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# EFFICACY OF PLANT EXTRACT FORMULATIONS TO SUPPRESS STEM ROT DISEASE ON VANILLA SEEDLINGS

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## ABSTRACT

Four plant species, i.e. *Eugenia aromatica* (Family Myrtaceae), *Piper betle* (Family Piperaceae), *Alpinia galanga* (Family Zingiberaceae) and *Sphaeranthus indicus* (Family Compositae) were used to develop extract formulations to confirm their antifungal activity. Of the six formulations tested, four formulation, F1,F2,F3 and F4, contained each extract of above mentioned plants, while F5 and F6 were consisted of the mixtures of *E.aromatica* and *P.betle* extract and *A.galanga* and *P.betle* extract, respectively. Treatment with 5% solution of F5 formulation showed the highest inhibitory effect against the radial growth of *F. oxysporum* f.sp. *vanillae* on PDA medium. Diameter of 5 days fungal colony on PDA treated with F5 was only 6.7 mm, while 87.9 mm on the non amended control. The development of stem rot disease was obviously suppressed on vanilla seedlings grown in the soil treated with 5% solution of each plant extract. The lowest disease incidence was attained by the treatment of F5, in which only 7% of the vanilla seedlings were infected. The low disease incidence in the plot treated with F5 was in line with the low population density of *F. oxysporum* f.sp. *vanillae* in the soil. Results of present study displayed that an extract formulation containing the mixture of *E. aromatica* and *P. betle* extracts was the most effective treatment to suppress the population growth of *F. oxysporum* f.sp. *vanillae* in the soil and in turn to suppress the stem rot disease on vanilla seedlings. This formulation can be used as an alternative measure to control stem rot disease on vanilla seedlings preparation.

**Key words** : Antifungal activity, disease incidence, fungal growth.

## INTRODUCTION

Vanilla is a crop of highly economic value for its pleasant flavor and the second expensive spice after saffron (Anonymous, 2004). The crop is grown in several parts of the world such as India, Indonesia, Madagascar, Comoros, Reunion and Mexico.

Bali Island is known as one of the important vanilla-growing areas in Indonesia that produces vanilla beans of the bourdon-like type. The total area of vanilla cultivation and vanilla production were 4,093 hectares and 279.7 tons in 1991, respectivbely. However, because of the devastating stem rot disease caused by a pathogenic fungus, *Fusarium oxysporum* f.sp. *vanilae*, the total cultivating area and the production of vanilla beans gradually decreased since 1992, and in 2001 the total area of vanilla plantation and the vanilla bean production were only 365 hectares and 5.5 tons, respectively (Anonymous, 2002). Stem rot disease is one of the important constraints for the vanilla cultivation, and responsible for the decreasing of vanilla production (Suprapta et al., 2006; Semangun, 2000; Jayasekar et al., 2008; Sulistyani, 2004).

Survey done by Suprapta et al. (2006) in three main vanilla-cultivating areas in Bali (Tabanan, Jembrana and Buleleng Regencies) showed that the stem rot disease caused by *F. oxysporum* f.sp. *vanillae* was the main disease on vanilla with the average disease incidence by 54.7%. The disease was also found to cause severe destruction on vanilla seedlings preparation with average disease incidence by 33.6%. Even the use of synthetic fungicides, the disease can not be controlled properly yet in Bali.

Several higher plants have been tested for their antifungal activities such as *Pometia pinnata* which is active against *Phytophthora infestans,* the causal agent of potato late blight disease (Suprapta et al., 2002), *Carica papaya* leaf extract active against *Ceratocystis* sp. (Suprapta et al., 2001), while *Piper betle*, *Alpinia galanga* and *C. papaya* extracts were found to be effective to suppress banana wilt disease in the field (Arya et al., 2001).

Jayawijaya (2003) showed that the 0.5% (w/v) of *Piper betle* leaf extract suppressed the growth of *F. oxysporum* f.sp. *vanillae* both on PDA medium and in vanilla seedlings effectively. Suprapta et al. (2006) also proved that the extracts of four plant species of 45 species inhabiting in Bali tested, namely *E.aromatica*, *Alpinia galanga*, *S. indicus* and *Piper betle*, inhibited the radial growth of *F. oxysporum* f.sp. *vanillae* on PDA more than 51%. Six extract formulations were developed and tested for their efficacy to suppress the growth of *F. oxysporum* f.sp. *vanillae* both on PDA medium and in the soil of vanilla seedlings.

## MATERIALS AND METHODS

### Extraction

Four plant species were used in this study to develop extract formulation, namely *E. aromatica* (flower bud), *A.galanga* (rhizome), *S.indicus* (leaf) and *P. betle* (leaf). All of these plants have been proven to posses antifungal activity against *F. oxysporum* f.sp. *vanillae* in previous works (Suprapta et al., 2006). Plant parts were chopped off into small pieces and air-dried for three days and then extracted using methanol three times, followed by filtration through No.2 Whatman filter paper prior to epavoration using a rotary vaccum evaporator (Iwaki, Tokyo). These extracts were used to develop formulations.

### Formulations

Extract formulations were developed using plant extracts, Tween-80, sticker and water. Six formulations were developed and tested in this study, those are :

1. Formulation 1 (F1), containing 10% (w/v) flower bud extract of *E.aromatica*, 5% (v/v) Tween-80 acting as emulsifier and distilled water.
2. Formulation 2 (F2), containing 10% (w/v) rhizome extract of *A.galanga*, 5% (v/v) Tween-80, and distilled water.
3. Formulation 3 (F3), containing 10% (w/v) leaf extract of *P.betle*, 5% (v/v) Tween-80, and distilled water.
4. Formulation 4 (F4), containing 10% (w/v) leaf extract of *S.indicus*, 5% Tween-80, and distilled water.
5. Formulation 5 (F5), containing 5% (w/v) flower bud extract of *E. aromatica*, 5% (w/v) leaf extract of *Piper betle*, 5% (v/v) Tween-80, and distilled water.
6. Formulation 6 (F6), containing 5% (w/v) rhizome extract of *A. galanga*, 5% (w/v) leaf extract of *Piper betle*, 5% (v/v) Tween-80 , and distilled water.

All formulations were packaged in 1-liter plastic bottles and stored in the dark at room temperature before use.

**Bioassay on PDA**

Treatment with extract formulation was done as follows: extract formulation (500 □l) was amended into 10 ml of melted PDA medium. PDA without extract formulation but with 500 □l distilled water with 5% (v/v) Tween-80 was prepared for control. After the PDA medium become solid, a mycelial plug (5 mm diam.) of *F. oxysporum* f.sp. *vanillae* taken from the edge of a 4-day old culture was put in the center of a Petri dish and incubated at room temperature for 5 days in the dark. Five Petri dishes were prepared for each formulation. Each diameter of fungal colony was measured everyday and the inhibitory activity was calculated by the data of colony diameter at the fifth day, according to the formula as follows :

$$\text{Inhibitory activity (\%)} = \frac{\text{Colony diam. on non-amended control - Colony diam.on amended medium}}{\text{Colony diameter on non-amended control}} \times 100\%$$

**Application of Extract Formulations**

Stems of vanilla for seedlings were obtained from the vanilla collection in a green house of the Laboratory of Biopesticide, Faculty of Agriculture, Udayana University, located at Denpasar, Bali. Firstly, the stems were washed in tap water and then with distilled water to remove all surface contaminants. Stem cuttings consisting of two nodes (approximately 20 cm length) were prepared by sharp scissor prior to the treatment with extract formulations. The stem cuttings were then soaked in each 5 % (v/v) water solution of the formulations for an hour. Stem cuttings soaked with 5% (v/v) distilled water solution of Tween-80 were prepared for control (F0). These stem cuttings were planted into the soil in polyethylene bags containing mixtures of sterile fertile soil: cow manure and saw dust (3:1:1) based on weight and inoculated with 10 ml spore suspension of *F. oxysporum* f.sp. *vanillae* ($10^6$ spores/ml). Average spore density was 2 x $10^3$ spores/gram of soil. Treatments with extract formulations were done five times, at 3 to 15 days after planting with three days interval, by dressing each 5% (v/v) of 100-ml extract formulation into the soil at the bottom of each stem-cutting in polyethylene bags. These cultures were maintained in a green house, with average daily temperature 29.4$^o$C and relative humidity 86.7% for 90 days.

This experiment was designed according to the randomized block design (RBD) with seven treatments (F0, F1, F2, F3, F4, F5 and F6). Each treatment was replicated five times, thus, there were 35 experimental units in this experiment. Each experimental unit was consisting of 20 vanilla seedlings.

Disease incidence of stem rot was observed every week and the population of *F. oxysporum* f.sp. *vanillae* in the soil was determined at the end of experiment (90 days after planting). Data obtained in this experiment was subjected to the statistical analysis, and the significance among the treatments were determined according to the least significant difference (LSD) test at 5%.

## RESULTS AND DISCUSSION

Treatment with plant extract formulations significantly ($P<0.05$) inhibited the growth of *F. oxysporum* f.sp. *vanilae* on PDA medium at formulation concentration 5% (v/v) with inhibitory activities varying from 52.5% to 92.4% (Table 1). F5 that consisted of the extracts of *E.aromatica* flower buds and *P.betle* leaves showed the strongest inhibitory activity among the all formulations tested. The radial fungal growth was suppressed 92.4% by this treatment. Fungal growth treated with F1 (containing the extract of *E. aromatica*) was significantly lower than those treated with F3 (containing extract of *P. betle*), suggesting that extract of *E. aromatica* possessed stronger fungicidal activity against *F. oxysporum* f.sp. *vanillae* than that of extract of *P. betle*. However, the mixture of the extracts of *E.aromatica* and *Piper betle* (F5) showed highly inhibiting activity than F1

and F3 independently. This data indicated that there must be synergy effect between extracts of *E. aromatica* and *Piper betle.* Similar phenomenon was observed by Suprapta et al. (2005) in which the mixture of *A. galanga* and *Piper betle* extracts could suppress banana wilt disease on banana seedlings better than that of *A. galanga* or *P. betle* alone.

**Table 1**. Inhibitory activities of plant extract formulations toward the growth of *Fusarium oxysporum* f.sp. *vanillae* on PDA medium.

| No. | Formulations | Diameter of colony at 5-day old (mm) | Inhibitory activity (%) |
|---|---|---|---|
| 1 | F0 | 87.9 a* | - |
| 2 | F1 | 8.5 e | 90.3 |
| 3 | F2 | 20.1 c | 77.1 |
| 4 | F3 | 11.6 d | 86.8 |
| 5 | F4 | 41.8 b | 52.5 |
| 6 | F5 | 6.7 f | 92.4 |
| 7 | F6 | 11.9 d | 86.5 |

*Figures with the same letter in the same column are not significantly different according to the least significant difference (LSD) test 5%.

Several substances present in the plant materials have been reported to posses antifungal activities against several plant pathogenic fungi. The essential oil of clove contained 87% eugenol, 8.01% eugenyl acetate and 3.56% beta caryophyllene (Alma et al., 2007). A study done by Nalina and Rahim (2007) showed that the extract of *P. betle* contained hydroxychavicol, fatty acids (stearic and palmitic) and hydroxyl fatty acid esters (stearic, palmitic and myristic). The important constituents of essential oil of *P. betle* are the phenols, eugenol, chavicol, methyl chavicol and betelphenol (van der Vossen and Wessel, 2000). The leaf extract of this plant has not only antibacterial but also antifungal activity that is related with its containing of eugenol (van der Vossen and Wessel, 2000). The presence of eugenol as the major constituent of *E. aromatica* and *P. betle* may be closely related to their antifungal activity against *F. oxysporum* f.sp. *vanillae.*

*Alpinia galanga* rhizhomes are commonly used as one of the most important spices in many Balinese traditional meat dishes. Rhizome of this plant contains several essential oils such as cineol, eugenol, galangin, galangol, pinene, camphor and methylcinamate (Anonymous, 1986). Dadang (1999) found that *A. galanga* contains 1-acetoxychavicol acetate. Bandara et al. (1989) tested the effect of the crude extract of rhizome of *Acorus calamus* (Araceae) and *Zingiber zerumbet* (Zingiberaceae) against the growth and sporulation of several pathogenic fungi. These plant extracts significantly inhibited the growth of *Cladosporium* sp., *Botryodiplodia theobromae, Fusarium solani, Phythophthora infestans, Phythium* sp., and *Pyricularia oryzae.* The inhibitory activity of *A. calamus* extract against the growth of *F. solani* was higher than that of benomyl, a synthetic fungicide.

Mares et al. (2005) studied the extract from the root of common vegetable, *Chichorium intybus* L. (Asteraceae) for its antifungal activity against various fungi isolated from various environments, including five plant pathogens namely *Botrytis cinerea, Fusarium moniliforme, Phoma betae Pythium ultimum* and *Alternaria* sp. The growth of *Pythium ultimum* and *Alternaria* sp. were inhibited by the extract treatment. The alcoholic extract of *Aloe vera* leaves was confirmed to inhibit

mycelial growth of *Botrytis gladiolorum*, *F.oxysporum* f.sp. *gladioli*, *Heterosporium pruneti* and *Penicillium gladioli*.

Several substances were identified and were thought to be responsible for the antifungal activity, namely 3-(4-hydroxyphenyl)-2(E)-propenoate isolated from *Costus specious* (Bandara et al., 1989); isobutyric acid, butyric acid, valeric acid and caproic acid from *Portulaca oleracea* (Park et al., 1986); tiliacorine from *Tiliacora racemosa* (Tripathi and Dwivedi, 1989); guaianolides from *Chichorium intybus* (Mares et al., 2005); acetoxychavicold acetate from *Alpinia galanga* (Janssen and Scheffer, 1985).

When the plant extract formulations were applied into the soils of vanilla seedlings, the disease incidence development became slow indicating the capacity of the extract to protect the seedling from fungal infection (Fig.1). The stem rot disease on vanilla seedlings in non treated control (F0) occured at two weeks after planting, while at 4 and 8 weeks after planting in F4 and F5 treated plots, respectively. The highest disease incidence was consistently displayed in the control plot while the lowest in the plot treated with F5.At the end of experiment (12 weeks after planting), the disease incidence in the control plot was 91%. The disease incidence in F5 treated plot was the lowest and only 7% of the vanilla seedlings were infected (Table 2).

**Table 2**. Incidence of stem rot disease on vanilla seedlings with or without treatment of plant extract formulations

| No. | Formulations | Disease incidence (%) | Inhibitory activity (%) |
|---|---|---|---|
| 1 | F0 | 91 a* | - |
| 2 | F1 | 14 e | 84.6 |
| 3 | F2 | 30 c | 67.0 |
| 4 | F3 | 21 d | 76.9 |
| 5 | F4 | 57 b | 37.4 |
| 6 | F5 | 7 f | 92.3 |
| 7 | F6 | 18 de | 80.2 |

*Values with the same letter in the same column are not significantly different according to the significant difference (LSD) test 5%.

The low disease incidence on vanilla seedlings treated with F5 was in accordance with the low population density of *F. oxysporum* f.sp. *vanillae* in the soil (Table 3). The population density of this fungus in the soil treated with F5 was only 3 x $10^3$ CFU/g of soil, and significantly ($P<0.05$) lower than the others, respectively. These results suggested that treatment with F5, containing the mixture of *E. aromatica* and *Piper betle* extracts, was significantly effective to suppress the population growth of *F. oxysporum* f.sp. *vanillae* in the soil as well as the incidence of stem rot disease on vanilla seedlings. This formulation can be used as an alternative measure to control stem rot disease on vanilla seedlings preparation.

**Table 3.** Population of *Fusarium. oxysporum* f.sp. *vanillae* in the soil with or without treatment of plant extract formulations

| No. | Formulations | Fungal population (CFU/g soil) x $10^3$ | Inhibitory activity (%) |
|---|---|---|---|
| 1 | F0 | 40.3 a | - |
| 2 | F1 | 7.8 d | 80.6 |
| 3 | F2 | 11.4 c | 71.7 |
| 4 | F3 | 8.7 cd | 78.4 |
| 5 | F4 | 14.7 b | 63.5 |
| 6 | F5 | 3.0 e | 92.6 |
| 7 | F6 | 9.2 cd | 77.2 |

*Values with the same letter in the same column are not significantly different according to the least significant difference (LSD) test 5%.

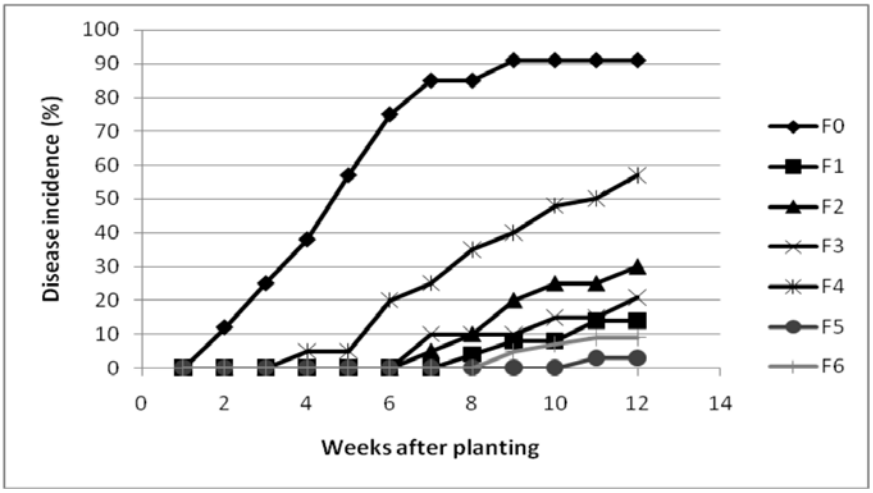


**Fig. 1.** Development of stem rot disease incidence on vanilla seedlings with or without extract formulation treatment. F0 is control plot, while F1, F2, F3, F4, F5 and F6 are treated plots.

## CONCLUSION

Plant extract formulation containing a mixture of *E. aromatica* and *Piper betle* extracts significantly suppressed the radial growth of *F. oxysprum* f.sp. *vanillae* on PDA medium, and in the soil of vanilla seedlings. This extract formulation resulted in the highest inhibitory activity toward stem rot disease incidence on vanilla seedlings. Most of the vanilla seedlings treated with this extract formulation were healthy, indicating that extract formulation containing a mixture of *E. aromatica* and *Piper betle* extracts effectively controlled the stem rot disease and can be used to produce healthy vanilla seedlings.

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