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Reduced phytotoxicity in biodegraded coir waste

(Kekurangan kesan fitotoksik gabus habuk kelapa yang telah reput)

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Key words: phytotoxicity, coir waste, cress bioassay, phenolic acids, biodegradation

Abstract

The raw coir waste extracts suppressed cress (*Lepidium sativum* L.) root development. However, when the coir waste was biodegraded between 1 and 3 months, this phytotoxicity effect was reduced when period of biodegradation was extended. Gas chromatography analysis demonstrated that raw coir waste contained substantial quantity of phenolic acids, namely ρ-coumaric (62 ppm), ferulic (31 ppm), ρ-hydroxybenzoic (24 ppm) and vanillic (12 ppm). The quantity of these phenolic acids was reduced substantially as biodegradation progressed. The reduction of these phenolic acids led to the improvement of cress root development. This showed that the presence of phenolic acids in the raw coir waste was one of the causes of phytotoxicity. Among the phenolic acids analysed, the higher concentration of ρ-coumaric acid was found to be the cause for cress root inhibition. Thus, coir waste should be biodegraded before being used as plant substrate to reduce the risk of phytotoxicity.

Introduction

The increasing demand and cost of peat as a plant growth medium had led to a search for a high quality but low-cost substitutes for peat soils. Furthermore, the disposal of biological wastes has become a major problem to mankind, both financially and environmentally. Various biological wastes such as barks, sawdust, leaf moulds, town refuse, sewage sludge, spent mushroom and animal excreta have been composted and evaluated as peat substitutes for growing plants (Cull 1981; Bik 1983; Lohr et al. 1984; Verdonck and Penninck 1985; Inbar et al. 1986, 1988; Raviv et al. 1986; Lopez-Real et al. 1989).

However, when these plant growth materials are used raw or as immature composts, they exhibit effects such as N immobilization or phytotoxic problems which suppress seed germination, root proliferation, plant growth and crop yields (Guenzi and McCalla 1962; Radjagukguk et al. 1983; Verdonck et al. 1983). Similarly, when raw coir waste is used as a plant growth medium, phytotoxicity and N immobilization problem occur (Radjagukguk et al 1983; Verdonck et al. 1983). Biodegraded coir waste is therefore recommended as a plant growth substrate to reduce the risk of plant phytotoxicity (Verdonck et al. 1983; Yau and Murphy 1999, 2000).

Composted substrates are known to result in better yields of crops than non composted substrates (Inbar et al. 1986; N'Dayegamiye and Isfan 1991; Yau and Murphy 2000). The improvement in plant growth and yield in composted substrates can be due, at least in part, to less N immobilization, reduction in phytotoxicity elements and improvement in cation

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exchange capacity in the composted substrates (Radjagukguk et al. 1983; Calvet et al. 1985; Richards et al. 1986). This study was carried out to examine the causes of phytotoxicity in raw coir waste and its amelioration through biodegradation.

Materials and methods

Raw waste material

Coir waste from a coir factory in Batu Pahat, Johor, Malaysia was used for the study. Before the experiment, the material was air-dried for 72 h in the laboratory at a temperature of 22 °C and 60% relative humidity. It was then weighed (100 g each) and sealed in polythene bags and sterilized by gamma radiation (25 kGy overnight).

Coir waste biodegradation

Two groups of microorganisms were used to enhance the biodegradation of coir waste. The first one was a proprietary microbial concoction used by Japanese farmers called EM (Effective Microorganisms). EM primarily consists of photosynthetic and lactic bacteria, yeast, actinomycetes and mould fungi such as *Aspergillus* and *Penicillium* (Shintani 1995). The second group of microorganisms consisted of some common mould and wood soft rotting fungi isolated from garden soil, viz, *Aspergillus niger, Penicillium citrinum, Trichoderma reesii, Humicola grisea* and *Chaetomium globosum*. Besides the microorganisms, N was also added to enhance the biodegradation process.

For the EM treatment, 10 ml of EM solution (at a concentration of 2 ml EM concoction/litre) and 350 ml of distilled water with 2.5 g urea were added to each 100 g sample. The sample was thoroughly mixed to ensure uniformity. For the microfungi treatment, a mixture of 10 ml spores suspension (2 ml per fungus with spores concentration of at least $x10^5$/ml) and 350 ml of distilled water with 2.5 g urea were added. The preparation of spore suspension followed that described in the European Prestandard For Wood Preservatives ENV 807 (Anon. 1993). The treated samples were incubated for 1, 2 and 3 months at a constant temperature of 30 °C. The treatments were replicated three times. The samples were turned weekly and a small quantity of water (mist) was added to ensure moisture content remained at about 80% (wet weight basis).

Phytotoxicity measurements

Cress phytotoxicity bioassay The water-soluble extracts from samples at various stages of biodegradation were taken. Each 5 g of moist coir waste sample was soaked in 10 ml of distilled water and the slurry was thoroughly shaken and then allowed to stand overnight (Kakezawa et al. 1992). The slurry was then filtered using a sintered glass filter (size No. 3) to obtain a clear water soluble extract. A filter paper (Whatman No. 1) was placed in a 90 mm Petri dish for the germination of cress (*Lepidium sativum* L.). A volume of 2 ml of each soluble extract was added to the filter paper to ensure that the filter paper was thoroughly wet and 100 cress seeds were placed on the wet filter paper. Similar preparation was carried out for the raw coir waste extract. As a control, a treatment with distilled water instead of coir waste extract was carried out. Each treatment was done in triplicates. The percentage of germination was recorded after 24 h and root length was recorded after 48 h. The root length was expressed as percentage of those growing using the distilled water (control treatment) as described by Zucconi and de Bertoldi (1987).

Gas chromatography study A 5 g sample of moist, biodegraded coir dust (80% moisture content, wet weight basis) taken from various stages of biodegradation was mixed with 5 ml of 50% acetonitrile solution (1 part of acetonitrile : 1 part of distilled water) and the thick slurry was shaken and allowed to stand overnight. The filtrate was obtained by passing through a sintered glass (size No. 3) filter. Similar

preparation was carried out for the raw coir waste sample. All determinations were carried out in duplicates. A volume of 4 µl of the soluble extract was injected into a Hewlett Packard GC (Model HP 6890) using a HP Innowax column. The flow rate used was 1 ml/min. The peaks and peak areas shown by the samples were recorded and these were identified and quantified by comparison with pure phenolic acid standards. The pure phenolic acids were ρ-coumaric (CA), ρ-hydroxybenzoic or 4-hydroxybenzoic (BHA), ferulic (FA) and vanillic (VA) acids. These were selected as they occurred widely in plant residues and soil solutions and often caused phytotoxicity to plants at relatively low concentrations (Guenzi ad McCalla 1962, 1966a,b; McCalla and Norstadt 1974).

Phenolic acids – cress confirmation bioassay The cress confirmation bioassay was conducted after the phenolic acids had been identified and quantified by gas chromatography. The range of phenolic acid concentrations used for this bioassay was based on the amount of phenolic acids present in the raw coir waste *(Table 1)*.

A control treatment using distilled water was carried out. Each treatment was replicated three times. The procedure of germinating the cress seeds and data collection followed that as described in the cress phytotoxicity bioassay. All data were analysed using analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT).

Table 1. Treatments and concentrations of phenolic acids

Treatment	Phenoloc acid	Concentration (ppm)
1	CA	7.5
2	CA	15.0
3	CA	30.0
4	CA	60.0
5	CA	120.0
6	BHA	0.83
7	BHA	2.5
8	BHA	7.5
9	BHA	22.5
10	BHA	67.5
11	FA	1.17
12	FA	1.17
13	FA	10.5
14	FA	31.5
15	FA	94.5
16	Mixture 1 (CA:HBA:FA)	60:22.5:3.15
17	Mixture 2 (CA:HBA:FA)	30:7.5:10.5
18	Mixture 3 (CA:HBA:FA)	15:2.5:3.5
19	Control	Distilled water

CA = ρ-coumaric acid; HBA = ρ-hydroxybenzoic acid; FA = ferulic acid

Results and discussion

Effects of biodegradation in reducing cress phytotoxicity

The germination of cress seeds was not affected by the raw coir waste extracts or those obtained from different stages of biodegradation. All the coir waste extracts (raw and biodegraded) resulted in 100% germination in comparison with the distilled water controls. This indicated that the raw coir waste extract and those obtained from all the stages of coir waste biodegradation did not impede the germination of cress seed. However, cress seed development was affected by several of the coir waste extracts taken from different stages of biodegradation.

Biodegradation of the coir waste led to progressive improvement in cress root development as shown by the increased root length *(Table 2)*. All the biodegraded samples resulted in significantly higher percentage of root length compared with that of the raw coir waste. The raw coir waste resulted in the lowest root length (61.4%) as a percentage of those growing using distilled water while all the biodegraded samples resulted in over 75% root length with the fungal mixture appearing superior to EM. The highest was 89% root length obtained from the coir waste biodegraded by the mixture of microfungi after 3 months.

The low percentage of root length from the raw coir waste extract (61.4%) indicated the presence of pyhtotoxic compounds

Table 2. Average percentage of root length of raw and biodegraded coir waste

Treatment	Average root length (%)
Raw coir waste	61.4c
EM-1 mth biodegradation (EM-1)	75.5b
EM-2 mth biodegradation (EM-2)	77.5ab
EM-3 mth biodegradation (EM-3)	81.9ab
Mixture of fungi – 1 mth biodegradation (Mix-1)	83.1ab
Mixture of fungi – 2 mth biodegradation (Mix-2)	87.8ab
Mixture of fungi – 3 mth biodegradation (Mix-3)	89.0a

Means of variables having the same letters are not significantly different from each other by DMRT at $p = 0.05$

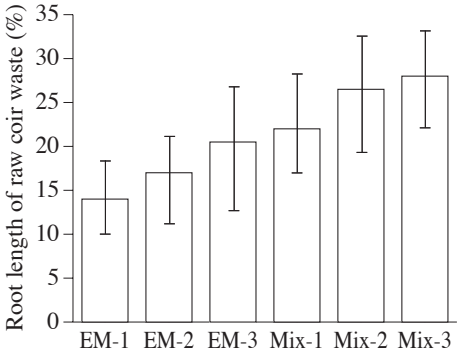

Figure 1. Increase in percentage of root length in relation to that in raw coir waste

which suppressed the normal root growth of cress. When the coir waste had undergone a period of biodegradation, this phytotoxicity effect was reduced as can be seen from the increase in the percentage of root length. After 3 months of biodegradation by the mixture of fungi, root length was restored to levels close to that of the distilled water control.

Among the two groups of microorganisms in the biodegradation of coir waste, the mixture of mould and soft rot fungi was found to be more efficient than the EM in reducing the phytotoxicity to cress root growth. As can be seen from *Figure 1,* the treatments of 1, 2 or 3 months biodegradation by EM resulted in a 15–20% increase in root length compared with that in raw coir waste. The treatment with a mixture of moulds and soft rot fungi resulted in growth increase of between 22% and 28% under the same conditions in relation to that of raw coir waste.

Generally, these results supported the suggestions that coir waste should be composted first before being used as a plant growth substrate (Verdonck et al, 1983; Yau and Murphy 1999, 2000). The decline in root inhibition/phytotoxicity with increasing periods of biodegradation had also been reported by other workers (Patrick et al. 1963; Toussoun et al. 1968; Kimber 1973; McCalla and Norstadt 1974). Tomato plants growing on the biodegraded coir waste had better root and shoot development and also higher fruit yield compared with those grown in untreated coir waste (Yau and Murphy 1999, 2000).

Gas chromatography study

The amount of phenolic acids present in the coir waste samples were quantified in *Table 3*. The raw coir waste contained substantial amounts of ρ-coumaric, ferulic, ρ-hydroxybenzoic and vanilli acids at 62.3, 30.9, 23.6 and 12.4 ppm respectively. The amount of ρ-coumaric acid was higher than the rest of phenolic acids. This latter observation was also found in plant residues and soil solutions as reported in Guenzi and McCalla (1966a,b). In the biodegraded samples, only ρ-coumaric, ρ-hydroxybenzoic and ferulic acids were found and vanillic acid was not detectable. The amount of ρ-coumaric acid was significantly lower than those present in the raw coir waste *(Table 3)*. After 1 month of biodegradation, the amount of ρ-coumaric acid were 36.5 ppm (EM-1 month) and 24 ppm (mixture of fungi-1 month). As biodegradation

Table 3. Amount of phenolic acids present in coir waste

Treatment	ρ-coumaric acid (ppm)	ρ-hydroxybenzoic (ppm)	Ferulic acid (ppm)	Vanillic acid (ppm)
Raw coir waste	62.3a	23.6a	30.9a	12.4
EM-1 mth	36.5b	13.0bc	8.9b	nd
Mix-1 mth	24.0bc	3.1c	6.6b	nd
EM-2 mth	35.4bc	2.7c	5.3b	nd
Mix-2 mth	25.7bc	2.8c	4.0b	nd
EM-3 mth	28.1bc	nd	5.0b	nd
Mix-3 mth	15.7c	1.3c	3.6b	nd

nd = not detectable
Means of variables having the same letters are not significantly different from each other by DMRT at $p = 0.05$

progressed, the amount of ρ-coumaric acid was reduced further. In the 2-month samples, the amount was reduced to 35.4 ppm in the EM treatment and 25.7 ppm by the mixture of fungi. After 3 months, ρ-coumaric acid was further reduced to 28.1 ppm (EM) and 15.7 ppm (mixture of fungi). This indicated that the mixture of mould and soft rot fungi was capable in lowering the ρ-coumaric acid at a faster rate than the EM.

Similarly, the amounts of ρ-hydroxybenzoic and ferulic acids were also significantly reduced during biodegradation compared with those present in the raw coir waste. The raw coir waste contained 23.6 ppm and 30.9 ppm of ρ-hydroxybenzoic acid and ferulic acid, respectively. After 1 month of biodegradation, the amount of ρ-hydroxybenzoic acid in the biodegraded coir waste were 13.0 ppm (EM) and 3.1 ppm (the mixture of fungi) and only traces of the acids were left after 2–3 months of biodegradation. The mixture of mould and soft rot fungi was more efficient in reducing the amount of ρ-hydroxybenzoic acid than the EM. In terms of ferulic acid, the amounts left in the 1-month biodegraded samples were 8.9 ppm (EM) and 6.6 ppm (the mixture of fungi). This ferulic acid continued to be degraded as biodegradation progressed until only traces of the acids were found after 3 months of biodegradation. These amounts of ferulic acids in the biodegraded coir waste were greatly lower than 30.9 ppm in the raw coir waste. Similarly, in olive marc composting, Estaun et al. (1985) and Calvet et al. (1985) also reported that the amount of fatty acids, organic acids and phenol levels decrease as composting progresses. These phytotoxic compounds were not detectable after 4 months of composting and this led to a high emergence of seedlings.

Phenolic acids – cress confirmation bioassay

The concentration of the phenolic acids used for cress confirmation bioassay was within the range of the soluble extracts. Generally, germination of the cress seeds was not affected by ρ-coumaric, ρ-hydroxybenzoic and ferulic acids at the tested range of concentrations. All the treatments resulted in 100% germination rate. Even at the highest concentration, all the cress seeds germinated. The results were consistent with that observed using the soluble extracts. However, the cress root length was affected by some phenolic acids especially at the higher range of concentrations.

In terms of ρ-coumaric acid, at high concentrations of 60 ppm and 120 ppm, the root length relative to those grown in distilled water was 61.8% and 54.8% respectively. These percentages of root length were significantly lower than those growing in the lower acid concentrations. At lower acid concentrations of between 7.5 ppm and 30 ppm, the root growth was about

90% of that grown in distilled water. This showed that root growth was retarded by ρ-coumaric acid concentration of above 60 ppm.

For the ρ-hydroxybenzoic acid, root growth was affected at 67.5 ppm as the root length was only 70.5% in relation to those grown in distilled water. At lower concentrations of between 22.5 ppm and 0.83 ppm, the root growth was satisfactory (varied from 79.3% to 93.5%). Similarly for the ferulic acid, at higher concentrations of 31.5 ppm and 94.5 ppm, the percentages of root length were 77.7% and 69.2% respectively. At lower ferulic acid concentrations of 10.5, 3.5 and 1.17 ppm, the root length was 85.8, 89.4 and 93.5% respectively. All these showed that as concentrations of ρ-hydroxybenzoic and ferulic acids increased, the cress root length development was inhibited.

In the treatment with the mixtures of pure ρ-coumaric (CA), ρ-hydroxybenzoic (HBA) and ferulic acids (FA), the percentage of root length decreased as the acid concentrations increased. Mixture 1 with combination of CA:HBA:FA (60:22.5:31.5 ppm) showed only 69.1% root length and this was significantly lower than mixture 2 with lower concentrations of CA:HBA:FA (30:7.5:3.5 ppm) which resulted in 90.4% root length and 93.1% in mixture 3 with CA:HBA:FA (15:2.5:3.5 ppm). Mixture 1 which had high concentration of CA:HBA:FA (60:22.5:31.5 ppm) reflected closely the major components of these acids in the raw coir waste. As can be seen in *Table 3,* the raw coir waste contained 62.3 ppm of CA, 23.6 ppm of HBA, 30.9 ppm of FA and 12.4 ppm of vanillic acids. In both instances *(Tables 2* and *4),* the cress root development was inhibited. The combination of pure acids (60 ppm CA, 22.5 ppm HBA and 31.5 ppm FA) resulted in 69.1% root length *(Table 4),* while in the raw coir waste extract, the root length was 61.4% *(Table 2).*

Table 4. Average percentage of root length of cress using pure phenolic acids

Phenolic acid	Concentration (ppm)	Mean (% root length)
ρ-coumaric	7.5	93.5a
ρ-coumaric	15.0	96.3a
ρ-coumaric	30.0	89.5abc
ρ-coumaric	60.0	61.8ef
ρ-coumaric	120.0	54.8f
ρ-hydroxybenzoic	0.83	93.5a
ρ-hydroxybenzoic	2.5	85.8abc
ρ-hydroxybenzoic	7.5	84.6abc
ρ-hydroxybenzoic	22.5	79.3bcd
ρ-hydroxybenzoic	67.5	70.5de
Ferulic	1.17	93.5a
Ferulic	3.5	89.4abc
Ferulic	10.5	85.8abc
Ferulic	31.5	77.7cd
Ferulic	94.5	69.2de
Mixture 1 (CA:HBA:FA)	60:22.5:31.5	69.1de
Mixture 2 (CA:HBA:FA)	30:7.5:10.5	90.4abc
Mixture 3 (CA:HBA:FA)	15:2.5:3.5	93.1ab

CA = ρ-coumaric acid; HBA = ρ-hydroxybenzoic acid; FA = ferulic acid
Means of variables having the same letters are not significantly different from each other by DMRT at $p = 0.05$

It appeared that about 60 ppm of CA found in the raw coir waste was the likely cause of the cress root inhibition. This could be inferred from cress root development using pure acids *(Table 4)*. At 60 ppm of pure CA, the root length was 61.8% while at 22.5 ppm and 31.5 ppm of HBA and FA, the root length was 79.3% and 77.7% respectively. The 61.8% root length obtained from the treatment with 60 ppm CA was very similar to the results obtained using the raw coir waste with 61.4% root length. This further suggested that the 62 ppm of CA found in the raw coir waste extract was probably the likely cause of phytotoxicity which inhibited cress root growth. In one report, a lower concentration of 50 ppm, coumarin causes toxicity to clover seedlings in a complete nutrient culture.

In the other two sets of acid combinations, the concentrations of acid selected were based quite closely to those coir waste samples which had been biodegraded for 1–3 months. As discussed earlier, as biodegradation progressed, the percentage of cress root length increased. This decrease in the content of phenolic acids was associated with a reduction in the root inhibitory effect. This can be seen from the combination of acids which showed that as their concentrations decreased, the percentage of root length increased. In the combination containing CA:HBA:FA in a ratio of 30:7.5:10.5 ppm, the root length was 90.4%. This root length increased to 93.1% when the ratio of acids was further reduced to a ratio of 15:2.5:3.5 ppm.

Conclusion

Raw coir waste suppressed cress root development but not its germination. However, when the coir waste had undergone a period of biodegradation, this root inhibitory effect was reduced and led to improvement in root growth. The presence of phenolic acids in the coir waste caused this phytotoxicity effect. However, the concentrations of these phenolic acids were reduced progressively as the coir waste underwent biodegradation. This provides a probable reason why the phytotoxicity was also reduced with increased periods of biodegradation. Among the phenolic acids present in the coir waste, ρ-coumaric acid was found to be the likely cause of cress root inhibitory effect. Thus, it is wise to initially biodegrade the coir waste before being used as a plant growth substrate to avoid any possible phytotoxic effect.

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Abstrak

Ekstrak gabus habuk kelapa asal didapati menghalang pertumbuhan akar 'cress' (*Lepidium sativum* L.). Walau bagaimanapun, apabila gabus habuk kelapa telah direputkan selama 1–3 bulan, kesan fitotoksik berkurangan apabila tempoh pereputan dilanjutkan. Analisis gas kromatografi menunjukkan gabus habuk kelapa asal mengandungi asid fenolik yang tinggi seperti asid ρ-koumarik (62 bsj), ferulik (31 bsj), ρ-hidroksibenzoik (24 bsj) dan vanilik (12 bsj). Kandungan asid fenolik ini berkurangan apabila tempoh pereputan dilanjutkan. Pengurangan kandungan asid fenolik ini menyebabkan peningkatan pertumbuhan akar 'cress'. Ini menunjukkan kandungan asid fenolik yang terdapat di dalam gabus habuk kelapa adalah salah satu punca terjadinya fitotoksik. Antara asid fenolik yang dianalisis, kandungan asid ρ-koumarik yang tinggi adalah penyebab perencatan akar 'cress'. Oleh yang demikian, gabus habuk kelapa perlu menjalani proses pereputan sebelum digunakan sebagai substrat tanaman untuk mengurangkan risiko fitotoksik.

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