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Effect of ultra-violet radiation protectants on indigenous isolates of entomopathogenic nematodes

KUM KUM WALIA, R. K. WALIA and S. N. NANDAL
Department of Nematology, CCS Haryana Agricultural University,
Hisar 125 004, Haryana, India.
E-mail: raman@hau.ernet.in

ABSTRACT: **The inability of most EPN isolates to withstand UV rays in solar radiation is one of the major impediments for their use against foliar insect pests. To overcome this, four chemicals *viz.*, PABA (para amino benzoic acid), Congo red, zinc oxide and titanium dioxide were tested at four concentrations (0.5, 0.25, 0.1 and 0.05%) to identify a suitable UV protectant and to standardize its concentration for use of indigenous isolates of *Heterorhabditis bacteriophora* and two isolates (Janti and RB-5) of *Steinernema* species. Nematode suspensions (1000 IJs/ml) treated with the above chemicals at different concentrations were exposed to UV radiation in a laminar flow bench for 30 and 60 min. PABA and Congo red provided nearly absolute protection from UV radiation in all the three EPN isolates. Zinc oxide was not effective (70-90% mortality in exposed IJs). Titanium dioxide protected *H. bacteriophora* completely at 0.5% concentration only, whereas in *Steinernema Janti* isolate protection was only 76% and in RB-5 isolate 40% IJs were alive even at 0.5% conc. The IJs exposed to PABA and Congo red remained infective to *Galleria mellonella* larvae whereas titanium dioxide hampered the infectivity. PABA and Congo red at 0.25%, and zinc oxide and titanium dioxide at 1% were not phytotoxic to mungbean. The effect of UV protectants was not nematode density-dependent, since they were equally effective at nematode populations of 1000, 2000 or 4000 IJs per ml.**

KEY WORDS: Congo red, entomopathogenic nematodes, *Heterorhabditis bacteriophora*, para amino benzoic acid, *Steinernema* spp., titanium dioxide, UV radiation, zinc oxide

INTRODUCTION

Entomopathogenic nematodes (EPNs) are effective bio-control agents of insect pests of many crops. However, their use as spray against foliar insect pests is restricted due to the sensitivity of infective juveniles (IJs) to ultraviolet (UV) radiation. UV radiation is conveniently divided into three groups – UV A (320-400 nm), UV B (280-320 nm) and UV C (200-280 nm). UV A constitutes about 90% of UV radiation reaching the earth's surface, whereas UV B constitutes only 10% but it is about 1000 times more potent than UV A. UV C is still more damaging than UV A and UV B but fortunately it does not reach earth's surface and is absorbed by the earth's atmosphere.

Para amino benzoic acid (PABA) is a UV B absorber and found effective against a number of EPNs (Gaugler and Boush, 1979; Hussaini *et al.*, 2003). Congo red, a dye, has been found effective in protecting gypsy moth nuclear polyhedrosis

virus from UV radiation (Shapiro, 1989) and has excellent absorbance of UV A and UV B radiation. Different populations of EPNs behave differently to UV radiation exposure (Grewal *et al.*, 2002). Hence, studies were carried out on indigenous isolate of *Heterorhabditis bacteriophora* and two isolates of *Steinernema* (Janti and RB-5) from Haryana soils.

MATERIALS AND METHODS

Screening Test

Indigenous isolates of *H. bacteriophora* (Sugarcane soil, Panipat) and two isolates *Steinernema* sp. (*Janti* soil, Sirsa and Paddy soil, Bhiwani) were used for the study. Two UV absorbers - PABA and Congo red, and two UV scatterers, zinc oxide and titanium dioxide were screened at four aqueous concentrations *viz.*, 0.05, 0.1, 0.25 and 0.5%. Zinc oxide and titanium dioxide were not soluble in water but dispersed well. One ml of nematode suspension containing 1000 IJs of respective nematode species/isolates and one ml of double strength UV protectants was added in 5 cm Petri plates. Non-irradiated IJs in UV protectants and in water alone, and irradiated IJs in water alone served as controls. The sets to be exposed to UV radiation were left on a laminar flow bench without lids under a UV lamp for 30 and 60 min. Lids were replaced after exposure and left overnight. Each treatment was replicated thrice and randomized completely. Per cent survival was calculated from number of dead and alive IJs. Data were angular transformed and analyzed statistically.

Infectivity Test

IJs surviving after exposure to UV radiation for 60 min were tested for infectivity to *Galleria mellonella* larvae (last instar) using 6-well tissue culture plates. The base of each well was lined with filter paper on to which 100 IJs were released in 0.5 ml of water. One *Galleria* larva was released in each of such six wells per treatment. Infectivity of IJs of all the nematode species/isolates exposed to PABA, Congo red and titanium dioxide at each concentration was tested. Zinc oxide was not found effective; hence the infectivity of IJs was not tested.

Effect of nematode density

Effect of nematode density on UV radiation was tested by adding 1000, 2000 or 4000 IJs per ml suspension to 0.1% concentration of Congo red and PABA and 0.5% concentration of zinc oxide and titanium dioxide. Appropriate control was maintained. Each treatment was replicated four times and randomized completely. UV exposure was given for 60 min and the number of dead and live IJs was counted 24h later.

Phytotoxicity Test

Congo red and PABA at 0.25% and zinc oxide and titanium dioxide at 1% concentration were sprayed on to 60-days-old mung bean plants in a screen house. Three replications were maintained. Observations on phytotoxic symptoms, if any, were recorded after 24h.

RESULTS AND DISCUSSION

PABA completely protected IJs of all the three nematode species even at lowest concentration (0.05%) and 60 min exposure in comparison to control (water alone) where 100% mortality was recorded in both the *Steinernema* isolates (Table 1). In *H. bacteriophora*, only negligible (6%) IJs could survive the exposure to UV radiation without any UV protectant. The IJs exposed to PABA and UV radiation were 100% infective to *Galleria* in comparison to irradiated IJs in water alone (Table 5). In Congo red, 100% survival was observed in *Steinernema* (RB-5 isolate) and *H. bacteriophora* and up to 96% in *Steinernema* (*Janti* isolate) at all the concentrations of the dye (Table 2). The Congo red-exposed juveniles were also 100% infective to *Galleria* larvae (Table 5).

Zinc oxide failed to protect IJs of any of the species/isolates from UV radiation. There was only 26% survival in *H. bacteriophora* IJs in 0.5% concentration after 60 min exposure to UV radiation whereas it was only 1.7 and 0.9% in *Steinernema* (*Janti* isolate) and *Steinernema* (RB-5 isolate), respectively at the same concentration and exposure time (Table 3). Since the zinc oxide was not effective, its infectivity to *Galleria* larvae was not tested.

Table 1. Effect of Para amino benzoic acid on the survival (%) of infective juveniles of entomopathogenic nematodes exposed to UV radiation

Concentration	EPN species/isolate											
	Steinernema sp. (*Janti* isolate)				*Steinernema* sp. (RB-5 isolate)				*Heterorhabditis bacteriophora*			
	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)
	0	30	60		0	30	60		0	30	60	
0.5	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0.25	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0.10	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0.05	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0 (control)	100 (90)	2.6 (9.2)	0 (0.0)	34.2 (33.0)	100 (90)	24.1 (29.2)	0 (0.0)	41.4 (39.7)	100 (90)	16.0 (23.6)	6.0 (11.7)	40.7 (41.7)
Mean (Exp. time)	100 (90)	80.5 (73.8)	80.0 (72.0)		100 (90)	84.8 (77.8)	80.0 (72.0)		100 (90)	83.2 (76.7)	81.2 (74.3)	
Factors	CD (5%)		SE (m)		CD (5%)		SE (m)		CD (5%)		SE (m)	
Conc.	0.67		0.23		1.37		0.47		2.55		0.88	
Exp. Time	0.52		0.18		1.06		0.37		1.98		0.68	
Conc. x Exp. Time	1.17		0.57		2.37		0.82		4.43		1.53	

Figures in parentheses are angular transformed values

Table 2. Effect of Congo red on the survival (%) of infective juveniles of entomopathogenic nematodes exposed to UV radiation

Concentration	EPN species/isolate											
	Steinernema sp. (*Janti* isolate)				*Steinernema* sp. (RB-5 isolate)				*Heterorhabditis bacteriophora*			
	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)
	0	30	60		0	30	60		0	30	60	
0.50	100 (90)	100 (90)	96.4 (79.2)	98.8 (86.4)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0.25	100 (90)	100 (90)	95.6 (78.1)	98.5 (86.0)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0.10	100 (90)	100 (90)	96.4 (79.1)	98.8 (86.4)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0.05	100 (90)	100 (90)	95.9 (78.6)	98.6 (86.2)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
0 (control)	100 (90)	14.6 (21.8)	0 (0)	38.2 (37.3)	100 (90)	2.4 (6.98)	0 (0.0)	34.1 (32.33)	100 (90)	8.0 (16.1)	4.9 (10.5)	37.6 (38.9)
Mean (Exp. time)	100 (90)	82.9 (76.4)	76.8 (63.0)		100 (90)	80.5 (73.4)	80.0 (72.0)		100 (90)	81.6 (75.2)	80.9 (74.1)	
Factors	CD (5%)		SE (m)		CD (5%)		SE (m)		CD (5%)		SE (m)	
Conc.	1.85		0.64		1.71		0.59		2.51		0.87	
Exp. Time	1.43		0.49		1.33		0.46		1.94		0.67	
Conc. x Exp. Time	3.20		1.10		2.96		1.02		4.35		2.12	

Figures in parentheses are angular transformed values

Table 3. Effect of Zinc oxide on the survival (%) of infective juveniles of entomopathogenic nematodes exposed to UV radiation

Concentration	EPN species/isolate											
	Steinernema sp. (*Janti* isolate)				*Steinernema* sp. (RB-5 isolate)				*Heterorhabditis bacteriophora*			
	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)
	0	30	60		0	30	60		0	30	60	
0.50	100 (90)	21.8 (27.4)	1.7 (4.4)	41.2 (40.6)	100 (90)	30.5 (33.4)	0.9 (3.1)	43.8 (42.1)	100 (90)	34.2 (35.8)	26.0 (30.7)	53.4 (52.2)
0.25	100 (90)	27.9 (31.4)	1.7 (4.4)	43.2 (41.9)	100 (90)	37.0 (37.3)	1.0 (3.3)	46.0 (43.5)	100 (90)	27.2 (31.3)	9.1 (13.8)	45.4 (45.0)
0.10	100 (90)	10.6 (17.6)	0.4 (2.1)	37.0 (36.6)	100 (90)	7.7 (15.9)	0.7 (2.9)	36.1 (36.3)	100 (90)	13.9 (21.0)	4.0 (6.8)	39.3 (39.2)
0.05	100 (90)	18.6 (25.5)	0.7 (3.9)	39.7 (39.8)	100 (90)	20.6 (26.4)	1.0 (3.3)	40.5 (39.9)	100 (90)	22.2 (28.1)	2.7 (7.8)	41.6 (42.0)
0 (control)	100 (90)	7.4 (14.9)	2.3 (8.1)	36.5 (37.7)	100 (90)	25.3 (30.1)	1.2 (3.7)	42.1 (41.3)	100 (90)	2.9 (8.1)	0 (0.0)	34.3 (32.7)
Mean (Exp. time)	100 (90)	17.3 (23.4)	1.4 (4.6)		100 (90)	24.2 (28.6)	0.9 (3.3)		100 (90)	20.0 (24.9)	8.4 (11.8)	
Factors	CD (5%)		SE (m)		CD (5%)		SE (m)		CD (5%)		SE (m)	
Conc.	N.S.		1.78		N.S.		1.83		5.76		1.98	
Exp. Time	4.01		1.38		4.11		1.41		4.46		1.54	
Conc. x Exp. Time	N.S.		4.37		N.S.		3.17		9.99		3.44	

Figures in parentheses are angular transformed values

Table 4. Effect of Titanium dioxide on the survival (%) of infective juveniles of entomopathogenic nematodes exposed to UV radiation

Concentration	EPN species/isolate											
	Steinernema sp. (*Janti* isolate)				*Steinernema* sp. (RB-5 isolate)				*Heterorhabditis bacteriophora*			
	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)	Duration of UV exposure (min)			Mean (Conc.)
	0	30	60		0	30	60		0	30	60	
0.5	100 (90)	94.1 (72.5)	76.2 (61.7)	90.1 (74.7)	100 (90)	52.9 (46.7)	40.4 (39.0)	64.4 (58.6)	100 (90)	100 (90)	100 (90)	100 (90)
0.25	100 (90)	95.0 (77.2)	65.5 (55.2)	86.8 (74.1)	100 (90)	35.2 (35.8)	3.2 (8.1)	46.1 (44.7)	100 (90)	100 (90)	51.6 (45.9)	83.8 (75.0)
0.10	100 (90)	85.3 (67.8)	3.6 (8.9)	62.9 (55.6)	100 (90)	13.0 (20.4)	3.8 (8.6)	38.9 (39.7)	100 (90)	87.8 (69.6)	32.6 (34.4)	62.6 (64.6)
0.05	100 (90)	11.9 (18.6)	0 (0.0)	37.3 (36.2)	100 (90)	7.3 (15.4)	2.6 (7.6)	36.6 (37.6)	100 (90)	10.5 (18.3)	4.9 (12.3)	38.5 (40.2)
0 (control)	100 (90)	0.9 (4.6)	0 (0.0)	33.6 (31.5)	100 (90)	14.3 (21.9)	0 (0.0)	38.1 (37.2)	100 (90)	0 (0.0)	0 (0.0)	33.3 (30.0)
Mean (Exp. time)	100 (90)	57.4 (48.2)	29.1 (25.2)		100 (90)	24.5 (28.0)	10.0 (12.7)		100 (90)	(53.6)	(36.5)	
Factors	CD (5%)		SE (m)		CD (5%)		SE (m)		CD (5%)		SE (m)	
Conc.	6.49		2.23		6.07		2.09		2.99		1.03	
Exp. Time	5.03		1.73		4.70		1.62		2.32		0.80	
Conc. x Exp. Time	11.24		3.87		10.53		3.63		5.19		1.78	

Figures in parentheses are angular transformed values

Table 5. Infectivity (%) of UV radiation exposed IJs of three EPN species/isolates to *Galleria mellonella* larvae

Concnetration (%)	*Heterorhabditis bacteriophora*			*Steinernema* sp.(*Janti* isolate)			*Steinernema* sp.(RB-5 isolate)		
	PABA	Congo Red	TiO_2	PABA	Congo Red	TiO_2	PABA	Congo Red	TiO_2
0.50	100	100	0	100	100	40	100	100	30
0.25	100	100	0	100	100	0	100	100	0
0.10	100	100	0	100	100	0	100	100	0
0.05	100	100	0	100	100	0	100	100	0
0.00 (Control-irradiated)	0	0	0	0	0	0	0	0	0
0.00 (Control-non irradiated)	100	100	100	100	100	100	100	100	100

Titanium dioxide gave 50-100% protection from UV radiation depending on nematode species and exposure time (Table 4). At highest concentration and 60 min exposure to UV radiation, survival was 100, 76.2 and 40.4% in *H. bacteriophora*, *Steinernema* sp. (*Janti* isolate) and *Steinernema* sp. (RB-5 isolate), respectively. The lower concentrations were ineffective in *Steinernema* (RB-5 isolate) whereas 50-65% survival was observed in other two species at 0.25% concentration. The concentrations lower than 0.25% were ineffective. When infectivity to *Galleria* larvae was tested, interestingly even after 100% survival, infectivity was nil in *H. bacteriophora*. In two *Steinernema* isolates where survival was less compared to *H. bacteriophora*, up to 30-40% infectivity was observed (Table 5).

Nematodes at population levels of 1000, 2000 or 4000 per ml could be equally protected at the highest concentration and 60 min exposure time for all the UV protectants tested i.e., there was no density dependent variation in the performance of UV protectants. No phytotoxicity symptoms on mungbean were recorded in any of the chemicals used.

PABA has earlier been found effective in mitigating the harmful effect of UV radiation on EPNs (Gaugler and Boush, 1979; Hussaini *et al.*, 2003). Congo red absorbs UV radiation over wide spectrum (280-400 nm). The absorbance is more in UV A (320-400 nm) than UV B (280-320 nm). Congo red absorbs UV rays in both the spectral regions and gives 100% protection without any deleterious effect on infectivity of IJs. Congo red provided complete protection to LdMNP virus at 1% concentration (Shapiro, 1989). Other workers have also reported that 100% original infectivity was retained by *Steinernema carpocapsae* in the presence of Stilbene brightener Tinopal LPW (Nickle & Shapiro, 1992), and some blankophore fluorescent brighteners BB 11, 11 RS and DML (Nickle & Shapiro, 1994). UV scatterers were not effective. UV protection was only 30-40% in zinc oxide and 100% in titanium dioxide only at high concentrations. The powdered chemicals stuck to the body of the IJs which perhaps hampered their infectivity. Even though these two chemicals were not phytotoxic, their use in foliar sprays is ruled out due to poor protection and low infectivity. From these studies, we could infer that UV absorbers are better UV protectants than UV scatterers.

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