NOTA CIENTÍFICA

Capacity of the terrestrial entomopathogenic nematode *Steinernema rarum* (Rhabditida: Steinernematidae) to parasite *Culex apicinus* larvae (Diptera: Culicidae)

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Capacidad del nemátodo terrestre entomopatógeno *Steinernema rarum* (Rhabditida: Steinernematidae) de parasitar larvas de *Culex apicinus* (Diptera: Culicidae)

■ **RESUMEN.** Los nemátodos entomopatógenos son considerados eficientes agentes de control de insectos plaga e inocuos para los humanos. Larvas de *Culex apicinus* Philippi fueron expuestas a seis dosis (1:1, 5:1, 10:1, 15:1, 100:1, 400:1) de juveniles infectivos de *Steinernema rarum* (aislado OLI). Se registró un incremento en la mortalidad de las larvas del mosquito con el aumento de la dosis del nematodo. El mayor porcentaje de mortalidad de larvas del mosquito (75%) se obtuvo con la dosis 400:1. Este es el primer reporte de parasitismo de un aislado de *S. rarum* de Córdoba, en larvas de *C. apicinus* con resultados promisorios. Por lo tanto, se debería profundizar su estudio para determinar si pueden resultar efectivos como agentes autóctonos para el control biológico de mosquitos *Culex* Linnaeus, y otros de interés sanitario en el país.

PALABRAS CLAVE. *Culex apicinus*. *Steinernema rarum* (aislado OLI). Control biológico.

■ **ABSTRACT.** Entomopathogenic nematodes can be considered effective agents for biocontrol of pest insects, resulting innocuous for humans. Larvae of *Culex apicinus* Philippi were exposed to infective juveniles of *Steinernema rarum* (OLI strain) under laboratory conditions, testing six doses (1:1, 5:1, 10:1, 15:1, 100:1, 400:1). An increasing percentage of mosquito larvae mortality was recorded with an increased dose. The highest percentage of mosquito larvae mortality (75%) was obtained with the dose 400:1. This is the first report of parasitism of an isolated of *S. rarum* from Córdoba against larvae of *C. apicinus,* with promising results. Therefore, further studies must be carried out to determine if these nematodes would be effective as autochthonous agents for the control of *Culex* Linnaeus and other mosquitoes of sanitary interest in the country.

KEY WORDS. *Culex apicinus*. *Steinernema rarum* (OLI strain). Biological control.

Recibido: 2-II-2010; aceptado: 17-V-2010

Mosquitoes are insects of great sanitary importance due to the transmission of several vector-borne human diseases. The vector control has involved an excessive used of chemical insecticides, substances that are toxic for humans, resulting in the development of vector resistance, affecting non target organisms mainly natural enemies of pests, and contributing to the environmental pollution (Santamarina *et al.,* 2000). Therefore, alternative methods to control mosquitoes are needed.

The biological control using nematodes that parasite insects represent a promising alternative, because they are innocuous for the flora as well as to humans and other vertebrates. Several assays were carried out using, for instance, *Romanomermis culicivorax* and *R. iyengari* (Nematoda: Mermithidae) against larvae of mosquitoes *Culex* Linnaeus and other genera (Santamarina, 1993; Pérez-Pacheco *et al.,* 2004). In Argentina, the pathogenic effect of the mermithid *Strelkovimermis spiculatus* isolated in our country, was tested against larvae of *Culex apicinus* Philippi, producing a 100% of mortality (Achinelly *et al.,* 2004).

Culex apicinus is known from Chile, Bolivia, Perú and Argentina (Martínez *et al.,* 1960; Knight & Stone, 1977). In Argentina it is listed for the provinces of Buenos Aires, Catamarca, Córdoba, Corrientes, Jujuy, La Pampa, La Rioja, Río Negro, Salta, San Luis and Santa Fe (Visintin *et al.,* 2010). Several mosquito species in the genus *Culex* are incriminated in the transmission of pathogens such as the filaria *Wuchereria bancrofti* that causes elephantiasis in humans, malarial parasites that affect hens, or arboviruses like the Saint Louis encephalitis virus (SLEV), and the West Nile virus, among others (Díaz *et al.,* 2003, 2008). *Culex apicinus* larvae were collected from natural breeding sites but also from artificial containers located inside or around houses, including swimming pools and domiciliary water tanks in Córdoba Province, Argentina (Almirón & Brewer, 1996). In spite of the fact that adults of *C. apicinus,* collected in Córdoba city during the 2001-2004 period, were detected naturally infected with the SLEV (Díaz, 2009), their role in the transmission of the virus is not yet clear .

Entomopathogenic nematodes belonging to the Steinernematidae Family parasite terrestrial insects and are naturally associated with a symbiotic bacteria. Testing these nematodes against mosquito larvae, the bacteria kill them within 24 to 48 hours once they are released by the nematode into the body host. These nematodes can be mass produced , applied with coventional equipment, and are also compatible with the use of chemical insecticides (Grewal *et al.,* 2001). Therefore, these nematodes could be used as an alternative tool in the pest integrated management. In our laboratory, from 4 infective juveniles (IJs) of nematodes applied to a wax moth larva (*Galleria mellonella* Linnaeus; Lepidoptera: Pyralidae), 70,000 new IJs were obtained after a parasitic cycle of 10 days.

The objective of this work was to evaluate the susceptibility of *C. apicinus* larvae to the parasitism by the terrestrial nematode *Steinernema rarum* (OLI strain). The nematode isolate was obtained from Oliva City (32° 02′ 24″ S; 63° 34′ 12″ W), Córdoba Province (Argentina).

Infective juveniles of *S. rarum* used in the assays were at least 30 days-old, and were stored at 25 °C. Nematodes were reared in the laboratory using *G. mellonella* larvae as hosts. Four days after the wax moth larvae death caused by the nematodes, they were placed on modified White traps (Kaya & Stock, 1997), and then into a culture stove at 25 ± 1 ºC. After the emergence, IJs were collected and stored previous to the assays (Shapiro-Ilan & Glaugler, 2002). Mosquito larvae were collected from artificial containers and kept in the laboratory according to conventional rearing techniques (Gerberg *et al.,* 1994).

Infections were performed exposing 10 second-instar mosquito larvae to six nematode doses (1:1, 5:1, 10:1, 15:1, 100:1, 400:1). Assays were carried out in containers with 2 ml of dechlorinate water, and incubated at 25 ºC. The exposure to the infective nematodes was continuous until the end of the assay. Dead mosquito larvae were transferred individually to Petri

dishes for observation. Three replicates by treatment and the respective control were performed. Mortality of mosquito larvae was analyzed by ANOVA, previous arcsin data transformation, to detect significant differences among treatments and controls.

There was no mortality in the controls and in the lowest doses tested. Five percent of mortality was recorded with dose 5:1 and up to 75% with dose 400:1, increasing the mortality with the dose (Fig. 1). Mosquito larva mortality was higher with doses 100:1 and 400:1 in comparison with the other doses tested ($F = 21.21$; $gl = 5$; $p < 0.0001$).

Although the IJs contacted the second-instar mosquito larvae, only the third-instar larvae were parasitized. Nematodes developed into the whole body of the mosquito larvae, even in the head and the siphon. In addition, melanization of IJs were observed as immune response by the mosquito larvae.

With the highest doses tested (100:1; 400:1), the nematodes infected the mosquito larvae, established inside them, and multiplied producing a new generation of IJs. This could constitute an advantageous characteristic of these nematodes because when applied they could continue living in the mosquito breeding sites treated, remaining in the environment and thus diminishing application costs.

In this work, it is demonstrated for the first time the capacity of the terrestrial nematode *S. rarum* to parasite *C. apicinus* larvae. In spite of being terrestrial nematodes they move in the interstitial water between the soil particles. This characteristic can be considered a possible disadvantage for these nematodes as agents for biological control in aquatic habitats. However, in the assays here performed, IJs were observed swimming actively looking for the mosquito larvae. Besides, mosquito larva movement favors the suspension of IJs into the water. Further studies are also needed to analyze the movement of these nematodes into the water. In the laboratory, using *G. mellonella* larvae in a substratum of wet sand, both cruising and ambushing host search strategies have been observed for *S. rarum* also OLI strain (unpublished data). Nevertheless, an appropriate experimental design with a higher volume of water would be necessary in order to know the behavior of this nematode in the aquatic environment.

Negative results were obtained in laboratory assays testing different entomopathogenic nematodes isolated from Argentina against mosquito larvae of *Culex pipiens* (Doucet *et al.*, 1999). Only the mermithids *Strelkovimermis spiculatus* (Poinar & Camino, 1986) and *Hydromermis* sp. (Camino, 1989) were isolated while searching for nematodes that parasite mosquito larvae in natural breeding sites. The biological characteristics of Mermithidae and Steinernematidae nematodes are quite different. In mermithid nematodes, a pre-parasitic (second instar juvenile) enters the host, develops into a juvenile parasitic, then into a juvenile post-parasitic that emerges and in the environment molts to adult; reproduction occurs in the environment. In steinernematid nematodes, two free living juveniles enter the host, develop into male and female adults, reproduce inside the host, and many infective juveniles emerge from the host.

In comparison, the mermithid nematode *Strelkovimermis spiculatus* caused a 100% of mortality in *C. apicinus* larvae with a lower dose (10:1 pre-parasites/larva) (Achinelly *et al.,* 2004) than the one used with *S. rarum*. Nevertheless, steinernematids would have better characteristics than mermithids since at the end of the parasitary cycle they produce a great number of new IJs originated in the first as well as in the second adult generations (Cagnolo *et al.,* 2004), so that mass production would be easier and at a lower cost. Recent studies have demonstrated higher infectivity of *S. rarum* at 23 ± 2 °C (Cagnolo & Campos, 2008), which would favour its application during the greater part of the year, considering the temperate climate of Córdoba province. However, field studies at greater scales are still needed.

This is the first report of parasitism of *S. rarum* from Cordoba against larvae of *C. apicinus* with promising results. Therefore, this terrestrial nematode could parasite both,

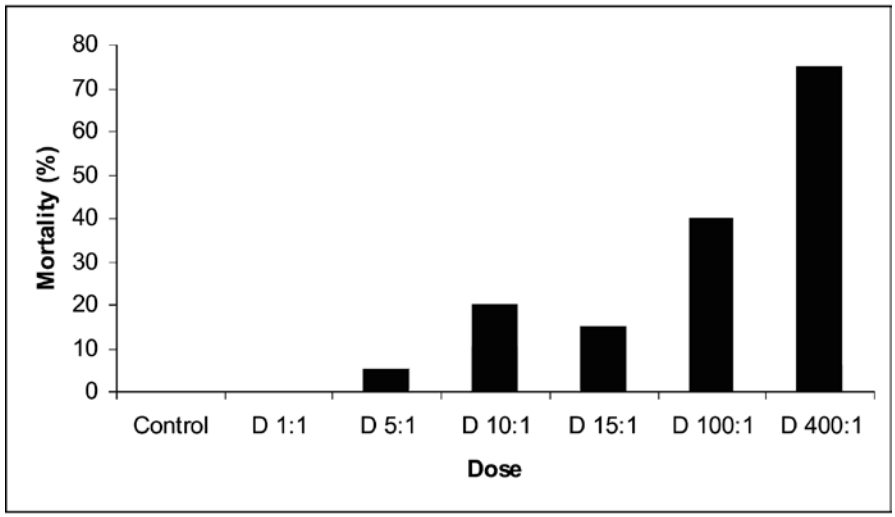

Fig. 1. Mortality of *Culex apicinus* larvae by infection with *Steinernema rarum* (OLI strain) according to different doses.

aquatic and terrestrial insects. Further studies are necessary in order to evaluate these nematodes as possible autochthonous agents for the control of *Culex* and other mosquitoes of sanitary interest in the country.

ACKNOWLEDGEMENTS

This work was partially supported by grants of the Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba, Argentina.

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