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Compost Extracts of Vegetable Wastes as Biopesticide to Control Cucumber Mosaic Virus

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In semiaerobic conditions, different composting processes of vegetable wastes have different characteristics. When compost extracts amended with the effective microorganism-4 (EM4, +E) and *Pseudomonas aeruginosa* Ch1 (+B) stored for 40 days, the bacteria population and P-content increased. Tobacco plants treated with compost extracts amended with +E+B and [+E+B] directly to organic materials and inoculated with *Cucumber mosaic virus* (CMV) both sprayed or watered applications reduced the disease severity. This is due to the higher bacteria population in the root and rhizosphere, particularly the activities of *P. aeruginosa* Ch1 as plant growth promoting rhizobacteria (PGPR) rather than the activities of bacteria from EM4. The role of *P. aeruginosa* Ch1 to induce resistance of the plants to CMV was suggested by producing siderophores under the limited Fe conditions,17-20 ppm.

Key words: vegetable waste, compost extract, *Pseudomonas aeruginosa* Ch1, biopesticide, CMV

INTRODUCTION

Several studies found that compost added to the growth medium of a plant may alter resistance of the plant to disease. Compost extracts have been used for topical sprays to control foliar disease (Zhang *et al.* 1996), such as grey mold caused by *Botrytis cinerea* on strawberries (Yohalem *et al.* 1995), late blight of potato caused by *Phytophtora infestans* (Elad *et al.* 1994) and apple scab caused by *Venturia inaequalis* (Yohalem *et al.* 1996). It was suggested that compost extracts contained biocontrol agents producing unidentified chemicals that played a role in inducing resistance of plant (De Brito-Alavaez *et al.*1995; Cronin *et al.* 1996). Zhang *et al.* (1996, 1998) found compost extracts contained microflora that can induce resistance of cucumber to *Colletotrichum orbiculare* by increasing â-1,3 glucanase activity. The increasing activity affected the PR-protein production which induced resistance on cucumber infected by pathogen (Zhang *et al.* 1996).

As the result of microbial activities, high C/N ratio in compost generally worked well to suppress plant disease (De Ceuster *et al.* 1999). However, if the C/N ratio was lower, some plant diseases became more severe like *Fusarium* wilt which has preference to excess of N (Hoitink *et al.* 1997). Compost processed aerobically having a higher diversity of microbes and pH below 5.0 will inhibits the growth of bacterial control agents (Hoitink *et al.* 1991). Some studies indicated that plant growth promoting rhizobacteria (PGPR) found in compost was acted as inducers of systemic resistance in plant (Zhang *et al*. 1998). *P. aeruginosa* 7NSK2 is PGPR and effective to control the root pathogen *Pythium splendens* on tomato (Buysens *et al.* 1996) and *Botrytis cinerea* on bean (De Meyer & Hofte 1997) by producing siderophores under limited iron conditions (Crowley 2001). PGPR increases the rate of plant growth (Kloepper *et al.* 1980) and is also able to induce systemic resistance to viral disease (Zender *et al.* 2001), such as *P. fluorescens* strain CHA0 to *Tobacco necrosis virus* (TNV), colonization of tobacco roots can increase salicylic acid concentration in leaf (Maurhofer *et al.* 1998). *P. fluorescens* strain 89B-27 induces systemic resistance to *Cucumber mosaic virus* (CMV) and increases the total leaf area and the length of main stem tomato plants (Raupach *et al.* 1996).

In this study, we compared the different compost-extracting processes of vegetable wastes amended with either the effective microbia-4 (EM4) or *P. aeruginosa* Ch1 to induce systemic resistance of tobacco to CMV. EM4 was used to accelerate the composting and *P. aeruginosa* Ch1 was used as PGPR.

MATERIALS AND METHODS

Composting Vegetable Wastes and Extracting the Compost. The effective microorganism-4 (EM4) was purchased from The Agricultural Shop, Jember. It contains many microfloras such as *Lactobacillus, Streptomyces*, phosphate-solubilized bacteria and yeast. Market vegetable wastes were composted in a closed plastic containers in several methods, i.e. (i) amended with EM4 and *P. aeruginosa* Ch1 directly at the same time as the composting ([C+E+B] directly), then extracted; (ii) amended with EM4 then extracted, and the extract was amended with *P. aeruginosa* Ch1 (C+E+B); (iii) amended with EM4 then extracted and the extract was not amended with *P. aeruginosa* Ch1 (C+E-B); (iv) without EM4 then extracted, and the extract was amended with *P. aeruginosa* Ch1 (C-

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E+B); (v) without EM4 and the extract was not amended with *P. aeruginosa* Ch1 (-C-E-B). During the composting, 50 g $CaCO_3$ was added per 10 kg vegetable wastes to increase the pH and to maintain the temperature, then it turned upside down for several times to maintain semiaerobic conditions. *P. aeruginosa* Ch1 (provided by TC. Setyawati, Jember University) was grown in peptone glucose (5 g peptone protease, Difco Labs. USA, and 10 g glucose per liter water, containing 100 ppm rifampicine) and used 100 ml of inoculums at 45×10^7 cfu ml^{-1} per 20 l of the compost extracts.

The compost extract was made according to Yohalem (1996) by adding the mature compost with well-water (1:3 w/v), followed by stirring, and straining. The CMV-48 inoculum was maintained on cigar tobacco H877 (hypersensitive to TMV) and used as inoculums at dilution 10^{-4} ml^{-1} 0.05 M PO_4 buffer, pH 7.0.

The Ability of PGPR in Compost Extract to Reduce CMV Infection. 37 days old of tobacco H-877 was transferred to 5 kg inceptisol soil per polybag as growth medium. The plastic polybags were without holes to avoid the added compost extract leaking from the medium. Seven days later the plants were inoculated with CMV. The compost extracts were applied (i) as leaf fertilizer, the extract was diluted in water (1:16), and sprayed on the leaf on 5, 10, 15, 20, 25, 30, 35, 40 days after inoculation of CMV (d.a.i.); (ii) as plant fertilizer, the extract was diluted in water (1:16) and watered to the medium on the same days as leaf fertilizer. About 100 µM salicylic acid was used as positive control, and sprayed on the leaf. The severity of CMV infection was observed on 14, 21, 28, 35, and 42 d.a.i. as described in Wahyuni (2005). Area under the disease progress curve (AUDPC) was calculated with the equation: $AUDPC = \Sigma [(0.5)(Y_{i+1}+Y_i)(T_{i+1}-T_i)]$. Y= disease severity at time T, i = the time of assessment (in days numbered sequentially beginning with the initial assessment).

The Ability of PGPR in Compost Extract to Solubilize Phosphate on Pikovskaya Medium. Compost extracts stored at 20, 40, 60 days was each diluted to 10^{-4} to 10^{-5} in 8.5% NaCl, and poured onto Pikovskaya medium. If the bacteria have the affinity to solubilize phosphate, the light zone appeared around the colony. Soluble P Index = diameter each colony with the halo/diameter colony.

The Ability of PGPR to Colonize Rhizosphere and the Root of Tobacco. When the extract was sprayed on leaf, some drops of compost extract fell down to the growth medium, therefore the bacteria were also isolated from the medium. To observe whether the bacteria able to colonize and dominate the plant root and rhizosphere, about 1 g soil of rhizosphere and 1 g root was randomly collected from each plant after treatment finished. Each sample was diluted to 10^{-7} and grown on King's B medium containing 100 ppm rifampicine.

Statistical Analyses. This experiment was designed as factorial with RCD (6 x 2 x 2). Each treatment was replicated five times. The first factor was six different treatments of plant with the kinds of compost extracts processed ([C+E+B] directly, C+E+B, C+E-B, C-E+B, C-E-B, and salicylic acid as positive control). The second factor was the application of extracts to the plants, i.e.(i) watered to the growth medium, and (ii) sprayed to the leaves. The third factor was plants inoculated and not inoculated with CMV. The difference among treatment values were tested by Duncan multiple range at 5%.

RESULTS

Description of the Compost Extracts. Organic materials amended with EM4 were composted two weeks faster than those without EM4. Compost extracts from different processes showed various color. One-two weeks after extraction, the extracts without addition of either EM4 or *P. aeruginosa* Ch1 (C-E-B) showed lightly brownish yellow in color (Figure 1b) compared to those amended with EM4 and *P. aeruginosa* Ch1 (C+E+B, [C+E+B] directly, Figure 1c,d), and they were all slightly smelly. However, after three weeks of extraction, all the compost extract color turned to dark brown to black and very smelly. This indicated that the microbes were still active degraded and composed the organic materials in compost extracts. It was evidence that after growing the microbes on Pikovskaya medium, one of them, i.e. *P. aeruginosa* Ch1 produced a larger halo zone when incubated for 72-75 hrs. The colony of *P. aeruginosa* Ch1 was differentiated from other microbes by growing in King's B medium. It produced a yellow fluorescent color on 48 hrs incubation period; and the growth was slow and small in size.

In semiaerobic conditions, the bacteria population increased as the storage got longer. The [C+E+B] directly and C+E+B (Figure 1d, 2a) showed the highest bacteria populations with dark brown to black in color. On the other hand, C-E-B which was lightly brownish yellow in color (Figure 1b), after 40 days of storage has the lowest bacteria population of (Figure 2a), it means the activity of

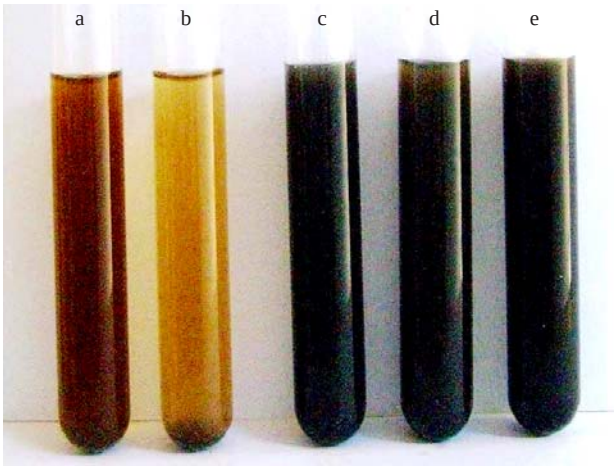

Figure 1. The color of compost extracts of vegetable wastes after being stored for 40 days. (a) Organic materials amended with *P. aeruginosa* Ch1 (B) without EM4 (E); [C-E+B]; (b) Organic materials with no amended either E or B, [C-E-B]; (c) Organic materials amended with E and B directly, then was extracted, [(C+E+B)] directly; (d) Organic materials amended with B and E, [C+E+B]; (e) Organic materials with E and without B, [C+E-B].

bacteria from organic materials was slow. Although soluble P Index of different compos extracts were relatively similar (Figure 2b), the P-content in the extracts increased after 40 days of storage (Figure 2c).

The biological activity of bacteria was higher in the extracts amended with EM4 and *P. aeruginosa* Ch1 (C+E+B, [C+E+B] directly) than in those of C–E+B or C+E-B, confirmed by the bacteria population on Pikovskaya medium. Both bacteria from EM4 and *P. aeruginosa* Ch1 in these previous compost extracts were capable to solubilize P on Pikovskaya medium, and these biological activities increased markedly as the extracts were stored longer. It seems that *P. aeruginosa* Ch1 in compost extracts has capability to solubilize P in (C-E+B) greater as in [C+E+B] directly.

Effect of Compost Extracts on Reducing CMV Infection. Treatments of plants inoculated with CMV either with C+E+B or [C+E+B] directly, both sprayed and watered have the lower percentage of CMV infection and AUDPC than other treatments ($P \leq 0.05$, Table 1). It seems that the synergism EM4 and *P. aeruginosa* Ch1 ([C+E+B] directly) or amended *P. aeruginosa* Ch1 after compost extraction

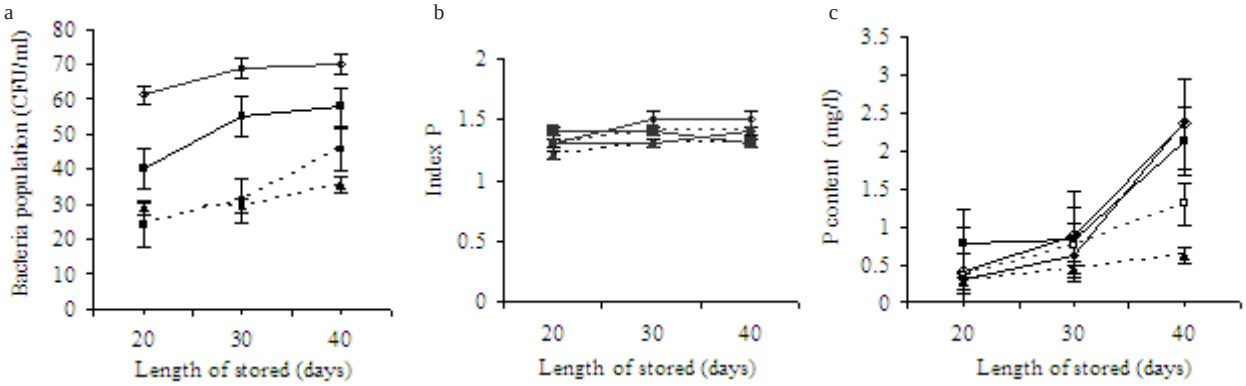

Figure 2. Relationship between population of bacteria (a) and Soluble P Index (b), with the content of P-available (c) in the compost extracts. A and B were the total numbers of bacteria grown on the Pikovskaya medium. C: compost of vegetable wastes, +E or -E = amended with or without EM4, + B or – B = amended with or without *P. aeruginosa* Ch1, [C+E+B] directly = Organic material amended with EM4 and *P. aeruginosa* Ch1 directly at the same times, composted then extracted. -▲-: C-E-B; -⊡-: C+E-B; —◇—: (C+E+B) directly; —◆—: C-E+B; —■—: C+E+B

Table 1. The relationship between bacteria population in the rhizosphere and the root and disease severity and AUPDC of CMV on 42 d.a.i.

Treatments	Disease severity (%)	AUDPC (%)	Bacteria population on King's B	
			Rhizosphere (x 10^6 cfu g^{-1} dry soil)	Root (x 10^6 cfu g^{-1} root)
Watery				
C-E-B-V	0.00a	0.00a	11.63a	10.75a
C-E-B+V	45.67d	41.97dc	14.35a	11.75a
C-E+B-V	0.00a	0.00a	62.05c	58.00bc
C-E+B+V	33.68c	28.48bc	92.78d	87.50c
C+E+B-V	6.76a	5.82a*	103.25d	97.00d
C+E+B+V	20.01b	17.71ab	177.30e	108.00d
C+E-B-V	0.00a	0.00a	62.36c	53.00b
C+E-B+V	40.52d	38.84cd	65.57c	48.00b
C[+E+B] –V	0.00a	0.00a	131.65de	61.50bc
C[+E+B] +V	23.02b	22.75b	152.50e	84.50c
SA – V	0.00a	0.00a	7.67a	6.10a
SA + V	35.71c	34.23c	8.90a	8.08a
Spray				
C-E-B-V	0.00a	0.00a	9.67a	8.25a
C-E-B+V	40.35d	39.70d	10.24a	10.25a
C-E+B-V	0.00a	0.00a	58.13bc	32.50ab
C-E+B+V	29.64c	24.39c	66.25c	48.00b
C+E+B-V	0.00a	0.00a	97.10d	87.00c
C+E+B+V	27.07b	24.36 b	118.75d	95.00d
C+E-B-V	0.00a	0.00a	42.70b	46.50b
C+E-B+V	32.42c	29.35b	42.25b	49.00b
C[+E+B]–V	0.00a	0.00a	98.69d	53.35b
C[+E+B]+V	22.73b	20.52b	102.25d	72.00c
SA – V	0.00a	0.00a	5.40a	2.06a
SA + V	32.93c	31.07c	6.16a	4.30a

d.a.i.: days after inoculation with CMV. + or -V: plant inoculated with or without virus, C: compost, +E or -E: extracts amended with or without EM4, +B or –B: extracts amended with or without *P. aeruginosa* Ch1, SA: salicylic acid, [+E+B]: organic materials amended with EM4 and *P. aeruginosa* Ch1directly, composted then extracted. *Infected naturally. Values within treatments followed with the same letter in one column do not significantly different according to Duncan multiple range test ($P \geq 0.05$).

(C+E+B) were more effective in decreasing the disease severity than with the extract amended with *P. aeruginosa* Ch1 itself without EM4 (C-E+B).

There was a relationship between disease severity with the bacteria population both from the rhizosphere and the root. The bacteria population was higher in the infected plants than that in non-infected plants. The bacteria population of infected plants watered with the compost extracts increased higher than that sprayed with it ($P \leq 0.05$, Table 1). The highest bacteria population of 177.30×10^6 cfu g^{-1} dry soils, and 108.00×10^6 cfu g^{-1} roots was on watered plant with C+E+B+V treatment. It was assumed that in the spray application, some drops of the extract fell down to the growth medium, therefore, the bacteria population was still high in C+E+B+V treatment, 118.75×10^6 cfu g^{-1} dry soil and 95×10^6 cfu g^{-1} root ($P \leq 0.05$, Table 1). Salicylic acid as the positive control also suppressed the disease severity, but the bacteria population was low similar to the negative control, these bacteria may be originally from the rhizosphere (Table 2).

The bacteria population from rhizosphere and root watered with the extracts was higher than that sprayed with it. There were three colonies of bacteria grown in King's B medium and counted as the total bacteria of *P. aeruginosa* Ch1 and bacteria either from EM4 or from the organic materials naturally. The number of fluorescence colony was much greater than others.

DISCUSSION

Under semiaerobic conditions, the different compost extracts have different colors, this was mainly due to the microbial activities. It seems that EM4 amended to the composts has dominant role to degrade and decompose organic materials and this shown with the dark brown to black color of the extract. Organic materials without either EM4 or *P. aeruginosa* Ch1 (E or B) form a clear and brownish yellow extract. When they are only amended with *P. aeruginosa* Ch1 it formed a clear and lightly brownish yellow color extract. However, our previous study shows that after three weeks extraction, vegetable wastes compost extract without EM4 but with *P. aeruginosa* Ch1 and processed anaerobically produced a lightly greyish yellow and slightly milky, whereas the color of aerobically processed extract is a clear and brownish yellow (Sriwardani 2006; Marisha-Gondjong 2006). This indicates that the biological activities of both bacteria of EM4 and *P. aeruginosa* Ch1 to decompose the organic materials under different conditions is different.

Table 2. Population of solubilize-P bacteria from rhizosphere (42 d.a.i) on Pikoskaya medium

Treatments	Population of bacteria ($x10^5$ cfu g^{-1} dry soil) on Pikovskaya medium		Soluble P Index	
	Watery	Spray	Watery	Spray
C-E-B-V	14.8a	12.7a	1.4a	1.3a
C-E-B+V	15.5a	18.8a	1.3a	1.3a
C-E+B-V	60.7c	57.0b	1.4a	1.5a
C-E+B+V	73.5c	57.8b	1.6a	1.4a
C+E-B-V	50.2b	53.3b	1.3a	1.5a
C+E-B+V	58.2b	54.0b	1.4a	1.8b
C+E+B-V	51.2b	52.0b	1.4a	1.3a
C+E+B+V	54.7b	54.7b	1.8b	1.8b
C[+E+B]-V	59.5c	56.3b	1.7b	2.1b
C[+E+B]+V	60.2c	57.8b	1.7b	2.1b
SA-V	2.0a	2.8a	0.3a	0.5a
SA+V	2.5a	2.0a	0.5a	0.4a

d.a.i. days after inoculation with CMV. The abbreviation on Table 2 was the same as on Table 1. Values within treatments followed with the same letter in one column do not significantly different according to Duncan multiple range test ($P \geq 0.05$).

The biological activities of these bacteria also affected C content. It was higher in the compost extract processed with [C+E+B] directly, C+E+B, and C–E+B (data was not shown) followed with the higher C/N ratio, while the N content was not significantly different in all compost extract. This would be because nitrogen have acted as a limiting factor in composting organic materials (Garcia *et al.* 1991). C content including water-soluble carbon may result from degraded and composted organic materials by the physical process and the biological activities (Garcia *et al*. 1993) involving these two bacteria and other microbes from organic materials.

In this study, under semiaerobic conditions the effect of both EM4 and *P. aeruginosa* Ch1 amended to the vegetable wastes was more synergistic to supress CMV development than other compost. The lower AUDPC could be caused by the activity *P. aeruginosa* Ch1 as PGPR (De Meyer & Hofte 1997) rather than caused by bacteria from either EM4 or organic materials naturally. The bacteria population was higher in the infected plants than that in uninfected plants as the result of better ability of *P. aeruginosa* Ch1 to colonize root and rhizosphere. This phenomenon is shown by the number of fluorescence colony was much greater than others when they grown on the King's B medium. In our previous study, compost amended or non-amended with either EM4 or *P. aeruginosa* Ch1 under the anaerob or aerob conditions showed that these two bacteria have different activities to supress the disease severity of anthracnose on *Capsicum annuum*. In the aerob process, the extract amended with both of EM4 and *P. aeruginosa* Ch1 was dominant to reduce the anthracnose severity. On the other hand, the role of *P. aeruginosa* Ch1 itself in the C–E+B compost extract from anaerob processed was dominant to reduce disease severity of anthracnose (Wahyuni *et al*. 2006). Futhermore, the role of *P. aeruginosa* Ch1 to induce resistance of tobacco to CMV was more reliable because the Fe content in the extract (C+E+B) and [C+E+B] directly was low (17-20 ppm), as in the plant growth medium watered with C-E+B (16-18 ppm). Under this limited Fe conditions, *P. aeruginosa* Ch1 may able to produce i.e. pioverdine, piochelin or salicylic acid siderophores (Hofte *et al.* 1994; De Meyer & Hofte 1997) which can induce resistance of the plant against pathogen.

The compost extract processed with E and B increased the number of bacteria colony in the rhizosphere and the root, because the root exudates attract the rhizobacteria to grow (Brimecombe *et al.* 2001). Adding the extract to the plant inoculated with virus increased the bacteria

population. It is possible that the bacteria in the extract forms siderophores as when the extract is sprayed to the plant. Crowley *et al.* (2001) and Ongena *et al*. (1999) suggested that siderophores was formed by fluorescent pseudomonad when the growth medium has limited Fe. *P. aeruginosa* Ch1 produced a yellow fluorescent color when it was grown on King's B medium (Siege 1993). Yellow fluorescent means that bacteria produces pioverdin under limited Fe (Tudor, K. 2008. *Pseudomonas aeruginosa*, www.textbookofbacteriology.net). The siderophores was abundance if the growth medium was watered with the extracts, as shown by the low of Fe content in C+E+B and [C+E+B] directly (17-20 ppm), as was also found by Wahyuni *et al*. (2003). The extracts stored more than 10 weeks has low Fe content (≤ 20 ppm), therefore *P. aeruginosa* Ch1 in C+E+B and [C+E+B] directly were more capable to chelate Fe than plant microbial pathogen (Leeman *et al.* 1996; Zender *et al.* 2001).

In conclusion, plants treated with compost extract increases the population of total bacteria both *P. aeruginosa* Ch1 and from EM4 in the root and rhizosphere. The population *P. aeruginosa* Ch1 is higher than bacteria from EM4. *P. aeruginosa* Ch1 is capable to solubilize P better than bacteria from EM4, however, the synergism of these two bacteria have the particular role in chelatting Fe. We suggest that plant treated with compost extracts amended with the bacteria (+E+B or [+E+B] directly) would induce plant resistance to CMV, whereas the mechanisms reponsible for systemic inducing resistance (Buysens *et al.* 1996; Zhang *et al.* 1998) such as â-1,3-glucanase activity (Maurhofer *et al.* 1998) and the mechanisms to improve the growth medium (Garcia *et al*. 1991; Islam *et al*. 2004) remain unknown.

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REFERENCES

Brimecombe MJ, de Lej FA, Lynch JM. 2001. The effect of root exudates on rhizosphere microbial populations. In: Pinton R, Varanini Z, Nannipieri P (eds). *The Rhizosphere*. New York: Marcell Dekker, Inc. p 95-140.

Buysens S, Heugens K, Poope J, Hofte M. 1996. Involvement of piochelin and pioverdin in suppression of *Pythium*-induced damping off of tomato by *Pseudomonas aeruginosa* 7NK2. *Appl Environ Microbiol* 62:865-871.

Cronin MJ, Yohalem DS, Harris RF, Andrew JH. 1996. Putative mechanism and dynamics of inhibition of the apple scab pathogen *Venturia inaequalis* by compost extracts. *Soil Biol Biochem* 28:1241-1249.

Crowley D. 2001. Function of siderophore in the plant rhizosphere. In: Pinton R, Varanini Z, Nannipieri P (eds). *The Rhizosphere*. New York: Marcell Dekker, Inc. p 223-261.

De Brito-Alavarez MA, Gagne S, Antoun H. 1995. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. *Appl Environ Microbiol* 61:194-199.

De Ceuster TJJ, Hoitink HAJ. 1999. Using compost to control plant disease. *Bio Cycle Mag* 6:61-64.

De Meyer G, Hofte M. 1997. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology* 87:588-593.

Elad Y, Shtienberg D. 1994. Effect of compost water extracts on grey mold (*Botrytis cinerea*). *Crop Prot* 13:109-114.

Garcia C, Hernandez T, Costa F. 1991. The influence of composting on the ferlizing value of an aerobic sewage sludge. *Plant Soil* 136:269-272.

Garcia C, Hernandez T, Costa C, Ceccanti B, Marciandaro G, Ciardi C. 1993. A study of biochemical parameters of composted and fresh municipal wastes. *Bioresources Tech* 44:17-23.

Hofte M, Buysens S, Koedam N, Cornelis P. 1994. Zinc affects siderophore-mediated high affinity iron uptake systems in the rhizosphere *Pseudomonas aeruginosa* 7NSK2. *Biometals* 6:85-91.

Hoitink HAJ, Inbar Y, Boehm MJ. 1991. Status of composted-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. *Plant Disease* 75:869-873.

Hoitink HAJ, Stoe AG, Han DY. 1997. Suppression of plant diseases by compost. *HortScience* 32:184-187.

Islam TMD, Toyota K. 2004. Suppression of bacterial wilt of tomato by *Ralstonia solaneacearum* by incorporation of composts in soil and possible mechanisms. *Microbes Environ* 19:53-60.

Kloepper JW, Leong J, Teintze M, Schroth MN. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* 286:885-886.

Leeman M, den Ouden FM, van Pelt JA, Dirkx FPM, Steijl H, Bakker PHAM, Schippers B. 1996. Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathology* 86:149-155.

Marisha-Gondjong MS. 2006. Effect of spraying of compost extract of vegetable wastes processed anaerobically to the development of anthracnose disease (*Gloeosporium piperarum*) on red chili [Thesis]. Jember: Jember Univ.

Maurhofer M, Reimman C, Schmidli-Sacherer P, Heep S, Defago G. 1998. Salysilic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against *Tobacco necrosis virus*. *Phytopathology* 88:678-684.

Ongena M, Daayl F, Jacques P, Thonart P, Benhamou N, Paulitz TC, Cornells P, Koedam N, Belanger RR. 1999. Protection cucumber against *Pythium* root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. *Plant Pathol* 48:66-76.

Raupach GS, Li Liu, Murphy JF, Tuzun S, Kloepper JW. 1996. Induced systemic resistance in cucumber and tomato against cucumber mosaic virus using plant growth-promoting rhizobacteria. *Plant Disease* 80:891-894.

Siege D. 1993. Bacterial Plant Pathology, Cell and Molecular Aspects. New York: Cambridge Univ Pr.

Sriwardani E. 2006. Effect of spraying of compost extract of vegetable wastes processed aerobically to the development of anthracnose disease (*Gloeosporium piperarum*) on red chili [Thesis]. Jember: Jember Univ.

Wahyuni WS, Iwan A, Purwiko H, Mudjiharjati A, Setyowati T. 2005. Penggunaan *P. putida* strain Pf-20 dan 24.7B untuk memperbaiki sifat kimia tanah dan penginduksi ketahanan tembakau terhadap CMV. *J Perlind Tan Ind* 11:77-87.

Wahyuni WS, Yutriono R, Winarso S. 2003. Pengaruh konsentrasi besi dalam media tanam pada aktivitas *Pseudomonas putida* Pf-20 untuk menginduksi ketahanan tembakau terhadap *Cucumber mosaic virus*. *Hayati* 10:130-133.

Yohalem DS, Harris RF, Andrews JH. 1995. Aquaeous extracts of spent mushroom substrate for foliar disease control. *Compost Scie Util* 4:67-74.

Yohalem DS, Nordheim EY, Andrews JH. 1996. The effect of water extracts of spent mushroom compost on apple scab in the field. *Phytopathology* 86:914-922.

Zender GW, Murphy JF, Sikora EJ, Klopper JW. 2001. Application of rhizobacteria for induced resistance. *Eur J Plant Pathol* 39:39-50.

Zhang W, Han DY, Dick WA, Davis KR, Holtink HAJ. 1998. Compost and compost water extract-induce systemic acquired resistance in cucumber and Arabidopsis. *Phytopathology* 88:450-455.

Zhang W, Hoitink HAJ, Dick WA. 1996. Compost-induced systemic acquired resistance in cucumber to *Pythium* root rot and anthracnose. *Phytopathology* 86:1006-2070.