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In vitro and in vivo evaluation of microbial-enriched compost tea on the development of powdery mildew on melon

Yuvarani Naidu · Sariah Meon · Yasmeen Siddiqui

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Abstract This study evaluated the effects of microbial-enriched compost tea (CT) on the conidial germination of *Golovinomyces cichoracearum* DC. and development of powdery mildew on melons in a time-dependent manner. In vitro conidial germination was significantly reduced by 94 % and 85 % upon treatment with Daconil® (fungicide) or microbial-enriched CT, respectively, 96 h after incubation (hai). Morphological analysis under light microscopy demonstrated that conidia co-incubated with microbial-enriched CT at 48 hai appeared ruptured, which contributed to higher inhibition of conidial germination, increased cell permeability and leakage of cellular contents. These observations may be explained by antibiosis. Moreover, different application time of microbial-enriched CT on melons significantly affected disease development. There was a delay in disease development by 12 days in plants treated with Daconil®, microbial-enriched CT applied 24 h after inoculation and microbial-enriched CT applied simultaneously with inoculation when compared to the control treatment. Curative application of microbial-enriched CT (24 h after inoculation) delayed the onset of disease, and the efficiency of inhibition was comparable to a fungicidal spray (Daconil®). Hence, microbial-enriched CT may be used to inhibit the development of powdery mildew on melons, thus reducing the dependency on chemical fertilisers.

Keywords Compost tea · Conidial germination · *Golovinomyces cichoracearum* DC. · Curative application · Biological control

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Y. Naidu (✉) · S. Meon · Y. Siddiqui
Laboratory of Food Crops and Floriculture, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia
e-mail: yuvarani_rajoo@yahoo.com

Y. Naidu
Malaysian Palm Oil Board, Bandar Baru Bangi, 43000 Kajang, Selangor D.E, Malaysia

Introduction

Powdery mildew caused by *G. cichoracearum* DC. is one of the most common diseases of melons (*Cucumis melo* L.) grown under both field and greenhouse conditions in Malaysia. The vast majority of melon growers presently rely on synthetic fungicides to control powdery mildew. Fungicides that contain active ingredients such as chlorothalonil, difenoconazole, triadimefon, triforine and benzimidazole are commonly used to control powdery mildew but with variable degrees of success. The frequent application of these fungicides is associated with an increase in environmental pollution, health hazards and development of resistance in microbial pathogens (McGrath 2001).

The application of different products based on organic matter, such as compost and organic manure, has been popular since the 1990s. Compost can provide natural biological control of mainly soil-associated diseases (Hoitink et al. 2001), but the application of compost is currently restricted to rhizosphere application. Thus, researchers and organic growers throughout the world have used aqueous extracts of compost or compost tea (CT) as foliar sprays to minimise foliar diseases on crops including apple scab disease (Cronin et al. 1996); powdery mildew diseases on rose and tomato (Scheuerell and Mahaffee 2000; Segarra et al. 2009; Koné et al. 2010); grey mould diseases on vegetables crops, strawberries, geranium and tomatoes (Welke 2004; Scheuerell and Mahaffee 2006; Koné et al. 2010); damping-off on cucumber seedling (Scheuerell and Mahaffee 2004); bacterial spot of tomato (Al-Dahmani et al. 2003); late blight of potato (Al-Mughrabi 2007); common scab of potato tubers (Al-Mughrabi et al. 2008); *Choanephora* wet rot on okra (Siddiqui et al. 2008, 2009) and anthracnose diseases on pepper and cucumber (Sang and Kim 2011).

CT are produced from composted organic matter by re-circulating water through loose compost bag brewed over or within a tank with the intention of maintaining aerobic conditions (Litterick et al. 2004). Primary goal of CT production is to increase the microbial populations. This can be achieved by providing nutrient additives for the microbes at the beginning or during the brewing process (Shrestha et al. 2011). According to Ingham (2000), the final balance between bacteria and fungi in CT can be predetermined by selecting an appropriate compost and fermentation nutrients.

A microbial starter was incorporated to enhance microbial proliferation of CT during the brewing process (Naidu et al. 2010). The dominant functional groups isolated from microbial-enriched CT were from the genera *Bacillus* sp., *Pseudomonas* sp., lactic acid bacteria (*Lactobacillus*), other bacteria species, actinomycetes, yeast, *Trichoderma* sp., *Penicillium* sp. and other fungi species. The most reported factor influencing the efficacy of CT's in inhibiting the development of plant pathogen is their microbial composition (Koné et al. 2010). Previous studies have demonstrated that beneficial microorganisms present in foliar-delivered CT effectively control foliar pathogens. These beneficial microorganisms compete with pathogens for infection sites and nutrients (Al-Mughrabi et al. 2008), secrete secondary metabolites on the plant surface and directly parasitise the pathogens (El-Masry et al. 2002). Moreover, CT treatment had been shown to induce systemic resistance in the host plant (Zhang et al. 1998; Siddiqui et al. 2009; Sang and Kim 2011).

A number of studies have indicated that the microbial community of CT is necessary for disease suppression (Hoitink et al. 1997; Siddiqui et al. 2009). However, it is unclear whether pathogen inhibition is due to parasitism or competition for nutrients and colonisation sites or of CT-associated microorganisms secreting antibiotics in situ on the plant surfaces. It is commonly difficult to determine the exact mechanism involved in the suppression of phytopathogens by CT because it is a consortium of microbial community rather than a single species. The mechanism of CT activity varies depending on the relationship between the host, the pathogen and the method of application. In light of the limited knowledge of how compost inhibits pathogen growth, a stepwise study was designed to address the effects of microbial-enriched CT on the development of powdery mildew and conidial germination of *G. cichoracearum* DC. in a time-dependent manner.

Materials and methods

Preparation of microbial-enriched CT

Three-month-old commercial compost ('Flora Mas' Bio-Organic Compost) prepared from empty fruit bunches and palm oil mill effluent was obtained from Asia Green Environmental Sdn. Bhd., Malaysia. CT was prepared according to the method described in Naidu et al. (2010). Compost and microbial starter were mixed in a muslin cloth bag. The compost was then soaked in water in a 1:5 w/v (compost:water) ratio for three days at 25 ± 2 °C using a 20 l brewing tank fitted with an aquarium pump for continuous aeration. Yeast extract (7g) and humic acid (4g) were incorporated into compost (100g) and were used as a carbon and nitrogen supplements respectively, for enrichment of bacterial and fungal growth in the CT. A combination of these two supplements (4 g 100 g^{-1} of compost) was used as a microbial starter. The microbial starter was incorporated during the brewing process to enhance microbial proliferation (Naidu et al. 2010).

Microbial diversity in CT

The microbial diversity in CT's prepared from different batches of the same compost was assessed by a serial dilution method on semi-selective and selective media (Naidu et al. 2010). Bacterial and fungal counts were enumerated as colony forming units per ml (CFUs ml^{-1}). Each batch was replicated five times, and each experimental replicate represents an average of ten readings.

Preparation of conidial suspension of *G. cichoracearum* DC.

Melon (*Cucumis melo* L.) leaves naturally infected with *G. cichoracearum* DC. were obtained from the Department of Agriculture, Serdang, Malaysia. A conidial suspension was prepared by scraping and washing the conidia from the leaf surface into sterile distilled water (SDW). The conidial suspension was centrifuged for 5 min at 4,000 rpm, and the conidial counts were adjusted to approximately 5×10^5 conidia ml^{-1} using a haemocytometer. Morphology of *G. cichoracearum* DC. was examined using light microscopy.

Effects of microbial-enriched CT on conidial germination of *G. cichoracearum* DC.

An in vitro study was performed to assess the role of microbial-enriched CT in inhibiting conidial germination. One-hundred-microlitre aliquots of each suspension namely, CT alone (brewed without a microbial starter), microbial-enriched CT (brewed with a microbial starter), and Daconil® (2 g l^{-1}) (active ingredient: Chlorothalonil), were centrifuged separately at 15,000 rpm for 10 min and filtered through Whatman No. 1 filter paper. A 40 µl aliquot of each suspension was pipetted onto each of the ten cavity slides (25.4 × 76.2 mm, 1.2–1.3 mm thick, China National Machinery Corporation, Shanghai, China). Ten microlitres of freshly harvested conidial suspension of *G. cichoracearum* DC. (5×10^5 conidia ml^{-1}) was pipetted onto the same cavity slide, and the mixture was incubated in the dark. The cavity slides containing SDW and the conidia suspension served as controls. The conidia were killed by adding 10 µl of 2 % sodium azide to each cavity at 6, 12, 24, 48, 72 or 96 hai. Conidia were then examined by light microscopy at 40× magnification. Approximately 100 conidia in each cavity slide were evaluated for the presence of germ tubes and appressorium. Conidia were considered to be germinated when it formed a primary germ tube with an initial appressorium (Li et al. 2005), or when the germ tube was equal in length or at least half the width of the conidia.

Inhibition was calculated based on the formula published by Cronin et al. (1996):

$$\%\text{Conidial inhibition} = 1 - (\text{Gr}/\text{Gc}) \times 100$$

Gr = Germination present in the treated sample, Gc = Germination present in the control sample

Efficacy of different application times of microbial-enriched CT on the development of powdery mildew

A glasshouse trial was conducted at field No. 2 at the Faculty of Agriculture, University Putra Malaysia, to evaluate the effect of different application times of microbial-enriched CT on the development of powdery mildew. The daily temperature of the glasshouse ranged from 25 to 30 °C, and the RH was 85 %. The photoperiod ranged between 8 and 10 h. The melon F-1 seed variety 'Emerald Jewel' was used as a test plant. The seeds were directly sown in a pot measuring 12 cm in diameter, which was filled with the soil mixture consisting of top soil, peat (Kosas-Peat, Kosas PROFIL Sdn. Bhd.) and sand at a ratio of 3:2:1 (v/v/v). Plants were watered daily and fertilised with the recommended dose of NPK Green at a ratio of 15:15:15. The following treatments were applied three weeks after the seedlings emerged and when the plants were at the 4–5 leaf stage: T1—water (Control), T2—microbial-enriched CT applied 24 h prior to inoculation, T3—microbial-enriched CT applied 24 h after inoculation, T4—microbial-enriched CT applied simultaneously with inoculation, T5—microbial-enriched CT applied one week after disease outbreak, and T6—Daconil® applied 24 h prior to inoculation (2 g l^{-1}). The experiment was conducted in a randomised complete block design with five replicates, and each replicate consisted of three plants. Each of the seedlings was sprayed with 200 ml of one of the six different treatments using a hand sprayer (Siddiqui et al. 2009). Powdery mildew inoculum was prepared as mentioned in "Preparation of conidial suspension of *G. cichoracearum* DC." and

plants were artificially inoculated with a conidial suspension as described above.

Disease assessment

The development of powdery mildew on melons was recorded at three-day intervals based on a disease rating scale of 0–5. In this scale, 0 indicates healthy; 1 indicates that <5 % of the total leaf surface was covered with subtle, small, round white spots; 2 indicates that 6–20 % of the total leaf surface was covered with enlarged and coalesced white spots; 3 indicates that 21–50 % of the total leaf surface was covered with lesions of white spores; 4 indicates that 51–75 % of the total leaf surface was covered with lesions of white spores; and 5 indicates that >76 % of the total leaf surface was covered with white spores of *G. cichoracearum* DC. demonstrating chlorotic symptoms and senescence of lower leaves.

Disease severity was expressed as a percentage (DS %) based on the formula described in Chaube and Pundhir 2005.

$$\text{DS } (\%) = \frac{\sum(\text{Number of plants in disease rating scale} \times \text{disease rating scale}) \times 100}{\text{Total number of plants assessed} \times \text{Highest disease rating scale}}$$

The extent of disease developed on melon plants was further expressed as the area under the disease progress curve (AUDPC) for each treatment, and the rate of disease development was expressed as an epidemic rate (R_L). Epidemic rates were calculated based on the formula outlined by Campbell and Madden (1990) and by transforming the DS % data using the logit model. This model was fitted to the severity values by non-linear regression analysis using sigma plot software (SPSS, Version 9.0, Systat Software Inc. (SSI), California, USA) (Siddiqui et al. 2008). Percentage of disease reduction was calculated based on control (T1).

Scanning electron microscopy (SEM)

The interaction of *G. cichoracearum* DC. and beneficial microorganisms present in microbial-enriched CT on the leaf surface was analysed by SEM. Leaf discs (14 mm diameter) from fully expanded melon leaves (three-week old plants) were cut with a cork borer. The leaf discs were oriented with the adaxial side up and placed in a 90 mm petri dish lined with double layer moistened Whatman No. 1 filter paper. Leaf discs were sprayed with one of the following solutions: water, microbial-enriched CT 24 h prior to inoculation with *G. cichoracearum* DC., microbial-enriched CT 24 h after inoculation with *G. cichoracearum* DC. or a conidial suspension of *G. cichoracearum* DC. All petri dishes were then incubated at 20 °C for 48 h. The treated leaf discs were dried and sputter-coated with gold palladium. Samples were stored in a desiccator until observation. The samples were observed under a scanning electron microscope (model-JEOL JSM-35CF).

Statistical analysis

All experiments were performed in duplicate unless otherwise specified. Since duplicated experiments yielded similar results, the data were pooled prior to statistical analyses. All percentages data were arcsine transformed and subjected to analysis of variance (ANOVA). Means were separated by Fisher's protected least significant difference (LSD) with $P \leq 0.05$ using SAS statistical software (PC-SAS software V8.2 (SAS) Institute, Cary, N.C. USA).

Results

Microbial diversity in CT

The microbial diversity in CT's (defined as CFUs ml^{-1}) of multiple batches of same compost is listed in Table 1. Separate batches of the same compost contained microbial populations of similar composition ($P \leq 0.05$). Therefore, only the Batch 1 of the same compost was utilized throughout this study.

Table 1 Assessing the microbial diversity (CFUs ml^{-1}) of multiple batches of compost tea after three days of brewing

Treatments	Other bacteria (CFUs ml^{-1}) (10^9)	*Pseudomonas* spp. (CFUs ml^{-1}) (10^8)	Lactic acid bacteria (CFUs ml^{-1}) (10^8)	Actinomycete (CFUs ml^{-1}) (10^7)	Yeasts (CFUs ml^{-1}) (10^6)	Other fungi (CFUs ml^{-1}) (10^5)	*Trichoderma* spp (CFUs ml^{-1}) (10^6)
Batch 1[a]	1.2 ± 0.02a	5.3 ± 0.03a	0.17 ± 0.29a	4.3 ± 0.79a	3.9 ± 0.035b	0.70 ± 0.56b	4.7 ± 0.032a
Batch 2	0.27 ± 0.05a	2.1 ± 0.01a	2.4 ± 0.26a	2.2 ± 0.84a	1.5 ± 0.024a	2.2 ± 0.43a	0.16 ± 0.041a
Batch 3	2.6 ± 0.24a	0.10 ± 0.03a	2.2 ± 0.37a	1.0 ± 0.78a	1.0 ± 0.023a	2.4 ± 0.67a	0.32 ± 0.026a
Batch 4	0.19 ± 0.05a	2.2 ± 0.04a	0.32 ± 0.42a	2.9 ± 0.76a	2.9 ± 0.012a	2.0 ± 0.70a	1.2 ± 0.049a

Means (±SE) with same letters within the same column are not significantly different from other numbers denoted by the same letter ($P \leq 0.05$) according to Fisher's protected LSD test on their transformed values ($F_{3,\ 12} = 1.00, 0.87, 0.40, 0.74, 5.16, 26.63$, and 0.04 and $P = 0.4250, 0.4828, 0.7522, 0.5476, 0.0161, <0.0001$ and 0.9895 for other bacteria, *Pseudomonas* spp., lactic acid bacteria, actinomycete, yeasts, other fungi and *Trichoderma* spp., respectively. Each value in computed on five replicates

[a] Source from Naidu et al. (2010)

Effects of microbial-enriched CT on the conidia germination of *G. cichoracearum* DC.

The percentage of conidial germination was significantly ($P \leq 0.05$) highest in the control (78 %) followed by CT alone (without microbial starter) (38.4 %), microbial-enriched CT (11.5 %) and Daconil® (4.7 %) at 96 hai (Fig. 1). Conidial germination of *G. cichoracearum* DC. was significantly ($P \leq 0.05$) inhibited by 94 %, 85 % and 51.3 % at 96 hai by Daconil®, microbial-enriched CT and CT alone, respectively (Table 2). *G. cichoracearum* DC. germinated by producing germ tube and appressorium after 6 hai in SDW samples (control) (Fig. 2a, b). These germ tubes were longer compared to the germ tubes that germinated in microbial-enriched CT and Daconil® treated samples. Additionally, germination was delayed in microbial-enriched CT (Fig. 2c). The germ tube length was significantly ($P \leq 0.05$) shorter in samples treated with Daconil® (10.20 µm) and microbial-enriched CT (15.52 µm) compared to the control sample (32.50 µm) at 24 hai (Table 2). Conidia in microbial-enriched CT appeared ruptured at 48 hai (Fig. 2d), which contributed to higher inhibition of conidial germination.

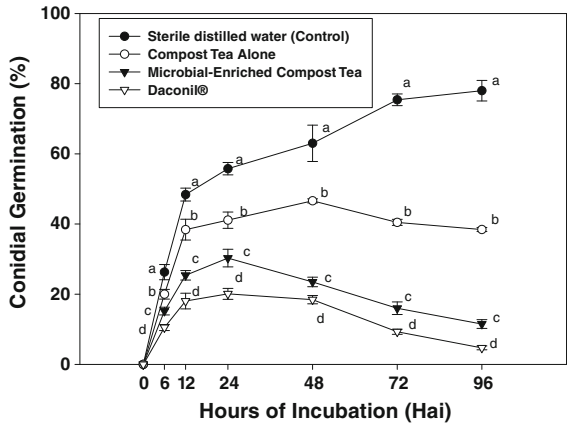

Fig. 1 The effect of microbial-enriched CT on conidial germination of *G. cichoracearum* DC. Values with the different letter at each time point are significantly different ($P \leq 0.05$) according Fisher's protected LSD test on their transformed values ($F_{3,\ 36} = 18.43, 20.01, 21.74, 60.96, 58.39$ and 146.46 and $P = < 0.0001, 0.0001, < 0.0001, < 0.0001, < 0.0001$ and 0.0001 at 6, 12, 24, 48, 72 and 96 h of incubation, respectively). Vertical bars indicate SE of ten replicates

Efficacy of different application times of microbial-enriched CT on the development of powdery mildew

Symptoms of powdery mildew initially appeared five days after inoculation (dai) with *G. cichoracearum* DC. on melon plants receiving only water (control), microbial-enriched CT applied 24 h prior to inoculation (T2) and microbial-enriched CT applied one week after disease outbreak (T5). The symptoms appeared as subtle, small, round, white spots, which subsequently grew in size and coalesced rapidly. The white spores of powdery mildew developed on lower leaves, which spread to new leaves, causing chlorosis and subsequently senescence of the lower leaves. The development of disease was delayed in plants treated with Daconil® (T6), microbial-enriched CT applied 24 h after inoculation (T3) and microbial-enriched CT

Table 2 Conidial inhibition, germ tubes length and appressorium formation of *G. cichoracearum* DC. conidia treated with SDW, CT alone, microbial-enriched compost tea or Daconil®

Treatments	Conidial inhibition (%)[a]	Germ tube length (μm)[b]	Appressorium formation[c]
SDW (control)	–	32.50 ± 2.71a	Detected
CT alone	51.3 ± 2.81c	20.79 ± 1.78b	Not detected
Microbial-enriched CT	85.0 ± 1.72b	15.52 ± 0.54c	Not detected
Daconil®	94.0 ± 1.02a	10.20 ± 1.50c	Not detected

Mean (±SE) with same letters within the same column are not significantly different ($P \leq 0.05$) according to Fisher's protected LSD test on their transformed values ($F_{2,27} = 49.39$ and $P = <0.0001$ for conidial inhibition (%) and $F_{3,27} = 23.06$ and $P = < 0.0001$ for germ tube length (μm). Each value in computed on ten replicates

[a] 96 h after incubation (hai)

[b] 24 hai

[c] Within 48 hai

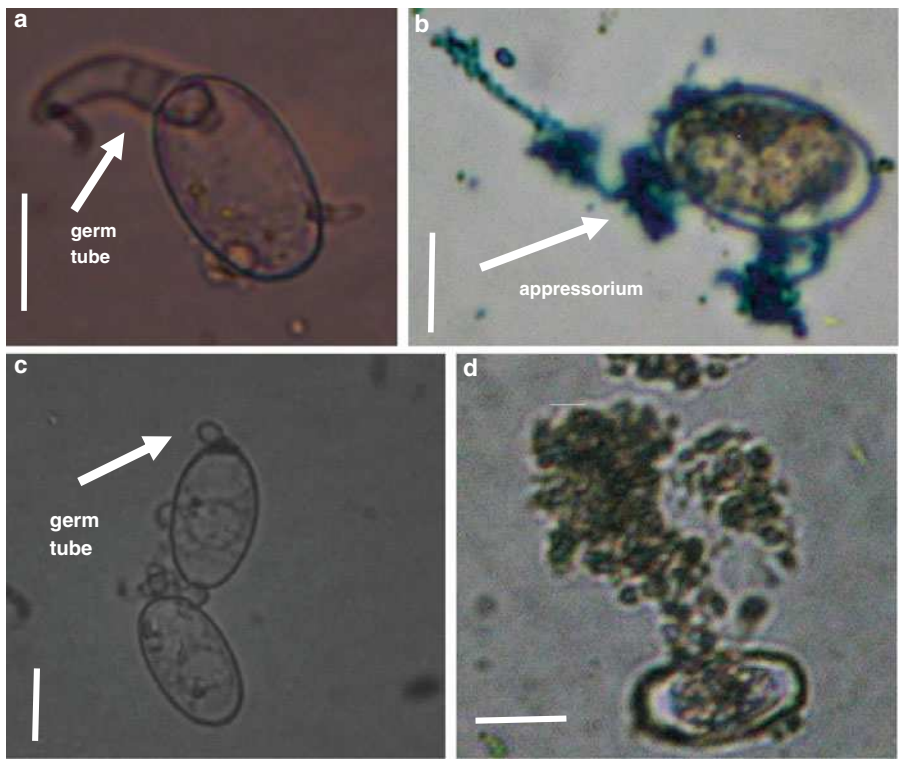

Fig. 2 Germinated conidia with germ tube treated with SDW (control) at 6 h after incubation (hai), magnification ×1,300 **a**; germinated conidia with appressorium treated with SDW (control) within 48 hai, magnification ×900 **b**; Germinated conidia with germ tube treated in microbial-enriched CT at 24 hai, magnification ×700 **c**; ruptured conidia with leaked cellular contents treated with microbial-enriched CT at 48 hai, magnification ×800 **d**. Bars represent 15 μm

applied simultaneously with inoculation (T4), and these symptoms were observed only on 12 dai.

There was a gradual increase in DS % over time. The percentage of disease severity was significantly highest ($P \leq 0.05$) in the control plants (T1), with a value of 85.94 %, and the lowest in plants treated with Daconil® followed by microbial-enriched CT applied 24 h after inoculation and microbial-enriched CT applied simultaneously with inoculation with values of 36.7, 42.36 and 57.14 %, respectively. At 27 dai, there was no significant difference ($P \leq 0.05$) in the DS % in plants treated with microbial-enriched CT

Table 3 Disease severity, area under disease progress curve (AUDPC), epidemic rate (R_L) and disease reduction for *G. cichoracearum* DC. present on melon plants treated with microbial-enriched compost tea at 27 days after inoculation

Treatments	Disease severity (%)	AUDPC (units^2)	Epidemic rate (R_L) (units day^{-1})	Disease reduction (%)
Water (control)	85.94 ± 0.29a	993.57 ± 6.53a	0.22 ± 0.013a	–
Microbial-enriched CT applied 24 h prior to inoculation	65.12 ± 2.17b	660.21 ± 5.42c	0.20 ± 0.012b	24.32
Microbial-enriched CT applied 24 h after inoculation	42.36 ± 0.65d	453.90 ± 9.21e	0.17 ± 0.02 b	50.71
Microbial-enriched CT applied simultaneously with inoculation	57.14 ± 0.99c	576.42 ± 6.97d	0.18 ± 0.032b	33.51
Microbial-enriched CT applied one week after disease outbreak	66.60 ± 2.17b	861.48 ± 5.87b	0.20 ± 0.015ab	22.50
Daconil® (2 g l^{-1})	36.70 ± 0.41e	371.52 ± 7.36f	0.13 ± 0.025c	57.30

Means (±SE) with different letters within the same column are significantly different ($P \leq 0.05$) according to Fisher's protected LSD test on their transformed values ($F_{5, 20} = 276.42$, 382.08 and 12.73 and $P = <0.0001$, <0.0001 and <0.0001 for disease severity (%), AUDPC and epidemic rate (RL), respectively. Each value in computed on five replicates

applied 24 h prior to inoculation (65.12 %) and microbial-enriched CT applied one week after disease outbreak (66.60 %) (Table 3).

Disease severity was further expressed as AUDPC and R_L. The AUDPC was significantly ($P \leq 0.05$) lower in plants treated with Daconil® (371.52 unit^2) and plants treated with microbial-enriched CT applied 24 h after inoculation (453.90 unit^2) compared to the control treatment (993.57 unit^2) (Table 3). Similarly, the epidemic rates (slope) were significantly lower ($P \leq 0.05$) in plants treated with Daconil® (R_L = 0.13 unit day^{-1}) followed by microbial-enriched CT applied 24 h after inoculation (R_L = 0.17 unit day^{-1}) and was recorded higher in the control plants (R_L = 0.22 unit day^{-1}), at 27 dai (Table 3). There was a significant reduction in the development of powdery mildew in plants treated with Daconil® followed by microbial-enriched CT applied 24 h after inoculation. There was a smaller, yet significant reduction in plants treated with microbial-enriched CT applied simultaneously with inoculation, microbial-enriched CT applied 24 h prior to inoculation and microbial-enriched CT applied one week after disease outbreak (Table 3).

Further, SEM revealed the presence of a large microbial population on the surface of melon leaves sprayed with microbial-enriched CT, which was nearly absent in control plants (Fig. 3a, b). Conidia of *G. cichoracearum* DC. appeared collapsed and colonized by a diverse genera of bacteria in leaf discs sprayed with microbial-enriched CT applied 24 h after the inoculation with *G. cichoracearum* DC. (Fig. 3c) in contrast to typical turgid conidia of *G. cichoracearum* DC. observed in the control (Fig. 3d).

Discussion

CTs are fermented watery extracts of composted materials that are used for their beneficial effects on disease suppression and plant growth of vegetable crops (Litterick et al. 2004). Present study demonstrated that CT alone (without microbial starter), microbial-enriched CT and Daconil® suppressed conidial germination of *G. cichoracearum* DC. in an in vitro assay. The suppressive activity of microbial-enriched CT observed in this study is likely biological in nature.

Previous reports have described the direct inhibition of both conidial germination and mycelia growth of various plant pathogens by beneficial microorganisms present in the water extract of compost (Stindt and Weltzien 1990; McQuilken et al. 1994; El-Masry et al. 2002). Similarly, non-aerated compost tea (NCT) inhibited the in vitro mycelial growth of tomato pathogens namely *Alternaria solani, Botrytis cinerea,* and *Phytophthora infestans* when compared to the water control (Koné et al. 2010). Water extracts from spent mushroom substrate inhibited in vitro germination of *Venturia inaequalis* conidia by 98 %, and microbial activity played a major role in the inhibition (Cronin et al. 1996). Similarly, beneficial microorganisms in non-sterilised

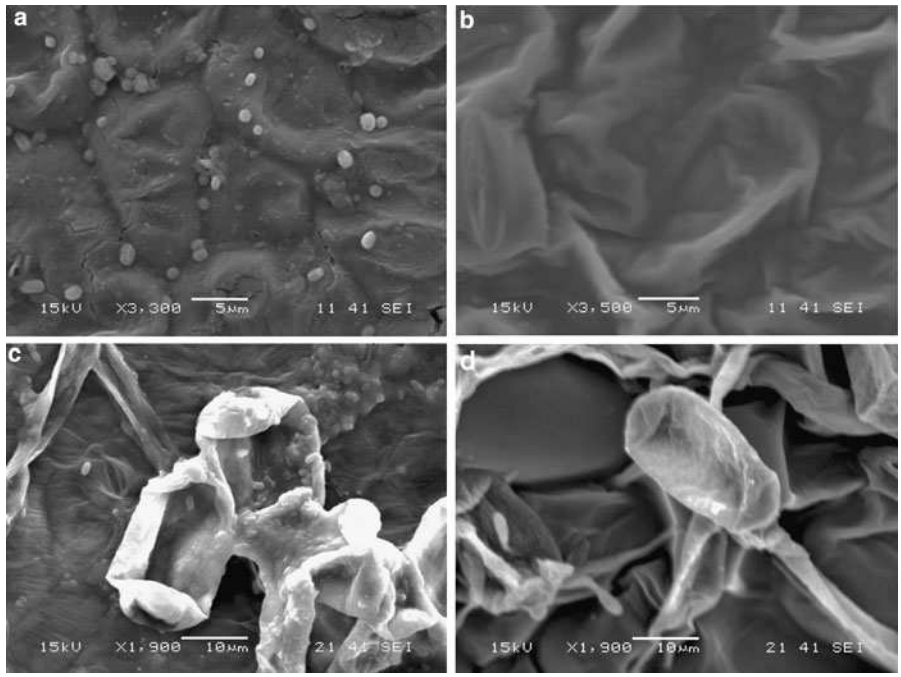

Fig. 3 Scanning electron microscopic micrograph of melon leaf surface sprayed with microbial-enriched CT 24 h prior to inoculation **a**; melon leaf sprayed with SDW (control) **b**; melon leaf surface sprayed with microbial-enriched CT 24 h after inoculation and showing collapsed conidia colonised by bacterial cells **c**; typical features of *G. cichoracearum* DC. conidia on melon leaf surface **d**

CT preferentially utilised the nutrients required for the germination of conidia. This prevented the formation of a germ tube and led to the lysis of *Choanephora cucurbitarum* conidia (Siddiqui et al. 2009). Recent findings by Sang and Kim (2011) demonstrated that, all the tested compost water extracts significantly inhibited in vitro conidial germination and appressorium formation of *Colletotrichum coccodes* and *C. orbiculare*, the causal pathogens of anthracnoses on pepper and cucumber. Koné et al. (2010) indicated that specific microorganisms (potential biological control agents) present in the CT would be more vital in the suppressive effect than the high total counts of bacteria. The beneficial microorganisms present in microbial-enriched CT that belong to different functional groups, such as *Bacillus*, *Pseudomonas*, lactic acid bacteria, actinomycetes and fungi (predominantly *Trichoderma* spp and *Penicillium* spp.) (Naidu et al. 2010) might have contributed to the conidial inhibition and disease suppression in the current study.

Previous findings by Siddiqui et al. (2009) found that mycelium of *Choanephora cucurbitarum* treated with non-sterilised CT showed alterations in mycelia morphology and lysis under light microscopy which resulted in the 100 % inhibition of mycelia growth. Observation under light microscopy in the present work identified ruptured *G. cichoracearum* DC. conidia that had been treated with microbial-enriched CT, which resulted in increased cell permeability and leakage of cellular contents. Mechanism of action underlying behind this observation could be through antibiosis. Antibiosis, which is a mechanism in which toxic metabolites penetrate the cell and inhibit cellular activity, may have caused these cytotoxic effects (Baker and Cook 1982).

Further in vivo study demonstrated the curative effect of microbial-enriched CT applied 24 h after inoculation, which delayed the onset of disease with an efficiency of inhibition comparable to a fungicidal spray (Daconil®). These finding are in agreement with the data published by Koné et al. (2010), who demonstrated that, curative application of NCT (3 dai) was shown to reduce the severity of powdery mildew on tomato (*Oidium neolycopersici*). Foliar application of CT eradicated 100 % powdery mildew pathogen (*Erysiphe polygoni*) on tomato leaves when applied as a curative treatment (Segarra et al. 2009). In vitro assessments revealed that four bacterial strains isolated from cattle manure compost extracts inhibited *B. cinerea* on detached bean leaves when applied 24 h after inoculation. Furthermore, these strains inhibited the growth of lesions for the next seven days (Stindt and Weltzien 1990). Significant reduction in the powdery mildew symptoms and development of *Sphaerotheca fusca* were observed when mycoparasites were applied to

melon leaves in the early stages of infection (3 dai) (Romero et al. 2003).

Conidial germination represents the first step in triggering an asexual life cycle and spreading the disease of several aerial phytopathogenic fungi, including powdery mildew (*Podosphaera fusca*) (Romero et al. 2007). Powdery mildew conidia transition through five developmental stages during pathogenesis: stage 1 includes the germination of conidia within 12 h of inoculation, stage 2 includes the formation of haustorium within 24 h, stage 3 includes the initiation of branching of the germ tube and elongation of hyphae within 48 h, stage 4 includes the formation of new conidia within 120 h and stage 5 includes the maturation of new conidia within 240 h (Kuzuya et al. 2006). Therefore, conidia germination for the present study was generally suppressed at the time of germ tube branching, hyphal elongation and haustorium formation (stage 2 and 3), and these germination steps are essential steps of pathogenesis of the fungus.

Microbial-enriched CT applied 24 h prior to inoculation or applied one week after disease outbreak was less effective at inhibiting the development of powdery mildew. This lack of inhibitory activity may reflect the inability of microorganisms in the CT to survive on the leaf surface (McQuilken et al. 1994). Previous reports have indicated that compost extracts are less effective in reducing lesion development on detached bean leaves when it is sprayed on leaves two days prior to inoculation with *B. cinerea*. A large community of microorganisms was identified by SEM on melon leaves treated with microbial-enriched CT, and these microorganisms may have played a major role in inhibiting germination of *G. cichoracearum* DC. conidia.

Therefore, it can be concluded that microbial-enriched CT can be applied as a curative treatment in inhibiting the development of powdery mildew on melon plants, hence reducing the dependency on chemical fertilisers. The powdery mildew colonization on melon leaf surface was suppressed apparently due to inhibition of conidial germination. Hence, it limits the number of penetration pegs produced, which in turn may prevent the spread of disease. However, the CT associated mechanism of suppression is difficult to determine because CT represents a microbial community rather than a single species.

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Author Biographies

Yuvarani Naidu is a Research Officer attached to the Malaysian Palm Oil Board. Her research area mainly focuses on utilization of organic wastes and identifying potential biocontrol agents for integrated management of plant diseases.

Sariah Meon is a Professor of Plant Pathology based at Universiti Putra Malaysia. Her research largely focuses on biointensive intergrated management of various plant diseases including *Fusarium* wilt on banana, powdery mildew on cucurbits, basal stem rot of oil palm (*Ganoderma boninense*) and *Chaenophora* blight of okra.

Yasmeen Siddiqui is a Senior lecturer at Centre of foundation studies for Agriculture based at University Putra Malaysia. Her research interest mainly focuses on development of botanical pesticides, organic farming and integrated disease management of horticultural crops.