# Pathogenicity of Three Entomopathogenic Nematodes to the Peach Fruit Fly, *Bacterocera zonata* (Saunders) and the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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## ABSTRACT

Pathogenicity of *Heterorhabditis bacteriophora* (native isolate), the foreign isolate HP88, and *Steinernema carpocapsae* All strain to Peach fruit fly, *Bactrocera zonata* (Saund.) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) was measured through their virulence, invasion and production rates. *H. bacteriophora* (native isolate) was more virulent than HP88 and *S. carpocapsae* to both insects. The same isolate was also faster in penetrating the host's body and its production rate was higher than the other two isolates.

**Key Words:** *Heterorhabditis bacteriophora* (native strain), HP88, *Steinernema carpocapsae* All strain, *Bactrocera zonata, Ceratitis capitata*

## INTRODUCTION

The Peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) are key pests of fruits in Egypt. Beside use of traps and chemical control methods, no other means are currently registered for the control of these pests in organic orchards. Hence, high infestation level can often be observed and severe yield loss can be an undesirable result. Entomopathogenic nematodes heterorhabditids and steinernematids containing mutualistic bacteria are lethal to many insect pests. They possess impressive attributes for the biological control of many soil-inhabiting insects in addition to their high virulence, ease of culture and application and high safety level (Gaugler, 2002).

Previous studies had demonstrated the susceptibility of different species of fruit flies (Tephritidae) to several strains of entomopathogenic nematodes (Lindegren and Vail, 1986; Lindegren *et al.*, 1990; Soliman, 2002; Toledo *et al.* 2001, 2005, 2006 and Hertz et al. 2007). Virulence, invasion and production rates of a nematode isolate are important criteria to measure the potential of such a nematode isolate for controlling the target pest. Shapiro *et al,* (2002) noted that if nematode did not possess a high level of virulence towards the target pest, there was a little hope of success. It was then suggested that invasion rate could serve as a criterion to measure the infectivity of different nematode isolate. Also, production rate (infective juveniles' recovery from the host) serve as a measure of success and suitability of used nematode species and or isolate against the target pest and increasing nematode population in soil may help in decreasing the target pest population along the time of control.

The aim of the present work is to measure the potential of *Heterorhabditis bacteriophora* (native isolate), HP88, and *Steinernema carpocapsae* All strain as bio-control agents against *B. zonata* and *C. capitata* larvae, particularly when they popped inside the soil for pupation expressed in determining their virulence, invasion and production rates.

## MATERIALS AND METHODS

### Insects and nematodes sources

*B. zonata* and *C. capitata* were obtained from the Insect Horticulture Department, Plant Protection Research Institute (PPRI), Dokki, Giza, Egypt. Both insects were reared using artificial diet for larval development according to the technique of Tanaka *et al.* (1969). The nematodes; *H. bacteriophora* (NS), HP88 and *S. carpocapsae* All strain were reared and multiplied using the Greater wax moth larvae, *Galleria mellonella* L. according to the method described by Kaya and Stock, (1997)

### Virulence

Nematode-host exposures were carried out in Petri dishes (5 cm) containing 15 gm of moistened sand. 5, 10, 15, 20, 25, 50, 75, 100, and 125 IJs /larva were distributed randomly on sand surface (final moisture reached 10% after adding IJs). Ten full-grown larvae of each insect species were introduced to nematodes. Each IJs concentration was replicated 5 times for each insect species. A parallel control experiment was carried out at the same time. All Petri dishes were kept at 25 ±°C and 65 ~ 70 RH. Mortality of exposed larvae was recorded after10 days (as the adult emerges from pupae after 9 days).

### Invasion rate

Invasion rate was determined by measuring the

effect of exposing insect larvae to IJs for 0.5, 1, 1.5, 2, 4, 6 and 8 hours on the level of insect mortality. Petri dishes measured 3.5 cm contained 9 gm of moistened sand were used for this purpose. Fifty IJs were randomly distributed on the sand surface. A single larva was introduced the IJs in each Petri dish and left for the previously mentioned time periods. Larvae were removed, washed with tap water to get rid of any attached nematode on their body surface and kept for 48 hrs in nematode free dishes. Each treatment was replicated 10 times for each insect species. Dead larvae were counted percentages of mortality were calculated.

**Production rate**

Production rate was measured by the mean number of juveniles produced from host cadaver. Petri dishes (5 cm) contained 15 gm of moistened sand acted as the nematode-host interaction stage. 25, 50 and 100 IJs/larva concentrations were distributed on sand surface, and then 10 fresh collected larvae were transferred to the infected sand. 48 hours later, every single larva was removed and washed using tap water and transferred to white-trap for the produced juveniles' extraction. Daily produced juveniles were harvested and counted till the end of production (no juveniles produced). Every nematode-host interaction was replicated 5 times for each insect species.

**Data analysis**

Data was subjected to analysis of variance (ANOVA) (SAS Institute, 1996). In the virulence test, all mortality values were corrected using Abbot's formula. LT50 and LT90 were calculated using Probit analysis.

## RESULTS AND DISCUSSION

All nematode strains showed increased mortality in parallel with increasing concentrations ($P<0.05$). Also, *C. capitata* displayed high sensitivity to nematode infection by the native isolate of H. *bacteriophora* ($F= 2.01$; $df=8$; $P<0.05$), HP88 ($F= 3.83$; $df = 8$ ; $P<0.05$) and *S. carpocapsae* ($F= 14.17$; $df = 8$; $P<0.05$).The native *H. bacteriophora* isolate was the most virulent isolate to both insects ($F=3.52$; $df= 8$; $P<0.05$) Table (1). *H. bacteriophora* (NS) was the fastest in penetrating the host's body ($F=2.81$; $df = 6$; $P<0.05$), followed by HP88 ($F=5.91$; $df = 6$; $P< 0.05$) while *S. carpocapsae* All strain was the slowest ($F=12.4$; $df=6$; $P<0.05$). LT50 and LT90 values of *H. bacteriophora* (NS) were the lowest while those of *S. carpocapsae* All strain were the highest, Table (2). Production rate of the three nematode strains at different concentrations for both insects showed significant differences among them. Also, production period of *B. zonata* was twice more than *C. capitata* (4 days and 2 days, respectively). The results showed that infective juveniles production increased by increasing concentrations Table (3). Emergence of IJs from the cadavers started 12 and 14days post infection from B. *zonata* and C. *capitata*, respectively. The total number of IJs recovered was considerably different in the two insects.

The main objective of the present work was to explore the potential of *H. bacteriophora* (NS), HP88 and *S. carpocapsae* All strain as bio-control agents expressed in determination of their virulence, invasion rate and reproduction ability inside *B. zonata* and *C. capitata*. It could be concluded that *H. bacteriophora* (NS) strain is the best candidate for control of both insects as it caused mortality to target pests. The high virulence of *H. bacteriophora* native isolates to the tested insects unclear but may be able to be attributed to the rapid penetration in or rapid bacterial growth inside host's body. Toledo *et al.* (2005) used *H. bacteriophora* strain and *S. feltiae* against *Anastrepha ludens* (Diptera: Tephritidae) and mentioned that the first caused high pathogenicity than the second. Previously, Campbell *et al.* (1996) reported that: *S. feltiae* tends to move horizontally close to the soil surface, whereas *H. bacteriophora* can disperse vertically and infect hosts at greater depths. Invasion or penetration rate at definite exposure time is used as a criterion to evaluate nematode activity in previous studies. Molta and Hominick (1989) measured the infectivity of *S. carpocapsae* and *H. bacteriophora* against third instars of yellowfever mosquito *Aedes aegypti* larvae by dose-response assay as well as exposure time assay; they found that larval mortality showed a positive linear correlation with both nematode dosage and duration of exposure. Glazer (1992) stated that *S. carpocapsae* All strain was less effective than *H. bacteriophora* HP88 when applied to different lepidopteran pests according to LD50 and LT50 values. The rate of IJs recovery per single larva varied between the tested hosts. Also, it seems that there is some sort of specificity between the pathogen and the host. Reproduction depends mainly upon the ability of the associated bacterium to establish itself inside the haemocoel of the dead hosts and inhibits other contaminants (Molyneaux *et al.* 1983; El-Bishry, 1994). The host's body contents may act as another reason for differences in production rate for IJs. Soliman (2002) found a destruction of cytoplasm fat cells appeared as large vacuoles in *C. capitata* larvae after infection with *H. bacteriophora* AS1 (a native strain). Yoo *et al.*

Table (1): Virulence of three nematode strains to *Bactrocera zonata* (Saund.) and *Ceratitis capitata* (Wied.) larvae at different concentrations

| | % of *B. zonata* larval mortality (SE) at IJs concentrations/larva | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 50 | 75 | 100 | 125 |
| *H. bacteriophora* (NS) | 18.58 (1.60) | 43.71 (1.57) | 46.43 (1.54) | 60.03 (1.51) | 68.79 (1.55) | 81.47 (1.58) | 85.68 (1.14) | 93.35 (1.42) | 100.0 (1.49) |
| *H. bacteriophora* HP88 | 7.41 (027) | 11.12 (0.39) | 25.92 (0.54) | 33.53 (0.67) | 41.19 (0.72) | 48.14 (0.82) | 56.77 (1.12) | 78.01 (1.24) | 83.33 (1.35) |
| *S. carpocapsae* All strain | 2.15 (0.18) | 3.42 (0.36) | 4.67 (0.31) | 5.39 (0.57) | 9.17 (0.23) | 16.28 (0.67) | 25.03 (0.79) | 37.98 (1.02) | 43.87 (0.94) |
| | % of *C. capitata* larval mortality (SE) at IJs concentrations/larva | | | | | | | | |
| | 5 | 10 | 15 | 20 | 25 | 50 | 75 | 100 | 125 |
| *H. bacteriophora* (NS) | 48.11 (1.44) | 51.98 (1.28) | 63.31 (1.15) | 66.73 (1.87) | 92.56 (2.26) | 100.0 (2.37) | 100.0 (1.26) | 100.0 (1.87) | 100.0 (2.09) |
| *H. bacteriophora* HP88 | 11.89 (0.12) | 26.67 (0.22) | 34.53 (0.36) | 36.13 (0.53) | 46.61 (0.68) | 73.19 (1.19) | 89.12 (1.42) | 96.30 (1.65) | 100.0 (1.76) |
| *S. carpocapsae* All strain | 3.25 (0.08) | 3.71 (0.21) | 3.85 (0.37) | 18.21 (0.87) | 19.31 (1.08) | 21.46 (0.65) | 29.01 (1.19) | 37.46 (0.88) | 52.47 (1.51) |

All mean mortality values were corrected using Abbot's formula (1925)

Table (2): LT 50 and LT 90 values of three nematode strains used against *Bactrocera zonata* (Saund.) and *Ceratitis capitata* (Wied.) larvae

| Nematode strain | *B. zonata* | | | | | |
|---|---|---|---|---|---|---|
| | LT50 | Fiducial limits | | LT90 | Fiducial limits | |
| | | lower | upper | | lower | upper |
| *H. bacteriophora* (NS) | 1.053 | 0.794 | 1.261 | 1.576 | 1.315 | 2.468 |
| HP88 | 2.870 | 2.347 | 3.445 | 4.731 | 3.883 | 6.633 |
| *S. carpocapsae* | 7.331 | 6.281 | 10.789 | 10.462 | 8.378 | 40.717 |
| | *C. capitata* | | | | | |
| Nematode strain | LT50 | Fiducial limits | | LT90 | Fiducial limits | |
| | | lower | upper | | lower | upper |
| *H. bacteriophora* (NS) | 1.184 | 0.915 | 1.434 | 1.841 | 1.510 | 3.173 |
| HP88 | 3.650 | 2.823 | 4.743 | 7.530 | 5.599 | 13.261 |
| *S. carpocapsae* | 6.044 | 4.814 | 8.197 | 10.28 | 7.752 | 28.588 |

Table (3): Infective juveniles' production of three nematode isolates penetrated *Bactrocera zonata* (Saund.) and *Ceratitis capitata* (Wied.) larvae

| Insect species | Mean numbers of produced IJs /10 larvae (SE) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *H. bacteriophora* (NS) | | | *H. bacteriophora* HP88 | | | *S. carpocapsae* | | |
| | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 |
| *B. zonata* | 1556.3 (0.42) | 3092.6 (0.68) | 6113.3 (3.53) | 912.66 (1.52) | 1611.3 (0.87) | 4863.1 (2.770) | 603.66 (0.85) | 1115.6 (1.61) | 2018.6 (0.92) |
| *C. capitata* | 979.66 (1.23) | 2013.6 (1.1) | 4326.6 (2.08) | 780.66 (0.71) | 1617.6 (2.04) | 3009.3 (0.57) | 507.33 (1.81) | 980.66 (1.64) | 1890.93 (1.37) |

(2001) reported that *H. bacteriophora* and its symbiont bacterium need lipids for growth. A comparison or analysis of the body contents of *B. zonata* and *C. capitata* larvae should be investigated in the near future to explain the difference in reproduction and IJs recovery from infected hosts. *H. bacteriophora* (NS) represented higher IJs recovery from *B. zonata* than C. *capitata*. In addition, it emerged from *B. zonata* 2 days earlier than those in *C.capitata*.

As a conclusion, *H. bacteriophora* (NS) seems to be a suitable highly pathogenic biological control agent for the control of *B. zonata* and *C. capitata*.

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