



## Effect of Ultra Violet (UV) radiation protectants on indigenous isolates of entomopathogenic nematode, *Steinernema dharanaii* (Nematoda: Steinernematidae)

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### Abstract

The paper reports the effect of Ultra-violet (UV) radiation on the survival and pathogenicity of new species of EPN, *Steinernema dharanaii* (TFRIEPN-15) in the laboratory. To investigate the effects of ultraviolet radiation exposure on *S. dharanaii* was taken with to PABA (Para Amino Benzoic Acid) and Ranipal (optical brighteners). The study was conducted on the effects of the survival (50 Hz-220 V) Ultra-Violet (UV) radiation for different time lengths (30, 60 min, 2, 4, 6, 16 and 24 hours. The pathogenicity of exposed EPN infective juveniles (IJs) to waxmoth larvae, *Galleria mellonella* were tested under laboratory conditions.

The result showed that the exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 30 min. indicated no immediate effect after evident by 100.0% survival at par with control without PABA ( $P > 0.05$ ). However, when the same batch of IJs was observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. The IJs exposed to UV light in presence of PABA did not show any deleterious effect on infectivity potential. The infectivity after 24hrs was at par with control unexposed to UV ( $P > 0.05$ ). The exposure of IJs to UV radiation in presence of 0.05 and 0.1% Ranipal® for 30 min. indicated no mortality effect after exposure, evident by 100.0% survival at par with control ( $P > 0.05$ ). Unlike PABA, there was no mortality observed even when the same batch of IJs were observed 12 and 24 hrs after exposure ( $P > 0.05$ ). There was 100.0% infectivity noted in all the IJs exposed to UV in presence of Ranipal®.

These findings will shed more light on using locally isolated entomopathogenic nematodes under ultraviolet radiation stress with different protectants.

**Keywords:** entomopathogenic nematodes, *Steinernema dharanaii*, biocontrol agents, infectivity, ultra violet radiation

### 1. Introduction

Entomopathogenic nematodes (EPNs) are obligate parasites of insects. The two genera of EPNs; *Steinernema* and *Heterorhabditis* are promising biocontrol agents used to target a variety of economically important insect pests (Gaugler and Kaya 1990; Grewal *et al.*, 2005; Kulkarni *et al.*, 2008; Paunekar and Kulkarni 2020) [7, 12, 21, 31]. EPNs kill the insect within 24-48 hours from infection. They are easy to reproduce and inexpensive to mass culture (Kaya and Gaugler 1993; Bedding 2006) [18, 3]. The application of EPNs does not require special personal protective equipment. They occur in a variety of soil types and environments (Hominick *et al.*, 1996; Kaya *et al.*, 2006) [15, 19]. Consequently, EPNs have great potential to suppress above ground level insect pests via foliar application (Mason *et al.*, 1998; Kulkarni 2011; Lacy and Geogris 2012; Paunekar, 2014; Platt *et al.*, 2019) [27, 22, 26, 32, 34]. They are not harmful to humans, other animals, or plants (Akhurst and Smith, 2002 [1]. However, the efficacy of different species/strains of entomopathogenic nematodes in biocontrol agents can be reduced by several adverse environmental conditions such as desiccation, temperature extremes, humidity, moisture and Ultra-Violet (UV) radiation (Gaugler and Boush 1978; Hussaini *et al.*, 2003; Shapiro-Ilan *et al.*, 2006; Kulkarni *et al.*, 2016; Sharmila *et al.*, 2018; Paunekar and Kulkarni 2019) [8, 16, 37, 23, 33, 39].

There are some studies on the impact of UV radiation indicates that exposure to UV radiation can affect EPN survival, virulence

and reproduction (Walia *et al.*, 2008; Shapiro-Ilan *et al.*, 2015; Mouniga *et al.*, 2018) [42, 38, 28]. Thus, in selecting a particular nematode species or strain for use in biocontrol programs, the relative ability to withstand exposure may be an important consideration, especially for applications that are likely to entail extensive exposure to UV radiation.

The data generated on effect of native isolate in terms of survival and infectivity under different length of time exposure to Ultra-violet radiation with different protectants will not only help in developing protocol for its economical mass multiplication, but also in bioassays for infectivity against the target foliage pests. These aspects have been experimented and results discussed for the indigenous new species of EPN, *S. dharanaii* (TFRIEPN-15) isolated from teak forest areas of Madhya Pradesh, central India, for which such exposure mechanism with respect to the Ultra-Violet (UV) radiation protectant was not known. The findings can be utilized in planning management of foliage insect pests in forest nurseries and plantations.

### 2. Materials and Methods

#### 2.1 Collection and Maintenance of EPN

The nematode species used in the current study, *Steinernema dharanaii* originated from samples that were collected locally, and maintained and cultured at Forest Entomology Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh

(Kulkarni *et al.*, 2012a) <sup>[24]</sup>. Infective juveniles (IJs) were cultured *in vivo* by infecting larvae of the waxmoth larvae, *Galleria mellonella* L. (Lepidoptera: Pyralidae) with IJs (Dutky *et al.*, 1964; Kulkarni *et al.*, 2012b) <sup>[5, 25]</sup>. Dead infected waxmoth were kept at 27°C in a petri dish lined with moist filter paper and sealed with Parafilm until IJ emergence, before being transferred to White traps (White, 1927) <sup>[43]</sup>. The freshly IJs harvested from the White traps were transferred to vented culture flasks, where they were kept at 27°C, in keeping with the guidelines set out by Kaya Stock (1997) <sup>[20]</sup> and. These flasks were gently agitated once a week to improve aeration. IJs for the experiment were used within one week of emergence. The experiment was repeated on a different test date, with a fresh batch of nematodes.

## 2.2 Effect of UV light on the survival of *S. dharanaii* and the infectivity to *Galleria mellonella*

The two concentrations PABA (Para Amino Benzoic Acid) and Ranipal (Optical brighteners) *viz*: 0.05% and 0.1% was prepared. The Petri dishes (10 cm dia x 1.5 cm depth) were taken and placed the prepared solution at the uniform quantity i.e. 12 ml in five replication and taken distilled water as a control. The fresh IJs of *Steinernema dharanaii* were released uniform quantity i.e. 1000 IJs/ml treated with above concentrations of PABA and Ranipal in the Petri dish. The all the prepared set of Petri dish were exposed with UV radiation in Acco Laminar Air Flow (50 Hz-220 V) for 30 and 60 min, 2, 4, 6, 16 and 24 hours without lid. The observations were taken for survival mortality in the treated chemicals and control. The exposed IJs were tested for infectivity against *G. mellonella*. The experiment was repeated thrice before compilation of data and statistical analysis.

## 2.3 Statistical Analysis

The data on surviving infective juveniles (IJs) was used to calculate mean percentage survival and subjected to Analysis of Variance (ANOVA) after transforming it to angular values (Gomez and Gomez, 1984) <sup>[11]</sup>. The multiple comparison of means was done using the Ryan, Eniot-Gabriel & Welsch (REGW) procedure (Quinn and Keough, 2002) <sup>[36]</sup>, using statistical software GenStat Discovery Version 3 and data presented.

## 3. Results and Discussion

The abiotic factors soil texture, moisture, desiccation, temperature including ultra-violet radiation also affect entomopathogenic nematode activity with different nematodes responding differently. The entomopathogenic nematodes can have their survival, infectivity, development and reproduction affected when exposed to adverse environmental conditions, such as ultra-violet radiation.

### 3.1 Effect of Ultra Violet (UV) Radiation on *Steinernema dharanaii* (TFRIEPN-15) in presence of UV protectants PABA

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 30 min. indicated no immediate effect after evident by 100.0% survival at par with control without PABA ( $P>0.05$ ). However, when the same batch of IJs was observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed 72.20 and 86.20% survival,

respectively. After 24 hrs, survival was reduced to 66.0 and 77.80%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% PABA, as compared to 100.0% mortality in IJs without PABA and 100.0% survival in IJs unexposed to UV radiation ( $P<0.05$ ) (Table 1). The IJs exposed to UV light in presence of PABA did not show any deleterious effect on infectivity potential. The infectivity after 24 hrs was at par with control unexposed to UV ( $P>0.05$ ).

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 60 min. also had no immediate effect, whereas 100.0% mortality in control ( $P<0.05$ ). However, when the same batches of IJs were observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed 64.0 and 78.40% survival, respectively. After 24 hrs, survival was reduced to 54.80 and 70.80%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% PABA, as compared to 100.0% mortality in IJs without PABA and 100.0% survival in IJs unexposed to UV radiation ( $P<0.05$ ) (Table 2). The IJs exposed to UV light in presence of PABA did not show any deleterious effect on infectivity potential. The infectivity after 24 hrs was at par with control unexposed to UV ( $P>0.05$ ).

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 120 min. (2 hrs) also had no immediate effect, as against 100.0% mortality in control set of EPN without PABA ( $P>0.05$ ). However, when the same batch of IJs was observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed 46.80 and 61.40% survival, respectively. After 24 hrs, survival was reduced to 41.0 and 56.20%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% PABA, as compared to 100.0% mortality in IJs without PABA and 100.0% survival in IJs unexposed to UV radiation ( $P<0.05$ ) (Table 3).

The IJs exposed to UV light in presence of PABA did not show any deleterious effect on infectivity potential. The infectivity after 24 hrs was at par with control unexposed to UV ( $P>0.05$ ).

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 240 min. (4 hrs) also had no immediate effect, against 100.0% mortality in control without PABA ( $P<0.05$ ). When the same batch of IJs was observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed 14.20 and 27.80% survival, respectively. After 24 hrs, survival was reduced to 11.00 and 23.60%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% PABA, as compared to 100.0% mortality in IJs without PABA and 100.0% survival in IJs unexposed to UV radiation ( $P<0.05$ ) (Table 4).

The IJs exposed to UV light in presence of PABA did not show any deleterious effect on infectivity potential. The infectivity after 24 hrs was at par with control unexposed to UV ( $P>0.05$ ). The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 360 min. (6 hrs) also had no immediate effect. When the same batch of IJs was observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed 12.20 and 26.80% survival, respectively. After 24 hrs, survival was reduced to 6.80 and 23.20%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% PABA, as compared

to 100.0% mortality in IJs without PABA and 100.0% survival in IJs unexposed to UV radiation ( $P < 0.05$ ) (Table 5).

The IJs exposed to UV light in presence of PABA did not show any deleterious effect on infectivity potential. The infectivity after 24 hrs was at par with control unexposed to UV ( $P > 0.05$ ). The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 16 hrs had immediate mortality effect, with 48.80 and 60.20% survival in IJs suspended respectively in 0.05 and 0.1% PABA, as compared to control 100.0% mortality in (without PABA under UV) ( $P < 0.05$ ). However, there was 100.0% survival in control without UV. When the same batch of IJs were observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed 7.40 and 15.80% survival, respectively. After 24 hrs, survival was reduced to 2.60 and 11.40%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% PABA, as compared to 100% mortality in IJs without PABA and 100.0% survival in IJs unexposed to UV radiation ( $P < 0.05$ ) (Table 6). Interestingly, The IJs exposed to UV light for 16 hrs in presence of PABA completely lost the infectivity potential ( $P > 0.05$ ).

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% PABA for 24 hrs had severe immediate mortality effect, evident by only 33.20 and 45.20% survival in IJs suspended respectively in 0.05 and 0.1% PABA, as compared to control (without PABA under UV) ( $P < 0.05$ ). However, there was 100.0% survival in control without UV. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% PABA concentration showed only 2.40 and 6.60% survival, respectively. After 24 hrs, no survival was observed ( $P > 0.05$ ) (Table 7). The IJs exposed to UV light in presence of PABA completely lost the infectivity ( $P > 0.05$ ).

### 3.2 Effect of Ultra Violet (UV) Radiation on *Steinernema dharanaii* (TFRIEPN-15) in presence of UV protectants or optical brighteners Ranipal

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% Ranipal® for 30 min. indicated no mortality effect after exposure, evident by 100.0% survival at par with control ( $P > 0.05$ ). Unlike PABA, there was no mortality observed even when the same batch of IJs were observed 12 and 24 hrs after exposure ( $P > 0.05$ ) (Table 8). There was 100.0% infectivity noted in all the IJs exposed to UV in presence of Ranipal®. The exposure of IJs to UV radiation in presence of 0.05 and 0.1% Ranipal® for 60 min. indicated no mortality effect after exposure, as against 100% mortality in control without Ranipal ( $P < 0.05$ ). Unlike PABA, there was no mortality observed even when the same batch of IJs were observed 12 and 24 hrs after exposure ( $P > 0.05$ ) (Table 9). There was 100.0% infectivity noted in all the IJs exposed to UV in presence of Ranipal®.

The result exhibited that both the concentrations (0.05% and 0.1%) of Ranipal® (Optical brighteners), 100.0% survival of IJs in 120 minutes (2 hrs) time exposure to ultra violet radiation in all the treatments except control without Ranipal®. It was observed also even after 12 hours and 24 IJs survived 100.0% in 0.05% and 0.1% concentration of Ranipal® under UV radiation in all treatments.

The IJs after 24 hours survival were further subjected to infectivity test against *G. mellonella* larvae. The 100.0 % was obtained by IJs suspended in both concentrations (0.05% and

0.10%) of Ranipal® for 72 hr exposure to ultra violet radiation as evident by mortality obtained in *G. mellonella*. (Table 10).

The result exhibited 100.0% survival of IJs when exposed to UV radiation while suspended for 240 minutes (4 hrs) in both the concentrations (0.05% and 0.10%) of Ranipal® (Optical brighteners). There was 100.0% mortality in control without Ranipal. However, mortality was observed when IJs suspended in 0.05 and 0.10% concentration of Ranipal® were observed after 12 and 24 hrs with survival being 87.40 and 96.40%, and 80.20 and 90.0%, respectively. The IJs after 24 hours were subjected to infectivity test against waxmoth larvae. No detrimental effect was observed on the infectivity of IJs of exposure to UV in presence of Ranipal® (Table 11).

Continuous exposure of IJs to UV radiation for 6 hrs in presence of 0.05% and 0.10% of Ranipal® did not have any immediate effect with 100.0% survival ( $P > 0.05$ ).

However, mortality was observed in UV exposed IJs suspended in 0.05 and 0.10% concentration of Ranipal® were observed after 12 and 24 hrs with survival being only 83.80 and 90.20%, 74.60 and 84.60%, respectively. The IJs after 24 hours were subjected to infectivity test against waxmoth larvae. No detrimental effect was observed on the infectivity of IJs of exposure to UV in presence of Ranipal® (Table 12).

The exposure of IJs to UV radiation in presence of 0.05 and 0.1% Ranipal for 16 hrs had immediate mortality effect, with 69.20 and 83.60 % survival in IJs suspended respectively in 0.05 and 0.1% Ranipal, as compared to control 100.0% mortality in (without Ranipal under UV) ( $P < 0.05$ ). When the same batch of IJs was observed 12 and 24 hrs after exposure, time-dependent and decrease in survival was noted. After 12 hrs of exposure, IJs exposed to 0.05 and 0.10% Ranipal concentration showed 61.60 and 72.80% survival, respectively. After 24 hrs, survival was reduced to 48.60 and 62.60%, respectively in IJ batch exposed to UV in presence of 0.05 and 0.1% Ranipal®, as compared to 100.0% mortality in IJs without Ranipal® and 100.0% survival in IJs unexposed to UV radiation ( $P < 0.05$ ) (Table 13).

The IJs exposed to UV light in presence of Ranipal® still displayed 100.0% infectivity at par with control (IJs without UV exposure) ( $P < 0.05$ ). The exposure of IJs to UV radiation in presence of 0.05 and 0.1% Ranipal® for 24 hrs had immediate mortality effect, with 44.40 and 64.40% survival in IJs suspended respectively in 0.05 and 0.1% Ranipal, as compared to control 100.0% mortality in (without Ranipal under UV) ( $P < 0.05$ ).

It was also observed that 12 hrs after the 24 hr-exposure, 35.04 and 51.20% IJs survived which were suspended in 0.05% and 0.10% Ranipal® as compared to Nil in control under UV radiation ( $F_{(P < 0.001)} = 1167, df = 12, SE(d) \pm = 1.53, LSD_{(P < 0.05)} = 3.33$ ). Twenty-four hours after the 24hr-exposure, 30.20 and 40.0% survival was exhibited in both concentrations of Ranipal® as compared to control where 100.0% mortality was observed ( $F_{(P < 0.001)} = 1253.10 df = 12, SE(d) \pm = 1.47, LSD_{(P < 0.05)} = 3.22$ ).

The IJs after 24 hours were used to infectivity test against *G. mellonella* larvae. The 100.0% larval mortality was obtained in both concentrations (0.05 and 0.10%) of Ranipal® for 72 hour exposure to ultra violet radiation and untreated control (unexposed). (Table 14).

The efforts to protect EPNs from UV rays have earlier been made using optical brighteners (OBs) like Ranipal® and Tinopal® and UV absorber, PABA (Para Amino Benzoic Acid) have been used as UV protectants earlier by a few workers (Gaugler and Boush

1979; Nickle and Shapiro 1992, 1994; Hussaini *et al.*, 2003; Grewal *et al.*, 2002; Walia *et al.*, 2008) [9, 29, 30, 16, 13, 42].

Gaugler and Boush (1978) [8] observed the effects of short UV radiation and natural sunlight on *S. carpocapsae*, in terms of their interaction with *G. mellonella* larvae. They found that exposure of IJs to short-term UV radiation for 7 min caused reduced pathogenicity and increased larval survival time post-infection. Exposure to direct sunlight also reduced their pathogenicity by as much as 95% after 60 min. Gaugler and Boush (1979) [9] reported that PABA (Para Amino Benzoic Acid) is a UV absorber and found effective against a number of EPNs. Nickle and Shapiro (1992) [29] have reported that 100% original infectivity was retained by *Steinernema carpocapsae* in the presences of Stilbene brightener Tinopal LPW. A stilbene fluorescent brightener, Tinopal LPW, was used as an ultraviolet (UV) protectant for the entomopathogenic nematode *Steinernema carpocapsae* (All strain). Gaugler *et al.* (1992) [10] found that *S. carpocapsae* IJs were rendered completely inactive after 10 min of moderate UV exposure, whereas *H. bacteriophora* was significantly affected after only 4 min, indicating that the susceptibility to UV light varies across species.

Nickle and Shapiro (1994) [30] have studied on effects of eight brighteners like blankophore fluorescent BB 11, 11 RS and DML as solar radiation protectant for *S. carpocapsae*. Seven commercially available Blankophor fluorescent brighteners were compared with the standard Tinopal LPW as solar radiation protectants for *S. carpocapsae* (All strain). Blankophor BBH and Tinopal LPW were the most successful UV screens, with 95% of the original nematode infectivity to larvae of the greater wax moth, *Galleria mellonella*, retained after 4 hours of exposure to direct sunlight. The Blankophor HRS and DML preserved 80 and 85% infectivity, and the P167 preserved 70% infectivity after the sunlight exposure. The other Blankophors (RKH, LPG, and BSU) were not as effective. Fujiie and Yokoyama (1998) [6] investigated that UV exposure harmed the nematode's symbiotic bacteria as well as the nematodes themselves. They may explain the differential impact of UV on EPN viability and virulence; the EPNs may remain alive while the bacteria (the primary virulence carrier) are killed or damaged

Hussaini *et al.* (2003) [16] have investigated on survival and pathogenicity of indigenous entomopathogenic nematodes in different UV protectants such as some optical brighteners (OBs) and Para Amino Benzoic Acid (PABA). They found that both the concentration (0.05% and 0.1%) of optical brighteners (OBs) and PABA tested offered excellent protection. The protection effect was density dependant and irradiation became lethal as the period exposure increased.

Grewal *et al.* (2002) [13] have reported that different populations of EPNs behave differently to UV radiation exposure.

Banu and Rajendran (2003) [2] recorded that 100 per cent mortality of IJ of *H. indica* and *S. glaseri* and *Steinernema* sp. from Kerala with continuous exposure to UV radiation for 120-135 min and the same Subramanian and Senthamizh (2004) [41] reported 100 per cent mortality of *H. indica* and *S. glaseri* after 120 and 210 min exposure to UV light respectively. Deepa and Subramanian (2006) [4] found 100 per cent mortality of *S. thermophilum* after 240 minutes of continuous exposure and failed to infect *C. cephalonica*. Grewal, (2001) [14] also reported that entomopathogenic nematodes are sensitive to UV light and their survival and infectivity on foliage is decreased by exposure

to sunlight. Jagdale and Grewal (2007) [17] reported that UV had no impact on the virulence of *S. feltiae* (SN), but did affect *S. carpocapsae* (All) and *S. riobrave* (TX-355 strain).

Wallia *et al.* (2008) [42] reported the four different concentrations (0.5, 0.25, 0.1 and 0.05%) of PABA with indigenous isolate of *Heterorhabditis bacteriophora* and two isolates (Janti and RB-5) of *Steinernema* species in UV protectant in a laminar flow bench for 30 and 60 min. They found that PABA completely protected IJs of all the three nematode species even at lowest (0.05%) and 60 min exposure in comparison to control (water alone) where 100% mortality was recorded in both the *Steinernema* isolates. The IJs exposed to PABA and UV radiation were 100% infective to *Galleria* in comparison to irradiate IJs in water alone.

Shapir-Ilan *et al.*, (2015) [38] investigated 9 different EPN species and 15 strains: *Heterorhabditis bacteriophora* (Baine, fl11, Oswego, and Vs strains), *H. floridensis* (332), *H. georgiana* (Kesha), *H. indica* (HOM1), *H. megidis* (UK211), *Steinernema carpocapsae* (All, Cxrd, DD136, and Sal strains), *S. feltiae* (SN), *S. rarum* (17C&E), and *S. riobrave* (355). In viability assessments, steinernematids, particularly strains of *S. carpocapsae*, generally exhibited superior UV tolerance compared with the heterorhabditids. However, some heterorhabditids tended to be more tolerant than others, e.g., *H. megidis* and *H. bacteriophora* (Baine) were most susceptible and *H. bacteriophora* (Vs) was the only heterorhabditid that did not exhibit a significant effect after 10 min of exposure. All heterorhabditids experienced reduced viability after 20 min exposure though several *S. carpocapsae* strains did not. In total, after 10 or 20min exposure, the viability of seven nematode strains did not differ from their non-UV exposed controls. In virulence assays, steinernematids (particularly *S. carpocapsae* strains) also tended to exhibit higher UV tolerance. However, in contrast to the viability measurements, all nematodes experienced a reduction in virulence relative to their controls.

Sharmila and Subramanian (2015) [40] studied effect of the ultraviolet (UV) radiation on the virulence of the entomopathogenic nematodes *viz.*, *Heterorhabditis indica* and *Steinernema glaseri*. UV radiations at 30 to 210 minutes were tested under laboratory conditions. Ultraviolet light emitted from UV lamp of 15W caused 100 per cent mortality of IJ within 120 minutes of continuous exposure. At the first observation interval of 30 minutes exposure to UV radiation, mortality of IJ was observed at 63.25 per cent. As the exposure period increased, the mortality of IJ was gradually increased. The highest mortality of 83.50 and 94.75 per cent of IJ was occurred between 60 and 90 minutes followed by 63.25 per cent at 30 minutes. Ultraviolet light caused 81.75, 88.75, 97.50 and 100 per cent mortality of IJ within 120, 150, 180 and 210 minutes of continuous exposure, respectively. The nematodes *H. indica* and *S. glaseri* exposed to UV light failed to infect *C. cephalonica*. There is no reproductivity of the nematodes of both species exposed to UV light.

Xi *et al.* (2019) [35] investigated the effect of 20 or 40 W UV-B radiation for different time lengths (30, 60, 90, or 120 min) on the virulence of EPN infective juveniles (IJs) to *Galleria mellonella*, including 9 strains from 4 species (*Steinernema feltiae*, *S. krausseii*, *Heterorhabditis brevicaudis*, and *H. megidis*) isolated from Gansu Province. The results showed that there were no differences among the strains in the survival rate of IJs with and without 30 min UV-B irradiation. The virulence of IJs varied

among strains after exposure to 20 W ultraviolet radiation for 30 min: virulence of two strains (*S. felitiae* 0639C and *S. felitiae* 0663TD) was increased ( $P < 0.05$ ), that of one strain (*S. felitiae* 0661YM) decreased ( $P < 0.05$ ), and that of all others (*H. brevicaudis* 0641TY, *H. megidis* 0627M, *S. felitiae* 0639C, *S.*

*felitiae* 0619HT, *S. kraussei* 0663HT, and *S. kraussei* 0657L) showed no significantly change ( $P > 0.05$ ). Hence, Ultra-violet radiation (UV) protectant is to be added to the nematode formulation before application on the foliage.

**Table 1:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 30 minutes exposure to PABA

Concentration (in %) PABA	Exposure Time (30 min)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	72.20 <sup>c</sup> (58.26)	66.00 <sup>c</sup> (54.37)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	86.20 <sup>b</sup> (68.27)	77.80 <sup>b</sup> (61.95)	100.00 <sup>a</sup> (90.04)
NO PABA (Control) Under UV	100.00 <sup>a</sup> (90.04)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( $P < 0.001$ )	NS	169.34	160.08	NS
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	1.33	1.31	-
<i>LSD</i> ( $P < 0.05$ )	-	2.89	2.87	-

\*Data in parentheses are Arc Sin√n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other ( $P > 0.05$ ).

**Table 2:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 60 minutes exposure to PABA

Concentration (in %)	Exposure Time (60 min)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	64.00 <sup>c</sup> (53.15)	54.80 <sup>c</sup> (47.76)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	78.40 <sup>b</sup> (62.42)	70.80 <sup>b</sup> (57.33)	100.00 <sup>a</sup> (90.04)
NO PABA (Control) Under UV	100.00 <sup>a</sup> (90.04)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( $P < 0.001$ )	NS	173.17	171.65	NS
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	1.26	1.12	-
<i>LSD</i> ( $P < 0.05$ )	-	2.74	2.46	-

\*Data in parentheses are Arc Sin√n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other ( $P > 0.05$ ).

**Table 3:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 120 minutes (2 hours) exposure to PABA

Concentration (in %)	Exposure Time (120 min)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	46.80 <sup>c</sup> (43.15)	41.00 <sup>c</sup> (39.80)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	61.40 <sup>b</sup> (51.62)	56.20 <sup>b</sup> (48.57)	100.00 <sup>a</sup> (90.04)
NO PABA (Control) Under UV	0.00 <sup>b</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( $P < 0.001$ )	NS	114.29	212.94	NS
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	1.63	1.04	-
<i>LSD</i> ( $P < 0.05$ )	-	3.57	2.26	-

\*Data in parentheses are Arc Sin√n transformation of percentage values.

a,b Values followed by similar alphabets do not differ significantly with each other ( $P > 0.05$ ).

**Table 4:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 4 hours exposure to PABA

Concentration (in %)	Exposure Time (4 hrs.)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	14.20 <sup>c</sup> (22.11)	11.00 <sup>c</sup> (19.34)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	27.80 <sup>b</sup> (31.81)	23.60 <sup>b</sup> (29.05)	100.00 <sup>a</sup> (90.04)
NO PABA (Control) Under UV	0.00 <sup>b</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( $P < 0.001$ )	NS	126.82	54.02	NS
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	0.47	0.59	-
<i>LSD</i> ( $P < 0.05$ )	-	4.79	1.21	-

\*Data in parenthesis are Arc Sin√n transformation of percentage values.

a,b Values followed by similar alphabets do not differ significantly with each other ( $P > 0.05$ ).

**Table 5:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 6 hours exposure to PABA

Concentration (in %)	Exposure Time (6hrs.)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	12.20 <sup>c</sup> (20.32)	6.80 <sup>c</sup> (14.94)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	26.20 <sup>b</sup> (30.69)	19.60 <sup>b</sup> (26.12)	100.00 <sup>a</sup> (90.04)
NO PABA (Control) Under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	NS	1703.84	1799.97	NS
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	1.32	1.32	-
<i>LSD</i> ( <i>P</i> <0.05)	-	2.88	2.87	-

\*Data in parentheses are Arc Sin√n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 6:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 16 hours exposure to PABA

Concentration (in %)	Exposure Time (16 hrs.)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	48.80 <sup>c</sup> (44.31)	7.40 <sup>c</sup> (15.75)	2.60 <sup>c</sup> (9.18)	0.00 <sup>a</sup> (0.00)
0.1%	60.20 <sup>b</sup> (50.92)	15.80 <sup>b</sup> (25.19)	11.40 <sup>b</sup> (19.68)	0.00 <sup>a</sup> (0.00)
NO PABA (Control) Under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>a</sup> (0.00)
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	0.00 <sup>a</sup> (0.00)
<i>F</i> ( <i>P</i> <0.001)	128.43	121.94	685.50	NS
<i>df</i>	12	12	12	-
<i>SE</i> ( <i>d</i> )±	1.43	1.34	0.62	-
<i>LSD</i> ( <i>P</i> <0.05)	3.11	2.93	1.35	-

\*Data in parentheses are Arc Sin√n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 7:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* exposure for 24 hours to PABA

Concentration (in %)	Exposure Time (24 hrs.)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	33.20 <sup>c</sup> (35.15)	2.40 <sup>c</sup> (8.70)	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)
0.1%	45.20 <sup>b</sup> (42.23)	6.60 <sup>b</sup> (14.77)	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)
NO PABA (Control) Under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)-
<i>F</i> ( <i>P</i> <0.001)	3075.23	8624	NS	NS
<i>Df</i>	12	12	-	-
<i>SE</i> ( <i>d</i> )±	0.94	0.93	-	-
<i>LSD</i> ( <i>P</i> <0.05)	2.05	9.33	-	-

\*Data in parenthesis are Arc Sin√n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 8:** Effect of UV (Ultra Violet) Radiation on the survival and infectivity of *S. dharanii* for 30 minutes exposure to Ranipal®

Concentration (in %)	Exposure Time (30 min)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	100.00 <sup>a</sup> (90.04)	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	NS	NS	NS	NS
<i>df</i>	-	-	-	-
<i>SE</i> ( <i>d</i> )±	-	-	-	-
<i>LSD</i> ( <i>P</i> <0.05)	-	-	-	-

\*Data in parenthesis are Arc Sin√n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 9:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanii* for 60 minutes exposure to Ranipal®

Concentration (in %)	Exposure Time (60 min)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	100.00 <sup>a</sup> (90.04)	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	NS	NS	NS	NS

<i>df</i>	-	-	-	-
<i>SE</i> ( <i>d</i> )±	-	-	-	-
<i>LSD</i> ( <i>P</i> <0.05)	-	-	-	-

\*Data in parentheses are Arc Sin√ n transformation of percentage values

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 10:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanaii* for (120 min) 2 hrs exposure to Ranipal

Concentration (in %)	Exposure Time (2 hrs.)	Observation after 12 (hrs.)	Observation after 24 (hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)	0.00 <sup>b</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>
<i>df</i>	-	-	-	-
<i>SE</i> ( <i>d</i> )±	-	-	-	-
<i>LSD</i> ( <i>P</i> <0.05)	-	-	-	-

\*Data in parentheses are Arc Sin√ n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 11:** Effect of Ultra Violet (UV) Radiation on survival and infectivity of *S. dharanaii* for 4 hours exposure to Ranipal®

Concentration (in %)	Exposure Time (4 hrs.)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	87.40 <sup>c</sup> (69.26)	80.20 <sup>b</sup> (66.94)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	96.40 <sup>b</sup> (79.17)	90.00 <sup>a</sup> (71.57)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>c</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	<i>NS</i>	4301.96	549.49	<i>NS</i>
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	0.87	2.37	-
<i>LSD</i> ( <i>P</i> <0.05)	-	1.90	5.17	-

\*Data in parentheses are Arc Sin√ n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 12:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanaii* for 6 hours exposure to Ranipal

Concentration (in %)	Exposure Time (6 hrs.)	Observation after (12 hrs.)	Observation after (24 hrs.)	Mean Infectivity 24 hrs IJs (in %)
0.05%	100.00 <sup>a</sup> (90.04)	83.80 <sup>c</sup> (66.33)	74.60 <sup>c</sup> (59.77)	100.00 <sup>a</sup> (90.04)
0.1%	100.00 <sup>a</sup> (90.04)	90.20 <sup>b</sup> (72.04)	84.60 <sup>b</sup> (66.93)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	<i>NS</i>	1341.06	3859.30	<i>NS</i>
<i>df</i>	-	12	12	-
<i>SE</i> ( <i>d</i> )±	-	1.52	1.90	-
<i>LSD</i> ( <i>P</i> <0.05)	-	3.31	0.87	-

\*Data in paranthesis are Arc Sin√ n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 13:** Effect of Ultra Violet (UV) Radiation on the survival and infectivity of *S. dharanaii* for 16 hrs exposure to Ranipal

Concentration (in %)	Exposure Time (16 hrs.)	Observation after 12 hours	Observation after 24 hour	Infectivity 24 hrs IJs (in %)
0.05%	69.20 <sup>c</sup> (56.41)	61.60 <sup>c</sup> (51.76)	48.60 (45.72)	100.00 <sup>a</sup> (90.04)
0.1%	83.60 <sup>b</sup> (66.36)	72.80 <sup>b</sup> (57.75)	62.60 <sup>b</sup> (52.34)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	859.06	924.33	1130.24	<i>NS</i>
<i>df</i>	12	12	12	-
<i>SE</i> ( <i>d</i> )±	1.84	1.73	1.55	-
<i>LSD</i> ( <i>P</i> <0.05)	4.01	3.78	3.38	-

\*Data in paranthesis are Arc Sin√ n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

**Table 14:** Effect of UV (Ultra Violet) Radiation on the survival and infectivity of *S. dharanaii* for 24 hours exposure to Ranipal

Concentration (in %)	Exposure Time (24 hrs.)	Observation after 12 hours	Observation after 24 hour	Infectivity 24 hrs IJs (in %)
0.05%	44.40 <sup>c</sup> (41.75)	35.04 <sup>c</sup> (36.42)	30.20 <sup>c</sup> (33.23)	100.00 <sup>a</sup> (90.04)
0.1%	64.40 <sup>b</sup> (53.48)	51.20 <sup>b</sup> (45.68)	40.00 <sup>b</sup> (39.22)	100.00 <sup>a</sup> (90.04)
No Ranipal under UV	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	0.00 <sup>d</sup> (0.00)	-
(Control) without UV	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)	100.00 <sup>a</sup> (90.04)
<i>F</i> ( <i>P</i> <0.001)	699.32	1167	1253.10	NS
<i>df</i>	12	12	12	-
<i>SE</i> ( <i>d</i> )±	1.98	1.53	1.47	-
<i>LSD</i> ( <i>P</i> <0.05)	4.32	3.33	3.22	-

\*Data in paranthesis are Arc Sin<sup>√</sup> n transformation of percentage values.

a, b Values followed by similar alphabets do not differ significantly with each other (*P*>0.05).

#### 4. Conclusion

To conclude, the findings of present investigation revealed that the ecological factor such as Ultra violet (UV) radiation exposure have positive influence on the survival and infectivity of *S. dharanaii* to protectants, PABA and Ranipal (Optical Brighteners). Hence UV protectant is to be added to the nematode formulation before application on the foliage to enhancing the potential of infectivity of native isolate species of entomopathogenic nematodes will be useful to management of foliage insect pests.

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