

SYNERGISTIC EFFECT OF NEMATOPHAGOUS FUNGI AND ANTAGONISTIC MICROORGANISMS TO CONTROL THE PATHOGEN CAUSED YELLOW LEAF AND ROOT ROT DISEASE OF COFFEE IN TAYNGUYEN

Pham Van Toan^{1*}, Dao Huu Hien², Nguyen Thi Hong Minh³, Ho Hanh²

¹Vietnam Academy of Agricultural Sciences, Hanoi, Vietnam;

²Western Highlands Agriculture and Forestry Science Institute, DakLak, Vietnam;

³Agricultural Genetics Institute, Hanoi, Vietnam.

*Corresponding author: toanvaas@gmail.com

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ABSTRACT

This study aimed to apply a new bioproduct containing beneficial microorganisms as biocontrol to the yellow leaf and root rot disease of coffee plants in Vietnam caused by pathogenic nematodes (*Meloidogyne spp.*, *Pratylenchus spp.*) and fungi (*Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola*) and focused on evaluating the synergistic effect of nematophagous fungi and antagonistic microorganisms to control the pathogens. The results showed that the population of parasitic nematodes in the coffee-growing soil was reduced by 69.82% when treated with both nematophagous fungi and antagonistic microorganisms, compared to 61% and 67.24% when treated with nematophagous fungi or with antagonistic microorganisms alone. The reduction of the *F. oxysporum* population was 76.44% when both nematophagous fungi and antagonistic microorganisms were used, compared to 52.17% and 75.53% when treated with nematophagous fungi or with antagonistic microorganisms after 6 months. By using the new bioproduct, the reduction of coffee plant-parasitic nematodes, pathogen fungi, and the rate of the diseased coffee plant was 77.16%, 78.04%, and 79.08% for the coffee plant at the young stage, and 78.12%, 79.26%, and 78.48% for the coffee plant at the maturity stage after 270 days of application. Field demonstrations on the application of new bioproducts showed a disease reduction of 82.3% compared to farmer practices and brought a benefit of 9,400,000 VND/ha, equivalent to an income increase of 21.7% for the farmer. This new bioproduct can be applied to control the yellow leaf and root rot disease complex of coffee plants caused by *Pratylenchus spp.* and *F. oxysporum* in Taynguyen.

Keywords: Antagonistic microorganisms, Coffee, Nematophagous fungi, Synergic effect, Yellow leaf and root rot disease

QUAN HỆ TƯƠNG HỖ CỦA NẤM ĐỐI KHÁNG TUYẾN TRÙNG VÀ VI SINH VẬT ĐỐI KHÁNG NẤM BỆNH TRONG KIỂM SOÁT BỆNH VÀNG LÁ, THỐI RỄ CÂY CÀ PHÊ Ở TÂY NGUYÊN

Phạm Văn Toàn^{1*}, Đào Hữu Hiền², Nguyễn Thị Hồng Minh³, Hồ Hạnh²

¹Viện Khoa học Nông nghiệp Việt Nam, Hà Nội, Việt Nam;

²Viện Khoa học, kỹ thuật nông, lâm nghiệp Tây Nguyên, Đắk Lắk, Việt Nam;

³Viện Di truyền nông nghiệp, Hà Nội, Việt Nam.

*Tác giả liên hệ: toanvaas@gmail.com

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TÓM TẮT

Nhằm ứng dụng chế phẩm sinh học mới từ các vi sinh vật có lợi như một biện pháp phòng trừ sinh học đối với bệnh vàng lá, thối rễ cây cà phê do tuyến trùng (*Meloidogyne spp.*, *Pratylenchus spp.*) và nấm (*Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Rhizoctonia bataticola*) gây ra tại Việt Nam, nghiên cứu tập trung vào việc đánh giá tác dụng tương hỗ của nấm đối kháng tuyến trùng và vi sinh vật đối kháng trong phòng trừ tác nhân gây bệnh. Kết quả nghiên cứu cho thấy quần thể tuyến trùng trong đất trồng cà phê giảm 69,82% khi xử lý bằng cả nấm đối kháng tuyến trùng và vi sinh vật đối kháng so với 61% và 67,24% khi xử lý bằng nấm đối kháng tuyến trùng hoặc chỉ xử lý bằng vi sinh vật đối kháng. Quần thể nấm *F. oxysporum* giảm 76,44% khi sử dụng cả nấm đối kháng tuyến trùng và

vi sinh vật đối kháng so với 52,17% và 75,53% khi sử dụng riêng lẻ nấm đối kháng tuyến trùng hoặc vi sinh vật đối kháng sau 6 tháng. Sử dụng chế phẩm sinh học mới, hiệu quả giảm tuyến trùng, nấm gây bệnh và tỷ lệ cây cà phê bị bệnh lần lượt là 77,16%, 78,04%, 79,08% đối với cây cà phê giai đoạn kiến thiết cơ bản và 78,12%, 79,26% và 78,48% đối với cây cà phê giai đoạn kinh doanh sau 270 ngày sử dụng. Kết quả mô hình trình diễn sử dụng chế phẩm sinh học mới cho thấy tỷ lệ bệnh vàng lá, thối rễ giảm 82,3% so với đối chứng và mang lại lợi nhuận 9.400.000 đồng/ha, tương đương với 21,7% thu nhập tăng thêm của người trồng cà phê. Chế phẩm sinh học mới có thể sử dụng trong kiểm soát bệnh vàng lá, thối rễ cây cà phê do *Pratylenchus spp* và *F. oxysporum* gây ra ở Tây Nguyên.

Từ khóa: Cà phê, Bệnh vàng lá thối rễ, Nấm đối kháng tuyến trùng, Quan hệ tương hỗ, Vi sinh vật đối kháng

1. INTRODUCTION

As a perennial industrial crop, coffee is cultivated in more than 50 countries, covering approximately 1,127 million hectares worldwide, in which Vietnam is one of the four big coffee-producing countries and ranked the 2nd coffee exporter in the world. In 2024, Vietnam coffee exports reached 1.32 million tonnes with a value of USD 5.48 billion (Ministry of Agriculture and Rural Development, 2024). The coffee yield in Vietnam had more than 3.5 tons/ha, while it is 0.8 tons/ha in Thailand, 0.5 tons/ha in Indonesia, and only 0.4 tons/ha in Lao PDR. Vietnam's production grew at an average annual rate of 19.8% from 1980/81 to 2019/20; however, annual growth has only been 2% in the last five years (International Coffee Organization, 2020), primarily due to falling coffee export prices, unfavorable weather conditions, and an increased incidence of coffee diseases.

Plant-parasitic nematodes are responsible for annual crop losses of around \$USD 157 billion and pose a particular problem to coffee growers. Phytoparasitic nematodes are obligate parasites, relying on the host plant as the nutrient source essential to their survival and reproduction, targeting plant roots of crops, and preventing water, nutrient uptake, and reducing agronomic performance, overall quality, and yields. Symptoms such as leaf yellowing, wilting, and loss of vigour may present at sufficiently high levels of

infection (Kawabata et al. 2018). Nematode populations persist in the soil for a long time without a host plant, and are difficult to control (Kung et al. 1991). Nematodes can parasitize coffee plants from the seedling to the mature stage. The symptoms of nematode-infected coffee plants are yellow leaves, rotting roots, or galling, stunted growth, and may die (Vu et al. 2014). Young coffee plants are more likely to become stunted and die as a result of nematode infection due to the undeveloped root system (Vovlas and Di Vito, 1991).

Root-knot nematode *Meloidogyne spp.* and the lesion nematode *Pratylenchus spp.* are serious challenges for coffee replanting in Vietnam (Ta Hong Linh et al., 2018; Villain et al., 2018; Trinh et al., 2019). In the coffee plantation, *Meloidogyne* and *Pratylenchus spp.* can coexist on the same plant, increasing the damage potential and the economic loss. Belonging *Meloidogyne* species, *M. incognita*, has been mainly documented in Vietnam. Subsequently, four other *Meloidogyne* species have also been found in coffee plantations in Vietnam, namely *M. exigua*, *M. coffeicola*, *M. hapla*, and *M. daklakensis* (Bell et al., 2018; Trinh et al., 2019). Ta Hong Linh et al. (2018) found that *Pratylenchus coffeae* dominated in the soil samples around the upper roots of coffee and is an important pathogen that damages coffee in the central highlands of Vietnam. Ha Hoang et al. (2020) reported

the most abundant *Pratylenchus* to be found in a 5-year-old coffee plantation.

In addition to nematodes, some major coffee root pathogens were identified as *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, and *Rhizoctonia bataticola* (Ta Hong Linh et.al.2017).

Numerous species of fungi and bacteria as biocontrol agents for pathogen nematode and fungi-damaged crops are reported (Stirling et al., 2017; Le et al., 2018; Aminuzzaman et al., 2018; Nguyen Viet Hiep et al., 2019).

To apply the beneficial microorganisms as biocontrol of pathogens caused by yellow leaf and root rot disease of coffee plants in Vietnam, the study concentrated on evaluating the synergistic effect of nematophagous fungi and antagonistic microorganisms to control the pathogenic nematodes and fungi on the coffee plants.

2. MATERIALS AND METHODS

Materials used in the experiments include beneficial microorganisms, pathogens, plant variety and commercial biopesticide are described in the Table 1.

Table 1. Materials used in the experiments

Beneficial microorganisms	Code	Provider
<i>Paecilomyces lilacinum</i> Pae	Pae	Plant Protection Research Institute (PPPI),
<i>Arthrobotrys oligospora</i> NVC7.4	NVC7.4	Soil and Fertilizer Research Institute,
<i>Bacillus velezensis</i> P9.1	P9.1	Agricultural Genetics Institute, Institute for
<i>Streptomyces enissocaesilis</i> 2P	2P	Agricultural Environment
<i>Chaetomium cochliodes</i> TQHT01	TQHT01	
Pathogens		
<i>Pratylenchus coffeae</i>	<i>P. coffeae</i>	PPRI
<i>Fusarium oxysporum</i>	<i>F. oxysporum</i>	
Crop		
Coffee plant variety Tr4	Tr4	Taynguyen Agriculture and Forestry Science Institute
Commercial biopesticide		
<i>Paecilomyces</i> and <i>Chaetomium</i> <i>spp</i> with a density of 10 ⁸ CFU/g	Padave	Agricultural Genetic Research Institute

The new bioproduct was produced by mixing the biomass of 5 selected microorganisms in a w/w ratio of 30%:25%:25%:10%:10% with steril

dextrin, containing a density of beneficial microorganisms of 10⁸ CFU/g at the time of 6 months stored at room temperature (Table 2).

Table 2. Components of new bioproduct

Beneficial microorganisms	Code	Mixing ration (% w/w)
<i>Paecilomyces lilacinum</i>	Pae	30
<i>Arthrobotrys oligospora</i>	NVC7.4	25
<i>Bacillus velezensis</i>	P9.1	25
<i>Streptomyces enissocaesilis</i>	2P	10
<i>Chaetomium cochliodes</i>	TQHT01	10

Three greenhouse experiments were carried out on the *Rhodic Ferralsols* soil. The control treatments were infected with *P. coffeae* at a dose of 250 nematodes per 100 g soil or with *F. oxysporum* at a density of 10^4 CFU/g soil or infected with both *P. coffeae* and *F. oxysporum* at the same density. The experiment treatments are infected with *P. coffeae* or *F. oxysporum* or both of them with the same density as the control, and inoculated with the nematophagous fungi and or with the antagonistic microorganism at a density of 10^4 CFU/g soil, depending on the treatments. Pathogen nematodes and fungi are mixed into the soil before making the pots. Beneficial microorganisms are inoculated through watering the solution containing biomass of selected microorganisms after planting the coffee in the pots. Coffee plants are cared for in the greenhouse according to the guidelines of the WASI. Each treatment was replicated 3 times with 5 pots/replication and randomized. Monitoring the density of nematodes and *F. oxysporum* in the soil, the yellow leaf coffee, and the coffee height after treatment of 30, 60, and 90 days.

The field experiments were carried out as a completely randomized design according to regulations of the Ministry of Agriculture and Rural Development in the integrated crop management system at the young and mature stages of the coffee plant. The experiment comprises 3 treatments: 1.

Negative control as farmer practices; 2. Using a new bioproduct with a dose of 50g /plant; 3. Using the commercialized bio product Padave with a dose of 25g /plant. The experiment was designed with 30 plants/treatment in the coffee plantation with a density of 1.110 plants/ha. All experiments were laid out with 3 replications. Evaluating the density of soil pathogen fungi, nematodes, and the coffee plant height at the times of 30, 90, 180, and 270 days after treatments. Summary of treatments of green house and small scale field experiments was described in the Table 3.

Field demonstrations were carried out at the 4-year-old coffee plantation of Mr. Phan Hong Luan in GiaLai province with the density of 1.100 plants/ ha. Two treatments are set up without replication, 1. Using chemical control methods as farmer practices; 2. Using a new bioproduct with a dose of 50g/plant. Evaluating the density of soil pathogen fungi, nematodes, and the coffee plant height at the times of 30, 90, 180, and 270 days after treatments.

The soil nematode density was determined following the method described by Ha Tran Thi Thu and Ton Nguyen Tang (2011). The pathogen nematodes and fungi reduction percentage was calculated by Henderson-Tilton's formula (Henderson and Tilton, 1955) according to the following equation:

$$\% \text{ population reduction} = \left(1 - \frac{T_a \times C_b}{C_a \times T_b} \right) \times 100$$

where: T_a =number of pathogen after treatment; T_b =number of pathogen before treatment; C_a =number of pathogen in the control after treatment; C_b =number of pathogen in the control before treatment

The rate of coffee plants damaged by the nematode was calculated following the QCVN01-38:2010/BNNPTNT: National

technical regulation on surveillance method of plant pests. The density of soil pathogen fungi was determined following the

methods described by Burgess et al. (2009). The IRRISTAT statistical and data processing program (ANOVA) is used for

the study data processing, and the difference between the mean values was determined with 95% confidence.

Table 3. Treatments of greenhouse and field experiments

Experiments	Treatment	Inoculated microorganisms
Greenhouse experiment on the synergistic effect of nematophagous fungi Pae and NVC7.4	I	<i>P. coffeae</i> (control)
	II	<i>P. coffeae</i> + Pae
	III	<i>P. coffeae</i> + NVC7.4
	IV	<i>P. coffeae</i> + Pae + NVC7.4
Greenhouse experiment on the synergistic effect of antagonistic microorganisms: P9.1, 2P, and TQHT01	I	<i>F. oxysporum</i> (control)
	II	<i>F. oxysporum</i> + TQHT01
	III	<i>F. oxysporum</i> + 2P
	IV	<i>F. oxysporum</i> + P9.1
	V	<i>F. oxysporum</i> + TQHT01 + 2P + P9.1
Greenhouse experiment on the synergistic effect of nematophagous fungi Pae and NVC7.4 and antagonistic microorganisms P9.1, 2P, and TQHT01	I	<i>P. coffeae</i> (control)
	II	<i>F. oxysporum</i> (control)
	III	<i>P. coffeae</i> + <i>F. oxysporum</i> + Pae + NVC7.4
	IV	<i>P. coffeae</i> + <i>F. oxysporum</i> + TQHT01 + 2P + P9.1
	V	<i>P. coffeae</i> + <i>F. oxysporum</i> + Pae + NVC7.4 + TQHT01 + 2P + P9.1
Small scale field experiment evaluating the effect of new bio product on the coffee production at young stage	I	FP (- control)
	II	New bio product
	III	Padave (+ control)
Small scale field experiment evaluating the effect of new bio product on the coffee production at the mature stage	I	FP (- control)
	II	New bio product
	III	Padave (+ control)

3. RESEARCH RESULTS AND DISCUSSION

3.1. Synergistic effect of nematophagous fungi against *Pratylenchus coffeae*

The data of the study on the effect of nematophagous fungi to control coffee parasitic nematode presented in Table 4 showed that nematophagous fungi strain A.

oligospora NCV 7.4 and *P. lilacinum* Pae alone reduced the population of *P. coffeae* by 76.43% and 70.68% and the combination of both nematophagous fungi strains reduced 83.01% population of *P. coffeae* after 90 days of infection.

Coffee plants inoculated with both nematophagous fungi strains had the yellow

leaf plants significantly lower than control and treatments inoculated with a single strain. (Figure 1)

Arthrobotrys is a nematophagous fungus, adapted to different environmental conditions, and can be used as a bioagent to control parasitic nematodes by the capability to trap the nematodes (Nguyen Viet Hiep et al., 2019). Singh et al. (2012) reported that *A. oligospora* can reduce by 44.0 - 66.8% population of nematodes in the soil. In our research, the nematode population is reduced by 76.4% in the

greenhouse after 3 months of treatment. According to Azam et al. (2013), *Paecilomyces lilacinus* (Thom) is ubiquitously distributed, especially in warm climates, and can be applied as a bioagent to control the plant-parasitic nematodes. Our findings are similar to reports by Ahmad et al. (2019) that *P. lilacinus* can reduce the population of nematodes, and the combined application of *P. lilacinus* and *M. lysipagum* reduced the population of nematodes more than the application of a single fungus.

Table 4. Synergistic effect of Paecilomyces lilacinum Pae and Arthrobotrys oligospora NCV 7.4 on the control of coffee parasitic nematodes

Treatments	Nematode density (nematodes/100 g soil) after treatment			Reduction of nematode population after 90 days (%)
	30 days	60 days	90 days	
<i>P.coffeae</i>	275.40	310.25	365.10	-
<i>P.coffeae</i> + Pae	160.25	138.25	107.15	70.68
<i>P.coffeae</i> + NCV 7.4	132.35	108.40	86.45	76.43
<i>P.coffeae</i> + NCV 7.4 + Pae	117.25	85.45	62.55	83.01

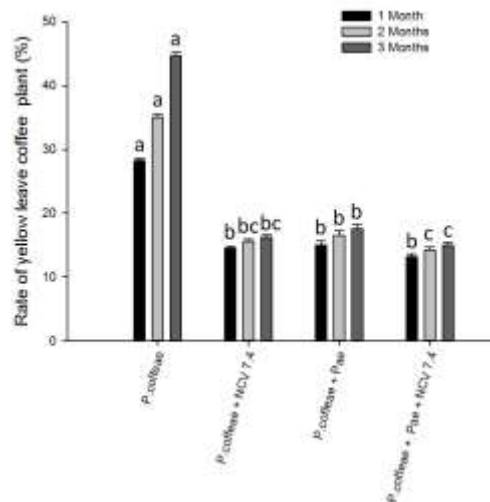


Figure 1. Effects of *P. lilacinum* Pae and *A. oligospora* NCV 7.4 on yellow leaf disease of coffee plant infected with the nematode

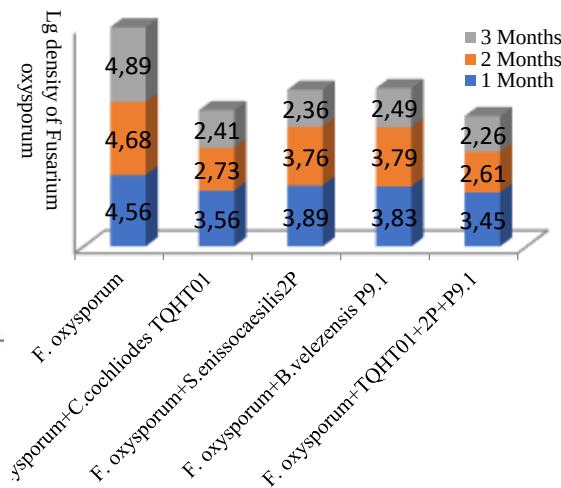


Figure 2. Synergistic effect of antagonistic microbes on the controlling the density of *Fusarium oxysporum* in coffee growing soil

3.2. Synergistic effects of antagonistic microbial strains against *Fusarium oxysporum*

The result of the experiment on the synergy effects of antagonistic microbial strains in figure 2 showed the *F. oxysporum* density in the coffee cultivation soil decreased from $7,8 \times 10^4$ CFU/g to $1,8 \times 10^2$ CFU/g after 90 days when a consortium of antagonistic microorganism used, compared to $2,6 \times 10^2$ CFU/g, $5,7 \times 10^2$ CFU/g and $6,2 \times 10^2$ CFU/g when *Chaetomium cochliodes* TQHT01, *Streptomyces enissocaesilis* 2P, and *Bacillus velezensis* P9.1 used alone.

The percentage ratio of yellow leaf plants, in this case, is significantly lower than the control without inoculation of antagonistic microbial strains and lower than the treatment used *Chaetomium cochliodes* TQHT01, *Streptomyces enissocaesilis* 2P and *Bacillus velezensis* P9.1 alone (Table 5).

3.3. Synergistic effects of nematophagous fungi and antagonistic microorganisms against *Pratylenchus coffeae* and *Fusarium oxysporum*

The greenhouse experiments to evaluate the synergistic effects of nematophagous fungi and antagonistic microorganisms on the population of parasitic nematode and pathogen fungi in

the soil summarized in Table 6 indicated that synergy effects of nematophagous fungi and antagonistic microorganisms can reduce the population of parasitic nematode and pathogen fungi in the soil better than nematophagous fungi or antagonistic microorganisms alone. The population of parasitic nematodes in the coffee-growing soil was reduced by 69.82% when treated with both nematophagous fungi and antagonistic microorganisms, compared to 61% when treated with nematophagous fungi or 67.24% when treated with antagonistic microorganisms. The same tendency in the reduction of *F. oxysporum* population, which was 76.44% when both nematophagous fungi and antagonistic microorganisms were used, compared to 52.17% when treated with nematophagous fungi or 75.53 % when treated with antagonistic microorganisms after 180 days.

The reduction in disease incidence of the plant by using both nematophagous fungi and antagonistic microorganisms after 180 days was 67.04% for *F. oxysporum* infection and 67.40% for *P. coffeae* infection, which is higher than the treatment using nematophagous fungi alone and same as antagonistic microbial strains alone. (table 6).

Table 5. Synergistic effect of antagonistic microbial strains on the control of yellow leaf of the coffee plant in the greenhouse experiment

Treatments	% yellow leaf coffee plant after inoculation		Reduction of yellow leaf coffee plant after 90 days of inoculation compared to a positive control
	30 days	90 days	
<i>F. oxysporum</i> (+ control)	27.54	35.26	-
<i>F. oxysporum</i> + 2P	17.56	18.26	48.21
<i>F. oxysporum</i> + P9.1	16.82	18.05	48.81
<i>F. oxysporum</i> + TQHT01	15.27	16.54	53.09
<i>F. oxysporum</i> + P9.1+ 2P+ TQHT01	14.27	15.42	56.27
Without inoculation (- Control)	2.06	10.20	-
LSD _{0,05}	0.63	0.77	-

Table 6. Synergistic effect of nematophagous fungi and antagonistic microbes on the population of parasitic nematode and pathogen fungi in the coffee-growing soil

Treatments	Nematode density			Population reduction compared to control after 180 days (%)
	No of nematodes per 100 g of soil after treatment			
	30 days	90 days	180 days	
<i>F. oxysporum</i> (control)	-	-	-	-
<i>P. coffeae</i> (control)	285.67	318.82	365.28	-
<i>F. oxysporum</i> + <i>P. coffeae</i> + Pae+ NVC7.4	186.34	165.24	142.45	61.00
<i>F. oxysporum</i> + <i>P. coffeae</i> + TQHT01+ 2P +P9.1	147.36	130.56	119.67	67.24
<i>F. oxysporum</i> + <i>P. coffeae</i> + TQHT01+ 2P +P9.1+ Pae+ NVC7.4	135.27	127.12	110.23	69.82
<i>LSD 0.05</i>	7.51	6.75	9.85	-
Treatments	<i>F. oxysporum</i> density			Population reduction compared to control after 180 days (%)
	10 ³ CFU/g soil after treatment			
	30 days	90 days	180 days	
<i>F.oxysporum</i> (control)	75.23	91.56	98.74	-
<i>P. coffeae</i> (control)	-	-	-	-
<i>F.oxysporum</i> + <i>P. coffeae</i> + Pae+ NVC7.4	52.26	49.14	47.23	52.17
<i>F.oxysporum</i> + <i>P. coffeae</i> + TQHT01+ 2P +P9.1	35.27	30.26	24.16	75.53
<i>F.oxysporum</i> + <i>P. coffeae</i> + TQHT01+ 2P +P9.1+ Pae+ NVC7.4	35.16	31.17	23.26	76.44
<i>LSD 0.05</i>	3.83	1.60	2.13	-

Ta Hong Linh et al. (2018) informed that *F. oxysporum* was the main pathogen agent damaging coffee plants in the replanting area of highland Taynguyen. Nematodes create mechanical wounds and damage the roots, then the fungus attacks, damaging and rotting the roots, leading to the plant's inability to absorb nutrients and water. Plants are weakened, with yellow leaves, wither, and die. Wanjohi et al. (2018) observed that parasitic nematodes in combination with the pathogen fungi enhanced the suppression of plant growth than that of the pathogen fungi and concluded that pathogen fungi and parasitic nematodes play important roles in reducing the quality and yield of host crops. Pal and

Gardener (2006) reported that biocontrol agents are antagonistic to the activities of plant pathogens as direct antagonism by hyperparasitism or predation, mixed-path antagonism by producing antibiotics, lytic enzymes, and secondary metabolites, direct antagonism by physical competition for resources, niche occupation, and induction of host resistance. Many papers presented the selection and use of *Bacillus spp.*, *Streptomyces spp.*, *Trichoderma spp.*, and *Chaetomium spp.* as antagonistic microorganisms to plant pathogen fungi (Mardanova et al. 2017; Bubici 2018; Boughalleb et al. 2018; Nguyen Thi Hong Minh et al. 2020). In our study, *Bacillus velezensis* P9.1, *Streptomyces*

enissocaesilis 2P, and *Chaetomium cochliodes* TQHT01 are used as antagonistic microorganisms to the coffee pathogen fungi. The research results indicated that the population of parasitic nematodes in the coffee-growing soil was reduced by 69.82% when treated with both nematophagous fungi and antagonistic microorganisms, compared to 61% when

treated with nematophagous fungi or 67.24% when treated with antagonistic microorganisms. The rate of yellow leaves in coffee of the treatment using a combination of microorganisms and using antagonistic microorganisms reached >67%, higher than the treatment using nematophagous fungi (table 7).

Table 7. Synergistic effect of nematophagous fungi and antagonistic microorganisms on yellow leaf of the coffee plant

Treatments	Rate of disease plant (%) after treatment			Reduction of rate of disease plant compared to control after 180 days (%)	
	30 days	90 days	180 days	<i>F. oxysporum</i>	<i>P. coffeae</i>
<i>F. oxysporum</i> (control)	27.54	35.26	43.57	-	
<i>P. coffeae</i> (control)	28.67	34.74	44.06		-
<i>F. oxysporum</i> + <i>P. coffeae</i> + Pae+ NVC7.4	18.36	19.36	20.35	53.29	53.81
<i>F. oxysporum</i> + <i>P. coffeae</i> + TQHT01+ 2P +P9.1	13.15	13.86	14.05	67.75	68.11
<i>F. oxysporum</i> + <i>P. coffeae</i> + TQHT01+ 2P +P9.1+ Pae+ NVC7.4	13.62	14.02	14.36	67.04	67.40
<i>LSD 0.05</i>	0.63	0.77	0.75	-	

3.4. Effects of new bioproduct on the coffee production

The small-scale field study on the effect of a new bioproduct containing both nematophagous fungi and antagonistic microorganisms showed that the nematode population was reduced by 77.16% in the coffee plantation at the young stage and 78.12% at the mature stage after 270 days

of treatment with the new bioproduct, compared to 70.22% and 70.97% by using Padave. The same tendency can be obtained with the reduction of *Fusarium spp.* population in the coffee plantation, which was 78.04% in the coffee plantation at the young stage and 79.26% at the mature stage, compared to 72.44% and 71.60% by Padave treating (Table 8).

Table 8. Effect of new bioproduct on the population of parasitic nematode and pathogen fungi in the coffee-growing soil

Coffee growing soil					
Treatments	Coffee plant at the young stage after treatment				Population reduction after 270 days (%)
	30 days	90 days	180 days	270 days	
	No of nematodes/100 g of soil (nematodes/g)				
FP (- control)	286.4	385.4	410.5	430.8	-
New bio product	121.1	108.5	100.6	98.4	77.16
Padave (+control)	150.7	128.5	120.6	128.3	70.22
<i>LsD 0.05</i>	6.67	7.40	1.07	1.32	
Treatments	<i>Fusarium. spp.</i> density (10 ³ CFU/g soil)				Population reduction after 270 days (%)
	30 days	90 days	180 days	270 days	
	No of nematodes/100 g of soil (nematodes/g)				
FP (- control)	74.8	85.9	92.37	95.73	-
New bio product	31.2	25.47	21.36	21.02	78.04
Padave (+control)	37.2	32.25	26.41	26.38	72.44
<i>LSD 0.05</i>	2.09	1.16	2.11	1.31	
Treatments	Coffee plant at the mature stage after treatment				Population reduction after 270 days (%)
	30 days	90 days	180 days	270 days	
	No of nematodes/100 g of soil (nematodes/g)				
FP (- control)	290.8	382.7	415.60	435.80	-
New bio product	136.5	118.2	106.37	95.36	78.12
Padave (+control)	158.3	142.7	132.60	126.50	70.97
<i>LsD 0.05</i>	4.61	3.27	5.27	4.55	
Treatments	<i>Fusarium. spp.</i> density (10 ³ CFU/g soil)				Population reduction after 270 days (%)
	30 days	90 days	180 days	270 days	
	No of nematodes/100 g of soil (nematodes/g)				
FP (- control)	41.28	46.27	52.74	61.52	-
New bio product	18.36	15.37	13.27	12.76	79.26
Padave (+control)	24.51	23.18	18.06	17.47	71.60
<i>LSD 0.05</i>	1.43	1.94	1.67	1.17	

The new bioproduct had a positive effect on the reduction of diseased coffee plants. The rate of yellow leaf coffee plants was 79.08% when the new bioproduct was

used in the coffee plantation at the young stage and 78.48% at the mature stage (Table 9).

Table 9. Effect of new bioproduct on yellow leaf disease of the coffee plant

Treatments	Coffee plant at the young stage				
	Rate of diseased plant (%) after treatment				Reduction of diseased plant compared to control after 270 days (%)
	30 days	90 days	180 days	270 days	
FP (- control)	49.35	74.38	83.26	87.25	-
New bio product	20.17	20	18.34	18.25	79.08
Padave (+ control)	24.35	25.17	24.57	23.26	73.34
<i>LSD 0.05</i>	1.28	2.47	0.98	1.75	-

Treatments	Coffee plant at the mature stage				
	Rate of diseased plant (%) after treatment				Reduction of diseased plant compared to control after 270 days (%)
	30 days	90 days	180 days	270 days	
FP (- control)	25.46	31.71	38.74	50.27	-
New bio product	10.26	11.57	11.52	10.82	78.48
Padave (+ control)	14.35	15.43	15.5	14.35	71.45
<i>LSD 0.05</i>	2.03	1.59	3.27	1.65	-

In the field demonstrations on the coffee plantation of Mr. Phan Hong Luan in Gialai province, the control effect on the yellow leaf disease of the coffee plant reached 82.3% after 9 months of treatment

with a new bioproduct (table 10). With a grain yield of 3.2 tons/ha, Mr. Luan gets a benefit of 9,400,000 VND/ha, equivalent to an income increase of 21.7% (Table 11).

Table 10. Control effect of new bioproduct on the yellow leaf disease of coffee plant (Field demonstration at coffee plantation of Mr. Phan Hong Luan in Gia Lai)

Time of evaluation (months)	Control		Demonstration		Control efficiency (%)
	Disease rate (%)	Disease index (%)	Disease rate (%)	Disease index (%)	
Before treatment	14.40	3.07	12.00	2.67	-
1 month after treatment	14.67	4.27	6.67	1.73	53.3
3 months after treatment	16.00	4.67	6.00	1.60	60.6
6 months after treatment	16.67	5.20	4.00	1.07	76.4
9 months after treatment	16.67	5.20	3.3	0.8	82.3

Using microbes to control root rot and root-knot nematode diseases is a promising method without negative effects on the environment and human health (Abd-Elgawad and Askary 2018). In a plant microbial ecosystem, fungi and bacteria play an important role in improving soil quality, plant growth, and plant resistance (Berendsen et al. 2018). The soil contains both pathogenic and nonpathogenic

microbes. Nonpathogenic plays roles in biodegradation, biocontrol, biotransformation, and phytoremediation (Dwibedi and Saxena 2019). Zhang et al. (2019) reported, the beneficial microbiomes can induce plant disease resistance, improve crop quality, suppress soil-borne disease, and improve the chemical properties of soil. The disease-suppressive soil is agricultural lands containing disease-

suppressing microbes that can pressure pathogens (Expósito et al. 2017). Various microbial inoculants have been developed by designing smart microbial consortia and have been successfully applied. Microbial consortia contain different traits that can combine different mechanisms for different effects, and tolerate different environmental

conditions (Berendsen et al. 2018). A new bioproduct containing both nematophagous fungi and antagonistic microorganisms is one of the microbial consortia and can be used as biocontrol for coffee yellow leaf and root rot disease complex in Tay Nguyen.

Table 11. Economical effect of new bioproduct on coffee production (Field demonstration at coffee plantation of Mr. Phan Hong Luan in Gia Lai)

Items	Control	Demonstration
Total input (1,000 VND)	55,800	53,000
Total outcome (1,000 VND)	99,000	105,600
Benefit (1,000 VND)	43,200	52,600
Benefit over control (1,000 VND)	-	9,400
Benefit over control (%)	-	21.75

Grain coffee price: 33,000 VND/kg; 1 USD = 25,000 VN

4. CONCLUSIONS

A combination of nematophagous fungi and antagonistic microorganisms reduced 69.82% the population of parasitic nematodes, compared to 61% and 67.24% when nematophagous fungi or antagonistic microorganisms were applied alone, and reduced 76.44% the *Fusarium oxysporum* population compared to 52.17% and 75.53% when nematophagous fungi or antagonistic microorganisms were applied after 6 months. By using the new bioproduct, the reduction of coffee plant-parasitic nematodes, pathogen fungi, and the rate of the diseased coffee plant were 77.16%, 78.04%, and 79.08% for the coffee plant at the young stage, and 78.12%, 79.26% and 78.48% for the coffee plant at the maturity stage after 270 days of application. Field demonstrations showed a disease reduction of 82.3% compared to the control, and brought a benefit of 9,400,000 VND/ha, equivalent to an income increase of 21.7% for farmers. The new bioproduct can be applied to control the yellow leaf and root rot disease complex of coffee plants in Tay Nguyen.

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