III. Short-term Experts' Report

1. Study on the causal agent of clove leaf fall and damping-off symptoms. M. Oniki *et al.* (1994)

2. Preliminary experiment on the measurement of quantity population of *Fusarium oxysporum* f. sp. *vanillae*. F. Namiki *et al.* (1994).

STUDY ON ON THE CAUSAL AGENT OF CLOVE LEAF FALL AND DAMPPNG-OFF SYMPTOM

Masaomi Oniki[1], Shizuo Mogi[2], Dono Wahyuno[3], Sukamto[3], and Alan Rachmat S[3].

Outline Of Research

1. Causal agent of clove leaf fall symptom.

To elucidate the causal agent of leaf fall symptom of clove occur at North Sulawesi mainly, we have examined the pathogenicity of *Guignardia* sp. to clove seedling trees by inoculation test method. We have collected diseased samples from Lake Tondano area of North Sulawesi and detected the causal agent from disease leaves.

We could isolate *Guignardia* sp. from disease leaves (yellowing leaf : having a part of hypertrophy of diseased petioles).

A kind of symptom of leaf fall is followed by the yellowing of clove leaf; it is high possible that this symptom is caused by *Guignardia* sp.

2. Causal agent of clove damping-off symptom.

Damping-off occurred abundantly damping-off in nursery bed of clove. Two kinds of *Cylindrocladium* spp. could be isolated from the diseased seedlings. We have examined the seed transimission of *Cylindrocladium* spp.

We observed that damping-off symptom occurred on seedlings grew from the seeds fell of the mother trees, and causal agent was detected around the diseased seedlings in natural field (West Java).

3. Collection of causal fungi of clove leaf fall and dampping off diseases and preparation of herbarium.

Isolation and collection from clove diseased samples, 19 isolates of *Guignardia* sp., 8 isolates of *Cylindrocladium* spp. are preserved. These isolates are kept in Balittro, and some part of these isolates will be sent to

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Japan, and will be registered in MAFF (Ministry of Agriculture, Forestry and Fisheries's Gene Bank). 6 specimens of leaf fall disease, 1 specimen of damping off disease and 3 specimens of CDC disease are preserved at BALITTRO.

We hold the seminar of Final report at 26th March, 1994 in BALITTRO, and it was the official forum to present the results of the collection, observation and experiment.

Experimental Results

Leaf fall disease

Leaf fall disease of clove is one of important disease especially in North Sulawesi and by this time the causal agent is not known yet. The disease was on clove cultivation when observation was conducted at Lake Tondano area, North Sulawesi.

Results of clove leaf petiole isolation showed that there were *Guignardia* sp. and other kinds of fungi. *Guignardia* sp. is suspected as the causal agent of leaf fall disease of clove, since there were *Phyllosticta* pycnidia on petiole of clove leaf from leaff fall dieased. The *Phyllosticta* sp. is the anamorph/ imperfect state of *Guignardia* sp.

The pathogenicity test were conducted by inoculated 14 days old *Guignardia* sp. isolates growth on PDA to unwounded and wounded clove petioles. The inoculated plants were incubated in room temperature (26 - 31 ^{o}C), kept in humid conditions and avoided from direct sun shine.

Observation at 14 to 21 days after inoculation, yellowing or leaf fall diseases were not found either on unwounded nor wounded petioles, so the research shall be continued in the future time with different in inoculation methods.

Clove damping-off symptom

Damping-off and root rot symptoms often observed on clove seedling, especially on clove nursery bed but the causal agent does not clear yet.

Observation in PT. Yanita Estate, Sukabumi, West Java on March 1994. There were damping-off and root rot diseases on clove seedlings grew by the mother plants.

Root tissues isolation was conducted in Plant Pathology Laboratory of Balittro, and the *Cylindrocladium* sp. were got besides other fungi.

Some species of *Cylindrocladium* spp. are known as causal agent of stem rot and root rot lession of some cultivated plants, i.e. *C. floridanum* on peanut and *C. scoparium* on azelae, red pine, rhododendron etc. On clove plant there is *C. quenqueseptatum* the causal agent of leaf rot diseases. Based on observation on number of septum of conidia and shape of vesicle the *Cylindrocladium* spp. were different with another one from clove.

The pathogenicity test was not conducted yet since there were few healthy clove seeds as material of research.

Herbarium

Fungi isolates from plant tissues isolation were trasferred from water agar (WA) to Potato Dextrose Agar (PDA) slant and kept them in low temperature incubator (12-13^{o} C). Besides kept in Balittro, the cultures were brough to Japan for registered in MAFF Gene Bank.

Diseased clove plant specimens showed leaf fall disease, leaf blister blight, dampind-off and root rot disease were dried by news paper as herbarium specimens and kept in herbarium collection boxes of Plant Pathology Division of Balittro.

Data 1. Origin of Isolates

No.	Name of fungus	Part of plant	Location	Collec.date
B-208	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-209	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-210	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-211	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-212	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-213	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-214	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-215	*Cylindrocladium* sp.	clove, seedl, root	P.ratu, West Java	94/3/11
B-216	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-217	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-216	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-217	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-218	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-219	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-220	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-221	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-222	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-223	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-224	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-225	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-226	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-227	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-228	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-229	*Guignardia* sp.	clove, leaf, petiole	Tinoor, North Sulawesi	94/3/5
B-230	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-231	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-232	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-233	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4
B-234	*Guignardia* sp.	clove, leaf, petiole	Sonder, North Sulawesi	94/3/4

Data 2. List of Herbarium

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No. : 219 HERBARIUM No. : (1)

Pathogen 1 : *Guignardia* sp.
(Leaf fall disease)
2 :

Host : Clove (*Syzigium aromaticum*)
Locality : Sonder, North Sulawesi Indonesia
Date : 1994/03/04
Collector : M. ONIKI, S. MOGI
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 220 HERBARIUM No : (2)

Pathogen 1 : *Guignardia* sp.
(Leaf fall disease)
2 :

Host : Clove (*Syzygium aromaticum*)
Locality : Sonder, North Sulawesi Indonesia
Date : 1994/03/04
Collector : M. ONIKI, S. MOGI
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 221 HERBARIUM No : (2)

Pathogen 1 : *Guignardia* sp.
(Leaf fall disease)
2 :

Host : Clove (*Syzygium aromaticum*)
Locality : Maumbi, North Sulawesi Indonesia
Date : 1994/03/04
Collector : M. ONIKI, S. MOGI
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 222 HERBARIUM No : (2)

Pathogen 1 : *Guignardia* sp.
(Leaf fall disease)
2 :

Host : Clove (*Syzygium aromaticum*)
Locality : Tinoor, North Sulawesi Indonesia
Date : 1994/03/05
Collector : M. ONIKI, S. MOGI
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 223 HERBARIUM No : (2)

Pathogen 1 : *Guignardia* sp.
(Leaf fall disease)
2 :

Host : Clove (*Syzygium aromaticum*)
Locality : Rumoong Atas, North Sulawesi Indonesia
Date : 1994/03/05
Collector : M. ONIKI, S. MOGI
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 224 HERBARIUM No : (2)

Pathogen 1 : *Guignardia* sp.
(Leaf fall disease)
2 :

Host : Clove (*Syzygium aromaticum*)
Locality : Ratahan, North Sulawesi Indonesia
Date : 1994/03/05
Collector : M. ONIKI, S. MOGI
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 225 HERBARIUM No : (2)

Pathogen 1 : *Phyllosticta syzygii*
(Leaf blister blight disease, CDC)
2 :
Host : Clove (*Syzygium aromaticum*)
Locality : Cibinong, West Java, Indonesia
Date : 1994/03/10
Collector : M. ONIKI, A. RACHMAT S, D. WAHYUNO
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 226 HERBARIUM No : (2)

Pathogen 1 : *Phyllosticta syzygii*
(Leaf fall disease)
2 :
Host : Clove (*Syzygium aromaticum*)
Locality : Cibinong,West Java, Indonesia
Date : 1994/03/10
Collector : M. ONIKI, A. RACHMAT S, D. WAHYUNO
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 227 HERBARIUM No : (2)

Pathogen 1 : *Phyllosticta syzygii*
(Leaf blister blight disease, CDC)
2 :
Host : Clove (*Syzygium aromaticum*)
Locality : Cibinong, West Java, Indonesia
Date : 1994/03/10
Collector : M. ONIKI, A. RACHMAT S, D. WAHYUNO
Identifier :

Isolate-No :

RESEARCH INSTITUTE FOR SPICES AND MEDICINAS CROPS (BALITTRO)

No : 228 HERBARIUM No : (2)

Pathogen 1 : *Cylindrocladium* sp.
(Seedlings stem rot disease)
2 :
Host : Clove (*Syzygium aromaticum*)
Locality : Pelabuhanratu, West Java, Indonesia
Date : 1994/03/10
Collector : M. ONIKI, D. WAHYUNO
Identifier :

Isolate-No :

Table of pathogens isolated from Indonesia registered in MAFF Gene Bank (micro-organisms), Japan

No. isolate	Scientific name	MAFF accesion no.
Cyl. quin, B-149	*Cylindrocladium quinqueseptum* Boedijn *et* Reitsma	236881*
Cyl. quin 2,B-150	*Cylindrocladium quinqueseptum* Boedijn *et* Reitsma	236882*
Cyl. sp., B-186	*Cylindrocladium* sp.	236883*
Cyl. sp. 2, B-187	*Cylindrocladium* sp.	236884*
Cyl. seed, B-188	*Cylindrocladium* sp.	236885*
Cyl. seed, B-189	*Cylindrocladium* sp.	236886*
Cyl. seed 2, B-190	*Cylindrocladium* sp.	236887*
Cyl. root, B-191	*Cylindrocladium* sp.	236888*
Cyl. root, B-192	*Cylindrocladium* sp.	236889*
B-157	*Rhizoctonia solani* Kuhn	236890*
B-158	*Rhizoctonia solani* Kuhn	236891*
58, B-174	*Rhizoctonia solani* Kuhn	236892*
88, B-175	*Rhizoctonia solani* Kuhn	236893*
B-117	*Colletotrichum gloeosporioides*	236904**
B-182	*Colletotrichum gloeosporioides*	236905**
B-183	*Colletotrichum gloeosporioides*	236906**
B-197	*Colletotrichum gloeosporioides*	236907**
B-198	*Colletotrichum gloeosporioides*	236908**
B-199	*Colletotrichum gloeosporioides*	236909**
B-200	*Colletotrichum gloeosporioides*	236910**
B-201	*Colletotrichum gloeosporioides*	236911**
B-126	*Coniella castaneicola*	236912**
B-131	*Coniella castaneicola*	236913**
B-133	*Coniella castaneicola*	236914**
B-137	*Coniella castaneicola*	236915**
B-140	*Coniella castaneicola*	236916**
B-169	*Fusarium oxysporum* f. sp. *vanillae*	236917**
B-170	*Fusarium oxysporum* f. sp. *vanillae*	236918**
B-171	*Fusarium oxysporum* f. sp. *vanillae*	236919**
B-172	*Pestalotiopsis versicolor*	236920**
B-173	*Pestalotiopsis versicolor*	236921**
B-193	*Pestalotiopsis versicolor*	236922**
B-194	*Pestalotiopsis versicolor*	236923**
B-195	*Pestalotiopsis versicolor*	236924**
B-196	*Pestalotiopsis versicolor*	236925**
B-202	*Marasmius tenuissimus*	236926**
B-203	*Marasmius tenuissimus*	236927**
B-204	*Marasmius tenuissimus*	236928**
B-205	*Marasmius tenuissimus*	236929**
B-206	*Marasmius tenuissimus*	236930**
B-207	*Marasmius tenuissimus*	236931**

No. isolate	Scientific name	MAFF accesion no.
B-127	*Botryodiplodia theobromae*	236969**
B-129	*Botryodiplodia theobromae*	236970**
B-135	*Botryodiplodia theobromae*	236971**
B-139	*Botryodiplodia theobromae*	236729**
P-9, B-109	*Phyllosticta syzigii*	236973**
P-13, B-113	*Phyllosticta syzigii*	236974**
T6-2	*Phyllosticta syzigii*	236975**
GM	*Guignardia* sp.	236976**
L1-2	*Guignardia* sp.	236977**
3-5	*Guignardia* sp.	236978**
E	*Guignardia* sp.	236979**
D-2	*Guignardia* sp.	236980**
GRj-1, B-166	*Guignardia* sp.	236981**
GRj-2, B-167	*Guignardia* sp.	236982**
Gomu 2, B-164	*Guignardia heveae*	236983**
Gomu 1, B-163	*Guignardia heveae*	236984**
R-3	*Guignardia heveae*	236985**
R-2-3	*Guignardia heveae*	236986**
R-2-4	*Guignardia heveae*	236987**
Sonder	*Guignardia* sp.	236988**
T-12	*Phyllosticta syzygii*	236989**
Maumbi	*Guignardia* sp.	236990**
Tareran	*Guignardia* sp.	236991**
Subang 2	*Phyllosticta syzygii*	236992**

*necessary condition : permission of Plant Protection Bureau, MAFF, Japan, AC (active collection)

**necessary condition : limitation for use, AC

PRELIMINARY EXPERIMENTS ON THE MEASUREMENT OF QUANTIFY POPULATION OF *FUSARIUM OXYSPORUM* F. SP. *VANILLAE*

Fumio Namiki[1)], Mesak Tombe[2)] and Sukamto[2)]
Shizuo Mogi[3)]

ABSTRACT

This experiment was carried out at the laboratory and glass house of Plant Pathology Division, Research Institute for Spice and Medicinal Crops. The objective of this study was to establish the method to selectively isolate and to quantify population of *F. oxysporum* including the causal pathogen of stem rot and other microorganisms from natural soil and soil amendment with clove powder. Estimation of population of soil microorganisms was made on selective culture media. The results indicated that the degree of disease occurrence did not correlate with population densities of bacteria, actinomycetes among these fields. The results suggest that activities of *F. oxysporum* were selectively inhibited by treatment with clove powder and comparatively narrow.

RINGKASAN

PENELITIAN PENDAHULUAN UNTUK MENGHITUNG POPULASI FUSARIUM OXYSPORUM *F. SP.* VANILLAE

Penelitian ini dilaksanakan di laboratorium dan rumah kaca, kelti Penyakit, Balai Penelitian Tanaman Rempah dan Obat. Tujuan penelitian adalah untuk menentukan metode isolasi dan penghitungan propagul F. oxysporum *secara kuantitatif termasuk patogen busuk batang dan mikroorganisme lain dari tanah alami dan tanah yang diberi tepung bunga cengkeh. Penghitungan populasi mikroorganisme tanah dilaksanakan dengan menggunakan media selektif. Hasil penelitian menunjukkan bahwa tidak ada korelasi antara tingkat serangan busuk batang dan populasi* F. oxysporum, *dan tidak terdapat perbedaan populasi bakteri dan aktinomycetes dari tanah yang dianalisa. Hasil percobaan menunjukkan bahwa pemberian tepung bunga cengkeh ke dalam tanah bersifat selektif terhadap aktivitas* F. oxysporum *dan mempunyai sepektrum yang sempit.*

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INTRODUCTION

Stem and foot rot of vanilla (*Vanilla planifolia* Andrews), caused by *Fusarium oxysporum* f. sp. *vanillae*, is one of the most destructive disease of vanilla in Indonesia. As the causal pathogen inhibits in soil, it is extremely difficult to control by chemical agents. Until now, effective control methods on this disease are not established. So, the pathogen causes serious damages to vanilla plantation. Major vanilla-producing centers were moved from Central Java to Bali and to North Sulawesi by severe occurrence of this disease. Bali is now facing a serious crisis for the collapse of producing center. Ecology of the causal pathogen remains to be unknown, and it spurs on the difficulties of controlling the disease.

The objective of this study is to establish the method to selectively isolate and to quantify populations of *F. oxysporum* including the causal pathogen of vanilla stem and foot rot from the divergent microflora in Indonesian natural soils, and to provide basic data on the epidemiology and the control of vanilla stem and foot rot.

MATERIAL AND METHODS

Infestation of soil with *F.oxysporum* f. sp. *vanillae*.

Erlenmeyer flasks containing PDB medium were seeded with a small cube of mycelium of *F. oxysporum* f. sp. *vanillae*. Flasks were incubated at room temperature in a reciprocal shaker for 5 days. Micro conidia were then separated from the mycelium by filtration through four layers of cheescloth. The conidia were collected by centrifugation at 2,000 rpm for 10 min. Sterilized soil was infested with microconidial suspension of the pathogen.

Treatments with clove powder or charcoal husk.

One kg of artificially or naturally infested soils were mixed with clove flower powder (2 g) or charcoal husk (200 g). After 1 day or 8 days of treatment, 5 cuttings per pot of vanilla stem were planted. Each pot contained approximately 500 g of soil. The experiments included 3 replicates. The external symptoms were checked regularly.

Quantification of soil microorganism.

Estimation of populations of soil microorganisms were made by using standard dilution-plating procedures on general and selective culture media. Inoculum densities of *F. oxysporum* were estimated by using Komada's medium. Five gram of soil samples were suspended in 45 ml of sterilized deionized-water and agitated on a shaker for 30 min, and 0,5 ml samples were spread over the surface of plates containing Komada's medium. Plates were incubated at room temperature for 7 days, and colonies were counted.

Fungal populations were determined on Martin's agar, and populations of bacteria and actinomycetes were estimated by using sodium albuminate agar. One ml of samples were poured into petridishes and then these general media were poured into them separately. Plates were incubated at room temperature for 4 days, and fungal colonies were counted. After 7 days incubation, total colonies of bacteria and actinomycetes were counted.

Pathogenicity test.

Colonies of *F. oxysporum* appeared on Komada's medium were randomly picked up and transferred onto PDA plate. Each *F. oxysporum* isolate was incubated at room temperature for 4 days. The mycelial disks (5 mm diameter) were cut from DPA plate, and put them on to slightly injured cuttings of vanilla stem which has two nods. The cuttings were maintained in a moist chamber. After 7 days, pathogenicity of tested isolates marked was checked by necrosis of the cuttings.

RESULTS AND DISCUSSION

Suppressive effects of treatment with clove powder or charcoal husk on the incidence of vanilla stem and foot rot, and variation of population density of inoculant.

Two gram of clove powder or 200 g of charcoal husk were treated with 1kg of artificially infested soil. After 8 days, vanilla cuttings were planted. The number of diseased cuttings and inoculum density were counted every few days. Inoculum density was extremely high (10^6 cfu/g dried soil). Symptoms appeared after 5 days, and disease incidence after 7 days was 100% in the control. Treatment with clove powder into soil reduced disease incidence as compared with control. Treatment with charcoal husk had no suppressive effect

on vanilla stem and foot rot (Fig. 1). Population density of pathogen in the soil treated with clove powder reduced to one-second, one-tenth and one-hundredth at 4, 7 and 21 days after treatment, respectively, compared with that in control. Population density in the soil treated with charcoal husk was the same as that in control (Fig.2).

Clove powder or charcoal husk were treated with artificially infested soil. After 24 hr, vanilla cuttings were planted. The same results as shown in Fig. 1 were obtained. Treatment with clove powder had remarkably suppressive effect on vanilla stem and foot rot (Fig. 3). Population density of pathogen in the soil treated with clove powder reduced to one hundredth, compared with that in control (Fig.4). It was clear that suppressiveness of treatment with clove powder on the pathogen was immediate effect. The isolates from soil treated with clove powder grew slowly on Komada's medium, compared with those from control. Abnormal growth of mycelia, for example, deformity and distortion, was observed on them. Population density of pathogen in the soil treated with clove powder was 10^5 to 10^6 cfu/g dried soil. This was very high level, but disease incidence was remarkably suppressed. It suggests that growth of pathogens was inhibited and their infection potential was declined by treatment with clove powder.

Effect of treatment with clove powder on microflora in non-sterilized soil.

One kg of natural soil collected from field was treated with 2 g of clove powder. The effect of clove powder on soil microflora was evaluated. Fungal populations, especially *Trichoderma* sp. and *Penicillium* sp., increased in the soil treated with clove powder (Fig.5) and bacterial populations also increased to 100 times after 10 days treatment compared with control (Fig. 6). These results suggests that activities of *F. oxysporum* were selectively inhibited by treatment with clove powder and it's antimicrobial spectrum was comparatively narrow. With increase of fungi and bacteria, they produced antibiotics and competed nutrients with *F. oxysporum*. It is possible that activities of pathogen were declined by these indirect effect.

Evaluation of soil microflora in the fields that vanilla stem and foot rot occurred.

Soil microflora from 11 fields was compared. The degree of disease occurrence in these fields was quite different. There was no difference with popu-

lation densities of bacteria and actinomyces among these fields. Total populations of fungi correlated with population density of *F. oxysporum*. The degree of disease occurrence did not correlate with population density of *F. oxysporum*. It is possible that the disease spread over if population density of *F. oxysporum* in soil reach to 10^3 cfu/g dried soil (Table 1).

It is impossible to differentiate whether colonies of *F. oxysporum* appeared on Komada's medium are those of pathogens or non-pathogens. Ten to 13 colonies of *F. oxysporum* appeared on Komada's medium were randomly isolated from each 11 field and transferred to PDA. After 7 days incubation, mycelial disks were cut from PDA and put on slightly injured vanilla stem cuttings. Inoculated stem cuttings were maintained in a moist chamber for 7 days, and then pathogenicity of each isolate was evaluated. The ratios of colonies pathogenic to vanilla to those tested were calculated in each field sample. The estimates of population density of *F. oxysporum* f. sp. *vanillae* in naturally infested soil was obtained from multiplying the above pathogen ratio by total population density (Table 2). Population densities of pathogen in the fields that vanilla stem and foot rot severely occurred tended to be high compared with those in the fields that the disease little occurred. In vanilla non-cultivated fields, population densities of *F. oxysporum* f. sp. *vanillae* were on the level with 10^2 cfu/g dried soil and increased to be 10^3 cfu/g dried soil by treatment with organic fertilizers. These results suggest that *F. oxysporum* f. sp. *vanillae* commonly exists in fields that vanilla is not cultivated.

Evidence of wind dispersal of *F. oxysporum*.

In the fields, the lesions similar to those caused by *F. oxysporum* f. sp. *vanillae* sometimes appeared on vanilla stems at the height of 0.5 to 1 m from the ground. Spores of *F. oxysporum* dispersed by wind were trapped by the apparatus indicated in Fig. 7. Petri dish containing 15 ml of deionized water sterilized by steam was placed at the height of 0.7 m from the ground. After 24 hr exposure, deionized water was collected and measured up to 10 ml by addition of deionized water sterilized by steam to compensate for evaporation. Sample water 0.5 ml was spread over Komada's medium. After 7 days incubation, total populations of *F. oxysporum* on the plate were counted. Colonies appeared on Komada's medium were tested their pathogenicity to vanilla. Thus, density of *F. oxysporum* f. sp. *vanillae* dispersing in the air was

evaluated. Population density of dispersing *F. oxysporum* in the air was 66.0-305.0 cfu/dish/24 hr. But colonies pathogenic to vanilla were not contained.

The problems to be solved in the future are as follows:

1. Suppressive effects of treatment with clove powder on the incidence of vanilla stem and foot rot and variation of population density of pathogen in natural soil.
2. To clarify mode of action of suppressive effects of clove powder on the incidence of the disease.
3. Development of monitoring system on *F. oxysporum* f.sp. *vanillae* by using *nit* mutants and DNA markers.
4. Reevaluation of wind dispersal of *F. oxysporum* f.sp. *vanillae* through out the year.

Table 1. Comparison of soil micro-flora in naturally infested soil from different locations.

Location	Disease occurrence	*F. oxysporum* (cfu/g dry soil)	Fungi (cfu/g dry soil)	Actinomycetes (cfu/g dry soil)	Bacteria (cfu/g dry soil)
West Java					
Bogor 1*)	-	5.0×10^3	7.5×10^4	3.2×10^6	1.7×10^7
Bogor 2*)	-	6.6×10^2	3.0×10^4	9.9×10^5	9.4×10^6
Sukamulia	High	5.1×10^3	8.3×10^4	3.2×10^6	1.3×10^7
Bali					
Bangli	Severe	7.1×10^3	1.2×10^5	4.9×10^6	1.6×10^7
Senganan	Severe	9.6×10^3	1.7×10^5	5.1×10^6	3.7×10^7
Seraberata	Severe	6.8×10^3	7.1×10^4	3.6×10^6	4.0×10^7
North Sulawesi					
Kiawi	Little	1.1×10^4	3.2×10^5	3.3×10^6	2.1×10^7
Kolongan Atas	Moderate	5.4×10^3	7.5×10^4	1.8×10^6	2.7×10^7
Maumbi	Little	1.1×10^4	2.8×10^5	3.1×10^6	2.5×10^7
Ratahan	Little	7.4×10^3	5.2×10^4	4.5×10^6	2.5×10^7
Ruscong Atas	Moderate	7.9×10^3	6.1×10^4	2.5×10^6	2.3×10^7
Tombatu	Moderate	3.6×10^3	5.1×10^4	2.7×10^6	1.6×10^7

*) : No cultivation of vanilla

Table 2. Estimated population density of *F. oxysporum* f. sp. *vanillae* in naturally infested soil.

Location	No. of pathogenic isolates/ No. of isolates tested*) (%)	Estimated *F. oxysporum* (cfu/g dry soil)
West Java		
Bogor 1 **)	9/10 (90.0)	4.5×10^3
Bogor 2 ***)	9/10 (90.0)	5.9×10^2
Sukamulya	8/13 (61.6)	3.2×10^3
Bali		
Bangli	4/12 (33.3)	2.3×10^3
Senganan	2/11 (18.2)	1.7×10^3
Seraberata	4/12 (33.3)	2.3×10^3
North Sulawesi		
Kiawi	1/12 (8.3)	8.7×10^2
Kolongan Atas	3/12 (25.0)	1.4×10^3
Maumbia	0/12 (0.0)	$< 10^3$
Ratahan	1/12 (8.3)	6.1×10^2
Rumong Atas	1/12 (8.3)	6.5×10^2
Tombatu	4/12 (33.3)	1.2×10^3

*): Isolates tested were randomly chosen from colonies appeared on Komada's medium.
**): Soil from vanilla non-cultivated field. Soil was treated with chicken manure and dried grass.
***): Soil from vanilla non-cultivated field. Soil was no treated with chicken manure and dried grass.

Table 3. Population density of *F. oxysporum* in the air by water trapping.

Period	Population density (cfu/dish/24 hour)
1993/03/07-1994/03/08	153.3
1994/03/11-1994/03/12	132.0
1994/03/12-1994/03/13	66.0
1994/03/13-1994/03/14	305.0

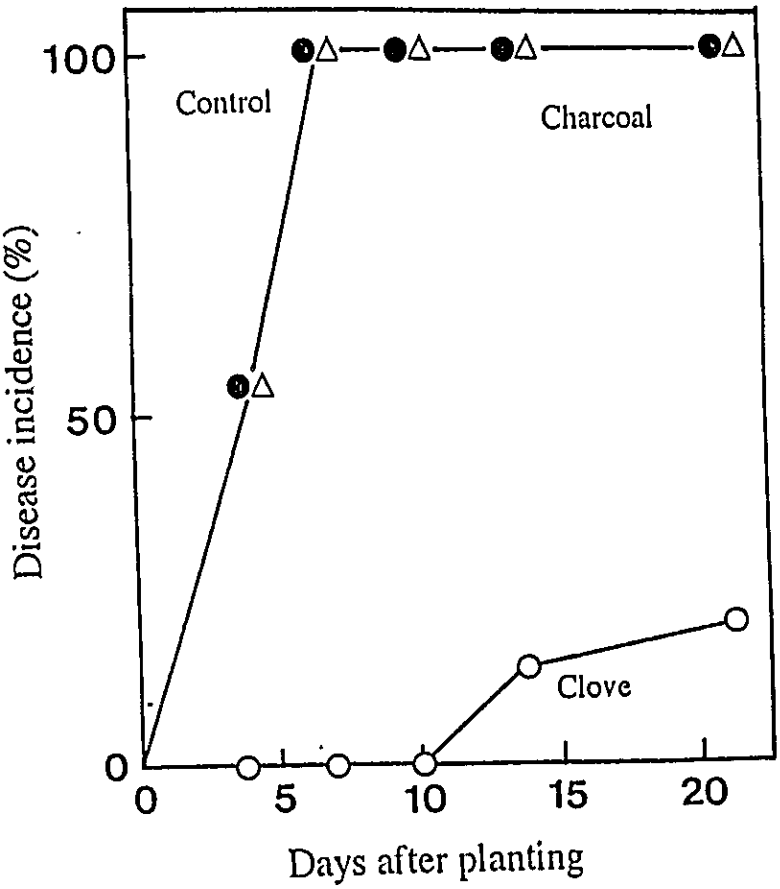


Figure 1: Disease incidence in soils treated with clove powder or charcoal husk.

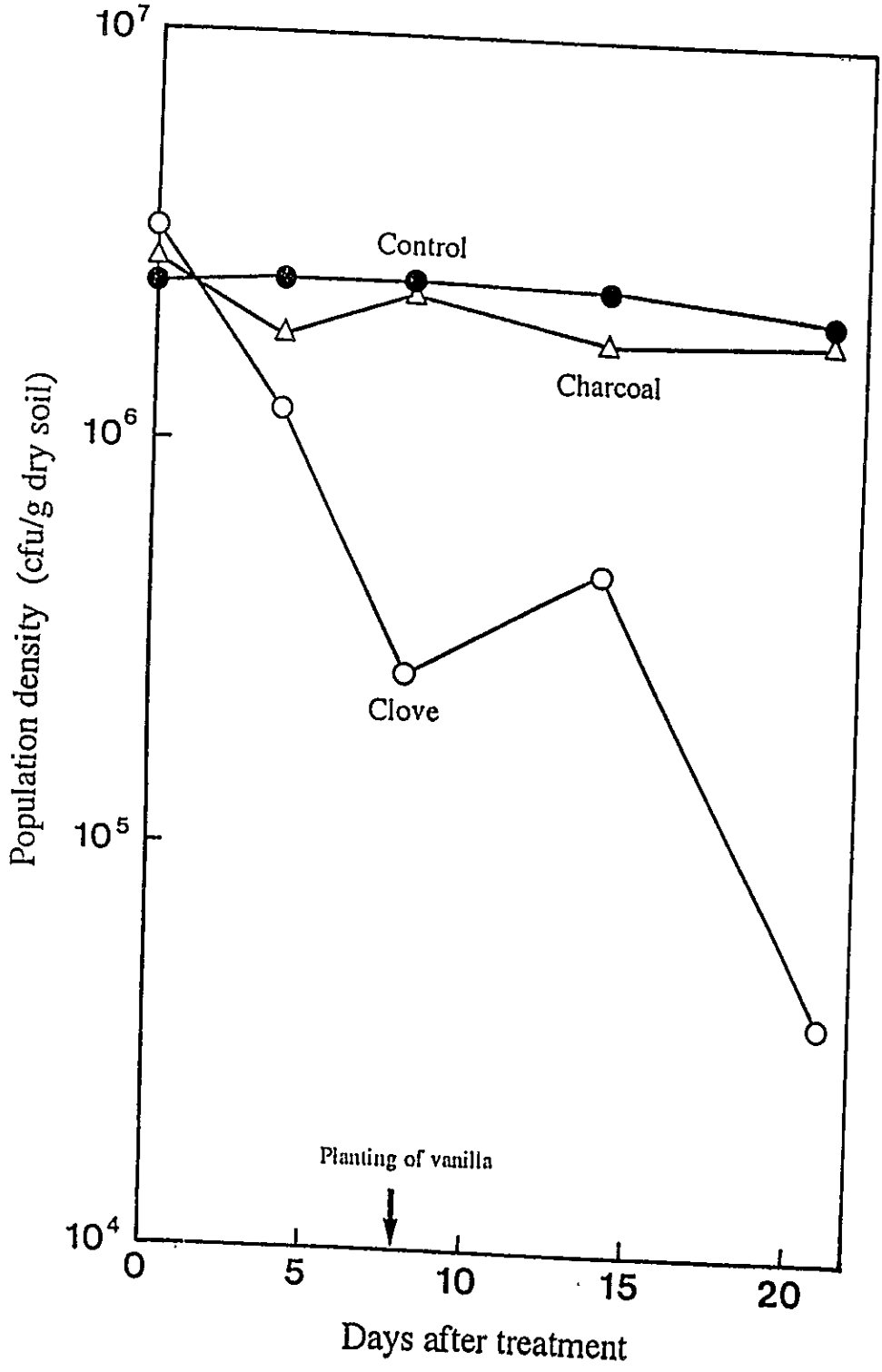


Figure 2 : Variation of population density of *Fusarium oxysporum* f.sp. *vanillae* in soils treated with clove powder or charcoal husk.

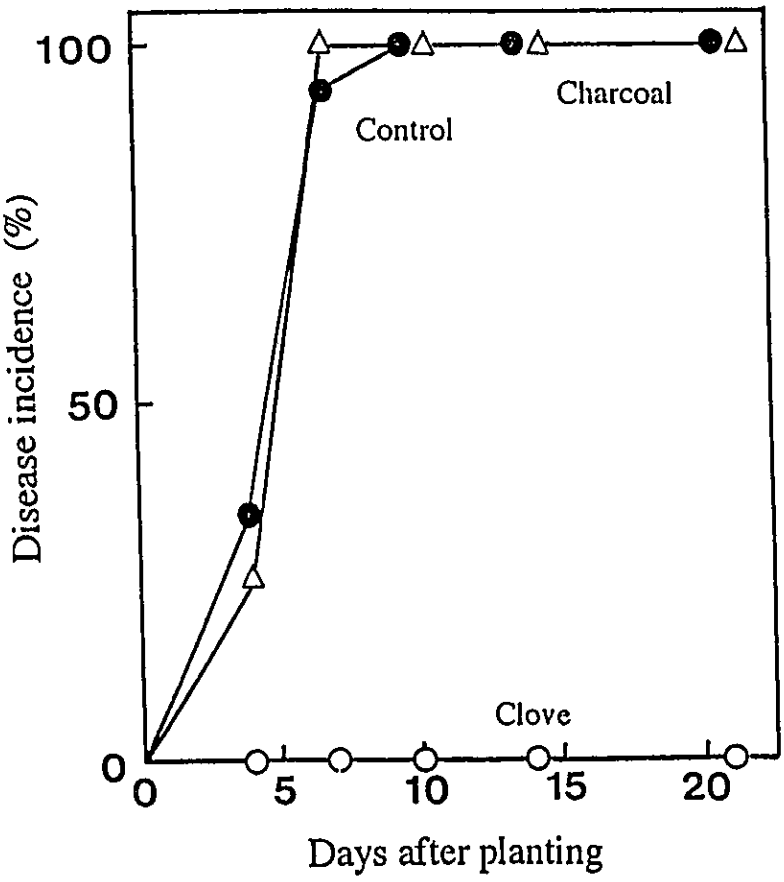


Figure 3 : Disease incidence in soils treated with clove powder or charcoal husk.

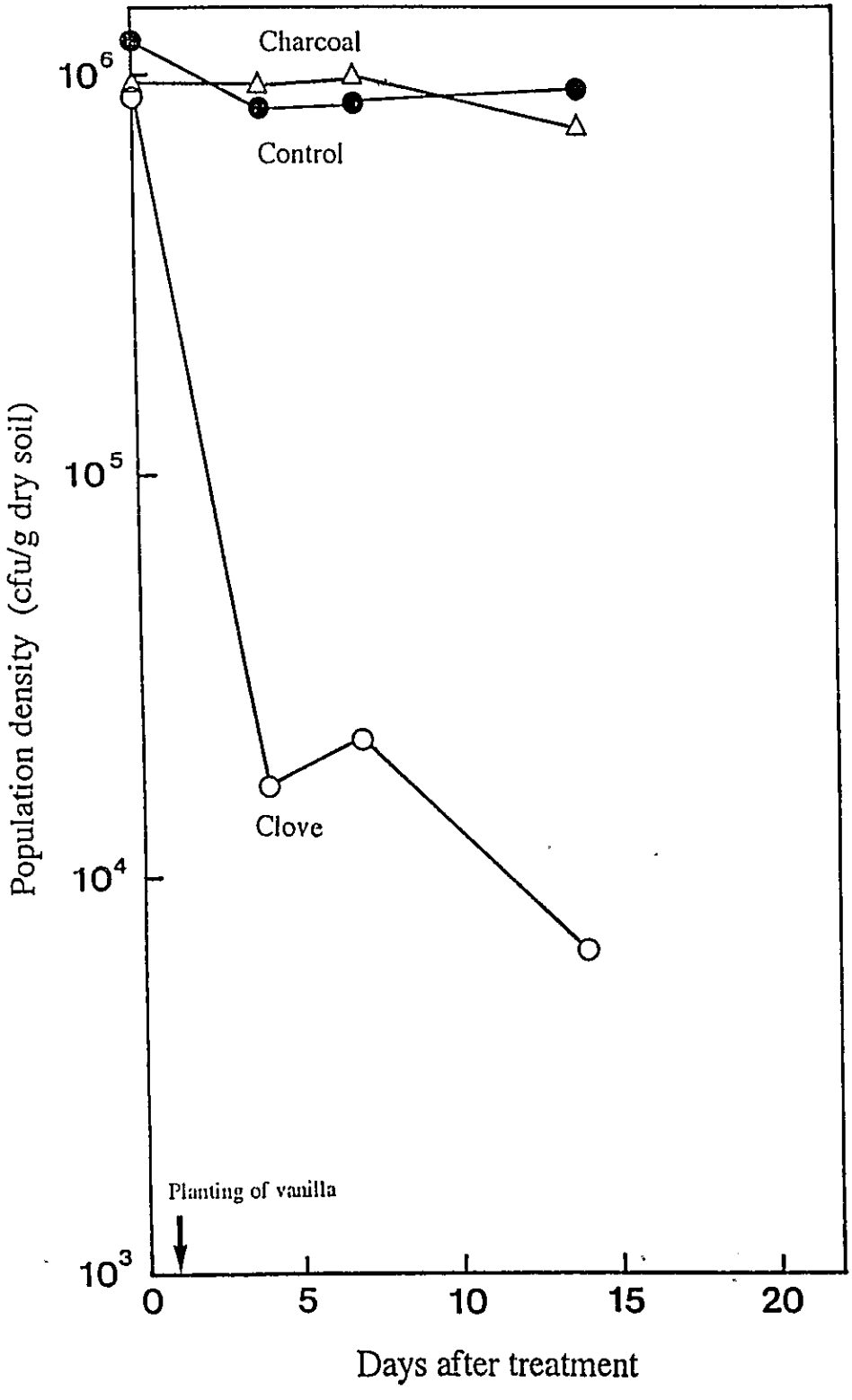


Figure 4 : Variation of population density of *Fusarium oxysporum* f. sp. *vanillae* in soils treated with clove powder or charcoal.

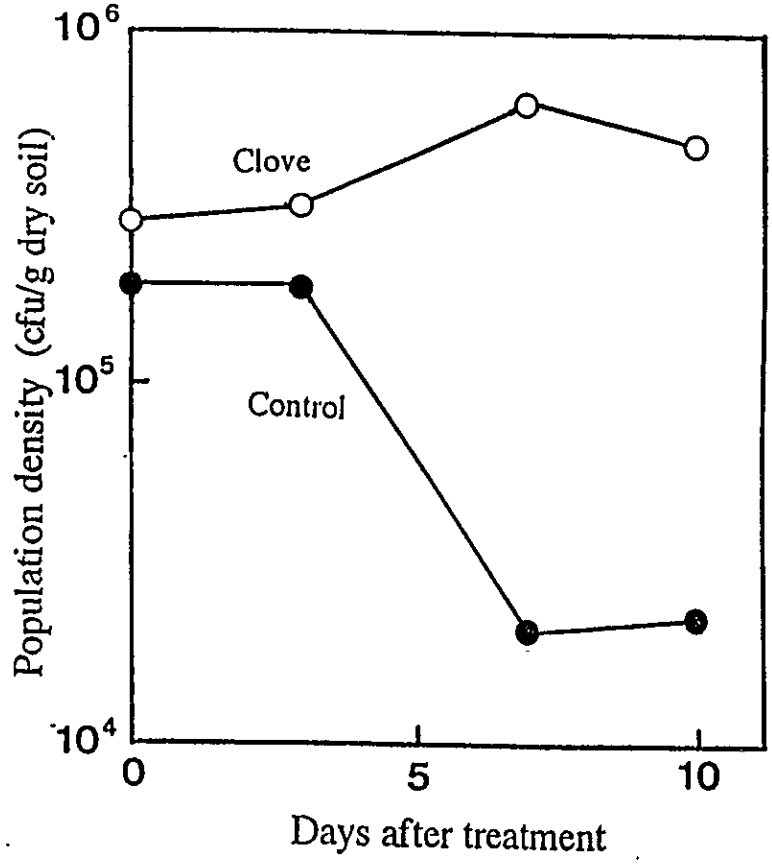


Figure 5 : Variation of fungal population density in non-sterilized soil treated with clove powder.

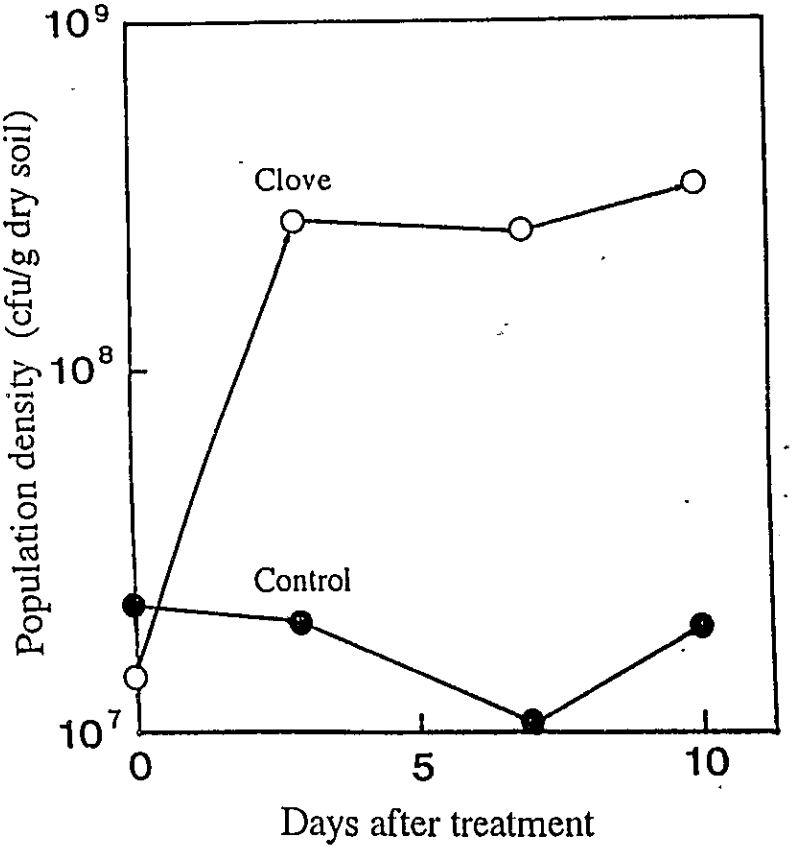


Figure 6 : Variation of bacterial population density in non-sterilized soil treated with clove powder.

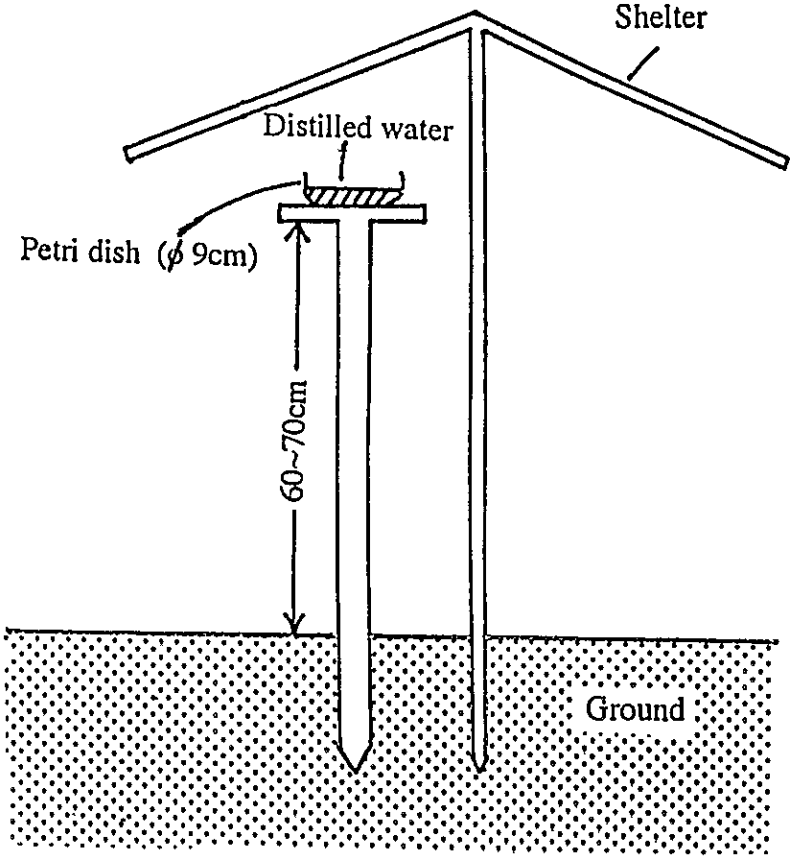


Figure 7 : Apparatus on trapping for wind-dispersed spores of *Fusarium oxysporum* f. sp. *vanillae*.

TARC (now JIRCAS) VISITING RESEARCH FELLOWSHIP 1993/94

Report
by
Nurliani Bermawie

JIRCAS (Japan International Research Centre for Agricultural Sciences) Visiting Research Fellowship Program was initiated in 1992. It invites 10 researches from developing countries to carry out research for a period of one year in the subtropical environment of Ishigaki island, Okinawa Prefecture. Research program under the fellowship consists of 4 themes as follows; Development techniques for environmental control by using plants and microorganisms specific to the tropics and sub-tropics) (No.1 theme), Studies on the mechanism of heat tolerance of tropical and subtropical srops (No. 2 theme), Identification and evaluation of salt-tolerance crops (No. 3 theme), Evaluation and development of long-term conservation techniques of genetic resources of vegetatively propagated crops in the tropics and subtropics (No. 4 theme). These researchers were carried out ender close cooperation with the Japanese counterparts.

From October 1993 till September 1994 I was invited as one of the visiting researchers. I selected research theme no. 3, analyzing the biochemical changes related to salt-tolerance in rice. The abstract of the research was follows:

SYNTHESIS OF SALT-STRESS RELATED PROTEINS IN TOLERANT AND SENSITIVE VARIETIES OF RICE (*Oryza sativa* L.)

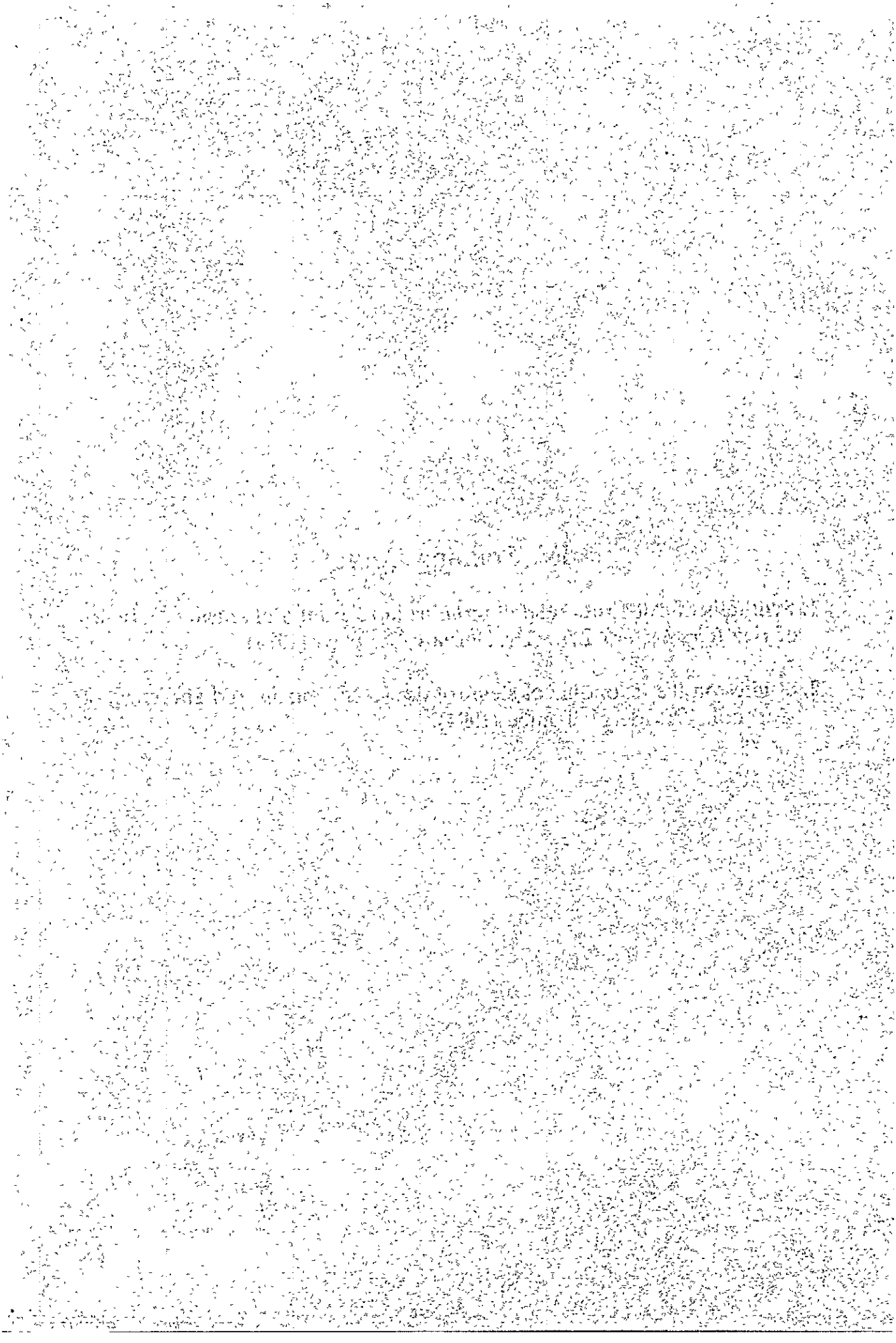
ABSTRACT

Two sets of experiments were conducted. In the first experiment, 3-week-old seedlings of five paddy rice varieties differing in salt-tolerance were subjected to NaCl solution (10 dS/m) for one week. To clarify possible changes in protein profiles of rice in reaction to salt stress which may associate with salt tolerance. The number of polypepetides induced by the treatment was more in salt-tolerant (17) and semitolerant varieties (15) than in sensetive ones (11). Although polypeptides having molecular weight ranging from 76-132 kD were accumulated in all varieties, the amount of the accumulated polypeptides was more in tolerant and semi-tolerant varieties than in sensitive ones. Low molecular weight protein of 21 kD was also found more abundance in tolerant and semi-tolerant than in sensitive ones. In the second set, Pokkali and IR28 were treated with NaCl solutin (18 dS/m) for 5 h, 1 day and 3 days. Three salt-responsive polypepetides of 15 kD, 26 kD and 28 kD were found more abundance in both Pokkali and IR28. These polypeptides accumulated only after a short-term exposure (5 h to 1 day) to NaCl.

IV. Training Course

1. Synthesis of salt-stress related proteins in tolerant and sensitive varieties of rice (*Oryza sativa* L.). Dr. Nurliani Bermawi (1994).

2. Studies on the taxonomy of stem rot disease on vanilla and antifungal of eugenol. Dr. Mesak Tombe (1994).



STUDIES ON THE TAXONOMY OF STEM ROT DISEASE ON VANILLA AND ANTIFUNGAL OF EUGENOL*)

Mesak Tombe

CONTENT

Isolation of *nit* mutants.

Nit mutant phenotype of *Fusarium oxysporum* f. sp. *vanillae* from Indonesia.

Vegetative compatibility groups (VCGs) of *F. oxysporum* f. sp. *vanillae* from Indonesia.

Antifungal activity of eugenol against several formae speciales of pathogenic *Fusarium oxysporum* from Japan.

Detection of eugenol contents in soil.

RINGKASAN

Penelitian ini dilaksanakan di Laboratorium penyakit, Fakultas Pertanian, Universitas Hokkaido, Jepan pada tahun 1993. Bertujuaan untuk mendeterminasi Vegetative compatibility groups (VCGs) *Fusarium oxysporum* f.sp. *vanillae* asal Indonesia dan aktivitas eugenol sebagai antijamur terhadap beberapa *F. oxysporum* patogenik asal Jepang. Hasil penelitian menunjukkan sensitifitas dari beberapa isolat *F. oxysporum* patogenik asal Jepang terhadap eugenol pada umumnya sama dengan *F. oxysporum* f. sp. *vanillae* asal tanaman panili di Indonesia. Dari 28 isolat *F. oxysporum* f.sp. *vanillae* yang digunakan dalam pengujian komplementasi, 24 isolate dapat dikelompokkan dalam 2 VCGs dan 4 isolat yang belum dikenal pasti kelompoknya.

ABSTRACT

The study was carried out at Laboratory of Plant Pathology, Faculty of Agriculture, Hokkaido University Japan in 1993. The aim of the study was to determine vegetative compatibility groups (VCGs) of *F. oxysporum* f.sp. *vanillae* and antifungal activity of eugenol on several pathogenic *F. oxysporum* from Japan. The results indicated that sensitivity of the isolates from different formae speciales of *F. oxysporum* in Japan to eugenol was almost the same as *F. oxysporum* f.sp. *vanillae*. Out 28 isolat *F. oxysporum* f.sp. *vanillae* used in complementation test, 24 isolates fell into 2 VCGs and 4 were single members of VCGs (artificial groups).

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*) Study was conducted at the Laboratory of Plant Pathology, Faculty of Agriculture, Hokkaido University and supervised by Prof. Dr. Akira Ogoshi and Dr. Kiroku Kobayashi.

INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) is one of the potensial export commodities in Indonesia. It provides incomes of farmers and stock exchange for the country. Growing vanilla in Indonesia has been always engaged with problems of disease and become more destructive and difficult to control from time to time.

In Indonesia stem rot disease of vanilla has constantly caused serious damages to many plantations of a large number of farmers and huge acreage of plants. Therefore it is important to study the possible strains in the disease development such as; *nit* mutant penothype, vegetative compatibility groups (VCGs) and their distribution in Indonesia. It is hoped that such information would help to establish the successful control strategy of the disease.

Control studies include the use of clove leaves and eugenol, which showed prospectve action for the control of the disease in the fields.

The objective of this study was to find out the *nit* muntant phenotype, VCGs of *F. oxysporum* f. sp. *vanillae*, anti fungal activity of eugenol against several pathogenic *F. oxysporum* and understanding of eugenol contents in soil.

MATERIAL AND METHODS

1. Isolation of *nit* mutant

A total of 28 *F. oxysporum* isolates obtained from vanilla plants showing typical symptoms of stem rot disease in 7 provinces throughout the vanilla growing regions in Indonesia were used (Table 1). The minimum medium (MM) and chlorate medium (MMC) described by Puhalla (1985) was used to recognize and generated *nit* mutant (Appendix 1). The chlorate medium (MMC) was prepared by adding the following to 1L of the minimal medium (MM): L-asparagine, 1,6g and KCLO3, 15g. Each strains of *F. oxysporum* was grown on MM for 5 - 7 days at 25 C. Two mycelial blocks (5mm in diameter) were then cut from these colonies and inoculated on each plate containing MMC medium. The culture were incubated at 25 C for 7 - 15 days and examined periodically for the appearance of fast growing sectors from the intially restricted colony. These sector were transferrd to a minimal medium that contained NaNO3 as the sole nitrogen source and

incubated at 25 C for 5 - 7 days. Very thin, but normally expansive growth on MM indicated that the sector were also unable to reduce nitrate.

2. Determination of *nit* mutant phenotypes

Class of *nit* mutant phenotypes of *F. oxysporum* were determined by colony morphology on media containing one of three different nitrogen sources ; nitrate, nitrite and hypoxanthine. The three medium on different nitrogen sources (Appendix 1), described by Correll *et al.* (1987) was used. Mycelial fragment 5 mm in diameter of the *nit* mutant from minimal medium was transferred on each of the three media. The culture were incubated at 25 C and the colony morphology was scored relative to the wilt - type parent after 5 - 7 days. Assignment of *nit* mutants to biochemical phenotype was based on nitrate metabolic pathways outline by Correll *et al.* (1987).

3. Complementation tests

Complementation between *nit* mutants was tested on minimal medium (MM). Five isolates of nit mutant were inoculated on each plate (9 cm in diameter) and the culture were incubated at 25 C and scored for complementation 7, 14 and 28 days. All of the *nit* mutants recovered from the same parent were paired with at least two *nit* 1, two *nit* 3 and two *nit* M from that parent. Also some *nit* M mutant the same phenotype were paired. A *nit* 1 or *nit* 3 and *nit* M mutant from each tester were then paired in all combination to determine the number of VCGs present among 28 strains of *F. oxysporum*. Complementation was evident by the formation of a dense aerial wild type mycelium where two mutants had met and formed a heterokaryon.

4. Determination of antifungal activity of eugenol

Twenty one isolates of pathogenic *F. oxysporum* originally obtained from several plants in Japan (Faculty of Agriculture, Hokkaido University Collection) and 2 isolates originated from vanilla plants in Indonesia were used in this study (Table 3). All the strains were grown on PDA and incubated at 25^{o} C for 7 days. Mycelial fragment 5mm in diameter obtained from the edge of active colony of each fungus tested were paired confrontly with paper disk (6 mm in diameter) containing 1 µg and 5 µg approximately 4 cm apart. The cul-

tures were incubated at 25 C^{O} for 7 days. The mycelial growth of *F. oxysporum* were measured to evaluate antifungal activity of eugenol against pathogenic *F. oxysporum*.

5. Detection of eugenol contents in soil

The natural soil collected from Hokkaido University Experimental Garden were used. One hundred grams of natural soil were mixed with clove leaf powder (5g and 10g) and eugenol (0.2g and 0.5g). The eugenol compound was dissolved in 10 ml aceton before treatment application.

Ten grams of soil sample from each treatment was then extracted with aceton on rotary shaker for 3 days. The extracts were filtered with Whatman Filter Paper and taken to dryness in rotavapor RE 120. For detection of eugenol content the residue was dissolved in aceton and then 2 µl of the solution was injected into gas-liquid chromotography (GLC).

RESULTS

1.*Nit* mutant phenotype

The 28 isolates of *F. oxysporum* readily formed chlorate resistant sectors on minimal chlorate medium (MMC). Chlorate resistant sectors appeared as a resumption of growth from the inoculum block, usually after 7 - 14 days of incubation.

The majority (55 - 90%) of the chlorate resistant sectors recovered were unable to utilize nitrate as a sole nitrogen source and consequently grew at the expensieve of colonies with no aerial mycelum on MM, these sectors were designated *nit* mutants. Five to 25 *nit* mutants were obtained from each strain of *F. oxysporum* f. sp. *vanillae*. Several sectors that were resistant to chlorate were recovered, but they had a wilt-type colony morphology on MM.

All the *nit* mutants obtained from the 28 isolates of *F. oxysporum* f. sp. *vanillae* isolates could be devided into three phenotype classes ; *Nit* 1, *Nit* 3 and *Nit* M (Figure 1). The majority of *nit* mutants recovered from MMC when paired on three diffrent nitrogen sources were *nit* 1 (60,7%). The *nit* 1 and *nit* 3 mutant were recovered from all 28 isolates of *F. oxysporum* f. sp. *vanillae*, but *nit* M mutant were recovered only from 7 strains (Table 1).

Table 1. Number and phenotype of *nit* mutants recovered from 28 isolates of *F. oxysporum* of vanilla.

Location (Indonesia)	Isolate No.	Number of *nit* mutant examined	*Nit* mutant class *Nit* 1	*Nit* 3	*Nit* M
Temanggung	94	9	6	3	0
	81	13	2	6	5
Cilacap	89	23	17	6	0
Brebes	68	10	5	5	0
	10A	22	6	2	14
Bogor	61	16	10	2	4
	42	12	1	11	0
	36	8	3	5	0
	64	12	2	2	8
Sukabumi	54	11	2	9	0
	59	8	2	6	0
Serang	22	25	20	5	0
	19	22	6	6	10
Sumedang	111	25	20	5	0
	110	15	7	8	0
Natar	101	11	9	2	0
	102	12	5	7	0
Tabanan	99	17	14	3	0
	18	6	2	4	0
Minahasa	119	21	16	5	0
	117	22	6	6	10
	121	5	4	1	0
Toraja	104M	17	15	2	0
	114	8	5	3	0
	115	23	12	6	5
Samarinda	122	5	3	2	0
	81M	17	11	6	0
	31	17	12	5	0
Total		402	244	116	42

2. Vegetative compatibility groups (VCGs)

Complementation between different mutants of *F. oxysporum* f. sp. *vanillae* was indicated by the development of dense aerial growth where the mycelia of the colonies grew together and anastomosed (Figure 2). Complementation occurred between *nit* mutants with different phenotypes. When *nit* M mutants were paired, complementation occurred more rapidly and resulted in heterokaryon that were more robust than those of other *nit* mutants pairs (Figure 3). When *nit* 1 and *nit* 3 mutants were paired, the complementation reaction was with even after 3 weeks.

In some pairings of *nit* 1 with *nit* 3 mutants recovered from the same parental strains, no complementation was observed. Some *nit* 1 mutant were able to complement one another, as could some *Nit* M mutants. No complementation was observed between any of the *nit* 3 mutants.

In vegetative compatibility tests 402 *nit* mutants from 28 isolates of *F. oxysporum* f. sp. *vanillae* obtained from vanilla at 7 provinces in Indonesia were assigned to two VCGs (Table 2). In addition, there were 4 isolates of *F. oxysporum* f. sp. *vanillae* that were found to be single member VCGs (Table 2). VCGs are numbered according to Puhalla (1985) and Leslie (1988).

3. Antifungal activity of eugenol

All the 23 isolates of *F. oxysporum* pathogenic formed inhibition zone when paired confrontly with paper disks containing 1 µg and 5 µg of eugenol (Figure 4). As shown in table 3, the width of inhibition zone of the 23 isolates of *F. oxysporum* range from 18 mm to 33.0 mm, indicated that sensitivity of the fungi from different formae speciales of *F. oxysporum* in Japan was almost the same with *F. oxysporum* f. sp. *vanillae* (21 mm to 25 mm).

The morphological abnormality of the mycelium of *F. oxysporum* pathogenic was seen under microscope indicated that eugenol inhibited not only radial growth but also sporulation. The mycelial tips of the pathogen generally were swelled, branched and distorted compared to normal mycelium without eugenol treatment.

Table 2. Geographical location and vegetative compatibility group (VCG) of *F. oxysporum* of vanilla from Indonesia.

VCGs	Geographical location		Isolate
	Province	District	
0201BI	Central Java	Temanggung	F-81, F-94
		Cilacap	F-89
		Brebes	F-68
	West Java	Bogor	F-42, F-61
		Serang	F-22
		Sumedang	F-110
		Sukabumi	F-59
	Lampung	Natar	F-101, F-102
	Bali	Tabanan	F-99
	North Sulawesi	Minahasa	F-117, F-119, F-121
	South Sulawesi	Tana Toraja	F-114, F-115
	East Kalimantan	Samarinda	F-81M, F-122, F-31M
0202BI	West Java	Sukabumi	F 54
		Serang	F-19
		Sumedang	F-111
	Bali	Tabanan	F-18
BIx*)	Central Java	Brebes	F-11, F-10A
	West Java	Bogor	F-64
	South Sulawesi	Tana Toraja	F-104M

*) Artficial Groups cantaining isolates that is single members of a VCG.

4. Detection of eugenol content in soil

Detection of eugenol compounds from soil was carried out by gas-liquid chromotography (GLC) after extraction with aceton. In GLC analysis, the residues of eugenol contents in natural soil treated with eugenol decreased about 80-90 % (Table 4) after one month treatment. Whereas residue of eugenol from clove leaves powder was almost the same level as control (extracted directly after treatment). From these it was found that eugenol was comparatively stable in soil.

Table 3. Host and formae speciales of *F. oxysporum* and antifungal activity of eugenol against the pathogens.

Isolate	Host	Formae speciales	Inhibition zone*)(mm)
HF8801	garlic	f. sp. *garlic*	28.0
SUF1343	garlic	f. sp. *garlic*	21.5
HUK-1	garlic	f. sp. *garlic*	33.0
OKF-6	lily	f. sp. *lilii*	21.0
OY-1	lily	f. sp. *lilii*	20.0
90 N-1	lily	f. sp. *lilii*	18.0
ATCC 18770	asparagus	f. sp. *asparagi*	20.0
R2-5	asparagus	f. sp. *asparagi*	30.0
F-11	allium bakari	f. sp. *allii*	24.5
F-21	allium bakari	f. sp. *allii*	21.0
A11	allium bakari	f. sp. *allii*	20.0
TUL	tulip	f. sp. *tulipae*	28.5
Y-2	onion	f. sp. *cepae*	26.5
Y-12	onion	f. sp. *cepae*	21.5
F-1	tomato	f. sp. *lycopersici*	20.0
J-2	tomato	f. sp. *lycopersici*	19.5
race 1	tomato	f. sp. *lycopersici*	23.5
Niv	water melon	f. sp. *niveum*	26.5
CUC	cucumber	f. sp. *cucumerinum*	25.0
Mel	melon	f. sp. *melonis*	24.0
SUF-221	sweet potato	f. sp. *batatas*	25.0
F-81M	vanilla	f. sp. *vanillae*	25.0
F-104M	vanilla	f. sp. *vanillae*	21.0

*) Inhibition zone by treatment with 5 ug of eugenol compared to control after 7 days of incubation.

Table 4. Detection of eugenol content in soil by GLC.

Treatment	Eugenol content (mg/100g soil)		
	week		
	0	5	10
Sterile soil			
1. 100 mg eugenol*)	30.15	29.30	3.45
2. 500 mg eugenol*)	142.00	225.10	10.30
3. 5 g clove leaves*)	417.50	244.40	279.05
4. 10 g clove leaves*)	1461.50	483.50	386.25
Natural soil			
5. 100 mg eugenol*)	21.72	1.65	
6. 500 mg eugenol*)	127.50	24.76	
7. 5 g clove leaves*)	483.50	559.50	
8. 10 g clove leaves*)	982.20	778.89	

* Mixed with 100 g soil

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Figure 1. Growth of wild type and three nitrate nonutilizing (*Nit*) mutant phenotypes of *F. oxysporum* f. sp. *vanillae* on media with one of three different nitrogen source. (A), wild type. (B, *Nit* M. (C), *Nit* 1 and (D), *Nit* 3. (MM), minimal medium. (NMM), nitrite medium. (HMM), hypoxanthine medium.

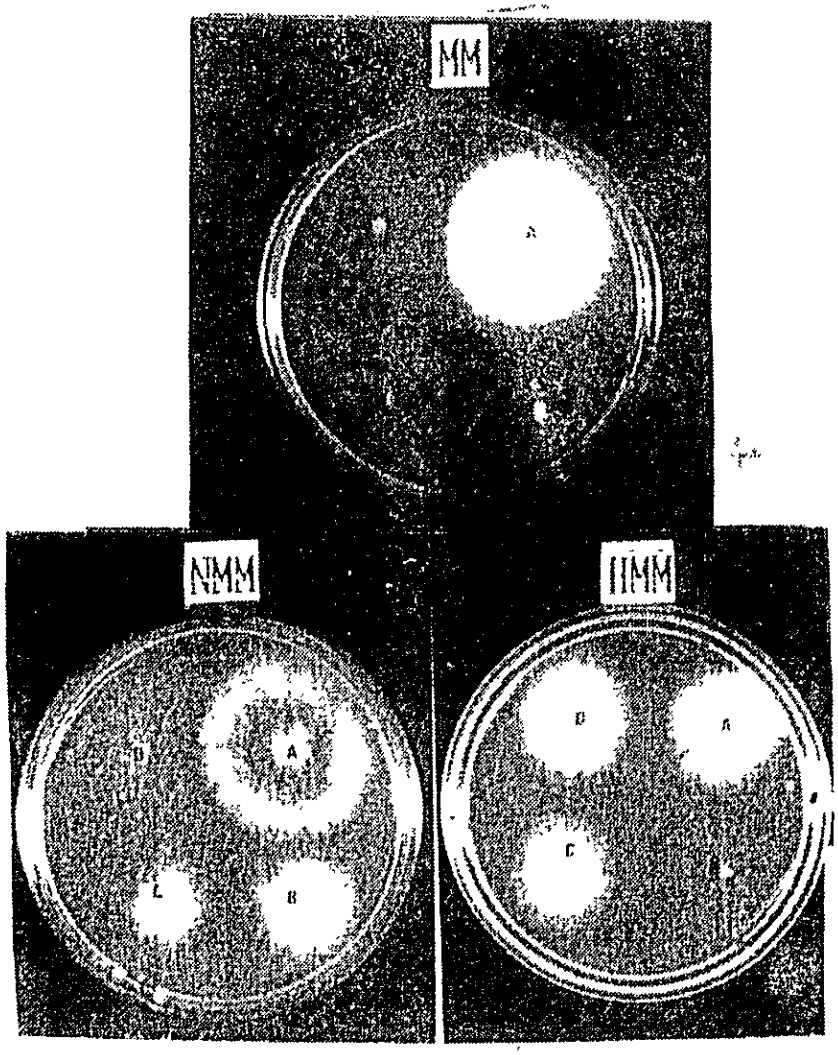


Figure 2. Complementation between mutant *nit* c and *nit* b or *nit* d is manifested by wilt-type growth of the heterokaryon formed where the mutant colonies met, whereas mutants b and a or d and a noncomplementary with each other.

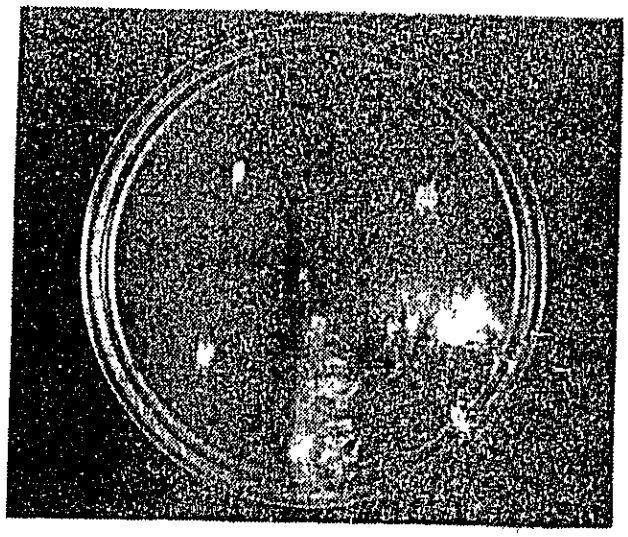


Figure 3. Complementation test between different *nit* mutant phenotypes. Left, *Nit* M in center (tester) surroundings with *Nit* 3. Right, *Nit* 1 in center (tester) surroundings with *Nit* 3.

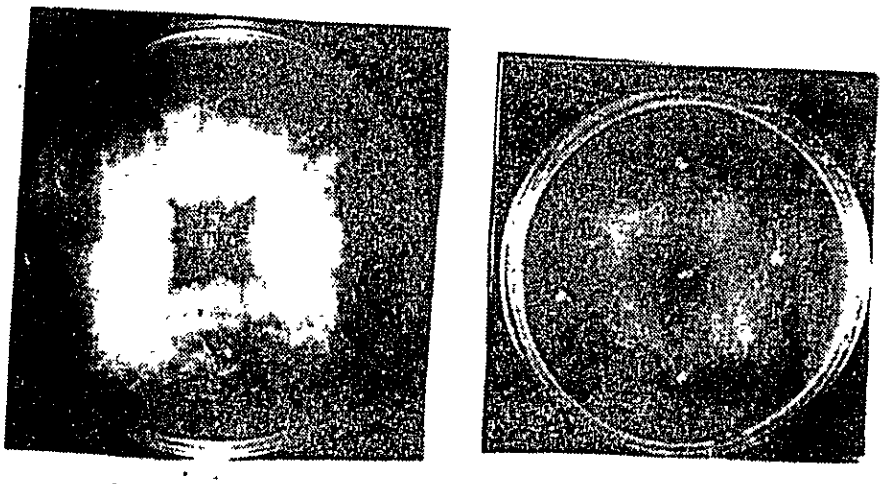
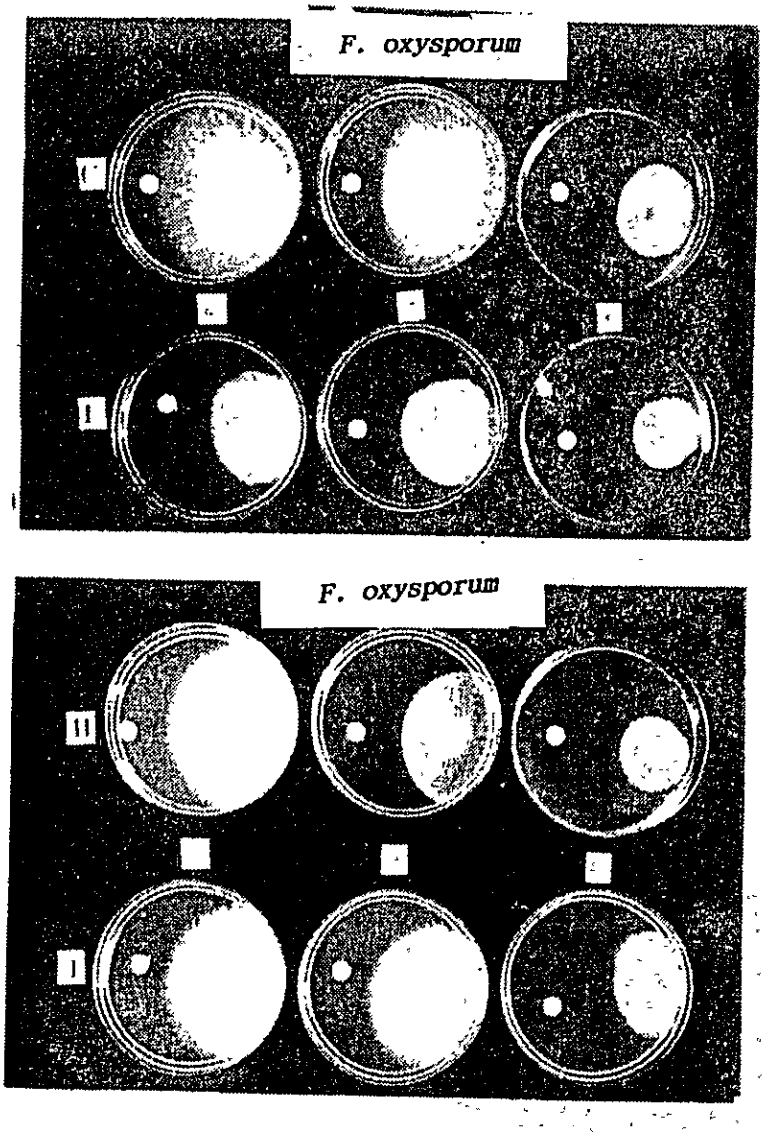
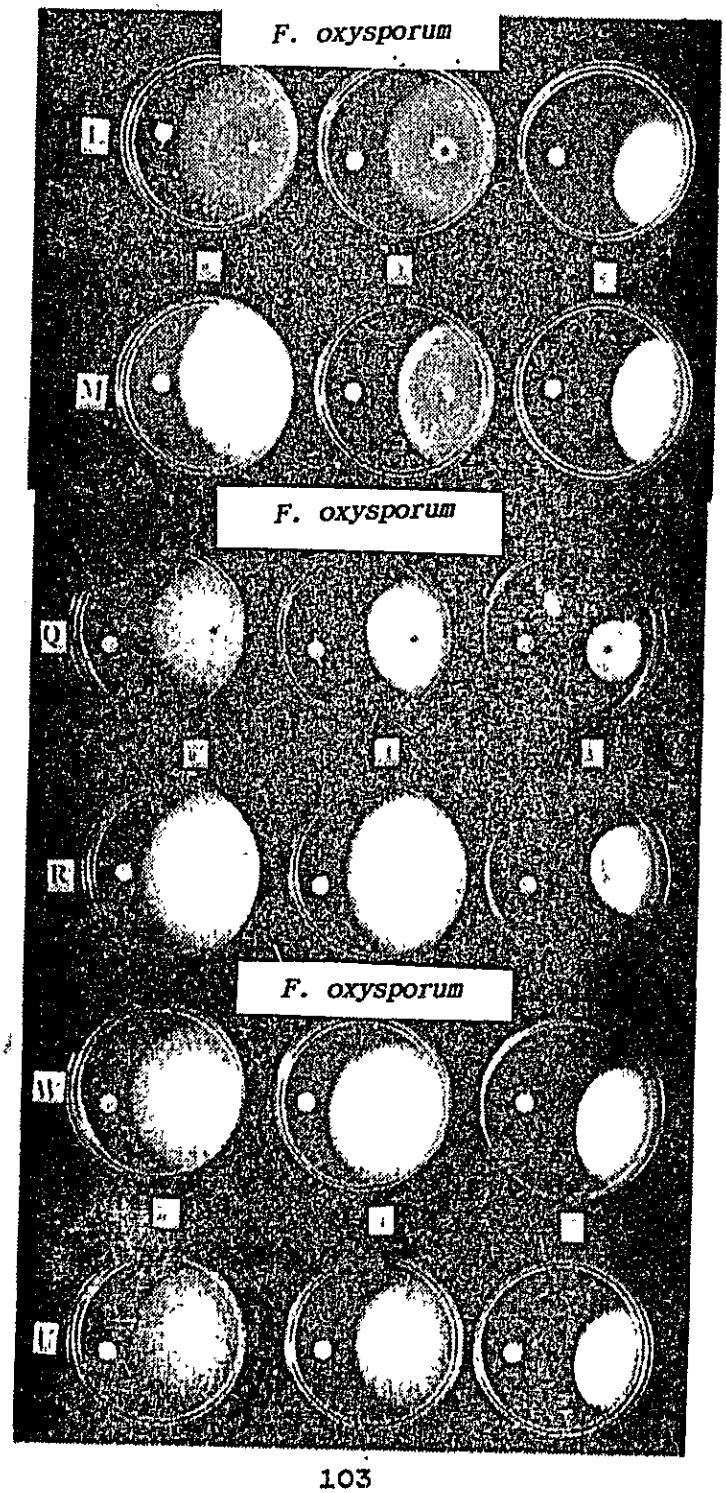


Figure 4. Antifungal activity of eugenol against several formae speciales of *Fusarium oxysporum*. (O), Control. (1), 1 µg of eugenol and (5), 5 µg of eugenol. (C), f. sp. *garlic*. (F), f. sp. *lilii*. (H), f. sp. *asparagi*. (I), f. sp. *allii*. (L), f. sp. *tulipae*. (M), f. sp. *cepae*. (Q), f. sp. *niveum*. (R), f. sp. *cucumerinum*. (W), f.sp. *vanillae*. (U), f. sp. *lycopersici*.




F. oxysporum
F. oxysporum
F. oxysporum

Appendix 1.

1. Minimal medium (MM), (Puhalla, 1985)

Sucrose	30	g
NaNO3	2	g
KH2PO4	1	g
MgSO4	0.5	g
KCl	0.5	g
Difco agar	20	g
Trace elements solution	0.2	ml
Distilled water	1000	ml

2. Chlorate medium (MMC), (Puhalla, 1985)

Minimal medium (MM)	1	l
L-asparagine	1.6	g
KCLO3	15	g

3. Basal medium (Correl *et. al.*, 1987)

Sucrose	30	g
KH2PO4	1	g
KCl	0.5	g
MgSO4	0.5	g
Fe2SO4	10	mg
Difco Agar	20	g
Trace elements solution	0.2	ml
Distilled water	1000	ml

V. ABSTRACT

THE ROLE OF EUGENOL IN SUPPRESSION OF STEM ROT DISEASE OF VANILLA

Mesak Tombe, Kiroku Kobayashi, Ma'mun, Triantoro
Masaomi Oniki and Kazuo Matsumoto

ABSTRACT

Stem rot of vanilla caused by *Fusarium oxysporum* f. sp. *vanillae* is an important and widely distributed disease in Indonesia. It is known that the disease is likely to be controlled by mixing clove leaves into the soil. The experiments were undertaken to extract and identify antifungal compounds from clove leaves and to clarify the relationship to disease suppression. The clove oil obtained by steam distillation of clove leaves (dried powder) was fractionated by solvent and column chromatography with salicic acid and each fraction and clove leaf oil were examined by GC, TLC and bioautography and analyzed with GC-MS. The main constituents of clove leaf oil were eugenol (78%), β-caryophyllene (18%). In antimicrobial tests, eugenol was active against the pathogen (MIC=300ug/ml). The antifungal activity was proportional to eugenol content of each fraction. By paper disk method, eugenol inhibited not only radial growth, but also sporulation and pigmentation of the pathogen. The mycelial tips were also swelled, branched and distorted. From the experiment, it was concluded that eugenol played a significant role in suppression of stem rot disease. The use of eugenol in the field is now being tested.

Industrial Crops Research Journal. 6 (1): 12-20. 1993.

IDENTIFICATION AND CULTURAL TYPES OF *FUSARIUM* ISOLATES FROM VANILLA IN INDONESIA

Mesak Tombe, Y. Komoto and N. Tezuka

ABSTRACT

The cultivation of vanilla, is seriously hampered by stem rot disease whenever it is grown in Indonesia. The pathogen of stem rot of vanilla isolated from various areas of Indonesia was identified as *Fusarium oxysporum* f.sp. *vanillae*. It could be divided into 9 cultural types, based on the colour of colony on PDA medium. The result indicated that there was no correlation between cultural types and geographical origin or infected portion of plants.

Industrial Crops Research Journal. 6 (1):1-5. 1993

ROOTING RESPONSE OF CLOVE STEM CUTTINGS TO VARIOUS PLANT GROWTH REGULATORS

Ireng Darwati, Rosita Sri Mulyati,
Maharani Hasanah and Masaomi Oniki

ABSTRACT

A study on vegetative propagation by cutting of clove was carried out, using several growth regulators. The objective of the present investigation was to determine the rooting response of clove stem cuttings to various types of plant growth regulator. The experiment was conducted at the Research Intitute for Spice and Medicinal Crops, Bogor from February 1991 to July 1991. Randomized bolck design with 5 treatments and 4 replications was used. The treatments consisted of 500 ppm IBA, 250 ppm Paclobutrazol, 100 ppm NK and 10 ppm Rootone-F, and control. The result showed that growth regulator was significantly affecting the percentage of shoot and root growth. The highest percentage was shown by NK treatment.

Journal of Spice and Medicinal Crops I (2) :18-22. 1993

THE EFFECT OF PLANT GROWTH REGULATOR ON THE ROOT GROWTH OF CLOVE MARCOTTING

Ireng Darwati, Rosita Sri Mulyati,
Maharani Hasanah and Masaomi Oniki.

ABSTRACT

A study on the vegetative propagation by marcotting in clove was conducted by using several growth regulators. The objective of the study was to determine the rooting response of marcotting to various types of plant growth regulators. The experiment was carried out at the Research Institute for Spice and Medicinal Crops, from November 1991 to May 1992. Randomized Block Design with 5 treatments and 4 replications was used. The treatments were control, IBA 500 ppm, Paclobutrazol 250 ppm. NK 100 ppm and Rootone-F. The result showed that Rootone-F significantly affected. IBA and Paclobutrazol did not show significant different with Rootone-F.

Journal of Spice and Medicinal Crops II(1): 31-35. 1993

SOOTY LEAF BLOTCH OF *CLAUSENA EXCAVATA*, A NEW DISEASE CAUSED BY *MYCOVELLOSIELLA CLAUSENAE* SP. NOV.

Dyah Manohara, Dono Wahyuno,
Takao Kobayashi and Masaomi Oniki

ABSTRACT

A new leaf disease was found on *Clausena excavata*, a medicinal and essential oil plant, in Java, Indonesia. The causal fungus was a species of *Mycovellosiella*. After confirmation of pathogenicity, the disease was named "sooty leaf blotch" and the causal fungus is described as a new species *Mycovellosiella claucenae*.

Trans. Mycol. Soc. Japan 34: 423-427, 1993.

ANGULAR LEAF SPOT OF RAMIE, *BOEHMERIA NIVEA*, CAUSED BY *PSEUDOCERCOSPORA BOEHMERIAE* IN INDONESIA

Alan Rachmat S., Takao Kobayashi and Masaomi Oniki

ABSTRACT

A serious leaf spot disease of ramie (*Boehmeria nivea*) was observed in Java, Indonesia. It occurs throughout the year, but spreads rapidly in rainy season and slightly in dry one. Decrease of yield is resulted from the marked growth retardation of ramie by the heavy diaease infestation. The causal fungus was identified as *Pseudocercospora boehmeriae* (PECK) GUO et LIU based on the accordance of its morphological characteristics. Conidial germination and mycelial growth of the fungus are well at 20 to 24°C. Incubation period was confirmed as 8 to 10 days through the inoculation experiments. The disease has been observed only on ramie in Indonesia.

Jpn. J. Trop. Agr. 38 (1): 59-64. 1994.

THE DISTRIBUTION OF *FUSARIUM OXYSPORUM*, ANTAGONISTIC AND OTHER MICROORGANISMS ON VANILLA PLANTATIONS

Sukamto, Mesak Tombe, Dono Wahyuno,
Fumio Namiki and Shizuo Mogi

ABSTRACT

Vanilla stem rot caused by *Fusarium oxysporum* f. sp. *vanillae* is the main disease on vanilla plantations in Indonesia. The soilborne pathogen can infect plant material when the pathogen exists at a certain amount in the soil, it can infect plant material if it is pathogenic and can compete with other microorganisms. The aim of this study was to detect population of *F. oxysporum*, other fungi, actinomycetes, bacteria and antagonistic microorganisms in some areas in Indonesia. The disease intensity of the sampled soils from Bali, Manado, Sukabumi and Bogor were severe, mild, light intensity and new area for vanilla plantation respectively. To count the microorganism population, dilution plate method on selective media was used. The results indicated that propagules of *F. oxysporum* significantly influenced disease intensity of vanilla stem rot. The population of *F. oxysporum*, other fungi, actinomycetes, bacteria and antagonistic microorganisms on severe infection was 95.55×10^2; 160.25×10^2; 51.19×10^5; 367.5×10^5 and 6.13×10^5 cfu/g soil respectively, while the population *F. oxysporum* on mild, light intensity of vanilla stem rot and new area for vanilla plantation was 55.23×10^2; 17.89×10^2 and 6.60×10^2 cfu/g soil respectively.

Seminar Regional II PFI Komda Jateng dan DIY, Purwokerto, 2 Juli 1994.

BIOLOGY AND INTEGRATED CONTROL OF *FUSARIUM OXYSPORUM* OF VANILLA STEM ROT

Mesak Tombe, Dyah Manohara and Kazuo Matsumoto

ABSTRACT

Stem rot, a very destructive disease of vanilla, is known to be a main constraint in almost all plantations in Indonesia. The causal fungus, a soil borne *Fusarium oxysporum* f.sp. *vanillae* is very virulent and spreads through various agents. Nitrogen and carbon of different source, stimulate the growth of the pathogen. The mycella of *F. oxysporum* grow well at a wide range of temperature, from 13°C to 30°C, with an optimum of 27-28°C. Its optimum pH is between 6-8. In attempts to control the disease, an integrated approach is suggested by applying compatible components such as planting good stem cuttings, proper cultivations, bioagents and suitable fungicides.

Biology and Control of Crop Pathogens. BIOTROP Special Publication. No. 54 : 159-168, 1994

BIOLOGY AND CONTROL OF LEAF BLISTER BLIGHT PATHOGEN ON CLOVE

Masaomi Oniki, Setyowati Retno Djiwanti and Djiman Sitepu

ABSTRACT

Clove in Indonesia has long been known to suffer from a leaf blister blight disease caused by *Phyllosticta syzygii*. The disease is characterized by blistered-spots on leaves. The pathogen infect leaves, petioles, fruit and young stems. Pathogenicity tests suggest that incubation period of the disease is about 7 days. The pathogen penetrates into the leaves by appressoria through stomata and spreads in the leaf tissues with intra and intercellular hyphae. The blister may be formed as a result of the hypertrophy of the host cells induced by the pathogen. In the petioles and young stems, the infections occur on the cortex and the vascular bundles. On PDA, the pathogen grows well at 20-27^{o}C. Plants may be protected with karbendazim and mancozeb, maneb and koptafol. Application should be started when the disease is first observed and continued at 10-14 day intervals as long as new infection is apparent. Sanitations of infected and fallen leaves and proper fertilizations are required for disease gardens.

Biology and Control of Crop Pathogens. BIOTROP Special Publication. No. 54 : 221-226, 1994

STUDY ON THE STEM ROT DISEASE OF VANILLA IN INDONESIA*)

Mesak Tombe

SUMMARY

Stem rot of vanilla is the main disease of vanilla in many plantation areas in Indonesia. Considerable damages due to the disease become serious problems and cause economic losses every year.

The isolates of *Fusarium* from vanilla produce asexual structures (microconidia, macroconidia and chlamydospores). Morphologically *Fusarium* isolates were variable but they were similar to each other and sufficiently capable of being identified as *Fusarium oxysporum* and the isolates identified as f.sp. vanillae from pathogenicity tests to vanilla and other crops. The nit mutants obtained from *F. oxysporum* f.sp. *vanillae* isolates could be divided into three phenotype classes i.e. Nit 1, Nit 3 and Nit M. In vegetative

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*) - Doctor thesis submitted to Faculty of Agriculture, Hokkaido University, JAPAN.

compatibility tests of nit mutants of *F. oxysporum* f.sp. *vanillae* obtained from vanilla at 12 regencies in Indonesia it was found that they were assigned to two vegetative compatibility groups (VCGs) and four isolates were single member of VCGs.

The growth of the pathogen of vanilla stem rot disease at different temperatures on PDA medium was optimum at 27 ^{0}C. No mycelial growth occurred at 35^{0}C and 40^0 C. The mycelia grew very well on media with pH ranged from 6 to 8. The optimum pH was 7 for the fungus and the growth of the pathogen was very poor in the pH 2 or 9. All the nitrogen and carbon sources produced better growth of *F. oxysporum* f.sp. *vanillae* compared with the basal medium. Potassium nitrate was the best nitrogen source for the growth of the pathogen in medium. The most fertile amount of growth of the pathogen was given by glucose, glycerol and mannitol.

Reduction number of population of *F. oxysporum* f.sp. *vanillae* determined by using a selective medium indicated poorer survival of inoculum in soils with low or no nitrogen than that grown in a high nitrogen content. The animal manure from goat and cow were effective to reduce population of *F. oxysporum* f.sp. *vanillae* in soils. On the contrary animal manure from chicken stimulated population of the pathogen in the soils compared with control. Soil amendment with rice husk charcoal in soils infested with *F. oxysporum* f.sp. *vanillae* indicated that charcoal reduced disease occurrence of stem rot.

Benomyl resistant isolates of *F. oxysporum* f.sp. *vanillae* were found in 5 of the 15 locations and were first observed in 1986 in Temanggung, Central Java and Minahasa, North Sulawesi. Pathogenicity test of the benomyl resistant isolates indicated that 8 isolates were pathogenic to vanilla plants and the other one was non-pathogenic. The minimal inhibitory concentration (MIC) values of resistant isolates of *F.oxysporum* f. sp. *vanillae* from vanilla ranged from 100 ppm to 1,000 ppm.

All 5 isolates of *Trichoderma harzianum* inhibited the growth of *F. oxysporum* f.sp. *vanillae* in vitro. Both the mycelial growth and population of the pathogen in control was significantly higher than in treatments with *T. harzianum*. On the contrary there was no significant effect on disease occurrence of stem rot. Among all the colonies of bacteria detected from rhizosphere of plants, about 10% produced a fluorescent pigment on the medium, and 24 isolates showed an antibiotic activity against the pathogen. Pre-treatment of non-pathogenic *F. oxysporum* by dipping method indicated that suppressed the disease occurrence of stem rot of vanilla compared with control.

Eugenol is the main antibiotic compound of clove leaves and played a significant role of disease suppression of stem rot of vanilla. Isolation of antibiotic compound from dried powder and essential oils of leaves showed that about 2 % essential oils of leaves were obtained from leaf powder. Antimicrobial spectra of the main compounds of clove leaves indicated that the growth of the pathogen of stem rot of vanilla was completely inhibited by eugenol at

300 ppm, *Phytopthora capsici* at 200 ppm, *Rhizoctonia solani* and *Sclerotium rolfsii* at 400 ppm.

Morphological abnormality of the mycelium of the pathogen of stem rot of vanilla was seen under microscope. Eugenol inhibited not only radial growth but also sporulation and pigmentation. The mycelia tips swelled, branched and distroted compared with normal mycelium without eugenol treatment.

Field experiments showed that eugenol application by spraying on vermiculate, and then mixing with infested soil were more effective to suppress the number of infected plants and population of *F. oxysporum* f.sp. *vanillae* in soils compared with clove leaf powder.

VEGETATIVE COMPATIBILITY GROUPS OF *FUSARIUM OXYSPORUM* F.SP. *VANILLAE* IN INDONESIA

Mesak Tombe, Kiroku Kobayashi and Akira Ogoshi

ABSTRACT

Since 1960's production of vanilla in Indonesia has been limited by a stem rot disease. The disease caused by *Fusarium oxysporum* f.sp. *vanillae* has been widely distributed on vanilla plantation in Indonesia. The study was carried out at the Laboratory of Plant Pathology, Hokkaido University, Japan.

Over 402 nitrate nonutilizing (*nit*) mutans were recovered from 28 isolates of *F. oxysporum* f.sp. *vanillae* cultured on *Fusarium* minimal medium, amended with 1.5% of potassium chlorate. The isolates were obtained from vanilla plants in 12 provinces in Indonesia. The *nit* mutants could be divided into three phenotype classes i.e, *nit* 1, *nit* 3 and *nit* M. These nitrate reduction mutants were used in complementation tests for vegetative compatibility. In vegetative compatibility tests of *nit* mutants of *F. oxysporum* f.sp. *vanillae*, it was found that they were assigned to two vegetative compatibility groups (VCGs) and four isolates were single member of VCG. Research on the vegetative compatibility using *nit*-mutan isolates open the possibility for identification of non-pathogenic *F. oxysporum* as biological control agents

Indonesia Journal Crops Science. 9 (2). 1994.

Brown Leaf Spot of Kapok, *Ceiba pentandra* GAERTN., Caused by *Pseudocerospora italica* (CURZI) DEIGHTON in Indonesia

Dono Wahyuno, Takao Kobayashi and Masaomi Oniki

ABSTRACT

Brown leaf spot disease of kapok, *Ceiba pentandra* GAERTN, was found in East Java, Indonesia. It was recorded to be prevalent on the seedlings and young trees of kapok. The causal fungus was identified as *Pseudocercospora italica* (CURZI) DEIGHTON through the etiological and mycological studies. It grew on PDA at 13 to 30° C with optimum temperature between 20 and 24° C. Employment to product conidia artificially on agar media was unsuccessful. Incubation period was confirmed as 4 weeks through the inoculation experiment.

Japanese Journal of Tropical Agriculture. 39 (1) : 35-38. 1995