Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2024.1004.02**

OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE AND HETERORHABDITIDAE) IN MADAGASCAR

Mandaniaina Andriamampianina [1*], Lala H. Raveloson-Ravaomanarivo [2],

Nirina C. Rakotoarimanga[1], Nomenjanahary F. Andriantefy[2], Victor Razafindranaivo[2]

1. Centre National de Recherches sur l'Environnement, Antananarivo, Madagascar.

2. Département d'Entomologie, Faculté des Sciences, Université d'Antananarivo, Madagascar.

ABSTRACT: The use of entomopathogenic nematodes (EPNs) as biological control agents against pests has been very developed over the few decades, but in Madagascar, no studies have been yet done on them. Thus the objective of this research is to determine the occurrence of EPNs in Madagascar and to evaluate the parameters that influence their distribution. Soil samples were collected from four distinct ecoregions across the country, totaling 510 samples from 17 sites. Using insect baiting techniques with *Galleria mellonella*, 38 samples (7.45%) tested positive for EPNs, with 30 being positive for *Steinernema* (5.88%) and 8 for *Heterorhabditis* (1.57%). Canonical Correspondence Analysis revealed ecological preferences for each nematode genus. *Steinernema* showed a preference for the central ecoregion with higher rainfall, woodland as habitat, and red ferralitic and loamy sand soil. *Heterorhabditis*, while less represented, were found in the eastern ecoregion with higher rainfall and temperature, forest habitat, and yellow red ferralitic and loamy sand soil. The southern ecoregion appears to be unfavorable for EPNs. Understanding these ecological nuances is crucial before implementing EPNs in biological pest management strategies in Madagascar, highlighting the importance of such research in guiding pest control efforts.

KEYWORDS: Entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*, distribution, Madagascar.

Article History: Received: July 28, 2024; Revised: August 04, 2024; Accepted: August 22, 2024.

Corresponding Author: Andriamampianina M.*

Laboratory of Entomology, Centre National de Recherches sur l'Environnement - Madagascar.

Email Address: manda.admp@gmail.com

1.INTRODUCTION

Entomopathogenic nematodes (EPNs) are soil-inhabiting microorganisms, that act as obligate parasites of a wide range of insects hence their ability to control pests [1] [2]. Although many families of nematodes have been isolated from insects, only two have attributes as effective biological control agents: Steinernematidae and Heterorhabditidae [3] [4]. Their use is currently highly recommended due to their ability to actively search for host resulting in a rapid mode of action. Additionally, their safety towards human health and the environment, along with the ease of production and formulation, further contribute to their recommendations [5] [6]. The occurrence and distribution of EPNs depend on various factors including geographical location, habitat types, soils parameters, etc [7]. Many surveys have been carried out, revealing the presence of several new species worldwide [8]. Among these surveys, Africa has already been a subject of studies; however, many regions remain unexplored, such as Madagascar. Being an island with diverse range of climates and habitats, the country may have a potential for a high frequency of recovery and richness of EPNs species. As far as the authors are concerned, EPNs have never been used in Madagascar to control pests, despite the ongoing threat the latter represent, particularly in agriculture. Indeed, cultural pests such as white grubs constitute one of the main causes of agricultural production loss, especially for rain-fed crops like rice and maize [9]. Unfortunately, chemical pesticides remain the primary method for controlling pests despite human and environmental health problems they generate. That is why studies of EPNs are important for the country and understanding of their biogeography is relevant to ensure their effectiveness. The objective of this study is then to determine the occurrence of EPNs in Madagascar and to evaluate the parameters that influence their presence. Surveys were conducted in each ecoregion of the country: Central, Eastern, Western and Southern ecoregions. Subsequently, EPNs were isolated and identified and the factors influencing their presence were analysed.

2. MATERIALS AND METHODS

Study area

The surveys were conducted in four ecoregions of Madagascar, as previously described by Burgess *et al.* [10]: the Central, the Eastern, the Western and the Southern ecoregion. The Central ecoregion is characterised by central highlands, with altitude ranging between 600-1800m. It features a humid subtropical climate and habitats dominated by subhumid forests, field crops, grasslands and savannahs [11]. The Eastern ecoregion is characterised by a warm and humid tropical climate with humid forest. The Western ecoregion is characterized by a dry tropical climate with dry deciduous forest. The Southern ecoregion is characterized by a very dry climate with deserts and xeric shrubs. From all ecoregions, seventeen sites were chosen with four sites from Central (Ambatobe, Fiadanana Tsimbazaza, Itasy and Antsirabe), five sites from Eastern (Ranomafana, Analalava Parc, Betampona, Vangaindrano and Midongy du Sud), four sites from Western (Mampikony, Malaimbandy, Majunga

and Diégo-Suarez), and four sites from Southern (Tuléar, Fort-Dauphin, Ambovombe and Amboasary Sud). For each site, three plots were also chosen randomly, resulting in a total of 51 plots. Surveys were conducted in various habitats including crop fields, forests, grasslands, woodlands and savannahs, spanning different altitudes, climates and soil characteristics.

Sampling methods and isolation of EPNs

Soil sampling was based on the methods outlined by Orozco *et al.* [12]. It consists on delimiting an area of 5 m^2 for each zone, then collecting ten soil samples randomly with a depth of at least 15 cm for each sample and placing them into a well-sealed plastic bag. For each sampling site, all necessary information was collected such as GPS coordinates, site name and code, date, habitat, associated vegetation, presence of insects if applicable, climate information, etc.

At the laboratory, EPNs were isolated from soil samples using the insect-baiting technique with *Galleria mellonella* [13]. Each soil sample was sieved, moistened, and then 250g were placed into a container and 5 last instar larvae of *G. mellonella* were placed on the surface of the soil, after which the container was cover with the lid and turned upside down. The containers were then placed in an insectarium, kept in the dark, with temperature of about 25°C. The status of *G. mellonella* was checked every day.

Recovery of EPNs

Death of *G. mellonella* could be attributed to any entomopathogens including bacteria, fungi, and virus, or it could occur naturally. Therefore, the appearance of dead larvae was checked; those that were hard, sometimes with mycelium visible externally on the cuticle, were considered infected by fungi; larvae that are very flaccid and liquefied were identified as infected by virus, while those with a putrid odour were considered to have dead naturally and were subsequently discarded. Only larvae that were more or less flaccid but not liquefied, and were infected with bacteria were collected as it maybe the symbiont bacteria of the EPNs that cause the death of the larvae. The appearance of the infected larvae can generally indicate the Family of the EPNs. Indeed, the symbiont bacteria *Xenorhabdus* and *Photorhabdus* associated with Steinernematidae and Heterorhabditidae, respectively, cause distinct coloration in the larvae of *G. mellonella*. Larvae infected with Steinernematidae exhibit a yellow-brown colour, while those infected with Heterorhabditidae show a brick-red colour [14]. The recovery of EPNs from dead larvae was based on the White trap method developed by White [15] and modified by Kaya and Stock [16]. It consists on placing a small Petri dish with moistened filter paper in a larger Petri dish containing distilled water. Infected larvae were placed in the small Petri dish and the large one was then sealed with parafilm. This device operates on the principle that nematodes are hydrophilic organisms, attracted by water and can be easily collected. Infected larvae from the same container, with the same morphological appearance, collected on the same day were placed on the same device. Petri dishes were kept in the laboratory at room temperature (25°C) and EPNs was collected after 15 days.

To be sure that collected nematodes were really entomopathogens, they were retested with larva of *G. mellonella*. Three last instar larvae of *G. mellonella* were placed into Petri dish of 50 mm with filter paper, then 50 nematodes were also put on the Petri dish. One Petri dish with only three larvae of *G. mellonella*, but no nematodes, was used as control.

Data analysis

The distribution of EPNs, based on different factors, was assessed using the recovery frequency calculated as the number of positive samples divided by the total number of samples. The relationship between the ecological factors and the presence of EPNs was determined using Canonical Correspondence Analysis (CCA) with R 4.3.3.

3. RESULTS AND DISCUSSION

Isolation of recovered nematodes

A total of 510 soil samples were collected from all ecoregions comprising 120 from the Centre, 150 from the East, 120 from the West and 120 from the South. Cadavers of *G. mellonella* from soil showed aspect of larvae infected by Steinernematidae and Heterorhabditidae, thus *Steinernema* and *Heterorhabditis* (Figure 1).

The test with *G. mellonella* has demonstrated that they were EPNs since 100% of *G. mellonella* tested were died and they showed the same colours as during the isolation. The controls were all alive.

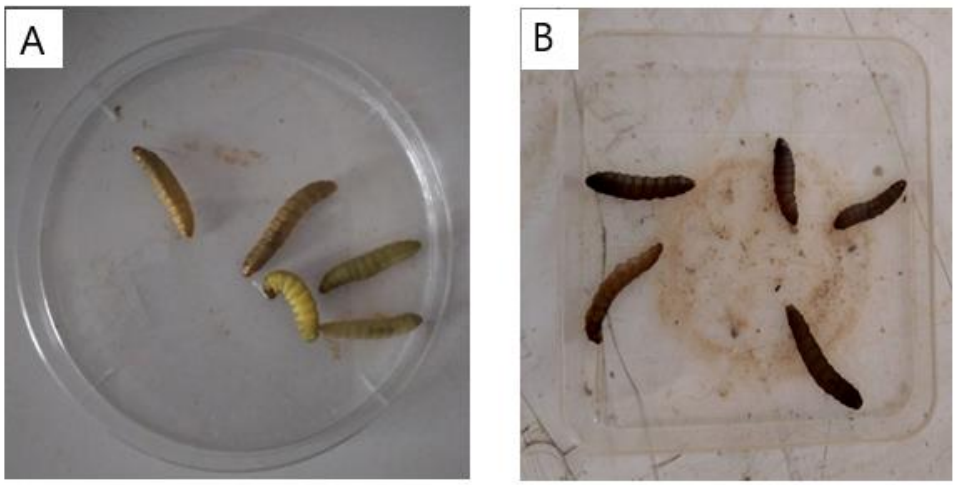

Figure 1. Larvae of Galleria mellonella infected by Steinernematidae (A) and Heterorhabditidae (B)

They were collected from all ecoregions except the Southern (Figure 2). 38 samples (7.45%) were positive, of which 30 (5.88%) where positive for *Steinernema* and 8 (1.57%) for *Heterorhabditis.* The positive samples were obtained from 10 sites (58.82%) with 8 sites for *Steinernema* and 2 for *Heterorhabditis*.

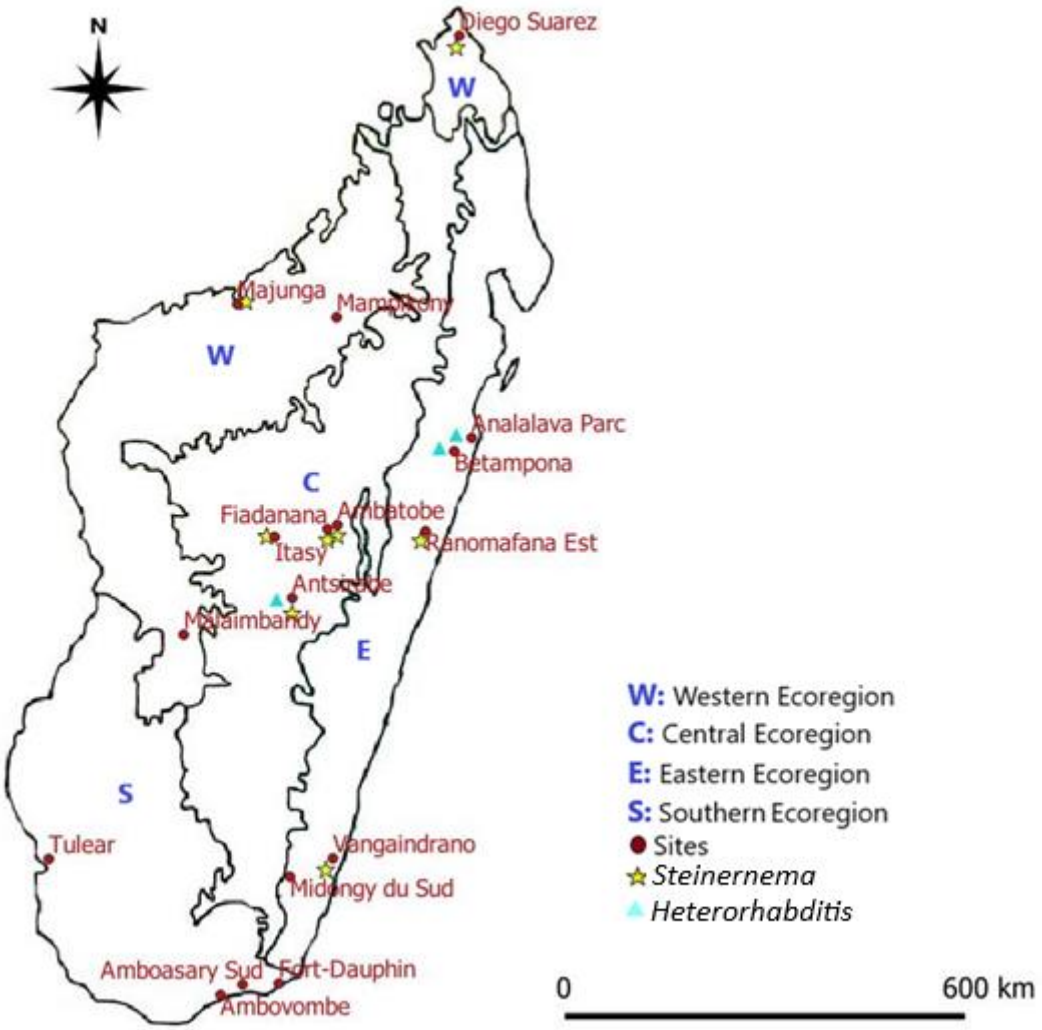

Figure 2. Map of Madagascar showing the distribution of entomopathogenic nematodes

Influence of ecological factors on the presence of entomopathogenic nematodes

Statistical tests were performed to assess the relation between the presence of *Steinernema* and *Heterorhabditis* and ecological factors. The following figure shows this relationship.

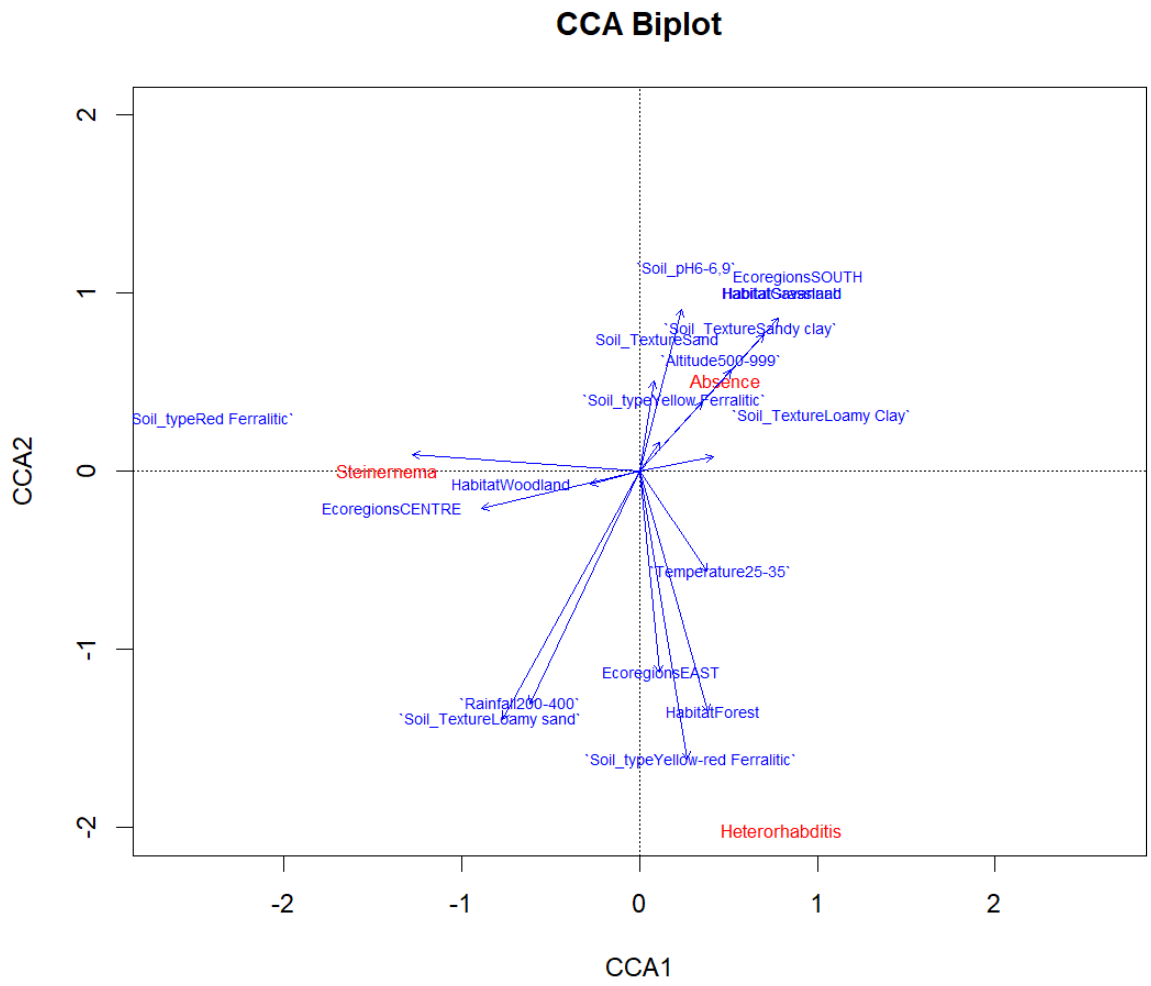

Figure 3. Canonical Correspondance Anlaysis of presence of *Steinernema* and *Heterorhabditis* and their absence with ecological factors variables

This graph illustrates the main ecological factors which are shown in blue with arrows, influencing the presence of EPNs and their absence. The position of *Steinernema* and *Heterorhabditis* far from the origin of the graph demonstrate that they are well represented and they are associated with certain environmental factor. Thus, the Central Ecoregion seems to be the most favorable to the presence of *Steinernema* with the following main factors: woodland as habitat; red ferralitic and loamy sand soil; and rainfall of 200 – 400mm. *Heterorhabditis* is more correlated with the Eastern ecoregion with forest as habitat; yellow red ferralitic soil and loamy sand soil texture; rainfall and temperature of 200-400mm and 25° – 35°C respectively. The Southern ecoregions are disfavorable for EPNs, with altitude of 500-999m, savannah and grassland, yellow ferralitic soil, sand, loamy clay and sandy clay soil, and soil pH of 6-6.9.

This is the first research on the distribution of EPNs in Madagascar and it reveals the presence of *Steinernema* and *Heterorhabditis*. They are present in all ecoregions except in the South. The recovery frequency was approximately 7.45%; while relatively low, it aligns with results observed in other tropical countries in Africa: 6.5% in Nigeria [17], 6.9% in Ethiopia [18] , and 7% in South

Africa [19], 7.3% in Fidji [20]. However, higher recovery rate were found in other tropical countries such as 50% in Kenya [21] and 20.5% in Costa-Rica [22]. These differences are probably due to the difference on number of soil samplings and nematodes isolation methods. *Steinernema* had a higher frequency than *Heterorhabditis* with respective recovery frequencies of 5.88% and 1.57%. This proportion is coherent with Hominick's findings [23], which specify that Steinernematidae are generally more frequent than Heterorhabditidae when soil samplings are not targeted since there are more species of Steinernematidae and they occupy more niches. Additionally, some researchers stipulate that Steinernematidae are more persistent and exhibit high recycling ability since they are amphimictics [24]. However, many studies reiterate that EPNs repartition differs on species [25] [8]. About ecological factors influencing the presence of EPNs, for *Steinernema*, result showed that they have a preference on higher rainfall. This is confirmed by the studies of [26] and [27], which reiterate that there is a seasonality in EPNs density and they appear to be more frequent in summer. In terms of soil characteristics, this study stipulates that *Steinernema* is more prevalent in loamy sand soil. This finding is in agreement with the Půža and Mráček researches [27], which also relate that EPNs in general are more prevalent in loamy sand soil. Also, according to Kung *et al.* [28], [29], [30], EPNs tend to be more abundant in soil with high sand content and less prevalent in soil with high clay content. This is because in contrast to sandy soil, clay soil has poor aeration and high water retention, which is not favourable for nematodes movement and survival. In this study, the limited presence of EPNs in sandy soil could be attributed to an inappropriate climatic condition like in the South where the soil is extensively dry and temperatures are too high, creating unfavourable conditions for EPNs. The relation of presence of EPNs and soil type is not very known but in this study, *Steinernema* is closely related to red ferralitic soil, it may be still related to texture of soil since the texture of red ferralitic soil with positive samples are loamy sand and loam. About habitats, *Steinernema* has a preference for woodland. This is in accordance with the some researches which found that woodlands support the largest Steinernematids biodiversity [31]; [32]. They were also found in some sites with crop field habitat. This result concord with other studies confirming that EPNs are more abundant in cultivated habitats, which typically have better soil. Crop plots with different crops in different seasons often host different pests, which is favourable for nematodes multiplication [21]. However, other surveys state that crop field might not be very suitable for Steinernematids, or EPNs in general because the high disturbance of these soils may cause disruption of soil microenvironment and expose the nematodes to UV light and desiccation, which may have detrimental effects [18]; [20]. Nevertheless, numerous studies indicate that Steinernematids are found in various type of habitats but it depends on species, soil texture and climate. So these results may suggest that Steinernematids collected in different habitat could belong to different species. Regarding *Heterorhabditis*, the limited proportion collected doesn't allow to firmly confirming their preferences. However, based on the results, they are exclusively present in

the eastern ecoregion with the following specific conditions: yellow red ferralitic soil, loamy sand, forest habitat and with 200-400mm of rainfall, temperature of 25-35°C, 1000-1500 of altitude. In many studies, *Heterorhabditis* is known to be abundant in coastal sandy soil in subtropical and tropical region [33]; [20], and several researches also underline that natural sites accommodating *Heterorhabditis spp* are essentially localised in coastal sites [34], [35], [29], [36], [30], [37] The southern ecoregion was not suitable for EPNs in our study; it includes altitude ranging from 500 – 900m, grassland and savannah habitats, sand, sandy clay, loamy sand soil texture, yellow ferralitic soil and soil with 6 – 6.9 pH. However, the absence of EPNs during surveys in these sites does not imply that they are completely absent. They may be present but not currently active, or the suitability of the insect bait, *G. mellonella,* may vary for different nematode species. It is especially attributes to the significant impact of climate on nematodes presence. Dry climate and high temperature, leading to soil dryness, are not suitable with nematodes prevalence as it restricts nematodes movement.

4. CONCLUSION

This study permitted to determine the presence of EPNs in Madagascar. However, it is just a preliminary study which gives basic results about EPNs distribution, but it is relevant for further researches. Identification of isolates is essential and should be done. Also, more surveys should be done in different seasons on same sites and different methods of extractions of EPNs should also be used.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to the Centre National de Recherches sur l' Environnement (CNRE) and its laboratory for their support and valuable collaboration throughout this study, and for graciously providing us with its facilities and equipment. We also thank the Department of Entomology at the University of Antananarivo for their significant contribution to our research. This exemplary collaboration has greatly enriched our research, and we are grateful to all those involved.

CONFLICT OF INTEREST

No author has any conflicts of interest to disclose.

REFERENCES

1. Poinar GOJ. Nematodes for Biological Control of Insects. Boca Raton: CRC Press; 1979. 277 p.
2. Shapiro-Ilan D, Hazir S, Glazer I. Basic and Applied Research: Entomopathogenic Nematodes. In: Lacey LA, éditeur. Microbial Control of Insect and Mite Pests. Cambridge: Academic Press; 2017. 91-105.
3. Vashisth S, Chandel YS, Sharma PK. Entomopathogenic nematodes - A review. Agri Rev. 2012; 34(3):163.
4. Sankaranarayanan C, Askary TH. Status of entomopathogenic nematodes in integrated pest management strategies in India. In: Biocontrol agents: entomopathogenic and slug parasitic nematodes. CABI; 2017. 362-382.
5. Shapiro-Ilan DI, Bruck DJ, Lacey LA. Principles of Epizootiology and Microbial Control. In: Insect Pathology. Elsevier; 2012. 29-72.
6. Koppenhöfer AM, Jackson TA, Klein MG. Bacteria for use against soil-inhabiting insects. In: Manual of Techniques in Invertebrate Pathology. London, UK: Academic Press: Elsevier; 2012. 373-426.
7. Campos-Herrera R, Escuer M, Labrador S, Robertson L, Barrios L, Gutiérrez C. Distribution of the entomopathogenic nematodes from La Rioja (Northern Spain). Journal of Invertebrate Pathology. 2007; 95(2):125-139.
8. Bhat AH, Chaubey AK, Askary TH. Global distribution of entomopathogenic nematodes, Steinernema and Heterorhabditis. Egypt J Biol Pest Control. 2020; 30(1):31.
9. Randriamanantsoa R, Quaranta B, Aberlenc HP, Rafamatanantsoa E, Ratnadass A, Vercambre B. Diversité de gestion des vers blancs (COLEOPTERE, SCARABAEOIDEA) en Riziculture pluviale sur les Hautes Terres Malgaches. Académie Malgache. 2011; 8.
10. Burgess N, Hales JD, Underwood E, Dinerstein E, Olson D, Itoua I, et al. Terrestrial ecoregions of Africa and Madagascar: a conservation assessment. Terrestrial ecoregions of Africa and Madagascar: a conservation assessment. 2004.
11. Collins NM, Harcourt CS, Sayer J, Whitmore TC. The conservation atlas of tropical forests : Africa. Springer. 1992.
12. Orozco RA, Lee MM, Stock SP. Soil Sampling and Isolation of Entomopathogenic Nematodes (Steinernematidae, Heterorhabditidae). JoVE. 11 2014; (89).
13. Bedding RA, Akhurst RJ. A simple technique for the detection of insect paristic rhabditid nematodes in soil. Nematol. 1975; 21(1):109-110.
14. Mauléon H, Boemare N, Denon D, Briand S. LES NEMATODES ENTOMOPATHOGENES. 2006; 31.
15. White GF. A method for obtaining infective nematode larvae from cultures. Science. 1927;

66(1709):302-303.
16. Kaya HK, Stock SP. Techniques in insect nematology. In: Manual of Techniques in Insect Pathology. Elsevier; 1997. 281-324.
17. Akyazi F, Ansari MA, Ahmed BI, Crow WT, Mekete T. First record of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Nigerian soil and their morphometrical and ribosomal DNA sequence analysis. Nematologia Mediterranea. 2012; 40(2):95-100.
18. Mekete T, Gaugler R, Nguyen KB, Mandefro W, Tessera M. Biogeography of entomopathogenic nematodes in Ethiopia. Nematropica. 2005; 31-6.
19. Malan AP, Nguyen KB, Addison MF. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the southwestern parts of South Africa | African Plant Protection. African Plant Protection. 2006 ; 12(1).
20. Kour S, Khurma U, Brodie G, Hazir S. Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in Viti Levu, Fiji Islands. Journal of Nematology. 2020; 52(1). 1-17.
21. Mwaniki SW, Nderitu JH, Olubayo F, Kimenju JW, Nguyen K. Factors influencing the occurrence of entomopathogenic nematodes in the Central Rift Valley Region of Kenya. African J Ecol. 2008; 46:79-84.
22. Uribe-Lorío L, Mora M, Stock SP. First record of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Costa Rica. Journal of Invertebrate Pathology. 2005; 88(3):226-231.
23. Hominick WM. Biogeography. In: Entomopathogenic nematology. Wallingford UK: Wallingford UK : CABI publishing; 2002. 115-143.
24. Nyasani JO, Kimenju JW, Olubayo FM, Shibairo SI, Mutua GK. Occurence of Entomopathogenic Nematodes and their Potential in the Management of Diamondback Moth in Kale. Asian Journal of Plant Sciences. 2008; 7(3):314-318.
25. Hominick WM, Reid AP, Bohan DA, Briscoe BR. Entomopathogenic Nematodes: Biodiversity, Geographical Distribution and the Convention on Biological Diversity. Biocontrol Science and Technology. 1996; 6(3):317-332.
26. Mráček Z. Horizontal distribution in soil, and seasonal dynamics of the nematode *Steinernema kraussei* , a parasite of *Cephalcia abietis*. Zeitschrift für Angewandte Entomologie. 1982; 94(1-5):110-112.
27. Půža V, Mráček Z. Seasonal dynamics of entomopathogenic nematodes of the genera Steinernema and Heterorhabditis as a response to abiotic factors and abundance of insect hosts. Journal of Invertebrate Pathology. 2005; 89(2):116-122.
28. Kung SP, Gaugler R, Kaya HK. Influence of Soil pH and oxygen on persistence of Steinernema

spp. Journal of Nematology. 1990; 22:440-445.

29. Hara AH, Gaugler R, Kaya HK, Lebeck LM. Natural populations of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) from the Hawaiian islands. Environmental Entomology. 1991; 20:211-216.
30. Amarasinghe LD, Hominick WM, Briscoe BR, Reid AP. Occurrence and distribution of entomopathogenic nematodes in Sri Lanka. J Helminthol. déc 1994; 68(4):277-286.
31. Stock SP, Pryor BM, Kaya HK. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. Biodiversity and Conservation. 1999; 8(4): 535-549.
32. Sturhan D, Liskova M. Occurrence and distribution of entomopathogenic nematodes in the Slovak Republic. Nematology. 1999; 1: 273-277.
33. Griffin CT, Chaerani R, Fallon D, Reid AP, Downes MJ. Occurrence and distribution of the entomopathogenic nematodes Steinernema spp. and Heterorhabditis indica in Indonesia. Journal of Helminthology. 2000; 74: 143-150.
34. Griffin CT, Moore JF, Downes MJ. Occurence of insect-parasitic nematodes (Steinernematidae, Heterorhabditidae) in Republic of Ireland. Nematologica. 1991; 37:92-100.
35. Griffin CT, Joyce SA, Dix I, Burnell AM, Downes MJ. Characterisation of entomopathogenic nematodes Heterorhabditis (Nematoda: Heterorhabditidae) from Ireland and Britain by molecular and crossbreeding techniques, and the occurrence of the genus in these islands. Fundamental and Applied Nematology. 1994; 17: 245-253.
36. Poinar GOJ. Origins and phylogenetic relationships of the entomophilic rhabditids, Heterorhabditis and Steinernema. Fundamental and Applied Nematology. 1993; 16: 332-338.
37. Mason JM, Razak AR, Wright DJ. The recovery of entomopathogenic nematodes from selected areas within Peninsular Malaysia. Journal of Helminthology. 1996; 70: 303-307.