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Effects of water extracts of a composted manure-straw mixture on the plant pathogen *Botrytis cinerea*

M.P. McQuilken,* J.M. Whipps and J.M. Lynch

Manure-straw mixtures were composted and water extracts, made by incubating compost in water for 3 to 18 days, were assessed for antagonistic activity against *Botrytis cinerea*, using a range of tests. Extracts of all ages inhibited conidial germination on glass slides and reduced mycelial growth on agar. Mixing extracts of all ages with droplets of suspensions of *B. cinerea* conidia on detached *Phaseolus* bean leaves suppressed lesion development, but only 3- to 8-day-old extracts had an effect when sprayed onto leaves 2 days before inoculation. Extracts contained a large and varied microbial population of actinomycetes (0.3 to 2.4 $\times$ 10^5 c.f.u. ml^{-1}), bacteria (1.5 to 5.6 $\times$ 10^{10} c.f.u. ml^{-1}), filamentous fungi (25.0 to 45.5 c.f.u. ml^{-1}) and yeasts (26.1 to 62.6 c.f.u. ml^{-1}). Eight- and 18-day-old extracts lost activity completely on filter sterilization or autoclaving. Weekly sprays of 8-day-old extracts onto lettuce in the glasshouse had no effect on the incidence of grey mould, but significantly reduced its severity and increased marketable yield. The use of compost extracts in biocontrol of plant diseases and their possible mode of action is discussed.

Key words: Biocontrol, *Botrytis cinerea*, compost, compost extract.

Grey mould, caused by *Botrytis cinerea* Pers.:Fr., is a major disease of vegetables, ornamentals, fruits and field crops grown in Europe and elsewhere (Verhoeff *et al.* 1988). The pathogen attacks old, injured or weakened plants in particular, causing serious yield losses (Palti 1981). Effective control of the disease has been achieved with a range of fungicides, but their widespread use has led to the development of pathogen resistance (Dennis & Davis 1979; Maude 1980; Fletcher & Griffin 1981; Panagiotaku & Malathrakis 1981; Katan 1982; Pommer & Lorenz 1982; Panayotakou & Malathrakis 1983; Wang *et al.* 1986; Katan *et al.* 1989; Malathrakis 1989; Elad *et al.* 1992). Currently recommended treatments, involving the alternate use of fungicides with different modes of action, are not always effective. Furthermore, public concern over fungicide residues on fruits and vegetables has increased markedly. Consequently, there is a need for satisfactory alternative methods for managing the disease, including biocontrol and cultural practices.

M.P. McQuilken and J.M. Whipps are and J.M. Lynch was with the Microbiology and Crop Protection Department, Horticulture Research International, Littlehampton BN17 6LP, UK; fax: 0903 726780. J.M. Lynch is now with the School of Biological Sciences, University of Surrey, Guildford GU2 5XH, UK. * Corresponding author.

There has been considerable interest in using composting to inactivate plant pathogens in crop residues (Bollen 1993). In addition, disease biocontrol agents such as *Trichoderma* spp. can colonize composts (Lynch 1993) and extracts of compost may therefore contain such microorganisms, or their metabolites. Foliar sprays of compost extracts of composted organic materials have shown some potential for controlling a number of diseases in Germany, including downy and powdery mildews, potato blight and Botrytis grey mould (Stindt & Weltzien 1988a,b; Weltzien & Ketterer 1986; Weltzien 1989, 1992; Tränkner 1992). However, in most cases, the extracts have been produced using undefined composts. This paper reports on the efficacy of extracts of a compost produced from a defined mixture of manure and straw to control *B. cinerea*, using simple laboratory and glasshouse experiments. A preliminary report has been published (McQuilken *et al.* 1993).

Materials and Methods

Production of Compost

The compost starting mixture consisted of: chopped horse stable bedding (wheat straw plus horse manure) [3000 kg fresh (f.) wt;

40 to 45% (w/w) moisture content (mc); 0.8 to 1.2% (w/w) N dry wt]; deep litter chicken manure [150 kg f. wt; 30 to 32% (w/w) mc; 3.0 to 3.5% (w/w) N dry wt]; molassed brewers' grains (Sporavite; ADCO, Somersham, UK) as compost activator [30 kg f. wt; 20 to 25% (w/w) mc; 5.5 to 7.0% (w/w) N dry wt; 25% (w/w) soluble carbohydrate dry wt]; gypsum (75 kg dry wt); and water. Initially (day 1), chopped horse stable bedding was stacked in windrows (2.5 to 3 m wide, 1.5 to 2 m high) in an open-sided shed, and a mechanical turner was used to mix and thoroughly wet the stack. Chicken manure and some of the molassed brewers' grains were added and the stack constituents were mixed for a second time. On day 2, more water was added during turning. The remaining molassed brewers' grains were added on days 4 and 6 before turning. The stack was turned again on day 8 (gypsum added) and on days 10, 12 and 14, when more water was incorporated to obtain a moisture level of 60 to 70% (w/w). The stack was then turned weekly for 8 weeks. During the first 4 weeks of composting, the temperature within the centre of the stack increased from 15 to 60 °C, after which it declined slowly to 12 to 15 °C over 6 weeks and subsequently remained at about 12 °C.

Preparation of Compost Extract

Ten- to 16-week-old compost (pH 7.3 to 7.5) was used for preparing compost extracts (CE). Random samples of compost were taken from the centre of the stack, and added to tap water (1 : 5, v/v) in open buckets. The mixtures were stirred for 2 min and then incubated at 20 to 22 °C for 3, 5, 8, 12 or 18 days without stirring. Following each incubation period, mixtures were filtered through two layers of cheesecloth and the filtrate (CE; pH 7.4 to 7.7) used immediately. Batches of 8- and 18-day-old CE were also autoclaved (A-CE) at 121 °C for 15 min, or filter-sterilized (FS-CE) through a 0.2 μm-pore membrane.

Source of Botrytis cinerea *and Production of Conidial Suspensions*

Botrytis cinerea (isolate BC13), originally isolated in the Netherlands from a flower of gerbera (*Gerbera jamesonii* cv. Rebecca), was used. The pathogen was stored in polypropylene straw ampoules in liquid N_2 (Challen & Elliott 1986) and routinely cultured at 20 °C (12 h light/12 h dark) on tomato leaf extract agar (TLEA), to maintain a supply of actively growing and sporulating cultures. To prepare tomato leaf extract (TLE), 300 g of frozen tomato leaves (cv. Moneymaker) were added to 1 l of distilled water, blended for 2 min, using a Waring blender operated at full speed, and heated at 50 °C for 30 min in a water bath. TLEA was prepared from fresh TLE by the addition of 1.5% (w/v) agar (Oxoid No. 3).

Determination of Microbial Populations in Compost Extracts

Actinomycete and bacterial populations were determined by surface plating 0.1 ml, respectively, on water yeast agar [WYA; containing (g l^{-1}): yeast extract (Oxoid), 0.25; K_2HPO_4, 0.5; cycloheximide (Sigma), 0.05; and agar (Oxoid No. 3), 18 g] and on nutrient agar (NA; Oxoid) containing cycloheximide (50 mg l^{-1}). Fungal populations were determined by plating 0.5 ml samples on potato dextrose agar (PDA; Oxoid) containing Aureomycin [0.32 g l^{-1} of a powder containing 5.5% (w/w) chlortetracycline hydrochloride]. Four replicate plates were used for each dilution. Colonies were counted after incubation at 20 °C for 3 (bacteria), 8 (fungi) or 10 days (actinomycetes). Only the fungi recovered on dilution plates were identified. Isolates were examined using standard mycological techniques, and tentative identifications made based on morphological and cultural characteristics (Domsch *et al.* 1980; Barnett *et al.* 1983). To induce sporulation, fungal cultures were irradiated with near u.v. (Leach 1971). Definitive identification of filamentous fungi and yeasts was provided by experts at the International Mycological Institute (Kew, UK) and Centraalbureau voor Schimmelcultures (Delft, The Netherlands), respectively.

Conidial Germination Tests

Seven 'treatments' [3-, 5-, 8-, 12- and 18-day-old CE, sterile distilled water (control) and iprodione (Rovral 50% WP; 0.5 g l^{-1}) (fungicide control)] were mixed 1 : 1 (v/v) with conidial suspensions (approx. 8.0×10^5 conidia ml^{-1}). One-ml samples of each conidial treatment were placed within a single fused ring (15 mm diameter, 3 to 5 mm high) on four replicate microscope slides and covered with a cover slip to prevent drying out. Slides were placed in damp chambers and incubated in light at 20 °C for 18 h, after which at least 250 conidia on each slide were scored for germination. Only conidia with germ-tubes of length greater than spore diameter were considered to have germinated. The experiment was repeated using batches of 8- and 18-day-old CE, A-CE and FS-CE; both water and fungicide control treatments were also included.

Mycelial Growth Tests

The same seven treatments were mixed (1 : 1 v/v) with tap water agar [TWA; 15 g agar (Oxoid No. 3) l^{-1} tap water) cooled to 45 °C, and 10-ml volumes of each dispensed into four 9 cm diameter Petri dishes. Agar mixtures were covered with PDA (approx. 10 ml per dish), and then inoculated centrally with a 3 mm diameter mycelial disc cut from a growing colony of BC13. Two perpendicular colony diameters were measured on each dish after incubation in the dark at 20 °C for 72 h.

Detached Phaseolus *Bean Leaf Bioassays*

Each of the seven treatments were tested against strain BC13, using a modification of the bioassay of Leone & Tonneijck (1990). Eight fully-expanded detached primary bean leaves (*Phaseolus vulgaris* L. cv. Groffy; 15- to 21-day-old) were sprayed to run-off with each treatment. Treated leaves were placed on plastic grids on a layer of wet filter paper in plastic trays. The grids avoided direct contact between the leaves and the filter paper. The cut end of each petiole was inserted in wet floral foam. Trays containing leaves were enclosed in transparent plastic bags and incubated at 18 to 20 °C (14 h light/10 h dark). Two days after spraying, leaves were inoculated by placing six 15-μl droplets of conidial suspension (approx. 2×10^6 conidia ml^{-1}) on the adaxial surface of each leaf. After a further 3 days incubation, lesion diameters were measured using a ruler magnified under a binocular low power microscope. The bioassay was repeated as described, except that treatments were mixed directly with conidial suspension (30 : 70, v/v) and applied together as droplets, instead of spraying.

Glasshouse Experiment

Pelleted seeds of lettuce (*Lactuca sativa* L., cv. Hudson) were sown into peat blocks in mid-July 1992 and propagated using normal horticultural practices. Eight plots (1.2 × 2.2 m) were marked out, separated by 0.5 m paths, to give an arrangement of two plots across and four plots down the north–south orientated glasshouse. Four weeks later, the blocks were planted to half their depth at 20 × 20 cm spacing, giving 84 plants per plot. It was unnecessary to infest the glasshouse artificially with *B. cinerea* because large inocula were already present in the soil. Glasshouse temperatures were maintained at a minimum of 10 °C, during the day, venting at 18 °C, with a 5 °C minimum night temperature. Immediately after planting, 8-day-old CE was sprayed onto four replicate plots (500 to 700 ml per plot) using a Hazelock Courier 8 portable sprayer (Hazelock Ltd, Haddenham, UK), and then at weekly intervals until harvest. Control plots were sprayed with water.

Table 1. Changes in the microbial population (total number of c.f.u. ml^{-1}) of compost extract (CE) with incubation time.*

Incubation time (days)	Actinomycetes ($\times 10^5$)	Bacteria ($\times 10^{10}$)	Filamentous fungi	Yeasts
3	0.3 (4.43)†	1.5 (10.17)	25.0 (1.39)	28.8 (1.45)
5	0.4 (4.60)	1.8 (10.26)	28.8 (1.45)	26.1 (1.39)
8	2.4 (5.38)	5.6 (10.74)	45.5 (1.65)	62.6 (1.79)
12	2.0 (5.28)	3.0 (10.46)	45.5 (1.64)	53.0 (1.72)
18	2.1 (5.30)	3.1 (10.49)	31.9 (1.50)	52.4 (1.72)
SED (15 df)‡	(0.081)	(0.060)	(0.062)	(0.069)

* Composted manure-straw mixture was incubated (20 to 22°C) with tap water (1:5, v/v) in open buckets. Mixtures were filtered and the microbial population of the filtrate (CE) determined using dilution plating on selective agar media. See text for details.
† Values in parentheses are means of four replicate samples calculated after $\log_{10}$ transformation of colony counts on agar media.
‡ The standard error of the difference between two means derived by analysis of variance (ANOVA). A significant difference between two means at the $P = 0.05$ level is given by the least significant difference (LSD). (LSD = $t_v \times$ SED, where t = critical value ($p = 0.05$) of Student's t-distribution for v degrees of freedom (df) in ANOVA.)

Plants were harvested 8 weeks later and assessed individually for grey mould disease, using a scale of 0 to 3, where 0 = no disease, 1 = slight disease (plant marketable after little or no trimming), 2 = moderate disease (plant marketable after extra trimming), and 3 = severe disease (plant killed or unmarketable after trimming). A Botrytis severity index (0 to 100) was calculated for each plot:

$$\frac{100}{3} \times \frac{\sum in_i}{\sum n_i}$$

where i = score 0, 1, 2, or 3 and n_i = no. of plants in category i.

Statistical Analyses
Data were analysed using an analysis of variance (ANOVA) or a Student's t-test. Colony counts were transformed to $\log_{10}$ before analysis. When necessary, percentages were angularly transformed.

Results

Microbial Populations in Compost Extract
Populations of actinomycetes, bacteria, filamentous fungi and yeasts in the compost extract increased during the incubation period to reach a maximum after 8 to 12 days (Table 1). Subsequently, populations of each type of microorganism either remained stable or declined slightly during further incubation up to 18 days. Counts of filamentous fungi and yeasts were similar at each incubation time, but always lower than counts for either actinomycetes or bacteria. Maximum colony counts of actinomycetes and bacteria were 2.4×10^5 and 5.6×10^{10} ml^{-1} CE, whereas those for filamentous fungi and yeasts were 45.5 and 62.6 ml^{-1} CE, respectively. *Penicillium chrysogenum*, *P. brevicompactum*, *Mucor hiemalis* and *Trichoderma* spp. were the most frequent filamentous fungi whereas *Debaryomyces hansenii* was the only yeast.

Conidial Germination and Mycelial Growth Tests
Three- to 18-day-old compost extracts significantly ($P < 0.05$) inhibited conidial germination and reduced mycelial growth of *B. cinerea* compared with the water control (Table 2). However, none of the extracts was as effective as the fungicide, iprodione. Age of extracts had

Table 2. Effect of incubation time of compost extract on conidial germination (on glass slides) and mycelial growth (on PDA) of *Botrytis cinerea*.*

Treatment	Germination after 18 h at 20°C (%)	Colony diameter after 72 h at 20°C (mm)
Iprodione†	5.8	0
Control (water)	62.4 (52.2)‡	60.6§
3-day extract	35.4 (36.3)	50.1
5-day extract	33.6 (35.3)	48.1
8-day extract	38.0 (37.9)	36.8
12-day extract	40.2 (39.3)	37.8
18-day extract	54.2 (47.4)	41.8
SED (18 df)¶	(3.09)	3.04

* See text for experimental details.
† Excluded from statistical analysis.
‡ Values in parentheses are means from four replicates each of 250 conidia, calculated after angular transformation of percentage data.
§ Values of colony diameter are means from four replicates.
¶ See footnote to Table 1 for definition.

Table 3. Effect of filter and heat sterilization of compost extracts (8- and 18-day-old) on conidial germination (on glass slides) and mycelial growth (on PDA) of *Botrytis cinerea*.*

Treatment	Germination after 18 h at 20°C (%)		Colony diameter after 72 h at 20°C (mm)	
	8-day	18-day	8-day	18-day
Iprodione†	4.5	4.2	0	0
Control (water)	70.4 (57.1)‡	74.7 (59.9)	60.1§	60.0
Compost extract (CE)	38.0 (37.9)	54.2 (47.4)	36.8	41.8
Autoclaved CE	62.1 (52.0)	71.4 (57.7)	60.1	58.5
Filter-sterilized CE	71.9 (58.0)	69.9 (56.7)	60.8	56.0
SED (12 df)¶	(2.36)	(1.67)	1.32	4.39

* See text for experimental details.
† Excluded from statistical analysis.
‡ Values in parentheses are means from four replicates each of 250 conidia, calculated after angular transformation of percentage data.
§ Values of colony diameter are means from four replicates.
¶ See footnote to Table 1 for definition.

some effect on subsequent activity against both germination and mycelial growth. Treatments with 3- to 12-day-old extracts were equally effective in inhibiting conidial germination, but there was a significant ($P < 0.05$) decline in inhibition with 18-day-old extracts. Eight- to 18-day-old extracts were equally effective in reducing mycelial growth and were significantly ($P < 0.05$) better than either 3- or 5-day-old extracts. Eight- and 18-day-old extracts sterilized by autoclaving or filtration lost their activity completely and had no effect on conidial germination nor mycelial growth of *B. cinerea* (Table 3).

Detached Phaseolus *Bean Leaf Bioassays*

Sprays of 3- to 8-day-old extracts on detached bean leaves, 2 days before inoculation with droplets of *B. cinerea* conidia, were equally effective in reducing lesion development and were all significantly ($P < 0.05$) better than the water control (Table 4). However, 12- and 18-day-old extracts had no effect. Eight-day-old extract sterilized by autoclaving or filtration had no effect on lesion development (Table 5). When extracts were mixed with conidial suspension and applied as droplets to bean leaves, extracts of all ages suppressed lesion development, but none of the treatments was as effective as iprodione (Table 4). Age of extracts had

Table 4. Effect of incubation time of compost extract on lesion development by *Botrytis cinerea* on detached *Phaseolus* (cv. Groffy) bean leaves.*

Treatment	Lesion diameter (mm)	
	Experiment 1†	Experiment 2‡
Iprodione§	1.5	1.0
Control (water)	21.1	18.7
3-day extract	9.9	3.2
5-day extract	11.2	3.4
8-day extract	11.2	6.7
12-day extract	19.4	9.8
18-day extract	19.0	9.2
SED (42 df)¶	0.86	0.71

* See text for experimental details. Values are means from eight replicates, each of six lesions.
† Droplets of conidial suspension applied 2 days after treatments.
‡ Treatments plus conidial suspension (30:70, v/v) applied together in droplets.
§ Excluded from statistical analysis.
¶ See footnote to Table 1 for definitions.

Table 5. Effect of filter and heat sterilization of compost extracts (8- and 18-day-old) on lesion development by *Botrytis cinerea* on detached *Phaseolus* (cv. Groffy) bean leaves.*

Treatment	Lesion diameter (mm)			
	Experiment 1†		Experiment 2‡	
	8-day	18-day	8-day	18-day
Iprodione§	2.4	2.7	0	2.1
Control (water)	20.1	19.9	18.0	20.0
Compost extract (CE)	12.0	19.7	5.3	9.8
Autoclaved CE	19.7	19.7	19.1	19.6
Filter-sterilized CE	19.8	19.3	19.6	18.9
SED (28 df)¶	0.41	0.89	0.91	0.72

* See text for experimental details. Values are means from eight replicates, each of six lesions.
† Droplets of conidial suspension applied 2 days after treatments.
‡ Treatments plus conidial suspension (30:70, v/v) applied together in droplets.
§ Excluded from statistical analysis.
¶ See footnote to Table 1 for definitions.

Table 6. Effect of weekly applications of 8-day-old compost extract on grey mould (*Botrytis cinerea*) in glasshouse-grown lettuce 8 weeks after planting.*

Treatment	Diseased plants (%)	Botrytis severity index (0 to 100)	Marketable plants (%)
Control (water)	97.9	82.8	39.3
Compost extract	93.8	60.0	76.5
SED (6 df)†	2.37 $P > 0.1$	4.06 $P < 0.002$	5.39 $P < 0.001$

* See text for experimental details.
† SED is the standard error of the difference between the means. The difference between the two means divided by the SED gives the appropriate *t* value. df—Degrees of freedom.

some effect on the subsequent activity of extracts in reducing lesion diameter. Treatments of 3- to 5-day-old extract were the most effective in reducing lesion development and were significantly ($P < 0.05$) better than the 8-to 18-day-old extracts. Eight-day-old extracts reduced lesion development but there was a significant ($P < 0.05$) decline in activity thereafter, with the 12- and 18-day-old extracts being the least effective. Eight- and 18-day-old extracts sterilized by autoclaving or filtration had no effect on lesion development (Table 5). In most cases, extracts were more effective in reducing lesion development when applied with a conidial suspension as a droplet, than when sprayed on leaves 2 days before inoculation.

Glasshouse Experiment

Spraying 8-day-old extracts onto glasshouse-grown lettuce at weekly intervals immediately after planting did not reduce the number of diseased plants recorded 8 weeks later (Table 6). However, the disease severity was significantly reduced ($P < 0.002$) and the number of marketable plants was increased ($P < 0.001$) by this treatment.

Discussion

These results are the first report from the UK that compost extracts are antagonistic against the plant pathogen *Botrytis cinerea* and confirm the findings of other workers (Stindt & Weltzien 1988a,b; Weltzien 1989, 1992; Tränkner 1992). However, this is the first time that the effect has been demonstrated with a compost extract prepared from a compost based on a defined starting mixture. Other starting mixtures varied considerably and differed in their use and efficacy. In the present experiments, the effects of the extract were reproducible in various tests, both in the laboratory and glasshouse.

Extracts sterilized by autoclaving or filtration lost their activity completely and had no effect on conidial germination, mycelial growth, or lesion development. These observations agree with those of Stindt (1990), confirming the involvement of microorganisms in the suppression of *B. cinerea*. The microorganisms within the extracts probably suppress *B. cinerea* by direct inhibition of both conidial germination and mycelial growth. Extracts were always less effective in reducing lesion development on detached bean leaves when sprayed on leaves 2 days before inoculation; this may be related to the inability of some microorganisms within the extracts to survive on the leaf surface.

The activity of extracts against specific plant pathogens has been shown to vary with incubation time (Ketterer 1990; Stindt 1990; Weltzien 1992). This was confirmed in our experiments. In general, extracts more than 8 days old were less effective than the younger ones, possibly due to the physiological state of the microorganisms within the extracts. Indeed, the compost extract contained a large and varied microbial population, with the predominant microorganisms being bacteria. This has also been observed with compost extracts produced from composted horse manure, cattle manure and grape marc (grape skin, seeds and stalks left over after wine processing) in Germany (Ketterer 1990; Stindt 1990).

Bacterial counts in the present extracts, produced from a composted chicken/horse manure-straw mixture, were similar to those obtained with extracts produced from composted cattle or horse manure in Germany, but counts of filamentous fungi and yeasts were considerably lower. In addition, actinomycetes were also isolated from our extracts. These slight differences in microbial population are most likely related to the starting mixture of the composts, the time at which the composts were sampled from the heap during composting, and the conditions under which the composts were incubated with water to produce the extracts. *Trichoderma* spp. were one of the main filamentous fungi isolated; these well-known antagonists of fungal plant pathogens have been studied quite extensively for biocontrol of aerial plant pathogens. For example, conidial sprays of a fungicide-resistant strain of *T. harzianum*, when applied to apple blossom, controlled *B. cinerea* dry eye rot of fruit better than the fungicide-sensitive parent strain when this was used alone or with reduced dosages of the fungicide, vinclozolin (Tronsmo 1991). Also. spraying *T. viride* onto grape vines controlled *B. cinerea* rot of fruit to

a level similar to that obtained with sprays of dichlofluanid applied at the same time (Dubos *et al.* 1982).

Debaryomyces hansenii was the only yeast isolated in the present study. Recently, this yeast has been used successfully in biocontrol of post-harvest diseases of top fruit (McLaughlin *et al.* 1992). It would be worthwhile to evaluate these isolates and other components of the compost extract community as potential biocontrol agents. In this respect, Stindt (1990) has already isolated a range of bacterial antagonists from extracts produced from composted cattle manure. Several of the isolates inhibited conidial germination of *B. cinerea* and suppressed lesion development when sprayed onto detached *Phaseolus* bean leaves. Similarly, Ketterer (1990) isolated a range of bacteria, filamentous fungi and yeasts with antagonistic properties from extracts of composted horse manure. When these antagonists were tested against *Phytophthora infestans* on detached potato leaves, they were as effective as the extracts in reducing the percentage leaf area diseased. Furthermore, field applications of a mixture of these antagonists in combination with extracts were highly effective in reducing the development of potato blight.

The present results indicate that weekly sprays of extracts onto lettuce in the glasshouse reduced the severity of Botrytis grey mould and increased the marketable yield. It is possible that, with improved efficacy, these extracts may have some potential for use in biocontrol, especially in organic agricultural systems. They are also likely to be useful in low input agricultural systems in developing countries, where fungicides are either too expensive for small farmers to buy or commercially unobtainable. The production of extracts from composted organic materials is simple and requires no specialized equipment. In addition, agricultural wastes can be used. However, it may be necessary to determine the possible environmental and toxicological hazards of using compost extracts based on municipal waste or sewage sludge to suppress diseases on edible crops before they can be widely accepted. Future research will concentrate on testing compost extracts against other foliar plant pathogens, and increasing their efficacy by optimizing incubation times and using improved formulations. It would also be useful to evaluate extracts in combination with known biocontrol agents.

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