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Identification, Virulence and Ecological Characterisation of Indigenous Entomopathogenic Nematodes as Biological Control Agents Against Fall Armyworm (*Spodoptera frugiperda*) in Nigeria

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Abstract

Smallholder agriculture in Nigeria faces persistent insect pest pressure and a widespread dependence on chemical insecticides in several crop sectors, alongside limited awareness of sustainable alternatives. This researched evaluated the potential of indigenous entomopathogenic nematodes as biological control agents within an integrated pest management approach (IPM), using the Fall armyworm (FAW) *Spodoptera frugiperda* as a target organism for benchmarking. The work combines three strands of enquiry that are required for practical deployment. These are the socioeconomic conditions for adoption, the biological identification and virulence of local isolates, and the ecological characterisation that determines field fitness.

A structured survey of 740 farmers across ten Nigerian states examined awareness, perceptions, and willingness to adopt. Awareness of entomopathogenic nematodes was 12.8%, while 70.9% expressed interest in training. Perceived effectiveness and safety were neutral to moderate, and education showed a modest positive association with willingness to adopt. Cost, availability, and application complexity were the most frequent barriers. Field facing training through videos and on-farm demonstration was preferred.

From 202 soil samples collected in Ibadan and Zaria, six nematode isolates were recovered and identified by morphology and molecular markers as *Heterorhabditis bacteriophora* (Ib-CRIN68), *Steinernema carpocapsae* (Ib-IART45, Ib-ITUC102), *Steinernema feltiae* (Za-SAM), *Steinernema nepalense* (Ib-HORT), and *Oscheius myriophilus* (Ib-FRIN32). Recovery rates were 3.9 % in Ibadan and 1.4 % in Zaria. Virulence against *S. frugiperda* was stage and dose dependent. *Steinernema carpocapsae* isolates produced more than 90% mortality in second instars at 200 infective juveniles per insect within 72 hours. Lethal concentration values for early instars were 40 to 75 infective juveniles, and greater than 150 for pupae. Time to 50% mortality in second instars was 24 to 36 hours for *S. carpocapsae*.

Ecological assays showed peak infectivity and reproduction at 25 to 30°C, with progeny yields above 100,000 infective juveniles per cadaver at 25°C. *Steinernema carpocapsae* displayed an ambusher strategy, while *H. bacteriophora* penetrated deeper soil layers with a cruiser profile. Desiccation survival extended to water activity 0.83 to 0.89 for the best isolates, with MW_{50} between 0.88 and 0.95. Under anoxia, 72-hour survival exceeded 60% for *S. carpocapsae* and *H. bacteriophora*. Oxidative stress tolerance was highest in *S. carpocapsae*. These results indicate robust ecological fitness for the two leading species.

The research demonstrates that indigenous *S. carpocapsae* and *H. bacteriophora* are strong candidates for biological control of *S. frugiperda* in Nigerian systems. The combination of farmer willingness to learn, confirmed pathogenicity, and favourable ecological traits outlines a practical pathway for integration into pest management. Future work should prioritise semi field and field validation, development of stable and affordable formulations, and delivery models that align with farmer preferences and local production capacity.

Identifizierung, Virulenz und ökologische Charakterisierung einheimischer entomopathogener Nematoden zur biologischen Bekämpfung des Herbstheerwurms (*Spodoptera frugiperda*) in Nigeria

Zusammenfassung

Die kleinbäuerliche Landwirtschaft in Nigeria steht unter anhaltendem Druck durch Insekten und weist in mehreren Anbausektoren eine weit verbreitete Abhängigkeit von synthetischen Insektiziden auf, bei gleichzeitig geringer Kenntnis über nachhaltige Alternativen. Diese Arbeit bewertete das Potenzial einheimischer entomopathogener Nematoden (EPN) als biologische Bekämpfungsorganismen im Rahmen eines integrierten Pflanzenschutzes. Als Zielorganismus zur Leistungsbewertung diente der Herbstheerwurm *Spodoptera frugiperda*. Die Studie vereint drei Untersuchungsstränge, die für eine praktische Umsetzung erforderlich sind. Diese betreffen die sozioökonomischen Bedingungen für die Einführung, die biologische Identifizierung und Virulenz lokaler Isolate sowie die ökologische Charakterisierung als Grundlage der Feldtauglichkeit von EPN in Nigeria.

Eine strukturierte Befragung von 740 Landwirtinnen und Landwirten in zehn nigerianischen Bundesstaaten erfasste Wissen, Wahrnehmungen und die Bereitschaft zur Einführung von EPN. Kenntnisse über EPN hatten 12,8% der Befragten, während 70,9% ein Interesse an Schulungen zur Nutzung von EPN äußerten. Die wahrgenommene Wirksamkeit und Sicherheit wurde neutral bis mäßig bewertet, und ein höherer Bildungsgrad stand in einem schwach positiven Zusammenhang mit der Einführungsbereitschaft von EPN. Kosten, Verfügbarkeit und Anwendungsaufwand waren die häufigsten Hemmnisse. Bevorzugt wurden praxisnahe Schulungen durch Videos und Vorführungen auf dem Feld.

Aus 202 Bodenproben aus Ibadan und Zaria wurden sechs EPN Isolate gewonnen und anhand morphologischer Merkmale sowie molekularer Marker identifiziert: *Heterorhabditis bacteriophora* (Ib-CRIN68), *Steinernema carpocapsae* (Ib-IART45, Ib-ITUC102), *Steinernema feltiae* (Za-SAM), *Steinernema nepalense* (Ib-HORT) und *Oscheius myriophilus* (Ib-FRIN32). Die Wiederfindungsraten betrugen 3,9% in Ibadan und 1,4% in Zaria. Die Virulenz gegenüber *S. frugiperda* war stadien- und dosisabhängig. *Steinernema carpocapsae* verursachte bei Zweitlarven mit 200 infektiösen Juvenilen je Insekt innerhalb von 72 Stunden mehr als 90% Mortalität. Lethalkonzentrationswerte für frühe Larven lagen bei 40 bis 75 infektiösen Juvenilen und bei Puppen über 150. Die Zeit bis zur 50%igen Mortalität in Zweitlarven betrug für *S. carpocapsae* 24 bis 36 Stunden.

Ökologische Versuche mit den EPN zeigten maximale Infektivität und Fortpflanzung bei 25 bis 30 °C, mit einer Nachkommenschaft von über 100.000 infektiösen Juvenilen je Kadaver bei 25 °C. *Steinernema carpocapsae* zeigte eine Ansitzstrategie, während *H. bacteriophora* mit aktivem Suchverhalten in größere Bodentiefen eindrang. Das Überleben unter Austrocknung reichte bei den besten EPN Isolaten bis zu einer Wasseraktivität von 0,83 bis 0,89, mit MW50 zwischen 0,88 und 0,95. Unter Anoxie überstieg die 72-Stunden-Überlebensrate bei *S. carpocapsae* und *H. bacteriophora* 60%. Die höchste Toleranz gegenüber oxidativem Stress wurde bei *S. carpocapsae* festgestellt. Diese Ergebnisse belegen eine ausgeprägte ökologische Eignung der beiden führenden EPN Arten.

Diese Untersuchungen zeigen, dass einheimische *S. carpocapsae* und *H. bacteriophora* Isolate vielversprechende Kandidaten für die biologische Bekämpfung von *S. frugiperda* in nigerianischen Anbausystemen sind. Die Kombination aus Lernbereitschaft der Landwirte, bestätigte Pathogenität und günstigen ökologischen Eigenschaften der getesteten EPN Isolate weist einen praktikablen Weg für die Integration dieser Ergebnisses in den Pflanzenschutz auf. Zukünftige Arbeiten sollten Validierungen unter halbfreilandund freiland Bedingungen, die Entwicklung stabiler und kostengünstiger Formulierungen sowie Bereitstellungsmodelle priorisieren, die sich an den Präferenzen der Landwirte und an der lokalen Produktionskapazität orientieren sollten.

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a_w — Water activity

BCA — Biological control agent

CABI — Centre for Agriculture and Bioscience International

CI — Confidence interval

COI — Cytochrome c oxidase subunit I mitochondrial gene

DDT — Dichlorodiphenyltrichloroethane

EPN — Entomopathogenic nematode

FAW — Fall armyworm

H_2O_2 — Hydrogen peroxide

HCH — Hexachlorocyclohexane

icipe — International Centre of Insect Physiology and Ecology

IITA — International Institute of Tropical Agriculture

IJ — Infective juvenile (nematode stage)

IPM — Integrated pest management

ITS — Internal transcribed spacer region of ribosomal DNA

LC_{50} — Median lethal concentration

LT_{50} — Median lethal time

MEGA — Molecular Evolutionary Genetics Analysis software

MUSCLE — Multiple sequence comparison by log expectation

MW_{50} — Water activity at which 50 percent mortality occurs (desiccation metric)

OCP — Organochlorine pesticide

PCA — Principal component analysis

PEG 600 — Polyethylene glycol 600

SD — Standard deviation

SE — Standard error

ST_{10} — Time to 10 percent survival under stress

ST_{50} — Time to 50 percent survival under stress

Chapter 1: General introduction

This dissertation is organised into six chapters. Chapter 1 outlines the problem context in Nigeria, the rationale for sustainable pest management, and the study aims within a three-part framework. Chapter 2 reviews the literature on pest pressures in Africa, integrated pest management (IPM), and the biology and ecology of entomopathogenic nematodes. Chapter 3 reports a nationwide survey of farmers that examines awareness, perceptions, and willingness to adopt biological control. Chapter 4 presents the collection, identification, and virulence assessment of indigenous isolates against *Spodoptera frugiperda*. Chapter 5 evaluates ecological fitness through assays on temperature response, foraging behaviour, and tolerance to desiccation, hypoxia, and oxidative stress. Chapter 6 integrates findings across phases, states limitations, and proposes directions for future research and practical application.

1.1 Background

The management of agricultural insect pests remains a critical challenge for food security and rural livelihoods across sub-Saharan Africa (SSA). Insect pest infestations contribute significantly to yield losses in staple and commercial crops, particularly maize, *Zea mays* (L.) (Poaceae), cassava, *Manihot esculenta* Crantz (Euphorbiaceae), cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae), and vegetables (Savary et al., 2019). In Nigeria, which spans multiple agroecological zones and is dominated by smallholder farming, pest pressure is shaped by production choices and climate variability. Monocropping can heighten vulnerability to insect pests compared with diversified systems (Mohammed, 2020). Climate variability and change further aggravate pest risks and losses (Omokaro et al., 2025; Savary et al., 2019). In many crop sectors, farmers rely on synthetic insecticides as the primary defence, and numerous Nigerian studies report indiscriminate application and limited adherence to safety or resistance-management practices (Adesuyi et al., 2018; Rahman, 2018; Sosan & Akingbohungbe, 2009). Limited access to and awareness of sustainable alternatives and structured IPM support have also been documented.

While the introduction of invasive pests such as the FAW, *Spodoptera frugiperda* (J. E. Smith) (Lep.: Noctuidae), triggered short-term emergency responses across SSA, it also exposed systemic gaps in pest management strategies (Goergen et al., 2016; Hruska, 2019). Documented weaknesses include limited local capacity for biological control research and extension support for non-chemical options, and the underuse of indigenous natural enemies in smallholder systems (Hruska, 2019; Kansiime et al., 2019). As a result, in Nigeria as well as in many other countries in SSA, the agricultural landscape

remains largely dependent on imported or poorly adapted pest control technologies that are not always suitable for local conditions or acceptable to end-users (Tambo et al., 2023).

Entomopathogenic nematodes have been widely integrated into integrated pest management as environmentally benign agents for soil and some foliar insect pests (Koppenhöfer et al., 2020; Lacey et al., 2015). Belonging primarily to the genera *Steinernema* Travassos (Steinernematidae) and *Heterorhabditis* Poinar (Heterorhabditidae), EPNs possess a unique combination of desirable traits, including host specificity, ease of mass production, and the ability to persist in the soil (Georgis et al., 2006; Kaya & Gaugler, 1993). Their mutualistic association with symbiotic bacteria (*Xenorhabdus* spp. in *Steinernema* spp. and *Photorhabdus* spp. in *Heterorhabditis* spp.) enables rapid killing of insect hosts through septicemia, often within 48 to 72 hours (Lacey et al., 2015; Lewis et al., 2006). Despite these advantages, uptake and field use across much of Africa remain limited, with applied research and local adaptation still developing (Koppenhöfer et al., 2020; Platt et al., 2020).

The present study is situated within this broader challenge. It seeks to explore the biological control potential of indigenous EPN isolates in Nigeria using *S. frugiperda* as a model pest. Although FAW is currently not the most devastating pest in all Nigerian regions, its rapid spread, high reproductive capacity, and extensive host range make it a suitable target for EPN efficacy screening. More importantly, the study aims to demonstrate how local biocontrol agents can be developed through a systematic approach that includes farmer perspectives, species identification, virulence profiling, and ecological characterisation. This approach is intended not only to generate scientific knowledge but also to inform future efforts at sustainable pest management within the Nigerian context.

1.2 The Burden of Insect Pests and Farmer Practices in Nigeria

Agricultural productivity in Nigeria is constrained by recurrent insect pest pressure on staple crops that are central to household food security and national economic stability. Major pests include the maize stem borer *Busseola fusca* Fuller (Lep.: Noctuidae), the legume pod borer *Maruca vitrata* Fab. (Lep.: Crambidae), the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Hem.: Pseudococcidae), and the tomato leafminer *Phthorimaea* (*Tuta*) *absoluta* Meyrick (Lep.: Gelechiidae), each associated with substantial yield loss in African production systems (Ba et al., 2019; Desneux et al., 2010; Kfir et al., 2002; Neuenschwander, 2001). These pressures have been compounded by invasions, including *S. frugiperda*, first reported in West and Central Africa in early 2016 and now a seasonal threat to maize production (Goergen et al., 2016).

The economic implications of such infestations are far-reaching. Studies have shown that uncontrolled insect pest outbreaks can reduce yields by 20 to 80%, depending on the pest species, crop type, and

agroecological conditions (Savary et al., 2019). For smallholder farmers, who constitute over 70% of Nigeria's farming population, these losses often translate into food insecurity, income instability, and reduced capacity to invest in inputs. In response to acute outbreaks, farmers frequently resort to chemical insecticides obtained through open markets that often provide limited advisory support and operate under uneven regulatory enforcement, patterns documented across West Africa and in Nigerian case studies (Adesuyi et al., 2018; Haggblade et al., 2021; Kansiime et al., 2019).

Across West Africa, pesticide imports have risen sharply, doubling from 218,948 tonnes in 2015 to 437,930 tonnes in 2020, with Nigeria alone importing 147,446 tonnes in 2020 (Heinrich Böll, 2022). Repeated applications of broad-spectrum products are linked to resistance risks, disruption of beneficial arthropods, and residue concerns in food and water (Onunkun et al., 2021). More many farmers also apply pesticides without adequate training or protective equipment, which raises the likelihood of acute and chronic health effects (Oluwole & Cheke, 2009; Yusuf et al., 2019).

In Nigeria, extension services that could support safe and sustainable pest management are frequently under-resourced or irregular in rural areas, which limits farmers' access to practical training on non-chemical options (Coyne et al., 2019; Isgren & Andersson, 2023). This situation contributes to persistent knowledge gaps about alternative strategies, including biological control, and where awareness exists it is often accompanied by uncertainty about efficacy or access (Adesuyi et al., 2018; Kansiime et al., 2019). At the market level, locally available and affordable biological control products remain scarce due to commercialisation barriers and weak distribution systems, a challenge noted across Africa and relevant to Nigeria (Grzywacz et al., 2014; van Lenteren et al., 2018). Together these constraints help explain why many Nigerian farmers rely on chemical options despite interest in safer and more sustainable approaches.

In this context, the development and promotion of indigenous biological control agents such as EPNs offer an opportunity to address both the pest burden and the structural deficiencies in current management practices. By leveraging organisms that are already adapted to local environmental conditions, and by aligning research with farmer realities, there is potential to build a more resilient and ecologically sustainable pest management system in Nigeria.

1.3 Sustainable Pest Management and the Role of Biological Control

The global shift towards more sustainable agriculture has brought IPM to the forefront as a systematic and environmentally responsible approach to crop protection. It involves the deliberate combination of biological, cultural, mechanical, and chemical control methods to manage pest populations at economically acceptable levels while reducing adverse effects on human health and the environment

(Barzman et al., 2015; Kogan, 1998). Within this framework, biological control is a central element because it uses natural enemies to prevent or suppress pest outbreaks and to support ecological balance in production systems (van Lenteren, 2012).

Biological control agents include parasitoids, predators, and microbial pathogens such as entomopathogenic fungi, bacteria, and nematodes. They suppress pest populations through parasitism, predation, or infection that results in mortality or inhibition of pests (Eilenberg et al., 2001; Hajek & Eilenberg, 2018). The benefits of biological control are well documented, including the potential for durable suppression with limited ecological disruption and reduced reliance on synthetic insecticides where programmes are well designed and supported (Hajek & Eilenberg, 2018; van Lenteren et al., 2018).

In African agricultural systems, biological control has not yet been widely adopted beyond a few classical successes. A notable example is the introduction of the parasitoid *Anagyrus lopezi* De Santis (Hym.: Encyrtidae) for suppression of the cassava mealybug, *P. manihoti* Matile-Ferrero across several countries in SSA, including Nigeria (Neuenschwander, 2001). Despite this achievement, adoption in annual cropping systems remains limited. Reported constraints include low awareness among farmers, limited research and development capacity, uneven regulatory and market frameworks, and the scarcity of commercially available biological control products (Dunn & Malan, 2025; Grzywacz et al., 2014; van Lenteren et al., 2018). Recent syntheses and field studies also highlight institutional and distribution barriers that restrict uptake in smallholder contexts (Durocher-Granger et al., 2023).

Among the microbial control options, EPNs have shown considerable potential due to their broad host range, effectiveness against soil and foliar pests, and compatibility with other pest management tools. Species belonging to the genera *Steinernema* and *Heterorhabditis* are of particular interest due to their obligate symbiosis with insect-pathogenic bacteria from the genera *Xenorhabdus* and *Photorhabdus*, respectively (Dillman et al., 2015; Kaya & Gaugler, 1993). Once the nematode gains entry into the insect host through natural body openings, it releases its bacterial symbionts into the haemocoel, causing rapid death of the host by septicemia, usually within two to three days (Lacey et al., 2015).

In addition to their mode of action, EPNs possess traits that make them highly suitable for sustainable pest management. They can be mass-cultured using *in vivo* or *in vitro* methods, formulated into various delivery systems, and applied with conventional farm equipment. EPNs pose minimal risks to humans, animals, and non-target organisms, and their use does not contribute to pesticide residue accumulation or environmental pollution (Lacey & Georgis, 2012; Shapiro-Ilan et al., 2006). These attributes make

them especially promising for smallholder farming contexts in SSA, where the need for environmentally acceptable and farmer-friendly pest management tools is urgent.

In the Nigerian setting, where pest outbreaks are recurrent and pesticide misuse is widespread, the integration of biological control into mainstream agricultural practice remains limited. Farmers often rely exclusively on chemical insecticides, applied with limited technical support or understanding of potential long-term consequences. The lack of awareness about biological alternatives, coupled with the unavailability of affordable biocontrol products, has further entrenched this dependence (Onunkun et al., 2021). Awareness of biological alternatives is uneven, and affordable products are often unavailable or poorly distributed, which reinforces chemical dependence (van Lenteren et al., 2018). In this context, the development of EPNs sourced from local agroecological zones represents a promising opportunity to introduce effective and adaptable biocontrol options into existing farming systems (Dunn & Malan, 2025; Lacey & Georgis, 2012).

1.4 The Biocontrol Potential of Indigenous EPNs

The development of biological control strategies using EPNs has traditionally relied on the selection of strains that exhibit high virulence, environmental persistence, and ease of mass production. While commercial strains of *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* have been widely tested and used in temperate regions, their performance in tropical and subtropical environments has often been inconsistent due to ecological mismatch and reduced stress tolerance under local conditions (Ehlers, 2005; Hazir et al., 2003). This has led to a growing recognition of the value of indigenous EPN isolates that are better adapted to the specific climatic, edaphic, and ecological conditions of their native habitats.

In tropical agricultural systems, locally sourced EPNs may provide significant advantages over introduced strains. Indigenous isolates are more likely to possess tolerance to high temperatures, fluctuating moisture regimes, and interactions with native soil microbiota (Hazir et al., 2003; Lewis et al., 2024; Mukuka et al., 2008). They may also be better adapted to locate hosts under local soil textures, crop residue levels, and pest behaviour patterns. These adaptations are crucial for achieving successful infection, reproduction, and recycling of nematode populations in the field.

The potential of indigenous entomopathogenic nematodes has been demonstrated in parts of Africa, although research remains limited in scope. In Kenya, surveys have identified native *Steinernema* and *Heterorhabditis* with promising attributes, including records from the Central Rift Valley and evaluations against local pests (Mwaniki et al., 2008). In South Africa, several studies report effective indigenous strains and show cases where local isolates perform as well as or better than commonly

used exotics in pathogenicity or suitability for local conditions (du Preez et al., 2022; Platt et al., 2020). These findings underscore the value of biocontrol pipelines that start with locally validated strains and move toward product development and commercialisation.

Nigeria, despite its ecological diversity and large agricultural base, remains comparatively understudied in terms of EPN biodiversity and functional potential. Confirmed records include the first report of EPNs from several northern states and more recent recoveries from southwestern Nigeria, with some laboratory evaluations against key pests (Akyazi et al., 2012; Ottun et al., 2021; Rufai et al., 2020). Little is known about the virulence, behavioural ecology, or environmental stress tolerance of these isolates. These knowledge gaps limit both scientific understanding and practical application, particularly in the face of growing interest in non-chemical pest control.

The use of *S. frugiperda* as a model target in the present study offers a valuable opportunity to assess the efficacy of indigenous EPNs under laboratory. Although FAW is not currently the most widespread or destructive pest across all regions of Nigeria, its lifecycle is well described, it is economically important, and it is already established as a target in screening trials for entomopathogenic nematodes, which makes it a suitable organism for benchmarking nematode performance. Moreover, FAW's behaviour, including surface feeding, burrowing, and pupation in the soil makes it accessible to EPN (Baur, Kaya, & Strong, 1998; Baur, Kaya, Tabashnik, et al., 1998; Kenis et al., 2023; Shapiro-Ilan et al., 2006).

By focusing on indigenous isolates, this study aims to generate ecologically relevant data to guide future selection and deployment. Characterising virulence, foraging behaviour, and stress tolerance is a prerequisite for understanding their practical utility in Nigerian farming systems. In addition, evaluating farmer perceptions and willingness to adopt EPNs adds a social dimension to the biological inquiry, helping to align research outputs with user needs. Together, these efforts contribute to a more grounded and locally adapted model of biological control development.

1.5 Research Objectives

This research was designed to explore the potential for developing and deploying indigenous EPNs as biological control agents within the Nigerian agricultural context. Recognising the limitations of chemical pesticide dependence and the need for locally adapted, ecologically sustainable alternatives, the study aimed to address key gaps in both the scientific understanding of native EPN strains and the social dimensions that shape their practical use.

To achieve this aim, the research was structured around three interrelated components. Each component reflects a different but complementary perspective necessary for a holistic assessment of EPN-based pest management. These components are summarised below:

1.5.1 Socioeconomic Component: Farmer Perceptions and Adoption Potential

The first component focused on assessing farmers' awareness, perceptions, and willingness to adopt EPNs as a component of IPM. It involved a structured survey across major maize-producing regions in Nigeria, examining the factors that influence behavioural intentions, perceived barriers to adoption, and preferred learning pathways. This component was intended to identify the social and institutional conditions under which EPN technologies could be successfully introduced and scaled.

1.5.2. Biological Component: Identification and Virulence of Indigenous EPN Isolates

The second component involved the collection, isolation, and taxonomic identification of EPNs from Nigerian soils. Both morphological and molecular techniques were used to confirm species identities. Laboratory virulence assays were conducted to evaluate the pathogenicity of each isolate against different developmental stages of *S. frugiperda*, providing baseline data on infection efficiency and lethal concentration thresholds. This component addressed the biological viability and insecticidal potential of local strains.

1.5.3. Ecological Component: Environmental Stress Tolerance and Behavioural Ecology

The third component examined the ecological fitness of the isolates through a series of abiotic stress assays. These included temperature-dependent infectivity and reproduction, foraging strategy and vertical movement in soil, tolerance to desiccation and oxidative stress, and survival under hypoxic conditions. The purpose was to understand how environmental variables influence EPN performance and persistence in typical Nigerian agroecosystems. This component provided insights into the ecological adaptability of the isolates and their potential field stability.

Together, these three components offer a comprehensive assessment of the feasibility of using indigenous EPNs in Nigerian agriculture. The study’s multi-dimensional framework integrates biological efficacy, environmental compatibility, and social acceptability, which are factors that are often studied in isolation but are fundamentally interconnected in real-world pest management scenarios.

Chapter 2: Literature Review

Effective pest management remains a cornerstone of sustainable agriculture, especially in tropical and subtropical regions where pest pressure is exacerbated by climatic variability, crop diversity, and limited access to advanced control technologies. Over recent decades, SSA has experienced multiple pest outbreaks of both native and invasive species that have significantly constrained productivity and threatened food security (Abrahams et al., 2017; Savary et al., 2019). These pressures have often encouraged overreliance on chemical pesticides with environmental, economic, and health costs.

A growing body of work supports ecologically based pest management suited to local agroecological conditions and resource realities. Within this approach, biological control is noteworthy for the potential to provide durable suppression without the externalities of synthetic insecticides (van Lenteren et al., 2018). Entomopathogenic nematodes have attracted research and commercial interest because of their broad host range, ease of application, and favourable environmental safety profile (Lacey et al., 2015). While their use is well documented in temperate systems their application in tropical Africa remains relatively underdeveloped, with key knowledge gaps in species diversity, field efficacy, and farmer acceptability.

This chapter presents a comprehensive review of the literature relevant to this study. It begins by contextualising insect pest invasions in Africa, with a focus on economic impacts and species profiles. It then examines current pest management practices, including the limitations of chemical control, and explores the conceptual foundations of biological control within IPM. The biology, taxonomy, and infection mechanisms of EPNs are discussed, followed by an overview of empirical evidence regarding their use against *S. frugiperda* and other major insect pests. The chapter also reviews barriers to EPN adoption in Africa, including technical, socioeconomic, and institutional factors. Finally, it explores recent advances in ecological adaptation and environmental stress tolerance of EPNs, highlighting the importance of local strain selection for field success.

By synthesising these thematic areas, the chapter provides a foundation for the research objectives outlined in Chapter 1. It identifies where scientific understanding is well established, where gaps persist, and how the present study aims to contribute to this evolving field of sustainable pest management in African agriculture.

2.1 Invasive and Indigenous Insect Pests in Africa: Global and Local Contexts

Insect pests are among the most damaging biotic constraints to agricultural productivity across Africa. Indigenous and invasive species cause extensive yield losses in cereals, legumes, root crops, and

horticultural produce, with particularly severe impacts on smallholder farmers who rely on limited inputs and have few alternative management options (Rwomushana et al., 2018; Savary et al., 2019). Over roughly the past two decades, evidence from global and regional assessments indicates an increase in pest risk and damaging outbreaks, driven by interacting factors that include climate change effects on pest biology and distribution, trade and globalisation that facilitate invasions, land-use change that alters crop health and pest pressure, and landscape simplification that reduces natural enemy abundance (Gullino et al., 2022; Heeb et al., 2019; Savary et al., 2017; Scott-Brown et al., 2025; Skendžić et al., 2021).

Several indigenous pests remain economically important. In maize-based systems, the maize stem borer *B. fusca* is a longstanding constraint, with losses commonly reported between 20 and 50% in untreated fields (Kfir et al., 2002). In legume systems, the pod borer *M. vitrata* is a major pest of cowpea (*V. unguiculata*), damaging flowers and pods and causing substantial yield reductions during peak infestations (Tamò et al., 2012). Cassava cultivation in Nigeria and neighbouring countries has been negatively affected by cassava mealybug *P. manihoti*, which caused severe outbreaks in the 1980s before classical biological control was implemented (Neuenschwander, 2001).

The impact of invasive pests has intensified in recent years, with high-profile introductions disrupting established management strategies. One of the most prominent is FAW, *S. frugiperda*, native to the Americas and first reported in Africa in 2016 (Goergen et al., 2016). Within three years of its introduction, FAW had spread to more than forty African countries and infested maize, sorghum, millet, rice, and other crops (Day et al., 2017). Its larvae feed voraciously on leaf tissue, tassels, and ears of maize. Early scenario analyses soon after the pest was detected in Africa suggested that, without effective control, fall armyworm could reduce maize production in 12 African countries by 8.3 to 20.6 million tonnes per year (Abrahams et al., 2017). Recent studies show that FAW is now reported in 47 of 54 African countries and accounts for 9-54% of maize field losses, which contributes to the annual economic losses of approximately USD 9.4 billion across Africa (Barkessa et al., 2024; Kansiime et al., 2023; Mlambo et al., 2024; Overton et al., 2021; Zanzana et al., 2024).

Another invasive species of growing concern is the tomato leafminer, *T. absoluta*, which originated in South America and has rapidly colonised tomato-growing regions across Africa since its first detection in 2008. It damages leaves, stems, and fruits and can cause total crop failure when unmanaged (Desneux et al., 2010). The larger grain borer, *Prostephanus truncatus* (Col.: Bostrichidae), introduced into Africa in the late 1970s, remains a persistent storage pest in maize and cassava, that drives substantial postharvest losses (Boxall, 2002; Quellhorst et al., 2021). These examples highlight the

dynamic nature of pest pressures in Africa, where endemic and exotic species co-exist and interact with farming practices, climatic conditions, and policy environments. The arrival of invasive species such as *S. frugiperda* has not only disrupted ecological balances but has also overwhelmed national plant protection systems, many of which lack the capacity for rapid surveillance, containment, and development of alternative control methods (Nboyine et al., 2020; Nwanze et al., 2021; Odeyemi & Ugwu, 2021; Odong et al., 2024). As a result, many countries, including Nigeria, have defaulted to emergency pesticide use with mixed results and unintended consequences.

Thus, insect pest management in Africa must contend with both longstanding indigenous pests and rapidly spreading invasive species. The economic losses associated with these pests are substantial, affecting crop yields, quality, and household incomes. More importantly, the reliance on chemical control in the absence of effective and accessible alternatives underscores the urgency of developing sustainable, locally adapted strategies such as biological control using native entomopathogens. The following sections will examine how pest management is currently practised across the continent and the limitations that have hindered the adoption of safer and more ecologically rational approaches.

2.2 Pest Management in Africa: Practices, Costs, and Limitations

Routine pesticide use in SSA is concentrated among smallholders who produce market-oriented crops such as vegetables, cotton and cocoa, while many subsistence farmers growing staple crops continue to operate with very low external inputs. Insecticide use increased during the initial fall armyworm response in several countries as governments distributed and subsidised sprays for maize, and this pattern is documented alongside rapid continental spread of the pest (Abrahams et al., 2017; Day et al., 2017). Many farmers view insecticides as the most immediate and accessible means of protecting crops during invasive outbreaks when yields and incomes are under acute threat. Intensive and risky use is well described in market-vegetable systems, including Ethiopia and Ghana, where farmers commonly access products with limited oversight and report handling practices that raise health and environmental concerns (Demi et al., 2021; Mengistie et al., 2017; Negatu et al., 2021).

In Nigeria, chemical insecticides are readily available through agro-dealer networks in urban and rural markets. Evidence indicates uneven advisory quality and variable compliance with good distribution practice, which shapes farmer knowledge and behaviour in places such as Ogun State and reflects broader features of the input trade (Madaki et al., 2024; Olomola, 2014). Residue studies add further context from Lagos and Port Harcourt. Assessments have reported organochlorine and other pesticide residues in commonly consumed fruits and vegetables and have estimated human health risks associated with dietary exposure to legacy compounds in foods grown in Nigeria (Adeleye et al., 2019;

Omokpariola et al., 2023; Omoyajowo et al., 2018; Oyeyiola et al., 2017). A strategic review of persistent organic pollutants in Ogun State also documents trade and regulatory gaps that can perpetuate availability of problematic chemistries (Sustainable & Action for Environmental, 2021).

The continued dominance of pesticide-based responses also reflects limited access to viable alternatives and under-resourced extension systems that constrain accurate diagnosis, safe handling guidance, and farmer awareness of integrated pest management. In the absence of strong advisory support, application decisions are often guided by peer advice and sales recommendations, which increases the likelihood of sublethal dosing, resistance selection, and non-target effects on beneficial organisms like natural enemies and pollinators (Jepson et al., 2020; Matova et al., 2020). Together these patterns explain why pesticide dependence persists in market-oriented smallholder systems even as the need for safer and more sustainable approaches grows.

Health and environmental risks linked to pesticide misuse are well documented. Farmers and farmworkers are frequently exposed to hazardous active ingredients when personal protective equipment is not used, labels are unclear, and safe application practices are poorly understood (Damalas & Koutroubas, 2016; Yusuf et al., 2019). Evidence from Nigeria, Ethiopia and Uganda links chronic or repeated exposure to organophosphates and pyrethroids with adverse outcomes that include respiratory, neurological and dermatological symptoms, as well as sleep-related disturbances in exposed communities (Fuhrimann et al., 2022; Negatu et al., 2021; Yusuf et al., 2019). Residues detected in food, soils and water further raise concerns about wider ecosystem and public health effects (Onunkun et al., 2021).

Despite these risks, the persistence of chemical control in African farming systems reflects structural and institutional factors. Regulatory enforcement is often uneven, market incentives for safer alternatives are weak, domestic biopesticide manufacture is limited, and investment in locally adapted technologies remains insufficient. Historical promotion of synthetic insecticides has also shaped farmer perceptions of pesticides as fast and reliable solutions, which can constrain behavioural change toward alternatives such as biological control (Akutse et al., 2020; Grzywacz et al., 2014). A transition to sustainable pest management will therefore require not only effective biocontrol agents but also deliberate efforts to build farmer awareness, strengthen extension services, and create supportive policy and market environments. Locally sourced and adapted EPNs may offer one such alternative, however, their successful integration into farming systems will depend on a detailed understanding of their biology, ecological behaviour, and practical use, as discussed in the following sections.

2.3 Biological Control in IPM

Biological control is a central pillar of IPM and is defined as the use of living organisms to suppress pest populations so that they are less damaging than they would otherwise be (Eilenberg et al., 2001). In contrast to chemical insecticides that often produce immediate but short-lived effects, biological control offers a more sustainable form of regulation by re-establishing or enhancing natural ecological processes. These include predation, parasitism, pathogenicity and competition, which together act to maintain pest populations below economic thresholds.

Biological control strategies are commonly grouped into classical, augmentative and conservation approaches. Classical biological control introduces a natural enemy from the pest's native range into a region where the pest has become invasive. This approach was used successfully in Africa with the introduction of *A. lopezi* to manage *P. manihoti*, the cassava mealybug, which delivered sustained suppression across West and Central Africa (Neuenschwander, 2001). Augmentative biological control, which is the mass production and periodic release of natural enemies, either to inoculate a system (inoculative release) or to rapidly reduce pest numbers (inundative release), while conservation biological control focuses on modifying the environment to protect and enhance the effectiveness of existing natural enemies through habitat management, reduced disruption and compatible agronomic practices (Barzman et al., 2015; Eilenberg et al., 2001; van Lenteren, 2012).

In African smallholder agriculture, the adoption of biological control has historically lagged behind its development, despite the ecological and economic rationale for its use. Several challenges persist. These include the limited availability of suitable biocontrol products, lack of regulatory frameworks that support their registration and commercialisation, and weak extension systems that fail to promote their benefits to farmers (Mawcha et al., 2025; Ratto et al., 2022; Stevenson et al., 2017). Additionally, biological control agents are often perceived as slow acting compared to chemical insecticides, which make them less attractive in emergency pest outbreak situations unless their benefits are clearly demonstrated and understood (Dunn & Malan, 2025).

Despite these constraints, successful biocontrol programmes in Africa provide compelling evidence of their potential. For instance, the introduction of *A. lopezi* against cassava mealybug, and the braconid parasitoid *Cotesia flavipes* (Cameron) against the lepidopteran stem borer *Chilo partellus* (Swinhoe) (Lep.: Crambidae), have had measurable impacts on pest suppression and yield recovery in cassava and cereal systems, respectively (Herren & Neuenschwander, 1991; Kipkoech et al., 2008; Overholt et al., 1997; Zeddies et al., 2001). These examples demonstrate that, with appropriate investment in research, delivery, and farmer training, biological control can be a viable and lasting solution.

Microbial control agents, including bacteria, fungi, viruses, and nematodes, are gaining particular attention for their potential in augmentative strategies. These organisms can be cultured under laboratory conditions, formulated for field application, and applied using standard farming equipment (Lacey & Kaya, 2007; Shapiro-Ilan et al., 2025; Zhang et al., 2025; Zulu et al., 2025). Among these, EPNs have proven especially promising due to their ability to seek out and infect insect hosts actively, their safety to non-target organisms, and their adaptability to various cropping systems and soil types.

The successful integration of biological control into pest management programmes requires a systems-based approach that considers both biological efficacy and the social, institutional, and economic environments in which these agents are to be deployed. EPNs, as the next section will explore, possess a number of biological characteristics that make them uniquely suited for this role, particularly in smallholder farming systems facing increasing pest pressure and ecological degradation.

2.4 EPNs: Biology, Taxonomy, and Mode of Action

EPNs are obligate insect parasites belonging primarily to two families: Steinernematidae and Heterorhabditidae (Kaya & Gaugler, 1993). These families comprise genera such as *Steinernema* and *Heterorhabditis*, which have been the focus of most biological control research and commercial development. EPNs are found naturally in soils across a wide range of habitats, where they act as important regulators of insect populations. Their capacity to actively seek out hosts, kill them rapidly, and reproduce within the host cadaver has made them attractive candidates for use in IPM programmes.

The infective juvenile (IJ) stage, which is developmentally analogous to the dauer stage in free-living nematodes such as *C. elegans*, is the only free-living and infective form in the EPN life cycle (Amrit et al., 2014; Wang et al., 2009). The IJs are non-feeding, environmentally resistant, and capable of surviving in the soil for extended periods while seeking out a suitable host. Host-seeking strategies vary across species and are broadly categorised into ambushers and cruisers. Ambusher species, such as *S. carpocapsae*, tend to wait near the soil surface and rely on contact with passing insects. Cruiser species, such as *H. bacteriophora*, actively move through the soil in search of sedentary or subterranean hosts (Lewis et al., 2006; Lewis et al., 2024).

Upon locating a host, IJs enter the insect body through natural openings such as the mouth, spiracles, or anus, or by penetrating thin regions of the cuticle. Once inside, they release symbiotic bacteria into the host haemocoel. In *Steinernema* species, the bacteria belong to the genus *Xenorhabdus*, while *Heterorhabditis* species are symbiotically associated with *Photorhabdus* bacteria (Půža & Machado, 2024; Sajnaga & Kazimierczak, 2020; Sajnaga et al., 2024). These bacteria rapidly multiply and produce toxins and enzymes that kill the host within 24 to 72 hours. The nematodes then feed on the

bacterial soup and degraded host tissues, completing several generations within the cadaver before emerging as a new cohort of IJs (Koppenhöfer et al., 2020; Lacey & Georgis, 2012; Sivaramakrishnan & Razia, 2021b).

The pathogenic process is a result of a highly evolved mutualistic relationship between the nematode and its bacterial partner. This symbiosis allows for rapid host mortality, suppression of competing microbes, and optimisation of the cadaver environment for nematode reproduction (X. Zhang et al., 2019). Moreover, both nematode and bacterial components contribute to the suppression of the host immune response, allowing for efficient colonisation and development (Sivaramakrishnan & Razia, 2021a).

Taxonomic identification of EPNs has historically relied on morphological features, including body length, tail shape, spicule morphology in males, and reproductive structures in females or hermaphrodites. However, due to high intraspecific variation and the existence of cryptic species, molecular tools have become increasingly important. Commonly used genetic markers include the internal transcribed spacer (ITS) region, the D2–D3 expansion segment of the 28S rRNA gene, and mitochondrial cytochrome oxidase subunit I (COI) (Adams et al., 2006; Nguyen & Hunt, 2007; Nguyen et al., 2006). These markers are used for phylogenetic analysis, species confirmation, and comparison of local isolates with known reference strains.

EPNs possess a number of ecological traits that make them suitable for field use. They are able to persist in the soil, tolerate a range of environmental conditions, and can be applied using conventional spraying or irrigation equipment. Importantly, they pose minimal risk to non-target organisms, including humans, vertebrates, and most beneficial arthropods, due to their narrow host range and the requirement for specific host conditions to complete their life cycle (Koppenhöfer & Kaya, 1999; Kour et al., 2021; Stefanovska et al., 2011).

EPNs represent a well-characterised group of biological control agents with demonstrated efficacy against a wide range of insect pests. Their complex, yet efficient host-pathogen system, combined with their adaptability to soil environments, make them particularly promising for use in smallholder farming systems where chemical control is often problematic. However, as discussed in the next section, field efficacy is strongly influenced by ecological conditions and the interaction between host behaviour, nematode foraging, and environmental stress factors.

2.5 EPNs in Pest Control: Evidence from FAW and Other Species

EPNs have been extensively studied for their potential in managing economically important insect pests across multiple cropping systems. Their use has been reported against a wide range of pest taxa, including Lepidoptera, Coleoptera, and Diptera, with varying degrees of success depending on nematode species, host susceptibility, environmental conditions, and application strategies (Bhat et al., 2020; Lacey et al., 2015; Shapiro-Ilan et al., 2006). In tropical agriculture, EPNs offer particular advantages due to their ability to function under field conditions that often limit the efficacy of other biocontrol agents.

In the context of *S. frugiperda*, several studies have demonstrated promising outcomes with different EPN species. Research in Brazil, the pest's native region, has shown that *S. carpocapsae* and *H. indica* can cause significant mortality in FAW early instar larvae when applied to infested maize plants or directly to the soil surface (Andaló et al., 2010; Garcia et al., 2008; Leite et al., 2017; Negrisoli et al., 2010). Similarly, Molina-Ochoa et al. (2003) reported that *H. bacteriophora* achieved over 80% larval mortality under controlled conditions. These findings have been corroborated by studies from other tropical countries, including India and Ghana, where EPNs have demonstrated activity against FAW under laboratory and greenhouse conditions (Acharya, Hwang, et al., 2020; Danso et al., 2021; Sayed et al., 2022; Shinde et al., 2022).

In Africa, research on the use of EPNs against FAW is still in its early stages, although several indigenous strains have shown encouraging results. Waturu et al. (1997) reported on locally isolated strains of *S. karii* and *H. zealandica* in Kenya, with notable virulence against FAW larvae. In Rwanda, Fallet et al. (2020) assessed the pathogenicity of two isolates of *H. bacteriophrora*, on FAW larvae and highlighted smallholder farmer interest in alternatives to synthetic pesticides following repeated FAW infestations, suggesting that EPN-based solutions may be well received if they demonstrate clear efficacy and economic viability.

Beyond FAW, EPNs have been deployed with success against several other major pests in African and Asian farming systems. These include the sweetpotato weevil *Cylas puncticollis* (Boheman) (Col.: Brentidae), tomato leafminer *P. absoluta*, the sugarcane stalk borer *Eldana saccharina* Walker (Lep.: Pyralidae), *Bactrocera dorsalis* (Hendel) (Dip.: Tephritidae) and the cabbage moth *P. xylostella* (L.) (Lep.: Plutellidae) (Godjo et al., 2018; Ramakuwela et al., 2015; Ramakuwela et al., 2025). In most of these cases, the success of EPNs has been influenced by the match between nematode foraging strategy and host location, the developmental stage of the target pest, and the microclimatic conditions at the time of application.

Despite their potential, the field use of EPNs remains limited in many low- and middle-income countries due to gaps in formulation technology, shelf-life constraints, and limited commercial production infrastructure (Ehlers, 2005). Often, promising results obtained under laboratory conditions have not been translated to consistent field efficacy, especially in open-field systems with high exposure to desiccation and UV radiation (Fatimah et al., 2025; Platt et al., 2020; Shapiro-Ilan et al., 2015). This underscores the need for ecological screening and local adaptation of isolates prior to deployment as well as the development of suitable formulations.

One advantage of EPNs is their compatibility with other biocontrol and chemical agents. Studies have shown that EPNs can be integrated with entomopathogenic fungi, such as *Beauveria bassiana* (Bals-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin (both Hypocreales), as well as selective insecticides that do not harm nematode populations (Shapiro-Ilan et al., 2012). Such compatibility enhances the scope for incorporating EPNs into broader IPM strategies, reducing the risk of resistance development and minimising non-target effects.

The evidence base for the use of EPNs against *S. frugiperda* and other pests is steadily growing. While most studies confirm the high virulence of *Steinernema* and *Heterorhabditis* species under controlled conditions, there is a clear need for locally adapted isolates and field-relevant data to inform sustainable adoption in African agriculture. The next section will explore the sociotechnical barriers that have hindered the wider uptake of EPN technologies in sub-Saharan Africa, despite their ecological promise.

2.6 Barriers to Adoption of EPNs in Africa

Although EPNs have demonstrated considerable potential for insect pest management in various cropping systems, their adoption in African agriculture remains limited. Unlike in Europe, North America, and parts of Asia, where EPN-based products have been commercialised and integrated into pest management programmes, SSA has yet to establish a functional EPN value chain from research to application (Becerra-Encinales et al., 2024; Dunn & Malan, 2025; Marrone, 2007). This gap is not only scientific but also institutional, economic, and behavioural.

A major barrier to adoption is the limited awareness and knowledge among farmers, extension agents, and even agricultural input suppliers regarding biological control in general and EPNs in particular. Studies across Nigeria, Kenya, Malawi, and Ghana consistently show that smallholder farmers are either unaware of EPNs or lack confidence in their effectiveness as pest control agents (Constantine et al., 2023; Kirui et al., 2023; Oyediran, 2023). Unlike chemical pesticides, which have long been

promoted and are readily available in both formal and informal markets, EPNs are rarely mentioned in extension programmes or agro-dealer recommendations.

Another constraint relates to the absence of local production facilities for EPN formulations. Unlike synthetic insecticides, EPNs are living organisms that require specific production, storage, and transport conditions. Most commercial EPNs used in international markets are produced using advanced in vitro fermentation systems that are not yet widely established in Africa (Ehlers, 2005). Where in vivo production is possible using insect hosts such as the wax moth *Galleria mellonella* (L.) (Lep.: Pyralidae), it is labour-intensive and unsuitable for large-scale deployment. These technological limitations make EPNs less competitive in commercial input markets, especially where economies of scale and shelf-life are critical for distribution.

The regulatory environment also poses challenges. In many African countries, including Nigeria, there is a lack of clear guidelines for the registration, quality assurance, and monitoring of biocontrol products. This regulatory uncertainty discourages private sector investment and hinders innovation in biopesticide development, even where national policies acknowledge the importance of IPM, the institutional frameworks to support biological control remain weak or fragmented (Akutse et al., 2020; Ashaolu et al., 2022; Bailey et al., 2010; Grzywacz et al., 2014).

Economic factors further constrain adoption. While EPNs can reduce pest pressure without the health and environmental costs of chemical pesticides, they often require up-front investment in training, application equipment, and in some cases, cold storage. These requirements may be unaffordable or impractical for smallholder farmers operating under severe financial constraints. Moreover, because EPNs do not produce immediate, visible effects like chemical insecticides, farmers may perceive them as less effective, especially when dealing with highly mobile or fast-reproducing pests such as *S. frugiperda* (Grewal et al., 2005; Kalyebi et al., 2023; Ramakuwela et al., 2015; Shapiro-Ilan et al., 2006).

Behavioural and cultural factors also play a role. Farmers' pest control decisions are often shaped by habit, risk aversion, and peer influence. In many rural areas, the application of pesticides is considered standard practice, while biological control is viewed as experimental or unfamiliar. Without positive demonstrations, peer adoption, or extension support, even the most effective EPN products may struggle to gain traction (Constantine et al., 2020; Fallet et al., 2024; Isgren & Andersson, 2023; Murage et al., 2015; Tambo et al., 2023). In addition, trust in biologicals may be undermined by inconsistent results due to poor matching of EPN species to local ecological conditions or inappropriate application timing and technique (Touray et al., 2025).

Despite these challenges, there is growing recognition of the need to invest in the development and promotion of EPNs in Africa. Several research institutions in Africa, including the International Centre of Insect Physiology and Ecology (*icipe*) and the International Institute of Tropical Agriculture (IITA), have initiated efforts to explore native EPN strains and develop protocols for their use in local farming systems (Daramola et al., 2021; Kanga et al., 2012; Ndereyimana et al., 2019; Rakubu et al., 2024). These efforts are laying the foundation for more context-specific applications of EPN technology and, ultimately, for the development of market-ready biological control products.

In essence, the low adoption of EPNs in Africa is not a reflection of their biological inefficacy but rather a consequence of systemic barriers in knowledge dissemination, production infrastructure, regulatory frameworks, and farmer perception. Addressing these barriers will require coordinated efforts across research, policy, and extension systems, along with the development of robust local isolates that are adapted to the environmental and economic realities of African farming contexts.

2.7 Environmental Stress Tolerance and Ecological Adaptation of EPNs

The successful application of EPNs in open-field environments depends not only on their pathogenicity but also on their ability to survive and function under variable abiotic conditions. Environmental factors such as temperature, moisture, oxygen availability, and oxidative stress influence nematode viability, host-seeking behaviour, and reproductive capacity (Grewal et al., 2011; Grewal et al., 1994; Labaude & Griffin, 2018). These variables are particularly important in tropical agricultural systems, where high temperatures, seasonal droughts, and fluctuating soil conditions can compromise the persistence and efficacy of applied nematodes.

Temperature is among the most critical determinants of EPN activity. Optimal infectivity for most *Steinernema* and *Heterorhabditis* species occurs between 20 and 30 °C, although significant variation exists among species and even among isolates of the same species (Hazir et al., 2003). Temperatures above 35 °C can lead to mortality or reduced infectivity in sensitive strains, while low temperatures may delay development and reproduction. For example, *S. feltiae* is more cold-tolerant than *S. carpocapsae*, which is in turn more heat-tolerant under tropical conditions (Glazer, 2022). These differences underscore the importance of selecting species and isolates adapted to the thermal regimes of target environments.

Desiccation is another major constraint for field-applied EPNs, particularly in sandy or poorly structured soils with low water retention. The IJ stage is prone to water loss due to its high surface area-to-volume ratio, and even moderate reductions in soil moisture can reduce mobility, host-finding capacity, and survival (Okolo et al., 2018; Patel et al., 1997; Perry, 1999). Some species exhibit greater

tolerance through physiological mechanisms such as anhydrobiosis, while others can survive at lower water activity levels (a_w) for limited periods. Qiu and Bedding (2002) demonstrated that different *S. carpocapsae* isolates could maintain viability at a_w values as low as 0.90, although reproduction was impaired. Selection of desiccation-tolerant isolates is therefore essential for successful deployment of EPNs in semi-arid and drought-prone regions (Strauch et al., 2004).

Hypoxia and flooding conditions also affect EPN survival, particularly in waterlogged soils or poorly drained fields. The absence of oxygen impairs nematode respiration and may affect the viability of symbiotic bacteria necessary for pathogenesis. Nonetheless, certain species, including *H. bacteriophora*, have demonstrated moderate tolerance to low oxygen conditions, surviving for 48 to 72 hours in anoxic environments with limited loss of infectivity. These traits may prove valuable in rice-growing areas and riverine farming systems, where transient flooding is common (Grewal et al., 2002; Kour et al., 2021).

Oxidative stress, although less frequently studied, is an important environmental factor that affects nematode survival in microbially active or chemically altered soils. Exposure to reactive oxygen species (ROS) may occur during host invasion or due to soil chemical properties. The ability of EPNs to resist oxidative stress depends on both nematode-derived enzymes and the protective role of their symbiotic bacteria (Sumaya et al., 2017; Sumaya et al., 2018; W. Zhang et al., 2019). Isolates that exhibit higher tolerance to oxidative environments may have enhanced field persistence, especially in intensively farmed or pesticide-impacted soils.

Ecological adaptation also extends to host-seeking behaviour. Nematode foraging strategies influence their ability to locate and infect hosts under different environmental conditions. Ambusher species such as *S. carpocapsae* rely on vertical positioning and jumping behaviour to intercept mobile surface-feeding insects, while cruiser species such as *H. bacteriophora* move horizontally and vertically through the soil to locate sedentary or subterranean hosts (Lewis et al., 2006; Lewis et al., 2015; Lewis et al., 2024). The foraging strategy must therefore align with the behaviour and spatial niche of the target pest. In the case of *S. frugiperda*, which has both foliar-feeding and soil-dwelling stages, the use of multiple foraging types may enhance control outcomes.

Local adaptation is increasingly recognised as a key factor in improving field performance. Isolates sourced from the same or similar agroecological zones as the target area are more likely to possess the physiological and behavioural traits necessary for survival and infectivity under real-world conditions (Dolinski et al., 2006). For this reason, efforts to recover, characterise, and deploy indigenous EPNs are gaining momentum in several African countries, including Benin, Kenya, South Africa, Benin and

Nigeria (Abate et al., 2018; Bhat et al., 2020; Daramola et al., 2021; Dlamini et al., 2019; Godjo et al., 2021; Nosa, 2024; van Niekerk & Malan, 2012).

Environmental stress tolerance and ecological adaptation are fundamental determinants of EPN efficacy in the field. Understanding these traits not only guides the selection of suitable isolates for specific environments but also informs formulation development, application timing, and integration with other pest management strategies. The next section examines the complex interactions between EPNs and their insect hosts, with emphasis on behavioural and physiological dynamics that influence infection success.

2.8 EPN–Host Interactions and Behavioural Ecology

The success of EPNs in insect pest management depends not only on their virulence or environmental tolerance but also on the intricate behavioural and physiological interactions between the nematode, its insect host, and the surrounding habitat. These host–parasite interactions are governed by complex ecological processes, including host location, recognition, immune evasion, and manipulation of host behaviour and physiology (Koppenhöfer & Fuzy, 2009; Lewis et al., 2006).

The first critical step in the infection process is host location. EPNs rely on a range of sensory cues to detect and orient towards suitable insect hosts. These cues include CO_2 gradients, temperature, vibration, and chemical exudates such as fatty acids, cuticular hydrocarbons, and faecal volatiles (Grewal et al., 1997). The IJs display considerable plasticity in their behavioural responses, often adjusting their search strategy depending on soil texture, moisture, and the presence of host-related cues. This behavioural flexibility is a key determinant of foraging efficiency and varies both between and within species.

As previously noted, EPNs are typically categorised into ambushers and cruisers based on their host-seeking behaviour. Ambushers such as *S. carpocapsae* display a sit-and-wait strategy, often positioning themselves near the soil surface or on plant stems where they can attach to passing insects, including mobile larvae like *S. frugiperda* (Grunseich et al., 2021; Kaya & Gaugler, 1993; Sivaramakrishnan & Razia, 2021a). Cruisers, including *H. bacteriophora*, engage in active searching behaviour and are more effective against sedentary or soil-dwelling pests such as root feeders and pupae (Grunseich et al., 2021; Rakubu et al., 2024). Some species exhibit intermediate strategies or context-dependent behaviour, making them suitable for broader pest management applications.

Once inside the host, EPNs must evade or suppress the insect immune response, which includes both cellular and humoral components. Insect haemocytes can encapsulate and melanise invading

organisms, while antimicrobial peptides may limit bacterial proliferation. To counteract this, both the nematode and its symbiotic bacteria produce immunomodulatory compounds that inhibit haemocyte activation, prevent coagulation, and suppress the production of reactive oxygen species (Bai et al., 2013; Ciche et al., 2006; Herbert et al., 2007). These mechanisms ensure that the nematode–bacterium complex can proliferate rapidly within the insect haemocoel and cause host death within 24 to 72 hours.

Host specificity in EPNs is typically broad, although there is evidence of interspecific variation in susceptibility due to differences in cuticle thickness, immune competence, and behaviour. Larval stages of Lepidoptera are generally more susceptible than adults or pupae, with early instars often displaying higher infection rates and faster mortality (Molina-Ochoa et al., 2003). For instance, in studies involving *S. frugiperda*, second and third instars are more readily infected than older larvae or pupae, partly due to their thinner cuticles and reduced behavioural defences (Alonso et al., 2018; Fuxa et al., 1988; Labaude & Griffin, 2018).

Host behaviour can also influence infection outcomes. Some insects exhibit behavioural fever by seeking warmer microhabitats to reduce nematode survival or increase their own immune activity (Eleftherianos et al., 2016). Others engage in grooming, burrowing, or avoidance behaviours that reduce the likelihood of nematode attachment or penetration. Conversely, EPN-infected hosts often exhibit manipulated behaviours, such as reduced movement or altered feeding, that may facilitate nematode development or dispersal. These dynamics are part of a broader co-evolutionary relationship in which hosts and parasites constantly adapt to each other's strategies.

EPN performance is also influenced by biotic interactions within the soil food web. Predation by mites, microarthropods, or other nematodes can reduce IJ populations, while microbial antagonism may limit bacterial proliferation in the host cadaver (Koppenhöfer et al., 2020). Conversely, certain microbial communities may enhance EPN persistence by improving soil structure, water retention, or host attraction. These ecological interactions underscore the need to consider whole-system dynamics when deploying EPNs in the field.

In summary, EPN–host interactions are shaped by a combination of nematode behaviour, host physiology, and environmental conditions. Effective biocontrol depends on aligning these factors to maximise host contact, infection efficiency, and progeny emergence. Understanding these dynamics not only informs isolate selection and application strategies but also improves predictive models for field performance.

Chapter 3: Can biological control become a scalable solution for crop pest management in Nigeria? A focus on entomopathogenic nematodes

3.1 Introduction

Insect pests continue to severely constrain crop production in Nigeria, where yield losses in major staples can exceed 50% during outbreaks (Banwo & Adamu, 2003; Benjamin et al., 2024). Key pests such as *S. frugiperda*, *Maruca vitrata*, and *Phthorimaea absoluta* affect maize, cowpea, and tomato respectively, with smallholder farmers depending almost entirely on chemical pesticides for control. The intensive and often indiscriminate use of these inputs contributes to pest resistance, environmental contamination, and health risks for farmers and consumers (Nwadike et al., 2021; Yami et al., 2025).

Biological control agents provide safer and more sustainable pest management alternatives. They include entomopathogenic fungi, nematodes, parasitoids, and predators, which are key components of integrated pest management (van Lenteren, 2012; van Lenteren et al., 2018). Among these, EPNs are particularly promising due to their broad host range, rapid action through symbiotic bacteria, and proven safety to non-target organisms. Commercial formulations are widely used in North America and Europe but remain unavailable in most African countries.

Research in Africa, including Nigeria, has identified several indigenous EPN species and demonstrated their efficacy against pests such as FAW and cutworms (Daramola et al., 2021; Toepfer, 2024). However, these findings have not translated into practical adoption, and no EPN products are currently distributed or produced locally. Farmers have little awareness or access, and appropriate formulations for aboveground pests are lacking. The disconnection between research progress and field-level uptake underscores the need to understand social and institutional factors shaping adoption.

This study examined Nigerian farmers' awareness, perceptions, and willingness to adopt EPNs as a case study of biological control innovation. A nationwide survey of 740 farmers across five agroecological zones was conducted to identify determinants of willingness to adopt, perceived barriers, and enabling factors. The study provides empirical evidence on how knowledge, institutional support, and perceptions influence technology acceptance, offering guidance for future scaling of sustainable pest management in Nigeria.

3.2 Materials and Methods

3.2.1 Study area and sampling

The study covered five major agroecological zones of Nigeria (Fig. 3. 1), Freshwater Swamp, Lowland Rainforest, Northern Guinea Savannah, Southern Guinea Savannah, and Sudan Savannah, to capture diverse farming systems and pest pressures. Representative towns included Port Harcourt and Lagos (Freshwater Swamp), Ibadan and Uyo (Lowland Rainforest), Kano and Kaduna (Northern Guinea Savannah), Enugu and Lokoja (Southern Guinea Savannah), and Sokoto and Bauchi (Sudan Savannah). FAW damage is most severe in the northern zones (Benjamin et al., 2024).

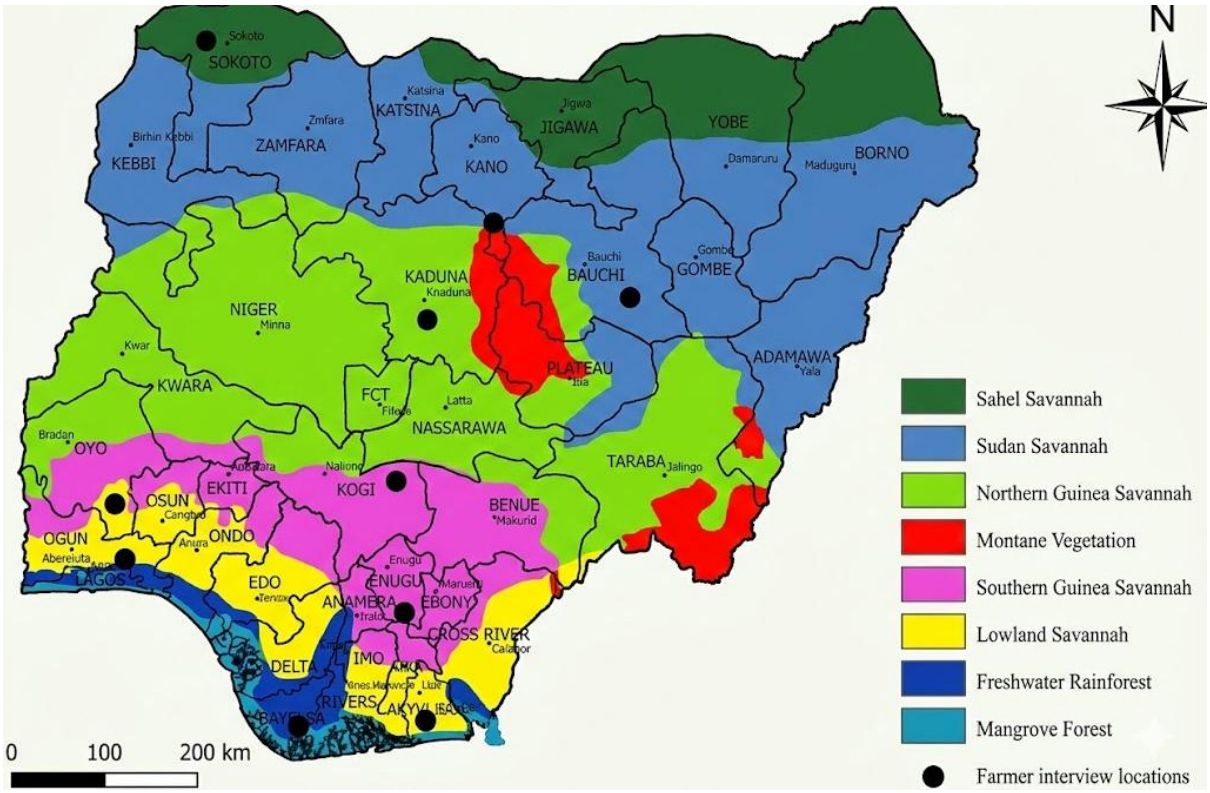


Fig. 3.1. Agroecological zones in Nigeria and survey sites.
Farmers' survey locations (marked with black dots) across the surveyed regions. Ten study locations (two towns per zone) served as statistical replicates (N= 10; $n_j = 74$ farmers per location; survey period: May–August 2022). Zones: Freshwater Swamp; Lowland Rainforest; Northern Guinea Savannah; Southern Guinea Savannah; Sudan Savannah.

A cross-sectional survey was conducted between May and August 2022. Using a multistage sampling procedure, farming districts were purposively selected within each zone, followed by random selection of villages and households. Participants were adult crop farmers (≥18 years) actively involved in pest management. In total, 740 farmers were interviewed. The sample size was derived assuming 50% adoption willingness, 5% margin of error, and 95% confidence, with allowance for non-response. Surveys were administered face-to-face, supported by local translation where required, following informed consent and confidentiality assurance.

3.2.2 Survey instrument

The questionnaire was developed by the research team and reviewed by entomologists and extension specialists for content validity and consisted of four sections.

3.2.2.1 Socio-demographic and farm characteristics

We gathered data on gender, age, education level, farming experience, farm size, main crops grown, and whether the farmer had access to extension services or belonged to any farmer groups/cooperatives. These variables provided context and allowed us to control for potential confounders in adoption propensity.

3.2.2.2 Pest management practices and awareness

We explored farmers' current pest control methods such as use of synthetic pesticides, cultural practices, their awareness of BCAs, and specifically their prior knowledge of EPNs. To gauge baseline awareness, farmers were asked questions such as: "Have you heard of entomopathogenic nematodes (insect-killing nematodes) before today?" (Yes/No), and "Have you ever used or seen any biological pest control products (such as microbial pesticides, beneficial insects, neem extracts)?" (Yes/No, with follow-up on which types). For those who answered "Yes" to knowing EPNs, further questions probed the source of their knowledge and whether they had ever tried them.

3.2.2.3 Perceptions and attitudes toward EPNs

Since most respondents were expected to be unfamiliar with EPNs, enumerators provided a brief, standardized explanation of what EPNs are and how they control insect pests (using simple terms and examples). Visual aids (illustrative pictures) were used to help explain the concept. After this introduction, farmers were presented with a series of statements to capture their perceptions of EPNs, using a 5-point Likert scale (1 = strongly disagree, 5 = strongly agree). These statements covered perceived benefits and concerns, for example: "Using entomopathogenic nematodes would be safer for my health and the environment than using chemical pesticides," "I believe EPNs can effectively control the pests on my farm," "Using EPNs would be too complicated or difficult for me," and "I am concerned about the cost of EPNs if they become available." We also asked how EPNs compare to farmers' current pest control methods: "Do you think using EPNs would be better, worse, or about the same as your current method in terms of effectiveness, cost, and ease?" This section allowed us to quantify farmers' attitudes (positive or negative) toward the idea of EPNs after they had a basic understanding of it. An index for "positive perception" was later constructed by combining several of these items (after reverse-coding negative phrasing), which showed good internal consistency (Cronbach's alpha = 0.78).

3.2.2.4 Willingness to adopt and perceived requirements

The final section focused on the farmers' willingness to adopt EPNs given certain conditions. The key question was: "If EPNs (insect-killing nematodes) were made available to you at an affordable cost and proven effective on your pest problem, would you be willing to use them on your farm?" with response options Yes, No, or Maybe/Not sure. We further asked those who said "No" or "Maybe" to explain their reservations (e.g., need more information, prefer current methods, fear of trying something new). Those who said "Yes" were asked why (e.g., desire to reduce chemical use, heard something positive, etc.). Importantly, all respondents were asked an open-ended question: "What would encourage or support you to use EPNs for pest control?" and conversely, "What do you see as the biggest challenges or obstacles if you were to use EPNs?" Enumerators recorded these responses verbatim, and later we categorized them into themes (e.g., needing training, availability of the product, cost issues, trust in effectiveness). We also inquired whether farmers would be willing to participate in training or field demonstrations on EPNs, and whether they would try EPNs first on a small portion of their land (to gauge their propensity for experimentation).

The questionnaire was pre-tested with 30 farmers in an area outside the study zone to ensure clarity and appropriate translation. Based on the pilot test, minor adjustments were made to question wording and length (for example, simplifying technical language around EPNs and ensuring the concept was understood).

3.2.3 Data analysis

All survey data were entered into a database and analysed using STATA 18 (StataCorp, College Station, TX). Prior to analysis, data cleaning was performed to check for consistency and handle any missing values. Simple imputation (using median values) was applied sparingly for a few missing demographic responses; however, for the key adoption-related questions (knowledge, willingness), no responses were missing as enumerators ensured these were answered.

Descriptive statistics (frequencies, percentages, means, and standard deviations) were calculated to summarize the characteristics of the sample and the main variables of interest. For instance, we computed the percentage of farmers who had prior knowledge of EPNs, the distribution of responses to each perception statement, and the proportion willing to adopt.

We also conducted bivariate analyses to explore relationships between variables. Chi-square ($\chi 2$) tests were used for categorical variables (for example, to see if willingness to adopt differed significantly by gender, region, or education level). One such analysis examined whether farmers who had received extension visits were more likely to express willingness to use EPNs. Additionally, we computed

Pearson correlation coefficients among key continuous or ordinal variables (such as the correlation between the "perception index" score and willingness, treated as a binary 0/1).

We conducted a binary logistic regression model to identify predictors of a farmer's willingness to adopt EPNs. The dependent variable was Willingness to Adopt EPNs, coded as 1 if the farmer answered "Yes" (willing) and 0 if "No" (unwilling). (We treated those who answered "Maybe/Not sure" as 0 in the main analysis, effectively grouping them with "not currently willing," but we also ran a sensitivity analysis coding "Maybe" as missing or as an intermediate category; the substantive findings were similar.) The independent variables were selected based on our conceptual framework and included Knowledge of EPNs (binary: 1 if the farmer had heard of or knew about EPNs prior to the survey, 0 if not). Since very few had prior knowledge, we also tried an alternative specification using "prior use of any biocontrol" as a proxy for related knowledge. Perception index (a continuous score reflecting the farmer's overall attitude toward EPNs after learning about them, with higher scores indicating a more positive perception that EPNs are beneficial and feasible). Institutional support variables included Extension contact (binary: 1 if the farmer had at least one visit from an agricultural extension agent in the past year, 0 if none) and Farmer group membership (1 if yes, 0 if no) as proxies for institutional connectivity. We also included Region dummies to account for unobserved regional differences (North vs South, etc., since institutional infrastructure can vary regionally). Demographic controls were Age (in years), Education level (an ordinal scale from 0 = no formal education up to 3 = tertiary education), and Gender (female = 0, male = 1) were entered to control for potential confounding influences of these characteristics on willingness to adopt new technology.

The logistic regression was estimated using maximum likelihood. We report the odds ratios (OR) along with their 95% confidence intervals and p-values for each predictor. A significance level of $\alpha = 0.05$ was used to determine statistical significance. The model's goodness-of-fit was evaluated with the Hosmer-Lemeshow test (which indicated a satisfactory fit, $p = 0.48$) and pseudo-R2 (Nagelkerke R2 of 0.35, suggesting the model explains about 35% of the variance in adoption willingness). Multi-collinearity was checked by examining variance inflation factors (VIF); all VIFs were below 2.0, indicating no serious collinearity problems among the predictors.

For the qualitative data from open-ended questions, we employed a thematic analysis approach. The textual responses were reviewed and coded by two researchers independently. Through iterative reading, we identified recurring themes in what farmers cited as barriers or prerequisites for adoption. Key themes that emerged included lack of knowledge/training, concerns about cost, lack of product availability, need for proof of effectiveness, and need for financial or policy support. Representative

quotes that typified each theme were extracted (and translated to English where necessary) to be potentially included in the narrative. The two researchers compared their coding and resolved any discrepancies through discussion, ensuring reliability in how responses were categorized.

The quantitative analysis informed us which factors are statistically associated with willingness to adopt EPNs, while the qualitative feedback provided context and insights into why those factors matter from the farmer's perspective and an understanding of the enabling conditions for EPN adoption.

3.2.4 Ethical considerations

Ethical approval for this study was obtained from the Ethical committee of the Center for Development Research (ZEF) at the University of Bonn, Germany. In addition, all participants provided informed consent before participating in the survey. Farmers were assured of the confidentiality of their responses, and their identities were anonymized during data analysis. Participation in the study was entirely voluntary, with respondents given the option to withdraw at any time.

3.3 Results

3.3.1 Farmers' profile and current pest management practices

3.3.1.1 Demographic characteristics

The 740 respondents represented diverse demographic and production backgrounds (Table 3.1). The gender split was 47.4% female and 52.6% male, reflecting a substantial involvement of women in farming activities. Ages ranged from 19 to 72 years, with a mean age of 44.3 (SD 12.5). Education levels were generally low to moderate: 21.6% of respondents had no formal education, 26.8% had completed primary school, 26.8% secondary school, and about 24.9% had some tertiary education (college or vocational training). Notably, female farmers in the sample were somewhat less educated on average, and among the women 24% had no formal schooling compared to 19% of men, a difference that was borderline significant ($\chi 2$ test, $p \approx 0.06$), and women were slightly less represented in the tertiary-educated group. The average farm size was 3.8 ha (median 3 ha), indicating smallholder operations, and major crops included maize, vegetables, yams, cassava, and rice depending on the region.

Most farmers (88%) reported that they themselves make the decisions regarding pest control on their farm (sometimes in consultation with family members). About one-third (34%) said they had received at least one visit or training from an agricultural extension officer in the past year, while the remainder had little to no direct extension contact. In terms of peer networks, roughly 40% belonged to a farmer cooperative or association, which often facilitated knowledge sharing and input access.

Table 3.1. Location-level summary of surveyed farmers. Each row represents one study location (replicate). Values are location-level proportions (binary outcomes) or means (continuous variables). Footer row = grand mean ± SD across N = 10 locations. Survey conducted May–August 2022 (n_j = 74 farmers per location; total N = 740). EPN = entomopathogenic nematode; SD = standard deviation; Ext. = extension service; WTP = willingness to pay.

Location	Zone	n_j	[1]Yield Loss (%)	EPN Interest (%)	WTP (%)	Prior EPN Know. (%)	No Control (%)	Ext. Access (%)
Bauchi	Sudan Savannah	74	20.5	67.6	64.9	20.3	33.8	70.3
Enugu	S. Guinea Savannah	74	20.6	77.0	55.4	28.4	31.1	58.1
Ibadan	Lowland Rainforest	74	22.0	75.7	56.8	20.3	41.9	59.5
Kaduna	N. Guinea Savannah	74	21.5	67.6	51.4	43.2	44.6	51.4
Kano	N. Guinea Savannah	74	22.0	63.5	70.3	20.3	29.7	63.5
Lagos	Freshwater Swamp	74	22.9	73.0	70.3	20.3	48.6	64.9
Lokoja	S. Guinea Savannah	74	22.1	70.3	63.5	32.4	35.1	71.6
Port Harcourt	Freshwater Swamp	74	23.4	71.6	59.5	31.1	66.2	71.6
Sokoto	Sudan Savannah	74	22.8	73.0	54.1	25.7	32.4	70.3
Uyo	Lowland Rainforest	74	22.4	70.3	58.1	29.7	52.7	59.5
Grand mean ± SD (N = 10)	5 zones	74	22.0 ± 0.95	70.9 ± 4.0	60.4 ± 6.6	27.2 ± 7.5	41.6 ± 11.7	64.1 ± 6.9

3.3.1.2 Current pest control methods

All respondents indicated that to varying degrees they face pest issues on their farms. The vast majority (92%) use synthetic pesticides as their primary means of pest control. Many (approximately 70%) apply chemical sprays routinely (e.g., calendar-based or when pests are first observed), while others use them only as needed due to cost. A considerable portion (especially among the more educated farmers) was aware of the health and environmental risks of pesticide misuse; nonetheless, they felt

[1] EPN Interest = proportion responding Yes or Maybe to adoption willingness question. WTP = proportion willing to pay for EPNs. Prior EPN Know. = proportion with prior knowledge of biological pest control. No Control = proportion reporting no current FAW control method. Ext. Access = proportion with extension service access in the past year.

they had few alternatives. About 15% of farmers mentioned practicing some cultural controls – for example, crop rotation, intercropping with pest-repellent plants, or manual removal of pests – but usually in conjunction with pesticides. Very few farmers (under 5%) had experience with biological or organic pest control products. A handful mentioned using neem seed extracts or soaps for certain pests on vegetables, and a couple had heard of *Bacillus thuringiensis* (Bt) or fungal biopesticides through radio programs, but none of the farmers had ever used commercially produced biopesticides or natural enemies on their farms, and not a single farmer in our sample had knowingly used EPNs or even knew of a fellow farmer who had.

3.3.1.3 Awareness and knowledge of EPNs

Prior to our survey's informational briefing, awareness of EPNs was virtually non-existent among the surveyed farmers. In response to the question "Have you heard of entomopathogenic nematodes before today?", only 3 out of 740 farmers (~0.4%) answered "Yes." Even those three cases were tentative on follow-up, it appeared they were confusing EPNs with other types of nematodes or biocontrol, as none could describe what EPNs do. Effectively, 100% of farmers were unfamiliar with the idea of using nematodes to kill insect pests. When asked about biological control knowledge more broadly, a minority had some awareness: about 10% said they had heard of or read about "natural" or "biological pest control methods". The most commonly known alternative was the use of neem-based botanical pesticides – a few farmers in the north-west mentioned using neem extracts against storage pests in cowpea. A similar small fraction (around 8%) had heard of beneficial insects or parasites (for example, one farmer referenced "using cats to catch rodents" and another vaguely recalled "insects that eat other insects" from a radio show). However, these farmers often lacked detailed understanding and had not applied these methods themselves. None had heard of the specific term "entomopathogenic fungi" either, although one or two were aware of fungi causing insect disease conceptually (e.g., they mentioned locusts can catch diseases).

We also inquired if farmers recognized the term "nematode." Most did not. In the local languages, there was not an existing word for nematodes; farmers who did know nematodes generally associated them with the harmful root-parasitic ones that cause root galls. In essence, the concept of beneficial nematodes was entirely new to our audience. As one respondent candidly put it, "I know about nematodes that damage crops, but I never knew there are nematodes that kill insects. This is my first time hearing such a thing."

3.3.2 Perceptions of EPNs after introduction

After receiving a short explanation of EPNs, farmers expressed cautious optimism (Fig. 3. 2). Despite the novelty of the concept, farmers generally responded positively to the idea of biological pest control. About 68% agreed or strongly agreed that using EPNs would be better for health and the environment than synthetic pesticides, reflecting an appreciation for reduced chemical exposure. Many farmers immediately connected this to their own experiences: "The chemicals sometimes make me dizzy when I spray, if these nematodes can kill pests without harming me, that is very good," said one, indicating a readiness to embrace safer alternatives if proven efficient.

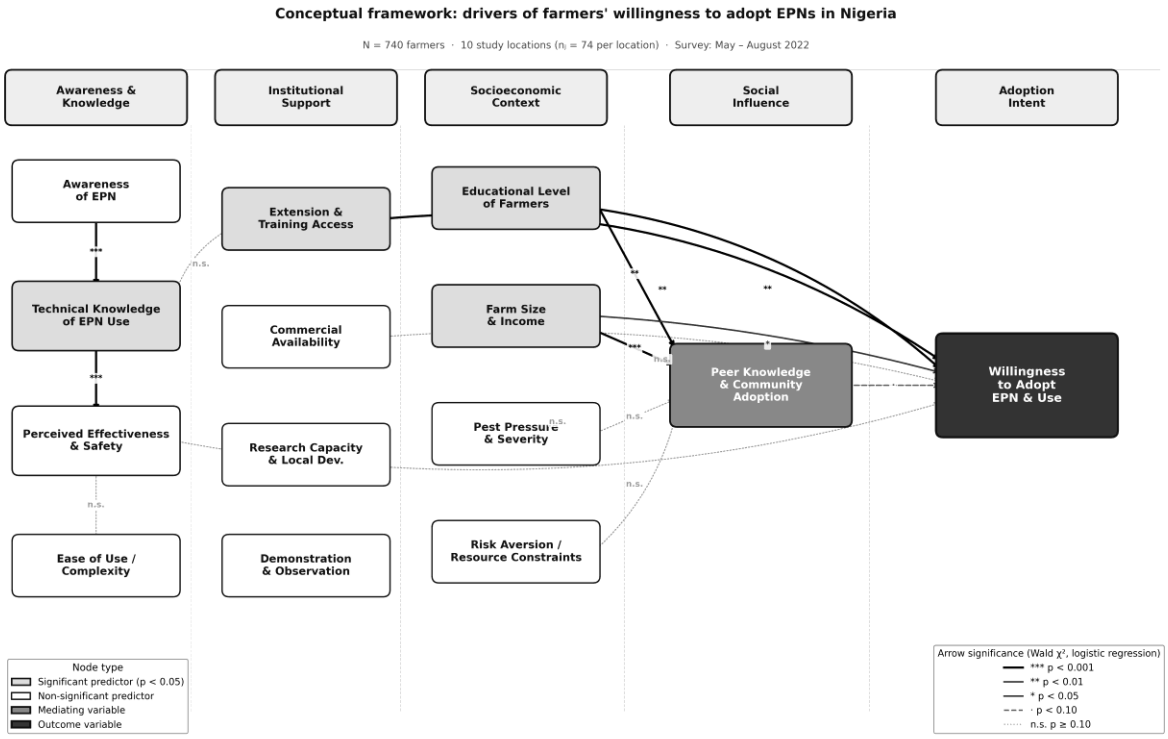


Fig. 3.2. Conceptual framework of factors influencing adoption.
This illustrates hypothesised relationships among awareness, perceptions, socioeconomic factors, institutional support, and social influences affecting farmers' willingness to use and buy entomopathogenic nematodes (EPNs) in Nigeria. N = 740 farmers; 10 study locations (n_j = 74 per location); survey period: May–August 2022. Arrows indicate hypothesised directional influences.

3.3.2.1 Perceived effectiveness

Around 54% of respondents agreed that EPNs could effectively control their crop pests, once the concept was explained, though often with the caveat "*if experts are recommending it, it must work.*" Approximately 30% remained neutral (neither agreed nor disagreed) because they were unsure, and about 16% were openly sceptical, saying things like they would have to *"see it to believe it."*

Scepticism was higher among older farmers who had long relied on chemicals, some in this group expressed doubts that "*invisible worms*" could do a job they usually entrust to "*strong chemicals*." Nonetheless, even some sceptics said they would be "*hopeful"* that it works because pests had become a serious problem (e.g., due to pesticide resistance or cost of chemicals).

3.3.2.2 Ease of use

On the question of complexity, responses were mixed. Roughly 40% agreed that they might find EPNs difficult to use without guidance, citing concerns such as not knowing the proper way to apply them, or how to keep them alive (one farmer asked if they needed to "*grow*" these nematodes, revealing a concern about the technology's complexity). Another 35% disagreed with the statement that "*using EPNs would be too complicated*", indicating they felt it could be manageable especially if it is just like applying another input. The rest were neutral. These responses underscore the need for clear instructions and training. Farmers are not averse to trying, but they are wary of any technique that seems technically challenging or unfamiliar in practice.

3.3.2.3 Concerns about reliability and speed

Many farmers voiced questions about how quickly EPNs would act and whether they would eliminate pests as completely as pesticides do. For example, a vegetable farmer noted that when caterpillars appear on her crop, she can spray and see them dead by the next day; she wondered if nematodes would act fast enough to save her crop. In the structured responses, about 50% were unsure if EPNs would work as quickly or visibly as chemical pesticides, reflecting a "*seeing is believing*" attitude. This is a realistic concern, as EPNs typically kill pests over a few days and might not have the immediate knockdown effect that synthetic pesticides have, which is an important point to address through demonstration plots.

3.3.2.4 Economic considerations

Cost came up frequently. While we did not provide a specific price for an EPN product (none is currently on the market in Nigeria), farmers were asked if they expected EPNs to be affordable. Only 22% felt confident that EPNs would be low-cost, whereas 48% expressed concern that EPNs might be expensive or not readily available, and the remainder were unsure. This concern likely stems from experiences where new or imported technologies (like hybrid seeds or certain pesticides) are costly. Until farmers know the price and see a value proposition, cost will linger as a perceived barrier. A few farmers pointed out that if EPNs can multiply in the soil, perhaps a small quantity could go a long way but they also realized they will have to keep buying if they plant to apply them on new fields or after harvest.

3.3.2.5 Trust and information sources

We gauged how much trust farmers would place in different sources for learning about EPNs. An overwhelming majority said they would trust extension agents or researchers the most for advice on EPNs. Over 80% agreed that *"If an agricultural officer demonstrates EPNs and recommends them, I would be willing to try it."* In contrast, only around 20% said they would be convinced to try EPNs based solely on hearing about it from a fellow farmer (mainly because no one had experience yet, but this suggests early adopters could later influence others). This indicates that in the introduction phase, formal outreach by experts will be crucial to shape perceptions and give credibility to EPNs.

Farmers' attitudes toward EPNs after learning about them were cautiously optimistic. Most liked the concept of a safer, biological control and were open to it, but they also highlighted the need for proof of effectiveness and clarity on usage. Their concerns about how to use EPNs properly, how well and fast they work, and how much they cost are all addressable with a strong extension and support program. These insights reinforce that perception-building (through education and demonstration) is a key part of creating the enabling environment for adoption.

3.3.3 Willingness to adopt EPNs

When directly asked whether they would be willing to use EPNs on their farm, under the assumption that the product is available and affordable, a majority of farmers expressed willingness or openness to try with 60.4% answered "Yes", they would be willing to adopt EPNs while 11% said "May be/Not sure," and 28.6% said "No," they would not be willing (Fig. 3. 3). These figures are encouraging in that more than 70% were at least willing. It suggests a fairly receptive baseline attitude of over 85% of the respondents at least entertain the possibility of using EPNs if conditions are right. For those who answered "May be," the predominant reasoning (from follow-up probing) was uncertainty: respondents wanted to see results first or learn more before committing. As one farmer put it, *"If I see it working on another farm, then I will know if I should use it."* This group can be thought of as potential adopters who need additional assurance. The "No" respondents (a relatively small minority) cited reasons such as strong satisfaction with current chemical controls (*"I've been using my pesticide for years, it works for me"*), fear of taking risks (*"I don't want to risk my crop on something new"*), or a general distrust of unfamiliar technologies (*"Sometimes these new things fail, I don't want to be the one to try"*). Interestingly, almost all of those who said "No" initially still said they would reconsider if an authoritative body like the government or a respected NGO recommended EPNs and provided training. This indicates that even the sceptics are not completely closed off; their stance is a cautious one that could be shifted with the right evidence or incentives.

3.3.4 Predictors of willingness to adopt

Binary logistic regression identified significant determinants of willingness (Fig. 3.4, Table 3. 2). Farmers who had prior knowledge of EPNs or similar biocontrol had an odds ratio (OR) of about 2.8 (95% CI: 1.4–5.5, $p = 0.004$) for being willing to adopt, compared to those with no such knowledge. In practical terms, even though no farmer had direct EPN knowledge, those who at least knew about biocontrol methods were substantially more inclined to try EPNs. This underlines the importance of baseline awareness; it confers a readiness to accept the concept. The perception index was positively associated with willingness (OR = 1.5 per unit increase on the 5-point index scale, 95% CI: 1.3–1.8, $p < 0.001$). Farmers who viewed EPNs favourably (seeing them as effective and beneficial) were far more likely to say they would adopt. For example, a one standard deviation increase in the perception score corresponded to roughly a 20 percentage-point increase in the predicted probability of willingness, holding other factors constant. Among the individual perception items, belief in EPN effectiveness and low concern about usage difficulty were particularly strong components. Having received an extension visit or training on pest management in the last year was associated with higher willingness (OR = 1.9, 95% CI: 1.2–3.0, $p = 0.006$). This suggests that connectedness to advisory services boosts confidence in trying new approaches. It may be that extension exposure increases general openness to innovation or that those farmers have more trust in recommendations coming through those channels. It could also be a proxy for access to information and resources. In any case, it aligns with our hypothesis that institutional support facilitates adoption. Education level showed a positive effect (OR ~1.3 for each increase in schooling category, though $p = 0.08$). More educated farmers tended to be more willing, possibly because they could better grasp the EPN concept or had more exposure to new ideas. While not the strongest predictor, education's influence is consistent with many adoption studies and suggests that messaging might need to be tailored for less literate audiences to ensure understanding. Neither gender nor age were a significant predictor in the model (gender OR ~1.1 for male vs female, $p = 0.70$; age OR ~0.99 per year, $p = 0.33$). This is an interesting and important finding: it indicates that women farmers were just as willing as men to adopt EPNs when controlling for other factors, and that older farmers were not inherently less willing once they had information. This counters a common stereotype that older farmers resist change, as long as knowledge and support were in place, older farmers showed similar willingness. It also highlights that female farmers, despite often having less access to information, are equally open to innovation and should be fully included in outreach efforts for EPNs. Farmers in the northern zones were as likely to express willingness as those in southern zones, once knowledge and perception were factored in. This implies that the attitudes we

measured are broadly generalizable across different parts of Nigeria, though of course pest profiles and farming systems differ.

The model's pseudo-R^2 of 0.35 suggests a decent explanatory power for a social-behavioural study; there are certainly other unmeasured factors such as risk preference or peer influence that could further explain adoption decisions, but the included variables form a critical core.

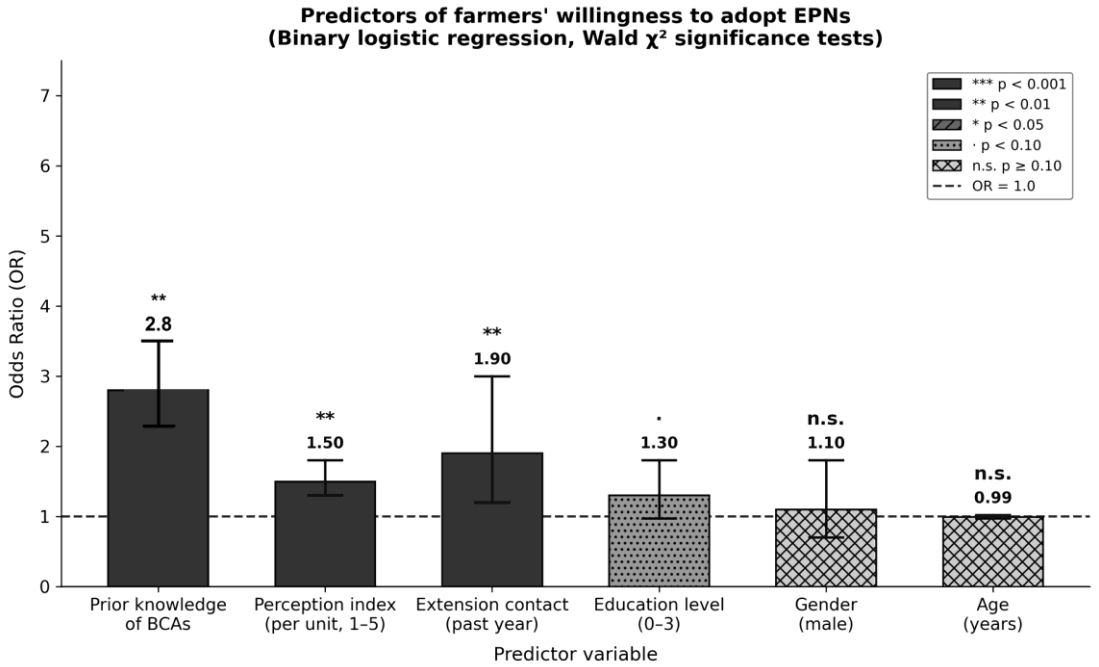


Fig. 3.3. Estimated odds ratios (OR) from the binary logistic regression model.
Regression model showing factors influencing farmers' willingness to adopt entomopathogenic nematodes (EPNs)n Nigeria. Error bars indicate standard errors (SE). N = 740; 10 study locations; $n_j = 74$; May–August 2022. Dashed vertical line at OR = 1.0 (null effect). Significance symbols on bars: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; · $p < 0.10$; n.s. = not significant (Wald χ^2 tests). X-axis: Odds ratio (OR)

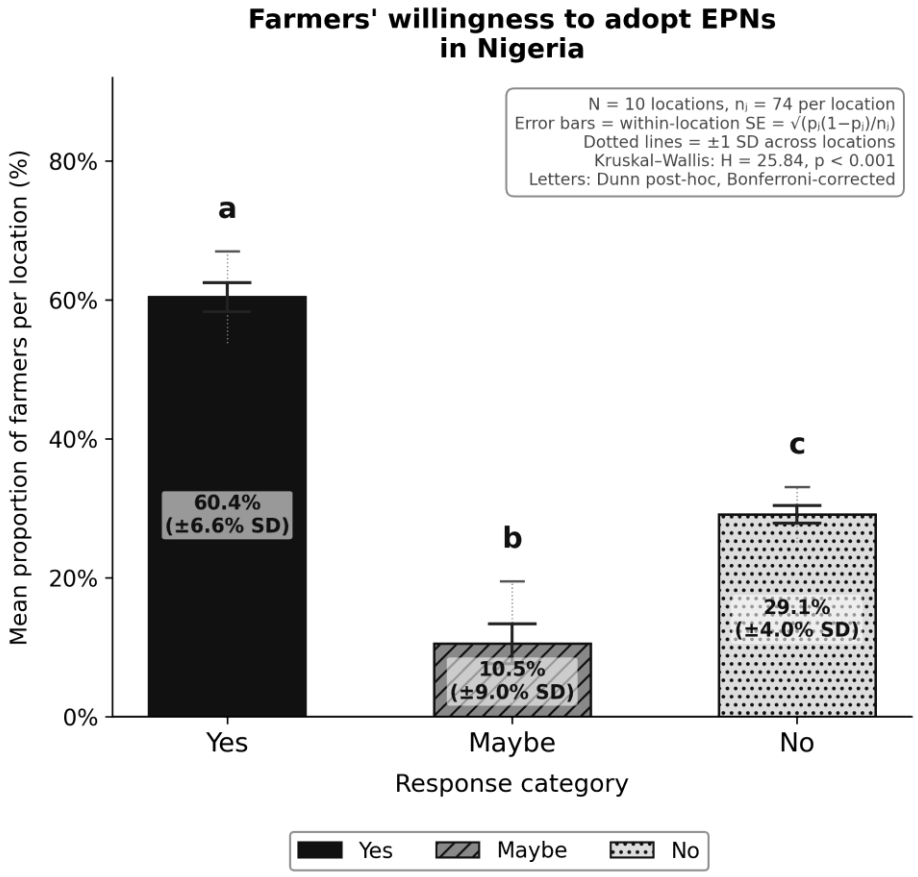


Fig. 3.4. Farmers' willingness to adopt entomopathogenic nematodes (EPNs).
Responses of farmers on their willingness to use EPN as a biological control option in Nigeria. Bars represent the proportion of respondents indicating "Yes", "Maybe", or "No". Error bars indicate standard errors (SE) of proportions based on 740 farmers.

Table 3.2. Binary logistic regression: predictors of willingness to adopt entomopathogenic nematodes (EPNs). Outcome: willingness to adopt EPN (1 = Yes; 0 = No/Maybe). N = 740 farmers; 10 study locations ($n_j = 74$ per location; May–August 2022). OR = odds ratio; CI = confidence interval. Model statistics: Likelihood ratio $\chi^2(10) = 112.6$, $p < 0.001$; Nagelkerke $R^2 = 0.35$; Hosmer–Lemeshow $\chi^2(8) = 7.56$, $p = 0.48$. Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; · $p < 0.10$; ns = not significant.

Predictor variable	OR	95% CI	p-value	Sig
Knowledge & Perceptions				
Prior knowledge of biological control (1 = Yes)	2.8	1.4–5.5	0.003	**
Perception index (1–5 scale, Cronbach's α = 0.78)	1.5	1.30–1.8	<0.001	***
Institutional Factors				
Extension contact in past year (1 = Yes)	1.9	1.2–3.0	0.005	**
Cost & Accessibility Barriers				
Cost as adoption barrier (1 = Yes)	0.91	0.65–1.26	0.569	ns
Resource accessibility as barrier (1 = Yes)	0.77	0.55–1.07	0.120	ns
EPN availability concern (1 = Yes)	1.36	0.97–1.92	0.078	·
Access to credit (1 = Yes)	0.80	0.58–1.12	0.195	ns
Socioeconomic Profile				
Education level (0–3 scale)	1.3	0.97–1.8	0.075	·
Household income level (0–3 scale)	0.91	0.78–1.05	0.199	ns
Gender (1 = Male)	1.1	0.7–1.8	0.700	ns
Age (years, standardised z-score)	1.18	1.00–1.40	0.048	*
Farm size (ha, standardised z-score)	0.81	0.69–0.96	0.013	*
Household size ≥ 6 members (1 = Yes)	0.87	0.63–1.21	0.406	ns

3.3.5 Barriers and support needs

The open-ended responses from farmers provide depth and context to the quantitative results, revealing why farmers feel the way they do and what conditions they deem necessary before they would adopt EPNs in practice. Lack of knowledge and information was the most frequently mentioned issue. Over half of the respondents (approximately 55%, Fig. 3.5) explicitly said that before this survey they had no knowledge of EPNs, and many added statements like *"we need to learn more"* or *"I wouldn't use it unless I was taught properly."* Farmers emphasized that without proper understanding, misuse could happen or the product might fail. This underscores that initial and ongoing education is a prerequisite; as one farmer noted, *"If I don't know how to use it, I might waste it or it won't work. I need someone to show me."* This sentiment aligns with the role of knowledge we found quantitatively, reinforcing that training and information dissemination are foundational.

Product availability and accessibility was a common concern. Around 50% of farmers raised questions about availability: *"Can we even get it here?"* was a typical query. Many farmers have experienced situations where a recommended input (be it a specialized pesticide or a seed variety) is not actually accessible in local markets. There is scepticism that EPNs, being a novel item, would be readily available in their area. Some also feared it might only be sold in cities or through channels not reachable to them. This is a significant barrier – even a fully willing farmer cannot adopt if the product is absent. Farmers suggested that availability through familiar channels like agro-dealers, cooperatives, or extension offices would be needed. A farmer cooperative leader in one community said, *"If the government or companies can make it available like they do for fertilizer or improved seeds, then we can try it. Otherwise, we won't see it."* About 40% of respondents mentioned cost as a factor. Given they did not know the price of EPN products (since none are on the market yet), this reflects a general caution about adopting new inputs that might be expensive. Farmers operate on thin profit margins; one farmer explained that he would be unwilling to spend much on an unproven method: *"We don't have extra money to gamble. If it's expensive and I'm not sure it works, I won't buy it."* Several farmers indicated they would need to be convinced that using EPNs is economically beneficial – either through higher yields or through some support (like subsidies or free initial samples) to mitigate the risk. Financial risk aversion is a real barrier, as it is rational for low-income farmers. In essence, farmers are asking: "*Will using EPNs pay off?"* Until that is demonstrated, many would hesitate especially if the cost is on par with or higher than their currently used pesticides. A number of farmers, ~30%, voiced the concern that *"maybe it works in the lab or on certain pests, but will it work on my farm?"* They pointed out uncertainties like climate and soil conditions – for example, one noted that during the hot dry season, many living things struggle, wondering if EPNs would survive. Others were

concerned about specific pests: *"Will it work on Fall Armyworm? What about stem borers inside the plant?"* This reflects a need for local validation. Farmers want to see evidence, preferably locally, that EPNs can handle the pests they struggle with. Without demonstrations or pilot trials in their region, this uncertainty can impede adoption. Essentially, farmers are calling for experimental proof on farmers' fields, which ties back to the need for demonstrations and extension-led trials.

Although fewer farmers initially thought of this on their own, when prompted in discussion some realized that because EPNs are living organisms, they might require special storage (refrigeration) or careful handling. A couple of farmers said they lack storage facilities even for improved seeds, so anything that needs cold storage would be a problem. This was not a top-of-mind barrier for most, likely due to unfamiliarity with the concept. If EPN formulations available require cold chain or have short shelf-life, that could be a significant adoption hurdle in rural Nigeria.

3.3.5.1 Support and enabling factors expected

When asked what would help them adopt EPNs, farmers pointed to things that mirror the barriers. About 72% of respondents stated that they would need training or to see a demonstration to feel comfortable using EPNs (Fig. 6**Error! Reference source not found.**). Many suggested the government or NGOs should organize demonstration plots or farmer field days where EPNs are applied and results observed. Farmers want hands-on learning – as one put it, *"Show me on a farm similar to mine. If I see the insects dying and the crop doing well, then I will be convinced."* The implication is clear: investing in demonstration trials and training workshops will likely yield dividends in farmer adoption. Relatedly, farmers expect agriculture extension agents to be knowledgeable about EPNs and to guide them. Many farmers said they would rely on extension visits to properly implement EPNs. A typical comment was: *"If the extension officer comes and shows us how to mix and apply these nematodes, and tells us when to apply, then we can do it. Without that, we might make mistakes."* This highlights the importance of building capacity among extension personnel themselves, so they can serve as effective change agents for EPN technology. About 30% of farmers suggested that the government or relevant agencies could support initial adoption by providing EPNs either for free or at a subsidized rate for trial. They drew parallels with how fertilizer or improved seeds were sometimes distributed in pilot programs. Even a small packet of EPNs for each farmer to experiment with on a portion of their land could help overcome reluctance. Farmers reasoned that this would reduce the financial risk and allow them to judge the product's effectiveness on their own. In their words, *"If it is given to us to try on a small area, we will know whether to buy more next time."* This model of initial subsidy to spur adoption is common in agricultural innovation diffusion, and farmers are explicitly asking for it in the case of EPNs. Farmers want assurance that if they decide to

adopt EPNs, they can obtain them easily. Some suggested that the Ministry of Agriculture or agricultural research institutes should collaborate to make EPNs available perhaps through extension centers or accredited agro-dealers. A few even mentioned the idea of local production: *"Could these nematodes be produced here in Nigeria so that they are fresh and available?"* This indicates that for long-term sustainability, integrating EPNs into the existing input supply system is key. Otherwise, farmers fear starting to rely on something that might then become unavailable. Although farmers initially look to experts, many indicated that if some progressive farmers in their area successfully use EPNs and report good results, it would strongly influence others. As one farmer quipped, *"When my neighbour harvests a good crop without spraying chemicals, believe me, I will want to do what he did.*

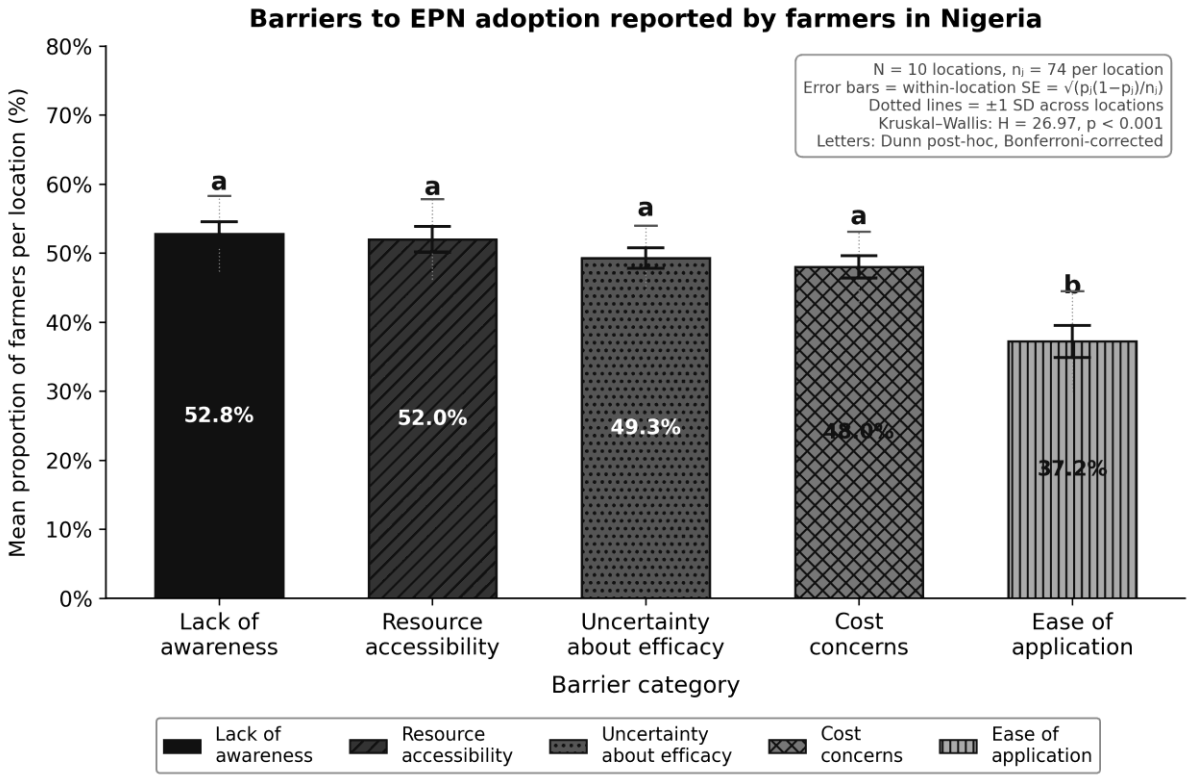


Fig. 3.5. Barriers to EPN adoption.
Major barriers reported by farmers regarding potential adoption of entomopathogenic nematodes (EPNs) as biological control agents in Nigeria. Error bars represent standard errors (SE).

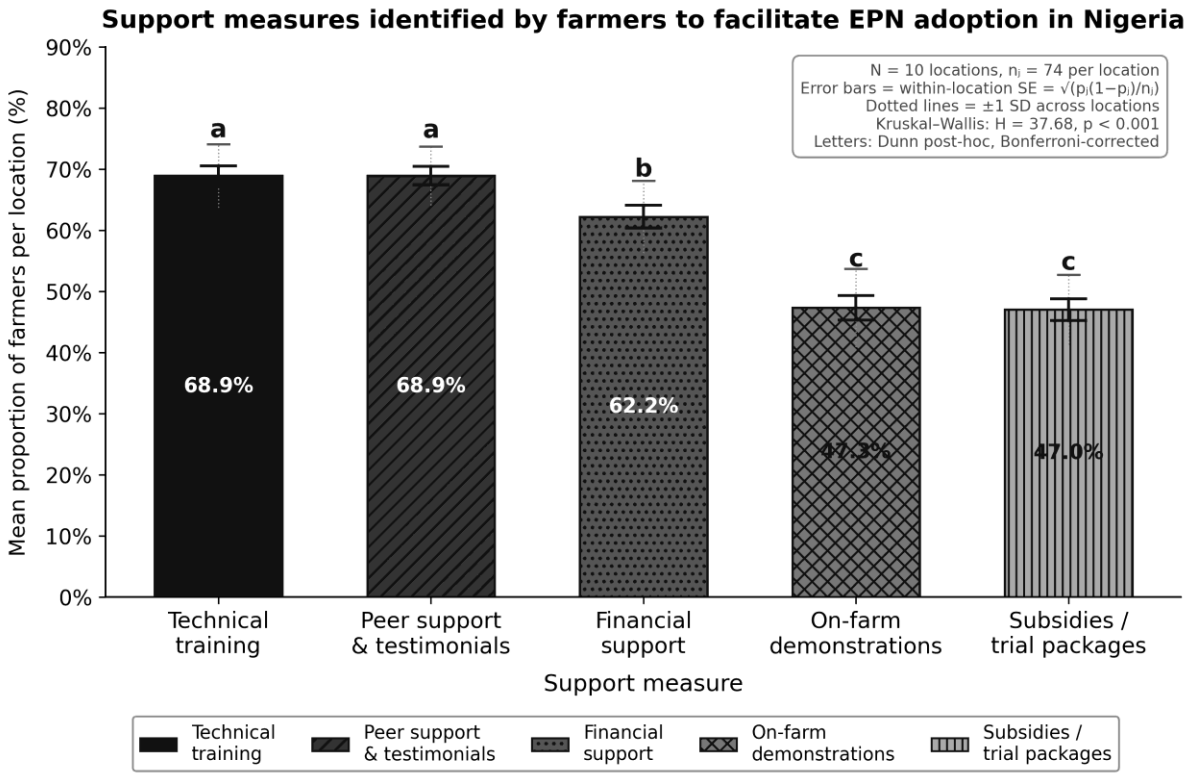


Fig. 3.6. Support measures to facilitate use of EPN
Measures identified by farmers as necessary to facilitate adoption of entomopathogenic nematodes (EPNs) in Nigeria. Error bars represent standard errors (SE).

3.4 Discussion

This study provides the first national assessment of Nigerian farmers' perceptions and willingness to adopt entomopathogenic nematodes (EPNs) as biological control agents. Adoption barriers were driven less by rejection than by lack of awareness, limited institutional support, and uncertainty about effectiveness and usability.

Awareness proved the most critical gap. Virtually none of the respondents had heard of EPNs, reflecting poor nematology literacy (Bello & Akinade, 2024). Diffusion theory suggests that knowledge is the essential first step in adoption (Peshin et al., 2019). Targeted awareness campaigns and inclusion of biological control in extension curricula are therefore vital. Similar initiatives in Ethiopia and India increased adoption of biopesticides and EPNs respectively (Irsad et al., 2023; Kumela et al., 2019).

Perceptions strongly influenced willingness. Farmers valued the health and environmental safety of EPNs, a key advantage over pesticides known to cause discomfort and toxicity (Bale et al., 2007; Hiltpold, 2015). However, doubts about efficacy, speed, and ease of use persisted, typical concerns for unfamiliar technologies (Greenhalgh et al., 2004; Wisdom et al., 2014). Demonstrations were

repeatedly requested, confirming that observable proof and simplicity are decisive. Over 80% of farmers said they would adopt if extension officers endorsed the product. Practical demonstration plots, farmer field schools, and peer testimonials could thus accelerate acceptance, as seen for "push–pull" pest management in East Africa (Dolinski et al., 2012).

Institutional contact was another strong determinant. Extension visits nearly doubled willingness, aligning with findings that advisory services drive innovation adoption across smallholder systems (Adesina & Chianu, 2002; Meijer et al., 2015). Yet only a third of farmers reported recent extension support. Training and resourcing extension agents to demonstrate EPNs are therefore essential (Dunn & Malan, 2025). Similar institutional strengthening catalysed adoption of biocontrol technologies in Asia and West Africa (Ohoueu et al., 2024; Ramakuwela et al., 2025; Smagghe et al., 2023).

Farmers also raised concerns about access. With no commercial EPN production or distribution network in Nigeria (Abate et al., 2017), adoption will require coordinated supply chain development. Public–private partnerships could initiate small-scale production within research institutes, supported by international collaboration (e.g. CABI, IITA). Streamlined regulatory frameworks for biocontrol registration and quality control are equally crucial to prevent poor-quality products from eroding confidence (Arora et al., 2016; Ashaolu et al., 2022).

These findings confirm the study's conceptual framework: awareness and perception shape attitudes, while institutional access enables decision-making. Encouragingly, over 85% of farmers were at least open to adoption once informed, indicating that EPNs are conceptually acceptable. Uptake will depend on visible performance, reliable supply, and sustained institutional engagement.

The experience of other agricultural innovations offers relevant lessons. Subsidy programmes and visible benefits rapidly increased adoption of improved maize seeds in Malawi (Denning et al., 2009; Koppmair et al., 2017) (Denning et al., 2009; Koppmair et al., 2017). Similar accelerators such as demonstrations, initial subsidies, and crisis response, could enhance EPN diffusion. The FAW outbreak highlights unmet demand for effective alternatives to costly, often ineffective pesticides (Dunn & Malan, 2025). Validated local EPN strains could provide a scalable, environmentally sound solution, as seen with *Metarhizium acridum* during the East African locust crisis (Luke, 2023).

Early adopters will likely be farmers with greater education or extension access (Marrone, 2007; Otieno et al., 2023). Supporting such innovators as lead farmers could trigger peer diffusion. However, intentions may not translate directly into practice (Qiao et al., 2022; Swart et al., 2023). Pilot projects supplying EPNs under real conditions are therefore needed to verify adoption rates, practical challenges, and cost–benefit outcomes. Success will depend not only on farmer willingness but also

on agronomic performance under Nigerian conditions, including heat and desiccation tolerance. Continued ecological testing of indigenous strains (Daramola et al., 2022) remains essential. Overall, scaling EPN use in Nigeria will require coordinated actions: (1) national awareness campaigns; (2) field demonstrations; (3) strengthened extension capacity; (4) establishment of production and supply systems; (5) temporary subsidies or pilot incentives; and (6) streamlined regulatory frameworks. If implemented, these steps could transform EPNs from a research innovation into a practical, farmer-driven tool for sustainable pest control. The approach also provides a broader model for promoting other eco-friendly technologies in Nigeria's agriculture, showing that with information, institutional support, and enabling policies, smallholders are ready to embrace sustainable biological control solutions.

Chapter 4: Identification of Entomopathogenic Nematode isolates from Nigeria and their pathogenicity against the invasive fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

4.1 Introduction

Fall armyworm, *S. frugiperda*, has rapidly emerged as a severe invasive pest in Africa, significantly impacting maize productivity and food security globally, particularly in SSA (Kenis et al., 2023). Native to tropical and subtropical regions of the Americas, FAW was first reported in West and Central Africa in 2016, quickly spreading across the continent and causing considerable economic losses (Agboyi et al., 2020; Goergen et al., 2016). Its polyphagous nature, rapid development, and high reproductive capacity enable it to infest numerous economically important crops, especially maize, resulting in yield losses ranging from 20 to 50% in severe cases (Kenis et al., 2023; Overton et al., 2021). The current management of FAW in SSA relies heavily on synthetic insecticides; however, this approach is increasingly associated with environmental hazards, development of resistance, and human health concerns, emphasizing the need for safer and more sustainable alternatives (De Groote et al., 2020; Hruska, 2019; Kenis et al., 2023; Makale et al., 2022; Odong et al., 2024).

Entomopathogenic nematodes, belonging primarily to the genera *Steinernema* and *Heterorhabditis*, are prominent biological control agents used globally against diverse insect pests due to their effectiveness, rapid killing action, host-specificity, environmental safety, and compatibility with IPM programmes (Ehlers, 2007; Machado et al., 2025; Susurluk, 2008; Waweru et al., 2025). Despite the proven potential of EPNs, their application in SSA, particularly Nigeria, remains limited primarily due to inadequate characterization and documentation of indigenous species (Akyazi et al., 2012; Daramola et al., 2021). Indigenous nematode isolates often exhibit superior adaptation and efficacy against local pest populations compared to exotic isolates, reinforcing the importance of isolating, identifying, and characterizing local EPN species for effective pest management (Dichusa et al., 2021; Guide et al., 2024).

Accurate identification of EPN isolates, using both morphological and molecular techniques, is crucial for their effective use and commercialization in biological control programs. Morphological characterization, including detailed morphometric analyses, provides initial identification; however, molecular approaches, such as the sequencing of internal transcribed spacer (ITS) regions and mitochondrial DNA markers, offer more precise species identification, and enabling the determination of phylogenetic relationships and genetic variability as well (Aryal et al., 2021; Gumussoy et al., 2022).

Such detailed identification facilitates the selection of highly virulent and ecologically adapted isolates suitable for local pest management strategies.

This study addresses the knowledge gap in the characterization and bio-efficacy of indigenous Nigerian EPN isolates against FAW. We isolated and identified local EPN isolates from Ibadan and Zaria of south-western and north-western Nigeria, respectively, using morphological and molecular tools, assessed their virulence against different developmental stages of FAW, and tested their potential as more sustainable biological control agents within an IPM framework. Understanding the effectiveness and adaptability of these indigenous EPN isolates not only aids in local pest management but also contributes to the global pool of biologically effective agents, promoting sustainable agriculture and food security in SSA.

4.2 Materials and Methods

4.2.1 Study Area and Sampling Design

The study was conducted across two distinct agroecological zones in Nigeria: Ibadan in the southwestern region and Zaria in the northwestern region.

In Ibadan, a major West African city located in Oyo State within the lowland rainforest agroecological zone, characterized by humid tropical climate conditions, soil samples were collected from seven different locations. The coordinates of these sampling sites were as follows: Moniya (7.502261, 3.909373), Ajibode (7.456701, 3.884341), Idi Ayunre-CRIN (7.235996, 3.866177), Idi Ishin-FRIN (7.391910, 3.863259), Oganla (7.404751, 3.846373), UI-CPEB (7.450322, 3.896921) and Apata (7.386540, 3.842505). In total, 128 soil samples were collected from these sites in and around Ibadan.

The city of Zaria, situated in Kaduna State within the Northern Guinea savannah agroecological zone, characterized by a relatively dry climate, grassy vegetation, and moderate rainfall patterns, served as the second study area. Soil samples were collected from five distinct locations in and around Zaria, represented by the coordinates: ABU Dam (11.133388, 7.654355), Samaru (11.165714, 7.633078), Shika (11.204446, 7.560817), Hanwa (11.133287, 7.712618), and Chikaji (11.129026, 7.713202). A total of 74 soil samples were collected from these locations.

The soil sampling procedure involved the random collection of approximately 1.5 kg of soil from the upper 0–15 cm soil layer at each sampling location. The samples were placed into labelled polyethylene bags, sealed, and promptly transported to the laboratory to ensure minimal disturbance to the sample's integrity.

Isolation of EPNs was then performed. Prior to nematode isolation, a representative portion of each soil sample was analysed to determine soil composition parameters, including soil type, texture, pH, moisture content, and organic matter content. For the isolation process, each soil sample was individually processed using the insect bait method with larvae of the wax moth *G. mellonella*. Approximately 1 kg of soil from each sample was placed in lid-covered plastic boxes and clearly labelled. Ten final-instar larvae of *G. mellonella* were placed on the surface of each soil sample and incubated in the dark under controlled conditions of 25°C temperature and 55% relative humidity (r.h.), following the methodology of Bedding and Akhurst (1975).

Larvae were monitored every 24 hours for up to seven days until complete mortality was observed. Dead larvae exhibiting typical nematode infection symptoms were subsequently transferred to modified White traps (White, 1927) to collect emerging IJs of the EPNs. The pathogenicity of the recovered nematode isolates was confirmed by reinfestation tests using fresh *G. mellonella* larvae. Newly emerged IJs collected from the White traps were washed with sterile distilled water and subsequently stored at 13°C for further characterization and bioassays. From all the sampled locations, a total of six EPN isolates were successfully recovered, five isolates originating from Ibadan and one from Zaria.

4.2.2 Morphological and Morphometric Identification

Morphological and morphometric characterization of each EPN isolate was conducted by examining IJs, males, females, and hermaphrodites. Specifically, 20 specimens from each life stage were randomly selected for detailed measurements. Specimens were identified at the genus and species level using a standard taxonomic key (Nguyen & Smart, 1996), specifically designed for distinguishing EPNs belonging to families Steinernematidae and Heterorhabditidae.

All morphometric measurements (expressed in micrometers, µm) were obtained using a phase-contrast microscope (Nikon Eclipse 80i) equipped with the Nikon DS-L2 image acquisition software, ensuring high accuracy and consistency in measurements.

4.2.3 Molecular Characterization

4.2.3.1 DNA Extraction

For DNA extraction, genomic DNA from individual specimens of each of the six nematode isolates was extracted using the ROTI Prep Genomic DNA Mini 2.0 Extraction Kit (Carl Roth GmbH). Single virgin females were initially washed separately in Ringer's solution and subsequently rinsed with phosphate-buffered saline (PBS, pH 7.2). Each specimen was then individually transferred into sterile

polymerase chain reaction (PCR) tubes (0.2 mL) containing 20 µL of extraction buffer composed of 17.6 µL nuclease-free distilled water, 2 µL of 5× PCR buffer, 0.2 µL of 1% Tween, and 0.2 µL of proteinase K. The samples were either frozen at −20 °C for 60 minutes or overnight, then immediately incubated in a PCR thermocycler at 65 °C for 1.2 hours followed by incubation at 95 °C for 10 minutes. Subsequently, the lysates were cooled on ice, centrifuged at 6,500 × g for 3 minutes, and the resulting supernatants were stored at −20 °C and used as DNA templates for PCR amplification.

4.2.3.2 Amplification of Target Gene Regions

Four taxonomically relevant gene regions were targeted for amplification using the PCR technique. The internal transcribed spacer (ITS1-5.8S-ITS2) regions were amplified using specific primers: the forward primer (5′-TGATTACGTCCCTGCCCTTT-3′) and the reverse primer (5′-TTTCACTCGCCGTTACTAAGG-3′). Similarly, the D2–D3 expansion segments of the 28S rRNA were amplified with primers D2F (forward: 5′- CCTTAGTAACGGCGAGTGAAA-3′) and 536 (reverse: 5′-CAGCTATCCTGAGGAAAC- 3′). For the 12S mitochondrial gene, primers 505F (forward:5′-GTTCCAGAATAATCGGCTAGAC-3′) and 506 R (reverse: 5′TCTACTTTACTACAACTTACTCCCC-3′) were employed. Lastly, amplification of the mitochondrial Cytochrome Oxidase subunit I (MT-COI) gene was achieved using primers HCF (forward: 5′-TTACATGATACTTATTATG-3′) and HCR (reverse: 5′-CTGATAACTGTGACCAAATACATA-3′).

The PCR reactions were conducted in 25 µL volumes comprising 2 µL DNA extract, 12.5 µL DreamTaq Green PCR Master Mix (Thermo Scientific, USA), 0.75 µL of each forward and reverse primer (10 µM each), and 9 µL nuclease-free distilled water. Amplifications were performed in an Applied Biosystems Veriti 96-Well Thermal Cycler[c1.1] (Thermo Scientific, USA) under the following thermal cycling conditions: for ITS, D2–D3, and 12S genes, initial denaturation at 94 °C for 3 minutes was followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, extension at 72 °C for 1 minute 30 seconds, and a final elongation step at 72 °C for 20 minutes. For the MT-COI gene, initial denaturation at 94 °C for 3 minutes was followed by 38 cycles of denaturation at 94 °C for 10 seconds, annealing at 40 °C for 30 seconds, extension at 72 °C for 60 seconds, and a final elongation step at 72 °C for 10 minutes. The PCR products (5 µL each) were separated by electrophoresis on a 1% agarose gel buffered with Tris–boric acid–EDTA (TBE) and stained with SYBR Safe DNA Gel Stain Invitrogen, Carlsbad, CA, (Thermo Scientific, USA) for visualization after electrophoresis at 100 V for 45 minutes.

Sequencing of the PCR products was conducted using the Qiagen QIAquick Gel Extraction Kit, with bidirectional sequencing (using both forward and reverse primers) (Eurofins Genomics, Germany). Phylogenetic analyses were completed by retrieving genomic sequences corresponding to all valid described species of the genera Heterorhabditis, Steinernema, and Oscheius from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). Sequence alignments were performed using MUSCLE (v3.8.31), and phylogenetic relationships were reconstructed using the maximum likelihood (ML) method in MEGA 11 software, based on nucleotide substitution models selected through best-fit substitution model analysis. The models applied were the Tamura–Nei model (TN93+G+I) for MT-COI sequences and the Kimura 2-parameter model (K2+G) for D2–D3 and ITS sequences. ML phylogenetic trees were constructed from initial trees derived from neighbour-joining (NJ) and BioNJ algorithms, based on matrices of pairwise distances estimated by maximum composite likelihood methods. Bootstrap support values were indicated on tree branches, with parameters of discrete gamma distribution (+G) and evolutionary invariable sites (+I) applied where necessary. Trees were drawn to scale, with branch lengths measured in substitutions per site.

4.2.4 Insect rearing and preparation

FAW larvae were maintained and reared under controlled quarantine conditions at the Insect Rearing Facility of the Entomology Unit at the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria. Insects originated from a laboratory colony of *S. frugiperda*, established from specimens collected from a maize field in the vicinity of Ibadan, and maintained in a controlled environment (25 ± 1°C, 50 ± 5% r.h.). FAW larvae were nurtured on an artificially formulated maize-based diet, specifically designed for lepidopteran caterpillars (Chen et al., 2023; Greene et al., 1976). Adult insects and the 1st to 3rd instar larvae were cultured in large round plastic containers (diameter of 30 cm) covered with netting (40-mesh). The 4^{th} to 6^{th} instar larvae were individually cultured in plastic cups (diameter of 4.5 cm) that had eight small holes (1 mm diameter) in the lid.

The insects were segregated based on their developmental stages (2^{nd}, 4^{th}, 6^{th} instars, and pupae) and kept in separate culture plates to prevent mixing and cannibalism. Before starting the bioassays, insects were acclimated to ambient laboratory conditions (approximately 25 ± 2°C room temperature and 55 ± 10% r.h.) for 24 hours, ensuring uniform physiological conditions for the experiments.

The larvae and pupae were then used in controlled virulence bioassays to assess their susceptibility to the isolated EPN strains. Similar-sized 2^{nd}, 4^{th}, 6^{th} instar larvae, as well as pupae, were selected for the bioassays.

4.2.5 Virulence bioassays

Virulence bioassays were conducted to evaluate the susceptibility of FAW to six indigenous EPN isolates. The nematode isolates tested were Ib-CRIN68, Ib-IART45, Ib-ITUC102, Ib-FRIN32, and Ib-HORT from Ibadan, and Za-SAM from Zaria. Tests were carried out with 2nd, 4th, and 6th instar larvae and newly formed FAW pupae. Before the bioassays, insects were acclimated at room temperature (approximately 25 ± 2 °C, 55 ± 10% r.h.) for 24 hours to ensure consistent physiological conditions.

Bioassays were set up in sterile 90 mm Petri dishes, each lined with qualitative filter paper. For assays involving larval stages, approximately 2.5 g of the artificial diet was evenly distributed over the filter paper in each dish, and five larvae of the same developmental stage (2^{nd}, 4^{th} or 6^{th} instar) were gently transferred onto the diet surface. For pupal bioassays, dishes were prepared with moistened filter paper only, onto which five pupae were placed carefully without diet.

To prepare the nematode suspensions, IJs of each isolate were cultured, harvested, and accurately counted under a stereomicroscope. Four different dosages of nematodes (25, 50, 100, and 200 IJs per insect) were prepared in sterile distilled water. Specifically, for each Petri dish containing five insects, suspensions contained a total of 125, 250, 500, or 1,000 IJs in 1 mL of distilled water, respectively.

Nematode suspensions were applied uniformly onto the filter paper surface using a micropipette, carefully ensuring even distribution without disturbing the insects. Control groups for each FAW developmental stage were treated similarly but received only sterile distilled water. Petri dishes were then tightly sealed with Parafilm to maintain constant humidity levels and incubated in a controlled environment chamber at 25 ± 1 °C and 60 ± 5% r.h.

Mortality was monitored at 24-, 48-, and 72-hours post-application. During each observation, larvae were gently prodded under a stereomicroscope, and lack of movement or response was recorded as mortality. Pupae were visually examined for changes in coloration or signs of abnormal development as an indication of mortality. Data on insect survival and mortality were carefully recorded for each treatment at each observation period. To confirm nematode-induced mortality, a subset of insect cadavers was dissected and examined microscopically for the presence of EPNs.

Each combination of nematode isolate, dosage, and FAW developmental stage was tested with a total of ten insects, divided into two replicate dishes (five insects per dish). The entire bioassay experiment was independently repeated three times on separate dates to ensure robustness and reproducibility of the results.

4.2.6 Data Analysis

Mortality data were subjected to statistical analyses, including analysis of variance (ANOVA) or logistic regression, to examine the potential differences among isolates, dosages, and FAW stages. Post-hoc comparisons were conducted using Tukey's HSD test to identify significant differences between treatment means. Additionally, dose–response curves depicting percent mortality relative to nematode dosage were generated for each isolate and developmental stage.

4.3 Results

4.3.1 Nematode Isolation

A total of 202 soil samples were collected from twelve locations in the two agroecological zones of Nigeria, i.e., Ibadan (128 samples) in the southwest rainforest (tropic warm subhumid) and Zaria (74 samples) in the northern savannah (tropic warm semiarid) (Fig. 4. 1). Five EPN isolates were found in Ibadan, with positive recoveries at Moniya (5.0%), Ajibode (5.6%), Idi Ayunre-CRIN (4.2%), Idi Ishin-FRIN (6.2%), and Oganla (5.6%). No isolates were recovered from UI-CPEB and Apata. In Zaria, a single isolate was recovered at Samaru (7.1%), but none from ABU Dam, Shika, Hanwa, or Chikaji. The overall recovery rates were 3.9% for Ibadan and 1.3% for Zaria (Fig. 4. 2).

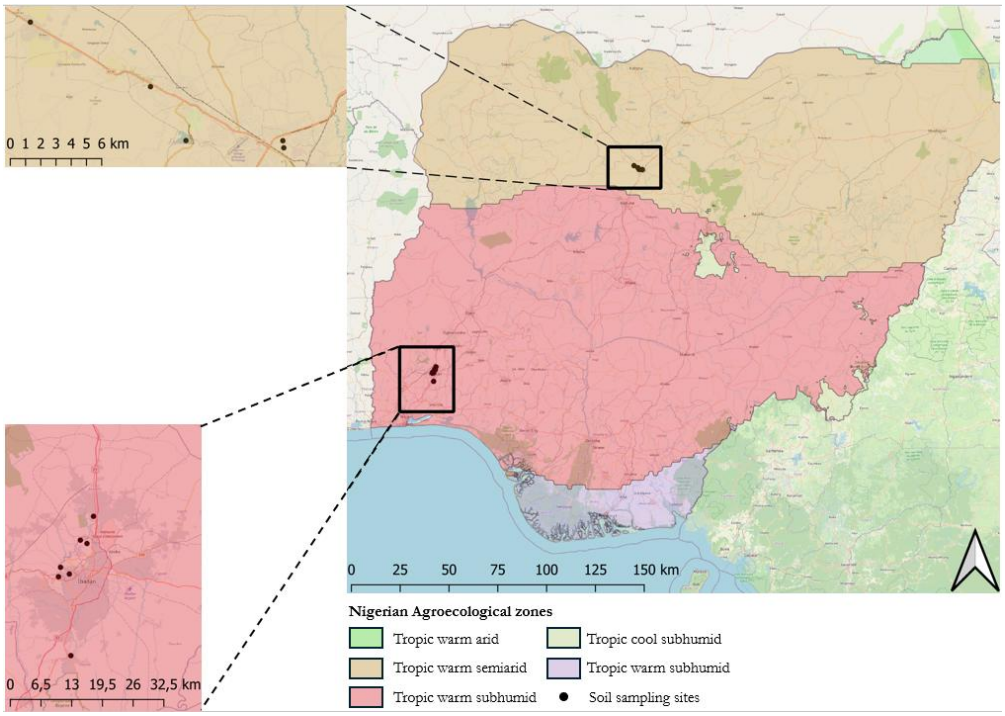


Fig. 4.1. Soil sampling sites for entomopathogenic nematode (EPN) isolation.
Map of Nigeria showing the agroecological zones and, with insets highlighting the specific sampling locations in each region.

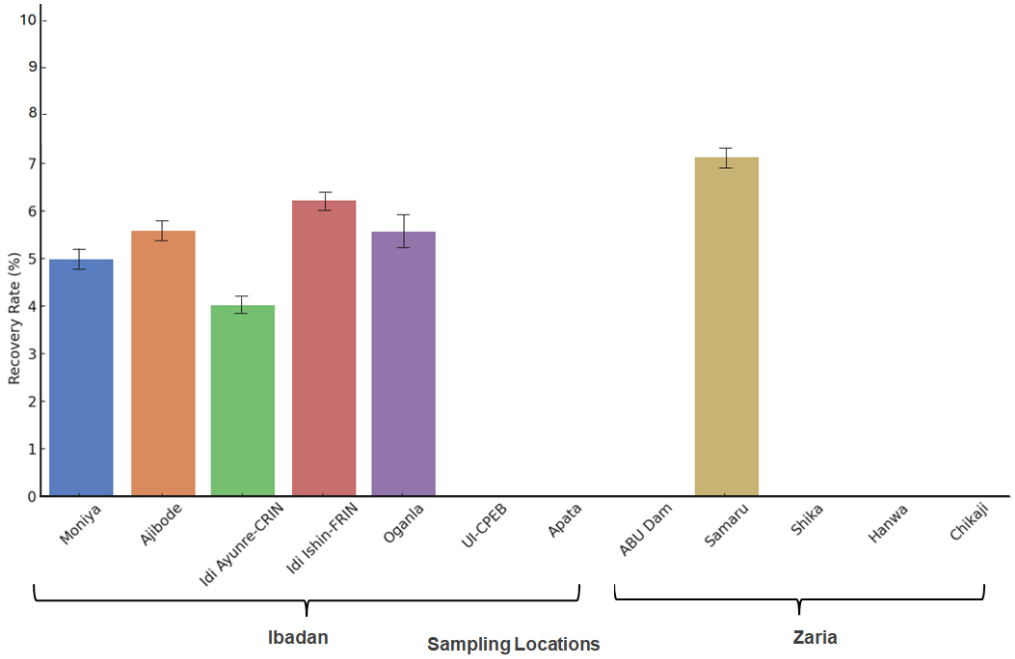


Fig. 4.2. Recovery rate of entomopathogenic nematode (EPN) isolates from soil samples. Soil samples were collected across twelve locations in Nigeria. Bars represent the percentage of soil samples from each site yielding at least one isolate.

4.3.2 Morphological and morphometric Identification

Morphological characterization of the six recovered EPN isolates was performed based on detailed morphometric analyses of IJs, males, amphimictic females, and hermaphrodites, averaging measurements from 20 specimens per life stage for each isolate. Examination focused on key morphological characters including body length (L), maximum body diameter (MBD), tail length (T), anal body diameter (ABD), position of the excretory pore (EP), nerve ring distance (NR), pharynx length (ES), and calculated morphometric ratios (a, b, c, D%, and E%) (Fig. 4. 3).

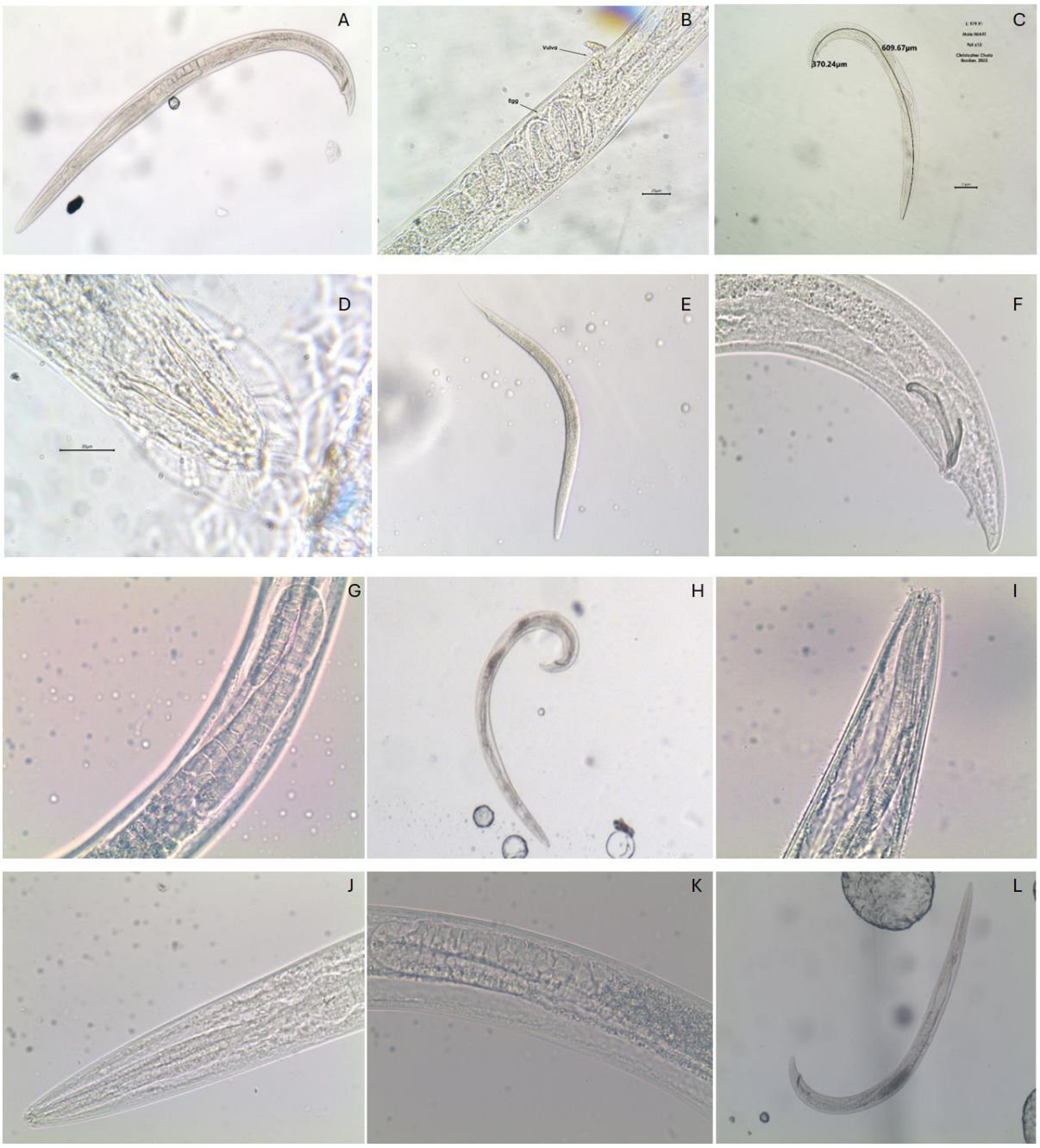
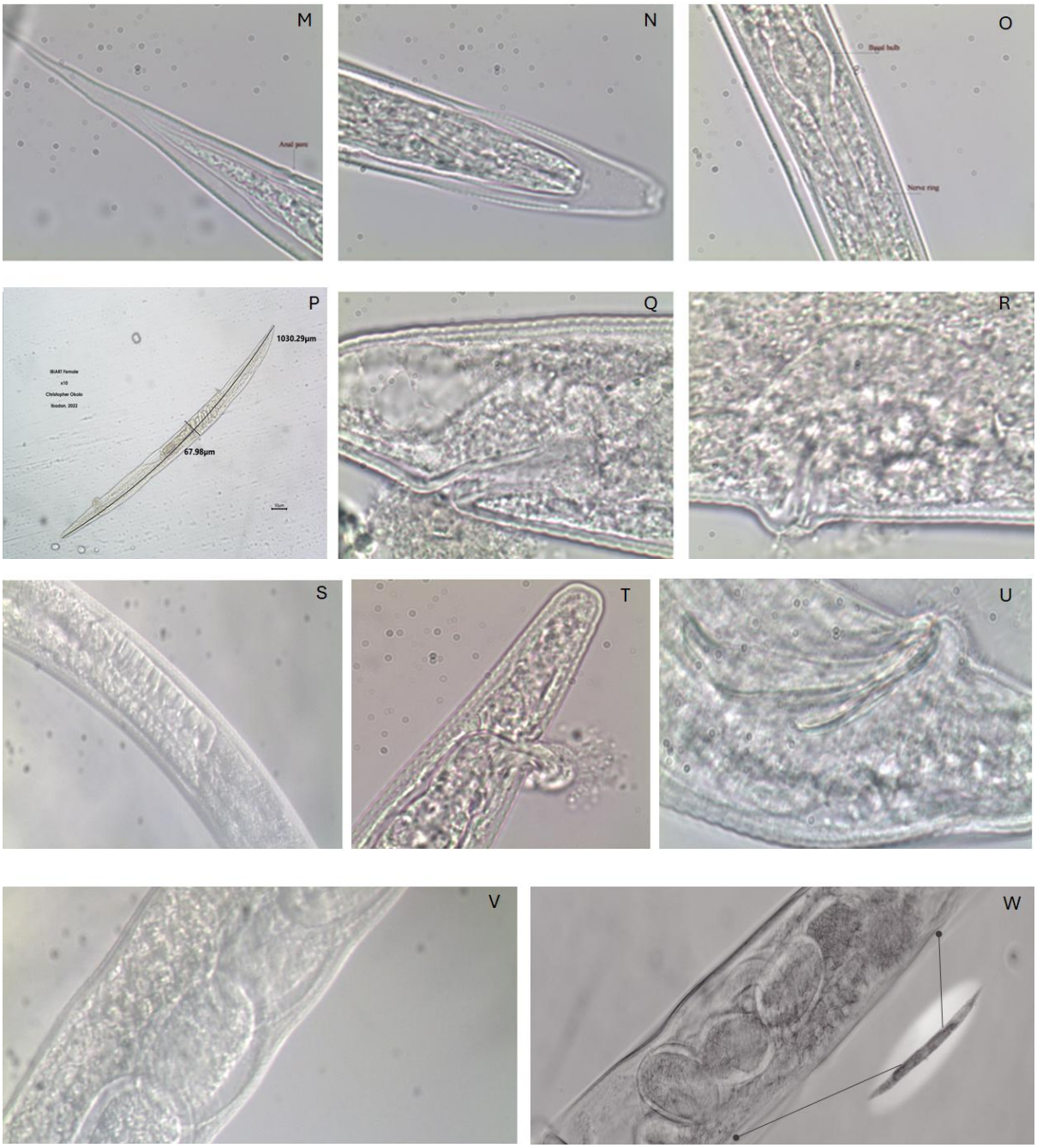


Fig. 4.3. Light micrographs of different life stages of entomopathogenic nematodes (EPN) isolated from Nigeria.

A: Full length male sample of Ib-ITUC102. **B**: Ib-IART45 female showing matured and vulva. **C**: Full length male Ib-IART45. **D**: Tail region of Ib-CRIN68 showing the distinct bursar of *Heterorhabditis* sp., **E**: Infective juvenile (IJ) of Ib-HORT. **F**: Tail region of Za-SAM showing the spicule and gubernaculum **G**: Testis reflex of Ib-HORT. **H**: Male of Ib-FRIN32. **I**: Anterior part Ib-HORT. **J**: Anterior part of Ib-IART45 showing median bulb. **K**: Testis reflex of IB-ITUC102. **L**: Full length male of Za-SAM.



M: Tail region of Ib-ITUC102 IJ showing hyalin**. N**: Head region of Ib-ITUC102 juvenile showing outer sheath. **O**: Median and nerve ring of Ib-FRIN32. **P**: Full length of female of Ib-IART45. **Q**: Anal region of Za-SAM showing excrete. **R**: Vulva region of ZA-SAM. **S**: Fat deposit in Ib-CRIN68 IJ. **T**: Tail region of Ib-FRIN32 showing excretes. **U**: Spicule and gubernaculum of IB-IART45 male. **V**: Eggs in female IB-CRIN68. **W**: Eggs in female Ib-FRIN32.

Morphometric analysis revealed distinct genus- and isolate-level traits across the three examined life stages. In the male stage (Table 4. 1), Ib-CRIN68 exhibited typical Heterorhabditis morphology, with elongate body form (~1,100 µm), strongly curved testis reflexion, and well-developed spicules (~50 µm). Males of Ib-FRIN32 were notably shorter (~780 µm) but had prominently curved testes and longer spicules (~42 µm), consistent with Oscheius diagnostics. Steinernema isolates showed

moderate variation in male body size and tail morphometrics, with Ib-IART45, Ib-ITUC102 showing body lengths of ~1,000 µm and high spicule-to-body diameter ratios, while Za-SAM and Ib-HORT presented longer males (up to 1,000 µm). In the female stage (Table 4. 2), females of Ib-CRIN68 were the longest among all isolates (~1,350 µm), with clearly defined vulval positioning and a long tail (~120 µm). Ib-HORT followed similar patterns with large body size (~1,200 µm) and cruiser-like tail characteristics. Other *Steinernema* isolates had more compact females, while Ib-FRIN32 showed distinctly smaller females (~900 µm) and reduced maximum body diameter, differentiating it clearly at the genus level. In the IJs stage (Table 4. 3), all Steinernema isolates displayed morphometrics typical of ambushers and intermediates. Ib-IART45, Ib-ITUC102 IJs were shorter (~525–540 µm) with high L/T ratios and elongated hyaline tail regions (~45 µm), consistent with ambusher behaviour. Za-SAM exhibited intermediate IJ length (~570 µm) and tail traits aligning with its foraging flexibility. Ib-CRIN68 had the longest IJs (~600–630 µm), while Ib-FRIN32 IJs were more compact (~600 µm) but had an extended hyaline region contributing nearly half the tail length, a key diagnostic trait of the genus *Oscheius.*

4.3.3 Molecular Identification

Molecular sequencing results and phylogenetic analyses supported and expanded upon the morphological identifications. The isolate Ib-CRIN68 demonstrated a high sequence similarity (>99%) to *H. bacteriophora*. Similarly, isolates Ib-IART45 and Ib-ITUC102 exhibited genetic congruence with *S. carpocapsae* sequences (>98% similarity), consistent with their morphological profiles. Ib-HORT was genetically closely aligned (>98% similarity) with *S. nepalense*, while Za-SAM showed high sequence similarity to *S. feltiae* (>98% identity). Molecular data for Ib-FRIN32 indicated close genetic affinity (>98%) with *O. myriophilus*, aligning well with morphological evidence.

Phylogenetic analyses performed using maximum-likelihood methods revealed clear and robust clustering of isolates within their respective genera. Ib-CRIN68 clustered definitively with *H. bacteriophora* reference sequences, closely grouping alongside related species such as *H. indica*. Within the genus *Steinernema*, isolates Ib-IART45 and Ib-ITUC102 formed a cohesive, monophyletic clade consistent with *S. carpocapsae*, while isolates Ib-HORT and Za-SAM occupied distinct, clearly separated branches aligned respectively with *S. nepalense* and *S. feltiae*. Isolate Ib-FRIN32 demonstrated clear separation within the genus *Oscheius*, clustering closely with reference sequences of *O. myriophilus* and related species like *O. tipulae.*

The phylogenetic results verify morphological identifications and offer additional molecular clarity, establishing species identity and taxonomic relationships among the six isolates.

4.3.4 Mortality Response Across Developmental Stages

The virulence of the six indigenous EPN was evaluated against four developmental stages of *S. frugiperda*: 2nd, 4th, 6th instar larvae, and pupae. Mortality rates increased consistently with both nematode dose and exposure duration for all developmental stages.

Among the life stages tested, FAW 2nd instar larvae were most susceptible. At 72 hours post-inoculation, all isolates achieved considerable dose-dependent mortality, with the highest mortality observed at 200 IJs/insect. Mean mortality ranged from 82.4 ± 5.6% (Ib-CRIN68) to 58.1 ± 7.2% (Ib-FRIN32) at this dose. Mortality at the lowest dose (25 IJs/insect) was modest, typically between 18% and 32% by 72 hours (Fig. 4. 4 A-C).

For the 4th instar larvae, mean mortality ranged from 65.2 ± 8.1% to 41.7 ± 7.5% at 200 IJs/insect after 72 hours. The 6th instar larvae showed lower mortality, with values between 52.5 ± 6.4% and 35.3 ± 8.7%. Pupae were the least affected, with mortality ranging from 55 ± 5.8% to 21.4 ± 6.1% across the isolates at the highest dose and latest time point.

Lethal concentration (LC_{50}) and lethal time (LT_{50}) values were calculated for the 2nd instar stage based on observed dose–response trends. The isolate Ib-CRIN68 exhibited the highest potency, with an LC_{50} of 61.8 IJs/insect and an LT_{50} of 38.5 hours. *Steinernema carpocapsae* isolates Ib-IART45 and Ib-ITUC102 also showed favourable performance with LC_{50} values of 72.5 and 66.0 IJs/insect, respectively. The lowest potency was recorded for the *O. myriophilus* isolate Ib-FRIN32, with an LC_{50} of 85.3 IJs/insect and an LT_{50} of 47.1 hours.

A three-way ANOVA confirmed that nematode isolate ($F_{5,120} = 28.34$, $p < 0.001$), dose ($F_{3,120} = 41.76$, $p < 0.001$), and time ($F_{2,120} = 19.21$, $p < 0.001$) significantly influenced larval mortality in 2nd instars. Significant interactions were also detected between isolate and dose ($F_{15,120} = 6.54$, $p < 0.001$), and between dose and time ($F_{6,120} = 5.02$, $p < 0.01$). For the 4th, 6th instars, and pupae, one-way ANOVA performed at 200 IJs/insect and 72 hours revealed no statistically significant differences among isolates (4th instar $F = 0.41$, $p = 0.8296$; 6th instar $F = 0.72$, $p = 0.6239$; and pupae $F = 0.95$, $p = 0.4868$).

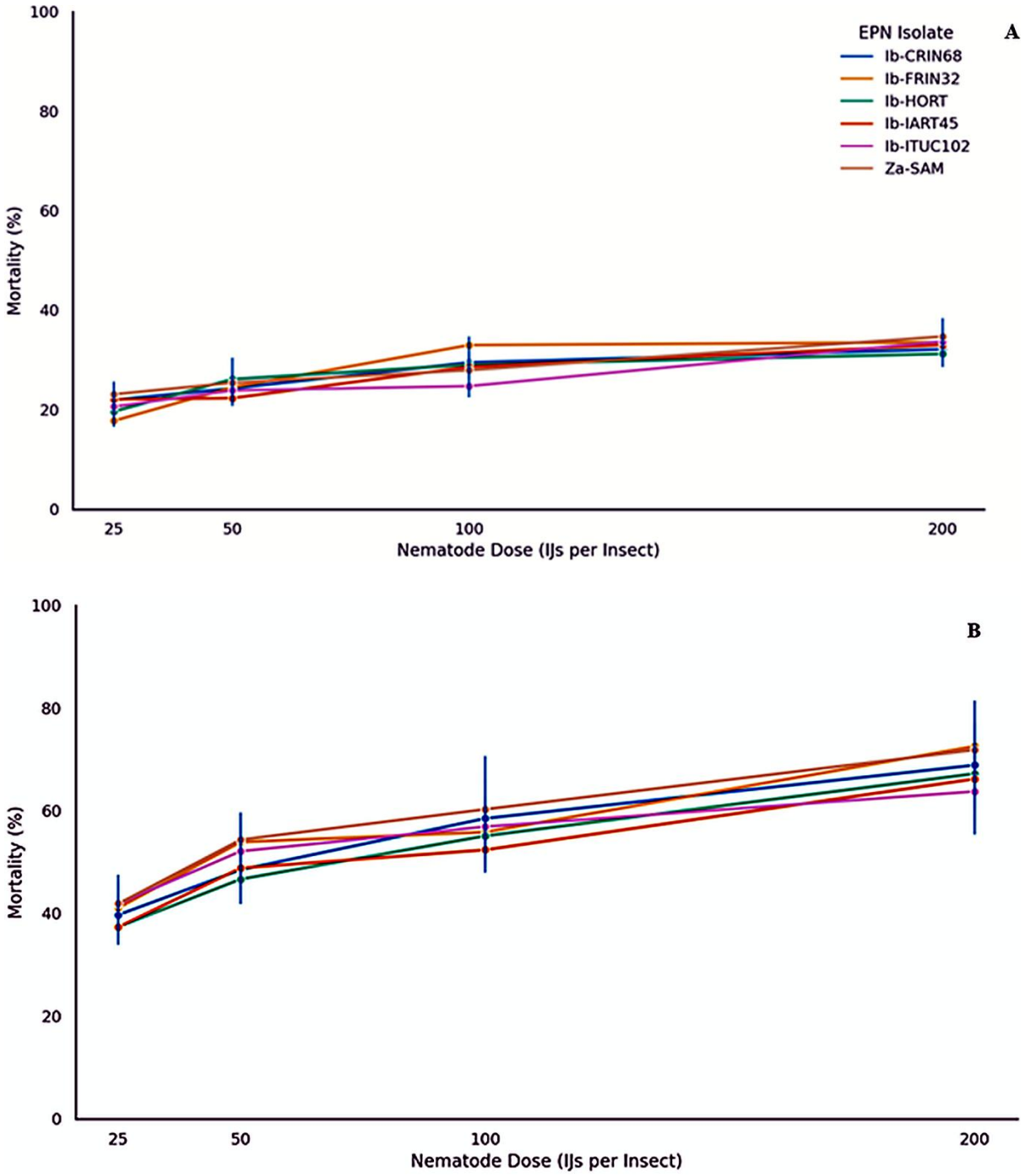
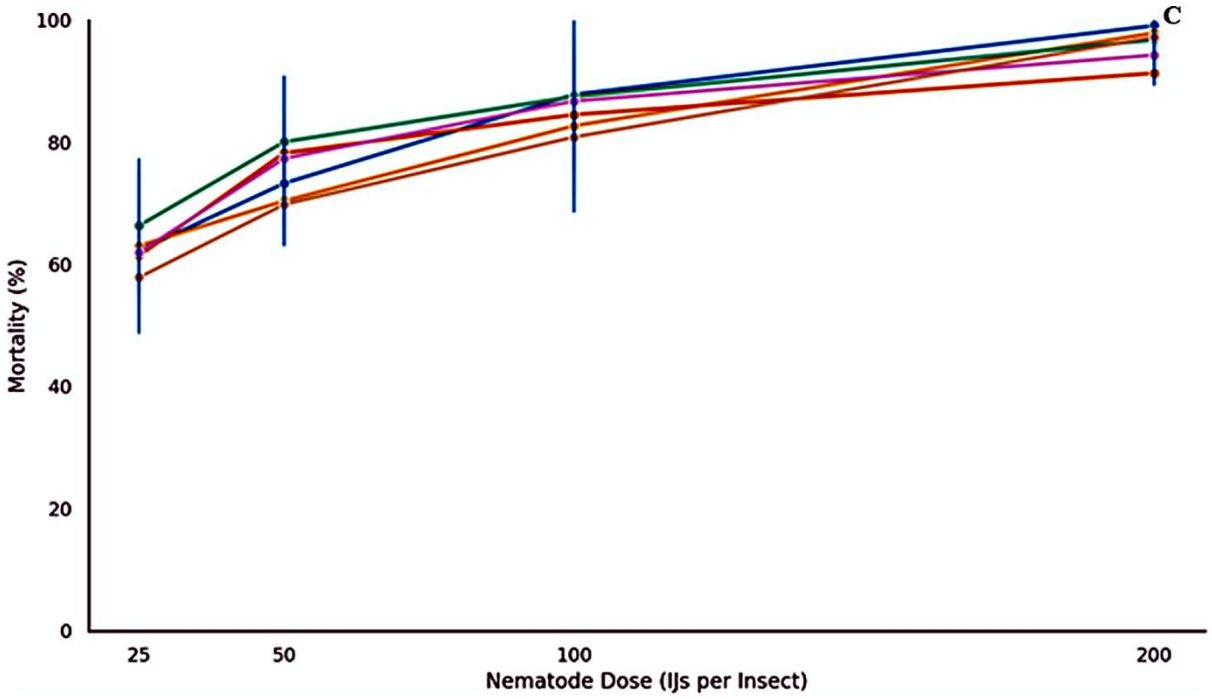
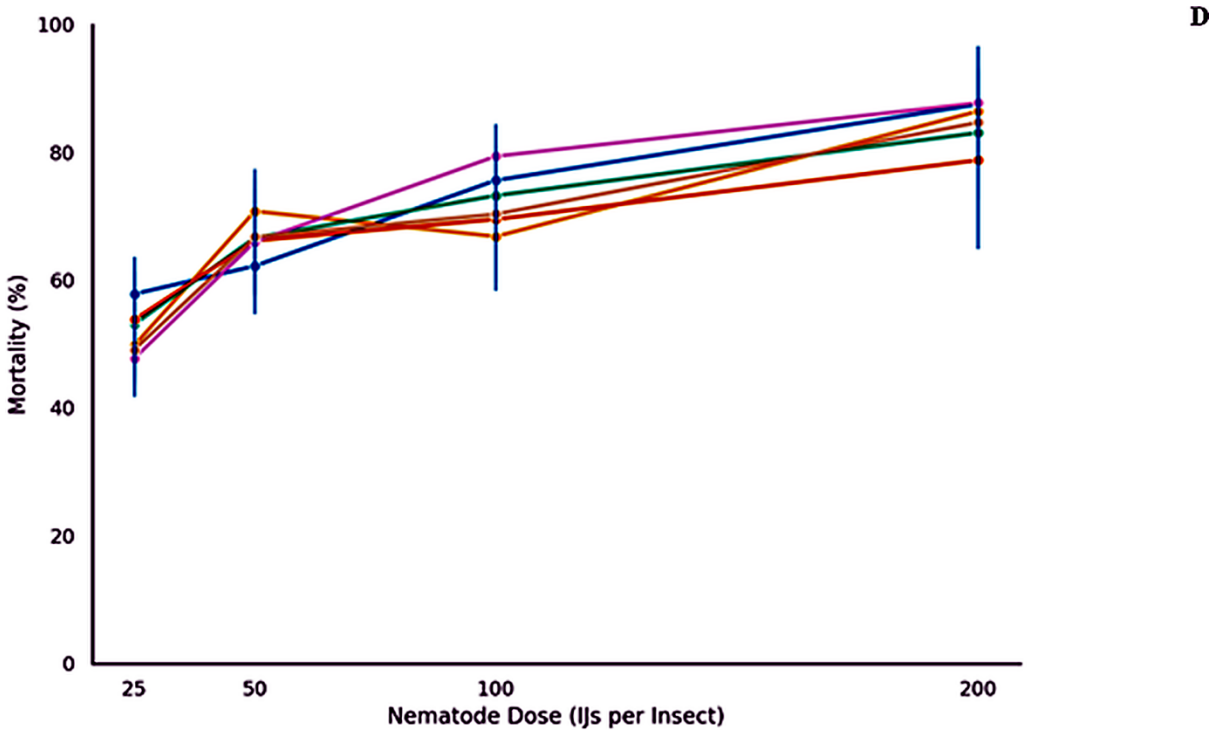
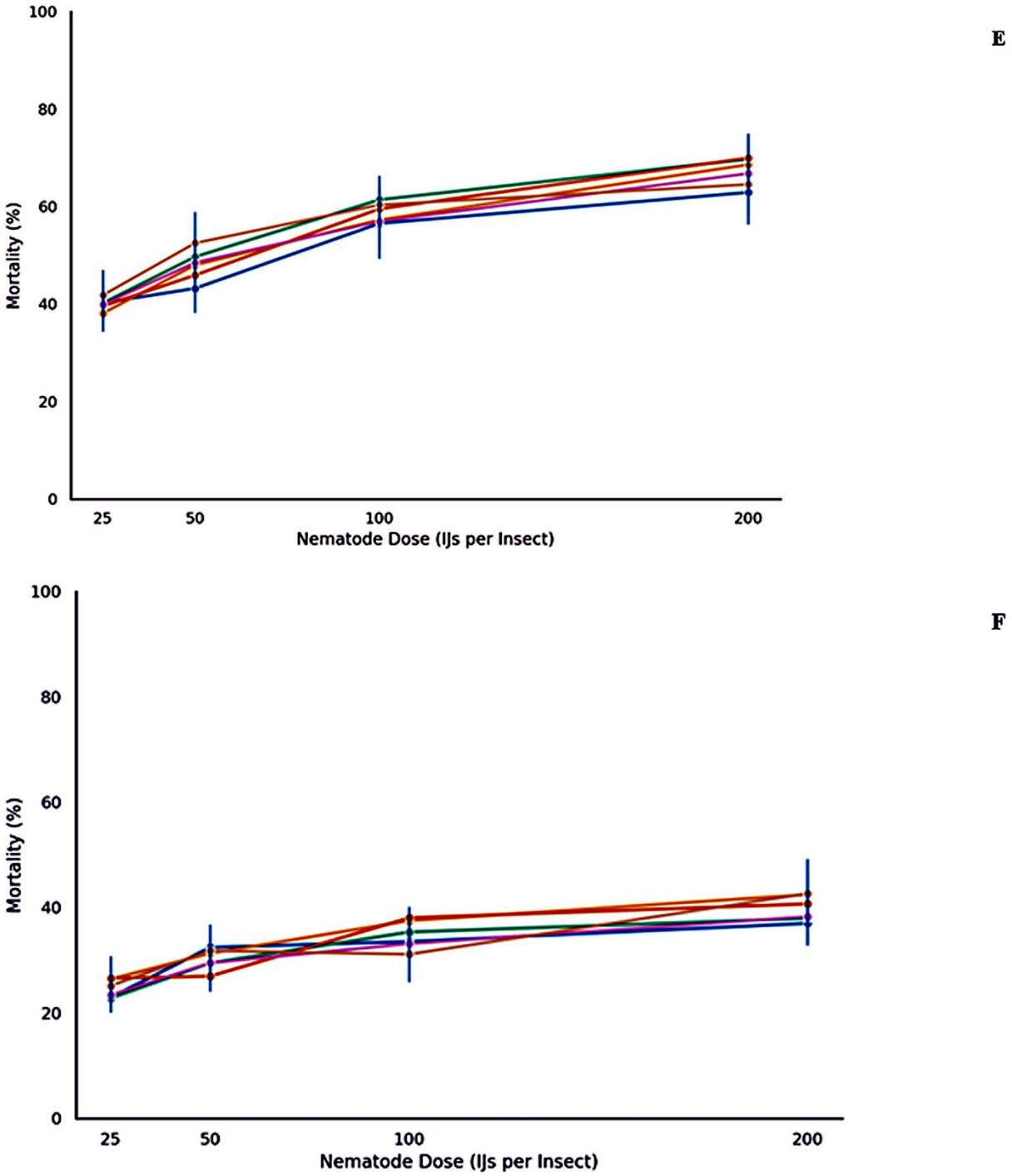


Fig. 4.4. Mean mortality (% ± SE) of *Spodoptera frugiperda* larvae exposed to different dosages. Infective juveniles (IJs) at the rate of 25, 50, 100, and 200 per insect of six indigenous entomopathogenic nematode (EPN) isolates applied to 2nd instar larva at 24 h (**A**) and 48 h (**B**) post-infection.





Infective juveniles (IJs) at the rate of 25, 50, 100, and 200 per insect of six indigenous entomopathogenic nematode (EPN) isolates applied to 2nd (**C**) and 4th (**D**) instar larvae at 72 h.



Infective juveniles (IJs) at the rate of 25, 50, 100, and 200 per insect of six indigenous entomopathogenic nematode (EPN) isolates applied to 6th instar larva (**E**) and pupal stage (**F**) at 72h post-infection.

For later larval and pupal stages of *S. frugiperda*, mortality also increased in a dose-dependent manner. However, the variability in isolate performance was less pronounced compared to FAW's 2nd instar stage (Fig. 4. 4. D-F).

At the highest dosage tested (200 IJs/insect) and 72 hours post-exposure, the efficacy of the isolates appeared to vary more notably at earlier insect developmental stages. Mortality differences among the

isolates for the 2nd instar larvae was not statistical significance (F = 1.93; p = 0.163), indicating negligible variability in isolate performance at this early stage. Also, mortality rates among isolates were statistically similar at later developmental stages, including the 4th instar (F = 0.41; p = 0.830), 6th instar (F = 0.72; p = 0.624), and pupal stage (F = 0.95; p = 0.487).

4.4 Discussion

This study represents the first comprehensive attempt to isolate, morphologically and molecularly characterize, and evaluate the infectivity of native EPN strains from Nigeria against multiple life stages of FAW under laboratory conditions.

The identification of isolates Ib-CRIN68 (*H. bacteriophora*), Ib-IART45 and Ib-ITUC102 (*S. carpocapsae*), Ib-HORT (*S. nepalense*), Za-SAM (*S. feltiae*), and Ib-FRIN32 (*O. myriophilus*), corroborates previous findings and provides valuable updates to the limited EPN taxonomic records in Nigeria. Previously, (Akyazi et al., 2012) recorded the first occurrence of EPNs from Nigerian soils, identifying isolates primarily belonging to the genera *Heterorhabditis* and *Steinernema*. Our findings reaffirm the presence of these genera and expand the diversity by including the less commonly reported genus of *Oscheius*. Our identification of *O. myriophilus* in Nigerian soils is critical, as *Oscheius* spp. are emerging globally as promising biocontrol agents, particularly against dipteran and lepidopteran pests (Dichusa et al., 2021; Suan et al., 2025).

Morphological variability observed among our isolates, demonstrated through comprehensive morphometric analyses, aligns closely with recent studies conducted in similar agroecological zones worldwide (Alotaibi et al., 2022; Daramola et al., 2021). Notably, the morphological parameters employed in our study were robust enough to differentiate effectively among genera, providing essential baseline data for future comparative studies. However, relying exclusively on morphological identification can be limiting due to phenotypic plasticity of EPNs as influenced by environmental conditions, host type, and geographic isolation (Caccia et al., 2017; Guide et al., 2018; Malan et al., 2016; Ngugi et al., 2019). Thus, our integration of molecular analyses using multiple genetic markers (ITS, 12S, COI, and D2–D3 regions) considerably enhanced species resolution and reduced potential taxonomic ambiguity. This dual approach, as underscored by recent literature (Gumussoy et al., 2022; Subbotin, 2021), is crucial for correctly delineating closely related nematode species, thereby providing a reliable basis for their subsequent application in pest management programs. The morphological and molecular characterization of EPNs isolated in this study contributes significantly to our understanding of indigenous nematode diversity in Nigerian agroecosystems and beyond.

The virulence assays conducted in this study clearly demonstrated that EPN isolates exhibit differential pathogenicity against various developmental stages of FAW. Our data revealed high susceptibility in the early instar larvae, with isolate Ib-CRIN68 *(H. bacteriophora*) achieving notably high mortality rates, consistent with prior reports by Alotaibi et al. (2022) and Guide et al. (2024). Our results also corroborate previous observations that earlier developmental stages of lepidopteran pests are typically more vulnerable to EPN attack due to their thinner cuticles, lower immune capabilities, and relatively less-developed defensive behaviours (Bai et al., 2016; Guo et al., 2023; Zhang et al., 2012). Conversely, reduced susceptibility observed in later instars and pupae of FAW in our study parallels findings from previous work that highlighted the increased physiological host defence against EPNs via thicker cuticles, enhanced immune responses, and behavioural resistance mechanisms in older FAW larvae (Acharya, Yu, et al., 2020; Fallet et al., 2022).

We found significant influences of nematode isolate, dosage, and exposure time on FAW mortality, particularly for early instars. Similar trends have been documented in several previous studies (Abd-Elgawad, 2019; Brusselman et al., 2007; Dillon et al., 2007; Kapranas et al., 2017), emphasizing the importance of optimizing dose and exposure parameters to maximize EPN efficacy under field conditions. The absence of significant differences in virulence among isolates at later FAW developmental stages further underscores the complex interaction dynamics between host defense strategies and nematode pathogenicity (Acharya, Hwang, et al., 2020; Castillo et al., 2011).

The presence of inter-isolate variation in pathogenicity among our Nigerian EPN isolates suggests potential genetic and ecological adaptations that could enhance or limit their practical effectiveness under field conditions. Thus, a more comprehensive ecological characterization, which extends beyond laboratory assays to encompass critical environmental factors such as temperature tolerance, desiccation survival, soil type preference, host-finding strategies, and compatibility with agricultural practices, is indispensable. Such detailed ecological studies have been advocated strongly in the recent literature as critical prerequisites for successful biocontrol implementation of EPNs (Koppenhöfer & Kaya, 1999; Kour et al., 2021). Indeed, isolates exhibiting broad environmental tolerance, effective dispersal capabilities, and high persistence in the soil are more likely to provide consistent biocontrol efficacy under varying field conditions (Aryal et al., 2025; Suan et al., 2025).

Furthermore, ecological profiling of indigenous isolates is essential in tailoring biological control agents for targeted applications within specific agroecological zones. Considering Nigeria's diverse agricultural environments, including lowland rainforest and the Guinea savannah zones represented in this study, the isolates' ecological adaptability and specificity could significantly influence their

biocontrol potential. Recent evidence from Brazilian orchards demonstrated that understanding nematode ecology substantially improved biocontrol outcomes against major pests, resulting in sustainable pest management strategies that effectively reduced chemical pesticide use (Barbosa-Negrisoli et al., 2010; Guide et al., 2024; Mejia-Torres & Sáenz, 2013). Possibly, our isolates could offer environmentally sustainable solutions tailored specifically to agricultural conditions in SSA where FAW represents a severe economic threat to maize production.

Hence, future research should emphasize more detailed ecological characterization of the EPN isolates identified in this study. Understanding their ecological interactions and environmental adaptability will not only optimize their biocontrol efficacy but also guide their integration into comprehensive IPM programs. Additionally, ecological characterization will help assess the feasibility of commercial-scale nematode formulations, ensuring consistency and reliability under varied field conditions.

Thus, in summary our study provides critical baseline data on indigenous Nigerian EPN isolates' taxonomic identities and pathogenic capabilities against the invasive FAW. These findings expand the limited existing knowledge base on EPNs in SSA (Akyazi et al., 2012). Moreover, our results highlight the necessity for ecological characterization of EPNs to translate promising laboratory results into reliable field applications, thereby possibly contributing to sustainable pest management strategies in Nigeria and similar agroecological regions in SSA and beyond.

Table 4.1. Male morphometrics of six isolates of entomopathogenic nematodes from Nigeria [2]

Characters	Ib-CRIN68	Ib-FRIN32	Ib-IART45	Ib-ITUC102	Za-SAM	Ib-HORT
n	15	17	20	20	18	20
L	1107.6 ± 57.8 (986.9–1199.0)	796.3 ± 44.9 (709.5–871.1)	992.3 ± 35.4 (936.8–1046.0)	1000.1 ± 48.5 (898.5–1085.9)	1001.3 ± 37.4 (909.4–1070.7)	1010.5 ± 47.0 (1081.1–1145.4)
a = L/MBD	22.8	18.9	21.9	21.4	21.0	22.1
b = L/ES	6.8	6.8	7.0	7.1	7.4	7.7
c = L/T	12.0	10.3	11.8	11.3	11.5	12.4
c´ = T/ABD	3.3	3.4	3.6	3.7	3.5	3.4
MBD	47.3 ± 2.4 (42.7–50.9)	41.9 ± 1.9 (37.2–45.2)	45.0 ± 2.3 (41.8–51.3)	46.3 ± 2.5 (41.7–50.2)	48.2 ± 2.2 (43.5–54.5)	50.7 ± 2.2 (47.9–54.9)
EP	186.9 ± 6.7 (174.7–198.5)	160.2 ± 7.0 (150.5–172.1)	181.7 ± 11.2 (157.6–196.4)	180.6 ± 10.8 (160.1–202.5)	186.6 ± 9.8 (167.3–206.3)	195.2 ± 9.0 (181.8–209.7)
NR	131.8 ± 5.5 (122.3–141.8)	100.2 ± 4.4 (93.6–109.8)	113.5 ± 4.6 (105.3–121.1)	118.0 ± 5.5 (107.6–128.7)	112.7 ± 4.5 (105.8–123.4)	126.5 ± 6.3 (114.7–138.6)
ES	162.1 ± 5.5 (151.0–171.1)	119.2 ± 7.2 (103.3–129.3)	139.3 ± 6.1 (129.5–151.2)	137.3 ± 6.9 (123.0–147.7)	140.5 ± 4.8 (133.5–150.8)	148.0 ± 7.1 (129.9–159.4)
Testis Reflexion	511.6 ± 22.1 (461.7–550.0)	458.7 ± 20.7 (401.0–487.7)	488.3 ± 30.5 (429.0–555.2)	498.5 ± 25.9 (439.5–538.4)	504.0 ± 27.2 (453.2–561.6)	529.0 ± 21.0 (500.0–580.9)
T	88.8 ± 4.4 (82.8–97.2)	77.4 ± 3.3 (71.1–82.7)	85.1 ± 3.9 (77.5–92.5)	89.9 ± 4.5 (82.7–100.6)	88.9 ± 4.3 (80.3–97.7)	93.2 ± 4.8 (79.0–98.7)
ABD	27.5 ± 1.4 (24.3–29.6)	23.4 ± 0.9 (22.1–25.7)	22.7 ± 0.9 (20.7–24.2)	23.9 ± 0.9 (22.5–26.2)	25.0 ± 1.3 (23.5–28.9)	27.3 ± 1.1 (25.0–29.4)
SL	52.5 ± 2.2 (48.8–57.4)	41.7 ± 1.8 (38.8–45.7)	48.2 ± 1.8 (43.8–52.4)	49.0 ± 2.3 (45.7–53.9)	49.5 ± 1.9 (45.9–54.2)	53.0 ± 2.7 (46.0–56.9)
GL	24.1 ± 1.0 (22.0–25.7)	20.4 ± 1.1 (17.9–22.9)	21.7 ± 1.0 (19.7–23.7)	23.5 ± 0.7 (22.1–24.7)	22.8 ± 1.2 (20.8–25.2)	24.3 ± 1.0 (22.6–26.7)
D% = EP/ES × 100	117.5	130.1	126.4	129.2	130.3	133.3
E% = EP/T × 100	372.4	204.7	206.3	201.9	210.7	211.4
SW% = SL/ABD × 100	191.1	182.6	208.8	206.1	198.2	190.6
GS% = GL/SL × 100	45.9	47.8	46.1	47.1	45.9	44.8

L: Body length. **MBD**: Maximum body diameter. **EP**: Position of the excretory pore. **NR**: Nerve ring distance. **ES**: Pharynx length. **T**: Tail length.

ABD: Anal body diameter. **SL**: Spicule length. **GL**: Gubernaculum Length. **a, b, c, c´, D%, E%, SW% and GS%**: calculated morphometric ratios.

[2] All measurements are in µm except n, ratio, and percentage), and in the form: mean ± s.d. (range).

Table 4.2. Female morphometrics of six isolates of entomopathogenic nematodes from Nigeria

Characters	Ib-CRIN68	Ib-FRIN32	Ib-IART45	Ib-ITUC102	Za-SAM	Ib-HORT
n	17	18	20	19	20	18
L	1347.8 ± 77.3 (1214.0–1458.7)	922.3 ± 40.8 (825.2–1007.3)	1132.1 ± 52.6 (1038.8–1215.2)	1114.6 ± 57.2 (995.6–1198.0)	1263.8 ± 59.5 (1164.5–1342.0)	1215.4 ± 71.8 (1202.0–1383.7)
a = L/MBD	14.1	14.7	15.7	14.9	14.2	15.1
b = L/ES	7.9	7.2	7.8	7.9	8.3	8.6
c = L/T	10.7	9.2	10.2	10.1	11.0	10.6
c´ = T/ABD	3.9	4.0	3.9	3.9	3.8	3.9
V´	698.7 ± 33.8 (631.5–767.0)	491.0 ± 23.4 (447.4–539.3)	590.7 ± 24.8 (555.6–636.6)	606.8 ± 30.7 (545.6–678.6)	672.9 ± 28.0 (617.6–729.4)	712.6 ± 30.0 (674.7–786.5)
V = V´/L *100	51.8	53.2	52.2	54.4	53.2	51.9
MBD	94.8 ± 4.9 (85.2–103.4)	62.7 ± 2.7 (58.1–67.1)	71.8 ± 4.0 (64.7–78.3)	75.2 ± 3.7 (67.6–82.4)	87.1 ± 3.6 (80.3–93.6)	91.5 ± 3.3 (86.4–98.5)
EP	213.1 ± 8.1 (195.6–225.3)	168.1 ± 8.3 (149.7–181.2)	184.7 ± 3.9 (177.7–191.6)	188.2 ± 7.1 (171.2–198.7)	202.7 ± 9.5 (183.9–220.5)	214.6 ± 9.4 (196.2–233.1)
NR	145.4 ± 6.2 (136.2–156.7)	110.5 ± 5.8 (96.2–121.6)	122.6 ± 5.8 (112.2–133.1)	126.4 ± 4.3 (118.6–133.8)	132.8 ± 5.8 (121.9–144.8)	140.3 ± 5.7 (127.4–148.2)
ES	168.7 ± 9.4 (154.5–185.3)	129.9 ± 7.5 (115.9–140.9)	144.6 ± 6.6 (128.6–155.7)	145.4 ± 7.0 (133.0–161.3)	147.9 ± 6.6 (139.1–163.9)	158.8 ± 7.2 (141.1–171.6)
T	125.5 ± 6.8 (107.6–134.9)	104.0 ± 4.7 (95.2–113.0)	107.7 ± 6.5 (93.0–119.5)	112.2 ± 6.0 (99.3–119.6)	114.7 ± 5.4 (101.7–123.3)	131.6 ± 7.8 (110.5–145.8)
ABD	31.6 ± 1.5 (28.7–35.6)	26.3 ± 1.3 (24.2–28.7)	28.1 ± 1.6 (25.8–31.2)	28.7 ± 1.0 (27.0–30.4)	30.2 ± 1.2 (27.8–31.8)	33.1 ± 1.8 (29.5–36.3)
D% = EP/ES × 100	122.5	131.3	126.2	129.4	135.7	133.7
E% = EP/T × 100	167.9	166.1	170.1	169.0	176.0	165.3

L: Body length. **V´**: Distance of anterior to vulva. **MBD**: Maximum body diameter. **EP**: Position of the excretory pore. **NR**: Nerve ring distance. **ES**: Pharynx length. **T**: Tail length. **ABD**: Anal body diameter. **a, b, c, c´, D%, E%, V**: calculated morphometric ratios.

Table 4.3. Infective juvenile morphometrics of six isolates of entomopathogenic nematodes from Nigeria

Characters	Ib-CRIN68	Ib-FRIN32	Ib-IART45	Ib-ITUC102	Za-SAM	Ib-HORT
n	20	20	20	20	20	20
L	569.4 ± 21.7 (530.4–615.6)	601.9 ± 23.3 (565.2–654.8)	536.4 ± 32.4 (474.1–590.5)	538.0 ± 29.0 (499.0–592.9)	570.4 ± 31.6 (514.7–617.0)	582.0 ± 23.7 (526.4–629.9)
a = L/MBD	21.2	21.5	22.3	21.7	23.1	22.4
b = L/ES	5.3	5.9	5.2	5.2	5.4	5.2
c = L/T	6.2	7.0	6.1	6.0	6.7	6.5
c´ = T/ABD	6.0	6.0	6.2	6.3	5.6	6.0
MBD	26.3 ± 1.6 (23.4–29.5)	28.0 ± 1.5 (25.3–31.4)	23.9 ± 1.1 (22.2–26.0)	25.3 ± 1.3 (22.3–27.1)	24.9 ± 0.9 (23.1–26.4)	26.1 ± 1.0 (24.6–27.5)
EP	117.6 ± 7.0 (105.0–136.2)	107.2 ± 4.8 (95.9–114.7)	111.7 ± 5.2 (101.0–121.8)	113.7 ± 3.9 (108.4–123.4)	114.3 ± 4.9 (107.0–127.2)	120.1 ± 6.0 (111.8–131.9)
NR	86.4 ± 3.9 (79.3–91.9)	81.6 ± 5.1 (71.2–91.1)	86.5 ± 3.3 (77.8–92.4)	87.8 ± 4.8 (79.2–95.9)	84.5 ± 4.8 (77.4–91.7)	89.9 ± 4.9 (81.4–99.1)
ES	107.0 ± 4.0 (101.0–115.6)	101.4 ± 6.0 (92.2–112.9)	102.7 ± 4.5 (94.9–113.3)	103.0 ± 5.0 (87.6–109.5)	105.5 ± 4.0 (97.0–114.9)	109.9 ± 4.2 (102.8–122.1)
T	89.2 ± 5.5 (76.4–98.2)	84.4 ± 3.2 (76.1–90.5)	87.0 ± 4.6 (73.6–93.6)	87.4 ± 4.9 (76.0–97.0)	86.6 ± 3.4 (82.0–94.3)	87.5 ± 4.6 (79.9–99.4)
ABD	14.9 ± 0.8 (13.3–16.4)	14.3 ± 0.7 (13.3–16.1)	13.7 ± 0.6 (12.5–14.8)	14.1 ± 1.0 (11.8–15.8)	15.3 ± 0.8 (14.0–16.8)	14.8 ± 0.8 (13.5–17.2)
H			42.3 ± 1.6 (38.8–45.3)	41.8 ± 1.9 (39.1–45.4)	41.0 ± 2.1 (37.4–45.9)	42.4 ± 2.9 (37.8–50.8)
D% = EP/ES × 100	109.4	105.6	106.5	109.6	110.7	107.5
E% = EP/T × 100	130.7	127.2	125.6	125.6	134.1	133.8
H% = H/T × 100			47.6	48.3	47.5	47.7

L: Body length. **MBD**: Maximum body diameter. **EP**: Position of the excretory pore. **NR**: Nerve ring distance. **ES**: Pharynx length. **T**: Tail length. **ABD**: Anal body diameter. **H**: Hyalin length of tail. **a, b, c, c´, D%, E%, SW% and GS%**: calculated morphometric ratios.

Chapter 5: Ecological Characterization and Efficacy of Indigenous Entomopathogenic Nematodes Against *Spodoptera frugiperda* in Nigeria

5.1 Introduction

Despite their proven laboratory virulence, the field performance of EPNs is often highly variable, particularly under tropical or sub-tropical climates, where environmental stresses such as temperature extremes, desiccation, hypoxia, and oxidative conditions can impair their infectivity, reproduction, and survival (Koppenhöfer & Kaya, 1999; Levy et al., 2020; John Mukuka, Olaf Strauch, Mohamed Hisham Al Zainab, et al., 2010; John Mukuka, Olaf Strauch, & Ralf-Udo Ehlers, 2010). EPN IJs, which are the only free-living and infective stage, are particularly vulnerable to these abiotic factors, often resulting in poor field establishment and inconsistent pest suppression, thus limiting the adoption of EPN-based products in many developing regions (Kour et al., 2021; Lalramnghaki et al., 2017).

Understanding the ecological adaptability and environmental resilience of EPN isolates for their effective implementation in biological control programs is therefore crucially important (Fatimah et al., 2025). Traits such as infectivity and reproduction under different thermal regimes (Levy et al., 2020), substrate adaptability (Matuska-Lyzwa et al., 2023), foraging depth, and tolerance to environmental stresses like desiccation (Nimkingrat et al., 2011; Nimkingrat et al., 2013), oxidative stress, and oxygen deprivation (hypoxia) (Sumaya et al., 2018; Zadji et al., 2014) are all key indicators of an isolate's ability to perform well under field conditions. Ecological characterization, thus, provides a deeper understanding of isolate resilience and adaptability, far beyond what can be inferred from virulence tests alone (Anbesse, Sumaya, Dörfler, et al., 2013; Puza et al., 2021).

Although substantial progress has been made in characterizing EPNs in temperate regions, there is still a paucity of ecological studies on native African isolates. So far, work in Nigeria was largely limited to isolation, morphological and molecular identification, and laboratory virulence assays (Akyazi et al., 2012; Daramola et al., 2021), confirming the presence of diverse EPN taxa, including *S. carpocapsae*, *H. bacteriophora*, and *O. myriophilus*. However, no published work to date has evaluated the abiotic stress tolerance or environmental adaptability of these isolates, which is essential for developing robust EPN-based pest control tools suited to African agroecological systems.

The present study addresses this gap by building upon earlier identification and virulence evaluations of six indigenous EPN isolates recovered from two agroecological zones in Nigeria lowland rainforest (Ibadan, Oyo State) and northern Guinea savannah (Zaria, Kaduna State) (Okolo et al., 2025). Using morphological and molecular identification methods, these isolates were confirmed as *H. bacteriophora* (Ib-CRIN68), *S. carpocapsae* (Ib-IART45, Ib-ITUC102), *S. nepalense* (Ib-HORT), *S. feltiae* (Za-SAM), and *O. myriophilus* (Ib-FRIN32). Having previously established their laboratory virulence against FAW, *S. frugiperda*, an invasive and economically destructive pest of maize in sub-Saharan Africa (Goergen et al., 2016; Kenis et al., 2023), we now extend the investigation by evaluating their ecological traits relevant to field performance.

By integrating a comprehensive suite of laboratory tests, this study aimed to generate a multi-dimensional profile of ecological fitness for each EPN isolate, with a view to identifying the most robust candidates for potential field evaluation. Specifically, we assessed the infectivity and reproductive success of these isolates to abiotic stresses such as temperature, desiccation, hypoxia (anaerobic storage conditions), and oxidative stress (via H_2O_2 exposure). Additional bioassays were conducted to evaluate foraging behaviour on various substrates and at different soil depths.

5.2 Materials and Methods

5.2.1 Infectivity and reproduction in a range of temperatures

The temperature tolerances of the six EPN isolates (Ib-CRIN68, Ib-IART45, Ib-ITUC102, Ib-FRIN32, and Ib-HORT from Ibadan, and Za-SAM from Zaria) were evaluated comprehensively through two experimental approaches designed to determine both their infectivity and reproductive capabilities across a range of temperatures.

In the first set of experiments, infectivity was assessed in a CRD with four replicates using second-stage larvae of FAW at six distinct temperatures (10, 15, 20, 25, 30, and 35°C). Each well of a 24-well tissue culture plate was filled with 0.5 g of air-dried soil, equilibrated at the respective temperatures for one hour prior to inoculation. Subsequently, each well in two sets received 50 IJs suspended in 60 µl sterilized deionized water, while a third set served as control without nematodes. Following nematode application, a single FAW larva, weighing between 200 and 300 mg, was carefully introduced to each well. Plates were monitored every 12 hours over a seven-day period, meticulously recording larval mortality, time to death, and quantifying the number of IJs established per larva. One day post-mortem, cadavers from one set of plates

were dissected to count established IJs. Concurrently, the second set was examined daily for progeny IJ emergence from a White trap set up. Cadavers failing to yield emerging IJs within 14 days following initial emergence observations were dissected to validate infection status.

The second experiment evaluated the EPNs reproductive potential at four selected temperatures (15, 20, 25, and 30°C). Nematodes (50 IJs per larva) were initially inoculated into tissue culture plates containing 0.5 g of sand and incubated at 25°C for 24 hours to standardize IJ penetration into FAW larvae (250–300 mg weight). Post-infection, individual cadavers were carefully transferred onto modified White traps consisting of a petri dish lid lined with filter paper floating on sterilized water in a larger petri dish. These were incubated at the specified temperatures. Emergence of IJs from cadavers was monitored daily, documenting the onset of emergence, total emergence duration, and intervals. Emerged IJs were periodically harvested and quantified by counting four representative subsamples from the suspension derived from each cadaver.

5.2.2 Foraging Behaviour

The foraging behaviour of EPN isolates was evaluated through two experiments designed to assess nematode attachment and depth penetration capabilities. Initially, approximately 1,000 IJs from each isolate were applied onto three different soil moisture conditions (0%, 10%, and 20%) and fresh maize leaf surfaces. IJs were allowed 15 minutes to disperse and acclimatize to each substrate before introducing a single actively crawling FAW L2 larva (200–300 mg). To ensure continuous larval movement, larvae were gently prodded whenever they ceased activity during the 30-minute exposure. Post-exposure, larvae were gently rinsed, and the number of nematodes attached was carefully counted under a dissecting microscope. Each moisture condition and leaf surface treatment were replicated ten times per isolate in two separate experimental trials to enhance robustness in a CRD. In an associated vertical distribution experiment, vertical plastic column arenas, measuring 5.5 cm in diameter and 10.5 cm in height, were filled with soil to investigate the nematodes' depth penetration ability. Individual larvae were placed at varying soil depths: on the surface, or at depths of 2, 5, and 10 cm. Each column received an inoculation of 1,000 IJs suspended in 1 ml sterilized water. After three days, columns were carefully disassembled, larvae were retrieved and dissected to confirm nematode establishment. In a CRD, each soil depth was replicated five times per isolate in two independent trials, providing a comprehensive assessment of nematode vertical mobility.

5.2.3 Desiccation Tolerance

Desiccation tolerance of the isolates was assessed by inducing desiccation using different concentrations of polyethylene glycol (PEG 600) (Anbesse, Sumaya, Dorfler, et al., 2013; John Mukuka, Olaf Strauch, & Ralf-Udo Ehlers, 2010). Desiccation stress was measured as water activity (a_w), which indicates the relative availability of unbound water to sustain the IJs. It is calculated as the ratio of the vapor pressure of water in a sample (p) to the vapor pressure of pure water (p_0) at the same temperature. Freshly propagated IJs, pooled from four different growth batches, were used for the desiccation test in 24-cell well plates. The IJs were kept for 72 h in an adaptation solution of 40.3% PEG 600, (a_w of 0.96), prepared from a concentrated PEG 600 stock solution (Carl Roth, Karlsruhe, Germany). To minimize evaporation, the 24-cell well plates were sealed with Parafilm (Pechiney "M" Plastic Packaging, Chicago, USA). After adaptation, batches of 1,500 IJs in three replicates were exposed to seven PEG 600 concentrations for 24 h: 20% (aw 0.98), 30% (a_w 0.97), 40.3% (a_w 0.96), 50% (a_w 0.93), 60% (a_w 0.89), 70% (a_w 0.83), and 80% (a_w 0.74). Control IJs were transferred to Ringer's solution after the adaptation period (a_w 0.99). After treatment, the mortality of IJs was assessed in the three replicates by counting the number of active and inactive nematodes using a counting chamber. Percentage IJ mortality in different replicates was used to calculate the mean water activity (MW) tolerated by 50% of the population (MW_{50}) and the MW tolerated by the most tolerant 10% of the IJs population (MW_{10}).

5.2.4 Hypoxia Tolerance

Hypoxia tolerance of the isolates was assessed based on methods detailed by Zadji et al. (2014). For this assessment, 5,000 IJs from each isolate were placed into sealed 0.5 ml Eppendorf tubes containing distilled water and incubated under hypoxic conditions at 25°C in darkness for 24 or 72 hours. After exposure, IJs were transferred to Petri dishes containing 15 ml distilled water and further incubated at 25°C for 24 hours to evaluate recovery and survival rates. Tubes maintained in open conditions throughout incubation periods served as controls. Treatments were conducted with four replicates in a CRD and the entire experiment was repeated twice for reproducibility and confirmation of observed responses.

5.2.5 Oxidative Stress Tolerance

The oxidative stress performance of the six isolates was assessed by storing pools of IJs in Ringer's solution in the presence of hydrogen peroxide (H_2O_2) at room temperature (25°C). Freshly harvested IJ suspensions were separately exposed to H_2O_2 in a 24-cell well plate in a

CRD with three replicates, each containing 1,500 IJs in 400 µl of Ringer's solution and sealed with Parafilm. For oxidative stress induction, 12.76 µl of 1.94 M H_2O_2 was added to each cell well to obtain a final H_2O_2 concentration of 60 mM. IJs kept under control conditions were left at 25°C in cell wells without H_2O_2. To assess the IJs mortality over time, 50 µl aliquots from each experimental replicate were counted in a counting chamber daily for two weeks. The percentage IJ mortality was used to determine differences in the mean survival time of 50% of the population (ST_{50}) and the survival time of the most tolerant 10% of the IJ population (ST_{10}) for each strain. The determination of the ST values followed the same procedure as that described for the desiccation test.

5.2.6 Data analysis

All statistical analyses were conducted using R version 4.4.3 (R Core Team, 2024) on R Studio (Posit team, 2024). Data were first assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. Where appropriate, percentage data were arcsine square root transformed to meet parametric assumptions. For temperature-dependent infectivity and reproduction assays, two-way ANOVA was used to examine the effects of isolate and temperature on larval mortality and IJ emergence. Post hoc comparisons were performed using Tukey's HSD test. In the desiccation tolerance assay, percentage IJ mortality from replicate treatments was used to estimate the mean water activity (MW_{50}) tolerated by 50% of the population and MW_{10} for the most tolerant 10% of IJs. The data were fitted to a cumulative normal distribution curve, and the mean and standard deviation from the fitted curve were used to derive MW values by minimizing the χ^2 value between experimental and expected values. Similarly, for oxidative stress tolerance, IJ mortality percentages were used to estimate the mean survival time (ST_{50}) and the survival time of the most tolerant 10% (ST_{10}) of the population. These values were also obtained from a cumulative normal distribution fitted to the data, using the same χ^2 minimization approach as in the desiccation assay. Hypoxia tolerance data were analyzed using Kaplan–Meier survival analysis, and isolate differences were assessed using the log-rank test. Median survival times (ST_{50}) were extracted from the survival curves. Foraging ability across substrates and soil depths was analysed using generalized linear models (GLMs), with isolate, substrate, and depth treated as fixed effects. The appropriate error distribution (Poisson or binomial) was applied based on the nature of the response variable. All statistical tests were considered significant at $P < 0.05$.

5.3 Results

5.3.1 Effect of temperature on infectivity of IJs

The four key parameters measured to assess nematode infectivity, larval mortality at 72 hours post-inoculation, time until death of infected larvae, number of IJs established per FAW larva, and the percentage of larvae producing IJs are presented in Fig. 5. 1 A-D. The percentage mortality of FAW larvae varied markedly across EPN isolates and temperature levels, as well as their interaction. Larval mortality exhibited strong temperature dependency. Mortality rates were negligible at 10°C across all isolates and peaked between 25°C and 30°C (Fig. 5. 1A), with values reaching above 90% in some isolates such as Ib-IART45 and Ib-CRIN68. A two-way ANOVA confirmed highly significant main effects of temperature ($F = 1017.94$, $p < 0.001$), isolate ($F = 119.89$, $p < 0.001$), and their interaction ($F = 16.73$, $p < 0.001$) on larval mortality. Correspondingly, the time until death of infected larvae significantly declined with increasing temperatures, reaching the shortest average duration (36–48 hours) at 25°C. At lower (10–15°C) and higher (35°C) extremes, larval death occurred more slowly, with mean times extending beyond 100 hours in some treatments (Fig. 5. 1B). ANOVA analysis indicated statistically significant effects of temperature ($F = 167.04$, $p < 0.001$), isolate ($F = 28.02$, $p < 0.001$), and temperature × isolate interaction ($F = 4.27$, $p < 0.001$), supporting the hypothesis that both thermal and genetic factors shape virulence dynamics. The number of IJs successfully establishing per larva mirrored the mortality trend, increasing significantly with temperature up to 25°C and subsequently declining. At 25°C, the highest establishment rates were observed, particularly in *S. carpocapsae* and *S. feltiae* isolates, with average IJ counts per larva exceeding 45. In contrast, establishment at 10°C was extremely low (<5 IJs per larva) across all isolates (Fig. 5. 1C). A highly significant effect of temperature ($F = 1070.14$, $p < 0.001$), isolate ($F = 113.19$, $p < 0.001$), and their interaction ($F = 13.18$, $p < 0.001$) was observed, indicating that both host penetration and survival are profoundly influenced by thermal environment and nematode identity. IJ ability to complete their life cycle and reproduce within the host, as measured by the percentage of FAW larvae producing IJs, also showed strong temperature dependency. IJ emergence was not observed at 10°C and 35°C for any isolate. Between 15°C and 30°C, however, emergence rates increased progressively, peaking at 25°C where values ranged between 85% and 90% for *H. bacteriophora*, *S. feltiae* and *O. myriophilus* (Fig. 5. 1D). ANOVA again demonstrated significant effects of temperature ($F = 1184.93$, $p < 0.001$), isolate ($F = 83.41$, $p < 0.001$), and their interaction ($F = 18.80$, $p < 0.001$), highlighting variation in reproductive success under fluctuating environmental conditions. These underscore the critical

influence of temperature and isolate identity on the infectivity, pathogenicity, and reproductive success of EPN.

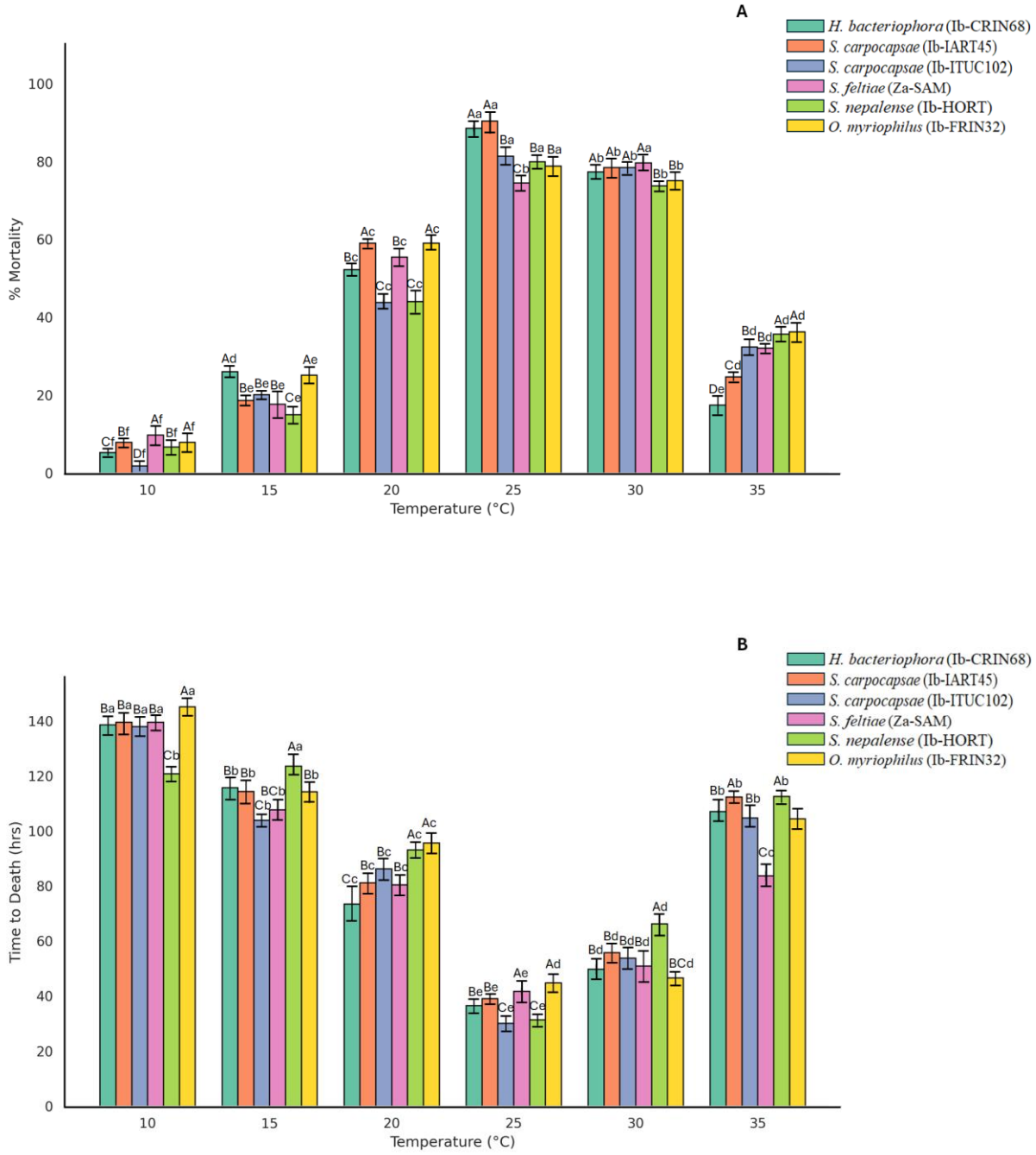
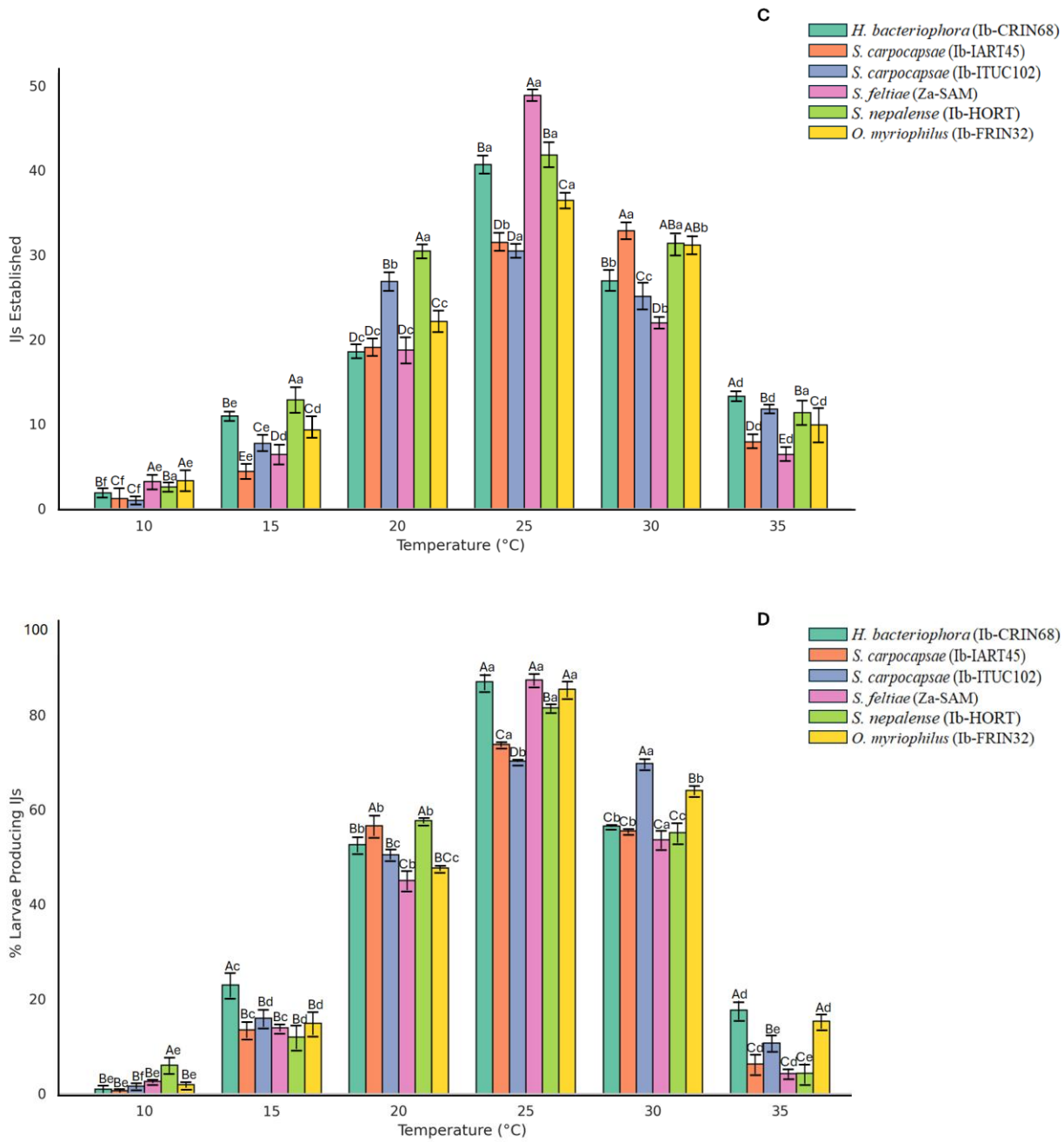


Fig. 5.1. Effect of temperature on infectivity traits of six entomopathogenic nematode (EPN) isolates against fall armyworm (FAW) 2nd instar larvae.

A. Percent mortality of infected larvae at 72 hours post-inoculation. **B.** Time to death of infected larvae (hours). Bars with different uppercase letters within each temperature represent significant differences among isolates, while different lowercase letters above the bars indicate significant differences across temperature for each isolate (Tukey's HSD, $p < 0.05$).



C. Number of Infected Juveniles (IJs) established per larva. **D.** Percentage of larvae producing IJs. Bars with different uppercase letters within each temperature represent significant differences among isolates, while different lowercase letters above the bars indicate significant differences across temperature for each isolate (Tukey's HSD, $p < 0.05$).

5.3 2 Effect of temperature on reproduction of IJs

The percentage of cadavers producing progeny varied significantly among isolates and across temperatures (Fig. 5. 2). A two-way ANOVA revealed significant main effects for both EPN isolate ($F(5, 96) = 11.28$, $p < 0.001$) and temperature ($F(3, 96) = 189.47$, $p < 0.001$), as well as a highly significant interaction effect between isolate and temperature ($F(15, 96) = 14.05$, $p < 0.001$). Overall, the proportion of cadavers yielding progeny increased progressively from 15°C to 25°C and declined slightly at 30°C, with marked variation in reproductive success between the isolates (Fig. 5. 2A). The timing of first IJ emergence from cadavers, measured in days post-infection (dpi), was also significantly influenced by both isolate identity and ambient temperature. Two-way ANOVA results indicated highly significant effects of isolate ($F(5, 96) = 15.05$, $p < 0.001$), temperature ($F(3, 96) = 151.66$, $p < 0.001$), and their interaction ($F(15, 96) = 18.48$, $p < 0.001$). On average, emergence commenced earlier at higher temperatures, with the earliest onset observed at 25°C and delayed emergence at lower temperatures (Fig. 5. 2B). Similarly, the duration of the IJ emergence period differed significantly among isolates and temperature treatments. The main effects of isolate ($F(5, 96) = 10.17$, $p < 0.001$) and temperature ($F(3, 96) = 155.25$, $p < 0.001$), along with the interaction between the two factors ($F(15, 96) = 12.61$, $p < 0.001$), were all statistically significant. The longest emergence durations were generally recorded at intermediate temperatures (20–25°C), whereas shorter durations were observed at the lower and upper extremes of the temperature range (Fig. 5. 2C). The total number of IJs emerging per cadaver (measured in thousands) also showed significant variability across treatments with highest progeny output clustered around 25°C for most isolates, and lower outputs observed at both 15°C and 30°C. Some isolates, particularly *H. bacteriophora* and the *S. carpocapsae* strains, maintained relatively high reproductive output across a broader temperature range (Fig. 5. 2D).

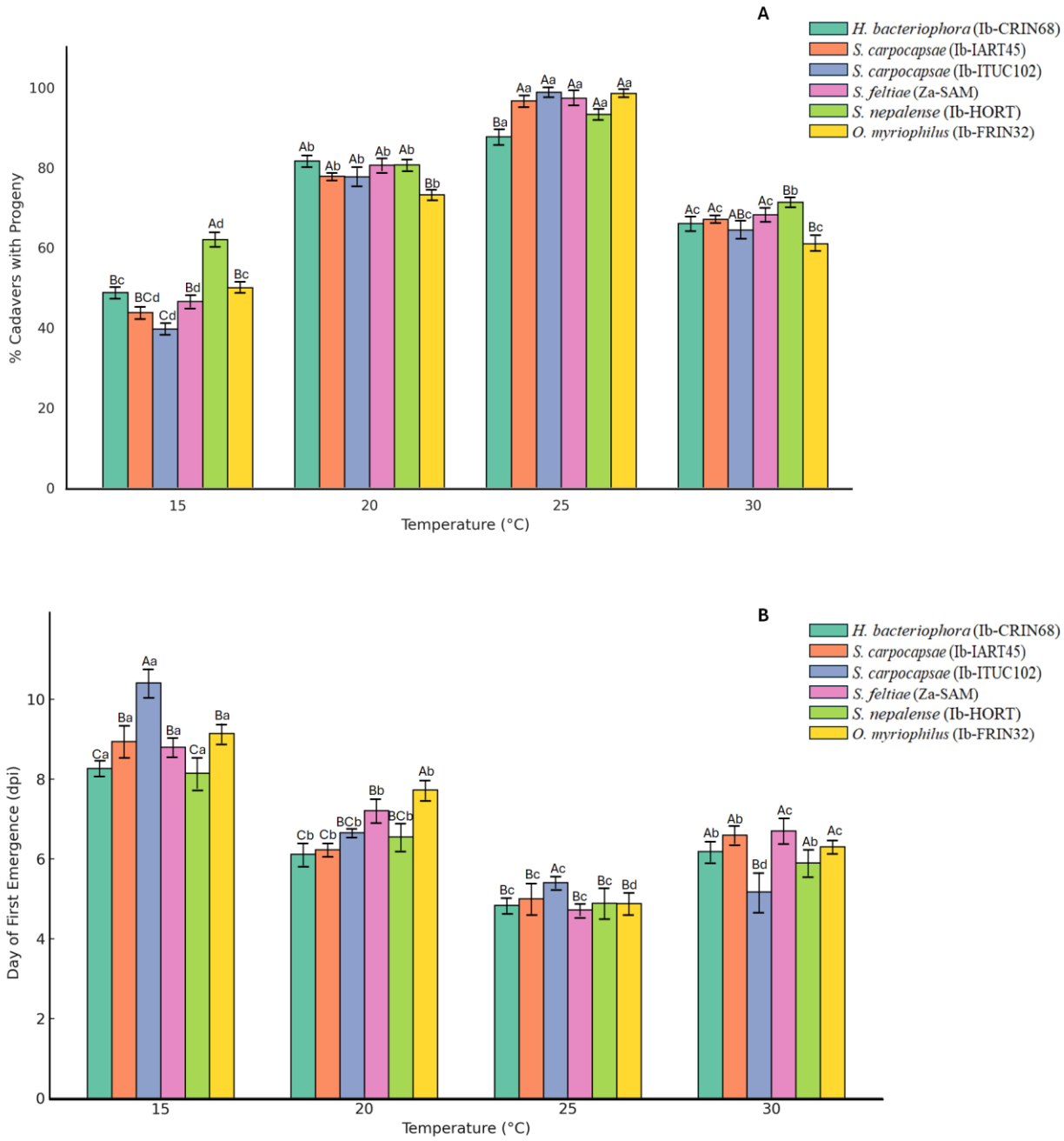
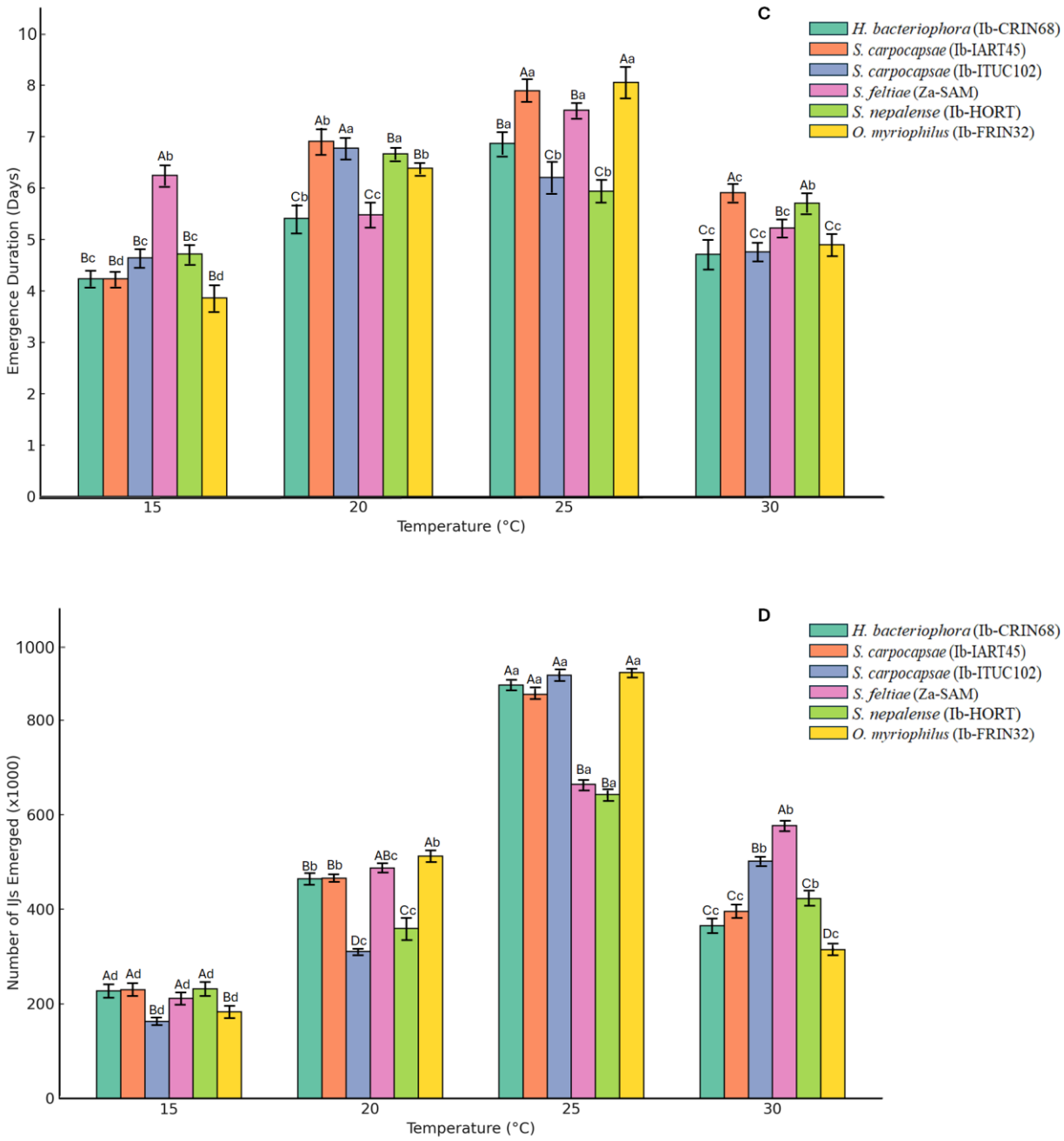


Fig. 5.2. Reproductive performance of six indigenous entomopathogenic nematode (EPN) isolates at four temperature regimes following infection of fall armyworm (FAW) larvae. **A.** Percentage of larval cadavers producing progeny. **B**. Day of first emergence of Infective Juveniles (IJs) from cadavers. Bars with different uppercase letters within each temperature represent significant differences among isolates, while different lowercase letters above the bars indicate significant differences across temperature for each isolate (Tukey's HSD, $p < 0.05$).



C. Duration of IJ emergence. **D.** Number of IJs emerged per cadaver (× 10^3). Bars with different uppercase letters within each temperature represent significant differences among isolates, while different lowercase letters above the bars indicate significant differences across temperature for each isolate (Tukey's HSD, $p < 0.05$).

5.3.3 Foraging Behaviour

The mean number of IJs attaching to FAW larvae varied significantly across substrate types and EPN isolates (ANOVA, $p < 0.05$). Substrate type had a strong effect on IJ attachment, with markedly lower attachment observed on dry soil (0% moisture) and significantly higher attachment under moist conditions (particularly at 20% soil moisture). Among the substrates, 20% soil moisture supported the highest attachment levels, with *S. carpocapsae* isolates Ib-IART45 and Ib-ITUC102 recording the highest mean IJ attachments (50 ± 5 and 46 ± 4.5, respectively). In contrast, 0% soil moisture yielded the lowest mean IJ counts, particularly for *O. myriophilus* (Ib-FRIN32) and *H. bacteriophora* (Ib-CRIN68), with values as low as 4 ± 1.1 and 5 ± 1.2, respectively (Fig. 5.3). The maize leaf surface presented an intermediate attachment potential. Notably, *S. nepalense* (Ib-HORT) and *S. feltiae* (Za-SAM) achieved attachment levels comparable to those on 10% soil moisture, averaging 33 ± 3.5 and 28 ± 3.0 IJs per larva, respectively. These findings underscore both substrate-specific differences in attachment efficiency and isolate-level variability in host-finding and infection initiation traits under varying environmental conditions.

The statistical analysis of the depth penetration assay evaluating the ability of six EPN IJs isolates to infect FAW larvae across four soil depths revealed significant isolate and depth dependent differences. A two-way ANOVA confirmed that isolate, depth, and their interaction significantly influenced penetration rates ($p < 0.001$ for all factors). The highest mean penetration rates were observed at the soil surface, where most isolates achieved infection levels exceeding 80%, with S. carpocapsae isolates Ib-IART45 and Ib-ITUC102 demonstrating superior performance even at increased depths (Fig. 5.4). Penetration rates declined progressively with soil depth, particularly at 10 cm, where all isolates recorded markedly lower effectiveness. Descriptive statistics underscored this trend, and post hoc Tukey HSD tests revealed statistically significant differences among isolates and depths, underscoring clear vertical stratification of infectivity potential among isolates.

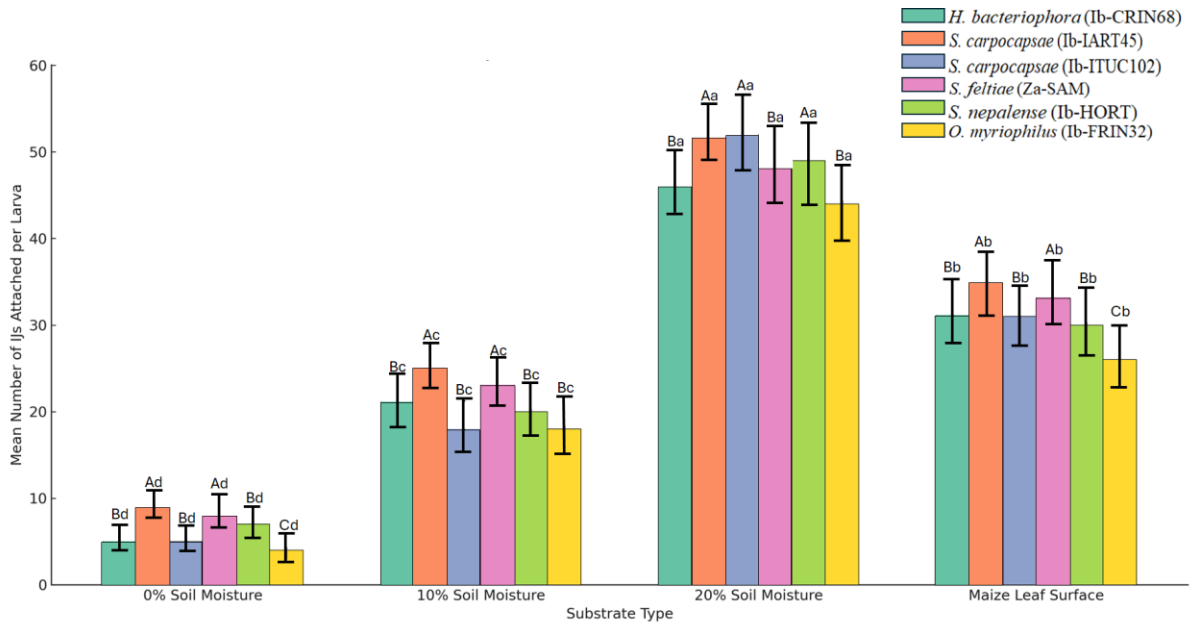


Fig. 5.3. Effect of substrate type on infective juvenile (IJ) attachment of six indigenous entomopathogenic nematode (EPN) isolates to larvae of fall armyworm (FAW).
Values are mean number of IJs attached per larva (± SE). Bars with different uppercase letters within each substrate type represent significant differences among isolates, while different lowercase letters above the bars indicate significant differences across substrate types for each isolate (Tukey's HSD, $p < 0.05$).

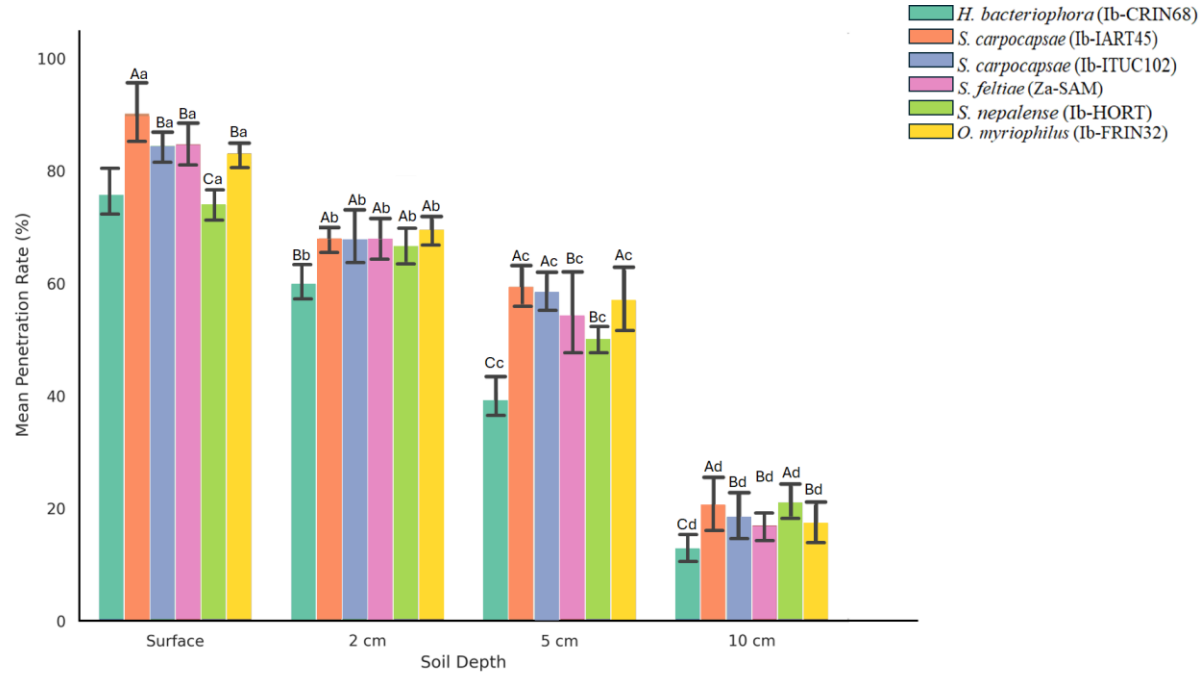


Fig. 5.4. Mean penetration rates (%) of six indigenous entomopathogenic nematode (EPN) isolates into fall armyworm (FAW) larvae at different soil depths (surface, 2 cm, 5 cm, 10 cm).
Bars represent mean ± standard deviation. Results are based on five replicates per depth per isolate. Bars with different uppercase letters within each soil depth represent significant differences among isolates (Tukey's HSD, $p < 0.05$), while different lowercase letters above the bars indicate significant differences across soil depths for each isolate.

5.3.4 Desiccation Tolerance

The minimum water activity required to maintain 50% (MW_{50}) and 10% (MW_{10}) IJ survival differed significantly among the six EPN isolates (Fig. 5.5). *Steinernema carpocapsae* isolates Ib-IART45 and Ib-ITUC102 recorded the least water activity (MW_{50} aw ~0.89), indicating superior overall desiccation resilience. In contrast, *H. bacteriophora* (Ib-CRIN68) exhibited the lowest desiccation tolerance (MW_{50} ~0.97), suggesting a need for more water to maintain survival and infectivity of 50% population and more rapid decline in population viability under drying conditions. Similarly, the 10% best performing IJs of Ib-IART45 and Ib-ITUC102 tolerated lower aw values (MW_{10} ~0.798), while *H. bacteriophora* lost 90% of their IJs at slightly higher aw (MW_{10} ~0.857). We also observed that at aw 0.99, IJ survival was highest in *S. carpocapsae* (Ib-IART45) (99.0 ± 2.3%) and lowest in *H. bacteriophora* (Ib-CRIN68) (88.0 ± 1.7%) (Table 5.1). At aw 0.98, survival remained above 84% for all isolates except *H. bacteriophora* (76.0 ± 2.3%). Subsequent reduction of aw showed *S. carpocapsae* isolates maintained high survival. At the lowest aw 0.74, survival dropped to <10% across all isolates, with *S. carpocapsae* (Ib-IART45) having the highest value (6.7 ± 1.7%) and *H. bacteriophora* showing complete mortality.

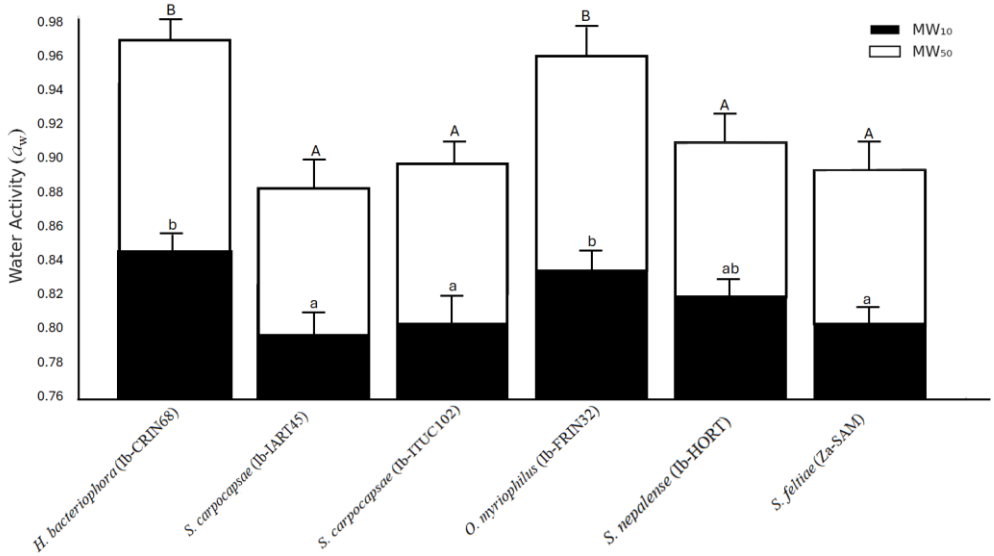


Fig. 5.5. Mean water activity a_w tolerated by 50% (MW_{50}) and 10% (MW_{10}) of the infective juvenile population

Stacked bar chart showing MW_{50} (white bar) and MW_{10} (black bar) a_w thresholds for desiccation tolerance of six entomopathogenic nematode (EPN) isolates. Error bars represent standard deviations. Bars with the same letter are not significantly different (Tukey's HSD, $p < 0.05$).

Table 5.1: Percentage survival of IJs of six EPN isolates to decreasing a_w levels[3]

Water Activity (a_w)	*H. bacteriophora* (Ib-CRIN68)	*S. carpocapsae* (Ib-IART45)	*S. carpocapsae* (Ib-ITUC102)	*O. myriophilus* (Ib-FRIN32)	*S. nepalense* (Ib-HORT)	*S. feltiae* (Za-SAM)
0.99	88.0 ± 1.7^{b}	99.0 ± 2.3^{a}	98.0 ± 2.9^{a}	95.0 ± 1.7^{a}	85.0 ± 2.3^{b}	95.0 ± 1.2^{a}
0.98	76.0 ± 2.3^{c}	94.8 ± 1.7^{a}	91.0 ± 2.3^{a}	87.0 ± 1.2^{b}	84.0 ± 2.3^{b}	85.0 ± 1.2^{b}
0.97	50.5 ± 2.9^{c}	87.85 ± 2.3^{a}	87.5 ± 1.7^{a}	77.0 ± 2.3^{b}	79.0 ± 2.3^{b}	74.0 ± 1.7^{b}
0.96	30.0 ± 3.5^{d}	85.0 ± 2.9^{a}	78.0 ± 2.3^{b}	50.8 ± 2.3^{c}	78.5 ± 2.3^{b}	62.0 ± 1.7^{c}
0.93	26.0 ± 2.9^{d}	65.0 ± 3.5^{a}	62.0 ± 2.9^{a}	47.0 ± 2.3^{c}	55.0 ± 2.9^{b}	58.0 ± 2.3^{b}
0.89	17.0 ± 2.3^{d}	52.0 ± 2.9^{a}	50.9 ± 2.9^{a}	32.0 ± 2.3^{c}	45.0 ± 2.3^{b}	49.0 ± 2.3^{ab}
0.83	7.0 ± 1.7^{c}	30.0 ± 2.3^{a}	25.0 ± 2.3^{a}	10.2 ± 1.7^{b}	12.0 ± 1.7^{b}	11.8 ± 1.7^{b}
0.74	00.00^{c}	6.7 ± 1.7^{a}	3.0 ± 1.2^{b}	2.3 ± 1.2^{b}	2.0 ± 1.2^{b}	4.0 ± 1.2^{ab}

5.3.5 Hypoxia Tolerance

Survival data demonstrated significant variability across isolates and exposure durations, as confirmed by analysis of variance (ANOVA). After 24 hours of exposure, survival rates ranged from approximately 56.3% to 76.2%, with *S. carpocapsae* (Ib-ITUC102) exhibiting the highest survival, followed closely by *S. carpocapsae* (Ib-IART45) (Fig. 5.6A). The lowest survival was recorded in O. myriophilus (Ib-FRIN32). We found statistically significant differences among isolates ($p < 0.05$), indicating distinct hypoxia tolerance profiles in the short term. Following 72 hours of hypoxia, a marked reduction in survival was observed across all isolates, with survival ranging from 27.9% to 63.4% (Fig. 5.6B). Again, S. carpocapsae (Ib-ITUC102) maintained the highest tolerance, whereas *O. myriophilus* (Ib-FRIN32) and *S. nepalense* (Ib-HORT) were the most adversely affected. Statistical analysis confirmed a significant isolate effect ($p < 0.05$), and visual comparison of both timepoints indicated time-dependent reduction in IJ viability under sustained oxygen deprivation.

[3] Values represent mean ± standard error (SE) of IJ survival at each water activity level. Different superscript letters within each row indicate statistically significant differences among EPN isolates at that specific water activity level (Tukey's HSD test, $p < 0.05$).

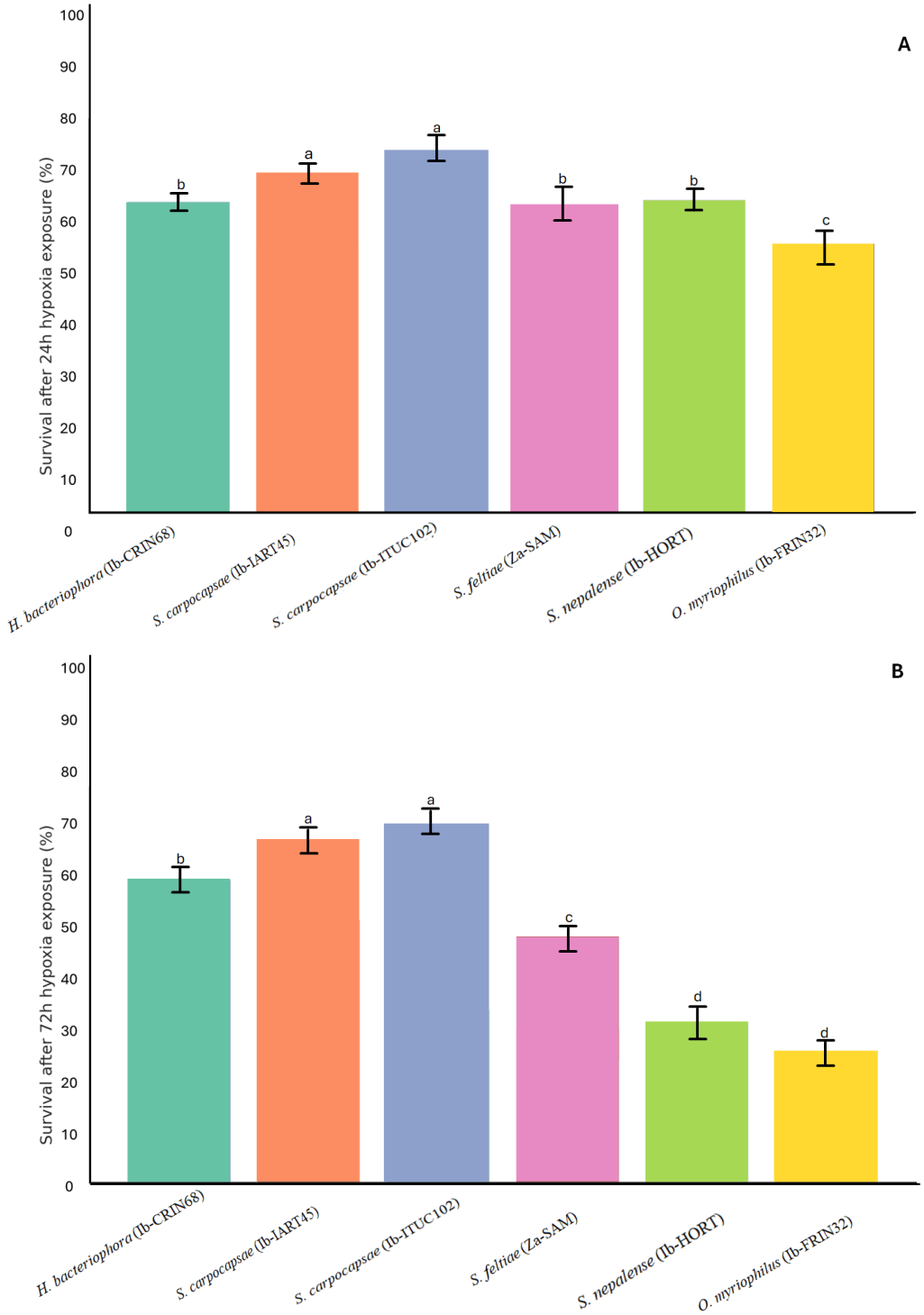


Fig. 5.6. Survival of Infective Juveniles (IJs) of six indigenous entomopathogenic nematode (EPN) isolates under hypoxic stress conditions at 25 °C.
A. Survival (%) after 24-hour sealed exposure. **B.** Survival (%) after 72-hour exposure. Bars represent mean values (± SE) from three independent replicates, with different letters above the bars indicating significant differences between isolates (Tukey's HSD test; $p < 0.05$).

5.3.6 Oxidative stress Tolerance

The oxidative stress tolerance was evaluated by assessing the survival time of the most tolerant 10% (ST_{10}) and 50% (ST_{50}) of IJs exposed to 60 mM H_2O_2. Survival time varied significantly among the isolates ($p < 0.05$), indicating differential capacities to withstand oxidative stress (Fig. 7). *Steinernema feltiae* (Za-SAM) and *S. carpocapsae* (Ib-IART45) exhibited the highest oxidative stress tolerance, with ST_{10} values of 16.8 ± 0.4 h and 16.5 ± 0.6 h, respectively, and corresponding ST_{50} values of 27.34 ± 0.8 h and 26.77 ± 0.9 h, significantly higher than those of other isolates. *Steinernema nepalense* (Ib-HORT) recorded the lowest oxidative stress resistance, with an ST_{10} of 13.1 ± 0.5 h and an ST_{50} of 18.93 ± 0.6 h, which were significantly lower ($p < 0.05$) than most other isolates. Moderate oxidative stress performance was observed for *H. bacteriophora* (Ib-CRIN68), *S. carpocapsae* (Ib-ITUC102), and *O. myriophilus* (Ib-FRIN32), which did not differ significantly from one another in ST_{10} and ST_{50} measures. The considerable variation in the oxidative resilience of the isolates suggest potential adaptive differences in physiological responses to environmental stress (Fig. 5.7).

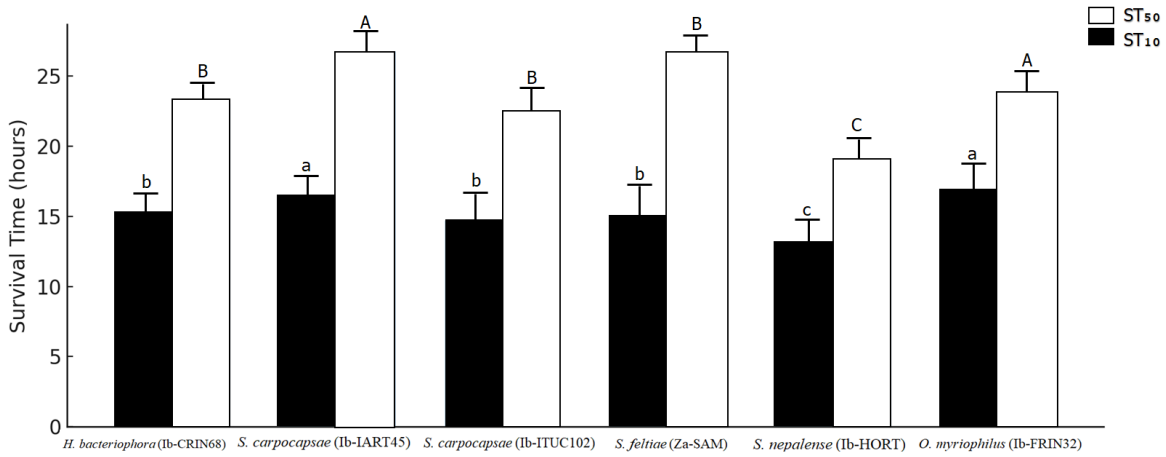


Fig. 5.7. Survival time of six entomopathogenic nematode (EPN) isolates under oxidative stress conditions induced by 60 mM H_2O_2.
The bar chart compares the mean survival time (ST) of the most tolerant 10% (ST_{10}) and 50% (ST_{50}) of Infective Juveniles (Ijs) for each isolate. Bars represent mean values ± standard error with lower and uppercase letters indicating statistically significant differences for ST_{10} and ST_{50}, respectively (Tukey's HSD test; $p < 0.05$).

5.4 Discussion

The ecological characterisation of EPN is a critical step in the development and deployment of effective biological control agents for sustainable pest management. This study evaluated six indigenous EPN isolates previously identified morphologically and molecularly from Nigeria;

H. bacteriophora (Ib-CRIN68), *S. carpocapsae* (Ib-IART45 and Ib-ITUC102), *O. myriophilus* (Ib-FRIN32), *S. nepalense* (Ib-HORT), and S. feltiae (Za-SAM), with respect to key ecological traits that influence their infectivity and persistence. These traits included thermal sensitivity, reproductive potential, tolerance to abiotic stresses (desiccation, hypoxia, oxidative stress) and host-seeking behaviour.

The six EPN isolates from Nigeria showed clear temperature-dependent patterns in both host infection and reproduction. Overall, moderate temperatures (in the mid-20°C range) supported the highest virulence and nematode progeny production, whereas extreme low or high temperatures constrained performance. These trends align with well-documented thermal optima for EPNs: for example, most EPN species achieve maximal infection and reproduction around 25°C and cannot reproduce below ~10–15°C (Koppenhöfer & Kaya, 1999; Raheel et al., 2017). In our study, *H. bacteriophora* (Ib-CRIN68) and *S. carpocapsae* (Ib-IART45, Ib-ITUC102) maintained high infectivity even at elevated temperatures, reflecting their adaptation to tropical climates. Notably, *H. bacteriophora* sustained strong virulence up to 30–35°C, consistent with reports that this species can perform robustly at the upper thermal limits of IJs activity (Aatif et al., 2020; Matuska-Lyzwa et al., 2024). By contrast, the *S. feltiae* isolate Za-SAM, a species typically native to cooler regions of Nigeria, showed reduced infectivity and yield at higher temperatures. This mirrors the known thermal sensitivity of *S. feltiae*, which exhibits diminished activity above ~30°C. In fact, storage of *S. feltiae* at 35°C for even 1 hour can dramatically curtail its motility, with lethal effects by 37°C (Matuska-Lyzwa et al., 2024). Such thermal intolerance likely explains the poorer performance of the Za-SAM isolate under hot conditions and underscores the importance of matching EPN strains to ambient thermal regimes.

Reproductive capacity followed similar temperature-related patterns. All isolates failed to recycle in hosts at very low temperatures (no reproduction occurred at 5°C, and only *S. feltiae* managed limited reproduction by 10°C). As temperatures rose, development accelerated and brood sizes increased for all species. The highest yields of IJs in our trials were produced by *H. bacteriophora* (Ib-CRIN68), consistent with previous studies showing *Heterorhabditis* can generate exceptionally large progenies in *Galleria* hosts (Levy et al., 2020; Raheel et al., 2017). We also observed that *H. bacteriophora* and the *S. carpocapsae* isolates had faster kill and emergence at 25–30°C than at cooler temperatures, whereas *S. feltiae* required longer intervals to produce IJs, especially at suboptimal temperatures. *Steinernema* species like *S. carpocapsae* can kill hosts and recycle more rapidly at 25–28°C (often within a week), whereas

Heterorhabditis typically takes a few days longer to emerge (Brown et al., 2002; Susurluk & Ulu, 2015). At the highest temperature tested in our study, some drop-off in IJ yield was noted for all isolates, suggesting that extreme heat imposes stress on nematode development and symbiont functioning. It is well known that sustained exposure to >32°C adversely affects EPN growth, reproduction and survival (Karanastasi et al., 2025; Lillis et al., 2023). Nonetheless, the indigenous tropical isolates in this study tolerated heat better than many temperate-strain EPNs reported in literature, reinforcing the ecological premise that local nematode populations are better adapted to the prevailing thermal conditions of their environments. The ability to adapt to varying thermal conditions is essential for biocontrol agents in SSA agroecosystems, where soil temperatures can vary considerably. Our results show that considering temperature optima and limits is crucial when choosing suitable EPN isolates for field evaluation. The Nigerian isolate *H. bacteriophora* Ib-CRIN68 has a broad thermal activity range, making it effective in warm climates.

The tested EPN isolates demonstrated distinct foraging strategies, following the ambusher versus cruiser host-finding behaviour. The two *S. carpocapsae* isolates Ib-IART45 and Ib-ITUC102 showed ambush foraging by staying near the soil surface and adopting a "sit-and-wait" tactic for mobile hosts. This was evident as they infected hosts near the soil surface with limited deep soil movement, consistent with *S. carpocapsae*'s ecology of targeting insects at or above the soil interface (Bal & Grewal, 2015; Raja et al., 2011). Ambushers like *S. carpocapsae* are known to conserve energy by nictating or standing upright near the surface, and only a small fraction of their population actively disperses far from the release point (Chen & Glazer, 2005; Wright et al., 1997). Interestingly, even ambushers can exhibit a subset of highly motile "sprinter" individuals that disperse rapidly when host cues (such as CO_2 or other volatiles) are detected. Our observations of *S. carpocapsae* reaching and infecting hosts a few tens of centimeters away concur with reports that a small proportion (~1–2%) of *S. carpocapsae* IJs can travel >10 cm in soil columns (Bal & Grewal, 2015). This dual strategy, mostly localized waiting with occasional long-distance forays, likely maximizes the chance of encountering a susceptible insect host in heterogenous soil environments.

In contrast, *H. bacteriophora* (Ib-CRIN68) and *O. myriophilus* (Ib-FRIN32) showed more cruiser-like foraging behaviour. They actively explored deeper soil layers and were able to locate hosts buried at greater depths or at further horizontal distances. *Heterorhabditis bacteriophora* in particular is known as an active cruiser that continuously moves through soil pore water in search of sedentary or subterranean hosts. In our depth-gradient assays, H.

bacteriophora IJs readily penetrated to lower strata (e.g. >15 cm depth) and successfully infected hosts there, whereas *S. carpocapsae* infections were concentrated in the upper 10 cm. Host-seeking observations show that *Heterorhabditis* spp. tend to distribute deeper in the soil profile than *Steinernema* ambushers. For example, in surveys *S. feltiae* were found mostly in the top 0–15 cm of soil, whereas *H. bacteriophora* can be recovered from much greater depths (Neumann & Shields, 2006; Williams et al., 2013). Our data similarly suggests that the Ib-CRIN68 isolate of *H. bacteriophora* is adept at vertical movement, an advantageous trait for targeting soil-dwelling stages of pests, such as pupating FAW. The ability to forage actively in the soil may also help cruisers find immobile hosts like cocoons or grubs, complementing the ambushers' strength against surface-active insects. Meanwhile, the behaviour of *S. nepalense* Ib-HORT and *S. feltiae* Za-SAM isolates appeared intermediate. They neither strictly waited at the surface nor ranged as widely as *H. bacteriophora*, suggesting a more flexible foraging strategy. Many *Steinernema* spp. are known to be intermediate strategists that can both ambush and cruise to some extent (Rakubu et al., 2024). This adaptable foraging strategy enables them to utilize various host niches, although it may not be as efficiently specialized as the extreme ambusher (*S. carpocapsae*) or the extreme cruiser (*H. bacteriophora*).

Soil moisture had a pronounced influence on foraging efficacy. In moderately moist soil, almost at field capacity, all isolates moved and located hosts with the highest success. However, under drier conditions, host-finding declined, particularly for the cruiser-type nematodes that rely on continuous water films for movement. Nematodes are essentially aquatic in locomotion, gliding along water-filled pores thus insufficient moisture breaks the continuity of those films, impeding IJ mobility (Kaspi et al., 2010). We observed that as the soil became drier, *H. bacteriophora* and *S. nepalense* showed reduced dispersal and tended to congregate in deeper, more humid layers if available. Nematodes will migrate downward as surface soil desiccates, accumulating at depths where humidity is higher (Cabanillas, 2003; Duncan & McCoy, 2001; Gouge et al., 2000; Yadav & Lalramliana, 2012). In one study, *S. riobrave* was seen to move 15–23 cm deep over four weeks of gradual surface drying, effectively tracking the receding moisture front (Gouge et al., 2000). A similar pattern in our experiments suggests that the Nigerian cruisers actively seek favourable moisture microhabitats, which would enhance their survival during dry spells. On the other hand, *S. carpocapsae* (ambusher) was less able to escape drying soil by migration. Instead, its strategy under low moisture may be to enter a quiescent state near the surface and wait for either a host or the return of moisture. Ambusher species like *S. carpocapsae* often have greater desiccation tolerance, enabling them to survive

near the surface until a host contacts them or rain rehydrates the soil (Shapiro-Ilan et al., 2014). Moreover, the virulence of *S. carpocapsae* in dry soil can be restored upon re-moistening of the soil, suggesting that the IJs remain viable in a dormant state and are capable of resuming their infective activity once favourable conditions are re-established (Grant & Villani, 2003). These behavioural differences mean in an applied context, that *S. carpocapsae* might be more effective when pests are on or near the soil surface (and intermittent dry periods occur), whereas *H. bacteriophora* could be superior for targets in the soil profile provided adequate moisture is present or irrigation is used. Overall, our foraging assays highlight that both moisture and soil depth interact with nematode behavioural traits. The most effective biocontrol may be achieved by matching isolate behaviour to pest ecology such as deploying ambushers for mobile foliar larvae and cruisers for soil-dwelling stages or by combining species to cover multiple strata and moisture conditions in the field (Bal & Grewal, 2015).

An important aspect of the ecological fitness of EPNs is their ability to withstand environmental stresses. We found considerable isolate-specific differences in tolerance to desiccation (dryness), low oxygen, and oxidative stress. *Steinernema carpocapsae* Ib-IART45/ITUC102 stood out for its superior desiccation tolerance, and its IJ survival after exposure to low humidity (or dry soil) was significantly higher than that of the *Heterorhabditis* and other *Steinernema* isolates. Generally, *S. carpocapsae* is considered to be one of the most desiccation-tolerant nematode species. For instance, Shapiro-Ilan et al. (2014) reported *that S. carpocapsae* IJs survived desiccating conditions far better than heterorhabditids, with *S. feltiae* also ranking high and *Heterorhabditis* generally the least tolerant. Our results mirror that pattern, the *S. feltiae* Za-SAM isolate had the second-highest desiccation survival, whereas *H. bacteriophora* was among the most sensitive to drying. Desiccation tolerance in EPNs is thought to be linked to behavioural and physiological adaptations; ambushers like *S. carpocapsae* often remain near the soil surface and have evolved mechanisms to survive transient drought (Glazer, 2022; Ramakrishnan et al., 2022). In contrast, cruisers avoid dry conditions by moving deeper, as earlier discussed, and consequently may not have invested in as strong desiccation-hardiness mechanisms which explains the lower survival of *H. bacteriophora* in our desiccation assays. It is encouraging that even the more sensitive isolates in our study still retained some viability after short dry exposures, suggesting that a fraction of the population can endure brief droughts. Additionally, we noted no obvious intraspecific variation in desiccation survival between the two *S. carpocapsae* strains, which is consistent with reports that different strains of *S. carpocapsae* tend to exhibit uniformly high desiccation

tolerance (Shapiro-Ilan et al., 2014). Overall, the ability of the *S. carpocapsae* and *S. feltiae* isolates from Nigeria to better survive drying conditions could be advantageous for use in regions with irregular rainfall or for above-ground applications where desiccation risk is high.

All isolates showed reduced survival under hypoxic (low oxygen) conditions, though with subtle differences. When subjected to oxygen-depleted environments (simulating waterlogged or compacted soils), *H. bacteriophora* and *S. nepalense* survived slightly longer than the *S. carpocapsae* and *Oscheius* isolates. This may reflect adaptation of cruisers to burrowing into less aerated soil pockets (Kung et al., 1990a, 1990b). Nonetheless, the overall intolerance of the nematodes to hypoxia was evident such that prolonged exposure to <1% O_2 led to high mortality across the board. This finding is expected since EPN IJs are aerobic organisms that rely on dissolved oxygen in soil water. As soil oxygen drops, nematode metabolism and survival sharply decline. For instance, oxygen levels near 1% drastically impair EPN viability and infectivity, and the survival of *S. carpocapsae* and *S. glaseri* IJs plummeted as oxygen was reduced from ambient (20%) to near-anoxic levels (Matuska-Lyzwa et al., 2024). Our isolates likely behave similarly, with heavy, water-saturated soils (common in the humid tropics during rains) posing a risk to their persistence. Interestingly, we did observe that nematodes in our experiments often sought out air pockets or moved to the soil surface in response to waterlogging, suggesting a behavioural escape from hypoxia. Such behaviour has been noted that IJs can sense gradients in oxygen or CO_2 and migrate toward more favourable conditions (Hoctor et al., 2013; Maushe et al., 2023). In practical terms, this means EPN applications should avoid fully waterlogged conditions; good soil drainage will promote nematode survival. Also, soil texture matters, coarse, well-aerated soils are more nematode-friendly than heavy clays that induce anaerobic micro-sites. While we did not identify a dramatically hypoxia-tolerant isolate, the slight edge of *H. bacteriophora* under low O_2 might relate to its natural occurrence in deeper soil. Still, hypoxia remains a limiting factor for all, reinforcing that EPNs work best under moderate moisture without oxygen starvation.

When exposed to oxidative challenges, such as hydrogen peroxide in our assays, some isolates fared noticeably better. In particular, *S. carpocapsae* Ib-IART45 showed higher survival and maintained mobility longer under oxidative stress than *H. bacteriophora* or *S. feltiae*. This could indicate a more robust antioxidant defense system in *S. carpocapsae*. Effective scavenging of reactive oxygen species (ROS) is critical for EPNs, both in the soil environment and during infection of the host. Insect hosts actively mount an oxidative immune response – generating superoxide, peroxide, and other ROS to attack invading nematodes (Lalitha et al.,

2018; Sumaya et al., 2018). Nematodes that can withstand this onslaught have a better chance to establish infection. *S. carpocapsae* is known for producing a lethal toxin (via its symbiont *Xenorhabdus*) that rapidly kills the host, which might shorten the window of exposure to host immune defense, indirectly reducing oxidative damage (Lu et al., 2017; Watanabe et al., 2019). Additionally, prior studies on the nematode models, *C. elegans*, show that exposure to peroxides causes immediate loss of mobility and depressed metabolism, but young nematodes can recover if their antioxidant enzymes like peroxiredoxins and catalases, are effective (Kumsta et al., 2011). The superior oxidative stress survival of *S. carpocapsae* could reflect such efficient detoxification systems. It is possible that this isolate constitutively expresses high levels of catalase, superoxide dismutase, or peroxiredoxin that neutralize ROS, a trait that would be beneficial during the early stages of infection when the insect's immune burst is highest. By contrast, *H. bacteriophora* Ib-CRIN68 showed more oxidative damage (lower survival) in our test, which might relate to its strategy of relying on a supportive mutualistic bacterium (*Photorhabdus*) to overcome host defense, *Photorhabdus* produces immunosuppressive factors but perhaps less in terms of ROS-scavengers. Another intriguing observation was that the *O. myriophilus* isolate had relatively good oxidative tolerance, almost on par with *S. carpocapsae*. *Oscheius* spp. are not symbiotically tied to *Xenorhabdus/Photorhabdus*; some are associated with other bacteria or can be facultative pathogens. Their pathogenicity often depends on releasing their own array of bacteria upon host entry (Onwong et al., 2023). The resilience of *O. myriophilus* to oxidative stress in our assays suggests it may possess inherent protective mechanisms (possibly due to its free-living lineage background) or perhaps carries bacteria that aid in detoxification. While literature on EPN oxidative stress tolerance is scant, our results suggest this trait could underlie differences in virulence and field persistence. Isolates that better endure oxidative stress might survive longer on foliage (exposed to UV and oxidative conditions) or overcome host immune reactions more successfully. This could partly explain why *S. carpocapsae* was so virulent in our trials, its physiological hardiness complements its aggressive infection strategy. In sum, screening for oxidative stress tolerance, alongside desiccation and hypoxia tolerance, provides a more complete picture of an EPN isolate's suitability for biocontrol deployment in challenging environments.

Trade-offs exist between virulence, environmental tolerance, and reproductive fitness (J. Mukuka et al., 2010). For instance, the most desiccation-tolerant species (*S. carpocapsae*) are not the most fecund reproducers, and the most fecund (*H. bacteriophora*) are not very

desiccation-tolerant. This necessitates a strategic approach to biocontrol to either formulate consortia of complementary EPN isolates or tailor the choice of isolate to the specific pest and environment. In practical IPM, one might apply *S. carpocapsae* for immediate knockdown of FAW larvae in the crop canopy, combined with *H. bacteriophora* for longer-lasting suppression of the next generation in the soil. There is evidence that mixed-species applications can sometimes yield additive benefits, as different nematodes occupy slightly different niches and timescales of action though careful consideration of competition and compatibility is needed. Our findings suggest that *H. bacteriophora* Ib-CRIN68 and *S. carpocapsae* Ib-IART45 together would make a formidable pair, the former ensuring persistence and recycling in soil and the latter providing quick action against active larvae. Additionally, *O. myriophilus* could be included to bolster resilience to stress, possibly as a stress-hardy backup that might sustain population when others wane. The demonstrated trait superiority of these isolates likely stems from their evolutionary history in Nigerian agroecosystems, for example Ib-CRIN68 coming from a farm soil that undergoes periodic drying and heating, selecting for a hardy yet virulent phenotype, and Ib-IART45 originating from an area with intense insect pressure, selecting for high pathogenicity. It would be valuable in future work to investigate the genetic or physiological basis of these traits. Previous and recent research are identifying genetic markers (heat-shock proteins, anhydrobiosis-related genes, antioxidant enzymes) that correlate with stress tolerance in nematodes (Grewal et al., 2006; Maushe et al., 2023; Segal & Glazer, 2000). Unravelling these mechanisms in our top isolates could enable marker-assisted selection or even bioengineering to further improve them. In essence, the diverse performances observed affirm that isolate selection is crucial and by choosing the right nematode combinations one can achieve reliable biocontrol even under the challenging conditions of SSA farmlands.

Chapter 6: General Discussion and Conclusion

The global shift towards more sustainable agricultural practices has intensified research into IPM strategies that reduce dependency on synthetic chemical pesticides. Among these, biological control using natural enemies such as parasitoids, predators, entomopathogenic fungi, bacteria, and nematodes has gained significant importance (Hajek & Eilenberg, 2018; van Lenteren et al., 2018). EPNs are recognised for their broad host range, ease of application, compatibility with existing farming systems, and favourable environmental profile (Campos-Herrera et al., 2021; Lacey et al., 2015). Within this context, the management of *S. frugiperda*, an invasive and economically devastating pest of maize and other cereals in SSA, presents a critical opportunity to explore localised biological control options (Goergen et al., 2016; Hruska, 2019).

This research was designed to develop a scientifically informed, ecologically relevant, and socially responsive framework for the potential utilisation of indigenous EPN isolates in the management of *S. frugiperda* in Nigeria. It addressed three complementary objectives. The first examined farmers' knowledge, perceptions, and willingness to adopt EPNs as part of a broader IPM strategy. The second involved the identification and virulence testing of indigenous EPN isolates collected from Nigerian soils, with a focus on their pathogenicity across different life stages of the pest. The third component explored the ecological traits of these isolates, including their thermal tolerance, desiccation and oxidative stress responses, and foraging behaviour under simulated environmental conditions.

Although each component represents a distinct layer of inquiry, their integration underscores the multifaceted nature of biocontrol development. Effective pest management using EPNs cannot be understood solely through laboratory efficacy. It must be embedded within a broader ecological and socioeconomic context that shapes both the performance of the organism in the field and the likelihood of its acceptance by end-users. This research thus contributes to current knowledge by bridging this interface, providing empirical evidence not only of biological potential but also of ecological adaptability and adoption constraints in the Nigerian setting.

The sections that follow synthesise the principal findings of the research and discuss them within the framework of existing literature on biological control, EPN ecology, and agricultural innovation in tropical contexts. The discussion moves from an analysis of farmers' perceptions and behavioural intentions to a technical assessment of EPN virulence and ecological

performance, concluding with an integrative appraisal of how these elements interact to inform future directions for research, development, and practical implementation.

6.1 Farmers Perception, Knowledge and Adoption Potential

Understanding farmer behaviour is critical for the success of any pest management innovation, particularly biological control technologies which often require shifts in established practices and knowledge systems (Peshin et al., 2019). The findings from the present study represent the first nationwide assessment of awareness and willingness to adopt EPNs for FAW management in Nigeria. Despite the relatively low awareness levels (12.8%) reported across the ten sampled states, the high expression of interest in EPN training (70.9%) reflects a strong latent demand for biological control innovations, provided they are accessible, affordable, and demonstrably effective.

These results are consistent with earlier research that has highlighted knowledge gaps as a primary barrier to the adoption of biopesticides in SSA (Constantine et al., 2020; Dunn & Malan, 2025; Kansiime et al., 2019). In Kenya and Uganda, for example, low awareness of biocontrol agents significantly limited uptake, even where field efficacy had been established (Constantine et al., 2023; Kalyebi et al., 2023). The present findings similarly suggest that awareness does not equate to resistance, and that farmers are not inherently sceptical of biological alternatives. Rather, knowledge dissemination, supported by practical demonstrations, plays a decisive role in shaping behavioural intentions. The preference for video materials and on-farm demonstrations as training formats further underscores the importance of participatory and visual learning in smallholder contexts.

The relatively neutral perception scores regarding EPN effectiveness (mean = 3.01) and safety (mean = 3.26) suggest ambivalence rather than opposition. These scores, derived from a 5-point Likert scale, may reflect a lack of familiarity rather than a definitive evaluation of EPNs themselves. Similar findings were reported by Harrison et al. (2022) in Malawi, where biocontrol products were often viewed as "experimental" due to their absence from mainstream extension narratives. Therefore, the data point to the importance of integrating EPN messaging within broader agricultural advisory services and not treating biocontrol as a fringe or experimental concept.

Regression and principal component analyses from the study offer further insight into the drivers of potential adoption. While formal education showed a modest positive correlation with willingness to adopt, larger farm size was weakly associated with lower willingness. This

is in contrast to findings from biopesticide adoption studies in Ghana and Ethiopia, where larger landholders were often more open to new inputs due to higher risk-bearing capacity (Bailey et al., 2010; Kirui et al., 2023). In the Nigerian case, it is plausible that smaller-scale farmers perceive biocontrol as a lower-cost alternative, or that larger-scale farmers have already committed to conventional pesticide regimes, making transitions more complex. These nuances highlight the need for disaggregated adoption strategies that do not assume uniform behaviour across farm sizes or regions.

Cost, availability, and application complexity were identified as the main barriers to adoption, consistent with the broader literature on non-chemical pest control (Otieno et al., 2023). The absence of locally produced EPN formulations and the lack of accessible distribution systems are structural constraints that cannot be addressed through awareness campaigns alone. Although policy implications were not the main focus of this research, these findings suggest that any future scaling of EPN technology in Nigeria will need to be supported by investment in production, formulation, and delivery systems that meet the logistical realities of smallholder farming.

A further insight from the survey is the alignment between perceived training needs and actual awareness levels. Farmers who were already aware of EPNs were more likely to indicate confidence in application, whereas those unaware expressed strong interest in experiential learning. This pattern supports the application of the Technology Acceptance Model (TAM), which posits that perceived usefulness and ease of use are significant predictors of behavioural intention to adopt new technologies (Davis, 1989; Venkatesh & Davis, 2000). The observed interest in EPNs, once explained and contextualised, aligns well with TAM-based predictors and affirms the potential for uptake through appropriate extension strategies.

Overall, this component of the research highlights the critical role of knowledge systems, perception shaping, and structural enablers in influencing the potential adoption of biological control agents such as EPNs. While technical efficacy remains central to adoption decisions, the social and institutional environment must be conducive to experimentation and trust-building. The low awareness but high willingness combination represents a favourable baseline from which adoption strategies can be launched, provided the scientific, extension, and regulatory communities coordinate their efforts.

6.2 Virulence and Biological Potential of Indigenous EPNs

The identification and virulence assessment of indigenous EPN isolates constitutes a foundational step in the development of a localised biological control programme. In this study, six nematode isolates recovered from soils in Ibadan and Zaria were subjected to detailed morphological, molecular, and bioassay evaluations against multiple developmental stages of *S. frugiperda*. The results confirm significant inter- and intra-species differences in pathogenicity, with *S. carpocapsae* isolates exhibiting consistently superior virulence, particularly against early larval instars. These findings are broadly consistent with previous studies that have characterised *S. carpocapsae* as an aggressive 'ambusher' species with rapid host invasion kinetics and high insecticidal potential (Labaude & Griffin, 2018; Lewis et al., 2006).

Mortality outcomes across dose and time intervals demonstrated both stage- and concentration-dependent effects. The highest levels of mortality were recorded in 2nd instar larvae at 200 IJs per insect, where *S. carpocapsae* isolates achieved over 90% mortality within 72 hours. This aligns with previous observations that early larval stages of Lepidoptera are more susceptible to nematode infection due to their thinner cuticle and lower behavioural defences (Baur, Kaya, Tabashnik, et al., 1998; Odendaal et al., 2016). Conversely, pupae exhibited the lowest levels of mortality, with LC_{50} values exceeding 150 IJs per insect, confirming the relative refractoriness of this stage to nematode penetration and establishment. This result is consistent with reports by Acharya, Yu, et al. (2020); Saleh (2017), who observed limited EPN efficacy against pupae of cotton bollworm *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae) and tobacco cutworm *S. litura* (Fab.), respectively.

The virulence profiles obtained in this study not only validate the selection of *S. carpocapsae* as a high-potential candidate but also reinforce the ecological logic of stage-targeted application. Application strategies that focus on the early larval instars, particularly within the first two weeks of infestation, are more likely to yield successful outcomes. This is particularly relevant for field implementation, where synchrony between pest phenology and biocontrol application is essential for effective suppression (Grewal et al., 2005).

Molecular identification through ITS, D2–D3, and COI sequencing provided robust species-level confirmation for the six isolates, supporting the validity of the morphological diagnostics and contributing new sequence data to the regional EPN biodiversity profile. Notably, the inclusion of *Oscheius myriophilus* (Poinar) as one of the isolates adds to the growing

recognition of this genus as an emerging group of facultative entomopathogens (Torres-Barragan et al., 2011). However, its relatively low virulence against *S. frugiperda* in this study suggests limited potential for standalone deployment, though it may warrant consideration in combination strategies or for use against other pest targets.

In addition to confirming the high virulence of *S. carpocapsae*, the findings underscore the importance of local bioprospecting efforts. The recovery of five isolates from Ibadan and only one from Zaria, despite similar sampling efforts, highlights possible spatial variability in EPN distribution. This may be influenced by soil texture, organic matter content, moisture levels, or cropping history which are known to affect EPN persistence and detectability (Campos-Herrera et al., 2021; Kaya et al., 2006). Although the study did not quantitatively assess soil parameters, the recovery pattern suggests that southern Nigerian soils may offer more favourable microhabitats for EPNs, a hypothesis that merits further investigation.

The relevance of these findings extends beyond laboratory virulence. The diversity and activity of indigenous isolates are essential to achieving sustainable biocontrol that is both effective and environmentally adapted. Unlike exotic strains, locally sourced EPNs are more likely to be pre-adapted to prevailing climatic and edaphic conditions, thereby increasing their survival, efficacy, and persistence under field conditions (Glazer, 2022). This study contributes to this paradigm by establishing a preliminary database of Nigerian isolates with verified taxonomic and pathogenic profiles, laying the groundwork for future ecological and formulation trials.

In summary, the virulence assays confirm the potential of *S. carpocapsae* as a lead candidate for biological control of *S. frugiperda* in Nigeria. The dose- and stage-specific findings support strategic targeting of larval stages, and the inter-isolate variation underscores the value of locally sourced strains over generic commercial formulations. These insights also provide the foundation upon which ecological performance needs to be evaluated to determine the practical viability of these isolates in real-world farming environments.

6.3 Ecological Fitness of Indigenous EPNs

The practical success of EPNs in biological control depends not only on their virulence but also on their ecological resilience under the variable and often harsh conditions encountered in agricultural soils. In this study, a comprehensive series of assays was conducted to evaluate the thermal tolerance, foraging ability, desiccation and oxidative stress survival, and hypoxia tolerance of six indigenous EPN isolates. These traits are considered essential indicators of

environmental adaptability and can influence both field persistence and infection success (Ehlers, 2005; Grewal et al., 2002).

The temperature-dependent infectivity and reproduction assays revealed clear species-specific and isolate-specific patterns. Peak infectivity occurred between 25 and 30°C across all isolates, consistent with the thermal optima reported for most *Steinernema* and *Heterorhabditis* species in tropical and subtropical climates (Hazir et al., 2003). At 35°C, infectivity dropped sharply and progeny emergence was markedly reduced, indicating that thermal stress may impair both host invasion and reproductive success. The reproductive output of *S. carpocapsae* isolates, which exceeded 100,000 IJs per cadaver at 25°C, suggests strong potential for in-field recycling under optimal temperature conditions. These values are comparable to those reported for commercial strains used in temperate systems (Shapiro-Ilan et al., 2006), suggesting that indigenous isolates can match or exceed established benchmarks when tested under ecologically relevant conditions.

The foraging behaviour assays revealed contrasting strategies among the isolates. *Steinernema carpocapsae* exhibited a pronounced ambusher strategy, showing high activity on dry substrates and maize leaf surfaces, while *H. bacteriophora* demonstrated cruiser-type behaviour, characterised by deeper vertical penetration in moist soils. These behavioural traits correspond with previously established dichotomies in EPN foraging ecology (Labaude & Griffin, 2018; Lewis et al., 2024), and are important for aligning isolate selection with pest habits and habitat structure. For instance, surface-active ambushers may be more effective against foliar feeders and exposed larvae, while cruisers may perform better in situations where larvae pupate or seek refuge below the soil surface. Given that *S. frugiperda* larvae often drop to the soil during later instars or for pupation, combining ambusher and cruiser isolates may enhance control by covering both microhabitats.

Desiccation tolerance, measured using PEG 600 to simulate low water activity, further differentiated isolate performance. *Steinernema carpocapsae* maintained survival at water activity levels as low as 0.83, while *S. feltiae* and *O. myriophilus* exhibited sharp declines below 0.93. These thresholds correspond with findings by Grewal et al. (1994), who demonstrated species-level differences in osmotic stress tolerance that affect nematode viability in arid or intermittently dry soils. The minimum water activity level required to kill 50% of the population (MW_{50}) ranged from 0.88 to 0.95 across isolates, indicating a spectrum of tolerance

relevant for deployment across Nigeria's diverse agroecological zones, particularly in savannah regions where periodic drought is common.

In the oxidative stress assays, *S. carpocapsae* isolates again demonstrated superior resilience. Survival times under hydrogen peroxide exposure (ST_{50} and ST_{10}) were significantly longer than those of other species, suggesting a greater capacity to withstand reactive oxygen species encountered during host invasion or in soils with high microbial activity. Oxidative stress tolerance has not traditionally been included in EPN screening protocols, but recent studies have highlighted its importance in mediating host-pathogen interactions and environmental survival (Machado et al., 2025). The results of this study support the inclusion of such physiological metrics in future assessments of EPN performance.

Hypoxia tolerance was evaluated as a proxy for survival in waterlogged or compacted soils, which are common in lowland farming systems during the rainy season. At 72 hours of anoxia, survival rates exceeded 60% for *S. carpocapsae* and *H. bacteriophora*, while falling below 40% for other isolates. These findings align with those of González-Paz et al. (2025), who showed that successful anoxia tolerance contributes to improved persistence and infectivity following flooding or poor drainage events. The relatively high tolerance observed in *H. bacteriophora* in this study suggests that it may be particularly suited to wetter soils, which are prevalent in southern Nigeria and riverine maize-growing areas.

Taken together, these ecological characterisation data underscore the robustness of *S. carpocapsae* and *H. bacteriophora* across multiple abiotic stress conditions. Their consistent performance across temperature, desiccation, oxidative stress, and hypoxia assays positions them as ecologically resilient candidates for field deployment. Moreover, the observed inter-isolate variability within species, especially among *S. carpocapsae*, highlights the importance of not treating commercial species designations as monolithic. Local isolates may exhibit traits that are more finely tuned to regional conditions, a point supported by similar findings from field surveys in Kenya Mutegi et al. (2018) and Brazil Andaló et al. (2018).

Ecological fitness must be viewed as complementary to virulence, rather than subordinate to it. An isolate that performs well in the laboratory but fails to persist or locate hosts under field conditions cannot contribute meaningfully to pest suppression. The comprehensive data generated in this study allow for a nuanced selection process that considers both biological efficacy and environmental adaptability. These criteria are particularly crucial in the context of

SSA, where climatic variability, soil diversity, and infrastructural constraints make consistent field performance a central requirement for adoption and scale-up.

6.4 Integrative Synthesis, Limitations, and Future Prospects

The findings from this research demonstrate that the development of indigenous EPNs for the biological control of *S. frugiperda* in Nigeria is scientifically viable, ecologically relevant, and potentially acceptable to end-users. The integration of socioeconomic, biological, and ecological data reveals that successful biocontrol interventions must not only be technically effective but also socially informed and environmentally resilient. This multi-dimensional approach, although resource intensive, is essential for generating solutions that can transition from experimental validation to practical implementation in smallholder farming systems.

From the socioeconomic analysis, it is evident that Nigerian farmers are not inherently resistant to biological alternatives. While baseline awareness of EPNs remains low, willingness to adopt is high, especially when accompanied by practical training and assurance of efficacy. These findings mirror behavioural adoption patterns reported for other biocontrol agents across SSA and underscore the value of participatory extension methods. Importantly, the divergence in adoption predictors across education levels and farm sizes cautions against one-size-fits-all promotion strategies. Instead, adoption pathways must be tailored to distinct demographic and agroecological contexts.

In the biological domain, the identification and virulence assays confirmed the high insecticidal potential of local *S. carpocapsae* isolates against early larval stages of *S. frugiperda*. These isolates exhibited rapid kill rates, low LC_{50} values, and high reproductive success under optimal conditions, positioning them as strong candidates for field application. The inclusion of other species, such as *H. bacteriophora* and *O. myriophilus*, provides additional perspectives on species diversity and performance variability. The documentation of these isolates contributes valuable taxonomic and functional data to Nigeria's largely under-explored EPN fauna.

Ecological characterisation, as the third pillar of this study, added critical depth to the biological assessments. Temperature, moisture, and oxygen availability are key determinants of EPN performance in the field. The ability of *S. carpocapsae* and *H. bacteriophora* to survive desiccation, oxidative stress, and anoxia under laboratory conditions provides evidence of their potential field persistence in tropical soils. Foraging assays further clarified niche preferences, offering practical insights into matching isolate behaviour with pest habitat. These traits, when considered together with virulence, support a more evidence-based approach to isolate

selection, rather than reliance on standard species identities or commercial strains developed in dissimilar environments.

Despite these advances, the research also faced several limitations. First, while the virulence and ecological assays were extensive, they were conducted under controlled laboratory conditions. Field environments introduce additional complexity, including microbial competition, fluctuating abiotic conditions, and predator–prey interactions that may affect EPN persistence and efficacy. Secondly, the survey component, while geographically broad, was limited to cross-sectional data. A longitudinal approach could provide deeper insights into how farmer perceptions evolve in response to exposure, extension, and observable outcomes. Moreover, while molecular diagnostics confirmed species identities, whole-genome sequencing or transcriptomic profiling could further elucidate mechanisms underlying stress tolerance and host-pathogen interactions, especially among high-performing isolates.

The future of EPN-based biocontrol in Nigeria depends on addressing these gaps while capitalising on the strong foundations established by this research. Further work should prioritise semi-field and field validation trials to test isolate performance under realistic agronomic conditions. These trials should be accompanied by formulation research to improve shelf life, ease of application, and compatibility with local farming practices. Liquid and granular formulations, developed with indigenous strains, would address one of the major constraints identified by farmers, namely, the unavailability of biocontrol products. Additionally, establishing a decentralised production network, possibly through community-based enterprises or research-extension partnerships, would help bridge the gap between laboratory success and farm-level adoption.

At the policy level, even basic engagement is warranted. Inclusion of EPNs within national IPM frameworks, integration into extension curricula, and regulatory recognition as viable biopesticides are critical first steps. While this study does not focus extensively on policy development, it highlights the need for coordinated action among researchers, extension agents, and regulatory bodies to ensure that scientific advances translate into practical impact. Experiences from Latin America and parts of Asia show that when locally adapted biocontrol agents are supported by institutional frameworks, adoption can scale rapidly (Lacey & Georgis, 2012; Maniania et al., 2016).

In conclusion, this research provides a holistic, data-driven foundation for the development of EPN-based biological control of *S. frugiperda* in Nigeria. Through the identification of

effective isolates, the assessment of their ecological suitability, and the understanding of farmer perceptions, it outlines a realistic pathway for integrating EPNs into sustainable pest management. The next phase of work should build on this foundation to develop cost-effective, field-tested solutions that are biologically sound, economically feasible, and institutionally supported.

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Appendix: Questionnaire

HOUSEHOLD SURVEY QUESTIONNAIRE
Farmers' Knowledge, Perception, and Willingness to Adopt Entomopathogenic Nematodes (EPN) for Fall Armyworm (FAW) Control in Nigeria
INSTRUCTIONS TO ENUMERATOR
Please read each question clearly to the respondent and record the answer accurately. For questions with response options, circle or tick the appropriate response(s). For open-ended questions, write the response in the space provided. Ensure the respondent gives informed consent before proceeding.
SECTION A: IDENTIFICATION AND LOCATION
Q1. Household ID (hhid): _____
Q2. GPS Coordinates — Latitude: _____
Q3. GPS Coordinates — Longitude: _____
Q4. Average Temperature in the area (°C): _____
Q5. Average Rainfall in the area (mm): _____
Q6. Agroecological Zone: [Select ONE]
☐ Freshwater Swamp
☐ Lowland Rainforest
☐ Northern Guinea Savannah
☐ Southern Guinea Savannah
☐ Sudan Savannah
☐ Derived Savannah
Q7. Town/City: [Select ONE]
☐ Bauchi ☐ Enugu ☐ Ibadan ☐ Kaduna ☐ Kano
☐ Lagos ☐ Lokoja ☐ Port Harcourt ☐ Sokoto ☐ Uyo
SECTION B: SOCIO-DEMOGRAPHIC CHARACTERISTICS
Q8. What is your age (in years)? _____
Q9. What is your gender? [Select ONE]

☐ Male ☐ Female
Q10. What is your highest level of education? [Select ONE]
☐ No formal education ☐ Primary ☐ Secondary ☐ Tertiary
Q11. What is your marital status? [Select ONE]
☐ Single ☐ Married ☐ Divorced ☐ Widowed
Q12. What is the size of your household (number of members)? _____
Q13. What is your annual household income (₦)? [Select ONE]
☐ Less than 50,000 ☐ 50,000–100,000 ☐ 100,000–200,000 ☐ Greater than 200,000
Q14. What is your primary source of income? [Select ONE]
☐ Farming ☐ Other (please specify): _____
Q15. Do you have access to credit? [Select ONE]
☐ Yes ☐ No
If Yes — (a) Source of credit: [Select ONE]
☐ Commercial Bank ☐ Cooperative ☐ Microfinance Bank ☐ Local lender
If Yes — (b) Amount of credit obtained (₦): [Select ONE]
☐ Less than 50,000 ☐ 50,000–100,000 ☐ 100,000–200,000 ☐ Greater than 200,000
SECTION C: FARM CHARACTERISTICS
Q16. What is the size of your farm (in hectares)? _____
SECTION D: FALL ARMYWORM (FAW) EXPERIENCE AND IMPACT
Q17. Have you experienced Fall Armyworm (FAW) infestation on your crops? [Select ONE]
☐ Yes ☐ No
Q18. How would you rate the severity of FAW infestation on your farm? [Rate 0–5]
(0 = No infestation; 5 = Very severe) 0 1 2 3 4 5
Q19. What is the estimated yield loss (%) due to FAW on your farm? _____%
Q20. What is the economic impact of FAW on your farming activities? [Select ONE]
☐ No impact ☐ Low (5–10% loss) ☐ Moderate (10–20% loss) ☐ High (>20% loss)

Q21. What is the economic impact of yield loss due to FAW on your household income (₦)? [Select ONE]
☐ Less than 50,000 ☐ 50,000–100,000 ☐ 100,000–200,000 ☐ Greater than 200,000
Q22. What is the impact of FAW on the quality of your crops? [Select ONE]
☐ No Impact ☐ Slight Reduction ☐ Significant Reduction
Q23. What method(s) do you currently use to control FAW? [Select ONE]
☐ None ☐ Chemical Pesticides ☐ Manual Methods (e.g., handpicking) ☐ Biological Control
☐ Other (please specify): _____
Q24. How would you rate the effectiveness of your current pest control measures? [Rate 1–5]
(1 = Very ineffective; 5 = Very effective) *1 2 3 4 5*
SECTION E: OTHER INSECT PESTS
Q25. What types of other insect pests have you encountered on your farm? [Select ALL that apply]
☐ Aphids ☐ Grasshoppers ☐ Stemborers ☐ Leafhoppers ☐ Cutworms ☐ Whiteflies
☐ Other (please specify): _____
Q26. How frequently do you experience infestations by other insect pests? [Select ONE]
☐ Rarely ☐ Occasionally ☐ Frequently ☐ Every season
Q27. What methods do you currently use to control other insect pests? [Select ONE]
☐ No Control ☐ Chemical Pesticides ☐ Manual Removal ☐ Biological Control
☐ Other (please specify): _____
Q28. Would you be interested in alternative (e.g., biological) solutions for other insect pests? [Select ONE]
☐ Yes ☐ No ☐ Maybe
SECTION F: KNOWLEDGE OF BIOLOGICAL PEST CONTROL AND EPN
Q29. Do you have any knowledge of biological pest control methods? [Select ONE]

☐ Yes ☐ No
Q30. If Yes, which other biological pest control methods have you known or used? [Select ALL that apply]
☐ Neem Extract ☐ Traps ☐ Biological Pesticides ☐ None
☐ Other (please specify): _____
Q31. Have you heard of Entomopathogenic Nematodes (EPN) before this survey? [Select ONE]
☐ Yes ☐ No
Q32. If Yes, what was your source of information on EPN? [Select ONE]
☐ Media (radio, TV, internet) ☐ Other Farmers ☐ Workshops/Seminars ☐ Extension Services ☐ N/A
Q33. How familiar are you with the function/mode of action of EPN? [Rate 0–5]
(0 = Not familiar at all; 5 = Very familiar) 0 1 2 3 4 5
SECTION G: PERCEPTION OF EPN
Q34. How would you rate the perceived effectiveness of EPN for pest control? [Rate 1–5]
(1 = Very ineffective; 5 = Very effective) 1 2 3 4 5
Q35. How would you rate the perceived safety of EPN for crops and soil? [Rate 1–5]
(1 = Very unsafe; 5 = Very safe) 1 2 3 4 5
Q36. What percentage increase in productivity do you expect from using EPN? _____%
(Record as percentage, e.g., 5, 10, 15, 20, 25, 30)
Q37. How would you compare EPN to chemical pesticides? [Select ONE]
☐ EPN are more effective ☐ EPN are safer ☐ EPN are less costly ☐ EPN are harder to apply
Q38. What are your concerns about EPN use? [Select ALL that apply]
☐ Cost ☐ Effectiveness ☐ Availability ☐ Ease of Application ☐ Other: _____
Q39. What potential benefits of EPN do you perceive? [Select ALL that apply]
☐ Cost-effective in the long term ☐ Environmentally friendly ☐ Safe for crops and soil

☐ Reduces pesticide use ☐ Other: _____
SECTION H: ENVIRONMENTAL ATTITUDES AND CONCERNS
Q40. How would you rate your level of concern about the environmental impact of current pest-control methods? [Rate 1–5]
(1 = Not concerned at all; 5 = Very concerned) *1 2 3 4 5*
Q41. How would you rate your preference for biological vs. chemical pest control methods? [Rate 1–5]
(1 = Strongly prefer chemical; 5 = Strongly prefer biological) *1 2 3 4 5*
Q42. Do you believe in the importance of environmentally friendly farming practices? [Select ONE]
☐ Yes ☐ No
Q43. Do you have concerns about the health impacts of chemical pesticides? [Select ONE]
☐ Yes ☐ No
SECTION I: WILLINGNESS TO ADOPT EPN
Q44. Are you interested in trying EPN for pest control on your farm? [Select ONE]
☐ Yes ☐ No
Q45. If Yes, what are your reasons for interest? [Select ALL that apply]
☐ Cost-effectiveness ☐ Pest control effectiveness ☐ Environmentally friendly
☐ Long-term soil health ☐ Other: _____
Q46. Are you willing to pay for EPN products? [Select ONE]
☐ Yes ☐ No
Q47. If Yes, what is the maximum amount you would be willing to pay for EPN (₦)? [Select ONE]
☐ Less than 100 ☐ 100–200 ☐ 200–500 ☐ Greater than 500 ☐ N/A
Q48. What conditions would you prefer for EPN adoption? [Select ALL that apply]
☐ Demonstrations ☐ Training ☐ Subsidies ☐ Success stories from other farmers
☐ Other: _____
Q49. What are the main barriers to adopting EPN? [Select ALL that apply]

☐ Lack of knowledge ☐ Cost ☐ Uncertainty about effectiveness ☐ Access to resources
☐ Other: _____
SECTION J: EXTENSION SERVICES AND TRAINING
Q50. How would you rate your level of trust in extension services recommendations? [Rate 1–5]
(1 = No trust; 5 = Very high trust) 1 2 3 4 5
Q51. Do you have access to agricultural extension services? [Select ONE]
☐ Yes ☐ No
Q52. How frequently do you have contact with extension agents? [Select ONE]
☐ Never ☐ Rarely ☐ Monthly ☐ Weekly
Q53. What topics are covered by the extension services you receive? [Select ALL that apply]
☐ Pest Control ☐ Crop Management ☐ EPN Information ☐ Other: _____
Q54. Are you interested in EPN demonstrations or training? [Select ONE]
☐ Yes ☐ No
Q55. What is your preferred training format? [Select ONE]
☐ Printed materials ☐ Videos ☐ In-person workshops ☐ On-farm demonstrations
Q56. Would you be interested in participating in field trials of EPN on your farm? [Select ONE]
☐ Yes ☐ No
SECTION K: SUPPORT NEEDS AND INSTITUTIONAL FACTORS
Q57. What types of support do you need for EPN adoption? [Select ALL that apply]
☐ Technical Training ☐ Peer Support ☐ Financial Support ☐ Other: _____
Q58. Do you expect government or NGO involvement in promoting EPN? [Select ONE]
☐ Yes ☐ No
Q59. What types of support from government/NGOs would you prefer? [Select ALL that apply]

☐ Training Programs ☐ Funding ☐ Provision of EPN ☐ Awareness Campaigns ☐ N/A
Q60. Would you be interested in joining farmer cooperatives for EPN purchase and usage? [Select ONE]
☐ Yes ☐ No
SECTION L: CONCERNS ABOUT NEW PEST CONTROL TECHNOLOGIES
Q61. What are your main concerns or hesitations about adopting new pest control technologies? [Select ALL that apply]
☐ Cost ☐ Effectiveness ☐ Ease of Use ☐ Safety ☐ Availability ☐ Environmental Impact
☐ Other: _____
Q62. What suggestions do you have for facilitating EPN adoption in your community? [Select ALL that apply]
☐ Training Programs ☐ On-Farm Demonstrations ☐ Success Stories from Other Farmers
☐ Peer Support ☐ Subsidies or Financial Support ☐ Other: _____
— END OF QUESTIONNAIRE —
Thank you for your time and participation in this survey.