Alim Isnansetyo · Tri Rini Nuringtyas
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Role of Extension Workers in Promoting Integrated Farming System Sustainability in Yogyakarta, Indonesia

Raden Ahmad Romadhoni Surya Putra and Fransiskus Trisakti Haryadi

Abstract Adoption of integrated farming systems can be a solution to many agricultural problems in Indonesia. The technology transfer model can be used to analyze the dissemination process, which emphasizes the role of extension to promote the innovation. This research aimed to examine the relationships among three variables that determine the role of extension workers in promoting integrated farming system sustainability. Those variables were attitude, leadership, and information access. A field survey using a closed-ended questionnaire was applied to collect data. There were 93 selected respondents from extension workers in the Food Security and Agricultural Extension Agency in four regencies in Yogyakarta Province. The results showed that all respondents had high levels of attitude, leadership, and information access toward integrated farming systems. A Pearson correlation test was applied to examine the relationships among attitude, leadership, and information access. The leadership of extension workers indicated significant relationships to attitude ($p < 0.01$) and information access ($p < 0.01$). This study showed that leadership of extension workers is an important variable to foster the sustainability of integrated farming systems. It could also generate effectiveness of technology transfer to farmers. Therefore, the leadership capacity of extension workers should be enhanced in order to ease the adoption of integrated farming systems at the grassroots level.

Keywords Attitude • Information access • Integrated farming system • Leadership • Sustainability

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1 Introduction

In 2007, BAPPENAS (Badan Perencanaan Pembangunan Nasional; National Development and Planning Agency) identified issues inhibiting the development of agriculture in Indonesia. The latent problems are limitations in resources and infrastructure, lack of knowledge and technology transfer, weak participation by farmers, and inefficiency of agricultural product marketing [1]. All of these problems ultimately lead to an increase in the number of poor people in Indonesia. The majority of poor people in Indonesia live in rural areas and cultivate land in the agricultural sector. This problem requires a solution that can break the chain of such problems as a cycle of positive solutions for farmers. Actually, the problems in the agricultural sector in general could be solved by adopting integrated farming systems [2]. Integrated farming practices themselves have been implemented in Indonesia through adoption of a model approach to integrating crops and livestock [3].

The principle of integrated farming system adoption is the outcome of the technology transfer process in the agricultural extension system. Transfer of technology itself is a process of technology transmission that starts with research and ends with farmers through a process of counseling, with extension workers as the main actors [4]. Within the process, the extension workers' role is very important in the transfer of agricultural technology. They are in a position to determine the effectiveness of the counseling and advising process. In general, the effectiveness of counseling is determined by access to good information [5]. The extension workers' performance in the field is affected by their leadership [6]. In addition, extension workers should have a positive attitude toward the concept of integrated farming systems [7]. These three variables are essential in the process of technology transfer at the farm level. Therefore, assessment of the relationships between access to information, leadership, and attitudes toward the sustainability of integrated farming systems is important to determine the priority for improving the quality of the human resources of extension workers.

1.1 Agricultural Extension

Agricultural extension can be defined as a process of assistance to farmers in order to identify and analyze problems in agricultural production and to create awareness of the opportunity to expand their businesses [8]. In the process of adopting and diffusing innovation, agricultural extension can also be equated with the process of technology transfer. This is in accordance with the technology transfer model developed by the Food and Agriculture Organization (FAO) [4] and indicates that agricultural extension is a very important activity in the flow of research results to be applied by farmers (Fig. 1).

In general, the agricultural extension process in Indonesia has not been as effective as the process of technology transfer to farmers because of the weakness of the
links between extension and research [9]. Furthermore, an effective relationship between research and extension depends on (1) internal efficiency in research and the extension system; (2) orientation of research and extension institutions; (3) the structure mechanism; and (4) the need for research and extension resources. Moreover, the quality of research and extension is an important factor to ensure that the technology will flow from research to farmers [10].

1.2 Integrated Farming Systems

Integration of all resources in agricultural systems has actually been practiced by many farmers around the world with different models for integration [11]. This is because conventional farming practices have impacts on economic problems because of overproduction, high costs, and low income levels of farm households. In addition, ecological problems—such as water and soil pollution, erosion, and low diversification of the ecosystem—are also faced by farmers because of the practices of conventional farming systems. Agricultural problems can be addressed by implementing integrated farming systems, which allow involvement of low-level input of fertilizers, pesticides, and product processing [2]. In Indonesia, integrated farming system practices are mostly based on crop and livestock production to meet consumption needs at the local level [24]. This means that the integration is expected to be protective against degradation of resources and inadequate food supply in the future [12].

Integrated farming systems have two basic components—namely, socioeconomic and biophysical components [13]. Both components are interconnected and form a spinning cycle (Fig. 2). Tipraqsa et al. [12] tried to simplify the model by designing an integrated farming system based on four principles:

- Food security function: this is defined as integration to increase the availability of food (crops and livestock).
Environmental function: this is focused on integration to improve support for resources (land, water, and perennials).

Economic function: this refers to integration to increase revenue for farmers (income generation).

Social function: this is intended as integration to involve farming communities (farmer participation).

These four principles are important components in the implementation of integrated farming systems to reflect the integration of social and technical aspects. Extension workers, who play a central role in the system of agricultural extension, should have
Role of Extension Workers in Promoting Integrated Farming System Sustainability...

awareness and understanding of the conceptual model of the integrated farming system. As a consequence, the responsibility of the extension workers is expanded from a training and visiting model to demand a driving perspective [24]. The extension workers should be polyvalent workers who are in charge of many competences in crops, livestock, fisheries, plantations, and even forestry. Therefore, the role of the extension worker as a facilitator in a farm community is more strongly emphasized with more responsibility in the farming system.

The extension worker is an agent of change in agriculture [15]. Rogers has explained that the function of an agent of change is a connection between two or more social systems. So it must be emphasized that extension agents should have both technical ability and good communication in the field. Moreover, in the context of technology transfer, extension workers must be able to access research to be disseminated at the farm level [4, 7].

The success of agricultural extension programs at the farm level is marked when farmers’ trust keeps on growing toward the extension workers’ leadership in optimizing the potential of human resources. Furthermore, a good extension agent is a leader who is able to understand the interests of farmers and to motivate them to achieve the success of the current proceedings [6]. In farming systems, the functions of the extension agent’s leadership are those of a catalyst with a solution-based approach, a motivator, and a facilitator [16]. Extension workers who have good leadership surely will continue to enhance the capabilities and skills of individuals in order to play a role in the success of extension programs in the field [24].

In addition, extension workers should also have good access to relevant information. Information is a raw material, which we can process to form a structure of knowledge. In order to get good material in the formation of the knowledge structure, we should be able to disclose any information messages that exist [17]. On this basis, access to information is very important to increase our knowledge. The information itself can be accessed through a variety of channels, such as interpersonal communication and mass media [15]. Interpersonal communication is an information channel that requires exchange of information by at least two individuals, while mass media allows dissemination of information from one source to a larger audience. Furthermore, new information channels are also able to provide unlimited information. In agricultural extension, the Internet is widely used to disseminate a demonstration plot or the results of applied research in agriculture [18].

In order to sustain the diffusion of innovation, attitudes toward technology or programs become important. Attitudes can be defined as positive or negative responses to objects, including people, objects, events, and current issues. Attitudes are shaped by beliefs, feelings, and behavioral intentions. An attitude reflects the intention and tendency for someone to respond to a certain incident, a certain idea, or a person’s behavior. In the context of agricultural development, attitudes, norms, and values became important factors in the behavior and performance of agricultural extension [19]. Attitudes toward integrated farming systems specifically can be defined as negative or positive responses based on the beliefs, opinions, and experiences of extension agents in disseminating ideas about the integrated farming system to the farmer. In some areas of Indonesia, such as Bantul and Sleman Regency,
integrated farming systems have become government policy to be implemented by farmers to overcome problems of lack of resources [7]. Although a positive attitude on the part of extension workers in those areas toward integrated farming systems is highly dependent on policy mechanisms and economic incentives, the interrelated factors of information access, leadership, and attitude are expected to be essential in the extension process. Therefore, this study aimed to examine the relationships among these three variables—information access, leadership, and attitude—in fostering integrated farming.

2 Methods

Data collection was conducted in four regencies in Yogyakarta Province—namely, Sleman, Bantul, Kulonprogo, and Gunungkidul—by use of a survey method. The survey involved 93 respondents who were chosen purposively. The respondents were required to be listed as agricultural extension workers who were both civil servants and contracted workers with more than 1 year of experience. Data were collected by use of a questionnaire containing statements to measure responses based on the Likert scale. The primary data included the characteristics of the respondent farmers and their independence level, based on functioning aspects of farmer groups as a unit of study, production, experimentation, and cooperation.

2.1 Analysis

The responses of the extension workers to statements in the questionnaire were graded using a five-point Likert scale ranging from strongly agree to strongly disagree. Prior to the main analysis, a validity and reliability analysis was conducted. The validity analysis was intended to measure the extent to which the questionnaire could measure the variables. Reliability could be used to see the extent to which the measurement results were relatively consistent when the questionnaire was used repeatedly. Validity was tested by use of a product moment correlation formula. The reliability was measured by use of Cronbach alpha estimation techniques, which can be processed with SPSS.

\[
\rho = \frac{N \sum (XY) - (\sum X)(\sum Y)}{\sqrt{[N \sum X^2 - (\sum X)^2][N \sum Y^2 - (\sum Y)^2]}}
\]

Description:

\( \rho \) = validity coefficient

\( X \) = item score
Y = total item score  
N = number of respondents  

With the provision of testing when \( r > r_{\text{Table}} (N-2; 5\%) \), the measurement results were valid. Furthermore, to test the reliability of the instrument, a Cronbach alpha formula was used as follows:

\[
\alpha = \frac{K}{K-1} \left( 1 - \frac{\sum S^2_j}{S^2X} \right)
\]

Description:

\(\alpha\) = alpha reliability coefficient  
K = number of hemisphere  
\(S^2_j\) = variance score of hemisphere  
\(S^2X\) = total variance score

As a result, the number of valid items for the attitude variable was seven. For the leadership variable, there were 12 valid items, as one item fell within the validation test. For access to information, all 18 items passed the validation test. Furthermore, the reliability test showed that the alpha (\(\alpha\)) coefficient had a value above 0.6. This indicated that the questionnaire was consistent and reliable for measurement in this study. The results of the validity and reliability tests of the questionnaire can be seen in Table 1.

Descriptive analysis of quantitative variables in rearing economic motivation and the degree of independence with its indicator were conducted by categorizing these variables into class intervals to be determined on the basis of the difference between the maximum value and the minimum in comparison with the number of categories. The criteria for the generated variable categories can be seen in Table 2.

On the basis of the objective of the study, this paper focuses only on the testing criteria for extension to support the effectiveness of technology transfer in accordance with the variables of the extension agents’ leadership, information access,

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of items</th>
<th>Number of invalid items</th>
<th>Number of valid items</th>
<th>Reliability ((\alpha))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitude</td>
<td>7</td>
<td>–</td>
<td>7</td>
<td>0.7312</td>
</tr>
<tr>
<td>Leadership</td>
<td>13</td>
<td>1 (item 10)</td>
<td>12</td>
<td>0.8715</td>
</tr>
<tr>
<td>Access to information</td>
<td>18</td>
<td>–</td>
<td>18</td>
<td>0.9164</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low category score</th>
<th>High category score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitude</td>
<td>7–21</td>
<td>22–35</td>
</tr>
<tr>
<td>Leadership</td>
<td>12–36</td>
<td>37–60</td>
</tr>
<tr>
<td>Access to information</td>
<td>18–54</td>
<td>55–90</td>
</tr>
</tbody>
</table>
and attitude toward the sustainability of integrated farming systems. Moreover, by use of statistical analysis, these variables were tested in three levels of relationships between the variables by use of a Pearson correlation test.

3 Results and Discussion

3.1 Characteristics of Respondents

The characteristics of the extension agents as respondents in Yogyakarta documented in this study were gender, age, education, years of work experience, income, and working area. The characteristics of the respondents can be seen in Table 3.

Table 3 shows that the gender distribution of the respondents was quite unequal: 64.5% were men and 35.5% were women. This indicates that the extension worker profession did not seem to hold special interest for women. This was most likely because this profession requires much time in the field. Moreover, the geographical conditions on farms are very broad and tend to be difficult, so women are not interested enough in this profession [7, 20-22].

The average age of the respondents was 40.48 years, showing that agricultural extension workers in the province are still in the productive age group. An average age of 40 years among agricultural extension workers is a common situation in developing countries [7, 23]. However, this condition also indicates that the younger generation are not interested in pursuing this profession [21].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64.5</td>
</tr>
<tr>
<td>Female</td>
<td>35.5</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>40.48 ± 10.10</td>
</tr>
<tr>
<td>Level of education (%)</td>
<td></td>
</tr>
<tr>
<td>Senior high school</td>
<td>24.7</td>
</tr>
<tr>
<td>Diploma</td>
<td>24.7</td>
</tr>
<tr>
<td>Undergraduate degree</td>
<td>48.4</td>
</tr>
<tr>
<td>Graduate degree</td>
<td>2.2</td>
</tr>
<tr>
<td>Average work experience (years)</td>
<td>12.52 ± 12.53</td>
</tr>
<tr>
<td>Average monthly income (Rp)</td>
<td>2,106,328.00 ± 979,219.59</td>
</tr>
<tr>
<td>Working unit (%)</td>
<td></td>
</tr>
<tr>
<td>Sleman</td>
<td>22.6</td>
</tr>
<tr>
<td>Bantul</td>
<td>30.1</td>
</tr>
<tr>
<td>Gunungkidul</td>
<td>22.6</td>
</tr>
<tr>
<td>Kulonprogo</td>
<td>24.7</td>
</tr>
</tbody>
</table>
The quality of the human resources of extension workers can be seen from their educational backgrounds and work experience. The respondents' educational backgrounds were high school (24.7%), diploma (24.7%), undergraduate degree (48.4%), and postgraduate degree (2.2%). From the data obtained, the majority of respondents held diplomas or degrees. This shows that the majority of extension agents in the province have higher education. This condition is very supportive in improving the quality of the human resources, especially with the average work experience reaching 12.52 years. The majority of extension workers in Indonesia do have higher educational backgrounds with practical work experience of more than 12 years [22]. However, this condition is not in accordance with the findings by Putra et al. [7], who stated that agricultural extension agents' work experience in the province reached 15.63 years. However, it shows that the work experience is getting longer and supports the argument that the younger generation are not interested in being agricultural extension workers [21].

The respondents in this study were spread across the districts in the province—namely, Sleman (22.6%), Bantul (30.1%), Gunungkidul (22.6%), and Kulonprogo (24.7%). The average monthly incomes of the extension workers reached Rp 2,106,328. This current condition represents an increase when compared with the findings of Putra et al. [7], who stated that the average income of agricultural extension workers reached Rp 2,039,992. Although this rise was not significant, it did indicate a visible improvement in the monthly incomes of extension agents.

### 3.2 Attitude, Leadership, and Access to Information

In this study, the roles of extension workers in supporting sustainable integrated farming systems were analyzed on the basis of the extension workers attitude, leadership, and access to information. These three components are able to support effective transfer of technology related to implementation of integrated farming systems. The distribution of attitude, leadership, and access to information can be seen in Table 4.

The attitude of extension agents in supporting the sustainability of the implementation of integrated farming systems at the farm level was very positive. This was proven by the data showing that 100% of the extension agents' attitude was categorized as high. Obviously, this may encourage the process of pushing technology dissemination in integrated farming systems. Every stakeholder in the development

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Attitude</td>
<td>100</td>
</tr>
<tr>
<td>Leadership</td>
<td>92.5</td>
</tr>
<tr>
<td>Access to information</td>
<td>87.1</td>
</tr>
</tbody>
</table>
of agriculture should have a progressive mental attitude toward innovation [24]. Moreover, a positive attitude on the part of agricultural extension workers may facilitate farmers' adoption of integrated farming systems [7, 25].

The results of the leadership analysis of the extension workers in Yogyakarta showed that 92.5% of them had the leadership spirit. This shows that the leadership functions of the extension workers in integrated farming systems are well developed. This situation certainly indicates high motivation to achieve success and become leaders for farmers in applying integrated farming systems [6, 16].

With regard to information access, Table 4 shows that the majority of extension agents had broad access to information (87.1%). Broad access to information is critical in building knowledge structures. In integrated farming systems, access to information become important to obtain new materials that will be disseminated to farmers and to improve the knowledge of the extension agents [7, 17].

3.3 Relationships Among Attitude, Leadership, and Access to Information

High values for extension workers' attitude, leadership, and access to information on integrated farming systems provide great hope for the sustainability of implementation at the farm level. However, strong relationships among these variables are needed to deepen the effectiveness of technology transfer by agricultural extension workers. The relationships between the extension workers' attitude, leadership, and access to information were analyzed by use of Pearson correlation, with the following results.

With regard to the three variables, it can be seen that the attitude of extension workers was significantly correlated ($p < 0.01$) with their leadership. However, the attitude of the extension workers was not correlated with their information access. The leadership of the agricultural extension workers was significantly correlated ($p < 0.01$) with their information access. Thus, the role of leadership becomes the core point of the relationships among these three variables. This is supported by the results of the analysis showing that leadership was significantly correlated with the other variables. It indicates that the primary function of the extension workers is as leaders for farmers [6]. Agricultural extension workers should be able to lead farmers to optimize the potential of their human and natural resources. Furthermore, in relation to the sustainability of integrated farming systems, it is necessary to develop good attitudes and leadership to ensure the success of extension programs at the farm level [14] (Table 5).
Table 5  Relationships among extension agents’ attitude, leadership, and access to information

<table>
<thead>
<tr>
<th>Variable</th>
<th>Attitude</th>
<th>Leadership</th>
<th>Access to information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>Significance</td>
<td>R</td>
</tr>
<tr>
<td>Attitude</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leadership</td>
<td>0.375</td>
<td>0.000**</td>
<td>-</td>
</tr>
<tr>
<td>Access to information</td>
<td>0.144</td>
<td>0.169</td>
<td>0.380</td>
</tr>
</tbody>
</table>

**Significant at $p < 0.01$

4 Conclusions

Agricultural extension workers’ attitude, leadership, and access to information in Yogyakarta are at high levels. It is expected that the adoption process at the farm level will be easier with the positive attitude on the part of the extension workers. This leadership indicates that extension agents have high motivation and can be leaders for farmers to support sustainable integrated farming systems. This is supported by the workers’ broad access to information to improve knowledge of innovations that support the implementation of integrated farming systems. The leadership of the extension agents in this study had significant relationships with their attitude and access to information. This means that their leadership has a major role in the success of the extension program. Therefore, leadership development training is a necessity for extension workers in order to ensure the success of innovation dissemination at the farm level. It certainly supports sustainable integrated farming systems because the technology transfer process becomes more effective.

References

Pathogenicity of Nematofagous Fungus for Control of *Pratylenchus coffeae* Nematodes on Coffee Plants

Rinda Fajrin Aldina, Siwi Indarti, and Arif Wibowo

Abstract *Pratylenchus coffeae* is a plant-parasitic nematode, which is a major cause in the decline of coffee plant yield in Indonesia. The loss caused by *P. coffeae* is up to 78%. This research is one of the efforts being made in biological control of *P. coffeae*. Isolates were screened by a pathogenicity test in vitro with a spore suspension at a density of $10^6$ spores $\cdot$ mL$^{-1}$ against *P. coffeae* nematodes at different life stages (J2, J3, J4 and adult). The pathogenicity test was done by choosing fungal isolates that had ability to cause $>50\%$ nematode mortality. *P. coffeae* nematodes were tested by an in vitro method with a fungal suspension and liquid culture filtrate (crude enzyme). Observations were done to determine the percentages of *P. coffeae* nematode destruction. Based on the test, isolate PIVB0402A had a very powerful mechanism of mortality in the pathogenicity process against *P. coffeae* nematodes. This work showed that isolate PIVB0402A caused the highest mortality of up to 100% in *P. coffeae*.

Keywords Biological control • Nematofagous fungus • Parasitic nematode

1 Introduction

The coffee plant is a plantation mainstay commodity, creating foreign exchange, a source of income for farmers, and industrial raw material; opening up job opportunities; and aiding in territorial development [1]. Two types of coffee plant are found in Indonesia: Robusta and Arabica.

In some coffee plantations, damaging disruptions have occurred. These are mostly caused by pests and diseases, which normally attack roots, rods, branches, flowers, fruit, and leaves. In addition to fungi in roots, attacks of nematodes on coffee roots have recently been identified as a dangerous threat against coffee plants because these can lower coffee plantation productivity in Indonesia.

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Nematode attacks can inhibit the growth of plants, reduce productivity, and reduce production quality. The most frequently found types of coffee plant-parasitic nematodes are the root-knot nematode *Meloidogyne* sp. [2] and the lesion nematode *Pratylenchus coffeae*, which is the most harmful nematode to coffee plants [3]. Losses caused by nematode attacks in coffee plantations in Indonesia are up to 78% [2].

Nowadays, use of organic materials, persistent varieties, and chemical materials are used to control nematodes. In implementation, farmers often choose only one of them – mainly the use of chemical materials – and the main target is only to control nematodes, with less regard to the effect on the whole agriculture ecosystem. To reduce the negative impact of the use of chemical materials or nematicides, it is necessary to use environmentally friendly controls. Some potential biological agents can be developed through the use of nematofagous fungi.

Nematofagous fungi are a group of microorganisms issuing toxins that can inhibit nematode growth and progress, and can kill nematodes [4]. Some fungi can be used as nematofagous fungi on nematodes, for example, *Trichoderma virens*, *Fusarium oxysporum*, *Fusarium lateritium*, *Penicillium triturn*, and *Talaromyces* sp., which have potential as biological control agents [5]. The pathogenic fungus penetrates the nematode, then the fungus proliferates in the nematode, and finally the nematode is killed; after that, the fungus eats the body content of the nematode. Fungal spores can enter or infect the cuticle of the nematode through mechanical force and the enzymatic activity from fungal combinations between two mechanisms [6, 7].

Research into biological control of coffee plant-parasitic nematodes has not been developed much. This research aimed to know the pathogenicity of nematofagous fungal isolates against the lesion nematode *P. coffeae*, before such an isolate is developed as a biological control agent for *P. coffeae* nematodes.

2 Methods

2.1 Isolate Fungi Selection with Pathogenicity Testing on P. coffeae Nematodes

2.1.1 Preparation of Nematofagous Fungal Isolates

Fungal isolates were identified in sub-lab nematology collections as nematofagous fungi (Table 1). Nematofagous fungal isolates were cultured beforehand, then spore suspension preparations were conducted. Next, spore suspension fungal isolates, isolates that had been cultured on a slant medium, were added with 10 mL of sterile Aqua Dest into the isolate tube and vortexed so the spore was released from the mycelium to form a spore suspension. Spore density calculations were done by using a hemocytometer. The suspension could be diluted to achieve density of $10^9 \text{ mL}^{-1}$. The densities of fungal spores that would be used for inoculation in the
Pathogenicity of Nematofagous Fungus for Control of Pratylenchus coffeae ...

Table 1 Tested
nematofagous fungal isolates

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Penicillium sp.</td>
<td>P Hib0402</td>
</tr>
<tr>
<td>2</td>
<td>Penicillium sp.</td>
<td>P Hib0402B</td>
</tr>
<tr>
<td>3</td>
<td>Penicillium sp.</td>
<td>Piva0403C</td>
</tr>
<tr>
<td>4</td>
<td>Penicillium sp.</td>
<td>Piva0402B</td>
</tr>
<tr>
<td>5</td>
<td>Penicillium sp.</td>
<td>Piva0402B</td>
</tr>
<tr>
<td>6</td>
<td>Penicillium sp.</td>
<td>PIVB0402A</td>
</tr>
<tr>
<td>7</td>
<td>Penicillium sp.</td>
<td>HI0202B</td>
</tr>
</tbody>
</table>

Species data were observed by morphology test and macroscopically and microscopically

nematode test were calculated so that the densities of fungal spores would be same as each other.

2.1.2 Isolation and Extraction of P. coffeae Nematodes

The diseased or infected roots of coffee plants were cleaned with water and cut into small pieces of ±2 cm, then mashed with a blender. The roots were filtered with a 40 μm filter and rinsed with water until clear, and then flattened on a filter coated with tissue paper. The filter was placed on a container bowl. The bowl was placed in a sieve, and sieving was done for 2 × 24 hours. Sieving was done to get the nematodes from the roots of the coffee plants into a container. Isolation and extraction of P. coffeae nematodes were conducted by using a sieving method [8].

The harvesting of nematodes was done by filtering the water in the container with a 35 μm filter, and then yields were observed with an Olympus SZX12 microscope. P. coffeae nematodes at different life stages (L2 until adult) were taken and collected in a Syracuse glass containing sterile Aqua Dest. Then the P. coffeae nematodes were reproduced on carrot slices.

Reproduction of P. coffeae Nematodes

Reproduction was done by using a modified carrot disk culture method [9]. Reproduction on carrot slices was done by inoculating P. coffeae onto carrot slices. Before inoculation of P. coffeae nematodes, carrot slices were prepared in advance. Carrots were washed and peeled and then soaked in a solvent of Clorox for ±1 hour. Then the carrots were sliced into pieces using a sterile blade. Carrot slices were inserted in a vial bottle and incubated at room temperature until a callus formed (2 weeks), and then 30 nematodes were inoculated at the center of the carrot pieces in a sterile vial bottle and then incubated for 2 months.
Preparation of Nematode Tests

The *P. coffeae* test nematodes were harvested from the carrot slices, and then the carrot slices were chopped by using a sterile blade. The extraction of *P. coffeae* nematodes was done by using a modified Whitehead technique [10]. Next, chopped carrots were added to Aqua Dest until they were soaked, then they were incubated for 24 hours. After 24 hours, the water was separated from the carrot slices and observed under an Olympus SZX12 microscope. *P. coffeae* nematodes were taken and collected in a Syracuse glass containing sterile Aqua Dest, with one Syracuse glass containing 30 nematodes [11].

### 2.2 Liquid Culture Filtrate (Crude Enzyme) Toxicity Test of Selected Isolates Against *P. coffeae* Nematodes

#### 2.2.1 Production of Crude Enzyme

The production of crude enzyme was done by growing the isolate in a liquid medium, referring to the method of Qureshi et al. [12]. The fungal isolate was inoculated in 20 mL liquid medium potato dextrose broth (PDB) in an Erlenmeyer flask with a volume of 100 mL. Isolates were incubated at a temperature of 20–25 °C in a shaker at a speed of 15.71 rad · s⁻¹ for 6 days, then filtered with Whatman paper 0.2 μm. Crude enzyme was harvested by using a centrifuge with a speed of 314.16 rad · s⁻¹ at 40 °C for 20 minutes to separate the fungal mycelium from the supernatant. The obtained supernatant was used for the ability test of the liquid culture filtrate in the destruction of *P. coffeae* nematodes.

#### 2.2.2 Incubation of *P. coffeae* Nematodes with Liquid Culture Filtrate

The incubation of nematodes with liquid culture filtrate was done by inserting 30 *P. coffeae* nematodes in 200 μL sterile Aqua Dest into each of 24 multiwell plate holes with three repetitions for each isolate. To each 24 multiwell plate hole was added 200 mL of crude enzyme filtrate and this was incubated at 20–25 °C for 7 days. Observations were done every 24 hours by using an Olympus SZX12 microscope at magnification of up to 200x.

Analysis of fungal nema-togous pathogenic ability against nematodes was determined by the percentage of dead *P. coffeae* nematodes, calculated by using the formula:

\[
\% \text{ mortality nematode} = \frac{\sum \text{mortality nematode}}{\sum \text{baseline nematode}} \times 100
\]  

\[ (1) \]
The treatment effect was analyzed by using an ANOVA statistical test (analysis of variance) and an LSD further test (least significant difference) in the SAS 9.1.3 program with a confidence interval of 95%.

3 Results and Discussion

3.1 Pathogenicity of Fungal Isolates with a Suspension of Spores Against P. coffeae Nematodes

The pathogenic ability of fungal isolates was tested by using a spore suspension. The observed parameters in this test were the numbers of dead P. coffeae nematodes. According to Cayrol et al. (1989), as reported by El-Ghany et al. [13], a nematode is dead if there is no response from it in the form of movement after contact with a special hook tool for nematodes.

The test suspensions of the fungi in seven different treatments showed no differences in the effects on mortality. Overall the test isolates showed mortality percentages above 50%. Based on the results of selected fungal isolates (Table 2), it is known that seven test isolates had the same ability, shown by more than 80% mortality. According to El-Ghany et al. [13], effective fungal isolates for nematode control are isolates that cause up to 50% mortality. The isolates tested included PIIIB0402, PIIIB0402B, PIVAO403C, PIVAO302B, PIVAO402B, PIVBO402A, and HI0202B, observed on the seventh day after treatment. The controls used in this selection were nematodes treated with the addition of a sterile Aqua Dest solution, which did not show any significant damage on the third and seventh days after treatment. In the control treatment, there was up to 3.3% nematode mortality, equivalent to one nematode death.

Table 2 Pathogenic damage levels of biological control isolates used on Pratylenchus coffeae nematodes

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolate</th>
<th>3 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PIIIB0402</td>
<td>66.7</td>
<td>100*</td>
</tr>
<tr>
<td>2</td>
<td>PIIIB0402B</td>
<td>50</td>
<td>83.3*</td>
</tr>
<tr>
<td>3</td>
<td>PIVAO403C</td>
<td>63.3</td>
<td>86.7*</td>
</tr>
<tr>
<td>4</td>
<td>PIVAO302B</td>
<td>56.7</td>
<td>96.7*</td>
</tr>
<tr>
<td>5</td>
<td>PIVAO402B</td>
<td>50</td>
<td>90*</td>
</tr>
<tr>
<td>6</td>
<td>PIVBO402A</td>
<td>46.7</td>
<td>100*</td>
</tr>
<tr>
<td>7</td>
<td>HI0202B</td>
<td>53.3</td>
<td>90*</td>
</tr>
<tr>
<td>8</td>
<td>Aqua Dest control</td>
<td>0</td>
<td>3.3*</td>
</tr>
</tbody>
</table>

Description: The different letter notations within columns show significantly different treatments with ANOVA test α 0.05%
In the treatment with a suspension of spores (Fig. 1), the observed hyphae attachment on the body was not so great. It was suspected that the involvement of mechanical mechanisms in the parasitism process toward the nematodes was not optimal. Hyphae attachments to the nematode body were seen on the whole bodies of the nematodes, but not on their heads and tails. Attachment patterns for nematode fungal hyphae (binding sites) can be divided into three patterns, namely: (1) attachment on the head and tail; (2) attachment to the entire surface of the body of the nematode; and (3) no attachment [7]. Attachment of hyphae to nematode bodies in the test with a spore suspension showed the existence of a mechanically antagonistic mechanism as shown by the attachment of hyphae on the body of the nematode.

Five isolates with the highest mortality percentages were taken from the fungal isolates that caused ≥50% damage. The fungal isolates selected as candidates were fungal isolates with above 89% mortality, including PIIIIB0402, PIVA0302B, PIVA0402B, PIVB0402A, and HI0202B, which then were used as candidates for the next test, i.e., the liquid culture filtrate (crude enzyme) toxicity test on *P. coffeae*.

### 3.2 Pathogenicity of Selected Isolates with a Liquid Culture Filtrate (Crude Enzyme) Against *P. coffeae* Nematodes

Crude enzymes or liquid culture filtrates of nematode biological control fungi contain substances that are toxic and deadly to nematodes. These substances can change extracellular enzymes or secondary metabolites [12]. This test aimed to determine the involvement of extracellular enzymes in the process of parasitism using selected fungal isolates against *P. coffeae*. Extracellular hydrolysis enzymes also determine
the level of virulence of nematofagous fungi and help the digestive process of the fungi in the nematode body cell as its host [14].

The test results for the liquid culture filtrates of selected fungal isolates (Table 3) showed significant effects on the mortality of *P. coffeae*. The highest percentage of nematode deaths reached 100% with isolate PIVB0402A, while the lowest percentage of mortality was 72.5% with isolate PIVA0402B. The five isolates showed high percentages of yield of more than 70%. The control treatment used was Aqua Dest solution. The results of these treatments did not provide any real evidence that treatment with Aqua Dest was not toxic to nematodes. According to Regaieg et al. [15], a supernatant of a crude enzyme of a biological control fungal isolate can cause nematode paralysis and mortality. Based on the toxicity test, the liquid culture filtrates of the selected isolates had nematicidal power against *P. coffeae*.

Treatment using liquid culture filtrate isolates (Fig. 2) on *P. coffeae* nematodes showed damage to the outermost layer of the bodies of the nematodes. This damage showed that the fungal isolates had high enzymatic ability to degrade the outermost layer of the bodies of the nematodes. The visible damage from the treatment with liquid culture filtrate fungal isolates was the formation of vacuoles in the bodies of nematodes. Treatment with liquid culture filtrates of these isolates against nematodes can cause nematode mortality of up to 100%.

Treatment using liquid culture filtrate isolates on nematode bodies caused clotting or shrinkage of the body content of the nematodes. Treatment with liquid culture filtrate fungal isolates gave the same effect as other isolates in the body structure of the nematode, i.e., vacuoles were formed in the bodies of the nematodes. Thus, the parasitic mechanism of these two isolates was allegedly use of a joint mechanical mechanism, i.e., enzymatic and toxic (a secondary metabolite). A liquid culture filtrate of a biological control fungus can cause mortality and parasitism in nematodes [15]. Based on the toxicity test of the liquid culture filtrate against *P. coffeae* nematodes, the five isolates had a nematicidal or destructive power against *P. coffeae* nematodes.

The PIVB0402A isolate had a very powerful mechanism of mortality in the pathogenicity process against *P. coffeae* nematodes. This proved that both treatments with the PIVB0402A isolate yielded the highest mortality of 100%. Based on

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate</th>
<th>Dead nematodes (%)</th>
<th>3 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PIVA0302B</td>
<td>50</td>
<td>81.76%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PIVA0402B</td>
<td>53.3</td>
<td>72.5%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>HI0202B</td>
<td>56.7</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PIHB0402</td>
<td>60</td>
<td>93.7%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PIVB0402A</td>
<td>53.3</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>3.3</td>
<td>3.33%</td>
<td></td>
</tr>
</tbody>
</table>

Description: The different letter notations within columns show significantly different treatments with ANOVA test at 0.05%.
the obtained test results, it can be concluded that the seven nematofagous fungal isolates were proved to be effective in inhibiting development of the nematode *P. coffeae* nematodes, as well as for potential biological control of plant nematodes, particularly *P. coffeae* nematodes.

4 Conclusion

The PIVB0402A isolate had a very powerful mechanism of mortality in the pathogenicity process against *P. coffeae* nematodes. The seven nematofagous fungal isolates were proved to be effective in inhibiting development of the nematode *P. coffeae* nematodes, as well as for potential biological control of plant nematodes, particularly *P. coffeae* nematodes.

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References

Pathogenicity of Nematofagous Fungus for Control of Pratylenchus coffeae...


