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Biocontrol of *Culex quinquefasciatus* using the insect parasitic nematode, *Romanomermis iyengari* (Nematoda: Mermithidae)

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Abstract. *Cx. quinquefasciatus* is a common nuisance mosquito widely distributed in tropical and subtropical areas. This mosquito is also a vector of urban filariasis. Control with chemicals has been hampered by the development of resistance against chemical insecticides and rising problems of environmental contamination associated with them. Therefore, it is important to adopt more integrated mosquito management approaches that include sustainable, non chemical solutions. The mermithid nematode *Romanomermis iyengari* is one of several natural control alternatives to synthetic pesticides for mosquito suppression. This study evaluated the effectiveness of the nematode *R. iyengari* for control of *Cx. quinquefasciatus*. The nematode *R. iyengari* was mass-produced, and pre-parasitics (J2) were used for laboratory and field experiments. In laboratory experiments, two concentrations of pre-parasitics (5 and 10 J2 per larva) were tested against L1, L2 and L3 instars larvae of *Cx. quinquefasciatus*. Infected larvae were observed daily to determine their mortality rate and the number of post-parasitic nematodes emerging from dead larvae. In field experiments, 1000, 2000 and 3000 J2/m^2 were sprayed in separate natural *Cx. quinquefasciatus* breeding sites. After treatment, the larval mosquito density in the breeding sites was assessed every 5 days. Laboratory results showed that all tested *Cx. quinquefasciatus* instars larvae were susceptible to nematode infection. The mortality rates observed for each larval stage indicated that the concentration of 10 J2 kills larvae faster, and that the L1 larvae died earlier than older larvae. The average number of post-parasitic nematodes emerging per larva increases with increasing nematode concentration; also more post-parasitic nematodes emerged from the L2 larvae. Field data showed that, in breeding site treated with 3000 J2 per square meter, larval mosquito reduction reached 97% after nematode application. The dosage of 1000 J2 per square meter did not reduce the larval density. The insect parasitic nematode *R. iyengari* could be easily used as component of integrated mosquitoes control program in lymphatic filariasis endemic countries.

INTRODUCTION

Unplanned urbanization is a factor that favours proliferation of mosquitoes. Rapid urbanization and unplanned growth of cities has resulted in proliferation of man made mosquito habitats promoting the breeding of a variety of disease vectors, and, consequently, enhanced disease transmission. Lack of adequate drainage, even the provision of drainage, and water stagnation has promoted the breeding of *Culex quinquefasciatus* (Alavo *et al*., 2010; Nazni *et al*., 2005). *Cx. quinquefasciatus* is a member of the *Culex pipiens* Linnaeus complex and one of the main subspecies found in Africa (Subra, 1981). *Cx. quinquefasciatus* is a common nuisance mosquito widely distributed in tropical and subtropical areas. *Cx. quinquefasciatus* larvae breed and thrive abundantly in stagnant dirty water. Because of the anthropophilic and endophilic blood feeding habits of the female, this mosquito is closely associated with human

habitations and is a continuous biting nuisance, mostly for those living close to larval habitats (Kumar *et al*., 2011; Sunaiyana *et al*., 2006; Hidayati *et al*., 2005; Vatandoost *et al*., 2004).

Cx. quinquefasciatus is also vector of urban filariasis. This mosquito is the main vector of the parasitic worm *Wuchereria bancrofti*, the agent of lymphatic filariasis. Interest in the control of *Cx. quinquefasciatus* lies in the fact that it acts as a vector of filarial fever, a serious public health problem in many developing countries (Kumar *et al*., 2011; Hidayati *et al*., 2005).

In some countries, the *Cx. quinquefasciatus* breeding sites have been sprayed with organophosphorus insecticides and this has resulted in the development of resistance (Hidayati *et al*., 2005). Even when no control programme is specially designated for *Culex* sp., this mosquito is highly resistant to organophosphates. The extensive use of chemicals for other disease vector mosquito control and also in agricultural pest control would have indirectly contributed to pressure for selection of resistance to organophosphorous and pyrethroid compounds in *Cx. quinquefasciatus* (Nazni *et al*., 2005).

The control of insects of medical importance with chemicals has been hampered by the development of resistance against chemical insecticides and rising problems of environmental contamination associated with them (Jones *et al*., 2012; Kumar *et al*., 2011; Sunaiyana *et al*., 2006; Hidayati *et al*., 2005).

Given these problems, it is urgent to consider the use of alternative means for vector control. For this purpose, natural enemies of mosquitoes can be considered. *Romanomermis iyengari* (Mermithidae) is one of several species of entomopathogenic nematodes which parasitize and kill mosquito larvae (Platzer, 2007).

Initial information on *R. iyengari* comes from observations made by Ross (1906) who reported the presence of mermithid nematodes in a sample of *Culex* larvae in India. Iyengar (1927) found juvenile mermithid nematode parasites in the larvae of seven different species of anopheles. These nematodes were later described by Welch (1964) as *R. iyengari*.

Various studies have demonstrated the effectiveness of these nematodes for mosquito control in some parts of the world. In Tajikistan, Vladimirova *et al.* (1990) demonstrated that *R. culicivorax* and *R. iyengari* were much more effective against *Anopheles* than *Culex*. In Uzbekistan, Pridantseva *et al*. (1990) demonstrated *Anopheles* infection by *R. iyengari*. Studies on mosquitoes conducted in Azerbaijan showed that *R. iyengari* infects both *An. sacharovi* and *Cx. theileri* (Alirzaev *et al*., 1990). The results of field works carried out in Cuba were particularly encouraging. Santamarina *et al.* (1992) demonstrated that for a concentration of 1000 pre-parasitic *R. iyengari* per square metre of surface area, the percentage of larval reduction was 80-100% for *Anopheles*. In Mexico, a dose of 2000 to 3000 pre-parasitic juvenile *R. iyengari* per square metre produced an infection rate of approximately 85-100% of *An. pseudopunctipennis* larvae (Santamarina *et al*., 1999). Similar results were also obtained in Brazil, where 12 natural sites of *Anopheles* were treated with 2000 infectious nematodes per square metre of surface. After a week, *An. albitarsis* and *An. rondoni* populations were reduced by 85-97% (Santamarina & Bellini, 2000).

The effectiveness of *R. iyengari* had been proved against malaria mosquito in Benin, West Africa (Abagli *et al.*, 2019). However, this insect parasitic nematode has never been tested against *Cx. quinquefasciatus* in tropical Africa.

The present work has evaluated the potential of the entomopathogenic nematode *R. iyengari* for the biological control of *Cx. quinquefasciatus* in Benin, West Africa.

MATERIALS AND METHODS

Nematode production

Production of *R. iyengari* in Benin (Alavo *et al*., 2015) was initiated with eggs obtained from the Department of Nematology (University of California, Riverside, USA).

Culex quinquefasciatus was used as host for the nematodes. Chickens (*Gallus domesticus*) were used to supply these mosquitoes with blood meals. A small plastic tray (12×8×6 cm) containing about 500 ml of water was provided in each mosquito cage for oviposition.

Four days after a blood meal the egg rafts, which contained about 120 eggs each, were collected and 6 of these were deposited in a plastic tray (25×15×12 cm) containing 2 L water. Five hundred plastic trays were installed on metal shelves in the nematode production room where temperature and relative humidity were 28±2°C and 70-90% RH, respectively. The containers were covered with a mesh screen to prevent oviposition by wild mosquitoes. When the larvae reached the second instar (2 days), they were infected with preparasitic nematodes (second stage juveniles, J2) of *R. iyengari*, by the addition of 3 J2 per mosquito larva. The nematodes were taken from cultures that had been stored for eight weeks. These cultures were flooded with sterilised (chlorine-free) water to induce the eclosion of eggs and emergence of infective preparasites from the substrate. Fourteen hours after flooding the cultures (overnight), the water was decanted, and the concentration of nematodes in the solution calculated by volumetric dilution. After 8 days, the mosquito larvae died and floated on the surface, indicating the end of the parasitic phase of the nematode. The water containing post-parasitic juveniles together with dead mosquito larvae and other debris was poured onto a sieve. The sieve containing the larvae and post-parasitic nematodes was then placed in a container with clean water. After a few minutes, the post-parasites passed through the sieve into the clean water so that when the sieve was removed, the dead mosquito larvae and debris were separated from the post-parasites that settled on the bottom of the container. The post-parasitic nematodes were collected using a syringe and transferred to a glass beaker with clean water. They were then washed thoroughly several times by sedimentation. Two grams (wet weight) of washed post-parasitic nematodes (composed of 457.6±1.38 females and 583±0.59 males, as determined in 25 samples) were deposited in round plastic containers (13 cm diameter) with previously sterilised coconut coir fibres (35 g) and 500 ml sterilized (chlorine-free) water. About 3 hours later (when all nematodes had moved into the substrate), the water was decanted and the containers covered and stored for eight weeks so that the nematodes could reach sexual maturity, mate and deposit eggs. They were kept in a climate-controlled room at 27±2°C and 85% RH. Every week, condensation water droplets were removed from the containers with cotton tissue to prevent premature hatching of nematodes eggs.

Laboratory test for larval mosquito susceptibility to *R. iyengari*

Bioassays were performed on the first three larval stages of *Cx. quinquefasciatus* (L1, L2 and L3). The larvae used for these tests were collected in natural breeding sites of *Cx. quinquefasciatus* in Cotonou, Benin. Wild larvae were used to ensure the results could be extrapolated to field conditions. The larvae were harvested on the day the tests were conducted. Once in the laboratory, the larvae were sorted and grouped by larval stage. For each stage, two batches of 100 larvae were established; one batch was infected with pre-parasitic nematodes and the other served as the untreated control group (zero nematode). Fish food was used to feed the experimental larvae throughout the trials. The larvae were maintained in 500 ml of distilled water.

The volumetric dilution method (Petersen and Willis, 1972) was used to determine the concentration of suspended pre-parasitic nematodes. Concentrations of 5 and 10 pre-parasitic juvenile nematodes per larva were used for the tests.

Four days after infection, 20 larvae were transferred individually into Petri dishes containing 30 ml of distilled water for daily observation. The experimental larvae were kept in a room where the temperature is maintained at 27°C±1. Experimental larvae were observed daily with a stereomicroscope

to determine the mortality rate and number of post-parasitic nematodes emerging from dead larvae.

Efficacy tests of *R. iyengari* against mosquito larvae in field

We treated 3 different *Culex* mosquito breeding sites located 500 meters from each other along a road in Cotonou, Benin. These breeding sites were manholes intended for the drainage of rain water from the road, but they contain stagnant water and wastes. They were square-shaped with 50 cm side and about 80 cm deep.

These 3 breeding sites were treated once with 1000 J2, 2000 J2 and 3000 J2 per square meter, respectively. The treatment was done by spraying the site uniformly with the suspension containing the required dose of J2 using a knapsack sprayer. Just before each application, the larval mosquito density was measured in each site. A total of 3 litres of water was collected in each breeding site with a 100 ml dipper. Using a Pasteur pipette, the number of larvae was counted and averaged to obtain the larval density expressed as the number of larvae per liter of water. After nematode spraying, the larval density in each site was measured at 5 days-intervals to determine the larval mosquito population dynamics over 15 days. In addition, five days after application, 20 mosquito larvae were collected at each treated site and subsequently examined in the laboratory to determine if sprayed nematode had effectively infected the larvae. The sampled larvae were transferred individually into Petri dishes and observed daily with a stereomicroscope to determine their mortality rate and the parasitism intensity (number of post-parasitic nematodes emerging per larva).

Statistical analyses

For the laboratory tests, probit analysis was performed to determine if there was a significant difference between tested nematodes concentrations. Pearson correlation test was also performed to determine the correlation between nematode concentration and number of post-parasitic nematodes emerging per larva. SPSS statistics package was used to perform probit analysis and correlation testing.

For field experiments, the average density of mosquito larvae was calculated for each day of observation and the population dynamics of larval mosquito in treated sites was determined. The percentage reduction of mosquito larvae compared to the situation before the first treatment was also calculated for each day of observation; for this, the following formula was used (Pérez-Pacheco, 2005):

$$\text{Percentage reduction} = [(LDpre - LDpost) / LDpre] \times 100$$

LDpre: Larval density before treatment
LDpost: Larval density after treatment

RESULTS

Susceptibility of *Cx. quinquefasciatus* larvae to nematode infection in laboratory experiments

Mortality rate of L1 larvae treated with 10 J2 was 40%, 75% and 100% respectively at the 5th, 6th and 7th day after treatment. For L2 larvae, the rates were 10%, 85%, and 95% respectively at the 5th, 6th and 7th day after treatment. Regarding L3 larvae, the rates were 15%, 80% and 90% respectively at the 5th, 6th and 7th day after treatment. Results show that all L1 and L2 larvae died respectively on 7th and 9th day after treatment. For L3 larvae, the highest mortality rate (which was 95%) occurred on day 9 after treatment. No mortality was recorded in the control larvae (Fig. 1). For the concentration of 5 J2 per larva, the mortality rate for L1 larvae was 5%, 15%, 70% and 95% respectively on day 5, 6, 7 and 8 post-treatment. This rate for L2 larvae was 0%, 10%, 70% and 100% respectively on day 5, 6, 7 and 8 post-treatment. Regarding L3 larvae, the mortality rate was 0%, 5%, 45%, 85% and 90% respectively on day 5, 6, 7, 8 and 9 post-treatment. No mortality was recorded in the control larvae (Fig. 2).

Results indicated that the concentration of 10 J2 per larva kills larvae faster and that the L1 larvae died earlier than older one.

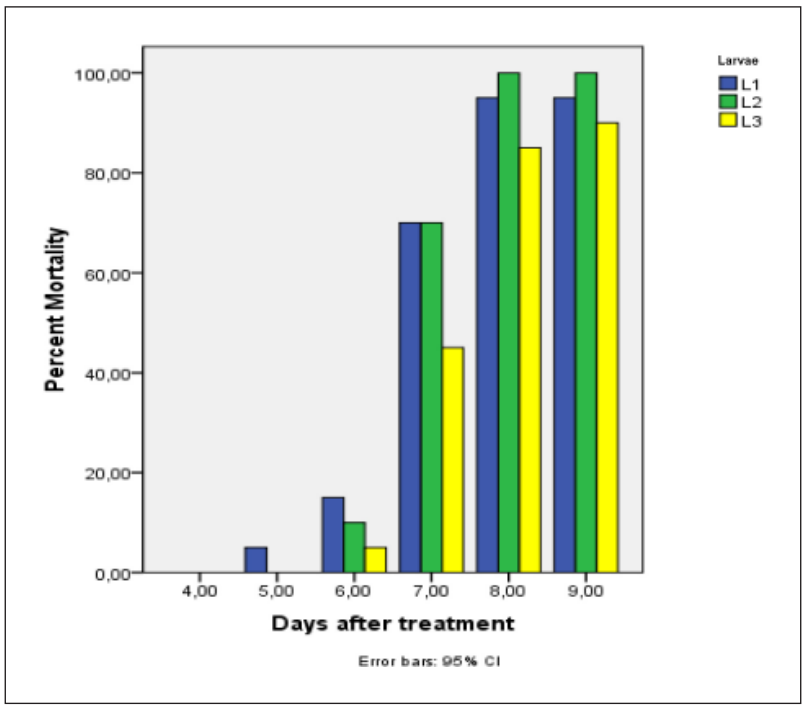

Figure 1. Mortality rates of *Cx. quinquefasciatus* at a concentration of 5 pre-parasitic nematodes per larva.

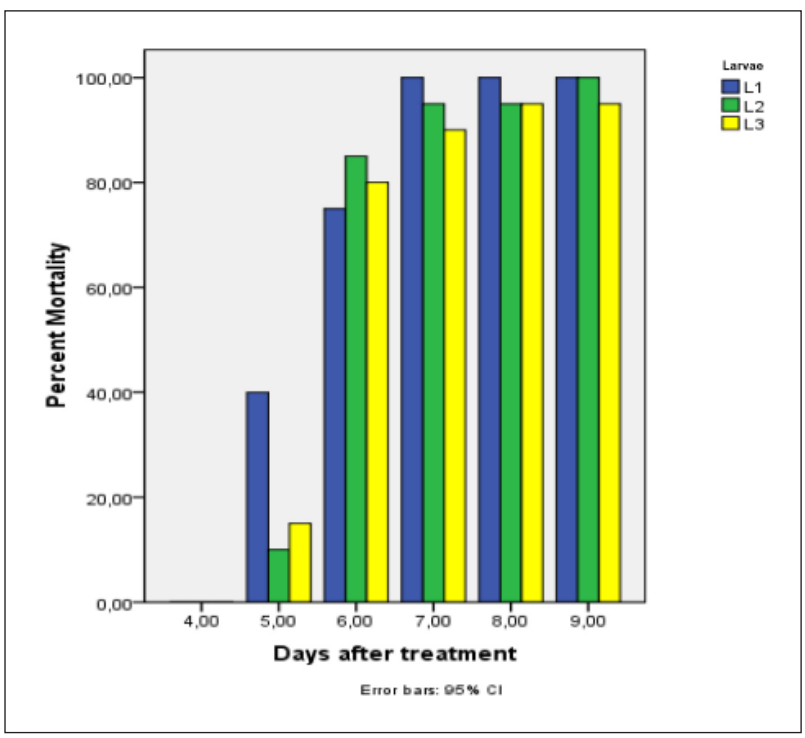

Figure 2. Mortality rates of *Cx. quinquefasciatus* at a concentration of 10 pre-parasitic nematodes per larva.

Statistical analysis revealed a significant difference between the two tested nematodes concentrations. Lethal times for 50% of the populations (LT_{50}) were 6.72, 6.69, and 7.28 days for the concentration of 5 J2 per larva respectively for L1, L2 and L3 larvae.

Regarding the concentration of 10 J2 per larva, the lethal times were 5.36, 5.70, 5.83 days respectively for L1, L2 and L3 larvae.

Table 1 shows the average number of post-parasitic nematodes emerging per larva for each larval stage of *Cx. quinquefasciatus*. Data in this table showed that parasitism intensity increased with increasing tested nematode concentration, and that more post-parasitic nematodes emerged from the L2 larvae.

Effectiveness of *R. iyengari* against *Cx. quinquefasciatus* in field experiments

In breeding site treated with 3000 J2 per square meter, larval density decreased from 295 (per liter of water) to 25, 5 days after treatment. This density was maintained at 20 larvae per liter on day 15 after treatment (Fig. 3). In this site, larval mosquito reduction reached 97% after nematode application. Throughout the test period, only L1 and L2 larvae were present in this breeding site (Table 2).

Regarding the site treated with 2000 J2 per square meter, larval mosquito density had dropped from 175 to 30 larvae, 5 days post application (Fig. 3). The percent reduction of larval density varied from 80 to 91% in this site over the experiment period. Throughout the test period, the majority of larvae collected at this site were also L1 and L2 larvae (Table 3).

The results at the site treated with 1000 J2 per square meter differed. The larval mosquito density slightly decreased 5 days after treatment but significantly increased thereafter (Fig. 3). Larvae of different stages and pupae were abundant at this site (Table 4).

Daily observations in the laboratory of larvae sampled at the breeding sites 5 days after treatment permitted to obtain Table 5 which shows the larval mortality rates and the average number of emerging post-parasitic nematodes. The mortality rate of sampled larvae as well as the average number of post-parasitic roundworms emerging per larva in the site treated with 3000 J2 were higher than those sampled in the 2 other sites.

Table 1. Average number of post-parasitic nematodes emerging per larval *Cx. quinquefasciatus* treated in laboratory

Infectious nematodes concentration (J2 per larva)	Mean number ± SD of post-parasitic nematodes emerging per larva at each larval stage		
	L1	L2	L3
10	3.35 ± 4.3	6.45 ± 3.2	3.95 ± 2.9
5	2.25 ± 1.2	3.05 ± 1.8	1.55 ± 1

Abbreviation: SD, standard deviation.

Table 2. Mean density of each larval stage in breeding site treated with 3000 J2 per m^2

Days after first treatment	Mean number of larvae per stage (± SD)			
	L1	L2	L3	L4 and pupae
5	10 ± 4.04	15 ± 4.54	0 ± 0.00	0 ± 0.00
10	10 ± 4.04	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	20 ± 7.37	0 ± 0.00	0 ± 0.00	0 ± 0.00

Abbreviation: SD, standard deviation.

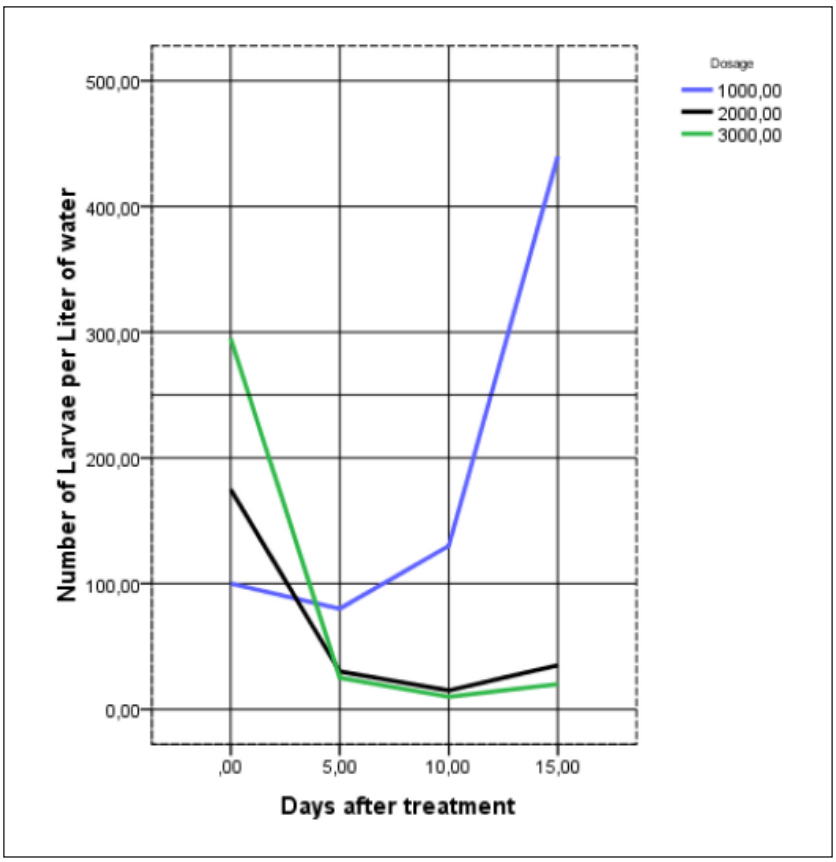

Figure 3. Larval mosquito population dynamics in natural breeding sites treated with different nematode dosages.

Table 3. Mean density of each larval stage in breeding site treated with 2000 J2 per m^2

Days after first treatment	Mean number of larvae per stage (± SD)			
	L1	L2	L3	L4 and pupae
5	25 ± 8.74	5 ± 3.37	0 ± 0.00	0 ± 0.00
10	15 ± 4.54	0 ± 0.00	0 ± 0.00	0 ± 0.00
15	20 ± 7.37	10 ± 4.04	0 ± 0.00	5 ± 3.37

Abbreviation: SD, standard deviation.

Table 4. Mean density of each larval stage in breeding site treated with 1000 J2 per m^2

Days after first treatment	Mean number of larvae per stage (± SD)			
	L1	L2	L3	L4 et pupae
5	65 ± 1.73	5 ± 3.37	5 ± 2.98	5 ± 3.42
10	15 ± 4.54	15 ± 5.02	90 ± 4.60	10 ± 4.04
15	120 ± 4.25	75 ± 2.92	125 ± 3.54	120 ± 5.43

Abbreviation: SD, standard deviation.

Table 5. Mortality rate and average number of post-parasitic roundworms emerging per larva from samples collected 5 days after first treatment in natural breeding sites treated with different nematode dosages

Days after first treatment	Mortality rate			Average number of post-parasitic roundworm emerging per larva		
	1000 J2	2000 J2	3000 J2	1000 J2	2000 J2	3000 J2
5	0%	0%	0%			
6	5%	5%	15%			
7	10%	15%	20%	0.2	0.35	0.55
8	20%	20%	35%			
9	25%	25%	45%			

DISCUSSION

This study has established the susceptibility of different larval instars of *Cx. quinquefasciatus* to *R. iyengari*, in Benin, West Africa. Results showed that this nematode parasitized and effectively killed mosquito larvae. The mortality rates observed for each larval stage indicate that L1 and L2 larvae are more susceptible to infection by the nematode, compared to higher stage larvae. Authors who obtained similar results have attributed this to the fact that the thin cuticle of young larvae facilitated the invasion of pre-parasitic nematodes which have more difficulty inserting their stylus in the thicker cuticles of older larvae (Pérez-Pacheco *et al*., 2004; Achinelly *et al*., 2004; Santamarina, 1994; Petersen, 1975).

Data regarding the average number of post-parasitic nematodes emerging per larva for each larval stage of *Cx. quinquefasciatus* indicated that parasitism intensity (the number of nematodes parasitizing a larva) increased with increasing nematode concentration.

Field data shows that with the dosage of 1000 J2 per square meter, larval mosquito density only slightly decreased 5 days after treatment but significantly increased thereafter. In contrast, with the dosage of 3000 J2 per square meter, larval mosquito reduction reached 97%. The laboratory data clearly support the results we obtained in the field. In general, larval population reduction increases with increasing nematode dosage. Therefore, we may conclude that, for more efficacy, increased dosage of infectious nematode should be used for the biocontrol of *Cx. quinquefasciatus*. To our knowledge, although *R. iyengari* has been tested in laboratory experiments against *Cx. quinquefasciatus* in many countries, no biocontrol trials in field were conducted against this mosquito species. Nevertheless, the dosage of 3000 infectious nematodes per square meter has been tested against *Anopheles pseudopunctipennis*, in Mexico (Pérez-Pacheco, 2004; 2005). Results of these studies were similar to that of present work, indicating that the dosage of at least 3000 J2 could be effectively used for the biocontrol of *Cx. quinquefasciatus*.

Rapid urbanization and unplanned growth of cities has resulted in proliferation of man-made mosquito habitats promoting the breeding of a variety of disease vectors, and consequently enhanced disease transmission (Alavo *et al*., 2010; Nazni *et al*., 2005). Lack of adequate drainage in many areas, even the provision of drainage, and water stagnation is promoting the breeding of *Cx. quinquefasciatus* and the spread of filariasis (WHO, 1992). Lymphatic filariasis puts at risk more than a billion people in more than 80 countries (WHO, 2005; Nazni *et al*., 2005). Resistance of *Cx. quinquefasciatus* to chemical insecticides has been reported in many parts of the world (Liu *et al*., 2004; Chandre *et al*., 1998; Bracco *et al*., 1997). *R. iyengari* is a parasite specific to mosquito larvae and its mass production is established (Petersen, 1985; Platzer; 2005; Popiel, 1992; Federici, 1995; Santamarina, 1997; Kerry, 2002). A large-scale production system of this worm has been developed

for tropical Africa. It uses endogenous and cost-effective materials which are locally available in tropical regions. The production system employs 3 technicians and can produce monthly a sufficient amount of nematodes to treat at least 75 000 square metres of breeding sites (Alavo *et al*., 2015). Therefore, the insect parasitic nematode *R. iyengari* could be easily used as component of integrated mosquitoes control program in lymphatic filariasis endemic countries.

Furthermore, *R. iyengari* is harmless for vertebrates. In experiments conducted by Gajanana *et al.* (1978), living infectious juveniles of *R. iyengari* were injected intravenously into animals such as mice, guinea pig, rabbit and chicken. Results showed that the overall health of these vertebrates remained unchanged. In treated animals, the histology of organs such as liver, spleen, kidneys, lungs and the gastro-intestinal system revealed no lesions linked to the nematode infection. In addition, *Gambusia affinis* fish exposed over several days to a large number of infectious juvenile worms were not affected in any way. Thus, the use of this biological control agent raises no concern for the health of vertebrates. Therefore, the use of *R. iyengari* for *Cx. quinquefasciatus* control may be considered.

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REFERENCES

Abagli, A.Z., Alavo, T.B.C., Perez-Pacheco, R. & Platzer, E.G. 2019. Efficacy of the mermithid nematode, *Romanomermis iyengari*, for the biocontrol of *Anopheles gambiae*, the major malaria vector in sub-Saharan Africa. *Parasites & Vectors* **12**: 253.

Alavo, T.B.C, Abagli, A.Z, Accodji, M. & Djouaka, R. 2010. Unplanned Urbanization Promotes the Proliferation of Disease Vector Mosquitoes (*Diptera: Culicidae*). *The Open Entomology Journal* **4**: 1-7.

Alavo, T.B.C., Abagli, A.Z., Pérez-Pacheco, R. & Platzer, E.G. 2015. Large-scale production of the malaria vector biocontrol agent *Romanomermis iyengari* (Nematoda: Mermithidae) in Benin, West Africa. *Malaria World Journal* **6**: 1.

Alirzaev, G.U., Pridantseva, E.P., Vladimirova, V.V. & Alekseev, A.N. 1990. Prospects for using *Romanomermis culicivorax* and *Romanomermis iyengari* Nematoda Mermithidae for mosquito control in Azerbaijan USSR. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **1**: 11-15.

Achinelly, M.F., Micieli, M.V., Marti, G.A. & Garcia, J.J. 2004. Susceptibility of neotropical mosquito larvae (*Diptera: Culicidae*) and non-target aquatic organisms to the entomoparasitic nematode *Strelkovimermis spiculatus;* Poinar & Camino, 1986 (Nematoda: Mermithidae). *Nematology* **6**: 299-302.

Bracco, J.E., Dalbon, M., Marinotti, O. & Barata, J.M. 1997. Resistance to organophosphorous and carbamates insecticides in a population of *Culex quinquefasciatus*. *Revista de Saude Publica* **31**(2): 182-183.

Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J.M., Pasteur, N. & Guillet, P. 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Medical and Veterinary Entomology* **12**(4): 359-366.

Jones, C.M., Machin, C., Mohammed, K., Majambere, S., Abdullah, S.A., Bakari, O.K., Juma, M., Hilary, R. & Kelly-Hope, L.A. 2012. Insecticide resistance in *Culex quinquefasciatus* from Zanzibar: implications for vector control programmes. *Parasites & Vectors* **5**: 78.

Federici, B.A. 1995. The future of microbial insecticides as vector control agents. *Journal of American Mosquito Control Association* **11**: 260-268.

Gajanana, A., Kazmi, S.J., Bheema, R.U.S., Suguna, S.G. & Chandrahas, R.K. 1978. Studies on a nematode parasite (*Romanomermis* sp.: Mermithidae) of mosquito larvae in Pondicherry. *Indian Journal of Medical Research* **68**: 242-247.

Hidayati, H., Mohd, S.A., Nazni, W.A. & Lee, H.L. 2005. Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae against malathion, permethrin and temephos. *Tropical Biomedicine* **22**(1): 45-52.

Iyengar, M.O.T. 1927. Parasitic nematodes of *Anopheles* in Bengal. *Transactions 7th Congress Far Eastern Association of Tropical Medicine* **3**: 128-135.

Kaushal, K., Abhay, K.S., Sarita, K., Sunita, P., Manas, S. & Chauhan, L.S. 2011. Multiple insecticide resistance/susceptibility status of *Culex quinquefasciatus*, principal vector of bancroftian filariasis from filarial endemic areas of northern India. *Asian Pacific Journal of Tropical Medicine* 426-429.

Kerry, B.R. & Hominick, W.M. 2002. Biological control. In: Lee DL, ed. *The Biology of Nematodes*. London: Taylor and Francis; 483-509.

Liu, H., Cupp, E.W., Micher, K.M., Guo, A. & Liu, N. 2004. Insecticide resistance and cross-resistance in Alabama and Florida strains of *Culex quinquefasciatus*. *Journal of Medical Entomology* **41**(3): 408-13.

Nazni, W.A., Lee, H.L. & Azahari, A.H. 2005. Adult and larval insecticide susceptibility status of *Culex quinquefasciatus* (Say) mosquitoes in Kuala Lumpur, Malaysia. *Tropical Biomedicine* **22**(1): 63-68.

Pridantseva, E.A., Lebedeva, N.I., Shcherban, Z.P. & Kadyrova, M.K. 1990. Assessment of the possibility of using the mermithid *Romanomermis iyengari* for the control of mosquitoes in Uzbekistan. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **1**: 15-17.

Platzer, E.G., Mullens, B.A. & Shamseldean, M.M. 2005. Mermithid Nematodes. In: Grewal P., Ehlers R., Shapiro-Ilan D. (eds). Nematodes as Biocontrol Agents. Wallinford, United Kingdom: CABI Publishing; 411-418.

Platzer, E.G. 2007. Mermithid nematodes. *Journal of American Mosquito Control Association* **23**(2): 58-64.

Petersen, J.J. & Willis, O.R. 1972. Procedures for the mass rearing of a mermithid parasite of mosquitoes. *Mosquito News* **32**: 226-230.

Petersen, J.J. 1975. Penetration and development of the mermithid nematode *Reesimermis nielseni* in eighteen species of mosquitoes. *Journal of Nematology* **17**: 207-10.

Petersen, J.J. 1985. Nematodes as biological control agents: Part I. Mermithidae. *Advances in Parasitology* **24**: 307-346.

Pérez-Pacheco, R., Rodriguez-Hernandez, C., Lara-Reyna, J., Montes-Belmont, R., Ruiz-Vega, J., Ramírez-Valverde, G. & Martínez-Martínez, L. 2004. Parasitism of *Romanomermis iyengari* in larvae of three species of mosquito in laboratory, and of *Anopheles pseudopunctipennis* in the field. *Agrociencia* **38**: 413-421.

Pérez-Pacheco, R., Rodriguez-Hernandez, C., Lara-Reyna, J., Montes-Belmont, R. & Ruiz-Vega, J. 2005. Control of the mosquito *Anopheles pseudopunctipennis* (*Diptera: Culicidae*) with *Romanomermis iyengari* (*Nematoda: Mermithidae*) in Oaxaca, Mexico. *Biological Control* **32**(1): 137-142.

Popiel, I. & Hominick, W.M. 1992. Nematodes as biological control agents: Part II. *Advances in Parasitology* **31**: 381-433.

Ross, R. 1906. Notes on the parasites of mosquitoes found in India between 1895 and 1899. *Journal of Hygiene* **6**: 101-108.

Samuel, T., Ravindran, K.J. & Arivoli, S. 2012. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine* S1130-S1134.

Santamarina, M.A., Garcia, A.I., Gonzalez, B.R., Mijares, A.S., Avila, I.G. & Broche, R.G. 1992. The ability of the nematode *Romanomermis iyengari* (Welch, 1964) (*Nematoda: Mermithidae*) to successfully parasitize mosquito larvae under natural conditions. *Rev Cubana Med Trop* **44**: 92-97.

Santamarina, M.A. 1994. Actividad parasitaria de *Romanomermis iyengari* (*Nematoda: Mermithidae*) en criaderos naturales de larvas de mosquitos. *Miscellània Zoològica* **17**: 59-65.

Santamarina, M.A. & Perez, P.R. 1997. Reduction of mosquito larval densities in natural sites after introduction of *Romanomermis culicivorax* (*Nematoda: Mermithidae*) in Cuba. *Journal of Medical Entomology* **34**: 1-4.

Santamarina, M.A., Perez, P.R., Tomas-Martinez, S.H., Enrique, C.L. & Flores, A.G. 1999. The *Romanomermis iyengari* parasite for *Anopheles pseudopunctipennis* suppression in natural habitats in Oaxaca State, Mexico. *Pan American Journal of Public Health* **5**: 23-28.

Santamarina, M.A. & Bellini, A.C. 2000. Mass produced *Romanomermis iyengari* (*Nematoda: Mermithidae*) applied to anopheline breeding sites in Boa Vista (Roraima), Brazil. *Pan American Journal of Public Health* **7**: 155-161.

Sunaiyana, S., Pungasem, P. & Kasin, S. 2006. Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* to four major groups of insecticides. *Tropical Biomedicine* **23**(1): 97-101.

Subra, R. 1981. Biology and control of *Culex pipiens quinquefasciatus* with special reference to Africa. *International Journal of Tropical Insect Science* **1**: 319-338.

Vatandoost, H., Ezeddinloo, L., Mahvi, A.H., Abai, M.R., Kia, E.B. & Mobedi, I. 2004. Enhanced Tolerance of House Mosquito to Different Insecticides due to Agricultural and Household Pesticides in Sewage System of Tehran, Iran. *Journal of Environmental Health Science & Engineering* **1**(1): 46-50.

Vladimirova, V.V., Pridantseva, E.A., Gafurov, A.K. & Muratova, M.E. 1990. Testing the mermithids *Romanomermis iyengari* and *R. culicivorax* for the control of blood-sucking mosquitoes in Tadznik SSR. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **3**: 42-45.

Welch, H.E. 1964. *Romanomermis iyengari* sp. nov. *Nematoda: Mermithidae* Braun, 1883. *Pilot Register of Zoology* Card No. 4.

WHO. 1992. Vector resistance to pesticides. Fifteenth report of the expert committee on vector biology and control. In *WHO Technical Report Series* **818**: 1-55.