STUDY ON NEW BIOPESTICIDES VAAS-AT1 AND VAAS - AT2 TO CONTROL THE PEPPER ROOT ROT AND ROOT - KNOT NEMATODE DISEASE AND COFFEE YELLOW LEAF AND ROOT ROT DISEASE IN VIETNAM

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ABSTRACT

This study aimed at evaluating the efficacy of biopesticides containing nematophagous fungi and antagonistic microorganisms, named VAAS-AT1 and VAAS-AT2, for controllingplant-parasitic nematodes and pathogenic fungi causing root rot and root-knot nematode disease complex in black pepper plants and yellow leaf and root rot diseases in coffee plants in Vietnam. A series of experiments were conducted in greenhouses, small-scale experiments, and field demonstrations. The results showed that both biopesticides effectively reduced the population of plant-parasitic nematodes and pathogenic fungi in the soils growing with black pepper and coffee plants. In greenhouse conditions, VAAS-AT1 demonstrated an efficacy of 83.3% against *Phytophthoracapsici,* 79.5% against *Fusarium solani*, 60.6% against *Rhizoctonia solani,* and 77.8% against plant-parasitic nematodes after 3 months of treatment. In small-scale field trials, VAAS-AT1 achieved efficacies ranging from 81.3 to 83.5% against *Phytophthora* spp., 82.3 to 83.7% against *Fusarium* spp., and 80.3 to 81.9% against plant-parasitic nematodes after 9 months of treatment. In field demonstrations, the efficacy of VAAS-AT1 reached up to 79.3% in Dak Lak province and 77.6% in Gia Lai province against the pepper root rot disease after 9 months of treatment. VAAS-AT2 exhibited its efficacy in greenhouse conditions by reducing the population of plant-parasitic nematodes by 69.82% and *F. oxysporum* by 76.44% after 3 months of treatments. In small-scale field experiments, VAAS-AT2 achieved efficacies of 76.16 to 78.12% against plant-parasitic nematode and 78.04 to 79.26% against *Fusarium* spp. after 9 months of treatment. In the field demonstrations, the efficacy of VAAS-AT2 against yellow leaf disease in coffee plants reached 72.73 to 79.3% after 9 months of treatment in Dak Lak and Gia Lai provinces, respectively. Furthermore, the economic benefits of using VAAS-AT1 and VAAS-AT2 were substantial, with income increases of 17.89 to 25.80% for farmers and a benefit over control ranging from 5.260 to 22.500 million VND/ha. These findings demonstrate the promise of VAAS-AT1 and VAAS-AT2 as effective biopesticides for enhancing crop health and income for farmers in Vietnam.

Keywords: *Bio-pesticide, black pepper, coffee, root rot and root-knot nematode, VAAS-AT1, VAAS-AT2, yellow leaf and root rot.*

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1. INTRODUCTION

Black pepper and coffee industries play a vital role in Vietnam's economy, with the export values reaching USD 1.422 billion and 2.862 billion in 2019 [1]. However, the production of black pepper and coffee in Vietnam faces numerous challenges, including price fluctuation in international markets, the impact of climate change, and, notably, the emergence of destructive soil-borne diseases.

One of the biggest obstacles and costly burdens in agricultural crop production is plant-parasitic nematodes, which result in substantial economic losses estimated at USD 80 to 118 billion annually [2]. As reported by Yadav in 2017 [3], these

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nematodes cause an average yield loss of over 10% globally; forcertain crops, this loss exceeds 20%. In tropical and sub-tropical climates, the impact of plant-parasitic nematode is particularly pronounced, causing a crop production loss of about 14.6%, in contrast to 8.8% in developed countries. Additionally, plant-parasitic nematode spose a significant challenge to coffee growers, as estimated by Youssef in 2013 [4], with annual crop losses amounting to approximately USD 157 billion.The damage inflicted by nematodes often paves the way for secondary infection by soil-borne pathogens, including *Fusarium* spp., *Phytophthora* spp., and *Rhizoctonia* spp., among others. This leads to the complete deterioration of the root system, resulting in root rot and, ultimately, plant death [5, 6, 7].

Various species of fungi and bacteria have been identified as biocontrol agents for combating plant-parasitic nematodes and pathogenic fungi, as documented in previous studies [8, 9, 10, 11, 12, 13]. Biopesticides represent a sustainable approach within integrated pest management, with minimal environmental impact and no adverse effects on human health or animals. However, despite these benefits, the commercialization of biopesticides still needs to be improved. In 2017, the proportion of biopesticides used in agriculture compared to conventional chemical pesticides was estimated at around 5% [14]. This indicates a significant opportunity for expanding and adopting biopesticide solutions in agricultural practices.

Several biopesticides have been registered and authorized in Vietnam as plant protection agents. According to Circular No. 19/2020/TT-BNNPTNT, there are 16 active ingredients with 20 trade names derived from various bio-agents, including *Trichoderma, Streptomyces, Bacillus, Metarhizium, Paecilomyces*, and *Chaetomium.* These biopesticides have been recommended for controlling plant-parasitic nematodes and pathogenic fungi in cultivatingblack pepper and coffee plants [15]. Most currently available commercial biopesticidesare produced from one or more strains of antagonistic or nematophagous microorganisms of the same species, with or without the addition ofbiologically active ingredients. However, they do not contain a wide variety of biological agents. Consequently, the effectiveness of these biopesticides in controlling plant-parasitic nematodes and pathogenic fungi remains relatively low,mainly due to the influence of numerous biotic and abiotic factors.

Within the framework of national research projects aimed at developing new biopesticide for controlling plant-parasitic nematodes and pathogenic fungi that damage coffee and black pepper in Vietnam, the Vietnam Academy of Agricultural Sciences, in collaboration with its member institutes (Soils and Fertilizers Research Institute - SFRI, Plant Protection Research Institute - PPRI, Institute for Agricultural Environment - IAE, Agricultural Genetics Institute – AGI and Western Highlands Agriculture and Forestry Science Institute - WASI), has undertaken a comprehensive effort. This initiative involved the selection, identification, and evaluation of the efficacy of a diverse group of antagonistic microorganisms and nematophagous fungi, including *Purpureocillium lilacinum* Pae, *Arthrobotrys oligospora* NVC7.4, *Bacillus velezensis* P9.1, *Streptomyces enissocaesilis* 2P, *Chaetomium cochliodes* TQHT01, *Trichoderma asperellum* Trivass. The research has culminated in developing a technology for producing novel biopesticides named VAAS-AT1 for black pepper plants and VAAS-AT2 for coffee plants. These biopesticides harbor a beneficial microorganism density of 10^8 CFU/g [16].

The objective of this study was to evaluate the effectiveness ofthe newly developed biopesticides, VAAS-AT1 and VAAS-AT2, in controllingroot rot and root-knot nematodes disease complex in black pepper plants, as well as the yellow leaf and root rot disease of coffee plants in Vietnam.

2. MATERIALS AND METHODS

VAAS-AT1 was applied toblack pepper plants, while VAAS-AT2 was used for coffee plants, and both were produced using selected nematophagous fungi and antagonistic microorganisms with a beneficial microorganism density of 10^8 CFU/g. *Pratylenchus coffeae, Meloidogyne incognita, Phytophthora capsisi, F. solani, Fusariumoxysporum,* and *R. solani* were provided by the Plant Protection Research Institute. Coffee plants of the Tr4 variety and Vinh Linh black pepper variety were sourced from the Western Highlands Agriculture and Forestry Science Institute.

Greenhouse experiments wereconducted using sterilized soil, which wassubsequently infected with pathogenic fungi including *P. capsisi, F. solani, F.*

oxysporum, R. solani, and nematodes such as *P. coffeae, M. incognita.* VAAS-AT1 or VAAS-AT2 was inoculated when planting black pepper and coffee, while the control treatment did not receive this inoculation.

The soil contained a density of 10^4 CFU/g soil of pathogenic fungi, antagonistic microorganisms, and nematophagous fungi, along with 250 total nematodes at the adult stageper gram of soil. The density of pathogenic fungi and nematodes in the soil was monitored one and three months after the treatments. The density of plant-parasitic nematodes in the black pepper and coffee growing soils was determined using the method described by Ha and Ton (2011) [17], while the density of pathogenic fungi in the soils was calculated following the approach outlined by Burgess *et al.* (2009) [18]. Additionally, the density of *Phytophthora* spp.. was evaluated using the method described by Erwin and Riberrio (1996) [19]. The reduction percentages of plant-parasitic nematodes and pathogenic fungi were calculated using Henderson Tilton's formula:

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

Where: T_a=number of plant-parasitic nematodes or pathogenic fungi after treatment; T_b=number of plant-parasitic nematodes or pathogenicfungi before treatment; C_a=number of plant-parasitic nematodes or pathogenicfungi in control plots after treatment; C_b=number of plant-parasitic nematodes or pathogenicfungi in control plots before treatment.

The small-scale field experiments were carried out according to the Ministry of Agriculture and Rural Development regulations, following an integrated crop management system at both the young and mature stages of the coffee and black pepper plants. The treatments included: 1) Negative control, reflectingtypical farmer practices. 2) Application of VAAS-AT1 or VAAS-AT2 at a dose of 30 g per plant. The experiments were designed with 30 plants per treatment and organized into 3 replications. The density of plant-parasitic nematodes and pathogenic fungi in the soil was monitored after 3, 6, and 9 months of treatment. The reduction percentages were calculated using Henderson-Tilton's formula.

Two field demonstrations for each crop were established in Gia Lai and Dak Lak provinces, with two treatmentswithout replication, including 1) Farmer practices and 2) Application of VAAS-AT1 or VAAS-AT2 at a dose of 30g per plant. The total area of the field demonstration was 1 ha per location. The rate of damaging plants (efficacy) was monitored 1, 3, 6, and 9 months after treatment following the guidelines of TCVN 12561: 2018: Pesticides - Bio-efficacy field trials [20]. The efficacy (E) was calculated using the formula:

$$E(\%) = (1 - \frac{Ta}{Ca}) \times 100$$

Where: E(%) = efficacy, Ca = disease severityin the farmer practices. Ta = disease severityin the treatment.

The disease severity was evaluated using a disease rating scale from 0 to 4 as follows: Scale 0: no symptom; scale 1(N_1): less than 25% of leaves turned yellow; Scale 2 (N_2): between 25 and less than 50% of leaves turned yellow; Scale 3 (N_3): Between 50 and 75% of leaves turned yellow; Scale 4 (N_4): more than 75% of leaves turned yellow. The disease severity in each treatment was calculated using the formula:

$$\text{Disease severity (\%)} = \frac{(N_1 \times 1) + (N_2 \times 2) + (N_3 \times 3) + (N_4 \times 4)}{4 \times \text{number of surveyed plants}} \times 100$$

Where: N_1, N_2, N_3, and N_4: number of damaging plants of each scale.

All optimization studies were carried out, and the data was analyzed using single-factor analysis of variance (ANOVA). The data were all summarized as mean ± SD (standard deviation). Mean separation was performed using the Duncan test at P=0.05 whenever a significant ANOVA ($P< 0.05$) result occurred or was processed according to the IRRISTAT statistical and data processing program.

3. RESULTS

3.1. Efficacy of VAAS-AT1 in controllingplant-parasitic nematodes and pathogenic fungion black pepper

The results of the greenhouse experiments evaluating the efficacy of VAAS-AT1 in controlling plant-parasitic nematodes and pathogenic fungi on black pepper are summarized in table 1. The results indicated that VAAS-AT1effectively controlled both plant-parasitic nematodes and pathogenic fungi. After 3 months of treatment, VAAS-AT1 achieved remarkable efficacy with 83.3% against *P. capsici,* 79.5% against *F. solani*, 60.6% against *R. solani,* and 77.8% against plant-parasitic nematodes.

Table 1. Efficacy of VAAS-AT1 in controlling plant-parasitic nematodes and pathogenic fungi in soil cultivating black pepper *(Thegreenhouse experiment, 2019)*

Treatments	The population of pathogens in the soil after treatment of		Efficacy (%) after 3 months of treatment
	01 month	03 months	
	Phytophthora spp. *		
P. capsici	60.0c	63.3c	-
P. capsici + M. incognita	63.3c	70.0d	-
P. capsici + M. incognita + VAAS-AT1	15.0a	11.7ab	83.3
	Fusarium spp.density (10^3CFU/g soil)		
F. solani	8.0b	12.3b	-
F. solani + M. incognita	8.3b	13.0b	-
F. solani + M. incognita + VAAS-AT1	3.0a	2.7a	79.5
	Rhizoctonia spp. density (10^3CFU/g soil)		
R. solani	5.3c	7.7b	-
R. solani + M. incognita	6.0d	11.0c	-
R. solani + M. incognita + VAAS-AT1	2.7ab	4.3a	60.6
	Nematode density (numbers of total nematodes/100 g soil)		
P. capsici + M. incognita	467.7c	717.0b	-
P. capsici + M. incognita + VAAS-AT1	177.3a	159.3a	77.8

*Statistical analysis indicated that averages not followed by the same lowercase letter within columns are significantly different at a significance level of $P<0.05$, as determined by Duncan's multiple range test. *The density of Phytophthora spp. was evaluated based on the number of rose petals losing their color.*

The field trials on black pepper plants in WASI indicated that VAAS-AT1 significantly reduced the density of plant-parasitic nematodes and pathogenic fungi. Notably, at the young stage, its efficacy was remarkable, achieving 81.3% against *Phytophthora* spp., 82.3% against *Fusarium* spp., 81.9% against plant-parasitic nematodes. Similarly, at the maturity stage, after 9 months of treatment, VAAS-AT1 continued to exhibit high efficacy of 83.5% against *Phytophthora* spp., 83.7% against *Fusarium* spp., and 80.3% againstplant - parasitic nematodes, all incomparison to the control (Table 2).

Table 2. Efficacy of VAAS-AT1 in controlling plant-parasitic nematodes and pathogenic fungi in soil-growing black pepper plants *(The small field trials, 2019)*

Pepper plant at the young stage				
Treatments	The population of pathogens in the soil after treatment of			Efficacy (%) after 9 months of treatment
	03 months	06 months	09 months	
	Phytophthora spp. *			
VAAS-AT1	21.0	13.0	15.0	81.3

Control (non-treatment)	71.0	74.0	80.0	
CV(%)	16.8	10.6	11.7	
$LSD_{0.05}$	7.8	4.0	4.1	
	Fusarium spp. density (10^3CFU/g soil)			
VAAS-AT1	4.4	4.2	4.4	82.3
Control (non-treatment)	17.2	23.2	24.8	-
CV(%)	28.2	21.3	21.0	
$LSD_{0.05}$	3.0	2.6	2.7	
	Nematode density (nematodes/100 g soil)			
VAAS-AT1	453.7	176.7	283.0	81.9
Control (non-treatment)	1291.3	1022.7	1564.0	-
CV(%)	13.3	14.1	8.0	
$LSD_{0.05}$	180.5	110.6	95.9	
Pepper plants at the maturity stage				
Treatments	The population of pathogens in the soil after treatment of			Efficacy (%) after 9 months of treatment
	03 months	06 months	09 months	
	Phytophthora spp. *			
VAAS-AT1	6.0	2.8	2.8	83.5
Control (non-treatment)	18.4	15.2	17.0	
CV(%)	9.7	7.5	6.0	
$LSD_{0.05}$	8.6	4.9	4.3	
	Fusarium spp. density (10^3CFU/g soil)			
VAAS-AT1	9.2	4.2	6.0	83.7
Control (non-treatment)	31.0	22.6	36.8	-
CV(%)	28.0	20.4	19.6	
$LSD_{0.05}$	8.2	4.0	6.1	
	Nematode density (nematodes/100 g soil)			
VAAS-AT1	571.0	234.3	262.7	80.3
Control (non-treatment)	1265.3	1099.3	1335.7	-
CV(%)	7.7	14.2	7.8	
$LSD_{0.05}$	159.5	217.0	141.5	

**The population of Phytophthora spp. was evaluated as the number of rose petals losing their color.*

In the field demonstration of black pepper plantations in Gia Lai and Dak Lak provinces, the effectiveness of VAAS-AT1 was markedly superior to the farmers' conventional practices. The application of VAAS-AT1 resulted in a notable reduction in disease incidence and severity. Specifically, in Dak Lak province, disease incidence decreased from 1.31 to 0.62%,while disease severity dropped from 0.56 to 0.17% after 9 months of treatment. In Gia Lai province,the impact was even more significant, as disease incidence decreased from 17.60 to 6.40%, and disease severity reduced from 6.40 to 1.6% over the same period. The efficacy of VAAS-AT1 reached 79.3% in Dak Lak province and 77.6% in Gia Lai province after 9 months of treatment (Table 3).

Table 3. The efficacy of VAAS-AT1 on the plant parasitic nematodes of black pepper plants (*The field demonstrations, 2019 – 2020*)

Time of survey	Farmers' practices		Demonstration (VAAS-AT1 treated)		
	Disease incidence	Disease severity	Disease incidence	Disease severity	Efficacy (%)
Dak Lak province					
Day before treatment (Jun., 2019)	0.15	0.04	0.23	0.06	
1 month after treatment (Jul., 2019)	0.23	0.08	0.23	0.06	50.0
3 months after treatment (Sep., 2019)	0.77	0.35	0.38	0.12	77.8
6 months after treatment (Dec., 2019)	1.08	0.50	0.46	0.13	82.1
9 months after treatment (Mar., 2020)	1.31	0.56	0.62	0.17	79.3
Gia Lai province					
Day before treatment (Jun., 2019)	10.40	2.72	11.20	3.04	-
1 month after treatment (Jul., 2019)	15.20	4.64	11.20	3.04	41.4
3 months after treatment (Sep., 2019)	12.00	4.16	4.80	1.12	75.9
6 months after treatment (Dec., 2019)	13.60	4.96	4.00	0.96	82.7
9 months after treatment (Mar., 2020)	17.60	6.40	6.40	1.60	77.6

In Gia Lai province, the pepper corns yield reached 4 tons per hectare, while in Dak Lak province, it was even more substantial at 5.6 tons per hectare. As a result of implementing VAAS-AT1, farmers got a significant economic benefit, with income increases ranging from 21.900 million to 22.500 million VND per hectare, which equated to a notable income rise of 17.89 to 25.8% when compared to traditional farmer practices (Table 4).

Table 4. Economic efficiency of VAAS-AT1application in black pepper production *(Field demonstrations, 2019 - 2020)*

Items	Gia Lai province		Dak Lak province	
	Farmers' practices	Demonstration	Farmers' practices	Demonstration
Total input (million VND)	55.200	52.300	68.000	64.500
Total outcome (million VND)	140.000	160.000	193.800	212.800
Benefit (million VND)	84.800	107.700	125.800	148.300
Benefit over control (million VND)	-	21.900	-	22.500
Benefit over control (%)	-	25.80		17.89

Notice: Peppercornsprice: 38,000 VND per kg

3.2. Efficacy of VAAS-AT2 on controlling plant-parasitic nematodes and pathogenic fungiin coffee plants

The greenhouse experiments aimed at evaluating the effect of VAAS-AT2 on the population of plant-parasitic nematodes and pathogenic fungi in soil-growing coffee plants are summarized in table 5. The results indicated that VAAS-AT2is highly effective in reducing the population of plant-parasitic nematodes and pathogenic fungi in the soil. Specifically, after just 3 months of treatment, VAAS-AT2 led to a remarkable 69.82% reduction in the population of plant-parasitic nematodes in the soil. A similar trend was observed in the reduction of *F. oxysporum* population, which reached an impressive 76.44% reduction following the treatments.

Table 5. The effect of the VAAS-AT2 on the population of pathogenic fungi and nematodes in the coffee plants-growing soil ***(Greenhouse experiments, 2019)***

Treatments	Nematode density		
	Number of nematodes per 100 gram of soil after treatment		Population reduction compared to control after 3 months (%)
	01 month	03 months	
F. oxysporum (control)	-	-	-
P. coffeae (control)	285.67	318.82	-
F. oxysporum + *P. coffeae* +VAAS-AT2	135.27	127.12	69.82
CV(%)	2.30	2.10	-
$LSD_{0.05}$	7.51	6.75	-
Treatments	*F. oxysporum* density		
	10^3 CFU per gram of soil after treatment		Population reduction compared to control after 3 months (%)
	01 month	03 months	
F. oxysporum (control)	75.23	91.56	-
P. coffeae (control)	-	-	-
F. oxysporum + *P. coffeae* +VAAS-AT2	35.16	31.17	76.44
CV(%)	4.60	2.00	-
$LSD_{0.05}$	3.83	1.60	-

The small-scale field study examining the impact of VAAS-AT2 showed that the plant-parasitic nematode population was significantly reduced. At the early stage of coffee plantations, there was a substantial reduction of 77.16%, and at the maturity stage, this reduction increased to 78.12% after 9 months of treatment. A similar positive trend was observed in the reduction of *Fusarium* spp. population in coffee plantations, with a reduction of 78.04% at the young stage and an even more significant reduction of 79.26% at the maturity stage (Table 6).

Table 6. Effect of VAAS-AT2 on the population of plant-parasitic nematode and pathogenic fungi in the coffee-growing soil ***(Small-scale field experiments, 2019)***

Treatments	Coffee plant at the young stage after treatment			
	03 months	06 months	09 months	Population reduction after 9 months (%)
	Nematode density (Number of nematodes/100 g of soil)			
Control	385.4	410.5	430.8	-
VAAS-AT2	108.5	100.6	98.4	77.16
CV(%)	1.8	3.1	3.3	
$LSD_{0.05}$	7.40	1.07	1.32	
	Fusarium spp. density (10^3CFU/g soil)			
Control	85.9	92.37	95.73	-
VAAS-AT2	25.47	21.36	21.02	78.04
CV(%)	1.3	2.6	1.6	
$LSD_{0.05}$	1.16	2.11	1.31	

Treatments	Coffee plant at the maturity stage after treatment			
	03 months	06 months	09 months	Population reduction after 9 months (%)
	Number nematodes/100 g of soil (nematodes/g)			
Control	382.7	415.60	435.80	-
VAAS-AT2	118.2	106.37	95.36	78.12
CV(%)	0.9	1.4	2.8	
$LSD_{0.05}$	3.27	5.27	4.55	
	Fusarium spp. density (10^3CFU/g soil)			
Control	46.27	52.74	61.52	-
VAAS-AT2	15.37	13.27	12.76	79.26
CV(%)	3.7	3.3	2.2	
$LSD_{0.05}$	1.94	1.67	1.17	

In the field demonstrations conducted on coffee plantations in Dak Lak and Gia Lai provinces, the efficacy of VAAS-AT2 in controlling the yellow leaf disease of coffee plants reached an impressive 72.73 to 79.3% after 9 months of treatment with VAAS-AT2 (Table 7).

Farmers who applied VAAS-AT2 enjoyed a substantial economic benefit over the control, with an income increase from 5.260 to 9.400 million VND per hectare, which equaled a remarkable income boost of 21.75 to 27.62% compared to the control practices (Table 8).

Table 7. Efficacy of VAAS-AT2in controlling yellow leaf disease in coffee plants (*Field demonstrations, 2019-2020*)

Time of survey	Disease incidence		Efficacy (%)
	Farmers' practices	Demonstration	
Dak Lak province			
Day before treatment (Jun., 2019)	0.15	0.23	-
1 month after treatment (Jul., 2019)	0.23	0.23	50.0
3 months after treatment (Sep., 2019)	0.77	0.38	77.8
6 months after treatment (Dec., 2019)	1.08	0.46	82.1
9 months after treatment (Mar., 2020)	1.31	0.62	79.3
Gia Lai province			
Day before treatment (Jun., 2019)	14.40	12.00	-
1 month after treatment (Jul., 2019)	14.67	6.67	53.3
3 months after treatment (Sep., 2019)	16.00	6.00	60.6
6 months after treatment (Dec., 2019)	16.67	4.00	76.4
9 months after treatment (Mar., 2020)	15.33	4.00	72.73

Table 8. Economic efficiency of VAAS-AT2 in coffee production *(Field demonstrations, 2019-2020)*

Items	Gia Lai province		Dak Lak province	
	Control	Demonstration	Control	Demonstration
Total input (million VND)	55.800	53.000	44.000	44.500
Total outcome (million VND)	99.000	105.600	63.040	68.800
Benefit (million VND)	43.200	52.600	19.040	24.300
Benefit over control (million VND)	-	9.400	-	5.260
Benefit over control (%)	-	21.75		27.62

Notice: Coffee bean price: 33,000 VND/kg;

4. DISCUSSION AND CONCLUSION

Beneficial microorganisms have emerged as valuable bio-agents for controlling plant diseases, offering a reliable and environmentally friendly alternative to chemical pesticides in agricultural production. Microbial biopesticide represents an economically viable and eco-friendly strategy for managinga range of plant diseases and insect pests affecting various crops [14]. Traditionally, microbial biopesticide typically consisted of a single strain of microorganisms. However, the effectiveness and persistence of a single microbial inoculant are profoundly influenced by factors such as soil microbiota and environmental conditions, resultingin inconsistency and inefficacy [21, 22, 23]. The development of microbial biopesticides has shifted toward microbial consortia as a promising solution to address these challenges. Combining compatible consortia of multifunctional microorganisms holds the potential to effectively control soil-borne pathogens through both direct and plant-mediated disease suppression. Synthetic microbial consortia offer a promising strategy to enhance the functionality and versatility of microbial biological control [22]. These microbial consortia, composed of multiple functional microorganisms, have found wide application in agricultural production. They have successfully controlled soil-borne diseases in valuable crops by competing for nutrient resources and niches with pathogenic microorganisms. Additionally, they producevarious antimicrobial compounds, facilitate the development of systemic resistance, and regulate soil microbial communities. Previous studies have reported enhanced stability and efficacy when using microbial consortia compared to individual microorganisms [24, 25, 26].

The efficacy of microbial consortia, which include combinations of different beneficial microorganisms, has been demonstrated in various studies. For example, a consortium of *T. asperellum* GDFS1009 and *B. amyloliquefaciens* ACCC1111060 strains showed higher efficacy against *Botrytis cinerea* than individualstrains [27]. Similarly, the combination of *T. virens* GI006 and *B. velezensis* Bs006 was more efficient in combating the *Fusarium* wilt of cape gooseberry when compared to usingthe individual strains [28].

The utilization of microbial consortia represents a significant advancement in biopesticides. These consortia, composed of multiple beneficial microorganisms, offer a more consistent and practical approach to disease management, providing a sustainable and eco-friendly solution for modern agricultural challenges. The findings presented in this study, particularly the efficacy of VAAS-AT1 and VAAS-AT2 in controlling plant-parasitic nematodes and pathogenic fungi in black pepper and coffee plants, demonstrate the potential of these microbial biopesticides to benefit crop production in Vietnam. This research lays the foundation for further exploration and development of microbial consortia for disease control and sustainable agriculture.

In the present study,we evaluated the new biopesticides named VAAS-AT1, and VAAS-AT2 for controlling plant-parasitic nematodes and pathogenic fungi on black pepper and coffee plants in Vietnam. The results indicated that VAAS-AT1 and VAAS-AT2 significantly reduce the population of plant-parasitic nematodes and pathogenic fungi in soils-growing black pepper and coffee plants. Forblack pepper plants, VAAS-AT1 exhibited a remarkable efficacy of 83.3% against *P. capsici*, 79.5% against *F. solani*, 60.6% against *R. solani*, and 77.8% against plant-parasitic nematodes after 3 months of treatment in the greenhouse condition. Its efficacy was 81.3 to 83.5% against *Phytophthora* spp., 82.3 to 83.7% against *Fusarium* spp., and 80.3 - 81.9% against plant-parasitic nematodes after 9 months of treatment in small-scale field experiments. In the field demonstration, the efficacy of VAAS-AT1 reached 79.3% in Dak Lak province and 82.7% in Gia Lai province against root-knot disease in black pepper plants after 9 months of treatment. VAAS-AT2 reduced the population of plant-parasitic nematodes by 69.82% and the *F. oxysporum* population by 76.44% after 3 months of treatments in greenhouse conditions. In small-scale field experiments, its efficacy remained high at 77.16 to 78.12% in the nematode population and 78.04 to 79.26% in *Fusarium* spp. population after 9 months of treatment. In the field demonstrations on coffee plantations in Dak Lak and Gia Lai provinces, VAAS-AT2 achieved an efficacy of 72.73 to 82.1% in controlling yellow leaf disease after 9 months of treatment. The economic benefits of using VAAS-AT1 and VAAS-AT2 were substantial, with income increases ranging from 21.900 to 22.500 million VND per hectare, equivalent to an income increase of 17.89-25.8% and 5.260 to 9.400 million VND per hectare, equivalent to an income growth of 21.75 to 27.62%, respectively. These research results strongly indicate that VAAS-AT1 and VAAS-AT2 can serve as effective new biopesticides for controlling the complex black pepper root rot, root-knot nematode diseases, and coffee yellow leaf and root rot disease. These findings promise to enhance crop production in Vietnam and present a significant advancement in sustainable agriculture practices.

Nevertheless, developing VAAS-AT1 and VAAS-AT2 for controlling soil-borne plant-parasitic nematodes and pathogenic fungi on black pepper and coffee plants requires additional research. This research should encompass various environmental properties, and climatic conditionsto understand the dynamic interrelationship of nematophagous fungi and antagonistic microorganisms in different ecosystems. Conducting official field trials, overseenby designated organizations, is essentialtoenhance the quality of VAAS-AT1 and VAAS-AT2by refining their production technology. These efforts will further contribute to the practical and sustainable managing diseases in black pepper and coffee plants.

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