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Searching for Better Methodologies for Successful Control of Termites Using Entomopathogenic Nematodes

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Additional information is available at the end of the chapter

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Abstract

Termites are social insects reported from many countries of the world. Some species of them are known to be beneficial to man, whereas some others cause substantial losses (billions of US dollars annually) of properties and amenities. Various preventive and remedial methods are used to control undesirable termite species. The current review paper gives an overview of beneficial and detrimental activities of termites. Methods of control of undesirable species of termites are given and their advantages and disadvantages are discussed. We emphasized on the use of entomopathogenic nematodes (EPNs) as effective, environmentally safe and sustainable biological control method against termites. Species of EPNs recovered in Africa are documented. Some techniques used to collect termites and to maintain them for experiments and also to propagate, to formulate, to store, and to check for the quality of EPNs for application in the laboratory and in the field are also discussed. The environmental factors affecting the potential of EPNs to control termites are discussed. The information provided in this chapter will help researchers to enhance their skills of the use of EPNs against termites by selecting from the methodologies described here the best ones to adapt to particular experimental conditions, especially in African soil conditions.

Keywords: termites, entomopathogenic nematodes, biological control, methodology, Africa

1. Introduction

Termites belong to the order Isoptera [1] and include more than 3500 species described in the world [2]. Some of them play beneficial roles to man [3, 4], but some cause to him significant economic losses [5]. In both cases, there are different termites considering their habitats and caste type. In recent years, there has been a large increase in the scientific literature concerning termites [6]. The control of species of termites with detrimental effects relies mainly on soil chemical termiticide applications, especially in African countries. But despite this reliance on chemical termiticides, termite control strategies need to conform to higher environmental standards [7]. In this regard, several research projects focus their activities on biological control methods which are environmentally safe. Among these methods is the use of entomopathogenic nematodes (EPNs). These nematodes have a worldwide distribution [8]. Apart from being environmentally safe, the use of EPN in pest control in general, and in termite control in particular, is rapid, sustainable, and cost effective. For the use of EPNs to control termites, different research methodologies are considered. But the results of these researches are sometimes controversial. This is related to the origin and biology of the species of the nematodes, to the type of termites, but also to the environment where the nematodes have been applied. Usually, newly described EPN species are first tested under laboratory conditions before best isolated are selected and tested under field conditions. Even under those conditions, methodologies used to evaluate the performance of the nematodes vary with researchers [9], leading to different results. The current review paper gives information on termites with focus on those with detrimental effects to man. It also discusses several methodologies commonly used to study the characteristics and also the performance of EPNs in the control of termites.

2. Termites

2.1. Classification and distribution of termites

Like ants, wasps, and bees, termites are social insects. They constitute 10% of all animal biomass in the tropics. Baker and Marchosky [10] divided termites into three general categories based on their habitat: dampwood, drywood, and subterranean termites. A typical termite colony contains larvae, nymphs, workers, soldiers, and reproductives, each type having its specific role inside the nest. Termites are grouped into seven families and 15 subfamilies [11], 281 genera [12], and over 3500 species identified worldwide [13]. Africa has the richest inter-continental diversity of termites with over 70% of all the identified species [14]. The family Termitidae alone comprises more than 664 African species grouped in four subfamilies: Apicotermitinae with 70 species [15], Termitinae with 272 species [16], Macrotermitinae with 165 African fungus-growing termites [17], and Nasutitermitinae with 56 species [18]. The total number of species of termites in the four subfamilies may surpass 90% of the world's known termite species [14]. These authors reported species richness and diversity (see below the formulas for their calculation) as a result of the friendly climatic conditions in Africa, and that, dry climate is a factor contributing for low numbers of termite species in some regions of the world. For example, termite species diversity is lower in Northern Africa compared

to Eastern, Western, and Southern regions of the continent [14]. Kemabonta et al. [19] also reported that termites are prominent in both tropical and subtropical ecosystems, but highest diversity is observed in tropical forests where they build very complex communities [16]. In recent years, there has been a large increase in the scientific literature concerning termites [6]. The different researches done on termites indicated their beneficial activities as well as detrimental effects to man.

Formulas are used to calculate termite species richness and diversity according to Ref. [19].

Termite species richness is calculated using the Shannon-Wiener diversity Index (H') as follows:

$H' = -\sum (P_i \ln P_i)$, where P_i is the proportion of individuals found in the i th species, while \ln is the natural logarithm.

Termite diversity D is calculated using the Simpson index as $D = \sum_{i=1}^S (-1)/N(N-1)$, where n_i is the number of individuals in the i th species, while N is the total number of entities in the dataset.

2.2. Beneficial activities of termites

Termites play a major role in peoples' lives, in physical as well as spiritual aspects [20]. Reis de Figueirêdo et al. [21] cataloged 43 species of termites, belonging to four families used in human diet and/or in livestock feeding and nine species used as a therapeutic resource. These authors registered termite use in 29 countries over three countries: Africa (19), America (5), and Asia (5). Authors of Refs. [4, 5] reported that termites are of highly nutritive value. Their soil is often eaten by pregnant women in Africa [20]. Termites also play a role as oracle, in superstitious beliefs, art, and literature [20]. Their mounds are often associated with the spiritual world, especially containing the spirits of ancestors. In agriculture, termites produce organic matter from dead wood and woody tissues of plants, thereby restore organic matter to the soil and to air, serve as ecological indicators [22]. They play significant role in subsistence agriculture as their mounds, with nutrient enriched soils, are incorporated into traditional cropping systems. Termite mound materials are also made hard and used to make roads, tennis court, and bricks used in buildings and are also source of pottery clay [23]. In this book chapter, we will focus on termites as pests and their control.

2.3. Detrimental activities of termites

More than 300 species of termites are known to be of economic importance [5] causing billions of dollars in damage worldwide. Since their food supply is mainly wood and woody tissues of plants, they feed on anything containing cellulose component including crop residues, mulches, and humus. They cause damage to agricultural crops such as cash crops and food crops [2], timbers in buildings, fences, clothes, books [24], removal of plant covers exposing soil surface to erosive forces [25]. They cause economic losses by directly injuring and destroying both living and dead vegetation and can damage right from sowing the crops till harvest [26].

Baker and Marchosky [10] reported drywood and subterranean termites as the most significant and costly termite pests. They feed on a wide range of living, dead, or decaying plant

material [16, 27], including the consumption and turnover of large volumes of soil rich in organic matter and fungi. These feeding habits make termites important ecosystem engineers, which over long periods of time can modify the physical properties of soil such as texture, water infiltration rates, and nutrient content [28]. They are among the most important insect pests in forests, and many destructive species live in the soil. For example, the forest termite *Coptotermes acinaciformis* causes more than 92% of total loss to Virgin *Eucalyptus pilularis*. In 2011, wood-eating termites consumed more than \$220,000 worth of Indian rupee notes [29].

In West Africa, several species of termites, including *Macrotermes bellicosus*, *Macrotermes natalensis*, *Coptotermes sjostedti*, and *Pseudocanthotermes militaris*, have been reported as general pests of living trees. The establishment of eucalyptus is limited by two termite species, i.e., *Ancistrotermes cavithorax* and *Amitermes evincifer* in drier areas of Ghana. In this country, termite attack of living trees is a potentially important problem facing the use of exotic forest species. In Nigeria, termite pest species of the genus *Macrotermes* are the most destructive to plants causing 5–18% yield losses [3]. Ten species of termites were found associated with citrus orchards in Benin: *Amitermes guineensis*, *Ancistrotermes crucifer*, *Angulitermes truncates*, *Coptotermes intermedius*, *Cubitermes* sp., *M. bellicosus*, *Microcerotermes progrediens*, *Pericapritermes* sp., *Trinervitermes occidentalis*, and *Trinervitermes trinervius* [30]. Among these, *M. bellicosus*, a fungus-growing termite, is the most important species that undermines citrus production and *T. occidentalis*, a grass-feeder termite, the most important to maize, cassava, groundnut, and bean grown under citrus canopies [31]. Abe et al. [2] also reported that the most troublesome termites in agriculture are the fungus-growing termites. In the absence of crop residues, mulches, and humus, these termites eat live plant material as groundnuts, millets, and maize. *Odontotermes erraticus*, *Macrotermes sibhyalinus*, *Amitermes evuncifer*, *Psammotermes hybostoma*, and *Microtermes lepidus* with a wide predominance of the *O. erraticus* were found ravaging cassava in Tivaouane, Senegal [32]. In South Africa, *Coptotermes* spp., *Cryptotermes* spp., and *Neotermes* spp. were observed undermining crop productivity [33]. But since termites make openings to the outside, farmers are aware of their presence only at an advanced stage of their invasion [34]. In regard of all this, the menace of termite activities is enormous. It is then important to bring these activities to a manageable level. For experimental purposes, termites are collected and used immediately or maintained for days before use.

2.4. Termite collection and maintenance

Termites are cryptic social insects. If some of them live in galleries made on the surface of wood products (examples of plant stems and trunks), some others live deep in the soil or inside wood products. Methods for collecting them will therefore depend on their habitat structures. Wang et al. [35] collected subterranean termites, *Reticulitermes flavipes* and *Coptotermes formosanus*, using cardboard bait buried in the field infested with termites. For the same type of termites, El-Bassiouny et al. [36] used El-Sebay's [37] modified trap. Baimey et al. [31] broke at the top nests made by *T. occidentalis* and *M. bellicosus* in citrus orchards to collect directly workers and soldiers of the termites. Alternatively, these authors covered broken nests with dried straws. The straws were left well colonized by termites for 3–4 h and then termites were easily collected. For experiments designed to evaluate the nest reconstruction

by termites following the break, it is advised to measure the height and surface denuded by the termites prior to breaking the nests.

Termites are usually collected in plastic containers, transferred to the laboratory where they are kept for given period of time before they are used for experiments. Authors of Refs. [31, 38] advised to put in the containers some moistened piece of paper as source of cellulose for the termites and also wet sand collected from termite nests. They also advised to keep the containers slightly open for aeration and in the dark at 25°C and 75–80% RH for 24 h before very active individuals are selected for experiments. El-Bassiouny et al. [36] rather kept termites at 25–28°C for 7 days in 9-cm diameter Petri dishes containing moistened corrugated cardboard before selecting active and vigorous individuals for use. Razia et al. [39] kept in the laboratory at 21–25°C workers of *R. flavipes* and *Odontotermis hornei* in plastic containers with 1–2 cm deep vermiculite sand and corrugated wood blocks added. Faye et al. [32] used sterilized soil (wetted soil heated to 80°C over a wood fire) on the surface of which vegetable debris was placed as culture media for *Odontotermes* spp.

2.5. Methods of control of termites

In response to the destructive activities of termites, man developed several preventative and remedial methods which are currently used against the pests [23]. Billions of dollars are spent annually throughout the world in this regard [26].

Chemical methods are practices frequently used against termites [40]. The methods rely on the use of synthetic chemicals such as dichloro diphenyl trichloroethane (DDT), benzene hexachloride (BHC), aldrin, dieldrin, soil barrier termiticides, dust and fumigant, treated zone termiticides [41]. These pesticides give quick control effects when they can reach termites but are costly, hazardous, and environmentally not safe. Therefore, despite this heavy reliance upon the application of chemical termiticides, future termite control technologies need to conform to higher environmental standards [7].

The most common nonchemical termite control method is the destruction of termite nests [42] because termites build epigeous mounds that affect cultivation and farm preparation [41]. This implies breaking and digging out the mound to reach and kill the reproductive queen and king of the nest [42]. But this method showed limitations as comeback is experienced after a period of time for some groups of termites that are capable of grooming new queen and king (*Cubitermes* and *Macrotermes*). Other nonchemical termite control methods include botanical termiticides [43], intercropping, crop rotation, planting of resistant crops [44], physical methods, i.e., debris removal, mechanical barriers, heat, high voltage electricity or electrocution, wood replacement, and biological control, i.e., use of predators [45], biological control agents such as fungi [46], bacteria, and nematodes [31, 35]. In a partial review, Myles [47] reported 2 viruses, 5 bacteria, 17 fungi, 5 nematodes, and 4 mites that have the potential to kill termites; the full list of these organisms being no doubt larger. But Weeks and Baker [48] reported that the behavior of termites affects the success of biological control. Lenz et al. [46] also reported that to be effective, biological control agents should be virulent, tolerate temperatures above 30°C, pose no health threats to man and higher animals, be easy mass produced and easy formulated,

applied, and stored. Lacey et al. [49] observed that fast host killing ability, increased environmental persistence, long shelf life, good fitness into integrated systems, acceptance by growers, and general public are also parameters to consider. This book chapter will focus on the use of entomopathogenic nematodes as biological control agents against termites of economic importance in agriculture.

3. Entomopathogenic nematodes

3.1. Classification and distribution of entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are soil-inhabiting microorganisms. They have been isolated from all continents (except the Antarctic) and from a wide range of soil habitats: fields, forests, grasslands, desert, and ocean beaches [50]. They have been described from more than 40 nematode families. But only the Steinernematidae and Heterorhabditidae families have received the most attention because they possess several attributes of effective biological control agents [51, 52]. The family Steinernematidae contains two genera, i.e., *Steinernema* with more than 100 species and *Neosteinerinema* with only one species, *Neosteinerinema longicurvicauda* as parasite of termites [53]. The family Heterorhabditidae contains one genus, *Heterorhabditis*, with more than 20 species. The list of EPN species described in the world being too long, we give here only those reported from Africa. In Africa, to our knowledge, EPNs have been observed in Algeria, Benin, Cameroon, Egypt, Ethiopia, Kenya, Morocco, Nigeria, Rwanda, South Africa, and Tanzania (**Table 1**).

3.2. Advantages of the use of entomopathogenic nematode

Entomopathogenic nematodes have several distinct advantages over other forms of pest control in that they have a broad host range are easy to mass produce *in vivo* and *in vitro* [83] and to store. The use of EPNs for insect pest control is a rapid, sustainable, environmentally safe, and cost-effective method [84]. The nematodes can be applied with standard spray equipment in open environment [83, 85]. They are effective against a number of insect pests that occur in cryptic habitats including termites, having a high degree of safety among vertebrates and other non-target organisms [86]. Also, they have the potential to recycle in the environment, are amenable to genetic selection for desirable traits, and are exempt from registration in many countries [86, 87]. They are compatible with many chemical pesticides: herbicides, fungicides, acaricides, insecticides, nematicides [88–91], azadirachtin [92], *Bacillus thuringiensis* products, and pesticidal soap [93]. They are also compatible with many biological pesticides [86, 87] and with some parasitoids [49, 94]. Synergistic interaction between EPNs and other control agents has been observed for various insecticides [95, 96] and pathogens [97, 98].

3.3. Characteristics of entomopathogenic nematodes

Species of EPNs of the genera *Steinernema* and *Heterorhabditis* are successfully used to control insect pests. The IJs of the nematodes (the stage used as biopesticide) live symbiotically

Country	Species of entomopathogenic nematodes	References
Algeria	<i>S. feltiae</i> <i>H. bacteriophora</i>	[54, 55] [55]
Benin	<i>Steinernema</i> sp, <i>H. sonorensis</i> and <i>H. indica</i>	[56, 57]
Cameroon	<i>H. amazoniensis</i> , <i>H. baujardi</i> , <i>S. cameroonense</i> and <i>S. nyetense</i>	[58]
Egypt	<i>H. indica</i> , <i>S. abbasi</i> , <i>S. carpocapsae</i> , <i>S. arenarium</i> <i>H. baujardi</i> <i>H. bacteriophora</i> , <i>H. taysearae</i>	[59] [60] [61]
Ethiopia	<i>S. ethiopiense</i> <i>H. bacteriophora</i> , and <i>S. yirgalemense</i>	[62] [63]
Kenya	<i>H. bacteriophora</i> , <i>S. arenarium</i> , <i>S. glaseri</i> <i>S. kari</i> <i>S. yirgalemense</i> , <i>S. weiserii</i> , <i>H. taysearae</i> <i>H. indica</i>	[64] [65] [66] [67]
Morocco	<i>S. feltiae</i>	[68]
Nigeria	<i>S. feltiae</i>	[69]
Rwanda	<i>S. carpocapsae</i> and <i>H. bacteriophora</i>	[70]
South Africa	<i>S. citrae</i> ; <i>S. khoisanae</i> ; <i>S. yirgalemense</i> , <i>H. zealandica</i> and <i>H. bacteriophora</i> <i>S. khoisanae</i> and <i>H. bacteriophora</i> <i>H. safricana</i> <i>S. beitlechemi</i> <i>S. fabii</i> <i>S. innovationi</i> <i>S. jeffreyense</i> <i>S. sacchari</i> <i>S. tophus</i> <i>H. noenieputensis</i> <i>S. nguyeni</i>	[71] [72] [73] [74] [75] [76] [77] [78] [79] [80] [81]
Tanzania	<i>S. pwaniensis</i>	[82]

S. = *Steinernema*; *H.* = *Heterorhabditis*.

Table 1 Species of entomopathogenic nematodes isolated in Africa.

with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively [99]. They are nonfeeding and the only stage observed in the soil. They rely solely on energy reserves for survival and infectivity [100]. Their efficacy in the control of insect hosts is dependent on their attack strategy, survival, and persistence [101]. They use “sit and wait” (ambush foragers = most *Steinernema* nematodes), cruise (most *Heterorhabditis* nematodes), or intermediate foraging (some *Steinernema* nematodes) strategies to attack their insect hosts. Once inside the host haemocoel, the IJs of the nematodes release their symbiotic bacteria which proliferate and kill the host by septicemia within 48 h postinfection. Proliferated bacteria serve as source of food for the nematodes [102]. Also, these bacteria protect the host cadaver from colonization by other microorganisms including late arriving nematodes. Zhou et al. [103] reported that bacterial

products from both *Xenorhabdus* and *Photorhabdus* make the infected insect repellent to ants. Fenton et al. [104] observed the protection of *Heterorhabditis bacteriophora*-infected cadavers from the avian predator, the European robin *Erithacus rubecula*. The authors reported that this protection was attributed to the red color reinforced by unpalatable taste of the cadavers and that the fact that the birds did not need to bite cadavers to reject them implies that some deterrent factor is emitted through the cadavers' cuticles. Thus, it is a nematode/bacterium complex that works together as a biological control unit to kill an insect host [85]. Insect susceptibility to EPN varies with insect species and is influenced by nematode species and strain [48]. Good knowledge of the IJs of EPNs and also of the relationships between IJs-insect-bacteria will allow increasing efficacy of treatment used to limit populations of pests [101]. Several researches are done in this regard using different protocols. The overall objective of these researches is to minimize pest populations to reduce losses they caused to crops. In countries where EPNs are observed and identified for the first time, researches usually start with the study of their biology under environmental extreme conditions in laboratory. This allows predicting which nematode isolate or species to use in target areas where environmental stress is expected.

3.4. Environmental stresses and their effect on the performance of entomopathogenic nematodes and their symbiotic bacteria

Authors of Refs. [48, 105] reported that the prevalence of infective juveniles (IJs) of EPNs in different habitats is affected by both intrinsic (behavioral, physiological, and genetic characteristics) and extrinsic (antibiotics, competition, natural enemies, temperature, soil moisture, pH, soil type, soil texture, relative humidity, UV radiation, and desiccation) factors. For experimental purposes, performance of EPNs is known by studying their ability to withstand conditions of drought, lack of oxygen, tolerance to heat [38, 106], capacity to search for targeted pests in the soil at specific concentration [107], to kill them, and to multiply inside them. The nematodes' tolerance to biotic factors is also studied under laboratory conditions. Most of the experiments designed in this regard are conducted using the larvae of the greater wax moth *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), a model insect for EPN biology and pathogenicity studies [108]. Nematode isolates that perform best under laboratory conditions are then taken to semi-controlled and fields conditions [31, 36, 45] and tested against insect pests in biological control programs [31, 36, 106, 109, 110]. Grewal et al. [111] observed greatest performance of indigenous EPN isolates as compared to exotic ones for the control of insect pests for being used in their natural environment.

To evaluate the tolerance of IJs of indigenous EPNs to environmental stresses, the nematodes are subjected to temperatures varying between -5 and 40°C [38, 112–116], to hypoxia [38, 117], to dehydration/desiccation for up to 75% RH [118, 119], and to ultraviolet radiation stress (for example, at 340 nm, [120]). The persistence or longevity of indigenous EPN species in the soil [121], their genetic improvement, their infectivity [118, 119, 122], trehalose content/accumulation [123], motility, development, virulence, and reproduction inside insect hosts [124] under environmental stresses are some traits that are often evaluated. Antagonists [125], soil type [126], cultural conditions [127], and nematode species of strain [128] also affect nematode

survival in soil. Studies on the symbiotic bacteria of EPNs include evaluation of growth and virulence of the bacteria under heat and cold temperatures [124]. All these different experiments are not only conducted mainly under laboratory [38, 106, 109, 118, 119] but also under greenhouse and field conditions [129, 130] either in open environments or in the dark [124].

The results from the different experiments are controversial and show variations for the potential of the nematode IJs to tolerate environmental stresses. This could be explained by differences among species and a great variability within species of EPNs, insect hosts, and also environmental stresses used in different experiments. Authors of Refs. [51, 131] reported moisture, temperature, foraging strategy, and pathogenicity for the targeted insect as the four most critical factors. Under adequate range of temperatures and moisture and with a susceptible host, EPNs with cruiser and intermediate foraging strategies are suitable for use in subterranean and certain aboveground habitats (foliar, epigeal, and cryptic habitats), while ambushers will be most effective in cryptic and soil surface habitats [132]. Authors of Refs. [122, 133] reported that temperatures of *ca.* 15–30°C provide highest and most stable survival (more than 95%) to nematodes' IJs than temperatures of *ca.* –5 to 10°C which reduce the nematodes' movement. Shapiro-Ilan et al. [134] reported significant contribution of the ability of EPNs to tolerate freezing conditions (–2°C for 6 or 24 h) to their biological control efficacy. But these authors did not observe any relationship between freezing and desiccation tolerance. This observation did not corroborate that of Solomon et al. [135] and Grewal et al. [136] who reported that tolerance to cold and desiccation is related in EPNs and that both stress factors cause an increase in trehalose levels, which is implicated as a physiological protectant. At high temperatures of *ca.* 35–40°C, nematode physiological activity is high, increasing the consumption of its stored energy and resulting in limited shelf life [112] and low searching [137] and pathogenicity [138] potential of the nematodes. Hang et al. [124] observed nematode IJs' development to adult at 13, 18, 24, 30, and 35°C and progeny production at 18, 24, and 30°C but not at 13 or 35°C. Zadji et al. [38] evaluated heat tolerance of 29 Benin isolates of *H. sonorensis* and one of *Heterorhabditis indica* under laboratory conditions using a method modified from Ref. [139]. Nematodes were subjected to 40°C for 2, 4, 6, and 8 h while being shaken at 70 rpm. The greatest survival of infective juveniles to heat (8 h), desiccation (8 h), and hypoxia (72 h) was observed with *H. sonorensis* isolates (72.8, 72.5, and 81.5%, respectively). Desiccation is important to conserve nematode IJ energy and improve their shelf life [140]. However, dehydration presents many challenges including difficulty in application because the carriers can block spray nozzles [141]. Genetic improvement of *H. bacteriophora* in beneficial traits as heat and desiccation tolerance by cross breeding and genetic selection is also reported. An overall increase in mean heat tolerance of 5.5°C by cross breeding five strains of the nematode species has been observed. But this enhanced heat tolerance and also tolerance to desiccation are often lost again during mass production. Fortunately, for *Heterorhabditid* nematodes, methods have now been developed to stabilize the traits by selection of tolerant inbred lines. This technique provides a pathway to genetic improvement of commercial strains which will maintain the improved characters also during *in vitro* mass production. For *Steinernematid* nematodes in contrast, the technique needs more investigation as these nematodes are amphimictic and production of inbred lines is much more laborious. Shapiro-Ilan et al. [139] reported that the effect of hypoxia on nematode IJs' survival varied significantly

with duration of exposure of the nematodes to stressed conditions and with nematode isolates from 33.2 to 81.5% and from 85.9 to 96.9% after 24 and 72 h of exposure, respectively. Entomopathogenic nematodes are sensitive to UV light. This is why they are usually applied to protected environments, particularly soil [86, 142]. But extended persistence of nematode IJs in the soil results in greater cumulative insect host mortality and reduced need for multiple nematode applications.

3.5. Mass production of entomopathogenic nematodes for laboratory and field application

Before EPN isolates with desirable characteristics such as tolerance to environmental stresses and virulence to insect hosts are used for experiments or for commercialization [143], they are cultured *in vivo* or *in vitro* at a small scale [144] or at a large scale [145].

3.5.1. *In vivo* production

For laboratory use and small-scale field experiments, *in vivo* production of EPNs appears to be appropriate method. Though various caterpillars and large beetle larvae are very susceptible insects to EPNs, for most laboratories and some field experiments, EPNs are mostly reared in last instar larvae of the greater wax moth, *G. mellonella* as described by Kaya et al. [144]. The larvae of *G. mellonella* can be produced using an artificial medium containing 22% ground wheat, 22% ground maize, 11% honey, 11% glycerol, 11% milk powder, 5.5% yeast extract, and 17.5% bee wax in a glass jar at 25°C in the laboratory [146]. The larvae of this insect are preferred because they are very susceptible to the nematodes and very easy to mass rear, they are commonly sold as fish bait. Nematode-infected larvae are incubated for around 72 h at 25–27°C before being transferred onto White trap. Hundreds of thousands of IJs of the nematodes emerge from infected *G. mellonella* larvae as progeny in few days [31, 109]. Emerged nematodes are collected [36, 39, 106] and used immediately [31] for experiments. They may be stored in tissue culture flask at 13°C [36, 115, 147] or at 19°C [39] and are used within 5 days [36, 39] or 2–6 weeks [109, 145] after collection. Though *in vivo* production of EPN is simple, reliable and results in high quality nematodes, the method is labor intensive and costly.

3.5.2. *In vitro* production

In vitro method of nematode production is used when large-scale production is needed at reasonable quality and cost. Two methods are used for *in vitro* production of EPN, i.e., solid media and liquid fermentation [148, 149]. The first method uses crumbed polyether polyurethane foam coated with a nutritive medium and inoculated first with symbiotic bacteria and then with nematodes. This method requires limited experience, its capital costs are low and logistics of production is flexible. The liquid fermentation method has the lowest mass production cost and is used by large companies with multiple products. The method relies on suitable medium composed of yeast extract as nitrogen source, a carbohydrate source as soy flour, glucose, or glycerol, lipids of plant or animal origin and salts and requires adequate oxygen [150, 151]. The following EPN species have been successfully produced using liquid fermentation method with yield capacity as high as 250,000 infective juveniles/ml: *Steinernema*

carpocapsae, *Steinernema riobrave*, *Steinernema kushidai*, *Steinernema feltiae*, *Steinernema glaseri*, *Steinernema scapterisci*, *H. bacteriophora*, and *Heterorhabditis megidis* in 7500–80,000 liter bioreactors. Ehlers [152] reported that industrial-scale *in vivo* EPN production is applicable in developing countries and the large-scale *in vitro* production best suited for countries with low labor costs or for serving high value markets.

3.6. Storage and formulation of entomopathogenic nematodes

When nematodes are not to be used immediately, they are kept in appropriate conditions for a while to avoid their deterioration. Several methods are used to store EPNs for extended periods or to formulate them immediately following their mass production. But before they are stored or formulated for successful control of insect pests, the quality (i.e., viability based on their movement, energy reserves, and infectivity) of IJs of the nematodes is checked. Authors of Refs. [100, 153] reported the one-on-one (one nematode to one *G. mellonella* larvae) sand-well assay. The energy reserves (dry weight and total lipid content) are predictors of nematode longevity. Because each nematode species has its specific requirements for temperature, moisture and oxygen [112, 140, 154, 155], it becomes difficult to obtain a formulation or storage condition suitable for all EPN species. Nematodes are stored on moist sponge, in formulations that contain alginate, vermiculite, clays, activated charcoals, polyacrylamide, and water dispersible granules or are partially desiccated in water dispersible granules [88, 149]. To be successful, any formulation method should consider reducing nematode metabolism by immobilization or partial desiccation. *Steinernema* species can be stored in aqueous suspension for 6–12 months at 4–15°C, while *Heterorhabditis* species can be stored only for 3–6 months at the same temperature. Partially-desiccated infective juveniles in water dispersible granules have a shelf life of 5–6 months for *S. carpocapsae* at 25°C and 2 months for *S. feltiae*, and 1 month for *S. riobrave* [156] at the same temperature.

3.7. Quality control of entomopathogenic nematodes

Before EPNs are used in the laboratory or in the field after being stored or formulated, they are acclimated at room temperature of *ca.* 25°C for 2 h. Their quality is then checked again, and their concentrations to be used in experiments are adjusted by volumetric dilutions in distilled water using the formula as given in Ref. [157].

4. Control of termites using entomopathogenic nematodes and their symbiotic bacteria

Authors of Refs. [158, 159] first reported the presence of parasitic head inhabiting nematodes in the termites *Reticulitermes lucifugus* and *C. formosanus*. But only 40 years later, Tamashiro [160] first proposed the use of nematodes against termites. Control of the pest based on the use of EPNs became a promising technology for future termite control option. Since then, a plethora of laboratory and in some extent field research efforts resulting in subsequent publications on biological control of termites have been observed [83, 161–164].

4.1. Control under laboratory conditions

Several experiments showed the effectiveness of EPNs to control termites under laboratory conditions. In the laboratory, bioassays with termites and EPNs are usually carried out in containers such as Petri dishes lined with wet filter paper or sterile sand [39, 109], PCV tubes, or Eppendorf tubes [109]. In all cases, piece of filter paper [165], straw [106], and also corrugated wood blocks [39] are usually used in the containers to serve as food for termites [48]. Nematodes strains used for inoculations are usually selected from a number of strains based on their greater virulence to *G. mellonella* larvae [39]. Selected strains are then mass reared [144] to have sufficient inoculums. Each container receives given population densities of nematodes, most of the time in the form of water suspension with appropriate water volumes. In the case of low population densities, nematodes are transferred into the containers using micropipettes [165].

According to the objectives of the experiment, termite caste (reproductive adults, soldiers, or workers) or developmental stage (larvae, nymphs, and adults) is selected and transferred into containers following nematode introduction [36]. Host-finding ability and nematode virulence (ability of the nematodes to kill their host and to produce offspring inside them) are recorded. Nematode mortality is recorded daily or at given intervals of time following inoculation to evaluate lethal dose (LD_{10} , LD_{50} , or LD_{90}) and lethal time (LT_{10} , LT_{50} , or LT_{90}). Insects that are killed are dissected 48 h postinoculation in Ringer solution under stereo-microscope to confirm parasitism and to record population density of infecting nematodes inside each termite and developmental stage of the nematodes. Also, part of termites killed by the nematodes is transferred to White traps (i.e., emerging from hosts and accumulating in water) for days to evaluate nematode progeny production [39, 109]. Because termites are very fragile, some usually die naturally during the course of the experiments. In this case, insect mortality data are corrected using the following formula of Ref. [166]: $M_c = [(M_o - M_c') / (100 - M_c')] \times 100$, where M_c = corrected mortality, M_o = Mortality caused by the nematodes, M_c' = Mortality observed in control treatments.

Wang et al. [35] showed that *S. carpocapsae* and *H. bacteriophora* were effective against workers of the subterranean termite *R. flavipes* under laboratory conditions. The same authors also reported that *H. indica* was more efficient than both *S. carpocapsae* and *H. bacteriophora* against *R. flavipes*. Razia et al. [39] studied in sand assay method the virulence of *S. siamkayai*, *S. pakistanense*, and *H. indica* applied at 100, 250, 300, 500, 700, and 900 IJs/ml suspension on workers of subterranean termites, *R. flavipes* and *O. hornei* (25 termites/Petri dish). The authors observed positive relationship between concentration and exposure time and mortality and variation between nematode and termite species for LD_{10} , LT_{50} , and LT_{90} . El-Sebay et al. [36] conducted similar experiment using Egyptian isolates of *Heterorhabditis baujardi* and *H. indica* to control *Psammotermes hypostoma* and *Anacanthotermes ochraceus* under laboratory conditions. The authors observed LC_{50} and LC_{90} values of, respectively, 15.03 and 361.53 for *P. hypostoma* and *H. baujardi* and 20.26 and 398.59 for *H. baujardi* and *A. ochraceus* at day 3 after inoculation. For the experiment, highest rate of insect mortality was observed at day 3 after inoculation. Zadji et al. [38] tested in 2-ml Eppendorf tubes (each with 50 nematodes and 1 insect) the pathogenicity of 29 Benin isolates of *H. sonorensis* and one *H. indica* against workers

of *M. bellicosus*. The results of the experiment showed that 73% of the nematode isolates killed more than 80% of the insects. In another study, Zadji et al. [106] evaluated the differential susceptibility of workers and soldiers of two termite species, *M. bellicosus* and *T. occidentalis*, to four Benin isolates of EPNs: one *H. indica*, two *H. sonorensis*, and one *Steinernema* sp. (5, 10, 25, 50, or 100 nematodes/well of tissue culture plates with one insect). A significant difference in termite mortality was recorded between termite castes but not between EPN isolates and termite species. Soldiers of both *M. bellicosus* and *T. occidentalis* were similarly susceptible but more susceptible than workers. The LD₅₀ varied with termite species and nematode isolates from 12 IJs (*T. occidentalis* with *Steinernema* sp.) to 23 IJs (*M. bellicosus* with *Steinernema* sp.).

The reproduction potential of EPNs inside termites varies not only with nematode species but also with termite species and caste. Zadji et al. [109] observed up to 20,213 *H. sonorensis* IJs per worker of *M. bellicosus* 10 days postinoculation with an average of six nematodes penetrating each insect. Wang et al. [35] similarly, but in much lower population densities, observed an average number of IJs of 289 ± 50 and 642 ± 93 per worker, respectively, produced from *R. flavipes* and *C. formosanus* (based on 11 and 8 workers, respectively). The nematodes were seen through the cuticle of dead termites 4–5 days postinoculation, and they began to emerge at day 5 after infestation. The authors concluded that EPNs have the potential to continue their infestation to termites after an initial treatment. But in the same experiments, they observed consumption of some nematode-killed termites by healthy termites or by a saprophagous mite, *Australhypopus* sp. This mite is very common on the body of *R. flavipes*, especially on the head. Once the termite dies, the mite reproduces quickly in large numbers and feeds on the dead termite. The consumption of dead termites by healthy ones and also by *Australhypopus* sp. is a cause for the failure of nematode recycling in termites.

Some others experiments are designed to evaluate the potential of the nematodes' symbiotic bacteria to kill termites or to evaluate the efficacy of combined effect of nematodes with other insect control methods on termites. *H. bacteriophora* and their associated bacteria were found to be effective against workers and nymphs of six different species of termites: *C. formosanus*, *Gnathamitermes perplexus*, *Heterotermes aureus*, *P. hybostoma*, *R. flavipes*, and *R. virginicus*. Meanwhile, *H. indica* and *Photorhabdus luminescens* complex were found to be effective against three species of termites: *C. formosanus*, *C. vastator*, and *R. flavipes*. *S. carpocapsae* together with their symbiotic bacteria, *X. nematophila*, are capable of suppressing population of eight different termite species including *C. formosanus*, *C. vastator*, *G. perplexus*, *H. aureus*, *P. hybostoma*, *R. flavipes*, *R. virginicus*, and *Zootermopsis angusticollis*.

Two-container choice device is used to evaluate the repellency of nematodes to termites [35] as described by Mauldin and Beal [167]. Wang et al. [35] used this method to study the repellency of four EPNs: *S. carpocapsae*, *Steinernema riobrave*, *H. bacteriophora*, and *H. indica* to two subterranean termites: *R. flavipes* and *C. formosanus*. *H. indica* repelled termites at high concentrations (90 nematodes/cm³ and above) in sand and vermiculite medium. The length of repellency varied (from 3 to 17 days postinoculation) with the nematode concentration and the size of the device used for the experiment. Similar experiment was conducted by Zadji et al. [106] with Benin nematode isolates: one *H. indica*, two *H. sonorensis*, and one *Steinernema* sp. (962.5 nematode IJs/cm³ of 40 cm³ sterilized sand) and termite species, *M. bellicosus* and *T.*

occidentalis. The experiment did not show evidence that *M. bellicosus* and *T. occidentalis* would be able to detect the presence of IJs of any EPN isolates. However, it was observed that nematode dispersal occurred by infected termites or phoresis.

The results of these experiments showed that, usually, under laboratory conditions, pathogenicity of nematodes to termites is certain as the host contact is certain, environmental conditions are optimal and no ecological or behavioral barriers to infection exist [168]. But under field conditions, successful termite control using nematodes is less certain.

4.2. Control under field conditions

In the world in general and in Africa in particular, field studies on the use of EPNs to control termites are limited [31, 169]. The few studies compared the effects of various formulations and methods of applications of the nematodes on the mortality of different casts and life stages of termites evaluated the performance of different nematode isolates on the progress of termite nests' reconstruction, the persistence of the nematodes in the nests, the percentage of nests for which the underground termite populations died, and the progeny production of the nematodes inside their host [31]. The ability of termites to detect the nematodes and to avoid them [48], and the overall behavior of termites following colonization of the nests by nematodes were also studied. The advantage of the use of EPNs over other methods, especially over chemical methods, for the control of termites under field conditions, is the capacity of nematodes to reach cryptic habitat of termites, difficultly reachable by chemical pesticides; termites live in an environment conducive to nematodes. A wide range of EPNs were identified in this regard as effective against various termite species [26, 31] under field conditions. But the nematode formulation used affects the success of the pest control.

Nematode water suspension (i.e., nematodes in water) or nematode-infected *G. mellonella* larvae are two nematode formulations mostly used in the fields to control termites [31, 46, 83]. In the case of nematode suspension, the nematode inoculum is applied using common nozzle type sprayers (hand and ground sprayers) with openings as small as 100 µm in diameter and with pressure of up to 1068 kPa on nematodes [170] in the field or using simple water cans on small areas against termites [171] successfully controlled *Neotermes* sp. associated with coconut palms and citrus trees. Meanwhile, Lenz et al. [172] injected *Heterorhabditis indicus* into cavity of mahogany tree against *Neotermes* sp. Also, Gouge [83] and Lenz [46] injected nematodes in tree trunks to control *Mastotermes darwiniensis* using *Heterorhabditis* sp. But the authors reported that the success of termite control is affected by the plant structure. For example, it is difficult to apply nematodes to the entire termite colony of branched trees, where termites find refuge in untreated branches of the plant.

For successful termite control, especially with nematode-infected *G. mellonella* larvae, the aboveground termite nests are first demolished before nematode suspension or infected *G. mellonella* larvae are applied. Baimey et al. [31] applied 52-week-old EPN-infected *G. mellonella* larvae per nest, each larva containing ca. 200,000 of IJs of Benin isolates of *H. sonorensis* and *H. indica*. At day 70 after inoculation, the underground populations of 71 and 60% treated nests were controlled by *H. sonorensis* and *H. indica*-infected *G. mellonella* larvae, respectively. When applied in infected *G. mellonella* larvae, nematodes will be protected for a while against

environmental stresses before emerging from the larvae and will certainly provide superior termite control as compared to nematodes applied in water suspension which will be rapidly affected by environmental stresses soon after their application.

Termite workers are able to reconstruct their nest after this is broken. Baimey et al. [31] reported that nest reconstruction as measured by the nest reconstruction rate (see formula below) differed significantly among nematode isolates and time of exposure of inocula to termites with significant correlation between the two parameters. The nest reconstruction rate (NRR) is estimated as followed: $NRR = (V_n/V_0) \times 100$, where V_n is the aboveground reconstructed nest volume n days after application of EPN-infected *G. mellonella* larvae and V_0 the volume of the aboveground nest before its demolition. The nest volume is calculated using the formula to calculate the volume of a cone, $V = 1/3\pi \times R^2 \times h$, where R (m) is the nest radius and h (m) the nest height.

Even though termite nest can be reconstructed after being broken or after nematode application, nematode persistence in the nest area is necessary to avoid frequent breaks and also frequent applications of the nematodes for successful control of termites. Nematode persistence in the nest can be assessed by randomly taking soil samples from treated nest at intervals of days and by baiting the samples with last instars *G. mellonella* larvae. Baimey et al. [31] took nest samples 10, 20, and 70 days postinoculation. The samples were baited with *G. mellonella* larvae for a week at $25 \pm 1^\circ\text{C}$ and dead larvae recorded daily from the 5th day to the 7th day. Cadavers of *G. mellonella* larvae were dissected to confirm EPN infection. Susurluk et al. [173] stated that the number of infected larvae found by sampling is related to the number of nematodes that were present in the soil.

Authors of Refs. [48, 174] reported an ability of some termite species (example of *Reticulitermes tibialis*) to detect EPN and avoid them or to detect other termites that have died from nematode infection. Nematode-killed individuals are walled off to avoid or reduce contamination to other individuals in the nest [175, 176]. Authors of Refs. [169, 177] stated that though nematodes appear to have a limited impact on subterranean and dampwood termites due to termite behavioral defense mechanisms, they successfully control drywood termites where colonies are contained within a single piece of wood or a single tree. Similarly, Fujii [178] reported that this walling-off behavior of certain termite species does not prevent the dispersal of nematodes inside the occlusion as, at least, nematodes that were produced from partially or loosely buried termites are often observed outside the occlusion. Wang et al. [35] reported a repellence of EPNs to termites and concluded that the repellence might be the main reason for the ineffectiveness of nematodes to termites in certain field experiments. Therefore, it is important to consider the species of termites before selecting the EPN isolates.

5. Limitations in the use of entomopathogenic nematodes as biological control agents of termites

The various information given in this chapter indicated that EPNs provides some successful control of termite. But the method presents some limitations that should be taken into account.

An example of limitation is the high nematode population densities needed for successful control of termites: approximately 23,000 infective juveniles of *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* nematodes are required to treat one square foot of termite infested area. Authors of Refs. [179, 180] stated that for successful control of drywood termites, all portions of the gallery system need to be located and treated. Nematodes were effective on the dampwood termite in the genus *Neotermes* infesting unbranched trunks of coconut palms, but their effectiveness was inferior in branched trees of citrus, cocoa, or mahogany [172]. The high numbers of termites in a nest, the wide foraging range of termites, the limited mobility of nematodes, the low reproduction rate of some nematode isolates in dead termites, and the repellence of some nematodes to termites are other examples of limitations for successful control of termites by nematodes, especially in field conditions. In this regard, Wang et al. [35] advised inundative release of EPNs rather than classical biological control for the control of subterranean termites. To increase termite susceptibility to EPNs, some researchers refer to sublethal doses of chemical termiticides, other biological control agents as fungi [181, 182] and bacteria [98] and imidacloprid [95, 183, 184] in an integrated pest management programs. However, this method encounters the problem of delivery of those insecticides to termite individuals at a distance from the application site [185]. Moreover, the method needs to provide cost effective protection against termite damage [35]. Temperatures of above 30°C in the center of the nests of *Coptotermes* species, where reproductives and brood are housed, are lethal for the nematodes. This means that different isolates or species of EPNs that are tolerant to higher temperatures are required for subterranean termite species. The diffuse nest system, the presence of multiple sets of reproductives, large territory size, and simultaneous use of many feeding sites also make the successful control of some termite species using EPNs difficult. Weeks and Baker [48] reported that the nematodes must be placed in environments congenial to their survival or they prove useless for control. More studies are then needed on these limitations for easier and better control of termites using EPNs.

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