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THE POTENTIAL OF CONTROLLING INSECT PESTS WITH NEMATODE-BACTERIA COMPLEX BASED BIOLOGICAL INSECTICIDES IN NIGERIA.

Aliyu H.U.[1], Tahir F.[2], Agbo E.B.[2] and Kela S.L.[1]

[1]Department of Biological Sciences, Federal University, Kashere, Gombe State.

[2]Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi.

ABSTRACT

Pesticides are widely used in Nigerian for pest control and they pose human and animal health problems. As a result of these challenges it became necessary for researchers to come up with better alternatives for the farmers. Entomopathogenic nematode-bacteria based biological insecticides possess balanced biological control attributes which qualify them as viable alternatives in the control of insect pests of crops. They carry bacteria in their intestines making them lethal to many important insects of crops. They have been isolated in Nigeria and their biological control potential are been tested and if properly used may act as an alternative to chemical pesticides in the future of Nigerian Agriculture or a component of integrated pest management system. This review is aimed as a gateway for researchers in Nigeria to have an insight into this interesting aspect of pest control and to undertake research in different aspects of nematode-bacteria complex so as to exploit them in crop pest management in Nigeria.

Keywords: Nigeria, synthetic pesticides, biopesticides, insect pests, entomopathogenic nematodes.

INTRODUCTION

The demand for biological control of insect pests in agriculture is on the rise due to increased awareness of the dangers posed by use of chemical pesticides. These chemical pesticides have been reported to contaminate the environment, drinking water sources, affect the health of humans and farm animals. In addition insects develop resistance to their effectiveness over long exposures leading to pest resurgence and invariably more damage to plants. These and other reasons have necessitated the need for search for alternative method of pest control especially

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those based on using the natural enemies of the pests to reduce their populations. This process when adopted will reduce significantly the over dependence on chemical pesticides thereby conserving natural ecosystems. In Nigeria there is no well documented literature on the menace of pests or pesticides in agriculture. However, Oscar (2016) noted that pesticides were an integral part of traditional agriculture where crops and livestock production relied exclusively on pesticide use. He also noted that data obtained from Kenya in 2008 and Senegal in 2011 showed that 70 to 80% of farmers interviewed used highly toxic or restricted use pesticides. The farmers also indicated that less pesticides will be used if they were provided with alternatives. Apart from indiscriminate use of toxic pesticides the concept of integrated pest management (IPM) was also strange to them.

Entomopathogenic (insect pathogenic) nematode-bacteria complexes also known as (EPNs) are obligate parasites of insects. They are grouped into two genera namely *Steinernema* and *Heterorhabditis*. They are mutualistically associated with bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus* respectively (Burnell and Stock, 2000). Their ability to locate, infect and kill insects have conferred better biological control potential on them and have attracted a lot of attention lately (Adams and Nguyen 2002; Boemare, 2002; Emeliahoff *et al*., 2008). These nematodes together with their bacterial symbionts have several deleterious effects on their insect hosts including sterility, reduced fecundity and flight activity as well as delayed development, and rapid mortality (Koppenhoffer and Kaya 2001). Other nematodes have been reported to cause insect death but are either difficult or too expensive to mass produce (Vashisth *et al*., 2013). EPNs on the other hand have a balanced biological control attribute. A number of species have been isolated and described in different countries of the world thus widening their biocontrol potentials.

Advantages of EPNs over synthetic pesticides:

The nematodes together with their bacteria symbionts form a nematode-bacteria complex that have activity against a wide range of insects including species in the order Hemiptera, Diptera, Hymenoptera, Lepidoptera, Orthopthera, Coleoptera, Thysaroptera, Siphonaptera as well as Isoptera. They are relatively safe to non target insects, vertebrates and plants (Boemare, 2002; Akhurst and Smith, 2002) as well as drinking water sources (Georgis *et al*., 1991). They have the ability to reach insect hosts in cryptic habitats (bark of trees)., they have high reproductive ability and do not require special application equipment as they can be applied using standard application equipments like backpacks, pressurized, mist, electrostatic, fan, and aerial as well as irrigation systems.

Life cycle of entomopathogenic nematode-bacteria complex

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The life cycle of the nematode-bacteria complex explains the mode of action of these potent biological insecticides. The third stage infective juvenile (IJ) is the only free living non-feeding stage that is able to persist in the soils for lengthy periods. During favourable environmental conditions, IJs are able to search for susceptible hosts in the environment and enter the host through natural openings such as the mouth, anus and respiratory spiracles or cuticle. Once inside the host, the nematodes penetrate into the haemocoel and release their associated bacteria. The bacteria produce secondary metabolites that depress the immune system of the insect larvae. The metabolites are also lethal to the larvae which succumb to septicaemia or toxaemia within 24-48 hours (Ehlers 2001) after infection. The bacterial endosymbionts also produce antimicrobial products that keep away other microorganisms thereby resulting in monoxenic conditions within the larval cadaver.

The bacteria as well as the contents of the host cadaver serve as sources of nutrients for nematode growth, development and reproduction. After two to three reproduction cycles and when the nutrient supply within the cadaver becomes limiting, the juvenile nematodes re-associate with bacterial symbionts and develop into non-feeding infective juveniles and emerge from the insect carcass in search for new susceptible hosts in the environment.

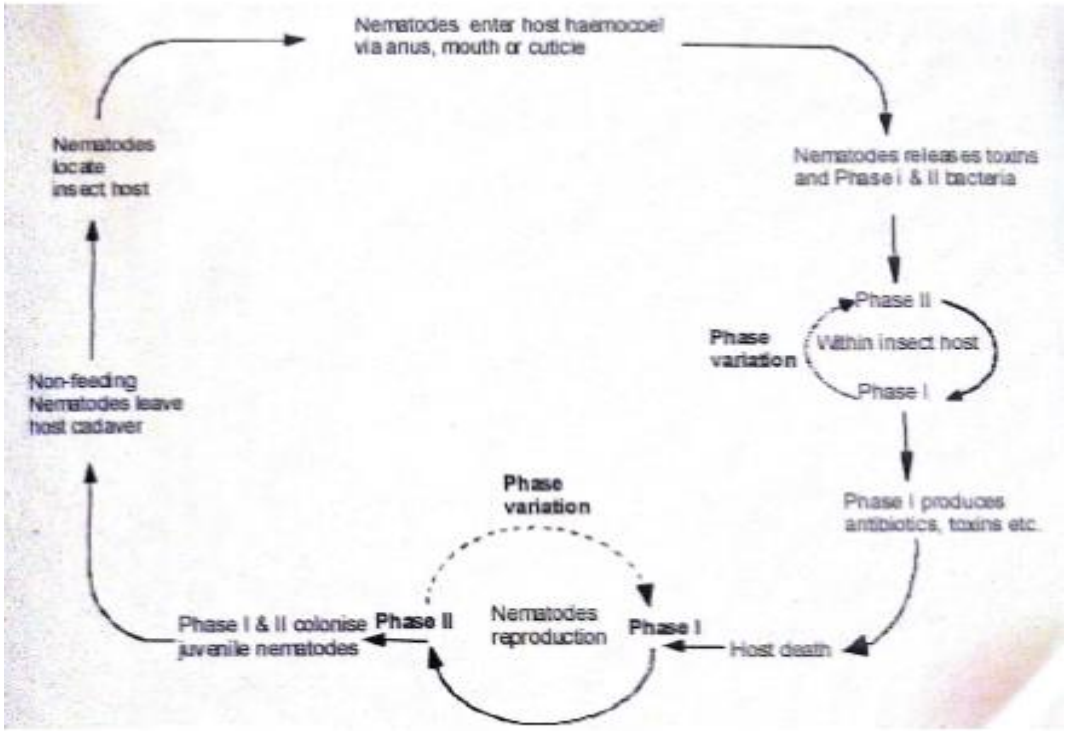

Figure 1. life cycle of nematode-bacteria complex (adapted from Owuama 2001).

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Distribution and commercial availability of EPNs:

They have been isolated from nearly all soil types however, their presence in cultivated areas is more frequent followed by forest ecosystems. These habitat represents the most probable habitat for finding insect hosts in large numbers. They have an ubiquitous distribution (Hominick *et al*., 1996). Most of the surveys that were conducted are in Europe and North America. As a result of their importance, however, more countries are conducting surveys with various levels of success for the occurrence of EPNs thus contributing to the number available. In addition, surveys have been conducted in South Africa, Ethiopia, Southeast Asia, Indo-Malaysian region and tropical areas (Hominick 2002) as well as parts of Nigeria (Aliyu, *et al*., 2015), DNA analysis have facilitated the accurate identification of the nematode-bacteria complexes and have also removed the ambiguity associated with similarities in morphological characteristics of the nematode progeny. Currently, there are about 92 species of steinernematids and 18 of heterorhabditids that have been described. In spite of these numbers, most species from third world countries including the ones from Nigeria have not been characterized due to expensive equipment and a lack of expertise. In order to exploit these EPNs as biological pesticides, especially in third world countries extensive surveys, proper identification and adequate understanding of the interaction between the nematode-bacteria complex and their host is a necessary prerequisite.

Despite the abundance of EPNs identified and classified, only a few species have been commercialized so far. Some commercially available nematode-bacteria complex based products are presented in table 1 below. The rate of application of EPNs depends on the target pest and usually one billion nematodes per acre (250,000/M^2) is recommended with higher rates being recommended for green house soils.

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Table 1: Nematode-bacteria complex used as biological control agents

Pest common name and crops targeted	Pest scientific name	Bacterial symbionts	Nematode species
Army worm (vegetables)	Lepidoptera: Noctuidae	*Xenorhabdus nematophilus* *X. bovienii*	*Steinernema carpocapsae* *S. feltiae*
Borer (fruit trees, ornamentals)	*Synanthedon* spp and other seslids	*Photorhabdus luminescens* *X. nematophilus* *X. bovienii*	*Heterorhabdus bacteriophora* *S. carpocapsae* *S. feltiae*
Leaf miner (vegetables, ornamentals)	*Liriomyza* spp. (Diptera:Agromyzidae)	*X. nematophilus* *X. bovienii*	*S. carpocapsae* *S. feltiae*
Small hive beetle (bee hives)	*Aethina tumida*	*P. luminescens*	*H. indica*
Sweet potato weevil	*Cylas formicarius*	*P. luminescens* *X. bovienii*	*H. bacteriophora* *S. feltiae*
Citrus root weevil (citrus, ornamentals)	*Pachnaeus* spp. (Coleoptera:Curculionidae)	*P. luminescens*	*H. bacteriophora*
Grape root borer (grapes)	*Vitacea polistiformis*	*Photorhabdus sp.* *X. nematophilus*	*H. zealandica* *S. carpocapsae*
Mole cricket (turf)	*Scapteriscus* species	*X. bovienii*	*S. feltiae*
Naval orangeworm (nut, fruit trees)	*Amyelois transitella*	*X. bovienii*	*S. feltiae*
Fungus gnat (mushroom)	Diptera:Sciaridae	*P. luminescens* *X. bovienii*	*H. bacteriophora* *S. feltiae*
Banana root borer (bananas)	*Cosmopolites sordidus*	*X. bovienii*	*S. feltiae*

(Owuama 2001; Shapiro-Ilan and Gaugler 2002; Shapiro-Ilan and Gaugler 2010).

Persistence of EPNs in the soil:

Soil is the most suitable habitat for targeting or isolating EPNs. Undisturbed soils like orchards and forests are the natural reservoir for both steinernematids and heterorhabditids. Several factors including soil texture, pH, moisture, heat, desication and ultraviolet radiation are some of the environmental factors which may impact on the efficacy of EPNs (Shapiro *et al*., 2000). Soil pH can also affect natural EPN distributions (Kanga *et al*., 2012). The effects of these factors

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also depend on the species, strain, or habitat of the EPNs. Their wide distribution throughout the world indicate their genetic ability to adapt to various environmental stresses that they experience in the soil environment (Hominick *et al*, 1996).

Infective juvenile persistence is higher in sandy loam with persistence lower in clay soils. The poor persistence in clay soil is attributed to lower oxygen concentration due to their small pore spaces and poor water draining capacity. Infective juveniles can persist in soils with pH values of 4 and 8, but persistence may decline with pH value of 10. Salinity does not affect EPNs since they have been isolated from soils near the sea shore (Griffin *et al*., 1994).

Biocontrol potential of EPNs:

Steinernema glaseri and *Steinernema carpocapsae* have extensively been tested for their potency against soil pests (Pionar, 1986). *S. glaseri* was first used against the Japanese beetle grubs, *Popillia japonica* Newman in the 1930s with varying degrees of success (Gaugler, 1988; Klein and Georgis, 1992).

Various degrees of success have also been recorded against soil inhabiting insects where *Heterorhabditis* spp successfully reduced black vine weevil population densities by 90% (Bedding and Miller, 1981). Successes have also been recorded in soil treatment against colorado potato beetles (Wright *et al*, 1987), and cutworms (Lossbroek and Theuiseen, 1985) among others. A number of surveys have been conducted which have provided evidence of EPNs in both natural and agricultural fields (Hominick, 2002). The effective use of EPNs as biocontrol agents requires knowledge and occurrence of native species. This is because using exotic species may induce exclusion of natural species due to displacement and they may not be suitable for the target local insect pest as a result of lack of adaption to local environmental conditions (Miller and Barbercheck, 2001).

Production of EPNs:

Entomopathogenic nematode-bacteria complexes can be mass produced as biopesticides using both *in vivo* (within hosts) and *in vitro* (outside hosts) methods respectively (Shapiro-Ilan and Gaugler, 2002). The *in vivo* culture method of nematode-bacteria complex involves production in insect hosts (Dutky *et al*., 1964; Poinar 1979 and Kaya and Stock, 1997). This method is based on the White trap (White, 1927), and it basically exploits the advantage of natural migration of infective juvenile nematodes away from their host insect cadavers when they emerge. The choice of nematode and host insect used dictates the quality and quantity of yield. As well, yield of nematode progeny generally is proportional to the size of host used in their propagation (Blinova and Ivanova, 1987; Flanders *et al*., 1996; Shapiro-Ilan and Gaugler, 2002). However, Dutky *et al*., (1964); Blinova and Ivanova, (1987) posited that yield per milligram insect and susceptibility

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to infection is usually inversely proportional to host size and age. The *in vivo* production method requires low startup capital and technical expertise (Gaugler and Han, 2002) hence it is the method of choice for most laboratory production assays and small scale industries with *Galleria mellonella* as the choice host due to its high susceptibility, to most EPN species, ease of rearing in the laboratory, availability and its ability to produce high yields (Woodring and Kaya, 1988). The *in vivo* method also produce the highest quality nematodes for biological control purposes (Shapiro and Gaugler, 2002).

In vitro production involves production of IJs either on solid or liquid artificial media. This complex process requires a proper understanding of the biology and behavior of the EPN species to be produced. *In vitro* production has advanced from solid media production developed by Glaser *et al*., 1940 to a three dimensional solid media production developed by (Bedding, 1981; Bedding, 1984), and the liquid fermentation method developed by Friedman, (1990). Presently, most commercial production of EPNs as biopesticides are based either on the solid media process or the liquid fermentation process. However, production of EPNs using solid media has been successful for both nematode species but with high labour costs as the major constraint. On the other hand, liquid fermentation process is best suited for steinernematids due to their ability to withstand high pressures but this method is poorly suited for heterorhbaditids.

EPN formulation and application:

The ability to immobilize EPNs on suitable substrates has enhanced its commercialization. Active nematodes are immobilized to prevent the depletion of their lipid and glycogen reserves. Various formulations have been developed in order to preserve the intergrity of stored EPNs, to facilitate their storage application. These formulations include activated charcoal, alginate and polyacrylamide gels, baits, clay, paste, peat, polyurethane sponge, vermiculite and water-dispersible granules. Successful storage under refrigeration conditions ranges from one-seven months depending on the EPN species. Temperatures as low as 2-5˚C reduces their metabolic activity and hence improves their shelf life, however, some species like *Heterorhabditis indica* and *Steinernema riobrave* do not store well at temperatures below 10˚C (Strauch, *et al*., 2000).

A strain of a specific nematode species can be used to control more than one type of insect pest in agriculture and other related fields. The efficacy of pest management and control depends on the methods employed for the application of EPNs and these methods include the use of spray equipments and several irrigation and pumping systems (Shapiro-Ilan *et al*., 2006). Equipments and techniques used to apply specific biological control agents influence and also increase the chances of proper contact between the nematode and the insect (Jagdale *et al*., 2009). They can be applied with most agronomic equipment like hand or ground sprayers, mist blowers and so on (Georgis *et al*., 1995). They can also be applied through irrigation systemusing microjet, drip,

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sprinkler (Cabnillas and Raulston 1996). They are usually applied at rates of 25Ijs/cm^2 of the treated area and the rates can either be increased or decreased depending on the nematode species and target pests (Shapiro-Ilan and Gaugler 2002).

Host range of EPNs:

The efficacy of EPNs have been evaluated against a large number of insect pest species with varying results from 0-100% control. Due to close contact in laboratory bioassays and optimal conditions, EPNs have a wider spectrum of activity compared to the field where a several ecological and behavioural barriers restrict host range (Gaugler, 1981; 1988). However, when nematode species are matched with their specific target pests, significant reduction in pests, significant reduction in pests are recorded (Shapiro-Ilan *et al*., 2002). Considerable evidence indicates that the host range of nematodes in their natural ecosystems is highly restricted and this attribute makes them safe to non target insect hosts and vertebrates compared to synthetic pesticides which usually have deleterious effect on both pests and beneficial insects in the environment (Akhurst 1990). Furthermore, EPNs have been observed to be effective against plant parasitic nematodes because they have the ability to displace them hence they have been used in the effective management of plant parasitic nematodes (Lewis and Grewal, 2005).

Compatibility of EPNs with chemicals and fertilizers:

Infective juveniles of EPNs have been reported to be able to withstand about 2-24hours exposure to many chemical and biological insecticides, as well as fungicides, herbicides, nematicides, fertilizers and growth factors. Hence, they can be mixed and applied simultaneously on the field offering a cost effective approach to pest management. Alumai and Grewal, (2004), noted that the concentration of chemicals to which the EPNs are mixed depends on the volume of the mixture to be used. EPNs have been found to be compatible with a number of chemical herbicides, fungicides and insecticides (Koppenhofer and Grewal, 2005). Koppenhofer, *et al*., (2000) noted that most EPNs are compatible with chemical pesticides with the interaction ranging from synergism, to augmentation. The nematode-bacteria complexes are also generally compatible with chemical fertilizers and composted manure but fresh manure on the other hand had detrimental effect on them.

The application of EPNs as biocontrol agents requires knowledge of the occurrence, species diversity, biology, ecology, distribution and insect host range of native EPN species. Application of native EPNs as insect biological control agents has superior advantage over exotic EPNs due to the fact that native species are better adapted to local environmental conditions (Ehlers, 2001). Non-native EPNs may target beneficial insect populations and may displace local EPNs. In addition, non-native EPNs may not be effective against local insect pests.

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Specificity and efficacy of EPNs:

EPNs are target specific and hence do not pose much threat to the environment compared to synthetic pesticides (Ehlers and Peters, 1995). Even though they are safe and target specific, in some cases, they have failed to compete favourably in the environment compared to synthetic pesticides (Georgis *et al*., 2006). However, with advances in their production processes, formulation, proper application timing and delivery of EPNs into suitable environments, their competitive abilities have been improved. In the Netherlands, England and Germany, S. feltiae has effectively replaced chemical pesticides in the control of insect pests of Horticulture (Jagdale, *et al*., 2004). Since they are living organisms they are constrained by factors such as desiccation and temperature extremes (Glazer, 2002), hence they are rapidly inactivated. As well, IJs in the soil are vulnerable to a variety of microbial and invertebrate antagonists (Kaya, 2002). Also they are only effective within a narrow range of temperature (20-30˚C), as well as soil type and irrigation frequency (Shapiro-Ilan *et al*., 2006). It was also observed that freshly harvested IJs were more virulent than those stored for 3months and more, (Hussaini *et al*., 2005; Aliyu *et al*., 2015).

The environment can be enhanced to achieve best results of EPNs in the soil environment. This can be achieved by augmenting moisture content of the environment through irrigation, mulching or applying EPNs early in the morning or evening and then treating the soil with irrigation to keep it moist for at least two weeks post application (Klein 1993). Application to foliage to target above ground pests has not been successful due to the sensitivity of the EPNs to UV radiation, however, their formulations have been improved to enhance their efficiency against above ground pests (Shapiro-Ilan *et al*., 2010).

CONCLUSION

In order to attain sustainable agriculture and food security in Nigeria as well as other third world countries, there is the need for the search in alternative pest control strategies. A strategy that will reduce the overdependence on conventional synthetic pesticides and embrace more environmentally safe alternatives like the one provided by the use of EPNs. These nematode-bacteria complexes have emerged as excellent alternatives as biological control agents attracting world-wide interests. This is mostly due to their safety to the environment, high reproducibility, excellent host seeking ability, fast at killing host, their safety to non target pests and most of all no case of pest resistance and resurgence have been reported with EPNs. More so, these EPNs have been isolated in Nigeria and their biological control potential is currently being tested against some important pests of crops with promising results. Extensive survey of other habitats and their successful production locally will target local pests for effective control and will exclude the need for importation of exotic nematodes.

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