Chapter 9
Microbes in Termite Management: Potential Role and Strategies

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Contents

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Abstract Several control methods like physical, chemical, and biological are adopted to control termites in various localities. Biological control methods are eco-friendly and target-specific; hence they could represent a suitable alternative to chemical control methods. Microbial biological control is based on the use, and proper adjustment, of natural enemies via microbial organisms, such as bacteria, fungi, virus, and nematodes with the aim of suppression and management of insect populations. A broad range of species, from different groups of microbial organisms, have strong association with termites, and some have been recorded as parasites, including species currently used as commercial biological control agents.

Keywords Biological control • Termite • Management • Microorganisms

9.1 Introduction

During the last three decades, research on alternative measures in termite control has increased. Various control methods like physical, chemical, and biological are effective in management. Although chemical insecticides are effectively used against termites, they are environmentally hazardous. Furthermore, the cryptic life style of termites makes their direct application difficult. Biological control agents appear as effective, eco-friendly, economically viable, and socially acceptable methods of management. Biological control is generally perceived as both providing more permanent insect control and as having less potential damage to the environment or nontarget organisms. Therefore, biocontrol should be considered as a long-range research goal rather than an immediate solution.

Recent prominences on biological processes to improve agricultural productivity are essential for sustainability in agriculture system. The shortcomings associated with conventional chemical control methods have prompted policy makers and scientists to evaluate the potential for natural enemies to suppress termite populations. Brazil has a history of success with biological control projects involving the use of insect pathogenic fungi and viruses (Campanhola et al. 1995). The use of biological control agents to hunt or to infect termites within their hidden galleries is appealing. Reviews by Grace (1997), Culliney and Grace (2000), and Verma et al. (2009) on biological control strategies for the suppression of termite or on biological alternatives for their control provide complete knowledge for nonchemical approaches.

The use of naturally occurring pathogens offers unique advantages over chemically based termiticides. The study of pathogens for termite control started as early as 1965 (Yendol and Paschke 1965). Since then there has been renewed interest in using pathogenic organisms, such as bacteria, viruses, nematodes, and most fungi for controlling termites in recent years. Several lineages of pathogenic fungi, such as *Beauveria*, *Metarhizium*, *Aspergillus*, and *Entomophthora*; bacteria, such as *Bacillus*, *Serratia*, *Pseudomonas*, *Photorhabdus*, and *Xenorhabdus*; and nematodes, such as genera *Heterorhabditis*, *Steinernema*, and *Neosteinernema*, have been reported as termite biocontrol agents (Kanzaki et al. 2010; Muralidhara et al. 2013).

Development of recombinant DNA techniques has made it possible to significantly improve the insecticidal efficacy of some microorganisms (Inceoglu et al. 2006; Wang and St. Leger 2007a, b, c). These new types of biological insecticides offer a range of environment eco-friendly options for cost-effective control of insect pests (Federici et al. 2008).

Phylogenetic sequence of termites is depicted in Fig. 9.1 with the Termitidae (the largest termite family) as responsible to damage most of crops (Fig. 9.2). The present chapter reviews the biological methods for termite management, focusing on its microbial share. The relationship between termites and microbes and their possible contribution toward termiticidal activity is reviewed and discussed.

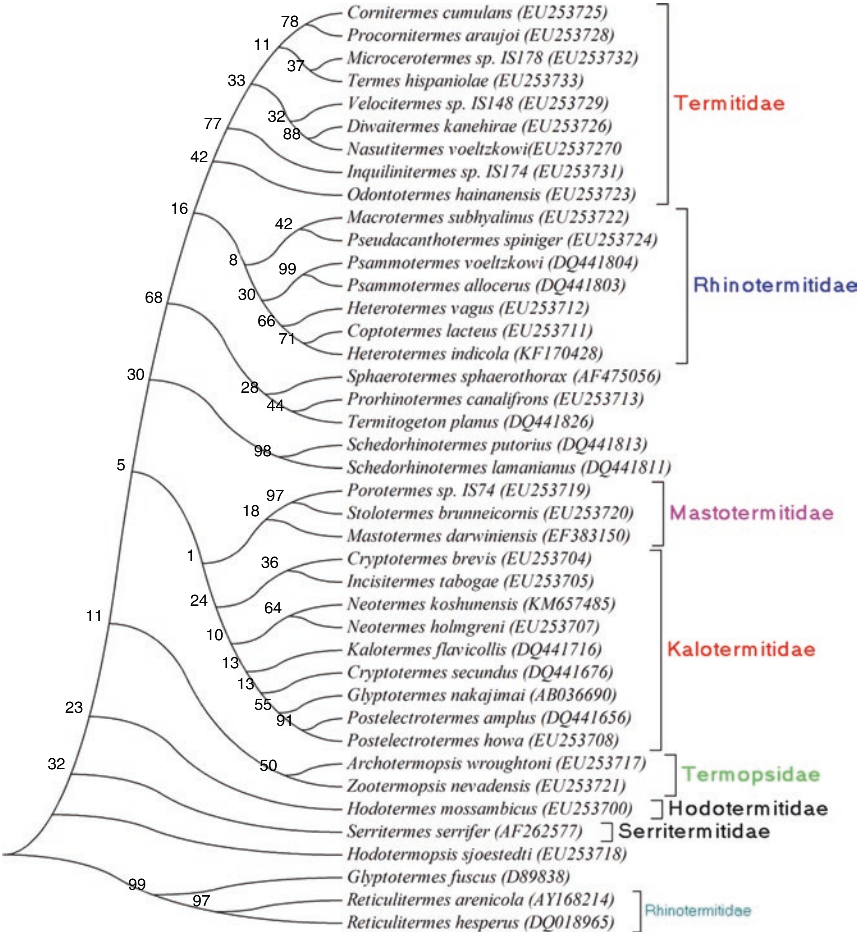

Fig. 9.1 Phylogenetic characterizations of termites, using 12S rRNA gene sequences obtained from NCBI GenBank databases. The sequence alignment was performed using the CLUSTAL W program, and tree was constructed using maximum likelihood method with algorithm using MEGA6 software

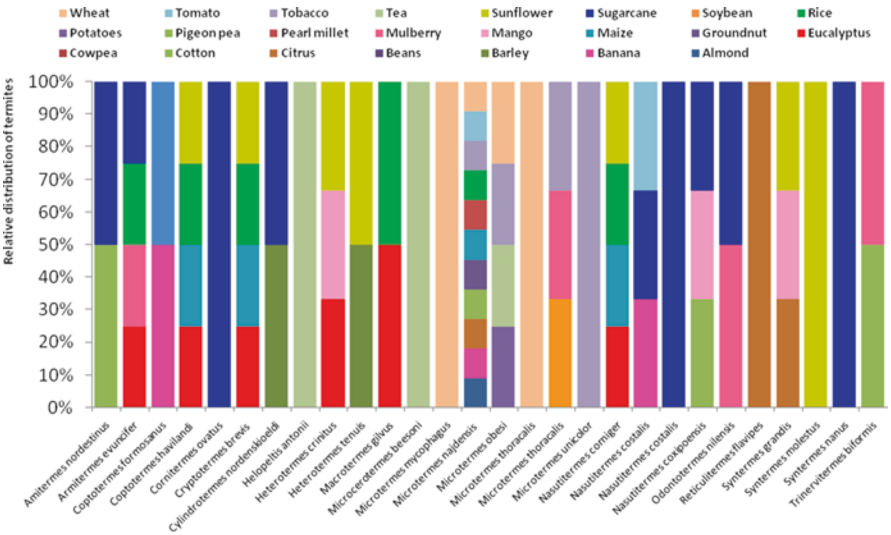

Fig. 9.2 Relative distributions of termites affecting different tropical crops (See for almond: Faragalla and Al Qhtani 2013; banana: Lai et al. 1983; Faragalla and Al Qhtani 2013; citrus: Stansly et al. 2001; Faragalla and Al Qhtani 2013; cotton: Wood et al. 1987; eucalyptus: Constantino 2002; Groundnut: Wood et al. 1987; Faragalla and Al Qhtani 2013; maize: Wood et al. 1987; Constantino 2002; Faragalla and Al Qhtani 2013; pearl millet: Wood et al. 1987; Faragalla and Al Qhtani 2013; Rathour et al. 2014; rice: Constantino 2002; Agunbiade et al. 2009; Maayiem et al. 2012; Togola et al. 2012b; Tomar 2013; Acda 2013; Oyetunji et al. 2014; sugarcane: Constantino 2002; Ahmed et al. 2007; Alam et al. 2012; sunflower: Aslam et al. 2000; Ashfaq and Aslam 2001; Sileshi et al. 2009; tea: Ahmed 2012; Singha et al. 2011; Singha et al. 2010; tobacco: Wood et al. 1987; Shah and Shah 2013; tomato: Wood et al. 1987; Pearce et al. 1995; Faragalla and Al Qhtani 2013; wheat: Wood et al. 1987; Sharma et al. 2004; Ahmed et al. 2004; Pardeshi et al. 2010; Rathour et al. 2014)

9.2 Damage in Tropical Crops

Termites are economic pests in tropical and subtropical environments where they destroy crops, forests along with wood and wooden products of human buildings (Meyer et al. 1999). Most tropical crops are susceptible to their attacks worldwide, including maize (Constantino 2002; Faragalla and Al Qhtani 2013), barley (Kharub and Chander 2012), beans (Sileshi et al. 2009), chickpea and citrus (Stansly et al. 2001; Faragalla and Al Qhtani 2013), cotton (Wood et al. 1987), cowpea (Mohammed et al. 2014), pigeon pea (Reddy et al. 1992), groundnut (Wood et al. 1987), pearl millet (Rathour et al. 2014), potatoes (Tomar 2013), rice (Agunbiade et al. 2009; Oyetunji et al. 2014), soybean and sugarcane (Ahmed et al. 2007; Alam et al. 2012), sunflower

(Ashfaq and Aslam 2001; Sileshi et al. 2009), tomato (Pearce et al. 1995), wheat (Ahmed et al. 2004; Pardeshi et al. 2010; Rathour et al. 2014), tea (Singha et al. 2011), tobacco (Shah and Shah 2013), eucalyptus (Faragalla and Al Qhtani 2013), mango (Tomar 2013), mulberry (Ahmed and Qasim 2011), and almond (Faragalla and Al Qhtani 2013) (Table 9.1).

Table 9.1 Crops attacked by termites and their regional distribution

Location	Banana	Barley	Beans	Chickpea	Citrus	Coconut	Cotton	Cowpea	Eucalyptus	Groundnut	Maize	Mango	Millet	Pigeon pea	Potatoes	Rice	Sesame	Sorghum	Soybean	Sugarcane	Sunflower	Tea	Tomato	Wheat
Afghanistan					■																			
Africa						■	■		■									■						
Algeria					■																			
America					■																			
Argentina																				■				
Australia					■				■	■					■					■				
Bangladesh										■										■		■		
Benin																■								
Brazil									■		■					■				■				
China							■			■			■	■										
China						■			■											■		■		
Colombia																				■				
Ethiopia	■	■			■					■	■	■					■							■
Ghana								■		■	■					■								
Hawaii	■											■												
India		■		■	■		■			■	■	■	■	■	■	■		■		■	■	■		■
Iran					■																			
Iraq					■																			
Israel					■																			
Kenya																			■	■		■		
Malawi	■						■			■	■							■						
Mexico																				■				
Nigeria								■					■	■						■				
Pakistan							■					■						■		■	■			■
Philippines																								
Saudi Arabia	■	■			■				■	■	■	■	■					■					■	
Sri Lanka																						■		
Sudan			■				■						■				■						■	
Tanzania			■			■	■												■			■		■

(continued)

Table 9.1 (continued)

Location	Banana	Barley	Beans	Chickpea	Citrus	Coconut	Cotton	Cowpea	Eucalyptus	Groundnut	Maize	Mango	Millet	Pigeon pea	Potatoes	Rice	Sesame	Sorghum	Soybean	Sugarcane	Sunflower	Tea	Tomato	Wheat
Uganda										■	■			■				■						
Uganda							■		■						■					■				
Yemen							■			■			■				■						■	■
Zambia			■				■	■						■					■		■			
Zimbabwe											■													

Sources:
Afghanistan: Stansly et al. (2001); Algeria: Stansly et al. (2001); America: Stansly et al. (2001); Africa: Zida et al. (2011); Tomar (2013); Rouland-Lefevre (2011); Argentina: Constantino (2002); Australia: Werner et al. (2008); Alam et al. (2012); Tomar (2013); Biswas (2014); Bangladesh: Alam et al. (2012); Biswas (2014); Benin: Togola et al. (2012a), b; Brazil: Rouland-Lefevre (2011); Constantino (2002); China: Muraleedharan (1992); Gui-Xiang et al. (1994); Rao et al. (2002); Zeng (2004); Tang et al. (2006); Tomar (2013); Maayiem et al. (2012); Colombia: Tomar (2013); Ethiopia: Cowie et al. (1990); Wood (1991); Maayiem et al. (2012); Ghana: Maayiem et al. (2012); Hawaii: Lai et al. (1983); India: Reddy et al. (1992); Basappa (2004); Kharub and Chander (2012); Tomar (2013); Pandey et al. (2013); Rathour et al. (2014); Iran: Faragalla and Al Qhtani (2013); Iraq: Faragalla and Al Qhtani (2013); Israel: Faragalla and Al Qhtani (2013); Kenya: Adoyo et al. (1997); Malawi: Munthali et al. (1999); Mexico: Collins (1984); Nigeria: Nwilene et al. (2008); Agunbiade et al. (2009); Pakistan: Wood (1991); Ahmed et al. (2007); Javaid and Afzal (2001); Aslam et al. (2000); Ahmed et al. (2004); Philippines: Orikiriza et al. (2012); Acda (2013); Saudi Arabia: Badawi et al. (1986); Faragalla and Al Qhtani (2013); Sri Lanka: Hemachandra et al. (2014); Sudan: Wood et al. (1987); Pearce et al. (1995); Tanzania: Bigger (1966); Mwalongo et al. (1999); Wood et al. (1987); Uganda: Wood et al. (1987); Nahdy et al. (1994); Nyeko and Nakabonge (2008); Orikiriza et al. (2012); Yemen: Wood et al. (1987); Zambia: Sileshi et al. (2008), (2009); Zimbabwe: Thierfelder et al. (2013)

9.3 Management

Different control methods (chemical, biological, and physical) have been adopted for termite management (Fig. 9.3). Majority of control practices are ineffective and ecologically unsustainable and, above all, do not address the root cause of termite infestation, merely providing a temporary relief to the problem (Mugerwa 2015). Most termite management practices are focused on total elimination rather than regulating their populations. Various natural enemies have shown the potential for use in biological control of termites and serve as an alternative to broad-spectrum chemical insecticides.

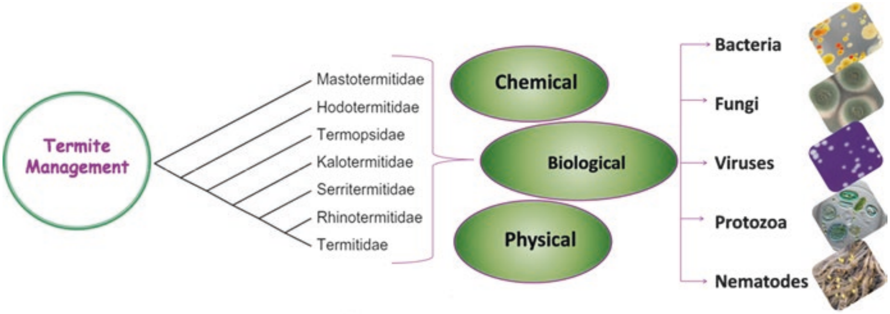

Fig. 9.3 Diagrammatic representation of different control measures of termites

9.3.1 Biological Control

With the growing realization of hazards and side effects associated with the extensive and indiscriminate use of synthetic chemical insecticides, entomologists have adopted a new concept of pest control, termed as integrated pest management (IPM). This term refers to a system that utilizes all suitable techniques and methods, in an as compatible manner as possible, in order to maintain the pest population at levels below a threshold causing significant economic losses (Mahtur and Kishor 1987). In this context, the role of biocontrol agents, viz., predators, parasitoids, and microbes, needs no emphasis due to their specificity, effectiveness, and safety to nontargeted organisms, besides other components in relation to man and biosphere. Microbial products have a long history of safety in use, and most of the microbial agents are compatible with other methods of pest control. In recent years, entomologists are leaning their attention on the exploration of microbial agents for pest suppression. Facultative pathogens of some insect species are commonly used as a biopesticide or microbial control agent. Interestingly, some of them have been widely tested and proved very effective against pernicious insect pests of agricultural crops. In certain developing and developed countries, a number of microbial biopesticides have been registered for field application on various vegetables, fruits, and other crops of agricultural, horticultural, and forest importance.

Microbial control includes all aspects of utilization of microorganisms or their by-products for pest control. Microbial biocontrol agents are relatively host specific and do not upset other biotic systems. They are safe to humans, vertebrates, and beneficial organisms and do not cause environmental pollution. The microorganisms isolated from termites mounds/nests or rhizosphere soil could be screened for their termiticidal activities for subsequent use in biocontrol plans. Due to their compatibility, the synergistic combinations of microbial control agents with other technologies will have excellent potential for use in IPM programs.

9.3.1.1 Bacteria

The large number of microbial species includes members of all major groups such as bacteria, virus, fungi, and nematodes. However, bacterial pathogens have been exploited the most and are recommended as potential biocontrol agent for the control of major insect pests. Bacteria are prokaryotic, unicellular organisms varying in size from less than 1 µm to several µm in length and are characterized by spherical, spiral, or rod-shaped cells. Most of the insect pathogenic bacteria occur under the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, and Streptococcaceae (Kalha et al. 2014). Members of Bacillaceae, particularly *Bacillus* spp., have received maximum attention as microbial control agents. *Bacillus thuringiensis* Berliner, which occupied 90% of the world biopesticide market, is pathogenic to more than 525 insect species belonging to various orders (Jayaraj 1986).

Bacillus thuringiensis is a rod-shaped, gram-positive, spore-forming, aerobic soil bacterium common in many ecosystems (Yadav et al. 2015c). It was first discovered in Japan in 1901 by Ishiwata and then reported in 1911 in Germany by Berliner (Baum et al. 1999). It is distributed worldwide in soil, stored products, insects, insect-breeding environments, and the phylloplane (Hofte and Whiteley 1989). Vegetative cells of *B. thuringiensis* are 0.2–5 µm in size with peritrichous flagella. They divide by binary fission and frequently occur in chains. During the sporulation phase of growth, this bacterium accumulates insecticidal crystal proteins (ICPs)/δ-endotoxin. They accumulate as inclusion bodies in the mother cell compartment and are finally released in the environment along with the spore, at the end of sporulation phase. Some ICPs have toxicity comparable to that of widely used organophosphate pesticides. There are around 34 recognized subspecies of *B. thuringiensis* with two distinct groups of toxin proteins: *Cry* (crystal delta-endotoxins) and *Cyt* (cytolysins), both pathogenic to insect pests (Schnepf et al. 1998).The insecticidal proteins of *B. thuringiensis* are highly specific as gut toxins with a superior safety record in regard to their effects on nontarget organisms (Lacey and Goettel 1995; Sarwar 2015). Castilhos-Fortes et al. (2002) evaluated the effects of *B. thuringiensis* subspecies against *Nasutitermes ehrhardti* under laboratory conditions. They reported that *B. thuringiensis* subspecies *kurstaki* registered <72% mortality at the seventh day after the bacterial application. Singha et al. (2010) evaluated *B. thuringiensis* and *B. thuringiensis* subsp. *israelensis* for their pathogenicity against two species of tea termites, viz., *Microtermes obesi* and *Microcerotermes beesoni*. They reported that *B. thuringiensis* strains caused >80% mortality in both the termite species. *Bacillus thuringiensis* subsp. *israelensis*, however, was noticed to be more virulent compared to other *B. thuringiensis*.

Similarly, other species showed similar efficacy level, such as *B. subtilis* that was also reported effective against termite species (Omoya and Kelly 2014). Natsir and Dali (2014) described the pathogenicity of *B. licheniformis* against termites, using feeding (baiting) and contact (spraying), which is further analyzed quantitatively by calculating host mortality during 2 weeks of observation. These authors reported that chitin deacetylase from *B. licheniformis* HSA3-1a induced a satisfactory level of pathogenicity, by inhibiting the growth of termites. The mortality obtained using spraying method reached 100% by the sixth day of observation. By the feeding method, mortality reached 100% by the eleventh day of observation. Data suggest that chitin deacetylase is effective against termite, to replace conventional termiticide.

Osbrink et al. (2001) isolated *Serratia* from dead termites and reported that three *Serratia* isolates induce >85% mortality within 19 days in petri dish tests. Connick et al. (2001) reported that *S. marcescens* strain T8 was highly virulent at the concentration of 3.4×10^{10} CFU/ml against *Coptotermes formosanus*. They reported termite mortality around 24% by 2 days, reaching 99% of termites after 19 days of assay. Omoya and Kelly (2014) also described that *S. marcescens* was much effective against termites.

Many rhizobacteria are known to produce and excrete hydrogen cyanide (HCN) into the rhizosphere (Verma et al. 2013; Verma et al. 2014; Yadav et al. 2016b). Release of HCN by rhizospheric bacteria into the soil can be toxic to subterranean termites. HCN-producing rhizobacteria could be useful for control by introducing them into termite mounds, thereby localizing cyanide production and minimizing potential deleterious effects on other soil fauna.

Nonparasitic rhizobacteria that produce harmful metabolites might also facilitate the biocontrol of termites. Three different species of HCN-producing rhizobacteria, *Rhizobium radiobacter*, *Alcaligenes latus*, and *Aeromonsa caviae*, were tested for their potential to kill *Odontotermes obesus*. The three bacterial species were found to be effective in killing the termites under in vitro conditions (Devi et al. 2007). Ivermectin is a metabolite produced by the bacterium *Streptomyces avermitilis*. Sublethal concentrations of ivermectin decreased the food consumption and tunneling capacity of *C. formosanus* (Mo et al. 2006). Further bacteria tested against termites include *Pseudomonas fluorescens* which blocked the pest respiratory system by producing hydrogen cyanide, resulting in a high termites mortality (Devi and Kothamasi 2009).

9.3.1.2 Fungi

Fungal diseases are known to cause in nature epidemics with high insect mortality levels (Vimaladevi and Prasad 2001). Entomopathogenic or disease-causing fungi have received considerable attentions as they are exceptionally virulent, being lethal parasites of insect pests. Fungi were among the first microorganisms to be used for the biological control of insect pests. They are cosmopolitan organisms and have been isolated from soils and infected insects from around the world. Conidia of entomopathogenic fungi could be spread through the colony by contact and grooming between contaminated and uncontaminated hosts. Entomopathogenic fungi possess added advantages over other microbial biocontrol agents, as they are capable of attacking all developmental stages of their hosts (Ferron 1978; Anand et al. 2009). None of the entomopathogenic fungi currently in use or under consideration are invasively pathogenic to humans (Kubicek and Druzhinina 2007).

Soil is a natural environment for entomopathogenic fungi, as many insects spend at least part of their life in soil. Such natural behavior is related to the insects' biology, due to accumulation in soil or leaf litter for wintering or pupation, a stage conducive to fungal infections and leading to natural regulation of many pests. Asexually produced fungal spores or conidia are generally responsible for infection and are dispersed throughout the environment in which the insect hosts are present.

More than 700 fungal species from around 90 genera are pathogenic to insects (Wraight et al. 2007; Hemasree 2013). However, only a few have been thoroughly investigated for their use against insect pests in agriculture. When a spore adheres to the host cuticle, a germ tube is generated which penetrates through the integument by mechanic and enzymatic processes (e.g., through the action of chitinases, proteases, and lipases). When the hyphae reach the insect hemocoel, they produce blastospores which are the final pathogenic step for completing the host infection (Vincent et al. 2007). The development of fungal infections in terrestrial insects is largely influenced by environmental conditions. High humidity is vital for germination of fungal spores and transmission of the pathogens from one insect to another.

Two fungal pathogens, *Metarhizium anisopliae* and *Beauveria bassiana*, have been extensively evaluated for termite control. *Metarhizium anisopliae* is a biological control agent that requires special application and handling techniques. Rath and Tidbury (1996) found that *Coptotermes acinaciformis* and *Nasutitermes exitiosus* were equally susceptible to direct conidial applications of both Australian and American strains of *M. anisopliae.* Ahmed et al. (2009) described that three strains of *M. anisopliae* were isolated from swarmed termite, *C. heimi* at Gujranwala. The exposure methods of these isolates against termites included soil substrate and filter paper treatments, at different concentrations of conidial suspension (1×10^4, 1×10^6, 1×10^8, 1×10^{10} propagules mL^{-1}). LT$_{50}$ for these strains against *C. heimi* were comparatively higher (65–106 h) in soil than on filter paper (50–83 h). Ravindran et al. (2015) isolated four strains of *M. anisopliae* using insect bait (*Galleria*) method and examined their sporulation characteristics and virulence against *C. formosanus. Metarhizium* sp. (Tk 4) was a high virulent strain identified showing 86.6% mortality rate on 4th day post inoculation (dpi). Wang and Powell (2003) reported six *B. bassiana* isolates obtained from *Reticulitermes flavipes* and *C. formosanus* termites in the USA and China. These isolates, along with *B. bassiana* isolate 26,037 from American Type Culture Collection, proceeding from a Colorado potato beetle, *Leptinotarsa decemlineata* (Say), were compared against *C. formosanus* and *R. flavipes* in the laboratory. Most *B. bassiana* isolates caused termite mortality within 4–8 days after treatment.

Conidiobolus coronatus isolates were found to be pathogenic against *C. formosanus*, *R. flavipes*, and *Nasutitermes exitiosus* (Wells et al. 1995). *Aspergillus* sp. has also been studied for its entomopathogenic activity against termites. In a study conducted by Muralidhara et al. (2013), *Aspergillus* sp. TK inoculation resulted in profuse colonization on the surface of *Microcerotermes beesoni*, with 100% mortality within 5 days, confirming the high potential of entomopathogenic fungus as biocontrol agents.

9.3.1.3 Viruses

A large number of viruses offer potential as microbial control agents of termites. Al Fazairy and Hassan (1993) reported that *Kalotermes flavicollis* died 2–10 dpi after inoculation by Nuclear polyhedrosis virus (NPV, Baculoviride), under laboratory conditions. Further authors suggested that termites control with NPV might be feasible. However, the potential of viruses for termite control has yet to be evaluating under field conditions. Accessibility of the target pest to control is the prime factor affecting the efficacy of viral pathogens. The efficacy, specificity, and production of secondary inocula make Baculoviruses an attractive alternative to broad-spectrum insecticides, and ideal components of IPM systems, due to their lack of effects on beneficial insects including other biological control organisms (Sindhu et al. 2011). Unfortunately, there are other drawbacks to the use of viruses to suppress pest populations: they kill their hosts slowly, when compared to other pathogens; environmental factors such as rainfall and solar radiation may reduce the viral persistence in soil; mass production of viruses is hampered by the need for living hosts or tissue culture; and finally, viral formulations experienced difficulties in competing successfully, on the basis of performance and cost, with other pest control products such as chemical insecticides or even other microbial agents (Fuxa 1990).

9.3.1.4 Nematode

Entomopathogenic nematodes (EPNs) are beneficial nematodes offering excellent potential for control of insects in soil habitats and commercially used to control many pests. EPNs like steinernematids and heterorhabditis are obligate insect parasites associated with symbiotic bacteria of the genera *Xenorhabdus* sp. and *Photorhabdus* sp., respectively (Akhurst and Boemare 1990). These bacteria are motile, gram-negative, facultative, non-spore-forming anaerobic rod members of the family *Enterobacteriaceae*. Together, nematodes and their symbiotic partners form an insecticidal complex that is effective against a wide range of hosts (Kaya and Gaugler 1993). Most *Photorhabdus* spp. are luminescent and catalase-positive, whereas *Xenorhabdus* spp. have no luminescence and are catalase-negative. Poinar and Thomas (1966) demonstrated the location of bacteria in the infective-stage juveniles, using light and electron microscopy. In *Steinernema*, the bacterial symbiont cells are harbored in a specialized structure known as “bacterial receptacle.” In

Heterorhabditis, the symbionts are distributed along a broad stretch of the anterior portion of the nematode intestine.

In laboratory tests, *S. carpocapsae* alone infected more than 250 species of insects from over 75 families in 11 orders (Poinar 1975). The broad host range and high virulence of entomopathogenic nematodes make them suitable for use as augmentative-release biocontrol agents (Hui and Webster 2000). The nematodes are compatible with many pesticides, can be mass produced and formulated, and are exempt from registration in many countries. Lacey and Georgis (2012) highlight EPN development for control of insect pests, above and below ground, including those from foliar, soil surface, cryptic, and subterranean habitats. Shahina and Tabassum (2010) reported higher mortalities in the subterranean termite *Macrotermes* caused by *S. pakistanense* in a filter paper and sand assay. Yu et al. (2010) compared virulence of three novel strains of *S. riobrave* (3-8b, 7-12, and TP) against subterranean termites *Heterotermes aureus*, *R. flavipes*, and *C. formosanus* workers. *Heterotermes aureus* was noticed as most susceptible to all the *S. riobrave* strains, and termites in all nematode treatments died after 4 days. Differential susceptibility of two termite species, *Macrotermes bellicosus* and *Trinervitermes occidentalis*, against EPNs isolates *H. indica* Ayogbe1, *H. sonorensis* Azohoue2, *H. sonorensis* Ze3, and *Steinernema* sp. Bembereke, from Benin (West Africa), was studied by Zadji et al. (2014). They reported that all tested EPN isolates can be recycled in both *M. bellicosus* and *T. occidentalis*, and the soldiers of both termites studied were noticed as more susceptible than workers. Current use of *Steinernema* and *Heterorhabditis* nematodes as biological control organisms has been summarized by Shapiro-Ilan and Gaugler (2010).

9.4 Biological Control Strategies

There are three main factors affecting microbial biological agent efficacy, reviewed as follows.

9.4.1 Toxin Production

Most of the insecticidal activity of *B. thuringiensis* is associated with the proteinaceous toxins located in the parasporal inclusion bodies, also known as parasporal crystals. Collectively, the toxins found in the parasporal crystals are referred to as δ-endotoxins. The *Cry*1 proteins which are found in the crystals are biologically inactive. Following ingestion and solubilization in the alkaline midgut, cleavage by gut proteases produces a 60–65 kDa activated protein that recognizes specific binding sites at the brush border membrane surface of the epithelial columnar cells, lining the host gut lumen (Lacey and Goettel 1995; Sarwar 2015).

Exposure of laboratory colonies of the subterranean species, *R. flavipes* and *R. hesperus*, to a mixture of soluble endotoxin, spores, and inclusion bodies of *B.*

thuringiensis resulted in 95% mortality after 6 days (Blaske and Hertel 2001; Lax and Osbrink 2003).

Pearce (1997) described that also the spores of entomopathogenic fungi may contain toxins, which would kill the termite host when ingested. Insecticidal cyclic depsipeptides were found to be produced by entomopathogenic fungi, including the destruxins from *M. anisopliae* var. *major*. It has been suggested that depsipeptides are localized on the surface of spores of *Beauveria* sp., whereas *Metarhizium* spp. destruxins are generally associated with in vivo or in vitro mycelial growth (Jegorov et al. 1989).

9.4.2 Siderophore Production

Siderophores are iron-chelating extracellular metabolites produced by different groups of bacteria and fungi (Verma et al. 2015a; Yadav et al. 2015a; Verma et al. 2016b; Suman et al. 2016). Extracellular siderophores of the brown-rot wood decay fungus *Gloeophyllum arabeum* were found to inhibit feeding of *C. formosanus* termite (Grace et al. 1992). Natural products, such as ant semiochemicals and fungal metabolites, or their synthetic analogues, might be valuable in termite control programs as repellents or insecticides in wood treatments or soil applications (Grace et al. 1992). However, the development of more stable formulations, such as microencapsulation, would be necessary to ensure their long-term, residual action.

9.4.3 Hydrolytic Enzymes Production

A large number of microorganisms including bacteria, fungi, and actinomycetes are capable of producing hydrolytic enzymes such as proteases, lipase, and chitinase from various types of natural resources (Pandey et al. 2013; Verma et al. 2015b; Yadav et al. 2015b; Yadav et al. 2017). Lysenko and Kucera (1971) described that *Serratia marcescens* produced extracellular proteases that could be underpin pathogenicity of these bacteria in termites. Osbrink et al. (2001) reported that 15 bacteria and 1 fungus were associated from dead termites as possible biological control agents against subterranean termites, *C. formosanus*. Bacterial isolates from dead termites were primarily *S. marcescens* that caused septicemia in *C. formosanus* and was found to contain proteolytic enzymes. Lipases (triacylglycerol acyl hydrolases) are one of the most important class of hydrolytic enzymes that catalyze both the hydrolysis and the synthesis of ester formed from glycerol and long-chain fatty acids. Lipases are ubiquitous enzymes produced by all animals, plants, and microorganisms (Yadav 2015; Yadav et al. 2016a). Lysis by hydrolytic enzymes excreted by microorganisms is a well-known feature of mycoparasitism.

Vaidya et al. (2003) isolated mutants of *Alcaligenes xylosoxidans* producing hyperchitinases and developed a rapid technique for screening chitinolytic bacteria using chitin-binding dye calcofluor, white M2R, in chitin agar. Microorganisms possessing high chitinolytic activity gave clear zone under ultraviolet light after 24–48 h of incubation. The mutant *A. xylosoxidans* EMS 33 was found to produce three to four times more chitinase then wild type. Chitinase production has also been reported in *S. marcescens*, *Pseudomonas* sp., *Bacillus* strains, *Paenibacillus* sp., and *Pseudomonas maltophilia* (Suyal et al. 2015; Verma et al. 2016a; Yadav et al. 2016a, b). Dua (2014) reported ten bacterial strains having termite killing ability along with two control strains for biocontrol against termites. Different bacterial strains showed >80% killing of termites at 5 days of incubation. Four bacterial strains (KBM79, KPM35, PPM147 and PBM195) caused 100% killing at 10 days of observation. The cell-free culture filtrate of these cultures showed that the antagonistic substance was extracellular. Bacterial strains of *B. subtilis* KBM79 and *Pseudomonas synxantha* KPM35 possessed proteolytic, lipolytic, and chitinolytic enzyme activities and caused 100% killing of termites at 10 dpi.

9.5 Conclusion

Termite management is a challenging task for researchers. While various approaches have been tried to manage termite populations, the relevant insecticides are associated with certain risks to the environment and human health. Biological control agents should be seen as one tool among others in an integrated approach to managing termite problems. Microbial control has been a component of IPM strategies in developing countries, enjoying particular success in Asia and South America. An increased understanding of the molecular basis of the various microbial pathogenic mechanisms on termites will lead not only to a rational management but also to the development of new biological control strategies. It is therefore worth to mention that the use of entomopathogens can provide a successful and environment friendly avenue for controlling termite pests.

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