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Effect of aerated and non-aerated compost tea against some fungal phytopathogens

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Abstract

Suppressive effect of aerated and non-aerated compost tea against different fungal phytopathogens, namely *Alternaria solani, Bipolaris sorokiniana, Fusarium oxysporum, Pestalotia palmarum* and *Sclerotium rolfsii* was evaluated in Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during 2016. Commercial compost, "Rastic Joibo Shar" was used to prepare aerated and non-aerated compost teas at 20% and 25% concentrations. All the treatments significantly inhibited the mycelial growth of tested five fungal phytopathogens. Among the five pathogens 25% concentration of non-aerated compost tea gave highest mycelial growth inhibition (76%) of *Sclerotium rolfsii*. Colony characteristics of five phytopathogens were significantly varied in all aspect by treating aerated and non-aerated compost teas at different concentrations. Finally it may be concluded that both aerated and non-aerated compost teas have suppressing effect on radial mycelial growth Thus compost tea may be used as alternatives to inorganic fertilizers/fungicides to suppress the pathogenic activity of the soil borne fungal phytopathogens. Thus based on the efficacy of i*n-vitro* experiments, efficacy in field condition can be assessed against different diseases.

Introduction

The majority of the fungal species are saprophytic, with around 10% (more or less 100 000 fungal species) can colonize plants and cause plant disease (Agrios, 1997). Among the fungal phytopatogens, soil-borne and seed-borne are most devastating to crops. Most *Alternaria* species are saprophytes that are commonly found in soil or on decaying plant tissues (Thomma, 2003). Early blight of potato and tomato are the most destructive foliar diseases caused by *Alternaria solani*. The yield loss due to early blight ranges from 5%- 78% in both tomato and potato (Waals *et al.*, 2004; Pasche *et al.*, 2005). Cereal production is seriously hampered by foliar diseases caused by *Bipolaris* spp**.** The pathogen reduced the average yield 70% to 80 % in barley and wheat respectively (Alam *et al*, 1994). The grain/ear in both crops is also reduced to 88.7% (Hossain *et al.*, 1998). *Fusarium* spp. is one of the most common soil born fungi. Vascular wilt of tomato caused by *F. oxysporum* may cause complete yield loss (Walker, 1971; Benhamou *et al*., 1998). *Pestalotia pulmarum* causes grey leaf spot of coconut and betel nut of the coastal area of Bangladesh. Every year leaf spot disease attacks the gardens of coconut and betel nut and causes as much as 14% yield loss (Islam *et al.*, 2004). *Sclerotium* spp. especially *Sclerotium rolfsii* is a serious soil-borne plant pathogen of many crops (Aycock, 1966). Seedling blight, foot rot, collar rot and southern blight etc. are the most common diseases incited by *S. rolfsii* (Khan and Kibria, 1973).

Generally, the control of soil-borne diseases is very difficult and chemical soil fumigation as the only possible way for remedy because of its effectiveness. However, the transition toward sustainable plant disease management required by the EU, through restrictive policies on the use of synthetic fungicides, has stimulated research into valid alternative methods equally capable of reducing losses due to plant diseases (Ariena *et al*., 2015). Use of aerated compost teas (ACTs) is becoming an attractive disease management option among producers who support sustainable protective methods. ACTs are liquid products generally derived from aerated aqueous extractions of composted biodegradable organic compounds (Ingham et al., 2003). Aerated and non-aerated compost teas, both products of compost, have also been shown to suppress soil-borne diseases, including damping-off and root rots caused by *Pythium ultimum, Rhizoctonia solani* (Scheuerell and Mahaffee, 2002; Dionne *et al.* 2012), *Phytophthora capcisi* (Sang *et al*., 2010) and wilts caused by *Fusarium oxysporum* and *Verticillium dahliae* (Alfano *et al.*, 2011). Hibar *et al.* (2005) demonstrated that transplanting inoculated tomato seedlings in compost extract-treated peat inhibited *Fusarium oxysporum*. In fact, there are a number of reports that demonstrate the ability of compost tea to suppress a wide range of both air- and soil-borne plant pathogens, when applied as foliar spray and soil drenching (Scheuerell and Mahaffee, 2002).

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A number of studies have indicated that the microbial community of CT is effective for disease suppression (Hoitink *et al*., 1997; Siddiqui *et al*., 2008). Reduction and elimination of the adverse effects of synthetic fertilizers and pesticides on human health and the environment is a strong indicator that organic agriculture is gaining worldwide attention. Because indiscriminate use of chemicals for controlling diseases of crop plants result environmental pollution and health hazards as well as reduce soil quality (Martin and Brathwaite, 2012). Different works have been reported as single or multiple mechanisms involving microbial antagonism through antibiosis, parasitism, competition for nutrients/space or induced plant resistance (Zhang *et al.* 1998; El-Masry *et al*. 2002; Al-Mughrabi *et al*. 2008), or suppressive physicochemical properties such as an improved nutrient status of the plant, toxic compounds, or induced resistance (Hoitink *et al*. 1997; Siddiqui *et al*. 2008). In this regard, compost teas are potential alternatives to the use of the common synthetic fungicides in response to environmental sustainability and food safety.

The present study has focused on the evaluation of the efficacy of aerated (ACT) and non-aerated compost teas for *in-vitro* suppression of different fungal phytopathogens such as *Alternaria solani, Bipolaris sorokiniana, Fusarium oxysporum, Pestalotia pulmarum* and *Sclerotium rolfsii*.

Materials and Methods

Sample collection and isolation of fungi

Infected tomato plants with typical symptoms of early blight and wilt were collected from Dumuria upazilla under Khulna district. The fungus was isolated following tissue planting method (Mian, 1995). It was identified observing morphological characters by using key described in Barnett and Hunter (1972). The pure culture of the fungus was obtained by Hyphal Tip Isolation Method. These fungal isolates were grown on PDA slants, stored at 4^0C in a refrigerator for further use. The preserved isolates of *Bipolaris sorokiniana, Pestalotia palmarum and Sclerotium rolfsii* were collected from the Plant Protection laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh and used in this experiment.

Laboratory experiment was conducted at Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during October, 2016 to November, 2016.

Multiplication of Fungi

Sterilized petridishes were placed in aseptic condition of a laminar air flow cabinet and 20ml PDA was poured in each petridish. After solidification of PDA, the plates were inoculated by placing 5mm disc of four days old pure culture of *Alternaria solani, Bipolaris sorokiniana, Fusarium oxysporum*, *Pestalotia palmarum* and *Sclerotium rolfsii* using flame sterilized cork-borer (5 mm diameter). The inoculated petridishes were marked and kept in the growth chamber at 24±1 $^{\circ}$C for few days.

Preparation of Aerated and Non-Aerated Compost Tea (ACT& NCT)

Commercially available compost named “Rastic Compost Joiboshar” was collected from the local market of Khulna city. For preparing ACT, 400 gm of compost was kept and equally distributed in eight conical flasks (50 gm/conical flask). Among them, four conical flasks, each of the flask was poured with 150 ml sterile distilled water and rest four conical flasks were poured with 200 ml sterile distilled water to prepare 25% and 20% concentrations respectively. The mixture was homogenized in orbital shaker at 250 rev/min and 25 ºC for 14 days under dark condition. After 14 days, the homogeneous solution was filtered with cheese cloth and the extracts were used to conduct the experiment (Martin *et al.,* 2013).

To obtain NCT, 400gm of compost was kept in an air-tight container at 25 $^{\circ}$C for 14 days under dark condition. After 14 days, above (ACT preparation) same procedures were followed to prepare 25% and 20% concentrations respectively. The mixture was homogenized in an orbital shaker at 250 rev/min and 25 $^{\circ}$C for 10 minutes for homogenize the solution.

Inoculation and Incubation

To get aerated or non-aerated compost tea media, the basic PDA media was modified by using aerated or non-aerated compost teas at 15% concentration. The fungi were grown on modified PDA (17 ml basic PDA media and 3 ml aerated compost tea) medium, each petridish containing 20ml of modified media. Basic PDA media with 15% sterilized distilled water was used as control. The composition of the inoculation media are presented in Table 1.

Table 1. Concentration of the inoculation media used in experiment (Martin *et al,* 2013)

Treatment	Concentration of treatment (%)	Concentration of media (%)	
		Treatment (mL)	PDA media (mL)
Aerated compost tea	20	20	80
	25	25	75
Non-aerated compost tea	20	20	80
	25	25	75
Sterilized distilled water (Control)	-	20 or 25	80 or 75

The pH of all the media were adjusted to 6.5 by adding alkaline solution (0.1% NaOH). The plates were inoculated and incubated at 24±1ºC. The radial mycelia growth of each plate was recorded considering 80% radial growth of control treatment.

Measurement of Radial Growth and Determination of Percentage Inhibition

The radial growth of mycelium of each plate was recorded as an average of two diameters measured at right angles to one another. The colony characters were recorded. The percentage inhibition of growth over control was calculated using the following formula (Naz *et al.*, 2006).

$$\% \text{ inhibition} = \frac{X-Y}{X} \times 100$$

Where,
X = Average growth of fungi in control petridish
Y= Average growth of fungi in each treated petridish

Observation of Colonial Character

After completion of 80% radial growth in control treatment, mycelial characters such as shape, structure, texture, and colony color of top view and reverse view were observed visually and recorded.

Experimental Design and Data Analysis

Completely randomized design was followed for conducting experiment. Five replications were used for each treatment. The data were statistically analyzed using STAR (Statistical Tool for Agricultural Research) computer program and means were compared following Duncan's Multiple Range Test (DMRT).

Results and Discussion

Effect of compost teas on radial mycelial growth

Radial mycelial growth significantly varied for both the aerated and non-aerated compost teas at different concentrations (Table 2). In case of *Alternaria solani* and *Bipolaris sorokiniana* aerated compost teas indicated lowest mycelia growth (30mm and 22mm, respectively). A similar effect was observed by Martin *et al*. (2014) who showed that aerated compost teas (ACT) from all sources generally gave rise to greater *in vitro* suppression than non-aerated compost teas (NCT), although both are considered effective. Haggag and Saber (2007), also found that both ACT and NCT from either plant residues or chicken manure compost inhibited conidium germination of *Alternaria porri* (purple blight) and *A. solani* (early blight) in *in vitro* experiments.

On the other hand, non-aerated compost teas reduced mycelia growth of *Fusarium oxysporum, Pestalotia palmarum* and *Sclerotium rolfsii* and lowest mycelia growth were 28 mm, 25 mm and 19 mm, respectively (Table 2). Cronin *et al,* (1996) concluded that NCT from manure-based spent mushroom compost effectively inhibited the *in vitro* conidium germination of *Venturia inaequalis* (apple scab), whereas ACT had no effect.

Previous reports have shown that compost teas affect the mycelial growth of numerous plant pathogens (e.g. Scheuerell and Mahaffee, 2004 and 2006; Haggag and Saber, 2007; Al-Mughrabi *et al*., 2008; Siddiqui *et al*., 2008; Koné *et al*., 2010). In particular, aerated grape marc compost tea greatly inhibited the mycelial growth of numerous soilborne fungi, including *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *radicis-cucumerinum*, *R. solani*, *V. dahliae* and *V. fungicola* (Diánez *et al*., 2006).

Effect of compost teas on inhibition percentage

The statistical bar (Figure 1) represents inhibition percentages were statistically significant for both the aerated and non-aerated compost teas at different concentrations. In case of *Alternaria solani* and *Bipolaris sorokiniana* aerated compost tea at 25% indicated highest inhibition percentage (61% and 70%, respectively) but non-aerated compost tea at 25% showed highest inhibition percentage in case of *Fusarium oxysporum* (64%), *Pestalotia palmarum* (68%) and *Sclerotium rolfsii* (76%). Among five tested fungal phytopathogens *Sclerotium rolfsii* at 25% aerated compost tea concentration showed highest inhibition percentage (76%) (Fig. 1).

Martin *et al.* (2013) showed that ACT and NCT filtrates inhibited the *in vitro* growth of all tested pathogens (*Botrytis cinerea, Sclerotinia sclerotiorum, Pythium aphanidermatum, Phytophthora parasitica, Fusarium oxysporum*) reaching 100 % inhibition in most cases. Akter *et al.* (2013) reported that using different aqueous plant extracts in combination with compost teas at 2.5% concentration gave 91% inhibition of conidial germination of *B. sorokiniana*. Kone *et al.* (2010) also found significant inhibition of the mycelial growth of *A. solani* (37–66%), *B. cinerea* (57–75%) and *P. infestans* (100%) for all non-aerated compost teas. Mechanisms of action underlying the efficacy of compost teas to control plant pathogens have been reported as single or multiple mechanisms involving microbial antagonism (through antibiosis, parasitism, competition for nutrients/space or induced plant resistance) (Zhang *et al*., 1998; El-Masry *et al*., 2002; Al Mughrabi *et al*., 2008) or suppressive physicochemical properties (improved nutrient status of the plant, toxic compounds or induced resistance) (Hoitink *et al*., 1997; Siddiqui *et al*., 2008).

Table 2. Effect of compost teas on radial mycelial growth of five fungal pathogens

Compost Tea	Mycelial growth				
	Alternaria solani	*Bipolaris sorokiniana*	*Fusarium oxysporum*	*Pestalotia palmarum*	*Sclerotium rolfsii*
Control	78 a	75 a	79 a	78 a	78 a
Aerated compost tea at 20%	35 b	22 d	40 b	34 b	36 b
Aerated compost tea at 25%	30 c	22 d	34 c	32 c	30 c
Non-aerated compost tea at 20%	36 b	31 b	31 d	27 d	28 c
Non-aerated compost tea at 25%	35 b	26 c	28 e	25 d	19 d
LSD	2.02	1.67	2.01	2.97	2.97
Level of significance (%)	0.01	0.01	0.01	0.01	0.01

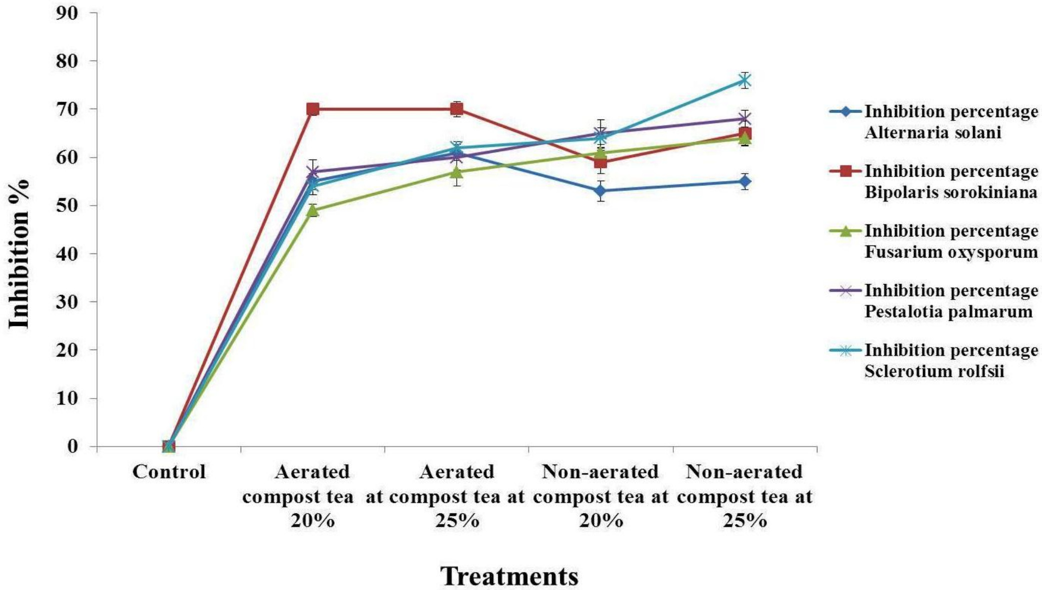

Fig. 1. Effect of compost teas on Inhibition percentage over control of five fungal pathogens

Table 3. Effect of compost teas on colony characters of five fungal pathogens

Name of fungus	Compost Tea	Colony characters				
		Shape	Texture	Margin	Color (upper surface)	Color (lower surface)
Alternaria solani	Control	Circular	Compact	Regular	Olive green	Dark green
	Aerated compost tea at 20%	Circular	Cottony	Irregular	Olive green	Black
	Aerated compost tea at 25%	Circular	Cottony	Irregular	Olive green	Black
	Non-aerated compost tea at 20%	Circular	Cottony	Regular	Olive green	Black
	Non-aerated compost tea at 25%	Circular	Cottony	Irregular	Olive green	Black
Bipolaris sorokiniana	Control	Irregular	Compact	Regular	Black	Black
	Aerated compost tea at 20%	Irregular	Compact	Irregular	Grayish	Black
	Aerated compost tea at 25%	Irregular	Compact	Irregular	Grayish	Black
	Non-aerated compost tea at 20%	Irregular	Compact	Irregular	Grayish	Black
	Non-aerated compost tea at 25%	Irregular	Compact	Irregular	Grayish	Black
Fusarium oxysporum	Control	Circular	Feathery	Regular	Pink	Creamy
	Aerated compost tea at 20%	Circular	Feathery	Regular	Pink	Creamy
	Aerated compost tea at 25%	Irregular	Feathery	Regular	Pink	Creamy
	Non-aerated compost tea at 20%	Circular	Feathery	Irregular	Pink	Creamy
	Non-aerated compost tea at 25%	Circular	Feathery	Regular	Pink	Creamy
Pestalotia palmarum	Control	Circular	Compact	Regular	White	White
	Aerated compost tea at 20%	Circular	Compact	Regular	White	White
	Aerated compost tea at 25%	Circular	Compact	Regular	White	White
	Non-aerated compost tea at 20%	Circular	Compact	Regular	White	White
	Non-aerated compost tea at 25%	Circular	Compact	Regular	White	White
Sclerotium rolfsii	Control	Circular	Compact	Regular	White	Reddish
	Aerated compost tea at 20%	Circular	Compact	Regular	White	Reddish
	Aerated compost tea at 25%	Circular	Compact	Irregular	White	Reddish
	Non-aerated compost tea at 20%	Irregular	Compact	Irregular	White	Reddish
	Non-aerated compost at 25%	Circular	Compact	Irregular	White	Reddish

Effect of compost teas on colony characters

Circular shaped mycelial colony was recorded in *Alternaria solani, Fusarium oxysporum, Pestalotia palmarum* and *Sclerotium rolfsii* at all concentration of compost teas except aerated compost tea 25% in case of *Fusarium oxysporum* and Non-aerated compost tea at 20% in case of *Sclerotium rolfsii* which showed irregular shape. *Bipolaris sorokiniana* showed irregular shape at all level of concentration.

Control plate of *Alternaria solani* and all concentration plate of *Bipolaris sorokiniana, Pestalotia palmarum* and *Sclerotium rolfsii* revealed compact texture; other plates of *Alternaria solani* showed cottony texture. Feathery texture was found from *Fusarium oxysporum*.

Regular margin were found from all the control plate of five fungi, all the concentration of compost tea of *Pestalotia palmarum*, Non-aerated compost tea at 20% of *Alternaria solani;* Aerated compost tea at 20% and 25%, Non-aerated compost tea at 25% of *Fusarium oxysporum* and aerated compost tea at 20% of *Sclerotium rolfsii.* Remaining others showed irregular margin.

The upper surface colony color of *Alternaria solani* was olive green, *Bipolaris sorokiniana* was grayish but the control plate was black, *Fusarium oxysporum* was pink and *Pestalotia palmarum* and *Sclerotium rolfsii* were white. That means application of compost tea affected only the upper surface colony color of *Bipolaris sorokiniana*.

Dark green lower surface colony color was observed on control plate of *Alternaria solani*; other treatment of *Alternaria solani* and *Bipolaris sorokiniana* showed black color. Creamy, white and reddish color were observed from *Fusarium oxysporum*, *Pestalotia palmarum* and *Sclerotium rolfsii*, respectively. So compost tea affected only the lower surface colony color of *Alternaria solani* (Table 3).

Overall, results from this study showed that both ACT and NCT provided a significant inhibition of the mycelial growth of soil-borne pathogens as well as seed-borne pathogens. Among the five Phytopathogens highest inhibition was found in *S. rolfsii*. So, compost tea could play important role to suppress the pathogenic activity of *S. rolfsii*. Future work will be attempted to identify more accurate concentration and application procedure to suppress diseases and enhancing plant growth in field condition.

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