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Effect of temperature on the survival and infectivity of new species of entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) from Madhya Pradesh, central India

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Abstract
The effect of different temperatures ranges on the survival and infectivity of indigenous isolated entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) was studied in the laboratory. The temperature ranges 0^0C to 40^0C were exposed to IJs for different time period. The exposed IJs was also assessed the infectivity in different temperature. The result indicated that exposure TFRIEPN-15 IJs to 10 to 34^0C for 72 hrs IJs did not show any marked effect, but temperature below 10^0C and above 34^0C exhibited significant mortality. The Infective Juveniles (IJs) of TFRIEPN-15, when exposed to 0^0C and 38^0C, could tolerate only up to 12 hrs, above which exposure time no survival was observed. The exposure to extreme temperature of 40^0C, allowed only 31.20 and 8.80% survival respectively at exposure period of 15 and 30 min., with finally 100.0% mortality after 60 min. of exposure. The infectivity of IJs pre-exposed to extreme temperature to waxmoth larvae was proportional to the exposure period. The period of exposure of IJs to 0^0C above 8 hrs affected infectivity potential of the pre-exposed IJs. IJs exposed for 2 hours were able to infect 100.0% *Galleria mellonella* larvae. Thereafter, the percentage of infectivity decreased. The IJs exposed for 4 and 6 hrs infected 80.0% and 32.0%, Further exposure to 8 hrs proved detrimental to the infectivity potential with only 16.0% infectivity to waxmoth larvae. Therefore, while temperature of $27\pm^0$C (control) proved optimum for mass-economical mass-multiplication purpose. However, this native population could sustain temperature range of 10 to 34^0C for 72 hrs, 0 and 38^0C for 12 hrs, which is a sufficient time for exploiting it as biological control agent, if planned judiciously.

Keywords: entomopathogenic nematodes, *Steinernema* sp., native species, temperature, infectivity, wax moth

1. Introduction
Entomopathogenic Nematodes (EPNs) are known to control a wide range of insect pest species (Hussaini *et al.*, 2003; Grewal *et al.*, 2004; Bedding 2006; Kulkarni *et al.*, 2008, 2017; Paunikar 2014; Kulkarni 2014,2017) [18, 14, 4, 27, 28, 40, 29, 30] some biotic and abiotic factors limit their use as bioinsecticides (Glazer and Golberg 1989) [12].
Biotic factors adversely affect the survival of EPNs and their symbiotic bacteria include; antibiosis, competition, natural enemies and host susceptibility (Kaya and Koppenhofer 1996; Klien 1990) [23, 25]. On the other hand, abiotic factors include extremes in soil conditions such as temperature, moisture, and texture, as well as ultraviolet light may influence nematode persistence, survival, movements, infectivity and reproduction (Kaya 1990) [24].
Infective juveniles (IJs) of entomopathogenic nematodes have been recovered from the soils of a wide variety of climatic regions (Bedding 2006) [4]. However, factors like atmospheric temperature effect the dispersal, infectivity, reproduction and development of entomopathogenic nematodes, families Steinernematidae and Heterorhabditidae (Molyneux 1985; Kaya 1990; Griffin 1993; Grewal *el al.*, 1994) [39, 24, 16, 15]. It directly influences host searching (Bilgrami and Gaugler, 2007; Susurluk 2008) [5, 47], pathogenicity (Aydin 2005; Pervez *et al.*, 2008) [2, 42] and survival (Ali *et al.*, 2007; Mason and Hominick 1995; Yadav and Lalramliana 2012; Lalramnghaki *et al.*, 2016; Lortkipanidze *et al.*, 2019) [1, 38, 48, 35, 37]. The success of nematode applications for insect control in soil depends on the infective juveniles (IJs) ability to disperse and persist until it can locate a host (Kaya 1990; Levis 2002; Koppenhofer and Fuzi 2007) [24, 36, 26]. The numbers of studies have revealed that IJs of different EPNs differ in their ecological and behavioural traits with regard to their persistence and survival in the soil.
Knowledge of the region from which the nematodes have originally been isolated appears to be very important in determining the temperature range to which it is best-adapted and an understanding of the effects of soil temperature on nematode survival and infectivity helps to improve the accuracy of field applications (Kaya 1990; Grewal *et al.*, 1994) [24, 15]. It is assumed that the EPNs, isolated in a region with a hot climate, can tolerate high temperatures for extended periods (Bedding 2006) [4]. However, species-specific variation in their susceptibilities is required to be investigated for its optimum practical utility.
Keeping in view, effect of temperature on a native population of entomopathogenic nematodes collected, isolated from Madhya Pradesh, Central India) and later identified as new-to-science, *Steinernema dharanaii* n.r. (Kulkarni *et al.*, 2012a) [31] has been investigated.

2. Material and Methods
The population of *Steinernema dharanaii* (TFRIEPN-15) was isolated under the environmental conditions of 28 to 36ºC and relative humidity 40-78%, as existing in nature during the month of June, 2007-2008. The habitat of collection was soil of forest floor of dense teak (*Tectona grandis* L.) plantation. The soil sample collections were made from 10-15 cm depth, baited with the mature last instar larvae of (Bedding and Akhurst, 1975) [3]. The

recovered infective juveniles (IJs) of EPN were multiplied in laboratory *in vivo* on larvae of waxmoth, *Galleria mellonella* reared on modified artificial diet (Kulkarni *et al.*, 2012b) [32]. The freshly emerged IJs of population of new species were used for experimental purpose for the present study.

For investigating the effect of temperature on survival and further infectivity, fresh counted number of infective juveniles (IJs) were exposed to the different ranges of temperature *viz*; 0^0C, 5^0C, 10^0C, 15^0C, 30^0C, 32^0C, 34^0C, 36^0C 38^0C, 40^0C and 45^0C in (5 dia x 1.5 cm depth) Petri dish. The mortality/ survival of the IJs was counted after 2,4,6,8,12,24,48,72,96 hrs experimental temperatures and control maintained in 27^0C±1. The exposed IJs which survival the temperature were tested against early last stage larvae of *Galleria mellonella* for testing their pathogenecity. The experiments were repeated three times, before statistical analysis. The data collected in the above manner were subjected to suitable statistical analysis. The data were transformed suitably in to Arcsin"n Transformation or Square Root Transformation or Log base 10 Transformations for removing the data error, before subjecting to the Analysis of Variance (ANOVA) (Gomez and Gomez, 1984) [13].

3. Results

3.1 Survival and infectivity at temperature 0 ^{0}C to 40 ^{0}C.

The temperatures extremes along with the exposure period exhibited negative effect on survival of the infective juveniles. A reduced survival was recorded in different exposure periods, 24, 48 and 72 in temperature 36^0C and above. After 24 hrs of exposure, significant survival 97 to 100.0% was recorded to except 36^0C, where only 40.79% IJs could survive (P<0.05), as compared to control. While, exposure period of 72 hrs did not show any marked effect in survival in IJs exposed to temperature 10^0C to 34^0C, below 10^0C and above 34^0C exhibited significant mortality (P<0.05) (Table-1).

3.2 Infectivity of *Steinernema dharanaii*, TFRIEPN-15 IJs to waxmoth larvae, survived exposure to different temperature regimes after 72 hrs.

Infectivity of *Steinernema dharanaii*, (TFRIEPN-15) IJs survived post exposure to different temperature indicated temperature of 10^0C to 34^0C did not affect with infectivity. The exposed IJs ranging from 84 to 100.0%. The result at this temperature were at par with 27^0C taken as control set (P>0.05) $F_{(P<0.001)}$ = 150.39, *df*= 25, $SE_{(d)}$± =2.99, $LSD_{(P< 0.005)}$ = 6.12. (Table-2)

3.3 Survival and infectivity at temperature 0^{0}C

The exposure to 0^0C temperature for 2 hrs showed negligible effect on survival (98.32%) Further, increase in the exposure period showed marked decrease in, survival (40.02, 30.87 and 9.90%) respectively at exposure time (4, 6 and 8 hrs). No survival was observed when IJs were exposed to 12 hrs. There was no mortality observed (100.0% survival) in control treatment (27 ^{0}C) ($F_{(P<0.001)}$ = 365.38, *df* = 45, $SE_{(d)}$± = 0.82, *LSD* ($_{P<0.05}$) = 1.65) (Table-3).

The period of exposure affected infectivity potential of the pre-exposed IJs. IJs exposed to 0^0C for 2 hrs were able to infect 100.0 % *G. mellonella* larvae. Thereafter, the percentage of infectivity decreased. The IJs exposed for 4 and 6 hrs infected 80.0% and 32.0%, Further exposure to 8 hrs proved detrimental to the infectivity potential with only 16.0% infectivity to waxmoth larvae. The control treatments showed 100.0% mortality (P<0.05) ($F_{(P<0.001)}$ = 33.00, *df* = 16, $SE_{(d)}$± = 8.46, $LSD_{(P<0.05)}$ = 17.95).

3.4 Survival and infectivity at temperature 38 ^{0}C

The infective juveniles when exposed to 38^0C, allowed 92.33, 88.84, 71.68 and 44.96 respectively at exposure periods of 2, 4, 6, 8 hrs. There was no survival recorded after 12 hrs with 100.0% mortality ($F_{(P<0.001)}$ = 207.90, *df* =45, $SE_{(d)}$± = 0.98, $LSD_{(P<0.05)}$ = 1.98). When IJs exposed to these exposure period were further subjected to infectivity bioassay IJs exposed up to 4 hours exposed IJs caused 100.0% mortality in waxmoth larvae. Further exposure to 6 hrs and 8 hrs had significant reduction in mortalities observed (P<0.05) with 56.0% and 20.0% mortalities, respectively ($F_{(P<0.001)}$ = 86.37, *df* = 16, $SE_{(d)}$± = 4.67, $LSD_{(P<0.05)}$ = 9.91). Since there was no survival of IJs in 12 hr exposure period no IJs were available to test for its infectivity (Table-4).

3.5 Survival and infectivity at temperature 40 ^{0}C

Result showed that the IJs when exposed to extreme temperature of 40^0C, allowed only 31.20 and 8.80% survival respectively at exposure period of 15 and 30 min. There was no survival recorded after 60 min. of exposure as compared to 100 per cent survival in control treatment ($F_{(P<0.001)}$ = 521.27, *df* =27, $SE_{(d)}$± = 0.71, $LSD_{(P<0.05)}$ = 1.47). The IJs surviving at this temperature after exposure upto 30 min. did not show any infectivity against *G. mellonella*, whereas control IJs caused 100% mortality (P<0.05) (Table-5).

Table 1: Survival of Infective Juveniles of *Steinernema dharanaii* (TFRIEPN-15) to exposure to different temperature regimes

Temperature Exposed (in ^{0}C)	Mean Survival of IJs (in %) after exposure to time periods (in hrs.)		
	24 hrs.	48 hrs.	72 hrs.
0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
5	97.20 (81.08)	48.91 (44.37)	0.00 (0.00)
10	100.00 (90.04)	96.23 (79.00)	88.59 (70.29)
15	100.00 (90.04)	98.51 (83.95)	95.24 (78.43)
27	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)
32	100.00 (90.04)	100.00 (90.04)	98.61 (84.15)
34	99.00 (85.60)	95.03 (78.44)	90.28 (71.87)
36	40.79 (39.76)	24.02 (29.33)	17.78 (24.90)
38	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
40	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$F_{(P<0.001)}$	Temperature.		149.37
	Exposure Time		132.59
	Temperature X Exp. Time		48.92
$SE_{(d)}$±	Temperature.		0.484
	Exposure Time		0.265
	Temperature X Exp. Time		0.839

$LSD_{(P<0.05)}$	Temperature.	0.953
	Exposure Time	0.522
	Temperature X Exp. Time	1.651

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

Table 2: Infectivity of (TFRIEPN-15) IJS to waxmoth larvae, survived exposure to different temperature regimes after 72 hrs.

Temperature Exposed (in ^{0}C)	Mean Infectivity of IJs (in %) survives after exposure to 72 hrs.
10	92.00^a (79.36)
15	96.00^a (84.68)
27	100.00^a (90.04)
32	100.00^a (90.04)
34	84.00^b (71.53)
36	28.00^c (31.62)
$F_{(P<0.001)}$	20.28
df	20
$SE_{(d)}\pm$	6.95
$LSD_{(P<0.05)}$	14.51

* Data in parentheses are Arc Sin√ n transformation of percentage values.
[a,b] Values with similar alphabets do not differ significantly with each other (P>0.05).

Table 3: Survival and infectivity of IJs of TFRIEPN-15 on exposure to 0 ^{0}C temperature

Infective juveniles exposure (time in hrs)	Mean Survival (in %)	Mean Infectivity (in %)
2	98.32^b (82.64)	100.00^a (90.04)
4	40.02^c (39.22)	80.00 ^{b} (68.99)
6	30.87^d (33.74)	32.00^c (26.31)
8	9.90^e (18.26)	16.00^c (18.47)
12	0.00^f (0.00)	-
Control (27 ^{0}C)	100.00^a (90.04)	100.00^a (90.04)
$F_{(P<0.001)}$	365.38	33.00
df	45	16
$SE_{(d)}\pm$	0.82	8.46
$LSD_{(P<0.05)}$	1.65	17.95

* 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: Survival and infectivity of IJs, TFRIEPN-15 on exposure to 38 ^{0}C temperature

Infective juveniles exposed time (In hrs.)	Mean Survival (in %)	Mean Infectivity (in %)
2	92. 33^b (74.03)	100. 00^a (90.04)
4	88.84^c (70.54)	100.00^a (90.04)
6	71.68^d (57.89)	56.00^b (48.68)
8	44.96^e (42.09)	20.00^c (23.78)
12	0.00^f (0.00)	-
Distilled water Control (27 ^{0}C)	100.00^a (90.04)	100.00^a (90.04)
$F_{(P<0.001)}$	207.90	86.37
df	45	16
$SE_{(d)}\pm$	0.98	4.67
$LSD_{(P<0.05)}$	1.98	9.91

* 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 5: Survival and infectivity of IJs of TFRIEPN-15 on exposure to 40 ^{0}C.

Time of exposure (in min.)	Mean Survival (in %)	Mean Infectivity (in %)
15	31.20^b (33.92)	0.0^b
30	8.80^c (17.12)	0.0^b
60	0.00^d (0.00)	-
Distilled water Control (27 ^{0}C)	100.00^a (90.04)	100.00^a (90.04)
$F_{(P<0.001)}$	521.27	*NS*
df	27	-
$SE_{(d)}\pm$	0.71	-
$LSD_{(P<0.05)}$	1.47	-

* 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).

4. Discussion

There are no reports available to compare the present results obtained with *Steinernema dharanaii* (TFRIEPN-15) however the significance of temperature conditions on EPN survival and infectivity has earlier been reported (Griffin 1993; Fan and Hominick, 1991; Jagdale, *et al.*, 1998; Karunakar *et al.*, 1999; Saunders and Webster 1999; Hazir *et al.*, 2001 and Cgnolo and Valeria, 2008; Parvez *et al.*, 2015; Kulkarni *et al.*, 2016) [16, 8, 21, 22, 44, 17, 6, 43, 33].

Karunakar *et al.* (1999) [22] studied the effect of eight different temperature levels, 10,12.5, 15, 25, 27.5, 30, 32.5 and 35^{0}C on infection, penetration and multiplication of IJs in *S. feltiae*, *S. glaseri* and *H. indicum* using larvae of *G. mellonella* as host in the laboratory. No host mortality was

observed at 10 and 35^{0}C. Hussaini *et al.* (2005) [19] studied the effect of different temperatures on the survival and infectivity of entomopathogenic nematodes *S. carpocapsae* and *S. tami*. At 35^{0}C of exposure, *S. carpocapsae, S. tami, S. feltiae* and *S. abbasi* did not exhibit significantly mortality up to 10 hours exposure period. The TFRIEPN-15 proves better EPN population, since temperature range of 10 to 34^{0}C did not significantly affect survival as well as infectivity even when exposed for 72 hrs. Hussaini *et al.* (2000) [20] reported that survival of IJs of the nematode species, *S. bicornutum* PDBC 3.2, *S. abbasi* 2.1 and *H. indica* PDBC 13.3 was not affected drastically even up to 6 weeks of storage at 8^{0}C, but in case of *S. bicornutum*, a loss of 50.0% was recorded at 30^{0}C. The virulence of *H. indica* was not significantly impaired even after storage at both the temperatures. The results with the *Steinernema dharanaii* have been better under more or less similar conditions, with slight experimental deviations.

The native population *Steinernema dharanaii* proved superior than the known *Steinernematids* in relation to the infectivity potential under experimented temperature extremes and appears similar to Ganguly and Singh (2001) [9] and Ganguly and Gavas (2004a) [10]. Ganguly and Singh (2001) [9] and Ganguly and Gavas (2004b) [11], who had described a high temperature tolerant new species *S. thermophilum* infected greater wax moth larvae at a temperature range from 10 to 35 ^{0}C and reproduced from 20 to 35 ^{0}C. However, EPN, *Steinernema dharanaii* population appears to be less tolerant to higher temperature than some of the species experimented by Ali *et al.* (2007) [1]. Ali *et al.* (2007) [1] have reported survival and infectivity of three indigenous EPNs, *S. seemae, S. masoodi* and *S. carpocapsae* (Ali strain) at different temperatures (15, 20, 25, 30, 35, 40 and 45^{0}C) against prepupa of *Helicoverpa armigera* (Hübner). They reported that survival of nematodes decreased with increases in temperature, similar in the present investigation. However, 46.6% of the populations were able to survive and tolerate the sub-lethal temperature (45^{0}C) treatment for 6 h. Out of the populations that survived, 43.3% infectivity was observed against *H. armigera* prepupa. Sunanda *et al.*, (2012) [46] reported highest survival of *S. abbasi and H. indica* at 30^{0}C up to 15 days (85.76 % survival in *S. abbasi* and 88.09% in *H. indica*). Further, storage up to 90 days resulted in to 70.22% in *S. abbasi* and 72.71% in *H. indica*.

Pervez *et al.* (2015) [43] studied effect of temperatures and infectivity of *Steinernema* sp. and *Heterorhabditis* sp. against shoot borer (*Conogethes punctiferalis* Guen.). They found that maximum mortality of larvae was found at 30°C followed by 25°C, whereas the least mortality was recorded at 20 and 35 °C. The maximum number of infective juveniles was multiplied at 30 ^{0}C, however minimum multiplication was recorded at 35°C. Among the test EPNs, no multiplication of *Heterorhabditis* sp. (IISR-EPN 01), *Steinernema* sp. (IISR-EPN 03) and *O. gingeri* was recorded at 20°C. IJs attached to larvae of *C. punctiferalis* in higher number after 6 h at 25 and 30 °C, whereas maximum number of IJs penetrated into *C. punctiferalis* larvae at 30 °C. Therefore, the optimal temperature for infection and development for all promising EPNs was 30°C.

Kulkarni *et al.* (2016) [33] reported effect of temperature and soil moisture on horizontal or vertical dispersal by infective juveniles (IJs) of native entomopathogenic isolate, sp. (TFRIEPN-57) from central India. The observations recorded 72 hrs after exposure of IJs to eleven temperature regimes (0, 5, 10, 15, 27, 30, 32, 34, 36, 38 and 40^{0}C) revealed survival of IJs from 10 to 36^{0}C temperature, below and above which, there was 100% mortality. Within the above range, IJs exposed to 10 and 36^{0}C showed 88.59% and 55.41% survival, respectively. The exposure of IJs to 0^{0}C revealed no survival at and above 12 hrs, whereas 5^{0}C allowed survival up to 48 hrs. IJs exposed to temperature extreme of 38^0 C exhibited 38.98% survival up to 8 hrs.

Lalramnghaki and Yadav (2016) [34] investigated the effect of temperature on the infectivity of two entomopathogenic nematodes, viz. *Steinernema* sp. and *Heterorhabditis indica*, locally isolated from Mizoram, northeastern India, using last instar larvae of greater wax moth, *Galleria mellonella*, as an insect host. They observed that temperature play a significant role in infectivity of the two nematodes. No establishment of IJs was observed at 10^{0}C, in addition 15^{0}C in *H. indica*, and 35^{0}C. *Steinernema* sp. appeared to be best adapted to temperatures between 15 and 30°C with an optimum temperature range of 25-30°C, whereas *H. indica* appeared to be adapted to temperatures between 20 and 30°C with an optimum temperature of 30^{0}C.

Sharmila and Subramanian (2016) [45] reported the effect of low temperature on the activity of the entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema glaseri*. They found survival of *H. indica* was significantly greater at the lowest temperature of 10^{0}C conversely survival of *S. glaseri* was significantly greater at a temperature of 5^0 and 10^{0}C. The infectivity of *H. indica* and *S. glaseri* was effective at temperature of 20^0 and 25^0 C (100 % and 100 %, respectively) for *S. glaseri* 10^0, 15^0 and 20^{0}C (74.00%, 100 % and 100 %, respectively). Paschapur *et al.* (2017) [41] have also studied that the mean time taken by the nematode to cause infection in the host insect was significantly less at three temperatures, *viz.*, 30^{0}C, room temperature (25-28^{0}C) and ambient atmospheric temperature (23-34^{0}C) which ranged from 24-30 hours and they were on par with each other and varied significantly with other three test temperatures. At 20^{0}C temperature, the infection occurred after 44 hrs of inoculation, indicating the maximum time required for *H. indica* to cause infection. At the test temperatures of 10^{0}C, and 40^{0}C nematode did not cause the infection to the host due to lethal high and lethal low temperature effects. The results indicated that, the most optimum temperature required for quicker infection was 30^{0}C, ambient atmospheric temperature (23-34^{0}C) and room temperature (25-28^{0}C), which took significantly less time to cause infection.

El Khoury *et al.* (2018) [7] reported seven strains of entomopathogenic nematodes (EPNs) belonging to three species (*Steinernema feltiae, S. ichnusae and Heterorhabditis bacteriophora*) naturally isolated from Mediterranean countries (Southern Italy and Lebanon) were evaluated for their potential to infest greater wax moth (*Galleria mellonella*) larvae at different temperatures under laboratory conditions. The higher mortalities were recorded at 15°C and 20°C. All species failed at lower temperatures except for *S. ichnusae* ItS-SAR4, which caused 7% mortality. At 35°C *S. ichnusae* maintained its infectious activity (24%) along *with H. bacteriophora* ItH-LU1 (38%); both were isolated from Italy and were more efficient at high temperatures than the remaining Lebanese isolate.

Lortkipanidze *et al.* (2019) [37] recorded effect of temperature on the virulence of three species of

entomopathogenic nematodes, *Steinernema thesami* and *Heterorhbatidis bacteriophora* infected *Tenebrio molitor* larvae at the wide temperature range between 8-35°C and 8-32°C, both nematode species infected and killed host insects between 10-33°C and 10-30°C, whereas *S. feltiae* infected host at the narrow temperature between 10-25°C, compare with *S. thesami* and *H. bacteriophora* and caused the highest mortality to larvae at 20°C. There was no significant difference between *S thesami* and *H. bacteriophora* species, significantly was observed between *S. thesami* and *S. feltiae* $P < 0.05$. The temperature ranges for establishment of *S. thesami* and *H. bacteriophora* in insects were between 12-30°C and 15-33°C, however *S. feltiae* between 12- 30 °C. No significant difference was found between *S. thesami* and *S. feltiae* species, difference was observed between *H. bacteriophora* and *S. feltiae* $P < 0.05$.

5. Conclusion

Hence, native species/strains of entomopathogenic nematode, *Steinernema dharanaii* are more tolerance to extreme temperature regimes of the region and for use as biocontrol agents under global concept of the IPM programme against locally target insect pests of forestry and agricultural importance.

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