



## Effect of Temperatures on the Pathogenicity of Entomopathogenic Nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) for Biocontrol of *Octodonta nipae* (Maulik)

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### Abstract

In the 21st century climate change has been recognized globally as the most impending, and critical issue that affects all animals including insects. In particular, temperature plays an important role in the development, reproduction, host searching, survival, pathogenicity, sex ratio and insect death. The Nipa palm hispid, *Octodonta nipae* (Maulik) is an important invasive pest of palm trees in Southern China. Knowledge of how this beetle can be controlled with entomopathogenic nematodes under different environmental temperature is scarce. Therefore, the aims of this study were to test the efficacy of two entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) at different development temperatures. The pathogenicity was tested at concentration of 100 IJs larva<sup>-1</sup> and treatment are kept at four temperatures of 20, 25, 30 and 36°C, 80 ± 5% RH. The results showed that, both nematodes species caused larval mortalities at all the tested temperatures except at 36°C. The highest larval mortalities of 85.3% and 40.6% were obtained at 30°C for both *S. carpocapsae* and *H. bacteriophora* respectively. Furthermore, both nematodes penetrated *O. nipae* larvae at all temperature conditions except at 36°C. Similarly, the highest penetrations of nematodes infective juveniles were recorded at 30°C for *S. carpocapsae* and *H. bacteriophora*. The study demonstrated that, warmer temperature enhanced the pathogenicity of nematodes, which in turns trade well with the unprecedented increase in environmental temperature under climate change, for integrated management of this beetle.

**Keywords:** Biocontrol; *Heterorhabditis bacteriophora*; *Octodonta nipae*; Penetration; *Steinernema carpocapsae*; Temperature.

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## INTRODUCTION

Nipa palm hispid beetle, *Octodonta nipae* (Maulik) (Coleoptera Chrysomelidae: Cassidinae), is an important pest of palm trees, that causes great economic loss by attacking leaf fronds of host trees. This is done by adults and young larvae; which are the most destructive stages of this pest (Hou *et al.*, 2014). These however resulted in shrinkage, curling, and in some situations death of the young leaves (Hou *et al.*, 2010; Tang and Hou, 2017). *O. nipae* is native to Malaysia, and was first reported in 2001 in Hainan island, China (Sun *et al.*, 2003) where it damaged California fan palm *Washingtonia filifera* (Linden ex André) H. Wendl. The main control method of this pest is the use of insecticides, which are usually inefficient. Because both adults and larvae lives together within the tight central leaves of the palm, thus, escaped from the exposure of the insecticide (Vassiliou *et al.*, 2011). However, *Tetrastichus brontispae*; an endoparasitoid of *O. nipae* was used for biocontrol of pupae (Howard *et al.*, 2001). Entomopathogenic fungus *Metarrhizium anisopliae* has also been reported as biocontrol agent of this pest (Xin *et al.*, 2009; Chen *et al.*, 2010; Nguyen *et al.*, 2012; Xu *et al.*, 2012). As part of integrated pest management, the use of suitable, cost effective and environmentally friendly methods for this pest is required.

Entomopathogenic nematodes (EPNs) are biological control agents for different economically important agricultural pests from laboratory to field levels (Lacey *et al.*, 2015). The most widely used EPNs are from the families' Steinernematidae and Heterorhabditidae, because of their ability to actively search for their hosts (Sanda *et al.*, 2018), they are recently shown to be effective for the control for leaf feeding insects (Labaude and Griffin, 2018). The

EPNs are reported to be used for control of quarantine leaf eating caterpillars and other insect pests on many crops such as adult western corn rootworm *Diabrotica virgifera* LeConte (Lavine and Strand, 2002), flea beetles (*Phyllotreta* spp.) (Yan *et al.*, 2013) and the Colorado potato beetle *Leptinotarsa decemlineata* found in leaf surfaces (Trdan *et al.*, 2009).

Temperature is one of the abiotic factors that play important roles in affecting the infectivity, time of death, development, reproduction and storage of EPNs (Susurluk 2008). However, temperature tolerance of EPNs is different between species and strains (Koppenhöfer *et al.*, 2000). Thus, EPNs isolates from specific regions have considerable variations in their abilities to infect the host, kill it and reproduce within it are all influenced by temperature characteristics of their original habitats (Mahar *et al.*, 2007). However, information of how this insect interacts with entomopathogenic nematodes and surrounding temperature especially due to climate change is needed. Thus, the study therefore, evaluated the pathogenicity and penetrations of two EPN species, *S. carpocapsae* All (Weiser) and *H. bacteriophora* H06 (Poinar), against third instar larvae of *O. nipae* in the laboratory at different developmental temperatures.

## MATERIALS AND METHODS

### Insects and Nematodes cultures

Adults of *O. nipae* were collected and reared with small piece of fortunes windmill palm, *Trachycarpus fortunei* (Hook) in the laboratory as reported by Sanda, *et al.*, (2018). The insects were maintained at  $25 \pm 1^\circ\text{C}$ ,  $80 \pm 5\%$  RH, and photoperiod of 12: 12 (Light: Dark) (Sanda *et al.*, 2019). *S. carpocapsae* All (Weiser) and *H. bacteriophora* H06 (Poinar) were obtained from Guangdong Institute of Applied

Biological Resources, China. Nematodes were cultured using the last instar larvae of greater wax moth, *Galleria mellonella*. Infective juveniles (IJs) were stored in distilled water at 13°C and were used in all experiments within 15 days of emergence from the host.

### **Effects of temperature on Mortality of *O. nipae* larvae**

Bioassays were conducted to determine the pathogenicity of *S. carpocapsae* All (Weiser) and *H. Bacteriophora* H06 (Poinar) at 100 IJs larvae<sup>-1</sup> concentration for the control of *O. nipae* larvae in 96-well plates (Costar®, Corning Incorporated Corning, New York 14831, USA) as described by Sanda *et al* (2018). Briefly, thirty larvae of *O. nipae* were placed individually in each well plate containing 1×2 cm of tissue paper with small piece of fortunes windmill palm, *T. fortunei* (Hook) (35, 1). IJs were diluted to 10 µl and applied to each well containing one larva. To prevent escape of the larvae, the micro titer plates were covered with the lid and kept at 20, 25, 30 and 36°C, 80 ± 5% RH, and a photoperiod of 12: 12 (Light: Dark) in incubators. For the control experiment, 10µl tap water was used. Larval mortality was recorded at 24 and 48 hours after treatments (HAT) and the experiments were run in triplicate to confirm the results.

### **Effects of temperatures on IJs penetration**

Bioassays were prepared as described above, to assess the penetration of the EPNs into the host larvae. The treatments were transferred to incubators sets at 20, 25, 30 and 36°C, 80 ± 5% RH after treating each well containing single larva with 10 µl IJs suspension. After 72 hours, ten dead larvae (cadavers) were picked from the well plates, rinsed with distilled water and dissected under dissecting Stereomicroscope. The number of penetrated

IJs were counted and recorded. The experiments were run in triplicate to confirm the results.

### **Statistical Analysis**

All statistical analyses were performed using IBM SPSS Statistics version 22 (IBM Corporation, New York, 10504-1722, United States) (SPSS, RRID: SCR\_002865). Data were corrected for control mortality using Abbott formula (1925) and percentage data were square-root transformed to meet assumptions of normality and homogeneity of variances. The percentage corrected cumulative mortalities data, Penetration of EPN on *O. nipae* larvae data were analyzed by one way Analysis of variance (ANOVA). When ANOVA showed a significance effect ( $P < 0.05$ ), means were compared or separated using least significance differences (LSD) at  $\alpha.5$ .

## **RESULTS**

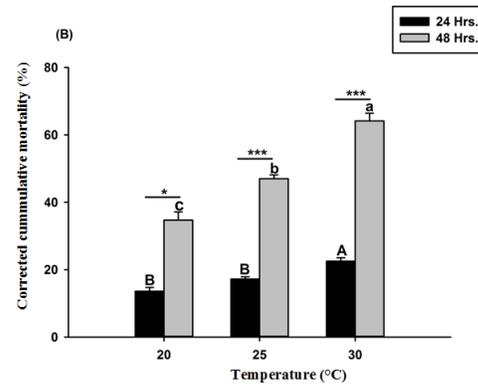
### **Effects of different temperatures on mortalities of *O. nipae***

The results revealed that, both nematodes species caused larval mortalities at all the tested temperatures except at 36°C treatments throughout the experiments. Therefore, the results were excluded from data analysis at 36°C. The results of the percentage cumulative mortality are presented in Figure 1 for *S. carpocapsae* and *H. bacteriophora*. There were significant differences among the three temperature conditions in mortality of third instar larvae caused by *S. carpocapsae* at 24 h ( $F_{2, 6} = 23.32$ ,  $P < 0.001$ ) and 48 h ( $F_{2, 6} = 39.27$ ,  $P < 0.001$ ) after treatment. Similarly, there were significant differences among the three temperature conditions in mortality of third instar larvae infected by *H. bacteriophora* at 24 h ( $F_{2, 6} = 21.13$ ,  $P < 0.001$ ) and 48 h ( $F_{2, 6} = 29.96$ ,  $P < 0.001$ ) after treatment. However,

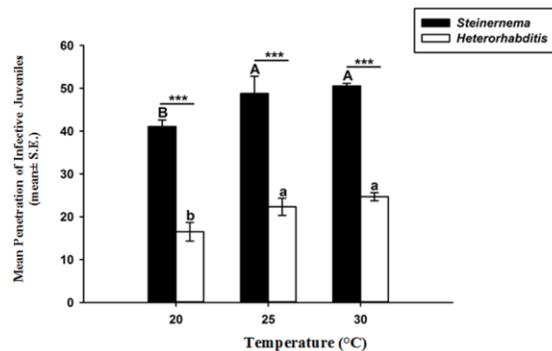
differences exist in the mortality of third instar larvae between the *S. carpocapsae* and *H. bacteriophora* treatments at both 24 h and 48 h after treatments. At 24 h, the *S. carpocapsae* and *H. bacteriophora* treatments differs significantly when conditioned at 20 °C ( $t_4 = 14.86$ ,  $P = 0.001$ ), 25 °C ( $t_4 = 18.65$ ,  $P = 0.001$ ) and 30 °C ( $t_4 = 37.26$ ,  $P = 0.001$ ) temperatures. In the same vein, the *S. carpocapsae* and *H. bacteriophora* treatments differs significantly at 20 °C ( $t_4 = 2.76$ ,  $P = 0.001$ ), 25 °C ( $t_4 = 5.85$ ,  $P = 0.001$ ) and 30 °C ( $t_4 = 16.36$ ,  $P = 0.005$ ) temperatures conditions 48 h after treatments. At 24 hours after inoculation (Figure 1A) the highest mortalities of  $85.3 \pm 6.9\%$  were recorded at 30°C for the *S. carpocapsae* treatment. Similarly, in the *H. bacteriophora*, the percentage larval mortalities of *O. nipae* were less than that recorded for *S. carpocapsae* treatments at all-time points (Figure 1 B). However, the highest mortalities of  $64.14 \pm 2.5\%$  were recorded at 30°C followed by 25°C ( $47.01 \pm 1.8\%$ ) at 48 h after *H. bacteriophora* treatments.

### Effects of different temperatures on IJs penetration

Temperatures had significant effects on the mean penetration of IJs for the *S. carpocapsae* ( $F_{2,6}=2.215$ ;  $P<0.008$ ) and *H. bacteriophora* ( $F_{2,6}=16.7$ ;  $P<0.004$ ) as presented in Figure 2. However, significant differences exist between *S. carpocapsae* and *H. bacteriophora* IJs penetration at 20°C ( $t_4 = 16.14$ ,  $P = 0.001$ ), 25 °C ( $t_4 = 10.25$ ,  $P = 0.001$ ) and 30 °C ( $t_4 = 40.11$ ,  $P = 0.001$ ). The highest mean penetrations rates of IJs of  $79.67 \pm 3.70$  and  $22.49 \pm 1.79$  IJs/larva were recorded at 30°C for *S. carpocapsae* and *H. bacteriophora* respectively.



**Figure 1:** Cumulative mortality of third instars larvae of *Octodonta nipae* at different temperature of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. (A) *Steinernema carpocapsae* at 24 and 48 hours after treatment (B) *Heterorhabditis bacteriophora* at 24 and 48 hours after treatment. Means with the same letter above bars are not significantly different using One-way ANOVA followed by least significance differences (LSD). Means shown by different letters are significantly different at 0.05%



**Figure 2:** Mean penetration of infective juveniles of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* at different temperatures into third instars of *Octodonta nipae*. Means with the same letter above bars are not significantly different using One-way ANOVA followed by least significance differences (LSD). Means shown by different letters are significantly different at 0.05%

## DISCUSSION

The host pathogen interactions can be affected by temperature as reported in nematodes and other agents such as fungi, viruses, bacteria and parasitoid (Menti *et al.*, 2000; Thomas and Blanford, 2003; Mahar *et al.*, 2007). This can either be directly or indirectly by triggering host defense mechanism. The results of this study indicated that, applications of two EPNs of *S. carpocapsae* and *H. bacteriophora* for the control of *O. nipae* larvae resulted in the higher larval percentage mortalities at 30°C. For Steinernematid species, different authors reported results with higher mortalities of insect hosts at higher temperatures. Lalramnghaki *et al.* (2016) reported *Steinernema* spp caused 100% percentage insects mortalities at 30°C. Similarly, 100% mortalities of *G. mellonella* and *Agrotis ipsilon* larvae caused by *S. carpocapsae* were reported by Bussaman *et al.*, (2009) at temperature range of 25 and 32°C. Maximum mortalities of *Conogethes punctiferalis* were reported for *Steinernema* spp and *Heterorhabditis* spp at 30°C (Miduturi *et al.*, 1994).

Additionally, maximum percentage mortality of 86.7% and 80.0% for *S. carpocapsae* and *Heterorhabditis* spp respectively at 31°C were reported for *Ceratitis capitata* larvae. Grewal *et al.* (1993) reported higher efficiency of *S. carpocapsae* at higher temperatures of 30°C and above (Tarasco *et al.*, 2015). This trend is unique to Steinernematids, as for most of the experiments using *H. bacteriophora* similar trends of higher percentage mortality were reported at higher temperatures Yadav (2016). It was speculated that, the higher mortalities of these EPNs might be traced to their origins, which is believed to be characterized by mild winter and hot summer regions (Shapiro-Ilan *et al.*, 2012). This study

also gains support from Mahar *et al.* (2007) reported higher mortalities of *P. cochleariae* larvae at 30°C by *H. bacteriophora*. Similarly, 98% *G. mellonella* larval mortality was highest by *H. bacteriophora* than other EPNs used in the experiment (Linder *et al.*, 2008; Lee *et al.*, 2016).

In our experiment IJs penetrations of *S. carpocapsae* and *H. bacteriophora* were also maximums at 30°C among the tested temperatures. Similarly according to Rashid, *et al.* (2015), higher IJs penetrations into *C. punctiferalis* larvae were reported for *Steinernema* spp and *Heterorhabditis* spp at 30°C (Linder *et al.*, 2008; Lee *et al.*, 2016). In addition to these, Mahar, *et al.* (2007) reported higher penetrations of *H. bacteriophora* and *H. indica* at 30°C in *Phaedon cochleariae* and contrarily higher penetration of *S. carpocapsae* and *S. feltiae* at 25°C. In similar host larvae, a UK strain of *S. carpocapsae* showed higher number of IJs during infectivity test at temperature range of 25-26°C (Rohde *et al.*, 2010). Maximum infections of *S. riobrave* were observed at 28.5°C against *Pectinophora gossypiella*, this is more or less similar to our finding (Shapiro-Ilan *et al.*, 2012). To sum it all, our result is in line with reports that, changes in temperature not only change the immune response of the insects, but also the degree of pathogen infectivity (Mahar *et al.*, 2007).

## CONCLUSION

Overall, in this study, our results revealed that, the warmer the temperature, the more the mortality of *O. nipae* larvae. Molecular and physiological data are also needed to find out why warmer temperature favoured infectivity of nematodes and makes *O. nipae* larvae susceptible at this condition. This study demonstrated the possibility of using EPNs against *O. nipae* in integrated

management programs in combination with other control methods under field conditions.

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