I. INTRODUCTION

1.1. Background

The use of chemical pesticides in agricultural pest control has been the primary solution for several decades; however, their negative impacts have become increasingly evident in recent years. Many farmers commonly apply synthetic chemical pesticides excessively, exceeding the recommended dosage, under the assumption that higher doses will eliminate pests more rapidly. This practice has led to the emergence of pest resistance, which has become a serious problem by reducing pesticide effectiveness and encouraging the use of even higher dosages, ultimately exacerbating ecosystem degradation (Meray *et al*., 2024). Therefore, sustainable and environmentally friendly pest control alternatives are urgently needed to minimize dependence on chemical pesticides. Integrated Pest Management (IPM) has emerged as an approach that combines various pest control methods to reduce environmental impacts while maintaining effectiveness.

IPM aims to keep pest populations below the economic threshold rather than completely eradicating them, thereby preserving ecosystem balance and food chains (Manueke *et al.*, 2017). One of the main components of IPM is the utilization of biological control agents, which are considered safe and sustainable. Biological control using formulated antagonistic microorganisms has been proven to effectively and efficiently suppress plant diseases (Wiyatiningsih *et al.*, 2021). Organisms capable of biologically controlling insect pests through pathogenic mechanisms are known as entomopathogens. A major advantage of entomopathogens is their host specificity, which reduces risks to non-target organisms and the environment compared to chemical pesticides (Suroto *et al.*, 2023). Research on biological control agents continues to develop in order to identify more effective and efficient organisms, including symbiotic bacteria that interact with entomopathogenic nematodes (EPNs).

Entomopathogenic nematodes (EPNs) are microscopic, worm-like organisms that parasitize insects and possess great potential as biological control agents (Erdiansyah & Fauziah, 2024). EPNs from genera such as *Steinernema* and *Heterorhabditis* are well known for their ability to infect and kill insect pests through a mutualistic association with symbiotic bacteria residing in their digestive tract (Sunarto & Irwan, 2019). Research by Alfarizi and Purnomo (2012) reported that entomopathogenic nematodes isolated from soils in several regions were able to cause up to 90% mortality of white grubs (*Lepidiota stigma*), a major sugarcane pest, within 48 hours. This finding highlights the strong potential of *Heterorhabditis* sp. as a natural enemy of sugarcane pests. EPNs from both genera induce insect larval mortality through cooperation with their symbiotic bacteria. *Heterorhabditis* sp. is associated with *Photorhabdus* sp., whereas *Steinernema* sp. is symbiotic with *Xenorhabdus* sp. (Maulida *et al*., 2021). Accurate identification of these symbiotic bacteria at the molecular level is essential to obtain reliable results.

Accurate identification of symbiotic bacteria is crucial for understanding their genetic diversity, biopesticide potential, and symbiotic interactions. Polymerase Chain Reaction (PCR) has become a standard method in molecular identification due to its ability to generate specific genetic profiles through amplification of target genes, such as the 16S rRNA gene. Analysis of the 16S rRNA gene offers high accuracy, efficiency, and relatively short processing time in bacterial identification (Noer, 2021). Precise identification supports efficient field application by ensuring the selection of the most effective nematode strains for specific target pests while minimizing unintended environmental impacts. Molecular identification of symbiotic bacteria associated with *Heterorhabditis* nematodes is particularly important because it enables accurate species determination, which is critical for their utilization as biological control agents against insect pests. Molecular techniques based on PCR amplification and sequencing of the 16S rRNA gene allow the detection of genetic differences among species that are difficult to distinguish based solely on morphological characteristics, thereby preventing misidentification. Therefore, this study aims to molecularly identify symbiotic bacteria from the genus *Photorhabdus* associated with *Heterorhabditis* sp., in order

to support the development of more efficient and environmentally friendly pest control strategies.

1.2. Problem Formulation

The research problem addressed in this study is whether molecular identification using PCR techniques can generate a genetic profile of symbiotic bacteria from the genus *Photorhabdus*

1.3. Research Objectives

The objective of this study is to identify bacteria symbiotically associated with *Heterorhabditis* based on 16S rRNA gene sequences and their phylogenetic relationships.

1.4. Research Benefits

The benefit of this study is to provide important information on entomopathogenic nematode (EPNs) species and their symbiotic bacteria based on a molecular approach, as an alternative pest control strategy that is more effective, environmentally friendly, and sustainable for agricultural practices.