



Biodiversity of Soil Microorganisms and their Effects on Disease Management at Black Pepper Farms in Gia Lai Province

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Authors' contributions

This work was carried out in collaboration among all authors. Author THN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ADN and NQV managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Research on soil ecological systems, such as the soil characteristics and properties of the soil micro-flora system, is essential in the sustainable production of black pepper (*Piper nigrum* L.). The role of using beneficial microorganisms in the sustainable production of black pepper is in increase because many people are being aware of the hazards associated with consuming products with chemical substances. Twenty-seven soil samples were collected from Chu Se, Chu Prong and Duc Co district of Gia Lai province to determine the relationships between the ecological system, pathogenic fungi and nematodes in black pepper farms. The obtained results showed that the soil micro flora community was quite diverse. The average total density microorganism in Duc Co district, Chu Prong district and Chu Se district was of 8.41×10^6 CFU/g, 6.76×10^6 CFU/g and 8.91×10^6 CFU/g, respectively. The density of average total beneficial microorganisms in Duc Co district, Chu Prong district and Chu Se district was of 20.03×10^4 CFU/g, 14.25×10^4 CFU/g and 17.62×10^4 CFU/g, respectively. The use of organic fertilizers is common to the farms where high microbial densities were recorded. The relationship between the density of beneficial

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microorganisms (Nitrogen fixing bacteria, Phosphorus soluble bacteria, Cellulose degradation bacteria) and the density of fungal pathogens and nematodes was negatively correlated with $R = -0.84$ and $R = -0.81$ respectively. The results of correlation analysis showed that the density of beneficial microorganisms in the soil and the incidence of diseases in orchards were negatively correlated ($R = -0.69$). This study proposes that the correlation between the basic factor of soil ecological system with pathogenic fungi and nematodes plays a very important role in biological control and the sustainable production of black pepper.

Keywords: *Microorganisms; beneficial microorganisms; pathogenic microorganisms; black pepper (General comment on abstract: rephrase sentences and follow basic arrangement).*

1. INTRODUCTION

Although plant physiologists sometimes view soil as simply a source of nutrients to plants, it is actually a complex ecosystem hosting bacteria, fungi, protists, and animals [1,2]. In the soil environment, there are countless species of organisms, of which microorganisms account for extremely large numbers. Microorganisms in soil play an important role in soil fertility and crop yields. They could metabolize large amounts of organic and inorganic substances forming a series of living active products including biologically active substances such as enzymes, antibiotics, vitamins and toxins [3]. Moreover, microorganisms have the ability to stimulate plant growth and inhibit pathogens [4,5]. Three mechanisms are usually put forward to explain how microbial activity can boost plant growth: (1) manipulating the hormonal signaling of plants [6]; (2) repelling or outcompeting pathogenic microbial strains [7]; and (3) increasing the bioavailability of soil-borne nutrients [8]. Therefore, studies on microorganism ecosystems in the soil and the correlation between groups of microorganisms are extremely necessary.

Gia Lai Province is located in the Central Highlands of Vietnam; within the range of geographic coordinates from $12^{\circ}58'20''N$ - $14^{\circ}36'30''N$ and from $107^{\circ}27'23''E$ - $108^{\circ}54'40''E$. Gia Lai has the second largest natural area in the whole country at $15,510 \text{ km}^2$, with 16,278 planted hectares of black pepper [9]. Black pepper is the key agricultural crop in Gia Lai Province, but black pepper farms do not follow the best cultivation practices and do not invest enough in soil fertility improvement and pathogens management [10]. Current trends in pepper cultivation, fertilizers, and wide-scale applications of broad-spectrum organophosphate pesticides could result in a degrading ecological environment besides shortening the lifespan of pepper vines [11]. As a result, the negative situation of pests and diseases on black pepper

plants is growing stronger and harder to control [11]. Rapid wilt disease caused by the fungus *Phytophthora* sp., and slow wilt caused by nematode and *Fusarium* sp., are two of the most serious diseases encountered in black pepper production [12].

Plant protection agrochemicals such as Fungicides kill, Nematicides are widely used around the world to control crop pests and diseases [13], but agricultural chemicals with broad spectrum also kill many beneficial microorganisms [14]. Following the old farming method, Farmers often have the habit of using overuse of chemical fertilizers and pesticides. Heavy treatment of soil with pesticides can cause populations of beneficial soil microorganisms to decline. Megir and Paulus observed that in most black pepper farms, intense use of farming chemicals causes the ecosystem to be degraded and therefore not suitable for microbial communities to live in soil [11]. Megir and Paulus added that these microbes may not be able to withstand the intolerable pH, moisture, and temperature conditions of degraded soils [11]. Replacing the old farming practices is the new trend of sustainable, environmentally safe agricultural production; this is the current general trend in agricultural production worldwide and in Vietnam. In particular, the trend includes researching and using useful microorganisms as an effective solution [15,16,17]. Therefore, the collection and conservation of microorganisms in black pepper cultivation land, exploitation and application in biological control and sustainable development of black pepper are very urgent challenges. Due to this fact, this project was to assess the level of soil microbiological biodiversity, in order to evaluate the relationship between the soil microorganism to the system, and soil ecological characteristics and factors causing diseases. (This section needs total overhaul and restructuring)

2. METHODS

2.1 The Study Area and Sampling

Twenty-seven soil samples were collected in the main black pepper growing areas in Gia Lai Province, nine farms for each district (Duc Co district: 13°48'3"N 107°40'46" E, Chu Prong district: 13°44'28"N 107°54'8"E, Chu Se district: 13°43'0"N 108°3'51"E). At each site, we collected five soil samples at a depth of 0-30 cm, took five diagonal points, and mixed them into one sample. Selected farms were 4 to 7 years old and exclusively grew black pepper. Soil samples were collected around tree roots. The samples were transported in polyethylene bags on ice packs to the laboratory. When samples could not be processed immediately, they were stored at 4°C for about 18-24 h.

2.2 Methods for Analysis of Soil Samples

2.2.1 Determination of microbiological density

Microbiological density was determined by culture-dependent method consisting of counting the number of colonies growing on agar. The soil samples were mixed, finely ground in a sterile porcelain mortar, and 1 g of sample was added to 9 ml of sterile physiological saline (sodium chloride 0.9%), homogenized and then diluted. Using a sterile pipette, 0.1 ml of the sample solution at different dilution rates was pipetted into a petri dish containing a suitable selective medium (PCA for Microorganism Total, PDA for fungi, Gause for actinomycetes...), and spread evenly with a glass rod, replicated three times per selective medium. Petri plates were incubated at room temperature for 2-3 d and colonies were counted [18,19]. Selected dishes with colonies numbering from 30-300 were chosen to calculate the results. The density of microorganisms in 1 g of sample is calculated as follows:

$$A(\text{CFU/g}) = N / (n_1 \cdot V \cdot f_1 + \dots + n_i \cdot V \cdot f_i)$$

Therein: -

A: colony forming unit

N: total count of colonies on the selected dishes

n_i: number of colonies at dilution i

f_i: equivalent dilution

V: volume of sample solution (ml) implanted in each dish

2.2.2 Determination of beneficial microorganism's density in the soil

The density of beneficial microorganisms was determined by the culture-dependent method of counting the number of colonies growing on a selective agar medium.

+ The density of nitrogen fixing bacteria was determined on Glucose Nitrogen Free Mineral Medium (GNFMM). The composition of the isolated medium was as follows (g/L): 1.0 K₂HPO₄, 1.0 CaCl₂, 0.5 NaCl, 0.25 MgSO₄·7H₂O, 0.01 FeSO₄·7H₂O, 0.01 Na₂MoO₄·2H₂O, 0.01 MnSO₄·5H₂O and the carbon source was glucose (7 g/L). Solid medium was produced by adding 2% agar [20].

+ The density of phosphorus soluble microorganism was determined on NBRIP medium. The NBRIP growth agar medium containing (per L) glucose, 10 g; Ca₃(PO₄)₂, 5 g; MgCl₂·6H₂O, 5 g; MgSO₄·7H₂O, 0.25 g; KCl, 0.2 g and (NH₄)₂SO₄, 0.1 g, agar 1.5%. The pH of the agar medium was adjusted to 7.0 before autoclaving. Tricalcium phosphate was autoclaved separately. The other sterile ingredients were then aseptically mixed after autoclaving [21].

+ *Actinomycetes* were inoculated on Gause I. Bacteria, and molds were cultivated on Vinogradski and Czapek media, respectively, supplemented with CMC (Carboxymethyl Cellulose) as the carbon resource [22,23].

2.2.3 Methods for determining the density of fungal pathogens in the soil

The soil samples were diluted with saline solution and then inoculated into PDA medium amended with 0.001% chloramphenicol and incubated at room temperature for 3-5 d. The density of *Fusarium* sp., *Phytophthora* sp., *Rhizoctonia* sp. was determined based on morphological identification for fungi [24].

2.2.4 Counting nematodes in the soil

Nematodes in the soil were filtered using the Berman method. A total of 50 g of soil was weighted and placed into a 10cm diameter sieve, with a filter cloth to prevent soil and plant residues from falling onto the Petri dish. The sieve was placed in a petri dish and filled with water, with the water level kept at half of the sieve height. The nematodes moved through the

membrane and fell down into the petri dish. Filter time was 24 h at room temperature. The sieve was then removed from the dish and the filtrate was collected inside the disc. We determined the composition and density of nematodes using microscopes and stereoscopes [25].

2.2.5 Methods of investigation and assessment of disease status

Investigating and evaluating pepper gardens in Chu Se district, Chu Prong district and Duc Co district, Gia Lai province. The survey method is based on the National Technical Regulation on the investigation method of detecting major pests of pepper plants QCVN 01 - 172: 2014 / BNNPTNT issued by the Ministry of Agriculture and Rural Development of Vietnam. In each garden, survey 10 points randomly or randomly located diagonally across the survey area. In each garden, survey 10 points randomly or randomly located diagonally across the survey area. The survey site must be located at least 5m from the outer edge of the garden. At each point, investigate 1 pillar. Observe remote recognition up close, then investigate directly on the tree or its parts. Collect infected parts of plants and bring them to the laboratory to identify the cause of the disease.

Pepper pathogens are determined by the method of making direct specimen observations of pathogenic microorganisms under a microscope or transplanting samples on the corresponding specific media for isolation, purification and observation. characteristics needed to identify pathogens by morphological method.

Disease incidence(%)=Number of infected plants/Total number of investigated plants x 100

2.2.6 Data analysis

Statistical analysis was performed using analysis of variance (ANOVA) and followed by Duncan's multiple range test with triplicate by SAS 9.4 software software, P-value ≤ 0.05 considered as significant.

3. RESULTS AND DISCUSSION

3.1 Microbial Biodiversity in the Black Pepper Soil

In the soil ecosystem, the diverse and abundant microorganisms play an important role in the process of soil formation, decomposition of

organic compounds, conversion cycle of important compounds, and provision of nutritional sources for plants, and as the indispensable link in the physical circulation of nature. Therefore, it is possible to evaluate soil quality, soil fertility based on soil microorganism density and soil microorganism activity, thereby offering suitable soil improvement measures [26].

Survey results and sample collection showed that the varieties of black pepper grown are quite diverse with many new black pepper varieties such as Sri Lanka, Loc Ninh, Ba Cang cultivated in this area. In particular, 74% of farms cultivate Vinh Linh pepper because it is suitable for the weather conditions in the Central Highlands with high drought tolerance and yield.

According to the results of our survey on the sampled farms, most farms use a harmonious combination of chemical and organic fertilizers (accounting for 92.6%). Among them, several farms use plant protection measures to prevent and treat diseases affecting black pepper plants. There are 41% of farms using pesticides with chemical origin for black pepper plant protection. The productivity of farms were quite high, from 3.0-3.5 tons/ha; especially farm productivity of 6.0 tons/ha was much higher than Vietnam's average productivity of 2.4 tons/ha in 2018.

Research results of soil microorganisms showed that the microorganisms in black pepper soil in Gia Lai Province were quite diverse and abundant. The average total microorganism density in Duc Co district, Chu Prong district and Chu Se district was of $8.41 \times 10^6 \pm 2.15$ CFU/g, $6.76 \times 10^6 \pm 2.07$ CFU/g and $8.91 \times 10^6 \pm 2.04$ CFU/g, respectively.

Among the surveyed farms in Gia Lai Province, the soil in V24 farm of Chu Se district was the one of the highest total aerobic microbial density of 11.82×10^6 CFU/g; which was 1.9 times higher than that of soil in the farm with the lowest density of microorganisms in the same district. The use of organic fertilizers is common to the farms where high microbial densities were recorded. In addition, the farm owners also pay attention to selecting seeds and improve farming techniques.

Farm V18 in Chu Prong district had the lowest total aerobic microorganism density in the surveyed gardens (4.61×10^6 CFU/g), with a low number of microorganisms. The farm owner use chemical fertilizers, do not add organic fertilizers,

use plant protection drugs of chemical origin. Intense use of farming chemicals causes the ecosystem to be degraded and therefore not suitable for microbial communities to live in soil [11]. The farm owner uses died stakes to plant black pepper but there are no measures to protect the trees in the dry season. Concrete pillars are neither waterproof nor beneficial for the growth of black pepper, especially in the hot dry season and the early period, thereby reducing the density of microorganisms in the soil. In addition, garden owners also use herbicides, pesticides and some chemical fungicides that adversely affect the soil microbiota. The herbicides in small or high doses affect the organisms living in the soil, causing an accumulation of toxins in the organisms, leading to their weakening or even death, thereby reducing their numbers [14,27]. For example, fungicides strongly affect useful microorganisms such as nitrate and nitrifying bacteria.

Different types of soil have different nutrient, moisture, aeration, and pH conditions; therefore, the distribution of microorganisms also differs. Among the 3 surveyed districts, the soil collected in Chu Se district has the highest average microorganism density, which was 1.32 times and 1.06 times higher than that in Chu Prong and Duc Co districts, respectively.

3.2 Diversity of Soil Microorganisms in Black Pepper Farms

Soil microorganisms are the driving factor behind soil organic C dynamics and decomposition, and soil microbial properties (such as microbial activity, microbial biomass, and microbial diversity) are recognized as sensitive indicators of soil health and quality [14]. High microbial density is the factor that makes the soil more fertile, helping plants to grow well. Microorganisms provide vitamins and beneficial growth substances for plants to grow and

develop; they also use plant secretions as nutrients for their growth.

Table 3 and Table 4 showed that the density of beneficial group microorganisms and fungal pathogens and nematodes varied among different regions. Among the soil in three surveyed districts, the soil collected in Duc Co Province possessed a higher density of beneficial microorganisms in compared to the soil collected in the other two districts. The average density of beneficial microorganisms was from 1.14 to 1.40 times higher than that of beneficial microorganisms in soils collected Chu Se and Chu Prong district. Soil in Farm V13 in Chu Prong district has the highest average density of beneficial microorganisms (total density of the 3 groups' beneficial microorganisms in soil is 20.03×10^4 CFU/g soil) . The farm owner avoiding chemical drugs, use microbial organic fertilizer, adding effective microorganisms to the soil, which help to increase the density of beneficial microorganisms in the soil. In addition, the farm owner also proactively protects the plants with biological products (chitosan oligomer, copper nanoparticles...), the density of fungal pathogens and nematodes in the soil is low; the black pepper plants grow healthy and have few diseases.

A previous work showed that, the use of effective microorganisms in agricultural soil suppress soil-borne pathogens [28]. The beneficial microorganisms decompose and ferment organic fraction of the soil system converting it into humus containing nutrients while releasing hormones that facilitate plant growth. They are responsible for providing hormones, nutrients and minerals in a useable form to the plants through the root system. In addition, they bring together soil particles in the soil structure enabling it to retain nutrients and moisture [29].

Table 1. Characteristics of sampling farms in Gia Lai province

Characteristics of sampling farms	Duc Co district	Chu Prong district	Chu Se district
Tree age (years)	4-7	4-7	5-7
The rate use of living plant stakes	33.33%	44.44%	33.33%
The rate use of died wood stakes	66.67%	55.56%	66.67%
Vinh Linh black pepper variety	66.67%	77.78%	77.78%
Fertilizing chemicals	11,11%	11,11%	0%
Applying organic fertilizer + chemical fertilizer	88,89%	88,89%	100%
The rate of chemical pesticide use	33.33%	55.56%	33.33%
Prevalence (rapid death, slow death)	18%	28%	20%
Productivity (ton/ha)	2.0-5.0	1.8-6.0	2.0-5.0

Table 2. Group Biodiversity of soil microorganisms in black pepper farms (±SD)

Location	Farm notation	Microorganism Total x10 ⁶ CFU/g	Bacteria x10 ⁶ CFU/g	Actinomycetes x10 ⁵ CFU/g	Fungi x10 ⁵ CFU/g	Number of species
Duc Co	V1	10.51 ± 0.13 ^a	10.11 ± 0.14 ^a	2.34 ± 0.06 ^c	1.68 ± 0.09 ^{ab}	30
	V2	10.37 ± 0.15 ^a	9.89 ± 0.13 ^a	3.24 ± 0.10 ^a	1.54 ± 0.19 ^{bc}	33
	V3	10.68 ± 0.35 ^a	10.23 ± 0.36 ^a	2.77 ± 0.21 ^b	1.77 ± 0.16 ^a	29
	V4	4.74 ± 0.25 ^f	4.57 ± 0.26 ^f	0.87 ± 0.11 ^f	0.82 ± 0.05 ^e	10
	V5	7.00 ± 0.24 ^d	6.78 ± 0.22 ^d	1.02 ± 0.13 ^f	1.16 ± 0.10 ^d	15
	V6	9.23 ± 0.13 ^b	8.89 ± 0.12 ^b	1.98 ± 0.08 ^{de}	1.45 ± 0.11 ^c	23
	V7	8.03 ± 0.23 ^c	7.72 ± 0.22 ^c	1.83 ± 0.06 ^e	1.23 ± 0.10 ^d	20
	V8	9.28 ± 0.26 ^b	8.92 ± 0.27 ^b	2.05 ± 0.11 ^d	1.56 ± 0.12 ^{bc}	26
	V9	5.83 ± 0.38 ^e	5.65 ± 0.39 ^e	0.99 ± 0.04 ^f	0.83 ± 0.09 ^e	16
	ANOVA ^z	**	**	**	**	
Chu Prong	V10	6.99 ± 0.13 ^c	6.77 ± 0.14 ^{bc}	1.13 ± 0.07 ^c	1.02 ± 0.07 ^c	14
	V11	4.73 ± 0.11 ^f	4.58 ± 0.10 ^f	0.79 ± 0.10 ^d	0.73 ± 0.06 ^d	11
	V12	7.10 ± 0.13 ^{bc}	6.90 ± 0.15 ^{bc}	0.98 ± 0.12 ^{cd}	0.98 ± 0.10 ^c	19
	V13	11.55 ± 0.22 ^a	11.07 ± 0.24 ^a	2.89 ± 0.2 ^a	1.91 ± 0.02 ^a	37
	V14	6.94 ± 0.11 ^c	6.67 ± 0.11 ^c	1.56 ± 0.11 ^b	1.09 ± 0.17 ^c	21
	V15	6.33 ± 0.25 ^d	6.07 ± 0.25 ^d	1.43 ± 0.16 ^b	1.12 ± 0.07 ^c	18
	V16	7.30 ± 0.15 ^b	7.02 ± 0.13 ^b	1.49 ± 0.13 ^b	1.30 ± 0.07 ^b	21
	V17	5.32 ± 0.13 ^e	5.11 ± 0.14 ^e	1.03 ± 0.06 ^c	1.02 ± 0.11 ^c	14
	V18	4.61 ± 0.09 ^f	4.45 ± 0.09 ^f	0.77 ± 0.10 ^d	0.81 ± 0.08 ^d	10
	ANOVA ^z	**	**	**	**	
Chu Se	V19	8.70 ± 0.22 ^d	8.33 ± 0.22 ^e	2.03 ± 0.05 ^b	1.66 ± 0.08 ^{bc}	24
	V20	10.18 ± 0.11 ^c	9.77 ± 0.11 ^c	2.14 ± 0.02 ^b	1.93 ± 0.06 ^a	30
	V21	10.10 ± 0.14 ^c	9.71 ± 0.15 ^c	2.09 ± 0.10 ^b	1.78 ± 0.1 ^b	31
	V22	6.33 ± 0.10 ^f	6.15 ± 0.10 ^g	0.93 ± 0.06 ^e	0.88 ± 0.03 ^e	15
	V23	6.99 ± 0.29 ^e	6.79 ± 0.28 ^f	1.03 ± 0.09 ^e	0.94 ± 0.05 ^e	17
	V24	11.82 ± 0.11 ^a	11.36 ± 0.11 ^a	2.60 ± 0.07 ^a	1.99 ± 0.12 ^a	35
	V25	10.94 ± 0.04 ^b	10.6 ± 0.04 ^b	1.78 ± 0.08 ^c	1.63 ± 0.05 ^c	28
	V26	8.89 ± 0.12 ^d	8.60 ± 0.14 ^d	1.47 ± 0.10 ^d	1.35 ± 0.09 ^d	21
	V27	6.24 ± 0.08 ^f	6.06 ± 0.09 ^g	0.91 ± 0.03 ^e	0.85 ± 0.07 ^e	16
	ANOVA ^z	**	**	**	**	

Where: **, *, significant differences at P 0.01, 0.05 and ns: no significant differences; Note: Different superscripts in the same column are significantly different between the treatments at 5% level according to Duncan's Multiple Range Test

Table 3. Average density of soil effective microorganisms in black pepper farms (±SD)

Groups of microorganisms	Duc Co	Chu Prong	Chu Se
Nitrogen fixing bacteria x10 ⁴ (CFU/g)	6.29 ± 2.20	4.57 ± 1.91	6.12 ± 2.17
Phosphorus soluble bacteria x10 ⁴ (CFU/g)	6.69 ± 2.21	4.76 ± 2.19	5.66 ± 2.10
Cellulose degradation bacteria x10 ⁴ (CFU/g)	7.05 ± 2.46	4.92 ± 2.22	5.84 ± 2.26

Results in Table 4 showed that the soil of black pepper farms in Chu Prong district had a higher density of fungal pathogens and nematodes compared to the other two regions (Duc Co and Chu Se). The average density of fungal pathogens was 4.10 x10¹ to 1.83 x10² (CFU/g), which was 1.55 to 1.6 times higher than that of Duc Co and Chu Se districts. Table 4 also showed that number of nematodes in Chu Prong district was 153 nematodes/50 g soil, which was 1.35 to 1.51 times higher than that of Chu Se and Duc Co districts. Based on investigated data, it is proposed that most farms applied too much chemical fertilizers and pesticides leading to high fungal pathogens and nematode densities present in the soils, which may have affected harmfully on soil microbiota, and beneficial microorganisms.

Results of the survey of pathogenic microorganisms also indicated that the disease status in several farms is already serious and easy to spread throughout the garden by germs. Fungal pathogens are spread by the contiguous layer of roots between diseased and non-diseased plants. On the other hand, the mature trees often have very far root-rot and the roots of diseased plants could be located nearby the roots of other plants. Therefore, it is necessary to dig deep trenches to isolate disease areas and deep trenches could separate the root circuits contacting with each other. Moreover, each disease area should be zoned to have appropriate treatment.

3.3 Correlation between Effective Microorganism and Pathogen Fungi and Nematodes

The soil microbial communities are known to affect plant fitness and soil quality [30]. Soil beneficial microorganisms play an important role in the metabolism and decomposition of complex organic compounds in the soil. Therefore, the growth of beneficial soil microorganisms is a contributing factor increasing soil fertility.

The results of correlation analysis in Fig. 2 showed that between the density of beneficial

microorganisms and fungal pathogens as well as nematodes were negatively correlated. The increase in beneficial microorganisms resulted in a decrease in pathogenic fungi and nematodes. The results in this study could be explained that beneficial microorganisms could invade through plant roots and stimulate plant growth as well as increase resistance to pathogens by antibiotics or enzymes that destroy cell walls [31,32]. In addition, these microbial groups are able to compete for nutrition and habitat with pathogens, thereby increasing the control and inhibition of pathogens [31,33,34].

The results of the current study indicated similarity with those of studies that showed correlation between beneficial microorganisms and soil pathogens. The diversity of *Actinomycetes* in the tomato root zone is inversely correlated with the severity of disease in tomato plants caused by *P. lycopersici* [35]. In another study, fungal diversity was also negatively correlated with the development of Adzuki stem rot disease caused by *A. gregatum* [36].

3.4 Correlation between Effective Microorganism and Disease Situation on Black Pepper

The results of correlation analysis showed that the density of beneficial microorganisms in the soil and the incidence of diseases in orchards were negatively correlated (Fig. 3). The beneficial microorganisms in soil through invading root plant roots, stimulate plant growth, and increase resistance to pathogens by antibiotics or enzymes that destroy cell walls [15,16,31,32]. In addition, these microbial groups are able to compete for nutrition and habitat with pathogens, thereby increasing the control and inhibition of pathogens [33,34].

Crop loss due to pest and disease incidence has been identified as one of the major pepper production constraints. This has resulted in a yearly reduction of about 2% of the total pepper area [37].

Table 4. Average density of pathogenic microorganisms in black pepper farms (±SD)

Pathogenic microorganisms	Value	Duc Co	Chu Prong	Chu Se
<i>Phytophthora</i> sp. x10 ² (CFU/g)	The average value	1.12 ± 0.35	1.83 ± 0.39	1.20 ± 0.29
<i>Fusarium</i> sp. x10 ¹ (CFU/g)	The average value	2.91 ± 1.03	4.36 ± 0.46	2.58 ± 0.71
<i>Rhizoctonia</i> sp. x10 ¹ (CFU/g)	The average value	2.46 ± 0.84	4.10 ± 0.76	2.68 ± 0.71
Nematodes (nematodes/50g soil)	The average value	101 ± 26	153 ± 27	113 ± 21

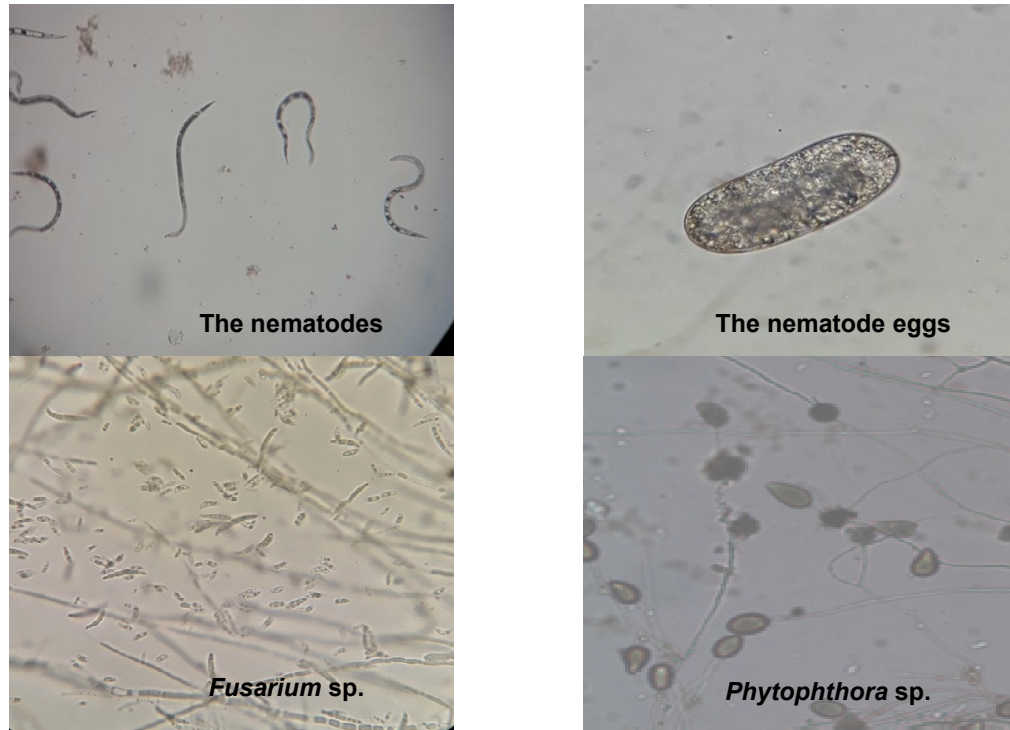


Fig. 1. The pathogenic microorganisms

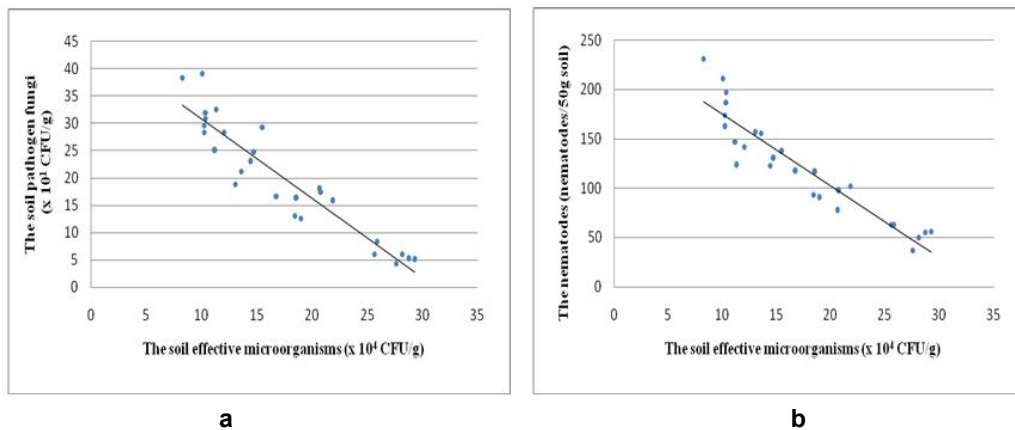


Fig. 2. The correlation analysis

- a. The correlation between the soil effective microorganisms and the soil pathogen fungi ($R = -0.84, n = 27$)
- b. The correlation between the soil effective microorganisms and the nematodes ($R = -0.81, n = 27$)

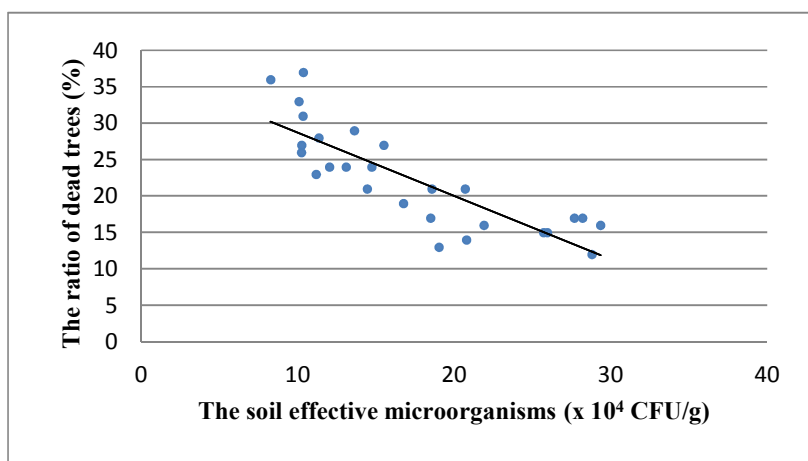


Fig. 3. Correlation between effective microorganism and disease situation on black pepper (R = - 0.69, n=27)

Firstly, black pepper live originally under forest and live supports such as *Cassia siamea*, *Wrightiaannamensis*, *Leucaenaleucocephala*, *Adenanthrapavonina*, *Glyricidiasepium* and *Gmelinaarborea*. These supports play important roles in yield, disease management, also improving quality. However, because of popular and available death supports and rapid growth of pepper areas, pepper growers tend to replace of live supports with dead standards [10]. Many scientists have emphasized that disease index and percentage of dead plant from foot rot were lower in the pepper fields with live support than use of dead wood standard, concrete and brick tower [38,39,40].

In Gia Lai province, the result investigation showed, 65% of black pepper areas applied dead wood support, the ate of disease in pepper farms ranged from 12% to 37%, the average rate was 22%, pepper plants suffer from diseases mainly quick wilt, slow wilt, anthracnose. Among the surveyed farmer households, there are a number of households who actively prevent disease in pepper gardens by applying lime, applying bio-products so that these households have a low rate of disease trees. When diseased trees were proactively zoned, ditched to isolate to avoid spreading to the whole garden so the disease rate was reduced.

4. CONCLUSION

Results of surveys and assessments of microbial density in soil at 27 black pepper farms showed that the soil microorganism system was quite diverse and abundant. Among the three districts

surveyed, Duc Co district is the region with the highest soil microbial density and low disease incidence. The gardens used a combination of inorganic fertilizer with organic fertilizer and used probiotics to prevent pathogens had a high density of microorganisms in the soil. In addition, there farms also pay attention to selecting seeds and farming techniques. The relationship between the density of beneficial microorganisms and the density of fungal pathogens and nematodes was negatively correlated with $R = - 0.84$ and $R = - 0.81$. The results of correlation analysis showed that the density of beneficial microorganisms in the soil and the incidence of diseases in orchards were negatively correlated ($R = - 0.69$).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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