

# Potential of compost tea for suppressing plant diseases

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## Abstract

Numerous studies have demonstrated that water-based compost preparations, referred to as compost tea and compost-water extract, can suppress phytopathogens and plant diseases. Despite its potential, compost tea has generally been considered as inadequate for use as a biocontrol agent in conventional cropping systems but important to organic producers who have limited disease control options. The major impediments to the use of compost tea have been the less-than-desirable and inconsistent levels of plant disease suppression as influenced by compost tea production and application factors including compost source and maturity, brewing time and aeration, dilution and application rate and application frequency. Although the mechanisms involved in disease suppression are not fully understood, sterilization of compost tea has generally resulted in a loss in disease suppressiveness. This indicates that the mechanisms of suppression are often, or predominantly, biological, although physico-chemical factors have also been implicated. Increasing the use of molecular approaches, such as metagenomics, metaproteomics, metatranscriptomics and metaproteogenomics should prove useful in better understanding the relationships between microbial abundance, diversity, functions and disease suppressive efficacy of compost tea. Such investigations are crucial in developing protocols for optimizing the compost tea production process so as to maximize disease suppressive effect without exposing the manufacturer or user to the risk of human pathogens. To this end, it is recommended that compost tea be used as part of an integrated disease management system.

**Keywords:** Compost, Biocontrol agent, Pathogens, Molecular tools, Metagenomics

**Review Methodology:** For literature regarding compost tea as a biocontrol agent, I searched the catalogue of the USDA National Agricultural Library, CAB Abstracts, Google Scholar, ScienceDirect and Scientific Electronic Library Online (SCIELO-Brazil) using the keyword search terms 'compost tea', 'compost extract', 'fermented extracts', 'water-based compost preparations', 'compost leachate', 'compost slurry', 'compost', 'non-aerated compost tea', 'aerated compost tea', 'organic tea' and 'steepages'. I used the references within books, theses, or articles, to search for additional relevant materials, to profile experts and researchers in the subject area and Google Scholar Alerts to keep track of the most recent publications relating to disease suppression using compost tea. Much of the work pertaining to plant disease suppression using compost tea is not published in peer-reviewed literature. I therefore used popular literature to discuss current practices and claims, emerging trends and to highlight the need for scientific research in specific areas.

## Introduction

The suppression of plant diseases using compost tea has received much attention in the last 20 years [1, 2]. Historically, the use of compost tea as a biological control agent (BCA) against phytopathogens and plant diseases has increased in parallel with anecdotal success stories mainly from professional landscapers and gardeners,

organic farmers and private companies [1, 3]. However, there is now considerable scientific evidence, which shows that various types of compost tea and/or compost-based liquid preparations can suppress phytopathogens and plant diseases [4–9]. Despite this evidence, the practical application of compost tea for plant disease management has been limited, and the paradigm for plant disease control in modern agriculture of using one active

ingredient to target one or multiple pathogens still persists [10]. In most cases, this approach to plant disease control has been translated into a heavy reliance on a single control strategy, which is often the widespread use of synthetic pesticides. Though not without successes, there have been some problems with the misuse of synthetic pesticides such as the emergence of pesticide-resistant strains in target pathogen worldwide [11–15]. This has somewhat served to strengthen the ideology that sustainable and environmentally acceptable farming should rely largely on the ability to reduce the need for hazardous agrochemicals to maintain plant health and productivity [16]. Such ideologies have become increasingly popular in the context of increasing concerns by the public over the use of synthetic pesticides and their potential health and environmental impact, as well as the increasing demand for organically produced foods [17].

In response to public concerns, much effort and resources have been expended to develop and adopt more sustainable plant disease management tools and practices [18]. Such practices, which are largely based on ecological principles, include the use of minimal tillage, soil solarization [19], cover crops, organic amendments such as, uncomposted and composted crop residues and manures, compost tea or compost water extract (CWE) and microbial biocontrol agents such *Trichoderma* [20], *Bacillus* and *Streptomyces* spp. [21, 22].

None of these practices has arguably received more attention than research on the development of BCAs. However, for the most part, research efforts for BCAs have generally followed a similar paradigm to synthetic pesticides, i.e. a single organism is identified and developed for delivery into an agroecosystem [10]. The use of this approach with BCAs has resulted in far less commercial successes [23] than with synthetic pesticides and has developed a reputation for inconsistent plant disease control [24, 25].

Mahaffee and Scheuerell [10] reported that this inconsistency results from the influence of numerous biotic and abiotic factors that affect the ability of the BCA to survive the application process, attach to, grow and reproduce on the plant and inhibit pathogenesis. Moreover, they concluded that the reasons suggested for the inconsistency of BCAs were all related to the inability of a single organism to be 'all things at all times' in an extremely dynamic and harsh environment. Deacon [26] and Mazzola [27] further suggested that the consistency and level of control of a BCA introduced in non-native soil ecosystems would be limited because the BCA rarely occupies the same niche as it would have in its natural habitat. In an effort to address these inconsistencies, three alternative types of approaches, which are based on the introduction of several microorganisms at the same time, have been explored: (i) the mixing of several known types of BCAs with diverse modes of action or that colonize different ecological niches [28–30]; (ii) the introduction of partially or uncharacterized microbial communities

(e.g. compost tea/extract) with no known activity [31, 32]; and (iii) the enhancement of resident populations existing on or around the plant [27]. Despite some success, these approaches still result in inconsistent plant disease control.

Compost tea is one means by which partially or uncharacterized microbial communities may be introduced in an agro-ecological system for plant disease control. The objective of this review is to collate current knowledge on plant disease suppression with compost tea and to discuss factors affecting its potential as a BCA. This review does not include to any great extent, the history of the use of compost tea or extracts, which has already been adequately addressed by Scheuerell and Mahaffee [1], Litterick and Wood [2] and Litterick *et al.* [33]. Instead, the review provides a discussion on the potential application of molecular tools and high-throughput sequencing (HTS) technologies to better understand the relationships between microbial abundance, diversity, functions and disease suppressive efficacy of compost tea. Such a discussion has either not been included or directly addressed in previous review articles on compost tea [1, 2, 33, 34].

In cases where scientific evidence is limited, reference to non-scientific reports are made to highlight current practices and claims, emerging trends and the need for research in specific areas.

## Definitions and Standards

Compost tea and compost extract have been used interchangeably to refer to liquid samples obtained from or through the use of compost by pressure, distillation evaporation or treatment with a solvent [35, 36]. However, compost tea is increasingly referred to as filtered products of compost fermented in water [33], whereas compost extracts are filtered products of compost mixed primarily with water (or any solvent) but not fermented or held for more than 1 h before use [1, 36]. Compost tea is further distinguished into aerated and non-aerated teas with respect to the fermentation method used to prepare them. Aerated compost teas (ACT) refer to products where the compost-water extract is actively aerated during the fermentation process and non-aerated compost teas (NCT) are products where the compost-water extract is not aerated, or receives minimal aeration only at the initial mixing stage of the fermentation process [2].

Though commonly used to describe the process by which both ACT and NCT are made, the term fermentation more appropriately describes or is related to the production of NCT. This is because, traditionally, fermentation has been defined as an anaerobic cellular process in which organic compounds are converted into simpler compounds and chemical energy [37]. As such, NCT are usually associated with microbial communities, which

are dominated by anaerobes and ACT with microbial communities that are dominated by aerobes. Although commonly implied, the association of ACT with aerobic and NCT with anaerobic environment or process has little scientific basis since there is no consensus on oxygen concentrations which define aerobic, micro-aerobic and anaerobic compost tea [38]. Moreover, it is unclear whether a minimum oxygen level needs to be set for NCT, since they are produced without aeration. Ingham [39] proposed a standard that dissolved oxygen (DO) concentration should remain above 5.5 mg/l at room temperature and sea level, during the production of ACT. This proposed standard seems congruent with the suggestion made by Davis [40], that a minimum DO level of 5 mg/l is needed to support a diverse population of aquatic organisms.

To date, Al-Dahmani *et al.* [41] are one of the few research teams to have produced appropriate and verifiable anaerobic conditions, using an anaerobic jar with O<sub>2</sub> absorbers and CO<sub>2</sub> indicators for 7 days, which is often assumed to exist during the production of NCT. In most disease suppression studies, the DO levels of NCT or ACT are often not measured or reported. Therefore, there is a dearth of scientific literature on the evolution of DO and other parameters, such as microbial populations, pH, electrical conductivity (EC) and nutrients during the compost tea production process. In one of the few scientific studies to investigate the evolution of compost tea properties during brewing, St. Martin *et al.* [42] reported that DO concentrations of NCT were < 4.1 mg/l and generally decreased as brewing time progressed. Whereas, DO levels of ACT were > 5 mg/l and fluctuated during brewing. Similar DO levels of > 6 mg/l were reported by Evans *et al.* [9] and Palmer *et al.* [43] for ACT.

In the absence of standard DO concentrations that distinguish ACT from NCT, and the close association of fermentation with the production of NCT, the term brewing, which has been used interchangeably with fermentation seems more appropriate to describe the production process of both NCT and ACT. For the purposes of this review, brewing will be used instead of fermentation, to mean a steeping process of compost in any solvent (usually water), which lasts for more than 1 h [36]. The end-product of which, is compost tea.

Various other terms, which have been used interchangeably with compost tea or are associated with the compost tea production process, are defined in Table 1. A more thorough review of these terms and others can be found in Scheuereil and Mahaffee [1] and Recycled organics Unit [44]. In an effort to standardize definitions and improve the clarity of research progress on disease suppression using compost tea, terms such as CWE that are used in many studies have been re-categorized as either ACT or NCT mainly in accordance with definitions presented in the Compost Tea Task Force Report [36].

## Production of Compost Tea

Both ACT and NCT production methods involve brewing well-characterized compost in water for a specific time period and require the use of a brewing vessel, compost, water, incubation and filtration prior to application with conventional pesticide spray equipment [1–3, 39].

Regardless of the brewing method used, dechlorination of water before compost is introduced, is recommended as a best practice to reduce the risk of the microbial propagation and growth being inhibited by chlorine or chloramines. This is usually done by allowing the potable water to sit in the brewing vessel for at least 24 h [51] or by aerating 20–120 min [42, 51].

Yohalem *et al.* [52] recommended that at least 500 g (FW) of compost be used to make NCT for *in vitro* efficacy testing against phytopathogens. This amount was found to reduce compost sampling error in experiments designed to screen the *in vitro* efficacy of NCT against *Venturia inaequalis*. However, Yohalem *et al.* [52] presented no data on the moisture levels of the compost used in the study, and though comparative investigations were not done for ACT, it is likely that the same amount of compost is required. To date, studies that directly address the standardization of compost amount based on end-use of compost tea and reducing compost sampling error are limited. The amount of compost used to make compost tea remains largely dependent on the size and type of the brewing vessel and equipment used [10]. Brewing vessel size used to produce compost tea varies from small buckets (approx. 15–19 litre) to units that can hold several thousand litres. Compost additives, including nutrient amendments and microbial inoculants may be added before, during, or after brewing, and adjuvants and UV stabilizers prior to application.

To this end, one part compost to 1–50 parts water ratio has been used to brew compost tea, with 1 : 5 v/v being the most commonly used ratio for brewing ACT and NCT. With the growing number of companies designing and selling apparatus to produce ACT, the compost to water ratio depends largely on the type of equipment and is often recommended by the supplier of compost tea brewing units. These units range from home-designed pieces to commercially available backyard or industrial equipment, which use different methods to inject air into the mixture. These include showering recirculated water through a porous bag of compost that is suspended over an open tank [53], recirculating water through a vortex nozzle mounted above a tank [54], injecting air through a hollow propeller shaft [55], venturi nozzles [56], aquarium stones [39] or fine bubble diffusion mats [57].

Despite the multitude of equipment used to make ACT, published scientific studies on the comparative evaluation of different brewing equipment as it relates to producing compost tea of a consistent quality for an intended use, are limited. In fact, most of the evidence in support of

**Table 1** Definitions of terms used interchangeably with compost tea or in association with the compost tea production or application process

Term	Definition	References
Composting	A biological process through which microorganisms convert organic materials into useful end-products, which may be used as soil conditioners and/or organic fertilizers, plant growth substrates and biological control agents	Modified from Buchanan and Gliessman [45]; Stoffella and Kahn [46]
Compost	The solid particulate products of composting, which are extracted during the maturation and curing phase are referred to as compost.	Paulin and O'Malley [47]; Litterick and Wood [2]
Vermicompost	The process of worms digesting organic matter to transform the material into a beneficial soil amendment.	NOSB [36]
Vermicompost tea	Filtered products of vermicompost fermented in water for more than 1 h.	Modified from Litterick <i>et al.</i> [33]
Compost leachate	Liquid that has leached through a compost pile and collects on the ground, compost pad, or collection ditches, puddles, and ponds.	NOSB [36]
Compost slurry	A term used to describe non-aerated compost tea prior to filtration.	Cronin <i>et al.</i> [48]
Compost tea additives	Materials apart from compost and water that are added in the process of making compost tea, which are presumed to sustain and enrich microbial growth.	NOSB [36]
Amended extracts	These compost extracts have been fermented with the addition of specific nutrients or combined with isolated microorganisms before application.	Weltzien [49]
Manure extract	Water suspension containing raw, non-disinfected manure; when the suspension is maintained for several hours or more it is sometimes referred to as manure tea.	NOSB [36]
Suppressive compost tea	A suppressive compost tea provides or changes the environment so that the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist.	Modified from Cook and Baker [50]
Spreader	An adjuvant that reduces the surface tension of spray droplets, thus allowing them to spread evenly over leaf surfaces rather than lying in beads.	Mahaffee and Scheuerell [10]
Sticker	An adjuvant that enhances the ability of compost teas to adhere to plant surfaces.	Mahaffee and Scheuerell [10]
Protectant	An adjuvant that protects microbes from stresses mainly due to desiccation and UV light.	Mahaffee and Scheuerell [10]

particular brewing machines has been in the form of anecdotal information provided by professional landscapers and from commercial sensitive data held by private companies [2, 58]. Moreover, in the absence of scientific consensus on the qualitative and quantitative factors, which define a suppressive compost tea or predict disease suppression, further clarification on the criteria to be used to evaluate brewing machines is urgently needed. In the context of a lack of scientific consensus on standards or protocols for evaluating brewing machines, many practitioners have partly or wholly adopted the 6-point evaluation criteria proposed by Ingham [58]. The criteria are based on the ability of the equipment to: (i) maintain aerobic conditions of  $>6$  mg/l, (ii) extract a diversity of microorganisms from compost including bacteria, fungi, protozoa and beneficial nematodes, (iii) extract soluble nutrients from compost, (iv) limit the compaction of compost, which can result in inadequate aeration and the proliferation of anaerobic microorganisms, (v) maintain adequate amounts of nutrients to grow beneficial microorganisms and (vi) the ease of cleaning the equipment to prevent the build-up of biofilms, which may negatively affect compost tea quality. According to Ingham [58], the basis of this evaluation criteria is the ability of the brewing machine to consistently produce compost tea that meets desired minimal ranges of active and total population of bacterial and fungi, protozoa and nematodes, which she claims is characteristic of a suppressive compost tea. These claims by Ingham [58] are clearly biased to ACT. More so, many of these claims concerning microbial populations and diversity thresholds, aeration levels, nutrient amendment and brewing duration associated with disease suppressive compost tea have been contradicted by scientific works of St. Martin *et al.* [42, 59], Scheuerell and Mahaffee [4], and Scheuerell and Mahaffee [60]. Nonetheless, further scientific studies are needed to obtain consensus, if possible, on the characteristics of suppressive compost tea. Unlike ACT, not much focus has been placed on developing brewers to improve the suppressive efficacy of NCT or to create a more anaerobic environment for brewing compost tea. NCT are generally produced by mixing one volume of compost with 4–10 volumes of water in an open container. Initially, the mixture is stirred, then allowed to stand undisturbed at 15–20 °C for at least 3 days [49] with no or minimal stirring. To facilitate the release of microbes from the compost particles, Brinton and Droffner [3] suggested stirring NCT every 2–3 days. In contrast, CWE is usually produced by vigorously shaking compost at ratio of a 1 : 2 (v/v) for 20–30 min in distilled water or phosphate buffer (PB). The suspension is then filtered through three layers of cheesecloth and/or centrifuged at 500–3000 rpm for approx. 10 min to remove large particles.

There has been continuous debate regarding the benefits of aeration during the compost tea production process [3]. NCT have been associated with low-cost,

low-energy input, longer brewing time and many documented reports of plant disease control [49]. In contrast, ACT have been associated with shorter brewing time, higher microbial mass and diversity, higher energy requirement due to continuous aeration and lower or no phytotoxicity [1, 2, 33, 34].

### Phytotoxicity of Compost Teas

An important but seldom reported step in the evaluation of compost tea as a BCA, is phytotoxicity testing. It is evident that compost teas that are highly suppressive against phytopathogens or plant diseases but are phytotoxic have very limited practical use in crop production systems [61]. Phytotoxicity refers to the deleterious effects that compounds, e.g. nitrate, heavy metals, organic contaminants, pesticides and physico-chemical factors such as, EC, and pH, have on seed germination and plant growth or performance.

Ingham [58] claimed that NCT are likely to be more phytotoxic than ACT because anaerobes, which are more abundant in NCT, produce potentially phytotoxic compounds. Conversely, Scheuerell and Mahaffee [1] reported that no phytotoxic symptoms were observed when NCT were used as foliar sprays or potting mix drenches. They therefore concluded that there is little evidence to substantiate the claims that NCT can cause phytotoxicity. However, recent studies by Xu *et al.* [62] found that ACT and NCT were more phytotoxic than direct extracts of compost in lettuce (*Lactuca sativa* L.) and cress (*Lepidium apetalum* Willd) bioassays, and that phytotoxicity was related to the properties of pig manure and rice straw compost used. These results suggest that the phytotoxic effect was predominantly related to the brewing process rather than compost source. In contrast, St. Martin *et al.* [42] reported that NCT brewed for 56 h using banana leaf compost (BLC) or lawn clipping compost (LCC), and ACT produced from BLC brewed for 18 h, significantly reduced seed germination of sweet pepper. Concentration of copper in compost tea was identified as the most significant factor inhibiting seed germination. These results suggest that phytotoxicity was brewing time and compost type specific. Further studies aimed at evaluating the effects and relationships of brewing parameters on the phytotoxicity of compost tea are needed. Moreover, since brewing parameters influence the microbial profile of the final product, and application parameters, the survivability of the applied microorganisms and the extent to which they cover and establish themselves on plant surfaces and in growth substrates, it is essential that research reports on the use of compost tea include detailed information on these parameters. Table 2, which was adapted from Scheuerell and Mahaffee [1], details the brewing and application parameters, which should be reported in compost tea-disease management studies.

**Table 2** Brewing and application parameters that affect compost tea production and disease suppressive efficacy

Brewing parameters	
Brewing vessel	Dimensions, manufacturer and model if applicable
Compost	Producer, feedstocks, age, stability, % moisture, available nutrients, microbial analysis, either volume and bulk density used or weight
Water source	Volume, initial and final temperature
Nutrient amendments	Source, quantity and timing
Oxygen content in ppm	Include any stirring, agitation or aeration; indicate time of reading(s) during production
Brewing duration	Method of storage if not used immediately.
Application parameters	
Filtration	Material used for filtering
Dilution ratio	Water source used
Adjuncts	Nutrients, microorganisms, surfactants, stickers and protectants, e.g. UV stabilizers
Application equipment	Make, model, nozzle specifications and PSI
Application	Rate, time of day, weather and interval between applications

Adapted from Scheuerell and Mahaffee [1]

### Effect of Compost Tea on Phytopathogens and Plant Diseases

Although the experimental evidence on the effect of compost tea on plant disease is increasing, the majority of the published work has been on phytopathogen suppression *in vitro* and plant disease management in containerized-production systems under controlled environments (greenhouses and growth rooms). As such, there is a paucity of information on the effect of compost tea on plant diseases under field conditions, particularly for soil-borne diseases. Field studies on the use of compost tea to control foliar and fruit diseases are much more common, and have been conducted predominantly under sub-tropical and temperate environmental conditions. Though common in popular literature, peer-reviewed studies on the effect of compost tea on turf are also limited. Table 3–6 provide a summary of studies done on the efficacy of compost tea (ACT and NCT) in suppressing soil-borne, foliar and fruit diseases under controlled and field environments.

### Soil-Borne Phytopathogens and Diseases

Table 3 provides a summary of studies examining the use of compost tea and extract to suppress soil-borne phytopathogens and diseases under controlled environments. The majority of studies show that compost tea suppressed soil-borne pathogens and diseases. In recent *in vitro* studies, Tian and Zheng [64] found that ACT made from manure composts, and vermicasting significantly suppressed the growth of six phytopathogens including *Pythium ultimum*, *Phytophthora cryptogea* and *Sclerotinia sclerotiorum*. They proposed that phytopathogen suppression was due to antagonistic microbes, particularly *Trichoderma* spp., which were abundantly present in compost tea. In a similar study, Diáñez *et al.* [82] reported that nine fungi including *Rhizoctonia solani*, *Phytophthora parasitica* and two races of *Fusarium oxysporum* f. sp.

*lycopersici* were controlled *in vitro* using ACT made from grape marc compost. Unlike Tian and Zheng [64], they found that growth inhibition of nine of the fungi tested was the result of siderophores excreted by microorganisms present in the grape marc compost.

NCT have also been shown to suppress the *in vitro* growth of several soil-borne phytopathogens [34, 69]. Kerkeni *et al.* [79] found that NCT made from various manure and vegetable-based composts suppressed the *in vitro* growth of several phytopathogenic fungi including *Fusarium graminearum*, *Rhizoctonia bataticola* and *Fusarium solani*. Kerkeni *et al.* [79] suggested that the presence of microorganisms in NCT is a prerequisite for inhibition of the phytopathogens. A similar conclusion was reported by Sabet *et al.* [65], who used CWEs made from crop residues and domestic waste composts to suppress *in vitro* growth of *F. solani*, *P. ultimum*, *R. solani* and *Sclerotium rolfsii*.

In contrast, St. Martin *et al.* [42] reported that the general lack of significant relationships between *P. ultimum* growth inhibition and total microbial population or specific subpopulations of NCT suggest that metrics of specific chemical compounds may better explain the variation of *P. ultimum* growth inhibition. The results of numerous studies, which showed that autoclaving NCT either did not reduce [42] or slightly reduced but did not eliminate phytopathogen suppressivity [48], imply that the mechanism of control is predominantly chemical in nature rather than biological.

Neutral and negative *in vitro* effects of compost teas have also been reported [62, 79, 81]. Xu *et al.* [62] showed that neither NCT, ACT nor CWE made from pig manure–straw compost suppressed *in vitro* growth of *R. solani*. They reported that the lack of a suppressive effect might be the result of the rapid growth of *R. solani* or the inability of bacteria in NCTs, ACTs or CWEs to outcompete *R. solani* for nutrients on the potato dextrose agar medium. Similarly, Bonanomi *et al.* [81] found that extracts of dry olive residue (DOR) did not suppress the growth of *F. oxysporum* f.sp. *lycopersici*. In contrast, the

**Table 3** Summary of in vitro, greenhouse and/or container experiments examining the use of compost teas and extracts to suppress soil-borne diseases in various plant species

Brewing method	Crop	Phytopathogens	Control <sup>1</sup>	Compost type	Brewing duration	Brewing nutrients	Dilution ratio <sup>2</sup>	Reference
CWE	Pea	<i>Fusarium solani</i>	+	NR <sup>3</sup>	18 h, 40 min	Alaskan humus, worm castings, rock, phosphate dust, fish hydrolysate, Kelp, and organic Turf	NR	Curlango-Rivera <i>et al.</i> [63]
CWE	Cucumber	<i>F. solani</i>	+	NR	18 h, 40 min	Alaskan humus, worm castings, rock, phosphate dust, fish hydrolysate, Kelp, and organic Turf	NR	Curlango-Rivera <i>et al.</i> [63]
ACT	IV	<i>F. foetens</i> , <i>Rhizoctonia solani</i> , <i>Sclerotinia sclerotiorum</i> , <i>Phytophthora cryptogea</i> , <i>Pythium intermedium</i> , <i>P. ultimum</i>	+ + + + +	Pine bark, manure and vermicasting	24 h	None	NR	Tian and Zheng [64]
CWE	IV	<i>R. solani</i> <i>S. minor</i>	+ +	Tomato, escarole, artichoke-derived composts and urban compost	NR	None	1: 2 w/v	Pane <i>et al.</i> [8]
CWE	IV	<i>F. solani</i> <i>P. ultimum</i> <i>R. solani</i> <i>Sclerotium rolfsii</i>	+ + + +	Crop residues and domestic waste composts	NR	None	1: 2 w/v	Sabet <i>et al.</i> [65]
ACT	IV	<i>Pyrenochaeta lycopersici</i>	+	Plant residues composts	14 days	Whey used as a solvent	1: 5 v/v	Pane <i>et al.</i> [7]
ACT	Tomato	<i>P. lycopersici</i>	+	Plant residues composts	14 days	Whey used as a solvent	1: 5 v/v	Pane <i>et al.</i> [7]
ACT	IV	<i>F. oxysporum</i>	+	Composts made from a combination of manures, agricultural and agro-industrial wastes	48 h	None	1: 2 v/v	Suárez-Estrella <i>et al.</i> [66]
ACT	Turf grass (Creeping bentgrass)	<i>S. homoeocarpa</i>	–	Cow manure	24	0.2 % (v/v) molasses	1 : 5 v/v	Kelloway [67]
ACT	IV	<i>Pythium ultimum</i>	+	Banana leaves and lawn clippings composts	18, 24, 36 h	None	1: 17 w/v	St. Martin <i>et al.</i> [42]
NCT	IV	<i>P. ultimum</i>	+	Banana leaves and lawn clippings composts	56, 112, 168 h	None	1: 17 w/v	St. Martin <i>et al.</i> [42]
CWE	IV	<i>F. oxysporum f.sp. niveum</i> , <i>F. oxysporum f.sp. cucumerinum</i> , <i>F. oxysporum f.sp. cubense and</i> <i>F. oxysporum f.sp. melonis</i>	+ + + +	Pig manure and straw compost	NR	None	1: 8 w/w	Xu <i>et al.</i> [62]
ACT	IV	<i>F. oxysporum f.sp. niveum</i> , <i>F. oxysporum f.sp. cucumerinum</i> , <i>F. oxysporum f.sp. cubense and</i> <i>F. oxysporum f.sp. melonis</i>	+ + + +	Pig manure and straw compost	NR	None	1: 8 w/w	Xu <i>et al.</i> [62]
NCT	IV	<i>R. solani</i> <i>F. oxysporum f.sp. niveum</i> , <i>F. oxysporum f.sp.</i> <i>cucumerinum</i> , <i>F. oxysporum f.sp.</i> <i>cubense and F. oxysporum f.sp.</i> <i>melonis</i> <i>R. solani</i>	– + + + + –	Pig manure and straw compost	NR	None	1: 8 w/w	Xu <i>et al.</i> [62]

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Table 3 (Continued)

Brewing method	Crop	Phytopathogens	Control <sup>1</sup>	Compost type	Brewing duration	Brewing nutrients	Dilution ratio <sup>2</sup>	Reference
NCT	IV	<i>Lecanicillium fungicola</i>	+	spent mushroom compost	18–20 days	None	1 : 2 v/v	Riahi <i>et al.</i> [68]
ACT	IV	<i>Phytophthora citrophthora</i>	+	Dog food	1, 2, 3, 5 days	None	1 : 10 v/v	Tateda <i>et al.</i> [69]
		<i>F. oxysporum</i>	+					
		<i>Fusarium sp.</i>	+					
		<i>Sclerotinia sp.</i>	+					
		<i>R. solani</i>	+					
		<i>P. cactorum</i>	+					
		<i>Pythium sylvaticum</i>	+					
		<i>F. proliferatum</i>	+					
		<i>Colletotrichum dematium</i>	+					
NCT	IV	<i>Phytophthora. Citrophthora</i>	+	Dog food	1 and 2 weeks	None	1 : 10 v/v	Tateda <i>et al.</i> [69]
		<i>F. oxysporum</i>	+					
		<i>Fusarium sp.</i>	+					
		<i>Sclerotinia sp.</i>	+					
		<i>R. solani</i>	+					
		<i>P. cactorum</i>	+					
		<i>Pythium sylvaticum</i>	+					
		<i>F. proliferatum</i>	+					
		<i>C. dematium</i>	+					
CWE	IV	<i>F. oxysporum f.sp. lycopersici</i> (Fol),	+	Olive mill waste	NR	None	1 : 2 w/v	Alfano <i>et al.</i> [70]
		<i>P. ultimum</i> ,	+					
		<i>S. sclerotiorum</i>	+					
		<i>Verticillium dahliae</i>	+					
CWE	IV	<i>Colletotrichum coccodes</i>	+	Pig and cow manure, and sawdust compost	NR	None	1 : 5 w/v	Sang and Kim [6]
		<i>Choanephora cucarumrbit</i>	+	Poultry manure and sawdust compost				
CWE	Pepper	<i>Collectotrichum coccodes</i>	+	Pig and cow manure, and sawdust compost	NR	None	1 : 5 w/v	Sang and Kim [6]
				Poultry manure and sawdust compost				
CWE	Cucumber	<i>C. cucarumrbit</i>	+	Pig and cow manure, and sawdust compost	NR	None	1 : 5 w/v	Sang and Kim [6]
				Poultry manure and sawdust compost				
CWE	IV	<i>Phytophthora capsici</i>	+	Pig and cow manure, and sawdust compost	30 min	None	1 : 5 w/v	Sang <i>et al.</i> [71]
				Poultry manure and sawdust compost				
CWE	Pepper	<i>P. capsici</i>	+	Pig and cow manure, and sawdust compost	30 min	None	1 : 5 w/v	Sang <i>et al.</i> [71]
				Poultry manure and sawdust compost				
ACT	Okra	<i>C. cucurbitarum</i>	+	Rice straw and empty fruit bunch of oil palm composts	12 days	None	1 : 5 w/v	Siddiqui <i>et al.</i> [72]
NCT	Tomato	<i>Pythium aphanidermatum</i>	+	Solid olive mill wastes (SOMW), <i>Posidonia oceanica</i> (Po) and chicken manure (CM),	6 days	None	1 : 5 v/v	Jenana <i>et al.</i> [73]
NCT	IV	<i>Armillaria mellea</i>	+	Green waste	14	None	1 : 1 v/v	Egwunatum and Lane [74]

NCT	Beech plants	<i>A. mellea</i>	+	Green waste	14	None	1:1 v/v	Egwunatum and Lane [74]
NCT	Beech timber	<i>A. mellea</i>	+	Green waste	14	None	1:1 v/v	Egwunatum and Lane [74]
NCT	IV	<i>Lecanicillium fungicola</i>	+	Spent mushroom substrate, olive Oil husk + cotton gin trash composted and mixed with rice husk, Grape marc compost and cork compost	1, 7 and 14 days	None	1:4 and 1:8 (w/v)	Gea <i>et al.</i> [75]
ACT	Okra	<i>C. cucurbitarum</i>	+	Rice straw and empty fruit bunch of oil palm composts	12 days	<i>Trichoderma</i> enriched	NR <sup>3</sup>	Siddiqui <i>et al.</i> [76]
ACT	IV	<i>S. rolfsii</i>	+	Municipal sewage sludge and yard waste compost	NR	None	1:2 w/w	Zmora-Nahum <i>et al.</i> [77]
ACT	IV	<i>P. ultimum</i> <i>P. capsici</i>	+	Two-phase olive mill waste	NR	None	1:10, 1:20 and 1:50 (w:v)	Cayuela <i>et al.</i> [78]
NCT	IV	<i>F. oxysporum</i> f. sp. <i>Radices-lycopersici</i> , <i>F. solani</i> , <i>Fusarium graminearum</i> , <i>S. sclerotiorum</i> , <i>R. solani</i> , <i>Rhizoctonia bataticola</i> , <i>Pythium</i> sp., <i>V. dahliae</i> , <i>C. coccodes</i> , <i>Aspergillus niger</i>	+	Cattle manure, sheep manure, vegetable based, Ground straw	5 days	None	1:5 v/v	Kerkeni <i>et al.</i> [79]
ACT	IV	<i>Sclerotinia homoeocarpa</i>	+	Turkey, mushroom, and cattle, sheep, Topdressing composts	8 days	None	1:2, 1:3 and 1:5	Hsiang and Tian [80]
Water extract	IV	<i>F. oxysporum</i> <i>Fusarium culmorum</i> <i>S. minor</i> <i>R. solani</i>	—	Olive mill residues	NR	None	5, 1.5, and 0.5%	Bonanomi <i>et al.</i> [81]
ACT	IV	<i>R. solani</i> , <i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i> , <i>F. oxysporum</i> f. sp. <i>lycopersici</i> race 0, <i>F. oxysporum</i> f. sp. <i>lycopersici</i> race 1, <i>F. oxysporum</i> f. sp. <i>Radicis-cucumerinum</i> , <i>P. aphanidermatum</i> , <i>Phytophthora parasitica</i> , <i>V. dahliae</i> <i>V. fungicola</i> <i>P. ultimum</i>	+	Grape marc compost	24 h	None	1:10 w/v	Diáñez <i>et al.</i> [82]
ACT	Cucumber	<i>P. ultimum</i>	+	Yard trimmings, mixed vegetation (vermicompost), Vegetative and animal manure-based composts	36 h for ACT	Kelp and humic acid Bacterial or fungal additive	1:30 w/v	Scheuerell and Mahaffee [4]
NCT	Cucumber	<i>P. ultimum</i>	+	Yard trimmings, mixed vegetation (vermicompost), Vegetative and animal manure-based composts	7–9 days for NCT	Kelp and humic acid Bacterial or fungal additive	1:30 w/v	Scheuerell and Mahaffee [4]

Table 3 (Continued)

Brewing method	Crop	Phytopathogens	Control <sup>1</sup>	Compost type	Brewing duration	Brewing nutrients	Dilution ratio <sup>2</sup>	Reference
CWE	IV	<i>Plythium debaryanum</i> , <i>F. oxysporum f.sp. lycopersici</i> , <i>Sclerotium bataticola</i>	+	Leafy fruit compost, garden compost, and crops compost	NR	None	1:2 w/v	El-Masry <i>et al.</i> [83]
CWE	Sweet pepper	<i>F. oxysporum f.sp. vasinfectum</i>	+	Pig, horse and cow manures	NR	None	NR	Ma <i>et al.</i> [84]
ACT	IV	<i>F. oxysporum</i>	+	Cattle manure vermicompost	2 days	None	1:2 v/v	Szczzech [85]
NCT	IV	<i>F. oxysporum</i>	+	Cattle manure vermicompost	7 or 14 days	None	1:2 v/v	Szczzech [85]
NCT	Cucumber	<i>C. orbiculare</i>	+	Composted pine bark mix, fortified with the <i>Trichoderma hamatum</i> 382 and <i>Flavobacterium balustinum</i>	7 days	None	1:1 v/v	Zhang <i>et al.</i> [86]
NCT	Pea	<i>P. ultimum</i>	+	Cattle manure or grape marc	5–10 days	None	NR	Trankner [87]
NCT	IV	<i>R. solani</i>	+	NR	NR	None	NR	Weltzien [88]

Notes: ACT, aerated compost tea; NCT, non-aerated compost tea; CWE, compost water extract; IV, *in vitro*.

<sup>1</sup>Control: + treatments statistically less disease (minimum  $P=0.05$ ) than control treatment; – treatment no difference from control treatment.

<sup>2</sup>Dilution ratio is expressed as compost/water.

<sup>3</sup>NR – not reported.

diametric growth rate of *Sclerotinia minor* had a positive relationship with DOR extract concentration rate.

Data from studies by Pane *et al.* [8] and Sabet *et al.* [65] showed that the level or even evidence of suppressiveness of compost tea against phytopathogens could be dependent on the type of *in vitro* antagonistic assays used. Pane *et al.* [8] found that all raw (unsterilized) CWEs inhibited *in vitro* growth of *R. solani* and *S. minor* in pouring plate and well-cut diffusion *in vitro* antagonistic assays. However, filter sterilized extracts inhibited the *in vitro* growth of only *S. minor*, and only when they were applied according to pouring method. Conversely, in well-cut diffusion assays, *R. solani* and *S. minor* treated with filter sterilized CWEs showed regular development not significantly different from that observed in control plates. Even with raw CWEs, mean *S. minor* suppression levels achieved in the well-cut diffusion assay ranged from 20.2 to 36.3% compared to 1.5–9.7% in the pouring-plate method. Similar inconsistencies in qualitative and quantitative measures of growth suppression of several phytopathogens across three types of antagonistic assays were reported by Sabet *et al.* [65].

As is evident from these findings, *in vitro* screening assays are not stand-alone tests to evaluate or predict the effectiveness of compost tea for plant disease control. This is because key elements and dynamics of a pathosystem including biota (plant and microorganisms), abiotic factors, and the interactions between biota and abiotic (ecological) factors are either absent or not appropriately represented or accounted for in *in vitro* assays. For instance, the use of a particular growth medium in *in vitro* plate challenge assays may allow for the proliferation of faster growing species of bacteria. This results in a gross misrepresentation of the complexity of microbial populations and possible interactions with pathogens. As such, though useful, results of pathogen inhibition obtained from *in vitro* assays must be interpreted with caution.

Table 4 provides a summary of studies examining the use of compost tea and extract to suppress soil-borne diseases under field conditions. Most *in vivo* and field trials have been done using tomato, pea, cucumber and potato (Tables 4 and 5). The theoretical basis for effectiveness of compost tea in controlling soil-borne diseases is argued to be its ability to alter the microbiota of the rhizosphere and/or plant growth substrate as a whole. However, it is highly debatable whether compost tea alters the microbiota of plant growth substrates as a whole [60, 92, 119]. Nonetheless, there is evidence that compost tea applied as a soil drench do have some plant disease suppressive effect. This means that at least one member of the plant growth substrate microbial community, i.e. the pathogen, is affected by the application of compost tea. In field trials, Kelloway [67] found that the efficacy of the mink compost tea (M-CT) in controlling *Sclerotinia homoeocarpa*, the causal agent of dollar spot disease, was site-specific and variable with only one location showing significant

**Table 4** Summary of field experiments examining the use of compost teas and extracts to suppress soil-borne diseases in various plant species

Brewing method	Crop	Phytopathogens	Disease	Control <sup>1</sup>	Compost type	Brewing duration	Brewing nutrients	Dilution ratio <sup>2</sup>	Reference
NCT	Eggplant	<i>Ralstonia solanacearum</i>	Bacterial wilt	+	NR <sup>3</sup>	7 days	Molasses	1:5 w/v	Islam <i>et al.</i> [89]
NCT	Potato	<i>Rhizoctonia solani</i>	Stem canker	+	NR	7 days	Molasses	1:5 w/v	Islam <i>et al.</i> [90]
ACT	Turf grass (creeping bentgrass)	<i>Sclerotinia homoeocarpa</i>	Dollar spot	+ –	Mink compost	24 h	0.2 % (v/v) molasses	1:5 v/v	Kelloway [67]
ACT	Apple	Complex of pathogens and soil factors	Apple replant disease	+	Wheat straw – chicken-cattle manure compost.	24 h	None	1:10 v/v	Van Schoor <i>et al.</i> [5]
NCT	French bean	<i>R. solani</i>	Root rot	+	Farmyard manure, poultry manure, vermicompost, spent mushroom, and <i>Lantana</i> and <i>urtica</i> composts	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
ACT	Irish potato	<i>R. solani</i>	Stem canker	+	Vermicompost made of crop residues, composted horse manure, paper and straw	24 h	Clay, blue-green algae, sugar, yeast, and kelp	NR	Larkin [92]
ACT	Irish potato	<i>Streptomyces scabiei</i>	Common scab	+	Vermicompost made of crop residues, composted horse manure, paper and straw	24 h	Clay, blue-green algae, sugar, yeast, and kelp	NR	Larkin [92]
ACT	Rice	<i>Fusarium moniliforme</i>	Foot rot	+	waste used to make compost was not specified	3 days	None	1:6 v/v	Manandhar and Yami [93]
NCT	Rice	<i>F. moniliforme</i>	Foot rot	+	Waste used to make compost was not specified	7–10 days	None	1:6 v/v	Manandhar and Yami [93]
AVCT	Rice	<i>F. moniliforme</i>	Foot rot	+	Fruit/vegetable wastes vermicompost	3 days	None	1:6 v/v	Manandhar and Yami [93]
NVCT	Rice	<i>F. moniliforme</i>	Foot rot	+	Fruit/vegetable wastes vermicompost	7–10 days	None	1:6 v/v	Manandhar and Yami [93]
ACT	Bentgrass	<i>S. homoeocarpa</i>	Dollar spot	+	Turkey, mushroom, and cattle, sheep and topdressing composts	7 days	None	1:2, 1:3 and 1:5	Hsiang and Tian [80]

Notes: ACT, aerated compost tea; NCT, non-aerated compost tea; AVCT, aerated vermicompost tea; NVCT, non-aerated vermicompost tea; CWE, compost water extract, IV, *in vitro*.

<sup>1</sup>Control: + treatments statistically less disease (minimum  $P=0.05$ ) than control treatment; – treatment no difference from control treatment.

<sup>2</sup>Dilution ratio is expressed as compost/water.

<sup>3</sup>NR, not reported.

**Table 5** Summary of *in vitro*, greenhouse and/or container experiments examining the use of compost teas and extracts to suppress foliar and fruit pathogens and diseases in various plant species

Brewing method	Crop	Phytopathogens	Control <sup>1</sup>	Compost type	Brewing duration	Brewing nutrients	Dilution ratio <sup>2</sup>	Reference
ACT	Blueberry	<i>Monilinia vaccinii-corymbosi</i>	–	Fish-farm compost	2–3 days	None	1:3 w/v	McGovern <i>et al.</i> [94]
ACT	Blueberry	<i>M. vaccinii-corymbosi</i>	–	Lobster compost	2–3 days	None	1:3 w/v	McGovern <i>et al.</i> [94]
NCT	Blueberry	<i>M. vaccinii-corymbosi</i>	–	Fish-farm compost	6–8 days	None	1:3 w/v	McGovern <i>et al.</i> [94]
NCT	Blueberry	<i>M. vaccinii-corymbosi</i>	–	Lobster composts	6–8 days	None	1:3 w/v	McGovern <i>et al.</i> [94]
ACT	IV	<i>Golovinomyces cichoracearum</i>	+	Empty fruit bunches and palm oil mill effluent composts	3 days	Yeast extract and humic acid microbial starter	1:5 v/v	Naidu <i>et al.</i> [95]
ACT	Melon	<i>G. cichoracearum</i>	+	Empty fruit bunches and palm oil mill effluent composts	3 days	Yeast extract and humic acid microbial starter	1:5 v/v	Naidu <i>et al.</i> [95]
ACT	IV	<i>Alternaria alternata</i> <i>Botrytis cinerea</i>	+ +	Plant residues composts	14 days	Whey used as a solvent	1:5 v/v	Pane <i>et al.</i> [7]
ACT	Tomato	<i>A. alternata</i> , <i>B. cinerea</i>	+ +	Plant residues composts	14 days	Whey used as a solvent	1:5 v/v	Pane <i>et al.</i> [7]
ACT	IV	<i>Pyricularia oryzae</i> <i>Botrytis tulipae</i> <i>Podosphaera xanthii</i>	+ + +	Dog food	1, 2, 3, or 5 days	None	1:10 v/v	Tateda <i>et al.</i> [69]
NCT	IV	<i>P. Oryzae</i> <i>B. tulipae</i> <i>P. xanthii</i>	+ + +	Dog food	1 and 2 weeks	None	1:10 v/v	Tateda <i>et al.</i> [69]
ACT	Cucumber	<i>P. xanthii</i>	+	Dog food	1 day	None	1:10 v/v	Tateda <i>et al.</i> [69]
CWE	IV	<i>Phytophthora infestans</i>	+	Olive mill waste	NR <sup>3</sup>	None	1:2 w/v	Alfano <i>et al.</i> [70]
NCT	IV	<i>P. infestans</i>	+	Chicken manure, sheep manure (four sources; SM1–SM4), bovine manure, shrimp powder, or seaweed	14 days	None	1:5 v/v	Koné <i>et al.</i> [96]
NCT	Tobacco	<i>Cucumber mosaic virus</i>	+	Market vegetable wastes	7–8 days	Use of effective microorganisms, <i>Lactobacillus Streptomyces</i> , phosphate-solubilized bacteria and yeast species, and <i>Pseudomonas aeruginosa</i>	1:3 w/v	Wahyuni <i>et al.</i> [97]
ACT	Tomato	<i>Erysiphe polygoni</i>	+	Market, urban and garden wastes compost	7 days	None	1:5 v/v	Segarra <i>et al.</i> [98]
ACT	IV	<i>B. cinerea</i>	+	two-phase olive mill waste	NR	None	1:10, 1:20 and 1:50 (w/v)	Cayuela <i>et al.</i> [78]
NCT	IV	<i>Alternaria</i> spp. <i>B. cinerea</i> <i>Phomopsis amygdali</i>	+ + +	Cattle manure, sheep manure, vegetable based, ground straw	5 days	None	1:5 v/v	Kerkeni <i>et al.</i> [79]
WE	IV	<i>B. cinerea</i>	–	Olive mill residues (not compost)	NR	NR	5, 1.5, and 0.5%	Bonanomi <i>et al.</i> [81]
ACT NCT	Geranium	<i>B. cinerea</i>	+	30 different types of compost	34–36 h 7–14 days	Either 0.3% molasses, 0.3% (w/v) hydrolysed yeast powder, 0.5%(v/v) bacterial nutrient solution, proprietary Blend of molasses, kelp and trace materials, or fungal nutrient mixture (1.2 g of powdered soluble kelp, 2.5 ml of liquid humic acids and 3 g of rock dust	1:25 v/v 1:5 v/v	Scheuerell and Mahaffee [60]

NCT	Tomato	<i>Xanthomonas vesicatoria</i>	+	Sawdust-bedded composted cow manure, pine bark, and cow manure and yard waste composts	7 days	None	1:5 v/v	Al-Dahmani <i>et al.</i> [41]
NCT	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> pv. <i>maculicola</i>	+	Composted pine bark mix, fortified with the <i>Trichoderma hamatum</i> 382 and <i>Flavobacterium balustinum</i>	7 days	None	1:1 v/v	Zhang <i>et al.</i> [86]
NCT	IV	<i>B. cinerea</i>	+	Manure-straw mixtures compost	3, 5, 8, 12 or 18 days	None	1:5 v/v	McQuilken <i>et al.</i> [99]
NCT	Lettuce	<i>B. cinerea</i>	+	Manure-straw mixtures compost	3, 5, 8, 12 or 18 days	None	1:5 v/v	McQuilken <i>et al.</i> [99]
NCT	Tomato	<i>B. cinerea</i>	+	Cattle and chicken-cattle manure, and grape marc composts	4 h, 7 days, 14 days.	Nutrient broth (Difco)	1:5 v/v	Elad and Shtienberg [100]
NCT	Tomato	<i>Leveillula taurica</i>	+	Cow manure	7 days	None		Miller <i>et al.</i> [101]
NCT	IV	<i>Xanthomonas campestris</i>	+	Spent mushroom substrates	7–8 days	None	1:2 w/v	Yohalem <i>et al.</i> [102]
NCT	Grape	<i>Venturia inaequalis</i>	+	animal manure (horse, pig, goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Grape	<i>Plasmopara viticola</i>	+	animal manure (horse, pig, goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Grape	<i>Uncinula necator</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Barley	<i>Erysiphe graminis f. sp. hordei</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Sugar beet	<i>Erysiphe betae</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Cucumber	<i>Sphaerotheca fuliginea</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Bean	<i>S. fuliginea</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Strawberry	<i>B. cinerea</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Yohalem <i>et al.</i> [102]
NCT	Potato	<i>P. infestans</i>	+	NR	NR	None	NR	Jongebloed <i>et al.</i> [103]
NCT	Tomato	<i>P. infestans</i>	+	NR	7–14 days	None	NR	Ketterer and Schwager [104]
NCT	Tomato	<i>P. infestans</i>	+	Horse-straw-soil	14 days	None		Ketterer [105]
NCT	Potato, Tomato	<i>P. infestans</i>	+	Animal manure (horse, pig and goat) with cereal straw and amendments of top soil	7, 14 days	None	1:5–1:8 v/v	Ketterer [105]

Notes: ACT, aerated compost tea; NCT, non-aerated compost tea; CWE, compost water extract; WE, water extract; IV, *in vitro*.

<sup>1</sup>Control: + treatments statistically less disease (minimum  $P=0.05$ ) than control treatment; – treatment no difference from control treatment.

<sup>2</sup>Dilution ratio is expressed as compost/water.

<sup>3</sup>NR – not reported.

**Table 6** Summary of field experiments examining the use of compost teas and extracts to suppress foliar and fruit diseases in various plant species

Brewing method	Crop	Phytopathogens	Diseases	Control <sup>1</sup>	Compost type	Brewing duration	Brewing nutrients	Dilution ratio <sup>2</sup>	Reference
ACT	Grape	<i>Botrytis cinerea</i>	Botrytis bunch rot	+	Cow or chicken manure and timber residues	48 h	None	1:3 w/v	Evans <i>et al.</i> [9]
ACT	Grape	<i>Erysiphe necator</i>	Powdery mildew	+	Cow or chicken manure and timber residues	48 h	None	1:3 w/v	Evans <i>et al.</i> [9]
NCT	Tomato	<i>Phytophthora infestans</i>	Late blight	+	NR <sup>3</sup>	7 days	Molasses	1:5 w/v	Islam <i>et al.</i> [106]
NCT	Potato	<i>P. infestans</i>	Late blight	+	NR	7 days	Molasses	1:5 w/v	Islam <i>et al.</i> [106]
NCT	Tomato	<i>Alternaria solani</i>	Early blight	+	NR	7 days	Molasses	1:5 w/v	Kabir <i>et al.</i> [107]
ACT	Blueberry	<i>Monilinia vaccinii-corymbosi</i>	Mummy berry	–	Fish and Farm and Lobster composts	2–3 days	None	1:3 w/v	McGovern <i>et al.</i> [94]
NCT	Blueberry	<i>M. vaccinii-corymbosi</i>	Mummy berry	–	Fish and Farm and Lobster composts	6–8 days	None	1:3 w/v	McGovern <i>et al.</i> [94]
NCT	Potato	Not specified		+	Kitchen waste	24 h	None	1:20 w/v	Albert <i>et al.</i> [108]
NCT	French bean	<i>Phaeoisariopsis griseola</i>	Angular leaf spot	+	Farmyard manure compost	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
NCT	French bean	<i>P. griseola</i>	Angular leaf spot	+	Poultry manure compost	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
NCT	French bean	<i>P. griseola</i>	Angular leaf spot	+	Vermicompost	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
NCT	French bean	<i>P. griseola</i>	Angular leaf spot	+	Spent mushroom compost	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
NCT	French bean	<i>P. griseola</i>	Angular leaf spot	+	<i>Lantana camara</i> composts	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
NCT	French bean	<i>P. griseola</i>	Angular leaf spot	+	<i>Urtica</i> spp. composts	10 days	None	1:5 v/v	Joshi <i>et al.</i> [91]
ACT	Strawberry	<i>B. cinerea</i>	Grey mould	+	Cattle and chicken manure composts	7 days	None	1:4 v/v 1:8 v/v	Welke [109]
NCT	Strawberry	<i>B. cinerea</i>	Grey mould	+	Cattle and chicken manure composts	7 days	None	1:4 v/v 1:8 v/v	Welke [109]
NCT	Tomato	<i>Xanthomonas vesicatoria</i>	Bacterial spot	–	Sawdust-bedded composted cow manure	7 days	None	1:5 v/v	Al-Dahmani <i>et al.</i> [41]
NCT	Tomato	<i>X. vesicatoria</i>	Bacterial spot	–	Composted pine bark	7 days	None	1:5 v/v	Al-Dahmani <i>et al.</i> [41]
NCT	Tomato	<i>X. vesicatoria</i>	Bacterial spot	–	Cow manure and yard waste composts	7 days	None	1:5 v/v	Al-Dahmani <i>et al.</i> [41]
NCT	Tomato	<i>X. vesicatoria</i>	Bacterial spot	–	Yard waste compost	7 days	None	1:5 v/v	Al-Dahmani <i>et al.</i> [41]
ACT	Rose	<i>Sphaerotheca pannosa</i>	Powdery mildew	+	Chicken manure Yard debris Mixed source Chicken manure Yard debris	24 h	0.3% molasses in all teas.	NR	Scheuerell and Mahaffee [110]
NCT	Rose	<i>S. pannosa</i>	Powdery mildew	+	Chicken manure Yard debris Mixed source Chicken manure Yard debris Mixed source	7–11 days	0.3% molasses in all teas.	NR	Scheuerell and Mahaffee [110]
NCT	Strawberry	<i>B. cinerea</i>	Grey mould	–	Cattle manure compost	7–21 days	None	1:4, 1:8 – units were not reported	Welke [111]
NCT	Strawberry	<i>B. cinerea</i>	Grey mould	–	Chicken manure compost	7–21 days	None	1:4, 1:8 – units were not reported	Welke [111]
NCT	Tomato	<i>A. solani</i>	Early blight	+	Cattle manure	7, 14 days	None	1:5 v/v	Tsrar [112]

ACT	Tomato	<i>A. solani</i>	Early blight		NR	24 h	1.25% molasses and rock flour	NR	Granatstein [113]
ACT	Apple	<i>Venturia inaequalis</i>	Apple scab	–	NR	24 h	1.25% molasses and rock flour	NR	Granatstein [113]
NCT	Tomato	<i>Xanthomonas campestris</i>	Bacterial leaf spot	–	Cow manure	7 days	None		Miller et al. [101]
NCT	Apple	<i>V. inaequalis</i>	Apple scab	+	Spent mushroom substrates	7–8 days	None	1:2 w/v	Yohalem et al. [114]
ACT	Cherry	<i>Blumeriella jaapii</i>	Cherry leaf spot	–	Compost type used not reported in study	24 h	0.5% molasses rock dust	NR	Pscheidt and Wittig [115]
ACT	Cherry	<i>Monilinia laxa</i>	Brown rot blossom blight	+	Compost type used not reported in study	24 h	0.5% molasses rock dust	NR	Pscheidt and Wittig [115]
ACT	Apple	<i>Podosphaera leucotricha</i>	Powdery mildew	–	Compost type used not reported in study	24 h	0.5% molasses rock dust	–	Pscheidt and Wittig [115]
ACT	Apple	<i>V. inaequalis</i>	Apple scab	–	Compost type used not reported in study	24 h	0.5% molasses rock dust	–	Pscheidt and Wittig [115]
ACT	Peach	<i>Taphrina deformans</i>	Peach leaf curl	–	Compost type used not reported in study	24 h	0.5% molasses rock dust	–	Pscheidt and Wittig [115]
ACT	Peach	<i>Venturia pirina</i>	Peach scab	–	Compost type used not reported in study	24 h	0.5% molasses rock dust	–	Pscheidt and Wittig [115]
NCT	Grape	<i>Uncinula necator</i>	Powdery mildew	+	Cattle manure Horse manure Horse manure	3 days for all NCT	No nutrients added in any NCT	–	Sackenheim et al. [116]
NCT	Potato	<i>P. infestans</i>	Potato blight	–	NR	NR	None	NR	Jongebloed et al. [103]
NCT	Apple	<i>V. inaequalis</i>	Apple scab	–	Spent mushroom Cattle manure Horse manure	7 days	None	NR	Andrews [117]
NCT	Grape berries	<i>B. cinerea</i>	Grey mould	+	Horse-straw-soil	2 and 4 months	None	NR	Ketterer [105]
NCT	Potato	<i>P. infestans</i>	Potato blight	–	horse-straw-soil compost	7 days	Adding pure culture of microbial antagonists to tea just before spraying.	NR	Ketterer [105]
NCT	Grape	<i>Plasmopara viticola</i>	Downey mildew	+	Horse-straw-soil	3 days	Pure cultures of microbial antagonists	–	Ketterer [105]
NCT	Strawberry	<i>B. cinerea</i>	Grey mould	+	Cattle manure compost Horse manure	16 days 12 weeks	None	–	Stindt [118]
NCT	Grape	<i>Pseudopeziza tracheiphila</i>	Brenner Rot	+	Horse-straw-soil	3 days	None	1:5–1:8 (units not reported)	Weltzien [88]

Notes: ACT, aerated compost tea, NCT, non-aerated compost tea, AVCT, aerated vermicompost tea, NVCT, non-aerated vermicompost tea, CWE, compost water extract, IV, *in vitro*.

<sup>1</sup>Control: + treatments statistically less disease (minimum  $P=0.05$ ) than control treatment; – treatment no difference from control treatment.

<sup>2</sup>Dilution ratio is expressed as compost/water.

<sup>3</sup>NR – not reported

control. M-CT did not alter the soil composition in terms of microbes or nutrients within the soil and there was no significant difference in dollar spot severity among infected treatments applied with cow manure compost tea or water (control), in *in vivo* studies. In contrast, Larkin [92] found that both crop rotation and biological amendments including ACT, significantly affected soil microbial community characteristics, but crop rotation effects were more dominant. Similarly, Fritz *et al.* [119] reported that minor changes to the soil microbial community occurred following foliar application of vermicompost tea, in both the laboratory and field-scale experiments.

The pioneering work by Scheuerell and Mahaffee [4] showed that the development of *Pythium* damping-off of cucumber grown in soil-less media was significantly reduced by ACT and NCT, with ACT fermented with kelp and humic acid nutrients displaying the most consistent disease suppression. Ma *et al.* [120] and Ma *et al.* [84] reported effective control of *Fusarium* wilt of greenhouse grown cucumber (*F. oxysporum* f. sp. *cucumerinum*) and sweet pepper (*F. oxysporum* f. sp. *vasinfectum*) using drench applications of NCT made from pig, horse and cow manures. They found that NCT had a mycolytic effect on *Fusarium* chlamydospores and microspores, which suggested that disease suppression was achieved through the destruction of the propagules of the pathogen. Sang *et al.* [71] showed that when applied as a root-drench, CWE made from manure and sawdust-based composts suppressed growth and activity of *Phytophthora capsici* in *in vitro* and *in vivo* trials. Moreover, these suppressions might result from direct inhibition of development and population of *P. capsici* for root infection, as well as indirect inhibition of foliar infection through induced systemic resistance (ISR) with broad-spectrum protection. Egwunatum and Lane [74] reported that NCT inhibited *in vitro* growth of *Armillaria mellea* and slowed rather than prevented the development of wilt symptoms in beech timber. They suggested changes in microbial activity and pH as factors related to *A. mellea* and root-rot disease suppression.

The results from these studies (Tables 3 and 4) indicate that in most cases compost tea applied as soil or root drench is useful in managing soil-borne diseases, particularly under soil-less and controlled environments. Further studies are needed to evaluate the disease suppressivity of compost tea under field conditions for various crop species. Moreover, investigations aimed at improving the disease suppressive consistency and predictability of compost tea are needed.

### Foliar and Fruit Phytopathogens and Diseases

Table 5 provides a summary of studies examining the use of compost tea and extract to suppress foliar and fruit phytopathogens and diseases under controlled environments. The majority of studies show that compost tea

suppressed aerial (foliar and fruit) plant pathogens and diseases. For example, in *in vitro* trials, Cayuela *et al.* [78] found ACT made from olive mill waste compost significantly inhibited the growth of *Botrytis cinerea* and suggested that inhibition was associated with micro-biologically based phenomena. In a similar *in vitro* study, Naidu *et al.* [95] reported both microbial-enriched and non-enriched ACT made from empty fruit bunches and palm oil mill effluent significantly reduced the conidial germination of *Golovinomyces cichoracearum*, a causal agent of powdery mildew on melon. Moreover, after 48 h of co-incubation, conidia in microbial-enriched ACT treatment appeared ruptured, which contributed to a significantly higher inhibition of conidial germination than non-enriched ACT, increased cell permeability, and leakage of cellular contents.

NCT have also been shown to suppress the *in vitro* growth of several aerial pathogens. Koné *et al.* [96] demonstrated that *Phytophthora infestans* can be controlled *in vitro* by NCT made from manure-based and shrimp powder or seaweed composts. Kerkeni *et al.* [79] found that NCT made from various manures and vegetable-based composts inhibited the growth of *Alternaria* spp., *B. cinerea* and *Phomopsis amygdali*. Likewise, Tateda *et al.* [69] demonstrated that NCT made from dog food (Aijo-Monogatari Beef taste, Yeaster, Japan) were effective in controlling the *in vitro* growth of *Pyricularia oryzae* and *Botrytis tulipae*. Both Kerkeni *et al.* [79] and Tateda *et al.* [69] purported that the microbes in NCT possibly played critical roles in the suppression of the phytopathogens. A similar postulation was provided by Koné *et al.* [96], although they reported that the overall relative efficacy of the various NCT did not correlate well with microbial communities or physico-chemical composition of the prepared NCT. Rather, results indicated that specific microorganisms from NCT made from various sheep manure composts were more important in the suppressive effect than the high total count of unspecified bacteria.

Though to a lesser extent, there have been reports which showed that compost teas failed to suppress the *in vitro* growth of some aerial phytopathogens. However, possible explanations for these negative results are often not provided or are vague. In contrast, Bonanomi *et al.* [81] reported that water extracts of DOR did not suppress the *in vitro* growth of *B. cinerea* but postulated that the presence of undecomposed DOR might have provided energy and nutrients for the growth of the phytopathogen.

Reports on the failure of compost tea to suppress aerial plant diseases are more common in field rather than *in vitro* studies. A possible reason, which relates to the dynamic nature of field environment, was explained in the previous subsection. Table 6 provides a summary of studies examining the use of compost tea and extracts to suppress foliar and fruit phytopathogens and diseases under field conditions.

The pioneering works of Stindt [118] and Samerski and Weltzien [121] suggested that the theoretical basis for effectiveness of compost tea in controlling aerial plant diseases is its ability to alter the microbiota of the phyllosphere and to induce resistance in plant hosts. To this end, McGovern *et al.* [94] reported that neither NCT nor ACT made from fish and farm, and lobster composts suppressed mummy berry disease in blueberries, which is caused by *Monilinia vaccinii-corymbosi*. They found that NCT did not affect the number of fungal and bacterial colony-forming units (CFU) on leaves compared to the controls. Although there is no consensus on what levels of bacterial or fungal populations are required on leaves for disease suppression [33, 49], McGovern *et al.* [94] concluded that fungal or bacterial CFU levels of leaves treated with NCT might not have been high enough for effective competition or inhibition of *M. vaccinii-corymbosi*. Sturz *et al.* [122] reported similar results with the use of a commercial ACT (Jolly Farmer, New Brunswick, Canada) for the control of potato late blight (*P. infestans*). Likewise, Plotkin [123] reported that ACT made from a commercial vermicompost (Microbial Magic, WA, USA) did not suppress *Alternaria* spp. blight or *Septoria* spp. leaf spot disease in tomato. Al-Dahmani *et al.* [41] found that NCT made from sawdust-bedded composted cow manure, pine bark, cow manure or yard waste composts were ineffective in the field against the foliar phase of the bacterial spot (*Xanthomonas vesicatoria*). However, a significant reduction of bacterial spot on fruit resulted from weekly and biweekly spray applications of NCT on plots not amended with compost. Al-Dahmani *et al.* [41] postulated that NCT possibly enhanced systemic resistance of host plants and that the filterable and heat-stable components in NCT played a role in disease suppression efficacy. Although executed using various crops, experimental protocols and under different environmental conditions, McGovern *et al.* [94], Sturz *et al.* [122], Plotkin [123] and Al-Dahmani *et al.* [41] all concluded that the use of compost teas to control diseases appears too unreliable to be agronomically effective compared to traditional chemical compounds.

In contrast, Evans *et al.* [9] found that multiple applications of ACT made from various animal manure and green waste composts were consistently as effective as standard fungicide spray programmes for managing grapevine powdery mildew (*Erysiphe necator*) and Botrytis bunch rot (*B. cinerea*). Suppression of powdery mildew on chardonnay leaves and bunches by ACT was achieved under conditions that were highly conducive to this disease. They reported that nutrients in ACT might have assisted in augmenting and sustaining microbial populations in some instances, which may be related to disease suppression effect. Such peer-reviewed reports, which show that compost tea had a consistent and comparable disease suppressive efficacy as synthetic pesticides or standard pesticide programmes are rare. Moreover, in studies in which such comparisons have been made, researchers reported that even in cases where compost

tea effectively suppressed plant pathogens and diseases, synthetic chemicals resulted in higher and more consistent levels of control [88, 91, 100, 114]. For example, Weltzien [88] noted that the significant reduction in the incidence of leaf blight (*Pseudopeziza tracheiphila*) in grape by NCT made from horse-manure compost deserved attention, as it was achieved under field conditions. However, the recommended fungicide treatments resulted in significantly higher disease control levels. To the best of the author's knowledge, no acknowledgement has been made that compost tea will rarely, if ever, give control comparable to that achieved with commercial synthetic pesticides as was suggested by Litterick *et al.* [33] regarding the use of compost to control nematodes. Interestingly though, most recent studies have tended to focus less on comparing the disease efficacy levels between compost teas and commercial synthetic pesticides [108, 109, 124]. Instead, greater focus is placed on comparing disease efficacy levels across compost tea types [124], other biocontrol agents [107], and inoculated and non-inoculated water controls. For example, Haggag and Saber [124] found that the incidence of early blight (*Alternaria solani*) in tomato and purple blight (*A. porri*) in onion was reduced in plants sprayed with NCT compared to those sprayed either with ACT or non-sprayed ones (water controls). While studies that exclude comparisons of disease efficacy levels between compost tea and commercial synthetic pesticides are useful in the academic sense and in the context of organic crop production, they limit the extent to which data generated can be used by farmers to make informed and practical decisions in conventional or sustainable crop production systems.

To this end, Mahaffee and Scheuerell [10] concluded that the level of disease suppression observed in most of these studies may not be sufficient for commercial production, however, there are numerous non-standard production systems (e.g. organic, biodynamic) for which these results are far better than the alternative of no control options.

## Factors Affecting Disease Suppression by Compost Teas

### Production Factors

#### *Compost source, quality and age*

Compost with high diversity of microbial populations is generally thought to be most appropriate for the production of compost tea with plant disease suppressive properties. Moreover, it can be argued that the brewing of suppressive compost is most likely to result in compost tea with plant disease suppressive properties [7]. In fact, it is increasingly being argued that due to a higher diversity of beneficial microorganisms, teas produced from vermicompost or vermicasting, will likely result in higher phytopathogen and plant disease suppression than those

made from compost [64, 93, 125–127]. For instance, Manandhar and Yami [93] showed that in *in vitro* and field trials, ACT produced from vermicompost consistently resulted in significantly higher levels of *Fusarium moniliforme* or foot root disease suppression in rice, compared to those made from thermophilic compost. A similar results pattern was observed with NCT produced from vermicompost or compost in the same study. In contrast, Tian and Zheng [64] reported that ACT made from pine bark compost significantly suppressed *in vitro* growth of all test pathogens (*Fusarium foetens*, *R. solani*, *S. sclerotiorum*, *P. cryptogea*, *Pythium intermedium*, and *P. ultimum*) with an inhibition over 50% after 10 days. Conversely, vermicasting tea (VT) showed biocidal activity against *S. sclerotiorum* (50%) and *F. foetens* (43%) after 10 days' incubation, whereas VT was initially effective against *R. solani* for the first 6 days but its effectiveness did not last the entire 9 days of the assay. VT did not inhibit the growth of *P. intermedium*. The findings from these studies seem to support the assertion that vermicomposting, composting processes and substrates select for specific microbial communities [125, 126], which might be useful indicators for the plant disease suppression potential of the finished products.

Several authors have reported that feedstock type and composting system, organic matter decomposition level and compost maturity, physical, chemical and biological attributes of compost, and inoculation of compost with biological control agents, all affect the disease suppressive ability of the compost [2, 128, 129]. Pane *et al.* [86] found that compost derived from animal manure showed the largest and most consistent suppression of *P. ultimum*, *R. solani* and *S. minor*. These findings are congruent with reports by Weltzien [49, 130], which indicated that NCT made with animal manure were more efficacious than those made with undigested vegetative matter. The disease suppressive consistency of compost tea made of animal manure has been attributed to a higher diversity in microbial populations [131].

In contrast, Erhart *et al.* [132] demonstrated that compost prepared from grape marc or 'biowaste' had neutral or promoting effects on *Pythium* rot diseases. However, Hadar and Gorodecki [133] reported that compost made from grape pomace, which contains high concentrations of sugars and relatively low levels of cellulose substances, tends to become colonized by *Aspergillus* and *Penicillium* spp., which have been shown to suppress *S. rolfii*. Other research indicated that undigested vegetative matter or composted materials were equally effective as compost tea made from animal manure at suppressing plant diseases [4, 60, 69, 100]. Reports also have shown that compost made from lignocellulosic substances, such as tree barks consistently suppress *Pythium* root rot [134, 135].

Conversely, more recent work by Scheuerell and Mahaffee [60] does not support the association of disease suppression with particular compost source and/or

feedstock. Scheuerell and Mahaffee [60] evaluated 30 different composts for the control of grey mould of geranium and found that disease suppression was associated with the particular batch of compost and not necessarily the feedstock used to make the compost. Though compost age, defined as duration from the establishment of compost pile to the time samples were collected, varied across compost types, Scheuerell and Mahaffee [60] did not evaluate compost age as a component of compost source. This may partly explain why they found a lack of association between compost source and disease suppression.

Compost age as a component of compost source has been investigated for its impact on disease suppression with NCT. Scheuerell [131] reported that studies done in Germany cited that composts should be cured for 2–6 months after processing for use in compost tea production. He reported that one of the research projects found that NCT made from 6-month-old horse-manure compost was significantly more effective than NCT made from 1-year-old horse manure compost in controlling cucumber downy mildew. According to Brinton and Droffner [3] and Dittmer *et al.* [136], 9–12-month-old horse- and dairy-manure compost can be used to make compost tea, whereas compost >3 months old, made of only plant material such as leaves, yard trimmings and straw is less useful for making compost tea [3, 136]. Egwunatum and Lane [74] suggested that as the type of assay shifts closer to an *in vivo* system, the effects of compost age become less pronounced, perhaps as a result of the increasing complexity of the system.

Most reports suggest that what constitutes a suitable age for brewing compost tea with disease suppressive properties is dependent on the feedstocks used to make the compost and storage conditions [49, 87, 137]. However, it is unclear whether the shifting microbial community or the reduction in biomass available to support microbial activity, i.e. compost stability, is the main factor in relating compost age with plant disease suppression. Al-Dahmani *et al.* [41] evaluated 3, 5, 10 and 16-month-old manure-based compost for the control of bacterial spot of tomato and found that compost age had no significant effect on disease suppressiveness. Palmer *et al.* [43] evaluated the suppressive effect of ACT made from four compost types at varying levels of maturity against *B. cinerea*. They found that all batches of ACT applied to detached bean leaflets reduced lesion development of *B. cinerea*. Furthermore, there was a significant linear, inverse relationship between the internal windrow temperature of compost ( $\leq 51^\circ\text{C}$ ) used to prepare ACT and the extent of lesion development. This was one of the earliest scientific studies, which reported on the use of immature compost to produce a pathogen-suppressive ACT and suggested that compost stage was an important production variable.

A recent review paper by Bonanomi *et al.* [138] showed that during organic matter decomposition, disease

suppression potential increased, decreased, was unchanged, or showed more complex responses, such as 'hump-shaped' dynamics with compost of decreasing organic matter content.

#### *Abiotic conditions, solvent, mixing and extracting*

It is known that temperature, humidity and other abiotic conditions influence the growth rate of microorganisms. However, peer-reviewed articles that examine the quantitative effects of abiotic conditions, type and properties of solvent, and mixing and extracting on the disease control efficacy of compost tea are limited. To this end, water or other solvents high in salts, heavy metals, nitrate, chlorine, sulfur, tannic acid, carbonates, or contaminated with pathogens (human, animal or plant disease-causing microorganisms) are not recommended for compost tea production.

Moreover, the effect of water or solvent temperature on suppressivity of compost tea is most likely related to the sensitivity of enzyme-catalysed reactions and membranes of antagonistic microbes to temperature. At temperatures below the optimum, enzymes cease to be catalytic and membranes solidify [139]. In contrast, at temperatures above the optimum, enzymes, transport carriers and other proteins begin to denature and lipid bilayer membranes melt and disintegrate [139]. At both extremes, microbial growth and metabolite production and function, which have been linked to disease suppression of compost tea [1, 16], are severely impaired [139]. To this end, high water (solvent) temperatures (>38 °C) are likely to be undesirable for brewing compost tea since they increase nutrient volatilization and cause evaporation that concentrate salts. There is no scientific consensus on minimum solvent temperature for brewing compost tea but it is expected to be not much lower than room temperature (20–25 °C), which is well within the range that mesophilic microorganisms in mature compost will proliferate.

Though not supported by much empirical evidence, solvent type used for brewing compost tea is another factor, which likely affects the efficacy compost tea. To date, potable or distilled water is the solvent most commonly used to brew compost tea. In one of the few peer-reviewed studies to directly compare the effects of solvent type on disease efficacy of compost tea, Pane *et al.* [7] found that all compost teas made from whey (whCTs) suppressed *in vitro* growth of *B. cinerea*, showing an inhibition zone about 50% larger than that produced by all compost tea made from water (waCTs). In the case of *Alternaria alternata*, *in vitro* suppression was independent of the solvent used and generally higher in waCTs. Compost tea made from whey produced from mature tomato residues – woodchips compost was most effective against *Pyrenochaeta lycopersici* whereas waCT made from tomato residues–escarole–woodchips compost was the least the effective treatment. They concluded that whey could be considered a viable solvent for suppressive compost-tea

production, although further dilution in dechlorinated water at a ratio of at least 1 : 5 proved a necessary method to avoid occurrence of root or foliar phytotoxicity, probably due to high salt concentrations and sub-acid pH of the relative teas. PBs are commonly used in the production of CWE [65]; the inoculum or compost is soaked and/or shaken in PB before being diluted with water. PBs are usually used to prevent cells from rupturing or shrivelling up due to osmosis [140]. Therefore, it is likely that this practice is done to maximize the number of live agents extracted from compost into compost tea.

Mixing, which assists with the physical extraction of microbes from compost, is an important part of the brewing process. In reference to mixing, Scheuerell [131] noted that too little energy can leave microorganisms in the compost whereas, too much energy can rupture cells. However, Scheuerell [131] assigned no quantitative values to these qualitative descriptions of energy, mixing speeds or force. More so, to the best of the author's knowledge there is no scientific consensus on such parameters. This highlights the need for systematic studies to investigate optimal mixing speeds and/or techniques as it relates to maximizing the diversity of live microorganisms in compost tea. Such studies should prove useful in developing production protocols for compost teas, which consistently suppressive plant diseases.

#### *Aeration*

Ingham [58] claims that NCT is less effective than ACT at controlling plant diseases because NCT tends to have lower microbial mass and diversity of beneficial microbes. However, the majority of scientific literature supports the suppression of phytopathogens and plant diseases by NCT [48, 87, 104]. Cronin *et al.* [48] reported that ACT did not suppress germination of *Venturia inaequalis* conidia whereas NCT did. Conidial suppression was however induced after ACT was allowed to incubate for an additional 7 days without aeration. In contrast, Manandhar and Yami [93] reported that while all compost tea significantly reduced the number of seeds infected with *F. moniliforme*, aerated compost and vermicompost teas resulted in higher disease reduction levels than non-aerated compost or vermicompost tea. Direct comparison of the efficacy of ACT and NCT within the same study has often shown that aeration has no effect on disease control [4, 41, 60]. St. Martin *et al.* [42] found that aerating compost tea made from banana leaf or lawn clippings composts did not consistently result in higher mycelial inhibition levels of *P. ultimum*. Al-Dahmani *et al.* [41] observed no differences in the control of bacterial spot when NCT or ACT was applied to tomato plants. Similarly, ACT and NCT were compared for control of powdery mildew, rust and blackspot of rose [141], *Pythium* damping-off of cucumber [4] and grey mould of geranium [60] and no differences in disease control in either of the pathosystems were observed. Moreover, numerous studies show that various

phytopathogens and plant diseases can be suppressed using CWE [71, 79, 83, 84]. This further questions the need for aeration or the relevance of any of the other brewing methods.

To date, the majority of the studies indicate that aeration does not increase disease control efficacy. Therefore, the factors influencing the choice of whether to brew (ACT or NCT) or not (CWE, compost leachates), are likely to depend on whether either time or cost of production equipment and labour are major constraints to using these BCAs.

#### Brewing time

Disease-suppressive properties of NCT and ACT have generally been reported to increase with brewing time to a maximum and then decline [104, 105]. Ingham and Alms [54] claimed that optimum brewing time is usually between 18 and 36 h at the point where active microbial biomass is at its highest. However, St. Martin *et al.* [42] found that increasing brewing time beyond 18 h for ACT and 56 h for NCT made from banana leaf or LCCs, did not increase growth inhibition level of *P. ultimum*. In contrast, McQuilken *et al.* [99] found that age of NCT (brewing time) had some effect on subsequent activity against both germination and mycelial growth of *B. cinerea*. They reported that 3- to 12-day-old NCT were equally effective in inhibiting conidial germination, but there was a significant decline in inhibition with 18-day-old NCT. Furthermore, 8- to 18-day-old NCT were equally effective in reducing mycelial growth of *B. cinerea* and were significantly better than either 3- or 5-day-old extracts. Other investigators suggested while 24 h brewing time is good for fertilization, brewing times of 7–14 days are better when producing compost teas with optimal disease suppressive properties [130, 142]. Although not substantiated by data, it is generally thought that optimum time is likely to depend on the compost source and brewing method [2]. However, the trend for brewing ACT remains 24 h and 5–7 days for NCT.

#### Nutrient amendments

Nutrient amendments are primarily added to increase overall microbial populations or the population of a specific group of microorganisms that are postulated to have beneficial effects, e.g. increased disease suppressive efficacy. To date, practitioners have used a plethora of nutrient amendment recipes, e.g. soyabean and feather meals, yucca powders, molasses, kelp, humic acids and rockdust, which they claim increases the disease suppressive efficacy of compost tea. Conversely, scientific studies have shown that nutrient amendments enhanced [64], reduced [4], or had no significant effect on the disease suppressive properties of compost teas [100]. For example, Elad and Shtienberg [100] reported that the addition of nutrients to the NCT did not generally improve the control of grey mould in tomato, pepper and grape plants. Scheuerell and Mahaffee [60] found that

although nutrient amendments generally increased bacterial populations in ACT, they did not consistently result in increased grey mould suppression in geranium. However, 67% of ACT batches made with a mixture of kelp extract, rock dust and humic acid significantly reduced the disease. In an earlier study, Scheuerell and Mahaffee [4] reported that the most consistent formulation for damping-off suppression was ACT produced with kelp and humic acid additives. They further found that producing ACT with a molasses-based additive inconsistently suppressed damping-off and suggested that residual nutrients can interfere with disease suppression.

Notwithstanding the effect of nutrient amendments on the disease suppressive properties of compost teas, there are mounting concerns on the regrowth potential of human pathogens in teas as it relates to the use of nutrient amendments and type of brewing method [36, 102]. NCT have been suggested to provide the optimal environment for human pathogen regrowth [58]. Contrastingly, ACT have been associated with less than ideal environment for the proliferation of human pathogen [58]. Contrary to claims concerning the suitability of environment of NCT for the proliferation of human pathogens, Ingram and Millner [143] reported that potential for regrowth of human pathogens *Escherichia coli* O157:H7, *Salmonella* and faecal coliforms was not compost-tea-brewing-method-specific but was greatly dependent on the addition of nutrient supplements at the beginning of the brewing process. In fact, they reported that ACT sustained higher concentrations of *E. coli* O157:H7, *Salmonella*, and faecal coliforms than did NCT when nutrient supplements were added. In contrast, Palmer *et al.* [144] found that a low oxygen concentration (3.4 mg/l) was not the only factor associated with increased *E. coli* M23 strep<sup>r</sup> populations in an NCT amended with 1% molasses; low pH and high conductivity also had a similar association. In contrast to Ingram and Millner [143], Palmer *et al.* [144] prepared ACT from compost in the early secondary mesophilic stage rather than the late mesophilic (mature) stage of aerobic composting and found that supplementing ACT with 0.8% fish hydrolysate or 1% molasses at 24 h during brewing cycle, resulted in a significant increase in *E. coli* M23 strep<sup>r</sup> populations at 72 h. *Escherichia coli* M23 strep<sup>r</sup>, a non-pathogenic strain, was used to indicate the potential behaviour of pathogenic strains of *E. coli*, since its growth characteristics are similar to strains of *E. coli* that are pathogenic to humans.

To date, molasses has been demonstrated to support the growth of *E. coli* and *Salmonella* if inadvertently present in compost tea, posing worker and consumer health concerns [144–146]. In contrast, recent investigations have shown that pathogen regrowth does not appear to be supported in compost tea brewing that does not contain added nutrients [144, 146, 147].

Further investigations are needed to test nutrient amendments for their effect on both targeted plant

pathogens and non-targeted human pathogens [1], and the overall disease suppressive efficacy of compost tea.

#### *Microbial amendments*

For the most part, research efforts for compost tea have followed a similar paradigm as compost, i.e. the use of microbial amendments to improve the disease suppressive efficacy and predictability of compost tea. Microbial amended composts are up to three times as suppressive as unamended, naturally suppressive composts [148, 149]. Likewise, Zhang *et al.* [86] showed that topical sprays with CWE prepared from a biocontrol agent-fortified compost mix reduced symptoms of bacterial speck and the population size of pathogenic KD4326 in *Arabidopsis* grown in the peat mix. Similarly, Wahyuni *et al.* [97] showed that tobacco plants treated with CWE made from compost amended with effective microorganisms-4 (EM-4) and *Pseudomonas aeruginosa* Ch1 at the same time or compost amended with EM4 only with the extract amended with *P. aeruginosa* Ch1, reduced the severity of cucumber mosaic virus. They concluded that higher bacteria population in the root and rhizosphere, particularly the activities of *P. aeruginosa* Ch1 as plant growth promoting rhizobacteria (PGPR) rather than the activities of bacteria from EM-4 was responsible for disease severity reduction. The increased efficacy of CWE or compost tea amended with microbes associated with disease suppressive effects means that application rates required for effective disease control are reduced. However, to fully exploit the benefit of enhanced disease suppression, further work is needed to assess the appropriateness of and manipulate the compost tea environment to promote the growth and reproduction of the microbial amendments.

#### **Application Factors**

##### *Dilution and application rate of compost tea*

The impact of diluting compost tea on disease control is increasingly being investigated. Scheuerell and Mahaffee [4] found that dilution decreased disease suppression of *Pythium* damping-off of cucumber. Similarly, Cayuela *et al.* [78] found that extracts made from stage I-compost, i.e. the undecomposed materials, were inhibitory against *P. capsici*, but the suppressiveness decreased with increasing dilution. They reported that inhibition was more effective with mature compost (stage IV) and very high even at 1 : 50 dilution. Conversely, Welke [109] reported that the effect of the CWE dilution on the incidence of *B. cinerea* was not significant at infection levels below 50%. However, at infection levels >50 %, CWE prepared at a dilution rate of 1 : 8 v/v (compost:water) significantly reduced the number of severely disease affected berries (Table 3), whereas suppression levels achieved with CWE prepared at a dilution rate of 1 : 4 v/v, did not differ significantly from control treatments. In contrast, Elad and Shtienberg [100] concluded that the ability to retain

suppression of *B. cinerea* upon dilution appeared to be compost source specific.

The practical and economic use of compost tea on a large-scale to suppress disease hinges on its ability to maintain suppressivity after diluting with water. Most practitioners have reported using the application rates of 40–150 litre compost tea/hectare for foliar and soil-borne diseases [44, 57, 58]. However, it seems impossible to produce enough tea to apply to thousands of hectares [10]. For soil applications, Scheuerell [131] noted that sufficient volume should be applied to reach the entire root zone to yield the desired effects. Research works, which evaluate the application rates required under different conditions, for various pathogens, which vary in their modes of survival, and infection processes are needed [131].

##### *Application frequency and time*

Studies on the systematic evaluation of application frequency as a variable has been lacking. Mahaffee and Scheuerell [10] noted that in most studies, compost tea is applied at intervals similar to those used for synthetic pesticides and traditional BCA. In this light, significant disease control has been observed where compost tea was applied at <14-day intervals and 5–10 total applications per year [49, 105, 112]. Application frequency and time are other factors, which must be evaluated to determine whether compost tea can be used economically to suppress plant diseases. It seems likely that application timing will depend on the disease that is being targeted and conditions that pose risks of infection by particular diseases. To this end, Grobe [150] suggested that the best time for application of compost tea is in the evening, when evaporation is minimum and ultraviolet (UV) light, which can be toxic to microorganisms, is minimal.

##### *Adjuvants*

The four main types of adjuvants, which have been evaluated with the aim of enhancing disease suppressiveness of compost tea, are spreaders, stickers, protectants and nutrients additives. All four types of adjuvants have been defined in Table 1. Although most researchers have reported increased disease suppression with the use of adjuvants such as spreaders and stickers including methylcellulose [1, 4, 116], further work is required to determine the best combinations of adjuvants to use for specific situations. Sackenheim *et al.* [116] found that under field conditions, the combination of brewing nutrients with methyl cellulose generated the greatest number of recovered organisms per leaf area, and only this treatment reduced disease significantly more than unamended NCT. In contrast, under severe disease pressure, Yohalem *et al.* [114] observed no further reduction in apple scab severity by NCT amended with either Latron B1956 (0.06% v:v) spreader-sticker or fish oil (0.025%) compared to unamended NCT. Moreover,

Brinton and Droffner [3] reported that spray adjuvants can inhibit microbial activity and this could affect the targeted pathogen and/or antagonists. The effect of adjuvants on microbial activity and targeted pathogen and/or antagonists, deserves more attention.

### Predictors and Mechanisms of Suppression

Understanding the predictors and mechanisms of suppression is crucial in addressing the inconsistent plant disease control, which has been associated with the use of compost tea. Most scientific literature acknowledges that several modes of activity are involved in the suppression of plant diseases with compost tea or extracts. These modes of activity appear to be mainly associated with live microorganisms, since sterilizing or micron filtering compost tea has generally resulted in the reduction or elimination of suppressive effects against phytopathogens and plant diseases [7, 83, 138, 151]. In some cases, the suppressive efficacy of compost tea has been unaffected by sterilization or micron filtration [42, 102]. According to Cronin *et al.* [48], these results indicate the likely role of heat-stable chemical compounds in suppressing the growth and activity of phytopathogens and disease incidence and severity.

Although other genera are involved, bacteria in the genera *Bacillus* and *Serratia* and fungi in the genera *Penicillium* and *Trichoderma*, are thought to be the main microbes responsible for the suppressive effects of compost tea [3, 33]. Unfortunately, most studies have focused almost exclusively on bacterial consortia as the live agents responsible for the disease suppressive effects of compost tea. This has resulted in very little knowledge of the fungal populations prevailing in suppressive compost tea. This is so, despite the isolation of effective fungal biocontrol agents from compost tea [16]. In this light, peer-reviewed literature on the yeast populations prevailing in suppressive compost tea is also limited. In a recent study, St. Martin *et al.* [42] found that yeast populations of ACTs were positively related to the growth inhibition of *P. ultimum*. To the best of the author's knowledge, viruses have never been considered as biological agents responsible or related to the disease suppression resulting from the application of compost tea. However, a recent study by Heringa *et al.* [152] may illustrate the potential role of viruses in disease suppression with compost and compost tea. They found that a five-strain bacteriophage mixture isolated from sewage effluent and applied to dairy manure-compost resulted in a greater than 2-log reduction in *Salmonella enterica* within 4 h at all moisture levels compared with controls. It is possible that strains of bacteriophage present in compost tea could play a similar role in plant disease suppression.

The relationship between microbial groups (total and active bacteria, fungi, yeast and actinomycetes) and the disease suppressive efficacy of compost tea has been

studied [4, 95, 153]. However, it is difficult to draw meaningful conclusions from the results. Ingham [58] suggested various thresholds or indices e.g. 150–300 µg/ml of total bacteria, 10–150 µg/ml active bacteria, 2–20 µg/ml total fungi and 2–10 µg/ml active fungi, which supposedly indicate a minimum range indicative of an effective compost tea. However, there is no scientific evidence to support these claims. In a review of the literature, Scheuerell and Mahaffee [1] reported that disease suppressive compost teas had total bacterial populations ranging from  $10^7$  to  $10^{10}$ . In fact, Scheuerell and Mahaffee [4] found that suppression of *Pythium* damping-off by a compost tea was related populations  $>10^7$  cfu/ml and  $10^6$  active bacterial cells/ml. They therefore concluded that these data appear to indicate that information on the bacterial populations of compost tea can be somewhat predictive of the disease suppression level of compost tea. This conclusion was supported by Bonanomi *et al.* [138] in their review paper, which identified the characteristics of organic soil amendments that suppress soilborne plant diseases.

In contrast, Pane *et al.* [7] found compost tea with total bacterial count of lower than  $10^{-3}$  cfu/ml inhibited *A. alternata*, *B. cinerea* and *P. lycopersici*. Alternatively, Palmer *et al.* [43] found that there was no relationship between the level of pathogen inhibition and the abundance of culturable bacteria or fungi (after 24 h incubation) in ACT. They concluded that the microbial diversity, more than abundance of culturable bacteria and fungi, was considered as a main factor contributing to the suppression of disease by compost tea, connected essentially to the presence of microbes exhibiting antagonistic activity. St. Martin *et al.* [42] further suggested an examination of the population metrics of specific microorganisms rather than total microbial populations may prove to be more reliable in rationalizing the efficacy between aerated and NCT. Mahaffee and Scheuerell [10], however, questioned the practical utility of microbial populations as predictors of the disease suppressive potential of compost tea. They stated that in all published studies, analyses of microbial population were conducted within a few hours of collecting samples of compost tea. However, compost tea samples sent to laboratories will incur unknown environmental conditions related to shipping and handling over the 24–48 h period between sample collection and analysis, which will affect the microbial populations present in the samples. There is still need for further investigations on the effect of compost tea application on the enzymatic (e.g. microbial activity, substrate respiration) and microbiological (fluorescent pseudomonads and *Trichoderma* populations) properties of substrates and their relationship to disease suppression.

To this end, though not fully understood, four main mechanisms have been described through which biological control agents suppress plant pathogens: antibiosis, competition for nutrients, parasitism or predation and ISR [154]. These mechanisms may exist separately or in

combinations. The first three affect the pathogen directly, reduce its survival while the latter act via the plant, and affect the disease cycle [16].

Most reports suggest that microbiostasis (antibiosis and/or competition for nutrients) and hyperparasitism are the principal mechanisms by which plant pathogens are suppressed. According to Hoitink and Ramos [155], suppression by microbiostasis seems to be more effective against pathogens with propagules <200 µmm diam. including coliforms, *Phytophthora* and *Pythium* spp.

Antibiosis refers to an association between organisms where the production of specific and/or non-toxic specific metabolites or antibiotics by one organism has a direct effect on other organisms [2]. Based on data, which showed no loss of suppressive activity due to the sterilization or micron filtration of NCT, Elad and Shtienberg [100], Yohalem et al. [102] and Cronin et al. [48] concluded that antibiosis can be partly responsible for phytopathogen or disease suppression. It is known that many of the microbes present in compost extracts and teas can produce compounds that are toxic to other microorganisms. For example, chitinolytic enzymes produced by *Enterobacter* strains were found to be antagonistic to several fungal pathogens including *R. solani* [156]. Lumsden et al. [157] and Roberts and Lumsden [158] reported that the toxin 'gliotoxin', which was isolated from *Gliocladium virens*, was found to be antagonistic against *P. ultimum*. Antagonistic activity of bacteria and fungi from horticultural compost against other plant pathogens including *F. oxysporum* also has been reported [159]. Potera [160] reported that some chemicals produced from *Pseudomonas* spp. (e.g. siderophores) exert a potent chemical effect against other organisms. *Bacillus subtilis* and other *Bacillus* spp. are known to produce antibiotics that can inhibit growth and germination of many fungal species [3].

Competition results when there is a demand by two or more microorganisms for a resource. Litterick and Wood [2] stated that competition occurs when a non-pathogen successfully out-competes a plant pathogen for a resource, which may lead to disease control. For example, Sivan and Chet [161] and Srivastava et al. [162] found that some microorganisms reduce the disease incidence by limiting iron availability for pathogens such as *Pythium* spp. through the production of low-molecular weight ferric-specific ligands (siderophores) under iron limiting conditions.

In contrast to antibiosis, parasitism has been observed with phytopathogens with propagules >200 µmm diam. Hoitink et al. [129] reported that the parasitic effect, which has been observed in <20% of uninoculated composts consists of four stages: chemotrophic growth, recognition, attachment and degradation of the host cell walls through the production of lytic enzymes [163]. All of these stages are affected by the organic matter decomposition level and the presence of glucose and other soluble nutrients, which repress the production and effect of lytic enzymes used to kill pathogens [129].

ISR triggered by beneficial microorganisms also has been proven to reduce disease severity in many crops [128, 164]. For example, Lievens et al. [165] showed that composts can induce systemic resistance to *Pythium* root-rot in cucumber when applied to a section of the root system using a split root system. Based on the detection of inducible resistance-related compounds, Siddiqui et al. [76] concluded that induced host resistance was stimulated in okra plants treated with non-sterilized and filter-sterilized compost teas.

Similar results have been reported by other authors, who have isolated microorganisms from compost, which trigger the systemic resistance effect [166, 167]. Most studies on ISR have involved the use of *Trichoderma* spp., microorganisms also known for their mycoparasitic and antibiosis effects [164, 166].

For example, Samerski and Weltzien [121] found that the germination of *Sphaerotheca fuliginea* conidia was not inhibited when treated with NCT in vitro. However, NCT-treated cucumber leaves demonstrated indicators of induced resistance including increased papilla formation, lignification and necrotic reactions when *S. fuliginea* began to infect. Further work is needed to determine consistently effective methods to predict disease suppressiveness of compost tea and to improve our understanding of mechanisms of disease suppression.

### Potential Application of Molecular Tools and High-Throughput Sequencing Technologies in Compost Tea Disease-Suppression Studies

To date, most published studies have relied on traditional culture-based methods or microscopic examination to address three fundamental questions related to the potential of compost tea to suppress plant diseases: (1) what type of microorganisms are present in compost tea? (2) what do these microorganisms do? and (3) how do the activities of these microorganisms relate to plant disease suppression? Though useful, culture-based methods are extremely biased in their evaluation of microbial genetic diversity by selecting particular populations of microorganisms, which represent <1% of the total number of prokaryotic species present in soils and an unknown percentage in compost and compost tea [16, 125, 168–170]. As such, these traditional methods provide limited information on the microbial ecology of compost tea. That is, microbial community structure and functional diversity in a sample and the interactions of microorganisms with biotic and abiotic factors. Such information is important as the suppressive effects of compost and compost tea are usually attributed to a diverse microbial community rather than to a population of a single defined species. Therefore, research that examines the functionality of microorganisms is needed. Such research can assist in identifying factors that govern each specific case of plant protection [16].

**Table 7** Summary of traditional compared to molecular microbiological research approaches used to elucidate the microbiological basis of disease-suppressive compost tea

Research approaches	
Traditional	Molecular microbiology
1. Selective autoclaving, heat, microfiltration, gamma radiation or biocidal treatments to eliminate specific microbial groups to infer whether suppressive effect may be biological in nature.	1. Metagenomic approaches such as the use of genetic fingerprinting techniques, fluorescence in situ hybridization, microbial lipid analysis and DNA–DNA hybridization kinetics for microbial community analysis and structure. Ribosomal DNA (rDNA) sequences of microorganisms that are more abundant in highly suppressive compost teas than in the less suppressive compost tea are considered candidate sequences.
2. Use of Petri dish, or media-based techniques to isolate specific microbial groups suspected to be related to suppression of phytopathogens.	2. Q-PCR, DNA microarrays and other such molecular techniques are used to verify the microbial community composition results. That is, selective PCR primers or probes for each of the candidate rDNA sequences are designed and used to determine the relative amounts of the candidate sequences in compost teas, which may vary in suppressivity levels depending on treatment factors, such as sterilization, diluting and amending compost tea with nutrient amendments or manipulation of abiotic factors, such as pH of compost tea. Culturable microorganisms that consistently correlate with suppressiveness are isolated and evaluated for their abilities to produce suppressiveness.
3. Evaluation of the suppressiveness of specific microbial groups.	3. Alternatively, and perhaps a more direct route, particularly if candidate sequences are not always apparent, is the use of metatranscriptomics and metaproteomics approaches to profile microbial function and link these data to microbial community structure. Metabolomics platforms can be used to identify the role of metabolites in disease suppression and gene expression analysis such as pathogenesis-related genes and defense-related enzymes assays to investigate plant defense responses to compost or microorganisms.
4. Introduction of microorganisms with the highest suppressive levels in disease conducive plant growth media or susceptible host plant.	
5. Phenotypic characterization of microbial microorganisms causing suppression.	
6. Genotypic diversity analysis among and within functional groups.	
7. Elucidation of disease suppression mechanism of microorganisms	

Table 7 provides a comparison between the traditional and possible molecular microbiological research approaches, which can be used to elucidate the microbiological basis of disease-suppressive compost tea. Some of these molecular tools and HTS technologies are discussed in the following sections.

### Microbial Community Structure Analysis

DNA-based molecular approaches and techniques such as genetic fingerprinting, fatty acid methyl ester (FAME) profiles (microbial lipid analysis), DNA microarrays, and metagenomics, offer an alternative to and complement cultivation-based techniques, which can assist in analysing microbial community structure (partial and whole) and

function. Unfortunately, limited use has been made of these molecular tools in studies that have examined the suppressivity of compost tea. Of the few compost tea-disease suppression studies in which these molecular tools have been used, focus was placed on the analysing and relating microbial abundance and diversity in the rhizosphere to disease suppression levels [43, 92]. Much less attention has been paid on the use of molecular tools to study the phyllosphere of plants treated with compost tea. As such, our knowledge on effect of compost tea on the microbiology of phyllosphere has lagged behind that of rhizosphere, and fundamental questions such as which microorganisms are present in phyllosphere of plants and what do they do, remain largely unanswered [171]. For instance, Palmer *et al.* [43] used terminal restriction fragment length polymorphisms (T-RFLPs) to assess the

microbial diversity and cultured-based methods for the microbial abundance of ACT produced from open-windrow composts, which were sampled weekly from the early secondary mesophilic stage until maturity. They found that there was a significant inverse linear relationship between the internal windrow temperature of compost ( $\leq 51^\circ\text{C}$ ) used to prepare ACT and the extent of lesion development caused by *B. cinerea*. More so, bacterial and fungal diversity were highest in ACT prepared using compost with an internal windrow temperature of  $48^\circ\text{C}$ . They therefore concluded that an abundant and diverse microbial community likely contributed to pathogen suppression. Similarly, Larkin [92] used soil dilution plating, substrate utilization (SU) profiles, and fatty acid methyl ester (FAME) profiles to evaluate the effect of ACT on soil microbial populations and communities, and soilborne diseases under various crop rotation systems. He found that ACT significantly affected soil microbial communities, reduced stem canker and black scurf (*R. solani* Kühn), and common scab (*Streptomyces scabiei* Lambert and Loriaon) of tubers, and improved yield under some crop rotations, but not others.

Fritz *et al.* [119] used denaturing-gradient gel electrophoresis (DGGE) to analyse bacterial and fungal community profiles of vermicompost teas amended with different carbon sources during the brewing process. They found that the microbial communities in the teas differed, with both DGGE band presence and intensity varying between the different extractions. Vermicompost tea amended with green leaf compost and sunflower press cake which had the highest microbial population and diversity, was found to best support plant growth in laboratory experiments, and changes in microbial communities was minimal after 1 week of storage at  $10^\circ\text{C}$ .

COMPOCHIP microarray [172] spotted with 369 probes was used for comparative analysis of bacterial community profiles of vermicompost tea amended with green leaf compost and sunflower press cake, which were produced at two different time points [119]. Results indicated that the genera *Xanthomonas* and *Stenotrophomonas*, which include species that are involved in plant disease suppression and are also known as plant and human pathogens [172], were present in vermicompost tea produced at both time points. Moreover, *Acinetobacter calcoaceticus*, which is suspected to trigger an autoimmune response related to bovine spongiform encephalopathy [173], was found in with vermicompost tea produced in May but not in those produced in August. In contrast, vermicompost tea made three months later had higher levels of ammonium-oxidizing bacteria of *Nitrosovibrio* and *Nitrosospora* spp.

Metagenomic investigations related to disease suppression with organic amendments have focused primarily on compost [174, 175], with limited studies on compost tea. In a study designed to evaluate the impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop, Ottesen *et al.* [176]

used clone library method to assess if increased biological food safety risks might be linked with the bacterial communities associated with either treatment. Compost tea was one of several nutrient amendments used as part of the organic management protocol of the crop. Ottesen *et al.* [176] reported that in pooled organic and conventional clone libraries, the identified diversity spanned eight bacterial phyla and 14 classes, with Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria being the most frequently encountered in apple phyllosphere samples. They found that 51 operational taxonomic units (OTUs) were unique to conventional, and 37 were unique to organic treatments. However, the abundance of these unique OTUs was very low, accounting for only 5.3% of the clone library for the organic treatment and 9.3% for that of the conventional treatment. They concluded that microorganisms identified were so diverse and complex that their implications are still uncertain. Moreover, despite the identification of significantly diverse microflorae, no detectable differences in the presence of potential enteric pathogens could be associated with either organic or conventional management. Neither of the bacterial genera most commonly associated with produce-related illness outbreaks (*Salmonella* and *Escherichia*) was observed in any of the libraries.

Other DNA-based molecular techniques including quantitative PCR (Q-PCR) or real time PCR, fluorescence in situ hybridization (FISH), DNA-DNA hybridization kinetics, and guanine-plus-cytosine content fractionation can be used to profile microbial community structure. It should however be noted that these molecular approaches and techniques are not without use-limitations. For instance eukaryotes, e.g. fungi and yeast in general have much larger genomes and a higher proportion of DNA that does not code for protein than prokaryotes [177]. Therefore, though possible, metagenomics analyses of eukaryotes are scarcely done due cost limitations. As with culture-based methods, this has resulted in almost exclusive focus on bacterial consortia as potential live agents responsible for the disease suppressive effects of compost tea. This means that the potential or contributing role of fungal strains in determining the suppressivity of compost tea is shadowed [16]. It is likely that the use of metagenomic analyses of eukaryotes in compost tea-disease suppression studies will increase as sequencing costs decrease, particularly with the advancement of higher-throughput sequencing technologies.

### High-Throughput Sequencing Technologies

According to Zwolinski [178], second and third-generation DNA sequencing technologies and techniques allow for investigating deeper layers of the microbial communities, which is essential in presenting an unbiased view of phylogenetic composition and functional diversity of environmental microbial communities. For example, the

454 pyrosequencing (second-generation) technique developed by 454Life Sciences, offers two to three orders of magnitude higher coverage of microbial diversity than the typical Sanger (first generation) sequencing technique [170], which has been used in most molecular microbial surveys. The major limiting factors of the Sanger technique were relatively high cost and low throughput [179]. The number of sequencing reactions required to achieve at least 50% coverage of the diversity in compost or compost tea samples has not been estimated. However, Dunbar *et al.* [180] estimated that over 40 000 sequencing reactions are required to reach 50% coverage of the diversity in a soil sample. This makes Sanger sequencing a slow and laborious process, which probably explains why only a few hundred 16S rRNA gene clones are sequenced in most studies [170, 181–183]. Rastogi and Sani [170] noted that sequencing of such a low number of clones captures only the dominant components of microbial communities that mask the detection of low-abundance microorganisms, which constitute a highly diverse 'rare biosphere' in almost every environmental sample [184]. According to Rastogi and Sani [170] the microbial populations, which constitute the 'rare biosphere' are largely unexplored and offer a potentially inexhaustible genetic reservoir that could be explored using next-generation sequencing techniques.

Other second-generation sequencing platforms such as HiSeq® (Illumina, CA, USA) and Applied Biosystems SOLiD® (Life Technologies, CA, USA) generally use a similar cyclic array-based sequencing method as 454 pyrosequencing, where strands of fragmented DNA are amplified, bases are added sequentially using DNA polymerase and imaging is used to identify bases incorporated [179, 185]. This allows for massive parallel HTS of hypervariable regions of 16S rRNA genes and are much faster and less expensive than traditional Sanger's dideoxy sequencing of cloned amplicons [170, 185]. However, all these platforms require PCR amplification of genome fragments with lengths of approximately 35–400 bp, which, because of clonal amplification, are error prone [179]. Moreover, they generally require complex sample preparation and chemistry such as fluorescent labelling and enzyme-substrate reaction, and greater proficiency in data analysis and interpretation [186, 187].

In contrast, third-generation sequencing platforms such as PacBio RS (Pacific BioSciences, CA, USA) and Heliscope sequencer (Helicos BioSciences, MA, USA) do not require PCR amplification and use single-molecule templates, which are less prone to error and require less starting material [179]. These single-molecule real time (SMRT) sequencing technologies are capable of single-molecule sequencing and producing reads exceeding >1 kb with an accuracy of >99.99% [185]. Platforms such as PacBio RS (Pacific BioSciences, CA, USA) are not without disadvantages, their raw read error rate is high (>5%), and throughput is substantially lower than that of second-generation and Helicos Biosciences true Single

Molecule Sequencing platforms [179]. Fourth-generation sequencing technologies such as Oxford Nanopore (Oxford Nanopore Technologies Ltd, Oxford, UK), also achieves sequencing by single-molecule sequencing without amplification, real-time sequencing without repeated cycles and synthesis can be eliminated [188]. The company purports that the Oxford Nanopore technology can execute a whole-genome scan in 15 min at a very low cost without having to modify or prepare samples [179]. However, there is scepticism about these claims since the machine is still not yet commercially available for testing by independent scientists.

In any case, the use of HTS technologies offers the opportunity for the generation and integration of more comprehensive (inclusive of prokaryotes and eukaryotes) data, which will allow for a more accurate interpretation of the complexity and dynamics of disease suppression using compost tea. For instance, it is interesting that although viral communities have been the subject of several metagenomic investigations and were among the earliest to be studied [177], to the best of the author's knowledge, they have not been considered as agents responsible or related to the disease suppression resulting from compost tea application. This represents an area, which deserves further attention.

### Microbial Functional Diversity Analysis

While metagenomic approaches are useful for indicating the genetic potential in environmental samples, they do not directly elucidate the functionality of microbial communities in ecosystems [189]. Unfortunately, very few researchers have used molecular techniques to systematically investigate the functionality of microorganisms in compost tea-disease studies. Research work on functionally active microbial populations, usually involves the extraction of RNA (mRNA and rRNA) rather than DNA from metagenomic samples. RNA is considered as indicators of functionally active microbial populations, primarily because they provide more valuable information than DNA, in distinguishing active microbial communities between dormant microbial communities in a sample [189, 190]. For example, Wellington *et al.* [190] reported that the amount of rRNA in a cell roughly correlates with the growth activity of bacteria, and mRNA of functional genes allows the detection and identification of bacteria actually expressing key enzyme activities under specific conditions. As such, it is possible to amplify several genes from DNA/RNA isolated from microbial communities of compost tea to obtain insights into key microbial processes that may enhance the expression of the pathogenesis-related genes, increase the production of specific enzymes, all of which, may improve plant defense. For instance, Sang *et al.* [71] found that the application of CWE enhanced the expression of the pathogenesis-related genes, *CABPR1*, *CABGLU CACHi2*, *CaPR-4*, *CAPO1* or

*CaPR-10* as well as  $\beta$ -1,3-glucanase, chitinase, and peroxidase activities, which resulted in enhanced plant defense against *P. capsici* in pepper plants. Moreover, they reported that CWE enhanced the chemical and structural defenses of the plants, including  $H_2O_2$  generation in the leaves and lignin accumulation in the stems. Due to the lack of significant differences between treatments of untreated, autoclaved, and filtered CWE in zoospore germination, disease incidence, and disease severity. Sang *et al.* [71] concluded that the suppressive effects of CWE may be due to a heat-stable chemical factor(s) in CWE but not a biological factor(s). Sang and Kim [6] reported similar results and conclusion when CWE was used to against anthracnose in pepper and cucumber caused by *C. coccodes* and *C. orbiculare*, respectively. However, without the identification of this specific heat-stable chemical factor(s), and the use of molecular tools to elucidate the community structure and functional role of microbes in the CWE, it is unclear whether this heat-stable chemical factor was produced by microorganisms. Moreover, the treatment designs used by Sang *et al.* [71] and Sang and Kim [6] does not allow one to make a more conclusive inference regarding the possible origin of the heat-stable chemical factor.

Microautoradiography (MAR), which is an efficient method to obtain reliable information about the eco-physiology of microorganisms at the single-cell level in mixed communities [191, 192] can be used to identify the iron reducing microbial communities in compost tea. Such studies are relevant since competition for nutrients and other resources has been identified as a possible mechanism by which microorganisms in compost tea suppress plant disease [34]. For instance, Clercq *et al.* [128] and Litterick and Wood [2] reported that microorganisms reduce the disease incidence by limiting iron availability for pathogens such as *Pythium* spp. through the production of low molecular weight ferric-specific ligands (siderophores) under iron limiting conditions. MAR is based on the premise that metabolically active cells utilizing radiolabeled substrate can be visualized by exposure to radiation-sensitive silver halide emulsion [193]. Kong *et al.* [194], Kong *et al.* [195], and Lee *et al.* [196] used MAR-FISH in wastewater treatment and marine systems to describe the functional properties of newly discovered species, and to identify microorganisms responsible for key physiological processes. Similar studies can be done with compost tea to describe the functional properties of species, and to identify microorganisms responsible for key physiological processes related to plant disease suppression.

Hesselsoe *et al.* [197] used a holistic strategy based on the isotope array approach to analyse the diversity and ecophysiology of Rhodocyclales in activated sludge from a full-scale wastewater treatment plant. Their results indicated that the functional redundancy of nitrate reduction and the functional versatility of substrate usage are important factors governing niche overlap and

differentiation of diverse Rhodocyclales members in this activated sludge. It is possible that such functional redundancy and versatility exist in compost tea with regard to physiological processes of microorganisms, which are possibly related to disease suppression. As such, compost tea-disease studies aimed at identifying and characterizing these functional redundancy and versatility using isotope arrays should prove useful in better understanding and predicting disease control and the relationship between nutrient amendments and the proliferation of human pathogens.

Though useful in discriminating active microbial populations from quiescent ones in environmental samples, methods such as MAR, isotope arrays and stable isotope probing, which are based on the premise of incorporating labelled markers in microbial biomass, only provide limited information on the microbial populations associated with specific processes rather than a complete description of their functional role within a community [198]. This shortcoming emphasises the need for greater use of postgenomic techniques to obtain detailed insights into the metabolic activities of microbial communities of compost tea and how these activities relate to plant disease suppression.

### Postgenomic Approaches

Postgenomic techniques including metaproteomics, metatranscriptomics, proteogenomics, and metabolomics can now be used to investigate relationships between genetic potential and functionality in microbial communities. This was made possible by the development and availability of comprehensive metagenomic databases, which includes genomic sequences from cultured and uncultured microorganisms [199]. This represents an important advancement since DNA-based techniques do not provide information on the gene expression (functionality) as it occurs under in situ conditions [200]. These postgenomic techniques are discussed in the following subsections and their potential applications in investigating functionality of microbial communities in compost tea are highlighted.

#### Metatranscriptomics

The potential expression of genes of microbes in complex communities such as compost tea can be examined using metatranscriptomics. Using this technique, a snapshot of transcriptional profiles that correspond to distinct populations within a microbial community at the time of sampling can be generated [201]. Such snapshots can be used to obtain greater insights into the potential activities of microbial communities in compost tea and the mechanisms that regulate them.

As with metagenomics, metatranscriptomics involves random sequencing of microbial community mRNA but does not involve the use of primers or probes as is characteristic in qPCR and microarrays, respectively. Therefore, as it relates to monitoring gene expression in compost tea or other such complex communities, metatranscriptomics overcomes constraints inherent in the use of qPCR and microarrays since it avoids the need to select beforehand, what genes should be studied and the number of genes that can be surveyed in a single study [201, 202]. On this basis, transcripts from microbial consortia are sequenced with less bias than qPCR or microarray techniques. Moreover, paralogous sequences which might cross-hybridize on a microarray can be distinguished [202].

Microbial expression profiles from diverse ecosystems are being generated from various studies [203–206], resulting in the development of more comprehensive and useful databases. The generation of microbial expression profiles for compost tea or substrates treated with compost tea resulting from comparative and experimental metatranscriptomics studies offers the opportunity to better understand the relationship between disease suppressive efficacy and compost tea production factors. This is because the technique is particularly amenable to controlled experimental studies, in which microbial community gene expression can be measured in direct response to a manipulated biotic or abiotic factor [202]. According to Moran [202], immediate regulatory responses to environmental changes may be better reflected by the metatranscriptome than the metaproteome. This is because mRNA has a much shorter half-life and lower inventory in cells compared to proteins [207–209], which makes it a more sensitive indicator of near-real-time conditions experienced by cells [210].

Carvalhais *et al.* [201] and Moran [202] provided a detailed review of the biases and main limitations of metatranscriptomics, which are related to technically difficult protocols for mRNA isolation and cDNA synthesis and amplification. These biases and limitations are related to mRNA instability (short half-life) [211, 212], relatively low amount of mRNA in environmental microbial communities [213], presence of impurities such as humic and fulvic acids during mRNA isolation [214], and the general lack of 3'-poly-A tails in prokaryotic microorganisms [202].

### **Metaproteomics and Metaproteogenomics**

Metaproteomics, which constitutes the large-scale characterization of the entire protein complement of environmental microbiota at a given point in time, is a more direct and arguably suitable way to profile microbial function in complex communities than metatranscriptomics [198, 202]. This is because proteins, more specifically enzymes, are the molecules that ultimately

perform the function in a cell [215]. In contrast, there is little correlation between the abundance of the transcripts that mediate the synthesis of related proteins [216, 217]. However, as is the case with mRNA in metatranscriptome analysis, there are technical issues with protein extraction, separation and identification as well as inorganic and organic contaminants that currently make metaproteomics more onerous than metatranscriptomics [202]. This is so, particularly in samples with a high microbial diversity, in which case, each protein is diluted in a complex mixture and only the most abundant proteins are therefore likely to be identified [218]. Such cases, usually results in an over- or under-representation of certain microorganisms. Moreover, microorganisms in environmental samples may include species, which may have never been studied *in vitro* and their genomes not sequenced. Hence, their protein sequences, which are required for mass spectrometry identification, are not available in public databases [218].

To address this limitation, metaproteogenomics, which is a combination of metaproteomics and metagenomic approaches has been used [219]. Rastogi and Sani [170] noted that the extraction of total DNA and proteins from the same environmental sample, allows linking of biological functions to phylogenetic identity with greater confidence. Delmotte *et al.* [220] used a culture-independent metaproteogenomic approach to investigate the physiology of phyllosphere bacteria associated with leaves of soybean, clover and *Arabidopsis thaliana* plants, under *in situ* conditions. They found that *Methylobacterium* and *Sphingomonas* spp. are abundant in the phyllosphere of these plants and Sphingomonads possess a particularly large substrate spectrum on plant leaves. In follow up work done in the same laboratory, Innerebner *et al.* [221] found that plant-colonizing *Sphingomonas* spp. displayed a significant plant-protective effect by suppressing disease symptoms resulting from infection with the foliar plant pathogen *Pseudomonas syringae* pv. tomato DC3000 on *Arabidopsis thaliana*. To the best of the author's knowledge such studies, involving the use metaproteogenomic approaches to characterize phyllosphere microbiota of plants treated with foliar applications of compost tea have not been done. Neither have compost tea-disease studies that have used molecular techniques to characterize rhizosphere and phyllosphere microbiota in a single study, as was done by Knief *et al.* [222] to profile microbiota associated with rice cultivars. Such studies are essential to gain insight into mechanisms of disease suppression since some authors have ascribed a phyllosphere disease suppressive-effect of compost tea [49, 118]. More specifically, the use of metaproteogenomic approaches in contrasted environmental situations such in aerobic and anaerobic compost teas should allow (1) tracking new functional genes and metabolic pathways (2) identifying proteins preferentially associated with specific stresses and more importantly and (3) revisiting microbial ecology concepts with a functional point of view [198].

## Metabolomics

Metabolomics, which is concerned with the study of naturally occurring, low molecular weight organic metabolites within a cell, tissue or biofluid [223] does not in itself directly characterize microbial functionality. In contrast, it focuses on the ultimate response of an organism to genetic alterations, disease, toxicants, or environmental influences [73]. As such, metabolomics is regarded as the end-point of the 'omics' cascade [224] since the metabolome is most predictive of phenotype [225].

The disease suppressiveness of compost tea has been reported to be associated with the production of secondary metabolites by native microorganisms [1, 158, 161]. However, most researchers have failed to clearly identify and purify these metabolites [6, 42, 48, 49, 71]. Consequently, data on the profiles and functions of metabolites present in compost tea is very limited. As such, opportunities exist to use metabolomics platforms to decipher the role of disease-suppressive and phytotoxic metabolites produced by the microbial consortia in compost tea or the metabolic response of a plant in treated with compost tea [16].

Cao *et al.* [226] used direct-infusion mass spectrometry to study the metabolic effects of the symbiosis between the endophytic fungus *Neotyphodium lolii* and its host perennial ryegrass (*Lolium perenne*) in three different tissues (immature leaf, blade, and sheath). They detected changes in the metabolome in infected plants, with compounds such as mannitol, peramine and perloline being key compounds in infected plants. Rasmussen *et al.* [227] did similar work and suggested that the effects of endophytes on metabolic profiles of *L. perenne* can be considerable, depending on host plant characteristics and nutrient supply. Furthermore, metabolomics techniques have been used in medicine to gain insights into the mechanisms of drug action. For example, in a disease pathophysiology study, Rozen *et al.* [228] used metabolomics techniques to characterize a metabolic signature in response to the drug Riluzole, which is used to treat patients with motor neuron disease. They found that the pharmacodynamics of the drug was not related to metabolism of the drug itself, but rather its effects on biochemical pathways. It was therefore possible to separate motor neuron diseased patients from controls based on their metabolomics signatures, and patients that were on-and-off the drug treatment. The aforementioned studies serve as examples of the potential application of metabolomics techniques in plant physiopathological studies in which compost tea is used as a biocontrol agent.

There are also opportunities to explore the use of metabolomics techniques in discovery-driven research, which focuses more on questions than hypotheses [229]. That is, metabolomics can discover unexpected relationships and metabolite responses, which in itself can lead to hypothesis generation [230]. This is particularly important as research into compost tea is at an early evolutionary

stage where investigations are needed to understand biotic–biotic interactions and organismal responses to abiotic stressors. In this regard, metabolomics can contribute to our biological understanding both in a mechanistic and predictive manner [224].

Miller [231] reported that the major limitations of metabolomics techniques are the lack of: (i) databases with comprehensive information for metabolite identification and (ii) software for automated identification and quantitation of metabolites. However, public metabolomics databases are becoming more comprehensive with the increased use of metabolomics techniques in fields of microbiology, medical, plant, animal and food science [232]. There have also been advances in the development of informatic and statistical approaches to handle large volumes of data [233, 234].

## Limitations of DNA-Based Techniques

Due to associated pitfalls and biases, none of the molecular techniques provides complete access to the phylogenetic and functional diversity of complex microbial communities, which are often present in compost tea, compost and soil. Major pitfalls and biases are mainly related to extraction of DNA and PCR amplification, which are often essential steps in most molecular techniques used for microbial community analysis and functional diversity. Biases associated with DNA extraction include recovery efficiency and the representativeness of DNA recovered from environmental samples. Both of which are associated with incomplete or preferential lysis of some microbial cells. For example, spores are generally more resistant to cell lysis than vegetative cells, and Gram-positive cells are less susceptible to lysis than Gram-negative cells [235]. This means that DNA recovery might be reduced by degradation or adsorption DNA to matrix materials. More so, with the total amount of DNA present in a sample is unknown; it makes it difficult to assess the recovery efficiency by any extraction method [235]. Therefore, the same lysis technique may give different results with different sample types.

As it relates to the representativeness of DNA recovered, populations resistant to breakage for example small cells (0.3–1.2  $\mu\text{m}$ ) [93, 236] in environmental samples, would be fractionally underrepresented, while microorganisms that are easily lysed such as larger cells (>1.2  $\mu\text{m}$ ) would be overrepresented. This may influence the recovery of sequences from environmental samples and distort results relating to the community composition, richness and microbial community structure. Feinstein *et al.* [237] found that to minimize the risk of bias associated with DNA extraction, validated extraction methods should be used and DNA obtained from three successive extractions should be pooled.

Main biases associated with PCR amplification include inhibition by compounds such as humic acids [238],

preferential amplification of certain templates due to hybridization efficiency and specificity of primers [239–241], and formations of PCR artifacts e.g., chimeric molecules, deletion mutants, and point mutants [242]. Rainey *et al.* [243] further suggested that templates with a high % G+C content are discriminated against due to low efficiency of strand separation during the denaturation step of the PCR reaction. All of these biases, which relate to the sensitivity and specificity of PCR reactions, can lead to misleading results and conclusion concerning microbial community structure and functional diversity.

## Conclusions and Future Work

The need to find more sustainable approaches for managing plant diseases have fuelled research into alternative strategies such as compost teas. Numerous studies have demonstrated that compost tea can be used to suppress soil-borne and foliar and fruit diseases. However, the level of disease control provided by compost tea has generally been viewed as inadequate for conventional agriculture [4, 60, 114, 141] but important to organic producers who have limited disease control options. Despite the increasing amount of information, research into compost tea as a BCA is at an early evolutionary stage and the overarching challenge remains integrating findings into commercial crop production systems. An important step towards application of suppressive compost tea could be the development of quality control tools that may reduce the variability in disease efficacy [244]. Unfortunately, there is no single chemical or physical, easy-to-perform parameter that could predict suppression, therefore quality control is dependent on bioassays designed for a specific pathogen or disease [244]. This emphasizes the need for a better understanding of the mechanisms and antagonistic microorganisms involved in disease suppression.

Traditional methodological tools such as culture-based techniques or microscopic examination have allowed only a limited view of the complexity of disease control using compost tea [16]. Molecular approaches such as metagenomics, metatranscriptomics, metaproteomics, meta-proteogenomics and metabolomics can be used to better understand the relationships between microbial abundance, diversity and functions and disease suppressive efficacy of compost tea. Such an understanding is crucial in developing protocols for optimizing the compost tea production process so as to maximize disease suppressive effect without exposing the manufacturer or user to the risk of human pathogens. Presently, the main challenge for all 'meta' approaches is that only a small percentage of the vast number of ecologically important genes has been correctly annotated [202]. More so, sequence datasets contain only the most abundant genes from a very limited number of natural microbial communities [202], with include limited data from compost tea environments. Even

so, Poretsky *et al.* [245] noted that only a few of sequences from environmental samples can be confidently assigned a function, as many sequences have no close matches in existing public sequence databases. There is an opportunity to contribute to the development of more comprehensive and useful public sequencing databases through the greater use of postgenomic approaches in compost tea-disease studies; particularly studies which examines both the rhizosphere and phyllosphere effects of compost tea.

In the absence datasets generated by postgenomic methodologies, much has been done to improve the consistency of disease suppression with compost tea. This has included the modification of compost tea production steps, by the addition of nutrient amendments to ensure the growth of specific groups of microbes [1]. However, there is a need to test nutrient supplements for their effect on both targeted plant pathogens and non-targeted human pathogens [1]. To date, molasses has been demonstrated to support the growth of *E. coli* and *Salmonella* if inadvertently present in compost tea, posing worker and consumer health concerns [145, 146]. Further field studies are needed on the interaction between aeration and nutrient and/or microbial amendments for optimizing disease suppression with compost tea. Studies examining the integrated and/or combined use of compost tea with compost, endomycorrhizal fungi e.g. *Glomus intraradices*, and other types of BCA are also needed. There is a paucity of scientific information on the cost-benefit analyses of compost tea as an alternative or complementary tool in plant disease management. As such, studies that examine the practical and economic use of compost tea on a large-scale to suppress plant diseases are needed.

To this end, it is recommended that compost tea must be used as part of an integrated disease management system with other strategies, including genetic disease resistance, fertility and water management, disease and pest forecasting and other cultural approaches to enhance plant health [10].

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