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Research to Produce Biological Products of Chaetonium to Control Fungal Diseases on Tea, Coffee and Rubber

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Two strains of *Chaetomium* that popular and high antagonistic activity from tea, coffee and rubber soil in Northern Vietnam were identified, these are *Chaetomium bostrychoides* and *Chaetomium globosum.* The strains are strongly antagonistic activity with *Fusarium sp*. causing root rot tea, coffee and rubber and *Colletotrichum sp.* causing tea anthracnose. New *Chaetomium* strains identified, have successfully produced biological products for disease called CP2-VMNPB. The composition is capable of excluding fungal diseases well such as *Fusarium sp.* (85.62%), *Colletotrichum sp.* (81.80%) after one month in laboratory conditions; in greenhouse conditions, the effect on root rot tea and coffee reached 83.30%, with powdery mildew disease on rubber *Oidium Hevea*, the effect reached 81.17% after 3 months.

Keywords: tea, coffee, rubber, bio-fungicide, *Chaetomium, Fusarium, Colletotrichum theae-sinensis, Oidium heveae.*

Introduction

Tea, coffee and rubber are perennial plants survive and grow throughout the year which are continuously damaged by many types of pestilent insect and diseases. According C.K. Jayasinghe (2001), there are more than 60 diseases for rubber trees in Sri Lanka. Popular diseases for rubber in South Asia and Southeast Asia inlcude: anthracnose *Colletotrichum acutatum Sim., C. gloeosporioides Sacc.,* blight *Doidium heveae Stain, Phytophthora palmivora* But, tree wilt *Fusarium solani Sacc*., white root disease *Ove Rigidoporus microporus.*, brown root disease *Phellinus noxius* Corner, black root disease *Xylaria thwaitesii* Cooke. In Vietnam, the popular diseases are powdery mildew disease *Oidium heveae* on young leaves, causing these leaves yellow, withered, defoliated earlier and poor growth, defoliation in rainy season *Phytophthora palmivora* causing defoliation or death of young shoot; in addition, there are cracked shell disease, root rot.

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For coffee, according to Mike A. Rutherford (2006), rust *Hemileia vastatrix* can reduce over 70% of the productivity. The cost of buying annual fungicides for rust control is estimated at VND1-3 billion worldwide; dried fruit disease *Colletotrichum diseases* damage widespread all over the coffee growing countries which can reduce 75% of the productivity in case the desease is not controlled well; dry tree disease *Fusarium xylarioides* damage both coffee, robusta coffee and wild varieties, 14 million coffee trees were killed in Uganda in 1993; rough bark disease, collar rot *Fusarium stilbioides* caused the stunting and low productivity. Coffee in our country encounters a lot of harmful diseases such as *Colletotrichum coffeanum* on leaves, stems and fruit; *Hemileia vastatrix* causes defoliation; *Botryobasidium rolfsii* damages the seedlings in the greenhouse; *Fusarium sp., Rhizoctonia* damage coffee, causing mushy roots, the roots dry inside, the whole system inside black and dry, lose branches and leave and then dead.

There are over 50 species of fungal diseases on tea in the northeast Indian tea. Popular diseases include *Colletotrichum theae - sinensis, Rosellinia arcuata, Corticium theae, Poria hypolateritia, Fusarium solani,* etc... Those diseases kill the plants and seriously degradate tea field. In Vietnam there are many diseases causing rot root, rot bud, rot leaves, dry twigs which have a serious impact on tea yields and productivity.

The control of disease on tea, coffee and rubber has mainly based on chemical methods which seems less effective and leave residues in the environment harmful to the health of consumer as well as producer. There have been studies and applications of beneficial microorganisms to control plant diseases, one of the microorganisms were *Chaetomium* (K. Soytong 1989). *Chaetomium* falls under the list of saprophytic fungi which have strong competitiveness against disease fungi, especially *Chaetomium globossum* and *Chaetomium bostrychoides* with strong resistance to pathogenic fungi of the *Fusarium* and *Helminthosporium* (Tveitand Moore, 1954), *Alternaria, Collectotrichum* (Vannacci *et al*, 1978; C. Talubnuc *et al*, 2010), and so on. The antagonistic activity of *Chaetomium* is due to the synthesis of antibiotic *Chaetoglobosin,* which breaks down cell membranes, making the cytopla broken and lose toxicity of fungal diseases (Di Petro *et al*, 1992; K. Soytong, 2007). In addition, *Chaetomium* helps stimulate growth and development, increase the resistance of plants (Le Thi Anh Hong, 2005; Doke *et al*, 1991, 1997).

Based on the useful characteristics of *Chaetomium*, the Northern Mountainous Agriculture and Forestry Science Institute (NOFMAFSI) has carried out the research and production of *Chaetomium* compositions to eliminate diseases on tea, coffe, rubber and other crops.

Materials and Methods

Research materials: Soil samples from the tea, coffee and rubber gardens for isolating *Chaetomium*; Fungal pathogens are *Fusarium sp*., *Colletotrichum theae-sinensis* and some other pathogenic fungi from sources stored at Department of Biotechnology and Plant Protection, NOFMAFSI; Potato, sugar, agar, vegetable oil, amino acid powder, autoclave sterilization, box for implanting microorganisms, microscope.

Research Content: Isolate antagonistic *Chaetomium;* Review of active resistant characteristics of *Chaetomium* against root rot for tea, coffee and rubber caused by *Fusarium sp*. Tea leave rot caused by *Colletotrichum camelliae* nursery (C. sinensis - theae); Research, manufacture and evaluate the effectiveness of disease control of the product.

Research Method: Isolate and purify *Chaetomium*; The soil samples were made small and put into a petri dish, put the pieces of filter paper with $1cm^2$ onto the surface, humidification and moisturizing regularly at room temperature to trap fungus by the method of K. Soytong 1989. The research samples tend to have different microbial species, therefore, it is needed to isolate for having the pure fungus. Separating each fungus by sterilized implants and implanted onto PDA to track the generation and development of spores .

To assess the activity of the Chaetomium against fungus disease: Perform the experiment by conducting transplants symmetry 2 antagonist fungus and pathogens on the surface of potato dextrose agar in petri dishes 9cm, transplanting in place 1cm from the edge of the petri dish:

Disc 1: Mushrooms antagonists (Chaetomium).

Disc 2: Mushrooms antagonists (Changing domain name) and fungal pathogens (NGB).

Disc 3: Pathogens (control).

The experiment was randomly arranged with 3 replications; Keep track of the daily growth of 2 fungi (NDK and NGB) and the crowding together by measuring the diameter of fungal growth and spore counts, of both NDK and NGB; The experiment by the method of Soytong 1989; SeiKetov 1982; Le Anh Hong 2005. Counting spore by MCV ruler; The inhibitory effect of NDK is calculated using the formula: PIRG (Percent Growth Inhibition of Radical) = (R1- R2) / R1 x 100. Of which:

R1: the development diameter of colony or number of fungus spores in the petri dish.

R2: the development diameter of colony or number of funga spores in the opposite dish.

Production and evaluation of compositions: Experiment to create compositions: study the proportion of material components of a composition, comprising cookingoil, water, sacaroze sugar, amino acids and fungal spores:

Treatment 1: Vegetable oil 50%; distilled water, sugar and fungal spores 49%; amino acid powder 1%; treatment 2: Vegetable oil 60%; distilled water, sugar and spores 39%; amino acid powder 1%; treatment 3: Vegetable oil 70%; distilled water, sugar and fungal spores 29%, amino acid powder 1%; treatment 4: Vegetable oil 80%; distilled water, sugar and fungal spores 19%, amino acid powder 1%; The amount entered Chaetomium spores reach 10^9 CFU/ml.

Experiment to evaluate effectiveness of compositions: planting tea, coffee and rubber 1 year old in a pot, put in the greenhouse, when they grow green, spread contagious tree root rot and powdery mildew disease. For tea and coffee, spread root rot, *Fusarium sp.,* For rubber trees, spread the powdery mildew disease (*Oidium Hevea* up leaves). After one month of spreading disease, process the composition. Dilute CP2-VMNPB composition with water and irrigate on crops (for root rot) and spray on foliar (for rubber powdery mildew disease) according to the dosage:

Treatment 1: 0.5ml of composition/ tree; treatment 2: 1ml of ml of composition/tree; treatment 3: 1,5ml ml of composition/trees; treatment 4: 2,0ml of composition/trees; treatment 5: control, spraying water.

Survey the disease index through disease symptoms expressed on foliar expression as follows: level 1: healthy plants, green leaves; level 2: 1-25% with yellow leaf; level 3: 26-50% with yellow leaf; level 4: 51-75% with yellow leaf; level 5: > 75% with yellow leaf;

Results and discussion

Resultof collecting and isolating Chaetomium

In order to have *Chaetomium* strains to produce composition, we collected soil samples from the tea, coffee and rubber gardens in the main growing areas such as Phu Tho, Thai Nguyen, Nghe An, Son La, Lai Chau, Yen Bai. From each soil sample, take about 30g, making the small and put into a petri dish 9cm to trap fungi. The trapped *Chaetomium* is purified and identified with scientific name in King Mongkut University, Thailand. 23 strains of *Chaetomium* have been identified, including 11 strains from tea soil, 8 strains from coffee soil and 4 strains from rubber soil. In which, two strains of *Chaetomium bostrychoides* CPT1 isolated from tea soil and *Chaetomium globosum* CFSL1 isolated from coffee soil is quite popular and vigorous, used for evaluation of antagonistic activity against fungal diseases on tea, coffee and ruber.

Antagonistic activity of Chaetomium against some fungal diseases

Two popular strains of *Chaetomium* isolated from tea and coffee soil were evaluated for antagonistic activity against root diseases caused by *Fusarium sp*. and anthracnose caused by *Colletotrichum sp.* in petri dishes with PDA in laboratory conditions.

Table 1. Antagonistic activity of *Chaetomium* strains against *Fusarium* sp. after a month

Treatment	Fungi colony condition (cm)	Inhibitant ability (%)
Fusarium sp. (control)	9,00 a	
C. bostrychoides against *Fusarium* sp.	3,74 b	58,44 b
C. globosum against *Fusarium* sp.	3,21 c	64,33 a
CV (%)	3,86	3,10
LSD_{05}	0,41	4,30

One month after implantation in the environment , in the control dish *Fusarium sp.* developed fully on petri dishes 9cm, Fungi colony is quite thick. In dish with antagonistic agent, *Fusarium sp.* is inhibited by *Chaetomium* colony and thus scaled down (3,21cm) being smaller than *Chaetomium* colony (5,79cm) . Also on the surface there appear *Chaetomium* bag (Picture 1) . That is because *Chaetomium* has grown, the mycelium spread to *Fusarium* colony encroaching and inhibiting the growth of fungal diseas causing the size of fungi reduce (Soytong et al , 2001). The *Chaetomium* mycelium coat on the surface of fungi diease develop and create bag on fungi disease surface.

In both *Chaetomium* strains surveyed, *Chaetomium globosum* (Cg) has stronger antagonist activity against *Fusarium* than *Chaetomium bostrychoides* (Cb). In disc having *Chaetomium globosum*, the size of fungi disease colony is 3,21cm and effectiveness of inhibitant activity against the development of fungi disease colony of Cg is 63.33 % Cg, while in disc having Cb the size of fungi disease colony is 3,74cm and Cb inhabits 58.44% the development of *Fusarium sp.*

For both control fungi and disease fungi, mycelium grow over time will produce spores, *Chaetomium* inhibit the growth of fiber system, and also inhibit the reproductive spores. We evaluate this activity through fungal spores number formed in the control experiment compared with the freely developed disease fungi, the results in Table 2

Table 2. Inhibitance against the development of *Fusarium sp.* Spores of newly isolated *Chaetomium*

Treatment	No. of spores (x 10^7)	Inhibitant ability (%)
Fusarium sp. (control)	67,46 a	
C. bostrychoides against *Fusarium* sp.	6,20 b	90,08 b
C. globosum against *Fusarium* sp.	4,18 c	93,80 a
CV (%)	8,03	6,24
LSD_{05}	1,76	3,12

It is shown from the experiement result that the number of root rot spores differ as opposed to different strains of *Chaetomium*. Number of spores of *Fusarium sp*. in formula against Cg (4:18 x 10^7 spores/g), less than the formula against Cb (6.2 x 10^7 spores). The effectiveness of inhibit against the growth of fungal spores of Cg reached 90.08%. From the experiments in Table 1 and 2, though the *Chaetomium* strains do not strongly inhibit the development of disease fungi colonies, it has the excellent inhibition against the formation of disease fungi spores. The result has a significant meaning on the inhibition of spread and epedimic of root rot trees caused by *Fusarium sp.*

Anthracnose damage foliar and twigs of many different crops. On the tea plants, *Colletotrichum camelliae* (C. sinensis - theae) causes anthracnose on leaves, buds, twigs of tea plants; on coffee trees, the disease caused by *Colletotrichum sp.*. We also assessed the antagonistic ability of the *Chaetomium* strains towards these two species of disease fungal. However, since the experiment results are similar, the presentation is *Colletotrichum sp* only.

Table 3. Antagonistic activity of several *Chaetomium* strains against *Colletotrichum* sp. after one month

Treatment	Fungi colony condition (cm)	Inhibition ability (%)
Colletotrichum sp. (control)	9,00 a	
C. bostrychodes against *Colletotrichum* sp.	3,61 b	59,88 b
C. globosum against *Colletotrichum* sp.	3,12 c	65,33a
CV (%)	5,06	9,83
LSD_{05}	0,42	3,24

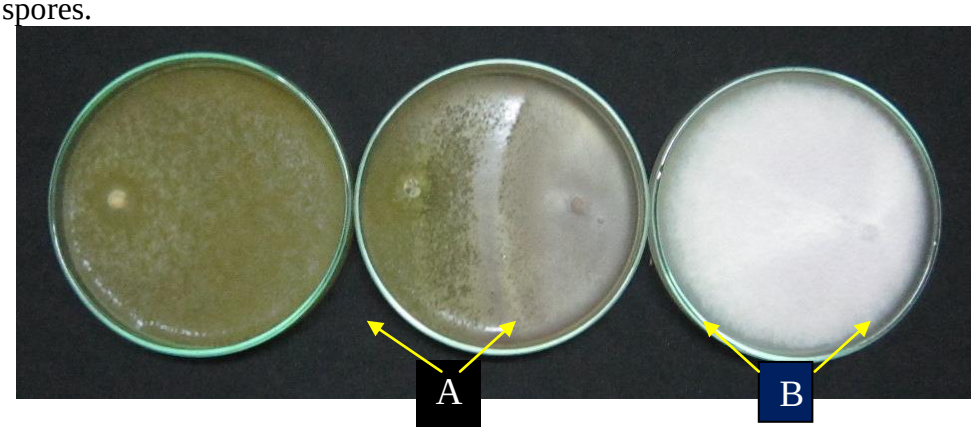
For *Colletotrichum sp.*, Fungal antagonists also inhibit the growth reducing the disease fungi size to 3,12-3,61cm only while in fungal control formula, the disease fungi fully cover the 9cm petri disc (Picture 2). Antagonist Cg inhibit disease fungal colonies (3,12cm) stronger than the inhibition ability of Cb (3,61cm). The effectiveness of inhibition against the disease fungal growth *Colletotrichum sp*. of Cg reach 65.33% of Cg, higher than Cb reached 59.88 %.

For reproductive spores of the fungus *Colletotrichum sp*. under the influence of antagonistic fungus *Chaetomium* was studied and recorded in the below table 4:

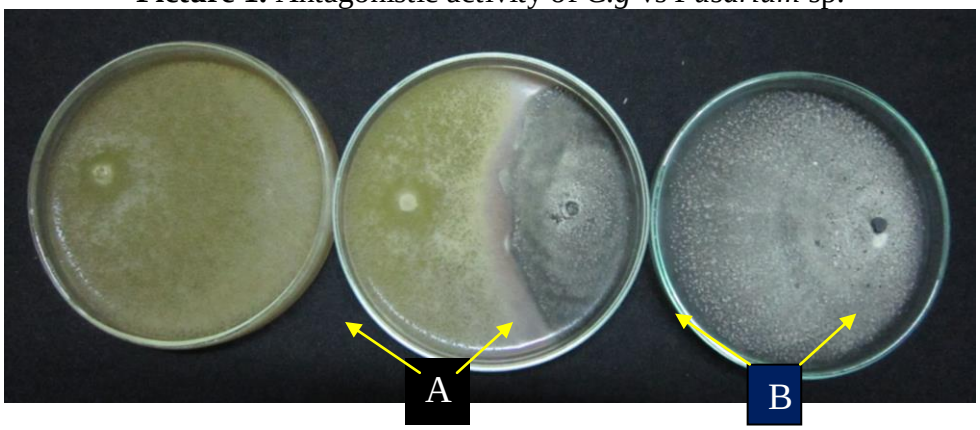
Table 4. Inhibition ability of newly isolated antagonistic *Chaetomium* strains against the reproductive spores of *Colletotrichum* sp.

Treatment	No. of spores (x 10^7)	Inhibition against the reproductive spores (%)
Colletotrichum sp. (Control)	82,64 a	
C. bostrychodes với *Colletotrichum* sp.	8,26 b	90,00 a
C. globosum với *Colletotrichum* sp.	8,15 b	90,13 a
CV (%)	13,23	14,43
LSD_{05}	3,35	6,95

For *Colletotrichum sp.* two strains of antagonistic *Chaetomium* strongly inhibited the reproductive spores. Two strains Cg and Cb are capable of inhibiting anthracnose in the same manner (number of fungal spores in the experiment from 8.15 to 8.26 x experiment only 10^7 spores/g); the effectiveness of inhibition against the fungal reproduction of Cg and Cb are similar and relatively high (reaching 90 to 90.13%). From the experiments, we observe that antagonistic *Chaetomium* inhibit the growth of anthracnose colonies is not high, but strongly restrict its reproduction of spores.



Picture 1. Antagonistic activity of *C.g* vs *Fusarium* sp.



Picture 2. Antagonistic activity of *C.g.* vs *Colletotrichum* sp. Note for Picture 1,2: A- *Chaetomium* colony; B- Disease fungal colony

With the goal of producing compositions capable of excluding diseases for both tea and coffee, the simultaneous use of antagonistic strains isolated from tea and coffee soil in the composition will be effective. However, the two strains of antagonists Cg and Cb have strongly effective antagonist against the disease fungi causing root rot and anthracnose on tea and coffee, but when combined whether they can influence to reduce the effectiveness of each other or not. The study result of the combined antagonistic activity of two strains of Cg isolated from coffee soil and Cb isolated from tea soil recognized in the following table .

Table 5. Antagonistic activity of the combination of two *Chaetomium* strains against the root rot and anthracnose on tea and coffee

Treatment	*Fusarium sp.*		*Colletotrichum* sp.	
	Fungal colony (cm)	No. of spores (10^7)	Fungal colony (cm)	No. of spores (10^7)
Control	9,00	7,82	9,00	7,18
Disease fungi	3,72	0,76	4,00	0,72
Inhibition effectiveness (%)	58,66	90,28	55,55	89,97

The experimental results have demonstrated the combination of two *Chaetomium* strains, the antagonistic activity against disease fungi remains strong as the individual strain. The combination of two *Chaetomium* strains inhibits the growth of fungal colony of the two diseases from 55.55 to 58.66%; inhibits the reproduction of spores from 89.97 to 90.28%. From the experiments, we can combine the *Chaetomium* strains together without having impact in inhibiting each other in the composition having *Chaetomium* strains, so the composition will have a broader effectiveness.

Sudy for production and evaluation of new compositions

Study for production of compositions

Based on the experiments for blending with the ratio of different components based on the four formulas as described in the method, the active ingredients are 2 strains of *C. bostrychoides* CPT1 and *C. globosum* CFSL1, collect the liquid composition having vegetable oil, sugar, amino acid powder, distilled water and *Chaetomium* spores. After having additive to dissolve vegetable oil, the treatment 2 (including vegetable oil: 60% + distilled water, sugar and fungal spores: 39% and amino acid powder 1%). become the liquid, not being precipitated and deposited, which meets the requirement to produce compositions. Fungal spores in the composition reach at least 1.0×10^9 spores/ml.

Evaluation of effect of composition is laboratory condition

To evaluate the effect of the newly produced compositions against disease fungi, the first experiments are performed in laboratory conditions. Fungus grown in PDA environment in petri disc 9cm and cultured in the opposite position, tracking the development of the fungal colonies after 1 month, calculate the inhibition effectiveness of the composition (PIRG) .

Table 6. Effect of compositions against *Fusarium sp.* và *Colletotrichum sp.*

Treatment	Effect to exclude fungi after one month (%)	
	Colletotrichum sp.	*Fusarium sp.*
Treament 1	43,34 d	44,90 d
Treament 2	81,80 a	85,62 a
Treament 3	69,75 b	71,73 b
Treament 4	56,78 c	65,57 c
CV%	4.55	4.10
LSD_{05}	5.39	5.17

In laboratory conditions, the experiment under formula 2 have the effect of excluding *Fusarium sp.* fungus and *Colletotrichum sp.* at the highest level of 85.62 % and 81.80 % . Based on the results of experiments, evaluate the effect of excluding disease fungi and uniformity of composition solution, the treatment 2 has been used to produce composition. The new compositin is named CP2 - VMNPB .

Evaluate the effect of excluding disease fungi of CP2-VMNPB composition in greenhouse

The seedlings used for experiment was planted in pots, after their roots become stablized, conducting the spread of disease, when the symptoms appear, produce the composition with certain different composition concentrations. The objective is in addition to the evaluation of the effect of composition, determine the reasonable dosage of composition. The evaluation results of fungicides of CP2 - VMNPB composition is in the following table:

Table 7. Effect of CP2-VMNPB against the root rot on tea and coffee in greehouse condition after 3 months

Treatment	Experiment on tea		Experiment on coffee	
	Disease index (%)	Fungicides (%)	Disease index (%)	Fungicides (%)
Treament 1- 0,5ml/tree	0,13 b	79,43 c	0,14 b	78,76 b
Treament 2- 1,0ml/ tree	0,13 b	80,71 bc	0,13 b	80,58 ab
Treament 3- 1,5ml/ tree	0,12 bc	82,34 ab	0,12 c	82,84 a
Treament 4- 2,0ml/ tree	0,11 c	83,43 a	0,12 c	83,30 a
Treament 5- Not process	0,68 a		0,71 a	
CV (%)	5,04	1,28	3,18	1,98
LSD_{05}	0,015	1,96	0,016	3,04

After the observation period, symptoms in the experimental tea are reduced. With the different dosages, disease indexs and the effect of excluding diseases are different. Statistical experiment results show dose treatment with 0.5-1.0 ml/pot (plants) have similar disease indicators and the effect of excluding disease are similar (from 79.43 to 80, 71% for tea and from 78.76 to 80.58% for coffee); treated with doses from 1.5-2.0 ml/pot, the effect of excluding disease are equivalent and achieve the highest results, from 82.34% to 83.43% on tea and from 82,84- 83.3% on coffee.

The results showed that the using of dose of 2ml CP2 - VMNPB/tree have excellent effect of excluding root rot for tea and coffee.

Rubber trees often appear many diseases, powdery mildew disease caused by the *Oidium heveae* causing deciduous, it takes long time for rubber trees to grow new leaves to replace, latex yield is therefore seriously affected. Especially disease in the nursery garden causes trees grow slowly, kill slips premature, reduce the incidence of the garden. The new composition was studied with the trial exclusion of powdery mildew disease in the following table:

Table 8. Effect of CP2-VMNPB against powdery mildew disease for rubber trees after 3 months

Treatment	Disease index (%)	Effect (%)
Treatment 1- 0,5ml/ tree	0,22 b	74,11 b
Treatment 2- 1,0ml/ tree	0,20 b	76,47 b
Treatment 3- 1,5ml/ tree	0,17 c	80,00 a
Treatment 4- 2,0ml/ tree	0,16 c	81,17 a
Treatment 5- No process	0,85 a	
CV (%)	7,43	11.34
LSD_{05}	0,022	2.96

For powdery mildew disease on rubber tree, handle compositions with different dosages having different results. At doses of 0.5-1.0 ml/tree, the effect of excluding disease reach from 74.11 to 76.47 similarly; increase the dose to 1,5ml composition, the effect is increased to 80%; increasing the treatment dose up 2,0ml/tree, the effect reach 81.17%, equivalent to the treatment dose of 1,5ml/tree. Assay on rubber powdery mildew disease has similar result to root rots on tea and coffee trees. It means that when handling compositions with the dose of 0.5-1.0 ml/pot , disease index and effect of excluding disease is similar. Handle with the treatment dose of 1.5-2.0 ml/tree, the effect is increased.

So, after 3 months of experiments, handle the composition with the dose of 1.5 and 2.0ml/pot (plants) have good effect of excluding disease for both root rot on tea and coffee and poweder mildew disease on rubber leaves, reach over 80%. Since the composition use the microorganisms, the effect lasts for longer period, from the experiment results can apply 1.5ml/tree for disease control will also achieve the effect from economic perspective.

Conclusion

Identify 2 strains of *Chaetomium* quite popular in tea, coffee and rubber soil in Northern Vietnam being *Chaetomium globosum* CPT1 and *Chaetomium bostrychoides* CFSL1.

New *Chaetomium* have strongly antagonistic activity with *Fusarium sp.* causing root rot tea, coffee and rubber (ability to inhibit the production of fungal spores reached 90.08 to 93.80%) and at the same time have strong resistance to *Colletotrichum sp.* causing anthracnose (90 to 90.13% inhibition of fungal spores reproduction).

CP2-VMNPB composition produced from two *Chaetomium* strains have the high effect of excluding fungi, with *Fusarium sp.* Reached 85.62% and *Colletotrichum sp*. reached 81.80% after one month in laboratory conditions, in greenhouse conditions, the effect on root rot tea and coffee reached 83.43% and 83.30%, with powdery mildew disease on rubber *Oidium Hevea*, the effect reached 81.17%.

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