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Bio-efficacy of entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the Cabbage butterfly (*Pieris brassicae* [L.]) under laboratory conditions

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Abstract

Background: Entomopathogenic nematodes (EPNs) have the potential to supersede larvicidal activity for the management of various insect pests.

Result: Lab experiments were conducted to test the pathogenicity of 2 EPNs local species; *Steinernema feltiae* and *Heterorhabditis bacteriophora* at different (IJs/cm²) concentrations against the cabbage butterfly, *Pieris brassicae* (L.). The native isolate was obtained from soil samples, collected from Rajgarh, Hamachi Pradesh, India. Petri dish bioassay used the EPNs species (*S. feltiae* HR1 and *H. bacteriophora* HR2) at the concentrations (0, 10, 20, 40, 80, 160 IJs/cm²). Based on the pathogenicity of the strains, only 2 isolates effectively showed larvicidal activity. The highest (%) (72.08 and 67.42%), at the 2nd instar larval mortality was recorded in the treatments with *H. bacteriophora* and *S. feltiae* at 160 IJs/cm², respectively. At the 4th instar larvae, respective larval mortality (85.38, 69.50%) was recorded in treatment with *H. bacteriophora*, and *S. feltiae*, respectively, at 160 IJs/cm². In case of pupae, the mortality rates were (62.12, 58.58%) for *H. bacteriophora* and *S. feltiae*, respectively, at 160 IJs/cm²; (74 and 12%) for both the tested EPNs, respectively, at 80 IJs/cm². Percent of *P. brassicae* larval mortality treated with the tested EPN isolates was significantly higher than the untreated control. Results revealed that the percent of larval mortality significantly increased with the increase in time periods, being maximum at 72 h. *S. feltiae* and *H. bacteriophora*, strains showed potent larvicidal activity at low concentration even at 48 and 72 h of exposure.

Conclusion: This study revealed that the local strains of EPNs (*S. feltiae* HR1 and *H. bacteriophora* HR2) were found as a biocontrol agent against *P. brassicae*.

Keywords: Biocontrol, *Pieris brassicae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, Pathogenicity

Background

Cabbage butterfly, *Pieris brassicae* (L.) (Lepidoptera: Pieridae), is one of the major limiting factors of cabbage production in the Himalayas, causing severe losses to the crop by larval feeding on leaves (Mazurkiewicz et al. 2017). Chemical insecticides have been a general practice used by cabbage growers for the management

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of *P. brassicae* in the Himalayas but the adverse effect of chemicals on the environment such as groundwater contamination, pesticide resistance, toxicity hazards and destruction of biodiversity of useful natural enemies demand an effective alternative method of crop insect pest management that should be eco-friendly and safe for non-target organisms. Entomopathogenic nematodes (EPNs) that belong to families, Steinernematidae and Heterorhabditidae are lethal parasites of insect pests, safe to humans, other vertebrates, non-target organisms, easy to apply, and cause no hazardous effect on the environment (Abbas et al. 2021). Besides, they are compatible with many chemical insecticides (Laznik and Trdan 2014).

Entomopathogenic nematodes (EPNs) that belong to the families Heterorhabditidae and Steinernematidae are soil-inhabiting organisms that are obligate insect parasites in nature (Kaya and Gaugler 1993). These nematodes have evolved a mutualistic association with bacteria in the genera *Photorhabdus* is associated with *Heterorhabditis* spp., is carried in the intestine of infective juveniles (IJs) (Arthurs et al. 2004). *Xenorhabdus* is connected with *Steinernema* spp. and confined to a specific vesicle within the intestine of the IJs. Nematodes locate their potential host by following insect cues (Lewis et al. 2006). After IJs locate a host, they infect it through an orifice such as the mouth, anus, or spiracles or by penetrating the cuticle (particularly in *Heterorhabditis* spp.). Once IJs enter the host, they shed their outer cuticle (Sicard et al. 2004) and begin ingesting hemolymph, which triggers the release of symbionts by defecation (in *Steinernema* spp.) or regurgitation (in *Heterorhabditis* spp.) (Grewal et al. 2005). The nematode–bacteria complex kills the host within 24–48 h through septicemia or toxemia (Forst and Clarke 2002). Bacteria recolonize the nematodes, which emerge as IJs from the depleted insect cadaver in search of fresh hosts (Poinar 1990).

More than 100 species of EPNs have been identified globally in which approximately 80% are steinernematid and 13% of these species have been commercialized (Abbas et al. 2021). EPNs have been broadly used in the biological control of a variety of economically important pests occupying different habitats (Grewal et al. 2005). However, EPN formulation to retard desiccation or the addition of adjuvants to increase leaf coverage and persistence of the IJs has enhanced the use of EPNs against foliar pests (Head et al. 2004).

The objective of the present study was to provide fundamental information necessary for the utilization of indigenously isolated EPNs as a biological control agent. The study dealt with 2 nematode species such as *S. feltiae* and *H. bacteriophora* (Poinar) and their pathogenicity against *P. brassicae* under laboratory conditions.

Methods

Rearing of *Pieris brassicae*

Pieris brassicae (L.) eggs, natural infestation, were collected from open fields represent the mid-hill zone of western Himalayas, Department of Entomology Research farm, UHF. The eggs were found in clusters on the lower sides of the leaves of few plants. Collected eggs were brought to the laboratory and placed in a B.O.D (Biological oxygen demand) incubator calibrated at 25 ± 1 °C coupled with 65 + 5% relative humidity and the photoperiod of 12 h L:12 h D. The eggs were maintained under Nematology laboratory at the Department of Entomology, UHF Nauni, Solan, HP, India. After calculating the percent of hatching from eggs, one hundred newly hatched larvae were collected and reared in plastic vials (10 × 12 cm) along with selective food in the form of leaves.

Entomopathogenic nematodes

In this study, the 2 EPNs, *S. feltiae* (HR1) and *H. bacteriophora* (HR2) were used directly in the experiments after isolation. The native isolate was obtained from fruit orchards soil samples, collected from 1682 m above mean sea level with 30° 53' 15 N latitude and 77° 16' 07 E longitude with the mid-hill zone of western Himalayas Rajgarh, Sirmaur district, Himachal Pradesh, India, using *Galleria mellonella* (L.) larvae as nematode baiting traps. The isolates were cultured based on the method (Kaya and Gaugler 1993) at 21 ± 1 °C on the last instar larvae of *G. mellonella*. Infective juveniles (IJs) that emerged during the first 10 days were collected from white traps stored at 4 °C in distilled water for up to 14 days. The nematodes were acclimatized at room temperature for about 30 min before being used in the experiments.

Effect of nematode concentrations

Bioassays were conducted in Petri dishes (9 cm). Each unit was filled by 20 g of sand soil (Table 1). Soil moisture was adjusted to 7% (w/w). IJs were uniformly applied to the soil surface at 0, 5, 10, 20, 40, 80, and 160 IJs/cm² in 1 ml of distilled water. The final soil moisture was 10% (w/w). The containers were then kept at room temperature for 1 h before every instar's 10 *P. brassicae* larvae per container were placed on the soil surface. There were 4 replicates for each concentration. The containers were kept for 72 h under controlled conditions in a growth chamber. Then, the larvae were separated from the substrate by gentle sieving and were individually maintained in controlled conditions until adult emergence. Three days later, 25% of the dead larvae were selected randomly and dissected under a stereomicroscope image analyzing software (Olympus Soft Imaging Solutions) to confirm nematode infection. The experiment was conducted twice.

Table 1 Mortality % of cabbage butterfly, *Pieris brassicae* stages after entomopathogenic nematode treatments

Treatments	Stages	<i>Pieris brassicae</i> mortality (%) after treatments					
		<i>Steinernema feltiae</i>			<i>Heterorhabditis bacteriophora</i>		
		24 h	48 h	72 h	24 h	48 h	72 h
Control	2nd instar larvae	0.00	0.00	0.00	0.00	0.00	0.00
	4th instar	0.00	0.00	0.00	0.00	0.00	0.00
	Pupa	0.00	0.00	0.00	0.00	0.00	0.00
10 IJs/cm ²	2nd instar	17.50	32.50	42.50	20.00	30.00	42.50
	4th instar	22.50	32.50	40.00	32.50	52.50	65.00
	Pupa	20.00	32.50	45.00	22.50	35.00	45.00
20 IJs/cm ²	2nd instar	25.00	37.50	50.00	27.50	40.00	50.00
	4th instar	35.00	45.00	55.00	45.00	67.50	77.50
	Pupa	27.50	37.50	52.50	35.00	47.50	57.50
40 IJs/cm ²	2nd instar	32.50	47.50	62.50	35.00	47.50	57.50
	4th instar	47.50	57.50	67.50	55.00	72.50	85.00
	Pupa	40.00	50.00	60.00	45.00	57.50	70.00
80 IJs/cm ²	2nd instar	37.50	55.00	70.00	42.50	60.00	70.00
	4th instar	55.00	67.50	77.50	67.50	80.00	90.00
	Pupa	47.50	62.50	72.50	52.50	65.00	77.50
160 IJs/cm ²	2nd instar	52.50	67.50	85.00	50.00	72.50	87.50
	4th instar	65.00	80.00	87.50	77.50	90.00	97.50
	Pupa	55.00	75.00	90.00	60.00	77.50	90.00
LSD	2nd instar	4.64	4.17	6.44	5.80	7.24	10.03
(<i>p</i> < 0.05)	4th instar	5.35	6.03	6.05	4.37	8.46	9.80
	Pupa	5.80	5.69	8.78	4.95	4.39	8.54

Larvicidal activity

Each nematode species was added at different concentrations (1.00, 1.30, 1.60, 1.90, 2.20 IJs/cm²) into the 9 cm Petri dish in triplicate with 2 ml of dechlorinated sterile water and 10 larvae of tested *P. brassicae* strains. The 2nd and 4th larval instars were provided by young age (vegetative stage) cabbage leaves. One Petri plate without EPNs suspension was used as a control. After 24, 48, and 72 h the number of dead larvae was calculated. The strains that killed more than 50% of the larvae were considered pathogenic. Both nematode isolates were examined quantitatively for larvicidal activity against *P. brassicae*, using various concentrations of EPNs' suspensions. The infected larvae were observed under a stereo zoom microscope for each concentration at 72 h exposure time.

Statistical analysis

Insect mortality was control-corrected (Abbott 1925) and Arcsine transformed when required to meet assumptions of normality and homogeneity of variances. In all experiments, control-corrected mortality was subjected to one-factor analysis of variance (ANOVA)

Corrected mortality (%)

$$= \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in the control}} \times 100$$

The corrected percent mortality data thus obtained for different concentrations of *P. brassicae* (L.) at different concentrations were subjected to probit analysis as per the method given by Finney 1971. Concentration-mortality response data was conducted. Also, LSD (*P* < 0.05) values were calculated to differentiate means among treatments.

Results

Bioassay of *P. brassicae*

Data of the efficacy of EPNs for the control of larvae *P. brassicae* are presented in Table 1. Percent of *P. brassicae* larval mortality treated with the tested EPN isolates was significantly higher than the untreated control. Results revealed that the percent of larval mortality significantly increased with the increase in time periods where it being maximum at 72 h. and followed at 48 and then 24 h. The highest per cent of larval mortality was recorded on the 2nd instar larvae for treatments

with *H. bacteriophora* (72.08%) and *S. feltiae* (67.42%) at 160 IJs/cm², followed by those at 80 IJs/cm² (56.92%) same results for both EPNs, while the larval mortality % by *H. bacteriophora* at 40 IJs/cm² was (49.37%) and by *S. feltiae* at 160 IJs/cm² was (52.31%) (Fig. 1). The

followed results were recorded on the 4th instar larval mortality at treatment with *H. bacteriophora* (85.38%) and then by *S. feltiae* (69.50%) at 160 IJs/cm² (Fig. 2). Pupal mortality was (74.12%) by *H. bacteriophora* and (74.12%) by *S. feltiae* at 160 IJs/cm². But at 80 IJs/cm²,

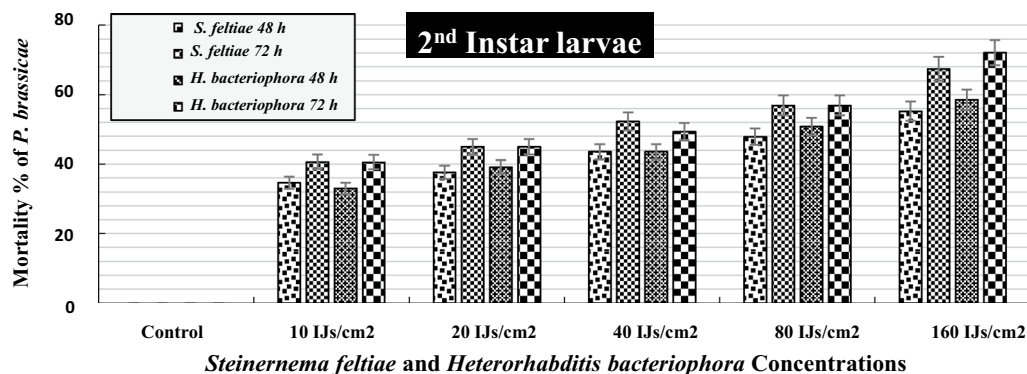


Fig. 1 Efficacy of *Steinernema feltiae* and *Heterorhabditis bacteriophora* against 2nd instar *P. brassicae* larvae

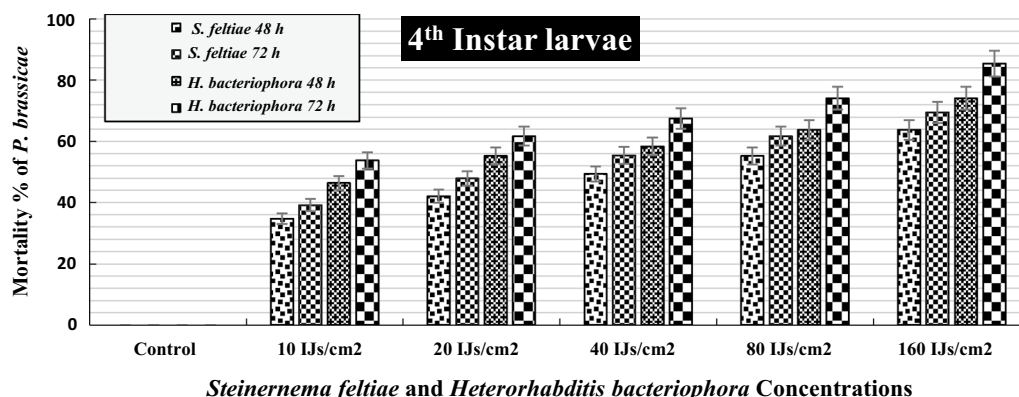


Fig. 2 Efficacy of *Steinernema feltiae* and *Heterorhabditis bacteriophora* against 4th instar *P. brassicae* larvae

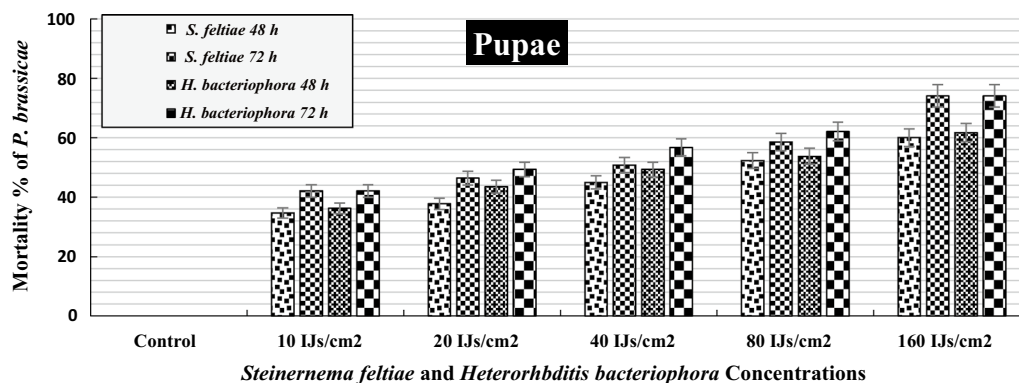


Fig. 3 Efficacy of *Steinernema feltiae* and *Heterorhabditis bacteriophora* against *P. brassicae* pupae

the larval mortality rates were (62.12%) and (58.58%) by *H. bacteriophora* and *S. feltiae*, respectively (Fig. 3). There was no larval mortality observed in the untreated control.

After 48 h, the treatment showed the highest percent of larval mortality (58.58%) at the 2nd instar for *H. bacteriophora* and (55.26%) for *S. feltiae* at 160 IJs/cm², followed by *H. bacteriophora* and *S. feltiae* at 80 IJs/cm² (50.81 and 47.86%, respectively). *H. bacteriophora* and *S. feltiae* caused mortality rate of (43.54%) for both at 40 IJs/cm² (Fig. 1). The treatments of the 4th instar larval mortality were (74.12%) for *H. bacteriophora*, followed by (63.78%) for *S. feltiae* at 160 IJs/cm² (Fig. 2). Pupal mortality was (63.78%) for *H. bacteriophora* and (55.26%) for *S. feltiae* at 80 IJs/cm². The efficacy of EPNs on pupal mortality recorded for *H. bacteriophora* (61.74%), and (60.08%) for *S. feltiae* at 160 IJs/cm². *H. bacteriophora* and *S. feltiae* caused (53.75%) and (52.31%) for larval mortality, respectively, at 80 IJs/cm². There was no larval mortality observed in the untreated control (Fig. 3).

After 24 h., the results showed that the highest mortality percent of the 2nd instar larvae was (46.42%) and (44.98%) that recorded in treatments with *S. feltiae* and, *H. bacteriophora*, respectively, at 160 IJs/cm², followed by those at 80 IJs/cm² (40.65 and 37.71%, respectively), and then at 40 IJs/cm² (36.20 and 34.70%, respectively). The next best result was (61.74%) for the mortality of 4th instar larvae by *H. bacteriophora* at 160 IJs/cm², followed by (53.75%) for *S. feltiae* at the same concentration. Pupal mortality was (50.81%) and (47.86%) by *H. bacteriophora* and *S. feltiae*, respectively, at 160 IJs/cm², but (46.12%)

and (43.54%) for EPNs at 80 IJs/cm². There was no larval mortality observed in the untreated control. Non-highly significant differences existed among the remaining treatments.

Bioassay of Log probit analysis larvicidal activity

The virulence was conducted to estimate the lethal concentrations of EPNs to different larval instars of *P. brassicae*. The data reflecting the efficacy of the two isolates of *S. feltiae* (HR1) and *H. bacteriophora* (HR2) against 2nd, 4th larval instars and pupae of *P. brassicae* are summarized in (Table 2). EPN was applied at 10, 20, 40, 80 and 160 IJs/cm², caused *P. brassicae* are recorded when the EPNs (at LC₅₀ level) were applied at 48 and 72 h after EPN treatment either LC₉₀ level (Table 2). An effect was observed when EPNs was applied at LC₉₀ in the best larvicidal activity was obtained during the 72 h of exposure. *S. feltiae* (HR1) and followed by *H. bacteriophora* (HR2) EPN strains showed potent larvicidal activity at low concentration even at 48 and 72 h of exposure (Table 2).

Discussion

Overall results on efficacy of the tested EPNs indicated that treatment of 4th instar larvae by *H. bacteriophora* at 160 IJs/cm² (85.38%) was found to be a superior than other treatments. However, treatment with *S. feltiae* at 160 IJs/cm² was found to be the next effective treatment. Hence, the results of the present study corroborate the finding of Sharma et al. (2018) who reported that *P. brassicae* caused larval net mortality of 86.2 and 66.5% under laboratory conditions, respectively. Similar results were also reported by (Askary and Ahmad, 2020).

Table 2 Log probit analysis of larval and pupal activity of cabbage butterfly, *Pieris brassicae* treated with entomopathogenic nematodes

Nematode species	Stages	Exposure time (hrs)	LC ₅₀ (IJs/cm ²) 95% LCI-UCL	LC ₉₀ (IJs/cm ²) 95% LCI-UCL	Intercept	Slope ± SE	χ ² value	P value
<i>Steinernema feltiae</i>	2nd instar larvae	48	46.96 (22.88–82.03)	69.06 (39.53–88.92)	−1.25	1.3 ± 0.22	0.57	0.005**
		72	23.02 (14.74–35.12)	51.62 (33.06–80.10)	−1.21	1.5 ± 0.22	1.54	0.006**
	4th instar larvae	48	35.47 (23.60–53.32)	79.91 (49.98–94.25)	−1.50	01 ± 0.23	0.16	0.003**
		72	22.02 (14.94–32.46)	59.58 (40.42–87.82)	−1.37	1.3 ± 0.23	0.11	0.004**
	Pupae	48	36.18 (23.28–56.24)	80.22 (51.61–97.08)	−1.49	1.5 ± 0.22	0.99	0.003**
		72	20.86 (13.76–29.93)	73.54 (48.51–94.49)	−1.28	01 ± 0.23	4.92	0.005**
<i>Heterorhabditis bacteriophora</i>	2nd instar larvae	48	39.94 (25.15–63.44)	75.72 (47.67–89.12)	−1.46	1.5 ± 0.22	0.47	0.003**
		72	20.38 (13.36–31.08)	48.82 (32.01–74.46)	−1.29	02 ± 0.23	2.87	0.005**
	4th instar larvae	48	29.66 (18.16–48.44)	78.70 (48.19–97.92)	−0.83	1.5 ± 0.23	1.15	0.024*
		72	22.11 (14.10–34.67)	49.84 (31.78–78.16)	−0.76	01 ± 0.26	1.25	0.046*
	Pupae	48	32.99 (20.63–56.76)	73.12 (45.72–95.20)	−1.27	1.5 ± 0.22	0.40	0.005**
		72	21.69 (14.46–32.52)	55.26 (36.85–82.86)	−1.24	1.5 ± 0.23	0.83	0.006**

EPNs suspensions were administered at a concentration of (IJs/cm²)

Lethal Concentrations are calculated by Probit Methods

Mantoo and Zaki (2014) obtained results confirm the findings of an increase in inoculum level of IJs, time consumed for larval mortality decreased but when the larval size increased the time consumed in larval mortality also increased, it was also shown that the EPNs could be an effective alternative to insecticides. Controlling insect pests with a foliar application is becoming a more widely-used practice Laznik et al. (2010).

The mortality was determined through different concentrations after 48 and 72 h exposure. The mortality rate depends on the concentration and exposure time. However, the highest mortality range was observed at *H. bacteriophora* treatment at very low concentrations after 48 and 72 h. Even though *S. feltiae* showed a slow mortality in 48 and 72 h of exposure time, they restrained the larval development at the early pupal stage. The results are similar to the findings of EPNs' pathogenicity of *H. bacteriophora* against vine mealy bug, in South African vineyards Sabry et al. (2016). The data revealed that the mortality rate increased with the increase in time intervals viz., 24, 48, and 72 h.

Obtained results confirm the findings of Mantoo and Zaki (2014) who observed that with the increase in inoculum level of IJs, time consumed for larval mortality decreased but when the larval size increased the time consumed in larval mortality also increased. Similar findings were also reported by other research workers Askary and Ahmad (2020) and Abbas et al. (2021).

Conclusions

The efficacy of local indigenous EPN isolates was significantly superior to that of the exotic species. High EPN efficacy obtained under laboratory conditions cannot easily be extrapolated to field efficacy; therefore, future field experiments are justified to fully determine the potential of local EPN isolates against *P. brassicae* in Himachal Pradesh and Indian, conditions.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; B.O.D: Biological oxygen demand; OPSTAT: Operational Status; cm²: Centimeter square; h: Hour; LSD: Least significant difference.

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Authors' contributions

All authors jointly designed the experiment. KI conducted the laboratory bioassays, performed data analysis and drafted the manuscript with inputs from all authors. MS, MW, MK, MW, DA and HG collaborated closely with KI in the whole process especially during data analysis. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate.

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Consent for publication

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Competing interests

We, the authors do not have competing interests.

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