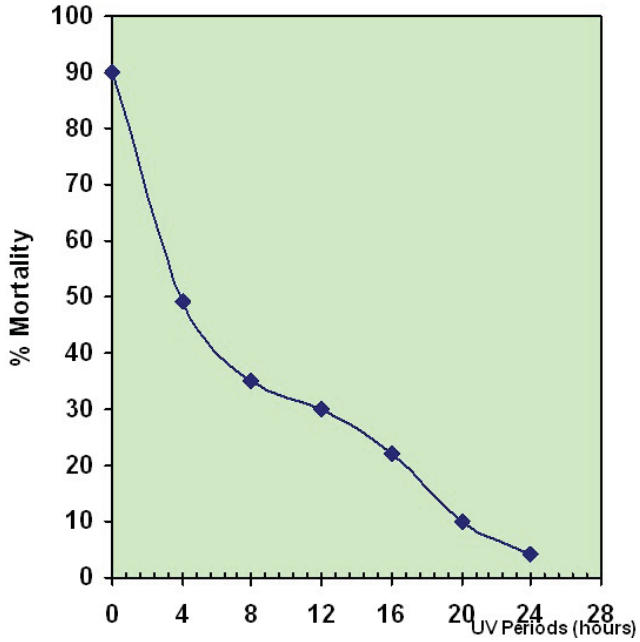

Fig. 1: % larval mortality of 1st instar larvae of *P.operculella* treated with *B. thuringiensis* var. *kurstaki* (HD-73) at (500 µg/ml) previously exposed to different periods of UV irradiation (artificial UV light).

Table 2: The absorbance values of *B. thuringiensis* var. *kurstaki* (HD-73) and photoprotectants using a spectrophotometer in the range of 200-700 nm

Tested photoprotectant	Peak picks	
	Wavelength (nm)	Corresponding absorbance values
B. thuringiensis suspension	216, 243.5, 262.5, 302.5, 466, 607	1.459, 1.164, 0.726, 0.665, 0.050, 0.045
Arabic gum	280.5, 602	1.115, 0.054
Vitamin (A)	208 , 325.5 , 475	1.971 , 1.513 , 0.033
Vitamin (E)	231.4 , 279 , 284.2 , 321.4	4.039 , 1.327 , 1.584 , 0.153
Vitamin (C)	216 , 266.6	0.231 , 3.642
Charcoal	206.5 , 216 , 223 , 229.5 , 243 , 278.5 , 492.5 , 663.5	0.236 , 0.224 , 0.183 , 0.165 , 0.140 , 0.075, 0.059 , 0.060
Selenium	216 , 222.5 , 243 , 271 , 602.5	0.631 , 0.577 , 0.484 , 0.399 , 0.234
Titanium dioxide	206.5 , 216 , 223 , 230 , 243.5 , 285.5 , 357.5 , 499.5	0.132 , 0.144 , 0.119 , 0.118 , 0.129 , 0.075 , 0.075 , 0.077
Vitamin(A)+ vitamin(E)	206, 569.5, 602.5	3.972, 0.099, 0.105
Vitamin(A)+vitamin(C)	216 , 222.6 , 266	0.375 , 0.293 , 1.909
Vitamin(E)+ vitamin (C)	211.2 , 273.4	3.053 , 2.336
Vitamin(A)+vitamin(E) + vitamin (C)	267.5 , 373 , 492.5	4.667 , 0.157 , 0.158
Vitamin(A)+vitamin(E) + vitamin (C) + Selenium	216.2 , 287.4 , 323.8	3.972 , 2.032 , 3.928
Vitamin(A)+vitamin(E) + vitamin (C) +Titanium dioxide	260, 331, 493	1.341, 0.142, 0.114

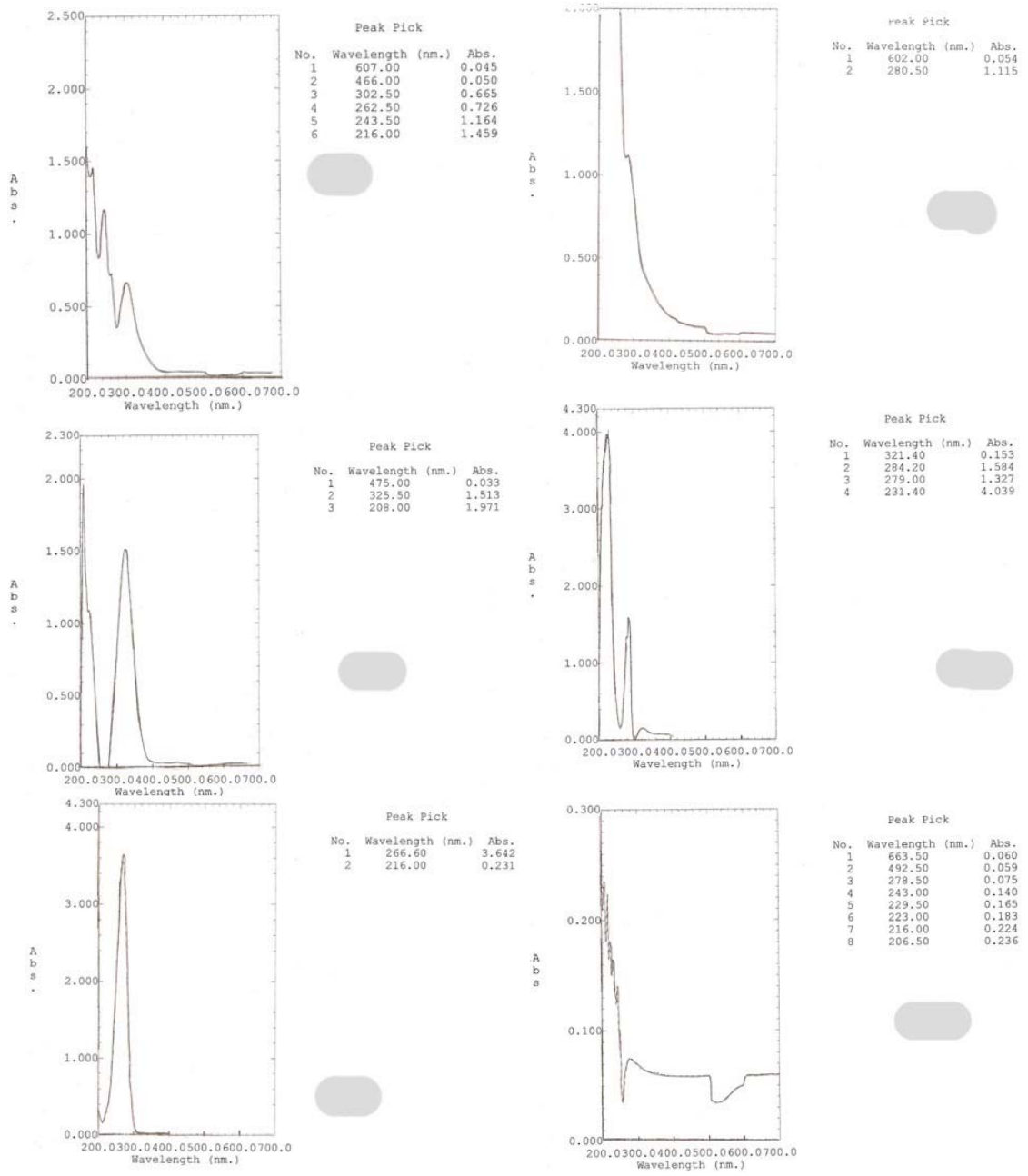

Fig. 2: The absorption rate of *B. thuringiensis* var. *kurstaki* (HD-73) and some photoprotectants to UV-light using Spectrophotometer at the wavelength range of 200-700nm.
i) *B. thuringiensis* var. *kurstaki* (HD-73), ii) Arabic gum, iii) Vitamin (A), iv) Vitamin (E), v) Vitamin (C), vi) Charcoal.

B. thuringiensis (HD-73) suspension used as control, data given in Table (2) and Figure (2 (i)) indicated that, *B. thuringiensis* (HD-73) has absorption at UV-C, UV-B and visible light regions but the maximum absorbance values of *B. thuringiensis* (HD-73) were 1.459 and 1.164 at wavelength of 216 and 243.5 nm (UV-C region).

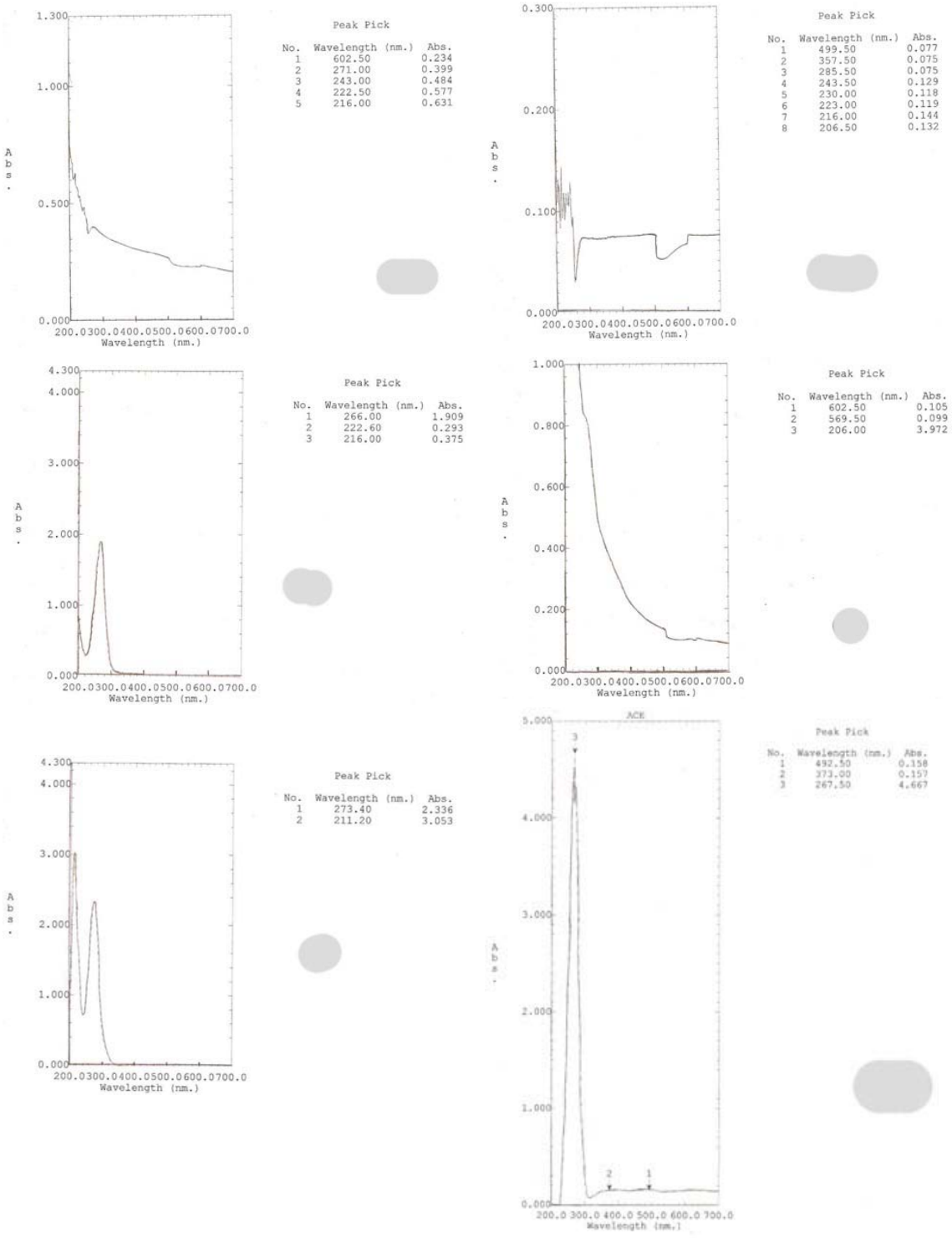

Fig. 3: The absorption rate of some photoprotectants to UV-light using Spectrophotometer at the wavelength range of 200-700nm. vii) Selenium, viii) Titanium dioxide, ix) Vitamin (A+E) mixture, x) Vitamin (A+C) mixture, xi) Vitamin (E+C) mixture, xii) Vitamin (A+E+C) mixture.

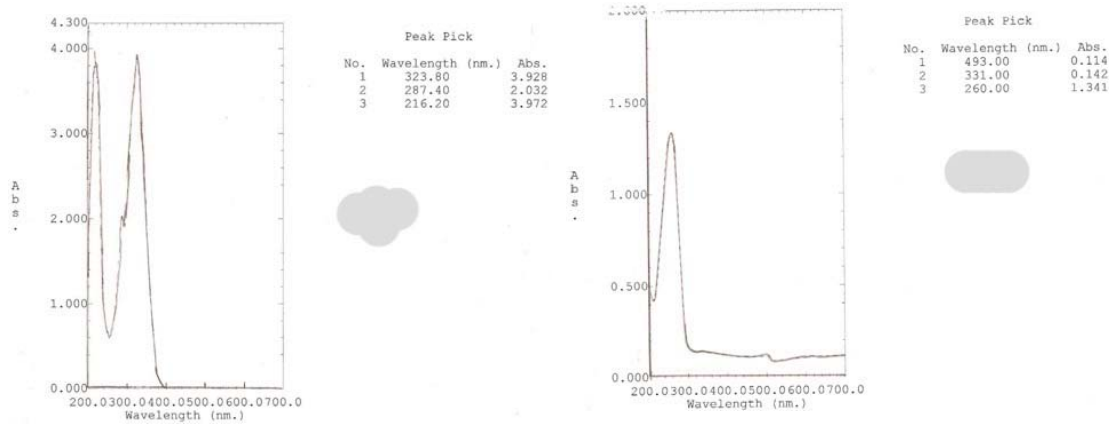

Fig. 4: The absorption rate of some photoprotectants combinations to UV-light using Spectrophotometer at the wavelength range of 200-700nm.
xiii) Vitamin (A+E+C+ Selenium) mixture, xiv) Vitamin (A+E+C+ Titanium dioxide) mixture

Data shown in Table (2) and Figure (2 (ii)) indicated that, arabic gum showed light absorption at UV-B and visible light regions, it absorbed at wavelengths of 280.5 and 602 nm with absorbance values of 1.115 and 0.054. Vitamin (A) showed absorption at UV-C, UV-A and visible light regions but its maximum absorbance was at wavelengths of 208 and 325.5 nm (UV-C and UV-A region) with absorbance values of 1.971 and 1.513 Figure (2 (iii)). Vitamin (E) absorbed radiation at UV-C, UV-B and UV-A regions but it has a highly absorbance value of 4.039 at wavelength of 231.4 nm Figure (2 (iv)). The maximum absorbance value of vitamin (C) was 3.642 at wavelength of 266.6 nm (UV-C region) Figure (2 (v)). Charcoal, selenium and titanium dioxide showed light absorbance at wavelengths of 200-700 nm Figures (2 (vi) -3 (vii, viii)). Vitamins (A+E) mixture showed to have high absorbance value which was 3.972 at wavelength of 267.5 nm (UV-C region) Figure (3 (ix)). Also results given in Table (2) and Figure (3 (x)) indicated that the maximum absorbance value of vitamins (A+C) mixture was 1.909 at wavelength of 266 nm (UV-C region). Vitamins (E+C) mixture showed to have high absorbance values which were 3.053 and 2.336 at wavelengths of 211.2 and 273.4 nm (UV-C region) Figure (3 (xi)).

The most highly absorbance value of the tested materials was obtained with vitamins (A+E+C) mixturethat was 4.667 at wavelength of 267.5 nm (UV-C region) Figure (3 (xii)). Vitamins (A+E+C) and selenium mixture showed a highly absorbance values at UV-C, UV-B and UV-A regions which were 3.972, 2.032 and 3.928 at wavelengths of 216.2, 287.4 and 323.8 nm, respectively Figure (4 (xiii)). Vitamins (A+E+C) and Titanium dioxide mixture showed a absorbance values at UV-C, UV-A and visible light regions which were 1.341, 0.142 and 0.114 at wavelengths of 260, 331 and 493 nm, respectively Figure (4 (xiv)). These results indicated that the use of vitamins (A), (E) and (C) and selenium in mixture will be the most effective as photoprotectants for the protection of *B. thuringiensis* (HD-73) against UV radiation because it absorb UV radiation at wavelengths of 216.2, 287.4 and 323.8 nm, i.e. absorption occurred at the three UV regions (UV-C, UV-B and UV-A).

Efficiency of B. thuringiensis var. kurstaki (HD-73) Mixed with Photoprotectants after Exposure to Artificial Uv Irradiation:

Data given in Table (3) showed that, before exposure to artificial UV irradiation, a significant insecticidal activity was achieved with the most of tested photoprotectants at (0.1%) mixed with *B. thuringiensis* var. *kurstaki* (HD-73) (500 µg/ml) against 1st instar larvae of *P.operculella*. After exposure to different periods of artificial UV irradiation, the persistence (half life) of *B. thuringiensis* var. *kurstaki* (HD-73) alone at 500µg/ml which used as a treated control was 4.712 hours (≈ 1.7 day in field) after exposure to UV irradiation. The persistence of *B. thuringiensis* var. *kurstaki* (HD-73) at 500µg/ml mixed with Arabic gum alone (1.0%) which also tested as a treated control, arabic gum used as a sticker for binding the photoprotectant material with *B. thuringiensis* var. *kurstaki* (HD-73) in granules together, was 6.563 hours (≈ 2.5 days in field) after exposure to UV irradiation.

Table 3: % Corrected larval mortality of *P.operculella* fed with *B. thuringiensis* var. *kurstaki* (HD-73) (500 µg/ml) mixed with 1.0%Arabic gum and some photoprotectants at (0.1%) individually and in combination previously exposed to different periods of artificial UV irradiation

Isolate code	Mean % Corrected larval mortality of *P.operculella* treated with *B. thuringiensis* previously exposed to artificial UV irradiation after indicated hours ± SE							Persistence of *B.thuringiensis* (hours)	% OAR*
	0	4 h	8 h	12 h	16 h	20 h	24 h		
B. thuringiensis alone (500 µg/ml)	90 ± 6.93	49 ± 4.05	35 ± 3.54	30 ± 3.11	22 ± 1.73	10 ± 1.15	4.0 ± 1.15	4.712	4.44
1.0% Arabic gum encapsulated *B. thuringiensis* at 500 µg/ml (G)	92 ± 5.20	69 ± 3.00	43 ± 2.75	35 ± 4.30	30 ± 3.36	16 ± 2.13	4.0 ± 0.58	6.563	4.44
(G) + 0.1% Vit. (A)	90 ± 4.62	71 ± 4.25	56 ± 4.05	42 ± 3.16	32 ± 3.54	18 ± 2.31	6.0 ± 1.73	7.386	6.67
(G) + 0.1% Vit. (E)	88 ± 6.93	75 ± 2.61	54 ± 2.83	41 ± 4.44	35 ± 2.55	22 ± 1.73	10 ± 2.47	7.751	11.11
(G) + 0.1% Vit. (C)	86 ± 4.04	82 ± 2.28	71 ± 4.05	59 ± 6.93	43 ± 4.49	30 ± 4.44	16 ± 3.10	11.321	17.78
(G) + 0.1% Selenium	88 ± 7.51	80 ± 6.93	70 ± 4.44	57 ± 5.20	49 ± 5.20	31 ± 3.10	12 ± 2.31	11.273	13.33
(G)+ 0.1% Charcoal	82 ± 5.20	78 ± 4.05	35 ± 3.61	29 ± 2.72	22 ± 2.31	12 ± 2.42	6.0 ± 1.15	5.381	6.67
(G) + 0.1% Titanium dioxide	90 ± 8.08	80 ± 3.69	69 ± 5.20	53 ± 4.44	37 ± 3.42	26 ± 2.83	8.0 ± 1.58	9.767	8.89
(G) + 0.1% Vit.(A)+ 0.1% Vit.(E)	90 ± 6.93	82 ± 4.25	63 ± 4.25	50 ± 2.24	33 ± 2.83	27 ± 3.61	20 ± 2.92	9.907	22.22
(G) + 0.1% Vit.(A)+ 0.1% Vit.(C)	88 ± 6.93	84 ± 5.20	69 ± 3.08	57 ± 3.69	45 ± 3.54	31 ± 2.55	22 ± 3.61	12.036	24.44
(G) + 0.1% Vit.(E)+ 0.1% Vit.(C)	88 ± 8.08	81 ± 3.86	74 ± 2.92	63 ± 2.83	57 ± 3.11	31 ± 1.73	27 ± 2.92	14.692	30.00
(G) + 0.1% Vit.(A)+ 0.1% Vit.(E)+ 0.1% Vit. (C)	89 ± 4.04	86 ± 5.20	75 ± 2.47	59 ± 4.49	49 ± 4.49	43 ± 5.20	35 ± 3.69	16.445	38.89
(G) + 0.1%Vit.(A)+ 0.1% Vit.(E)+ 0.1% Vit.(C) + 0.1% selenium	91 ± 9.81	86 ± 6.93	76 ± 6.93	59 ± 3.54	53 ± 7.51	51 ± 7.51	43 ± 4.44	20.605	47.78
(G) + 0.1% Vit.(A) + 0.1% Vit.(E)+ 0.1% Vit.(C) + 0.1% Titanium dioxide	90 ± 4.04	82 ± 3.54	75 ± 2.12	53 ± 2.55	47 ± 6.93	41 ± 4.49	33 ± 3.42	14.687	36.67

*% of Original Activity Remaining of *B. thuringiensis* var. *kurstaki* (HD-73) = (% cause-specific mortality from exposed *B. thuringiensis* for 24 h of UV irradiation ÷ % cause-specific mortality from unexposed *B. thuringiensis*) ×100

After the addition of vit.(A) at 0.1% to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum, the persistence of *B. thuringiensis* was 7.386 hours of artificial UV irradiation. The addition of each of vit.(E), vit. (C) and selenium at 0.1% to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum caused more persistence of *B. thuringiensis* to be 7.751, 11.321 and 11.273 hours (≈ 2.9, 4.2 and 4.2 respectively days in field) after exposure to UV irradiation, respectively. The persistence of *B. thuringiensis* was 5.381 and 9.767 hours (≈ 2.02 and 3.7 days in field) after exposure to UV irradiation after the addition of charcoal and titanium dioxide at 0.1%, respectively, to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum.

The addition of 0.1% vit.(A)+ 0.1% vit.(E) mixture, 0.1% vit.(A)+ 0.1% vit.(C) mixture and 0.1% vit.(E)+ 0.1% vit.(C) mixture to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum prolonged the persistence of *B. thuringiensis* to be 9.907, 12.036 and 14.692 hours (≈ 3.7, 4.5 and 5.5 respectively days in field) after exposure to UV irradiation, respectively. 0.1% vit.(A)+ 0.1% vit.(E)+ 0.1% vit. (C) mixture prolonged the persistence of *B. thuringiensis* to be 16.445 hours (≈ 6.2 days in field) after exposure to UV irradiation when added to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum.

The persistence of *B. thuringiensis* was 20.605 hours (≈ 7.7 days in field) after exposure to UV irradiation after the addition of 0.1% vit.(A)+ 0.1% vit. (E)+ 0.1% vit.(C)+ 0.1% selenium mixture to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum, and it was 14.687 hours of artificial UV irradiation after the addition of 0.1% vit.(A)+ 0.1% vit.(E)+ 0.1% vit.(C)+ 0.1%titanium dioxide mixture.

The percentage of Original Activity Remaining (%OAR) of *B. thuringiensis* (HD-73) alone at 500µg/ml was 4.44% against *P.operculella* larvae after 24 hours of exposure to UV irradation. The OAR of *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% arabic gum was 4.44% against *P.operculella* larvae after 24 hours of exposure to UV irradation. The addition of 0.1% vit.(A) to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% arabic gum kept about 6.67% of its OAR against *P.operculella* larvae after 24 hours of exposure to UV irradation.

The addition of each of vit.(E), vit. (C) and selenium at 0.1% to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum caused OAR to be 11.11, 17.78 and 13.33% against *P.operculella* larvae after 24 hours of exposure to UV irradation, respectively. The OAR of *B. thuringiensis* was 6.67 and 8.89% against *P.operculella* larvae after the addition of charcoal and titanium dioxide at 0.1%, respectively, to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum after 24 hours of exposure to UV irradation.

The addition of 0.1% vit.(A)+ 0.1% vit.(E) mixture, 0.1% vit.(A)+ 0.1% vit.(C) mixture and 0.1% vit.(E)+ 0.1% vit.(C) mixture to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum kept about 22.22, 24.44 and 30.00% of OAR of *B. thuringiensis* , respectively, against *P.operculella* larvae after 24 hours of exposure to UV irradation. 0.1% vit.(A)+ 0.1% vit.(E)+ 0.1% vit. (C) increased the OAR of *B. thuringiensis* to be 38.89% against *P.operculella* larvae when added to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum after 24 hours of exposure to UVirradation.

The OAR of *B. thuringiensis* was 47.78% against *P.operculella* larvae after the addition of 0.1% vit.(A)+ 0.1% vit.(E)+ 0.1% vit. (C)+ 0.1% selenium mixture to *B. thuringiensis* (HD-73) at 500µg/ml mixed with 1.0% Arabic gum after 24 hours of exposure to UVirradation, and it was 36.67% after the addition of 0.1% vit.(A)+ 0.1% vit.(E)+ 0.1% vit. (C)+ 0.1%titanium dioxide mixture.

Discussion:

Ultraviolet and ionizing radiation can cause *B. thuringiensis* insecticides to lose toxicity when used in the field. These irradiations can cause water and other substances in the cell to ionize. Then chemical free radicals are formed, and the most important one is hydroxyl radical (- OH). Free radicals react with macromolecules, such as DNA and proteins in the cell to inactivate them. The main active substances of *B. thuringiensis* formulations are toxic proteins and spores. The loss of the toxicity to insect larvae is due to UV damage to the spores and to their δ-endotoxin (Griego and Spence, 1978; Ignoffo and Garcia, 1978; Pozsgay *et al.,* 1987; Pusztai *et al.,* 1991; Chen *et al.*, 2004). It was also reported that tryptophan is essential for the interaction of the δ-endotoxin with insect midgut cell receptors and that the destruction of tryptophan residues may result in some changes in the three-dimensional configuration of the toxic protein and consequently the loss of its toxicity (Cohen *et al.,* 1991). Exposure of δ-endotoxin to UV irradiation resulted in the destruction of tryptophan and histidine residues (Pozsgay *et al.,* 1987). In order to overcome the short persistence of *B. thuringiensis*, some UV photoprotectants have been tested in the laboratory and field for their ability to inhibit or delay inactivation of *B. thuringiensis* by UV radiation. in this concern, several natural and synthetic organic chemicals such as, congo red dye, ranipal, berberine, tinopal, acriflavin, methyl green, egg albumen, Indian ink, carbon, shellac, Erio Acid Red, folic acid and p-amino benzoic acid have been evaluated as sunlight protectants for pathogens such as bacteria (Ramirez-Lepe *et al.,* 2003; Prasad et *al.,* 2003; Cohen *et al*., 2001; Xu *et al*., 2001; Tamez-Guerra *et al.,* 2000; Prabakaran *et al*., 2000; Ridgway *et al*., 1999; Justin *et al*., 1999; Ragaei, 1998 and 1990; Behle *et al.,* 1996 and 1997; Ignoffo and Garcia, 1994; McGuire *et al*., 1991; Cohen *et al.*, 1991; Dunkle and Shasha, 1989; Morris, 1983; Ignoffo and Garcia, 1978 and Bull *et al.*, 1976).

Sunlight is composed of UV-C (between 200– 280 nm), UV-B (between 280– 320 nm), UV-A (320–390 nm), visible (between 390– 780 nm), and infrared radiation (longer than 780 nm). The present investigations indicate that, the exposure of *B. thuringiensis* var. *kurstaki* (HD-73) to artificial UV light resulted in a gradual deterioration in virulence. The activity of *B. thuringiensis* var. *kurstaki* (HD-73) decreased sharply as a result of the exposure to the artificial UV light. The persistence of *B. thuringiensis* was 4.44 hours of artificial UV light.

The efficiency of any sunscreen depends on the depth of the layer of screen covering the pathogen which, in turn, depends on drop size. It is needed for the sun screen to be easy-to-handle, water soluble protectant, effective at low concentration and miscible with most products during storage, sunscreens act by selectively absorbing (absorbents convert damaging UV to harmless visible wavelengths), blocking or reflecting UV radiation, or negating active oxygen radicals (Burges, 1998). The effectiveness of a screen covering the entomopathogen depends on the concentration of both the screen and the pathogen in a product (Ignoffo and Garcia, 1996).

The tested UV photoprotectants were vitamin A, vitamin E, vitamin C and Selenium as antioxidant materials, also, charcoal and titanium dioxide were tested.

Relative Absorbance of B. Thuringiensis Var. Kurstaki (HD-73) and Photoprotectant Materials to Different Light Wavelengths:

Substances with a high degree of ultraviolet absorbance were able to show in laboratory experiments a good degree of protection and to retain the viability of *B. thuringiensis* (HD-73) but in varying degrees, the mode of action of protectant additives is the good absorption of the ultraviolet (UV-B region, 280-320 nm; UV-A region, 320-400 nm or both UV-B and UV-A) (Shapiro, 1989). Many natural and chemical additives have been evaluated as sunlight protectants for entomopathogens. The success of these substances was thought to be due to good absorption in the ultraviolet (UV-B region, 280-320 nm) as well as absorption in (UV-A region, 320-400) (Shapiro, 1985). Shapiro (1989) demonstrated that dyes that absorb both UV-B and UV-A radiation were more effective as radiation protectants than those dyes that absorb UV-B alone.

In the present study, the UV and visible absorption spectra of *B. thuringiensis* (HD-73) and tested photoprotectants are obtained using spectrophotometer scanning at wavelengths of 200-700 nm. The present investigations indicate that, the maximum absorbance values of *B. thuringiensis* (HD-73) were 1.459 and 1.164 at wavelength of 216 and 243.5 nm, respectively.

In the present study, the action of the tested photoprotectants was investigated by measuring the absorbance range of them individually and in combination, and the results obtained showed that some of these compounds may be effective in combination for practical use; most of the tested photoprotectants showed a high rate of absorption (from 200-700 nm) especially in the effective UV region (UV-C and UV-B) of *B. thuringiensis* inactivation.

The maximum absorbance value of the tested materials was obtained with vitamins (A+E+C) mixture that was 4.667 at wavelength of 267.5 nm (UV-C region). Also, vitamins (A+E+C) and selenium mixture showed a highly absorbance values which were 3.972, 2.032 and 3.928 at wavelengths of 216.2, 287.4 and 323.8 nm (UV-C, UV-B and UV-A), respectively.

Effect of Photoprotectants on Persistence of B. Thuringiensis (HD-73):

Some of the tested photoprotectants are antioxidants such as vitamin (A), vitamin (E), vitamin (C) and selenium, the mode of action of these antioxidants as photoprotectants is the prevention or inhibition of oxidation by: decreasing localized O_2 concentrations so that oxidation is less likely to occur; preventing initiation reactions by scavenging free radicals which are capable of directly abstracting H^+ from molecules; decomposing peroxides to prevent their conversion to further active and initiating radical species; reacting with chain-propagating radicals such as the peroxyl and alkoxyl radicals to prevent continued H^+ abstraction from fatty acid side chains (Anstey, 2002 and Duthie, 1999). Sies *et al.,* (1995) used Vitamins E and C, β-carotene, and other carotenoids as antioxidants and reported that photoprotective activity of them is related to their antioxidant properties, efficiently scavenging singlet molecular oxygen and peroxyl radicals.

Obtained results in present investigations proved that, the highest rate of UV-protection for *B. thuringiensis* (HD-73) was achieved with vitamins (A+E+C) and selenium mixture as it gave absorption rate at the three UV regions (UV-C, UV-B and UV-A), so, it may absorb the damaging UV energy and converting it to harmless visible light. The activity of *B. thuringiensis* (HD-73) mixed with vitamins (A+E+C) and selenium mixture slightly decreased as a result of the exposure to the artificial UV light measured by the persistence of *B. thuringiensis* (HD-73) which was 20.605 hours of artificial UV irradiation compared to 4.712 hours in the control (*B. thuringiensis* (HD-73) alone).

Arabic gum was used to adhere *B. thuringiensis* powder and photoprotectants particles together; however, it was tested alone with *B. thuringiensis,* it was found that, it has no UV protection effect to *B. thuringiensis*.

Charcoal gave slightly photoprotection to *B. thuringiensis* (HD-73), the persistence of *B. thuringiensis* (HD73) mixed with charcoal was 5.381 hours compared to 4.712 hours in the control. It formed suspension of black particles and resulted in a dark residue, probably protected *B. thuringiensis* by impeding all light including ultra violet (Ragaei, 1990), it was believed that carbon products have the ability to act as an oxygen sink, preventing the formation of free active radicals (Burges, 1998 and Ignoffo and Garcia, 1978). Charcoal was used as UV protectant during the preparation of *B. thuringiensis* formulation named 'Pusa *B.t.'* which gave good results against S. litura (Srivastava *et al.,* 2000).

The persistence of *B. thuringiensis* (HD-73) mixed with titanium dioxide after exposure to the artificial UV light for 24 hours was 9.767 hours compared to 4.712 hours in the control. It was used as reflector that reflects sunlight radiation from *B. thuringiensis.* Our results are similar to Medeiros *et al.* (2005). Bull *et al.* (1976) used titanium dioxide as a reflector for protection of nuclear polyhedrosis virus and it gave good protection in the laboratory and on cotton field.

The use of each of photoprotectants individually gave protection to *B. thuringiensis* (HD-73) to some extent; *B. thuringiensis* (HD-73) mixed with vitamin (A) gave a persistence to *B. thuringiensis* (HD-73) about 7.386 hours of artificial UV irradiation compared to 4.712 hours in the control (*B. thuringiensis* (HD-73) alone). Vitamin (A) is efficient in photoprotection, scavenging singlet oxygen and peroxyl radicals (Helmut *et al.,* 2004; Stahl *et al.,* 2003; Sies *et al.,* 1995 and Burton *et al.,* 1984).

B. thuringiensis (HD-73) mixed with vitamin (E) gave a persistence to *B. thuringiensis* (HD-73) about 7.751 hours of artificial UV irradiation compared to 4.712 hours in the control (*B. thuringiensis* (HD-73) alone). Vitamin (E) as antioxidant has photoprotective activity and is efficiently scavenging singlet molecular oxygen and peroxyl radicals (Sies *et al.,* 1995).

Vitamin (C) gave persistence to *B. thuringiensis* (HD-73) about 11.321 hours of artificial UV irradiation when mixed with it compared to 4.712 hours in the control. Vitamin (C) as antioxidant is efficiently scavenging singlet molecular oxygen and peroxyl radicals (Sies *et al.,* 1995). Ignoffo and Garcia (1994) used vitamin (C) as a photoprotectant for protection of nuclear polyhedrosis virus and it showed to be an effective UV protectant. Antioxidant mechanism of Vitamin (C) is that, it acts as an antioxidant by being available for energetically favourable oxidation. Many oxidants (typically, reactive oxygen species) such as the hydroxyl radical (formed from hydrogen peroxide), contain an unpaired electron, and thus, are highly reactive and damaging to humans and plants at the molecular level. This is due to their interaction with nucleic acid, proteins, and lipids. Reactive oxygen species oxidize (take electrons from) ascorbate first to monodehydroascorbate and then dehydroascorbate. The reactive oxygen species are reduced to water, while the oxidized forms of ascorbate are relatively stable and unreactive, and do not cause cellular damage

(wikipedia, 2008). Wefers *et al.* (1998) found that, in vitro studies suggest that vitamin C regenerates tocopherol from the tocopheroxyl radical and transfers the radical load to the aqueous compartment where it is finally eliminated by antioxidant enzymes.

A combination of topical vitamins C and E is better for UV protection than an equivalent concentration of topical vitamin C or E alone. In addition, the combination of vitamins C and E provided protection against thymine dimer formation (Lin *et al.,* 2003).

Using antioxidant complex - vitamins (lycopene, β-carotene, α-tocopherol), selenium- protect B. thuringiensis against UV and could provide a safe, daylong and efficient complement to photo-protective measures provided by topical and physical agents (Cesarini *et al.,* 2003).

The persistence of *B. thuringiensis* (HD-73) mixed with selenium after exposure to the artificial UV light for 24 hours was 11.273 hours compared to 4.712 hours in the control. It provided protection because it may scavenge or catalyze the degradation of reactive radicals (Burges, 1998).

The combinations between the tested antioxidants increased the protection of *B. thuringiensis* against UV radiation, The persistence of *B. thuringiensis* (HD-73) mixed with vit.(A+E), vit.(A+C), vit.(E+C) and with vit. (A+E+C) mixtures, after exposure to the artificial UV light for 24 hours was 9.907, 12.036, 14.692 and 16.445 hours, respectively, compared to 4.712 hours in the control.When vit. (A+E+C)+ titanium dioxide mixture mixed with *B. thuringiensis* (HD-73), it gave a persistence of 14.687 of artificial UV irradiation compared to 4.712 hours in the control this means that, vit. (A+E+C)+ selenium mixture gave the best protection to *B. thuringiensis* (HD-73).

The persistence of *B. thuringiensis* (HD-73) mixed with selenium after exposure to the artificial UV light for 24 hours was 11.273 hours compared to 4.712 hours in the control. It provided protection because it may scavenge or catalyze the degradation of reactive radicals (Burges, 1998).

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