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Study of the Application of Mixed Entomopathogen Formula *Streptomyces Griseorubens* and *Trichoderma Harzianum* on Non-Pest Insects Diversity in Rice Crops

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Abstract

This study explores the effects of applying a combination of biological agents, particularly Streptomyces sp. and Trichoderma sp., which produce chitinase enzymes that can control insect pests. The research focuses on how this mixture influences the species composition, population, and diversity index of predatory and parasitoid insects in rice fields in Gresik Regency, East Java. Data collection involved surveying both fields treated with biological control agents and conventional rice fields, using direct observation and various traps, including yellow tarp traps, pitfall traps, and light traps. The research on rice cultivation revealed that fields treated with biological agents had a total of 1191 natural enemy insects, compared to 448 individuals in fields without biological agents. This application of biological agents was effective in controlling pest populations. The application of a mixture of biological agents, Streptomyces sp. and Trichoderma sp., also influences the insect index value, with the land treated with these agents showing a higher index value compared to land without the application of these agents.

***Keywords**: Pest Population, Diversity, Evenness Index, Dominance Index.*

INTRODUCTION

Rice (*Oryza sativa* L) is one of the most widely cultivated food crops by farmers (Kurniasih et al. 2019). Rice plants are often disturbed by plant-disturbing organisms which result in a decrease in the quality and quantity of rice production (Purbiati et al., 2024; Zahro, Agustin, and Ridlo, 2023). The use of chemical pesticides that are not wise can cause new problems, such as environmental pollution, detrimental to the health of humans and other animals, besides that the target insect population becomes resistant to insecticides that are used continuously, resurgence occurs after insecticide treatment, many non-target organisms die such as predators, parasitoids, antagonistic agents and pollinators (Kaur et al., 2024; Mishra et al., 2021; Tanda, 2024; Sahayaraj and Hassan, 2023). The use of pesticides leads to a reduction

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in populations of beneficial insects, including pollinators, parasitoids, and predators (Serrão et al., 2022; Sánchez-Bayo, 2021).

Plant pest control by using natural enemies or entomopathogens is expected to be effective, environmentally friendly and sustainable (Archana et al., 2022). Diversity of *Streptomyces* sp. and its use needs to be done because of the growing need for antibiotics or other products that can be applied in the world of agriculture, pharmaceuticals, food, and industry (Alam et al., 2022). The application of the biological agent Streptomyces sp. significantly impacts the mortality of third instar larvae of *Oryctes rhinoceros* L. Meanwhile, the fungus Trichoderma sp. produces secondary metabolites that contain various enzymes, including protease, cellulase, cellobiase, chitinase, and 1,3-β-glucanase (Rahmadhini et al., 2022). Utilization of *Trichoderma* sp. for biological control of parasitic nematodes on coffee plants is considered more effective and environmentally friendly (Raina et al., 2020; Ahmad et al., 2021). Research (Wicaksono, Windriyanti, and Suryaminarsih, 2024) shows that *Streptomyces* can degrade and cause death symptoms of fruit fly insect larvae.

The use of entomopathogen for plant pests and their impact on predators and parasitoids is still being debated because of the possibility that the secondary metabolites produced can also parasitize insect natural enemies of plant pests (Sedaratian-Jahromi, 2021; Jensen et al., 2020). *Aphidius matricariae*, parasitoids and entomopathogen *B. bassiana* can be used together in the successful biological control of the green peach aphid, *Myzus persicae* (Abbas, 2020; Rashki et al., 2009). *Trybliographa rapae* is a parasitoid that targets the larvae of the cabbage root fly, *Delia radicum.* The use of entomopathogenic fungi, specifically the *Metarhizium brunneum KVL 04-57* and *Beauveria bassiana* KVL 03-90 isolates, has shown pathogenic effects on both *D. radicum larvae* and *adult T. rapae* when used either as infected hosts or as infective propagules in the environment. (Rännbäck et al., 2015). In general, several studies have shown a positive nature of the interaction between natural enemies of arthropods and fungal pathogens with respect to controlling insect pest populations (Roy and Pell, 2000; Thompson et al., 2022; Quesada-Moraga et al., 2022). This study aims to evaluate the impact of applying entomopathogenic Streptomyces sp. and Trichoderma sp. as biological agents on the species composition and population of insects, as well as the diversity index of insect natural enemies in rice fields.

MATERIALS AND METHODS

This research began in November 2019 - July 2020, at the Rice Plantation Land in Gresik Regency, East Java Province, Indonesia. Preparation of the biological agents was carried out at the Plant Health Laboratory, Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The research was conducted on 2 fields. The first field involves rice planting without the use of biological agents, while the second field involves rice planting with the application of biological agent sprays. The area of each land is 5m x 7m.

The tools and materials used are sweep nets, pitfall traps, light traps, yellow traps, label paper, 240 ml plastic cups, LAF(laminar air flow), bunsen, petri dishes, test tubes, loop needles, stationery, alcohol 70%, *Streptomyces* sp. isolate, *Trichoderma* sp. isolate, PDA media (potato dextrose agar), potatoes, granulated sugar, soapy water, sterile distilled water.

The application of a mixture of biological agents, Streptomyces sp. and Trichoderma sp., was conducted in the morning. A suspension containing 250 ml of Streptomyces sp. and 50 ml of Trichoderma sp., which had been shaken for 14 days, was mixed into 2 liters of water. This mixture was then sprayed onto the paddy fields four times.

There are four sampling methods of data collection, such as : direct observation, sweep nets, yellow sticky traps and pitfall traps. Direct observations were carried out along the paddy fields with the application of biological agents and without application for 20 minutes each. Observations were made in the morning at 06.00-08.00, afternoon at 11.00-13.00 and in the afternoon at 15.00-17.00. Installation of a yellow sticky trap using a stake with a length of 1 meter above the ground and a yellow sticky trap measuring 20 x 12.5 cm. The traps were placed 3 meters apart between the refugia plants. The pitfall trap is inserted into the ground hole, so that the bottle is parallel to the soil surface. Each pitfall trap is filled with 100 ml of soapy water.

The collected insect samples will be counted and identified by examining their external morphology. Identification will involve comparing the insects' characteristics with information from online resources such as Bugguide.net and iNaturalist, with the identification performed at the morphospecies level. The research results will be organized using Microsoft Excel. Quantitative data analysis for diversity will include calculating the Shannon-Wiener Diversity Index, Evenness Index, Species Richness Index, and Dominance Index.

Diversity Index

The diversity of insect species is known by using the Shannon-Wiener Diversity Index (Wilson and Bosset, 1971 in Tscharntke, 2000) with the formula:

$$H' = -\sum \frac{n_i}{N} log \frac{n_i}{N} \qquad [1]$$

Information:

H' = Diversity Index
Pi = ni/N (proportion of individuals in the sample belonging to the i species).
ni = Number of individuals of species i
N = Total number of individuals of all species

Index value:
1.5 : Low Diversity
1.5-3.5 : Medium Diversity
>3.5 : High Diversity

Evenness Index (E)

The evenness index can be calculated using the formula adopted by Hill (1973):

$$E = \frac{H'}{\ln S} \quad [2]$$

Information:
E = Evenness Index (value range: 0-10)
H' = Diversity Index
ln = Natural Logarithm
S = Species Count

Species Richness Index (Dmg)

The richness index can be calculated using the formula adopted from Margalef (1958) (Fedor and Zvaríková, 2019):

$$R = \frac{S-1}{\ln(N)} \quad [3]$$

Information:
R = Richness Index
S = Total number of genera
In = Natural Logarithm
N = Total Number of individuals in the samples
The total number of individuals in a habitat with criteria:
R < 2.5 = Low species richness
2.5 > R > 4 = Medium species richness
R > 4 = High species richness

Dominance Index

Simpson's dominance index values range from 0-1. Dominance is indicated by the Simpson dominance index formula (C) (Kwak and Peterson, 2007; A Rizal et al., 2022):

$$D = \sum\left(\frac{n_i}{N}\right)^2 \quad [4]$$

Information:
D = Simpson Dominance Index
ni = Number of individuals of species i
N = Number of individuals in total population

RESULT AND DISCUSSION

Types and populations of natural enemy insects

Insects found in rice plantations in Gresik among others, act as natural enemies. The species, roles, and populations that have been observed in rice fields with and without the application of mixed biological agents are presented in Table 1. The results obtained from 8 weeks observation were 1,191 individual pests found on rice field with the application of biological agents and 448 individual pests found on field without the application of mixed biological agents.

The results of research on rice fields, there were 7 orders consisting of Hemiptera, Hymenoptera, Coleoptera, Anisoptera, Hymenoptera, Zygoptera, Coleoptera, and Araneae indicating that the types of natural enemies of the two plantations were not much different.

However, there were differences in the insect population numbers between the fields treated with the mixed biological agents Streptomyces sp. and Trichoderma sp. and those without the application of these agents. As supporting information, this research was carried out in the same area, so that the same environmental factors are thought to be the cause of the similarity of insect species found in the two observed fields. However, there are differences in the number of populations of each type of insect between the two fields. The results of the research showed that natural enemy population in rice field without mixed biological agents application were lower than populations of natural enemies on land with the application of mixed biological agents. According to the use of chemical pesticides can cause a decrease in beneficial insect populations such as pollinators, parasitoids, and predators (Ara and Haque, 2021; Kannan et al., 2020).

The results of the ANOVA test showed that the treatment using biological agents had a significant effect on natural enemy populations. According to the 5% error level of t test, the application of biological agents had an effect on the number of individual insect natural enemies in rice planting and showed a significant difference with the number of insect populations of natural enemies on land with and without the application of mixed biological agents. Overall, the fields treated with the biological agents *Streptomyces sp.* and *Trichoderma sp.* exhibited a higher population of natural enemy insects, including *Microvelia douglasi atrolineta*, *Sphecidae sp.*, *Harmonia octomaculata*, *Pantala flavascens*, *Ischnura senegalensis*, *Agriocnemis femina*, *Paederus riparius, Tetragnatha nitens, Oxyopes javanus, and Trichogramma sp.* The populations of these natural enemies were consistently higher in the treated rice fields compared to those in conventional fields without the application of biological agents as shown in Figure 1.

The abundance of natural enemy populations in land with entomopathogens application also affected the number of individual natural enemies in rice fields without biological agent application which was 1,191 compared to 448 in land with biological agent application. This means that the *Streptomyces* sp. and *Trichoderma* sp. as entomopathogens worked together to reduce the insect pest population. In general, several studies have shown the positive nature of the interaction between natural enemies of arthropods and fungal pathogens in controlling insect pest populations (Roy and Pell, 2000).

Through this synergy, it can improve natural control processes and reduce the use of chemical pesticides. According to the use of chemical pesticides causes the death of insect pests and natural enemies (Overton et al., 2021). Thus, the use of biological control can prevent the decline in natural enemy populations (Culshaw-Maurer, Sih, and Rosenheim, 2020). Biological agents have the advantage of being able to parasitize insects without disturbing natural enemy populations due to the selective ability of these biological agents to control insect pests (Sentis et al., 2022; Kumari, Srivastava, and Sah, 2022). The selective nature of biological agents (entomopathogens) is due to genetics playing a role in the selectivity of biological agents against certain insect pests (Ortiz-Urquiza and Keyhani, 2013).

The biological agent *Trichoderma* sp. as an entomopathogen that attaches to the insect's body, performs signaling activities to detect and confirm if the insect where *Trichoderma* is attached is a host or not. If the host (insect pest) is confirmed to be true, then the biological agent forms hyphae on the surface of the host's body and will produce enzymes to degrade the host's cuticle. Hyphae that penetrate the cuticle will parasitize the soft tissue inside the host/insect pest (Singh, Raina, and Singh, 2017). In addition, *Streptomyces* is also known to have the ability as an entomopathogen but with a different mode of action. *Streptomyces* can produce secondary metabolites which are antifeedant, larvacidal, and growth inhibitor to control the insect pest. These antifeedant properties can control pest attacks by reducing pest preferences for plants coated with *Streptomyces* secondary metabolites, so that natural enemies that are not herbivores are not affected by these secondary metabolites (Vijayabharathi et al., 2014).

Diversity Index of Insect on Rice Fields

The calculated diversity index (H') values are 2.2 for fields without biological agents and 2.3 for fields with biological agents. With diversity index values ranging from 1.5 to 3.5, the species diversity on both types of land is deemed to be at a moderate level (Fig. 2).

Based on the Shanon-Winner diversity index, it shows that the diversity of species on agricultural land can be said to be quite high (H'> 3). A community is said to have high species diversity if the community is composed of many species with almost the same abundance of species. Conversely, if the community is composed of very few dominant species, then the species diversity is low (Achmad Rizal, Wirawan, and Pratiwy, 2022; Roswell, Dushoff, and Winfree, 2021).

The results of calculating the evenness index value (E) on land without the application of biological agents and the application of biological agents are 0.4 and 0.3. This figure is closer to 1, which means that the distribution of this type of insect in the two fields is evenly distributed. This shows that the application of biological agents does not have an effect on the evenness value of the species because there is no difference in the evenness index value in the two fields. Evenness index values range from 0-1. If the value of E <0.20 it can be said that the condition of the distribution of species is unstable, whereas if the value of E is 0.21, <E <1 it can be said that the condition of the distribution is stable (Krebs, 1972).

The results of calculating the dominance index value of the land without the application of mixed biological agents and the application of biological agents show values of 0.1 and 0.1 which are then in the low category, meaning that no insect species dominate the land. A lower C value indicates that these diverse species have relatively balanced opportunities to maintain species sustainability. Under low dominance conditions, it is anticipated that pest populations in the field will decrease, allowing for natural pest control to take place. This reduction can help prevent economic losses from pest damage and avoid extra costs associated with pest control measures (Ratto et al., 2022). Dominance index values range from 0-1. If the value is close to 0 then no individual dominates, and if the value is close to 1 it means that one species dominates (Odum and Barrett, 1971).

The Species Richness Index (R) provides a basic measure of biodiversity by counting the number of species present in a habitat. The analysis indicated that species richness was categorized as low, with insect species being evenly distributed across both types of rice fields. Additionally, the evenness of insect species distribution in both conventional and biologically treated fields was categorized as stable. The diversity index for both types of land fell into the moderate range, with values between 1 and 3 (H'). Biodiversity is thus influenced by both the number of species (species richness) and the uniformity of their distribution (Evenness) (Magurran, 2003).

The spread of insects is quite even in the rice fields due to the habitat that supports the survival of various types of insects there. All types of insects both harmful (pests) and beneficial insects (natural enemies and pollinators) can use or take advantage of these habitats to find food, reproduce and maintain their survival.

Ecological services carried by biodiversity in an agricultural agro-ecosystem, including pollination services, decomposition services, and biological control services (predators, parasitoids, and pathogens) to control pests, are very important for sustainable agriculture (Altieri and Nicholls, 2018; Jasrotia et al., 2023; Kremsa, 2021).

CONCLUSION

The study concluded that insect populations were higher on fields treated with biological agents, totaling 1,191 individuals compared to 488 individuals on fields without biological agents. The use of biological agents, specifically Streptomyces sp. and Trichoderma sp., significantly affected pest populations. Additionally, these agents influenced various biodiversity metrics: the species diversity index was categorized as high, the evenness index was medium on both types of land, the species richness index was high on both types of land, and the dominance index was low.

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