

IMPACT OF COMPOST TEA IN CONTROLLING CHOCOLATE LEAF SPOT DISEASE AND SOIL MICROORGANISMS' DENSITY IN FABA BEAN (*VICIA FABA* L.)

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This study was conducted to determine the effect of compost tea (CT) as an alternative biological fungicide for controlling chocolate leaf spot disease in faba bean, with different application methods, in addition to the effects on soil biota, yield and growth parameters of faba bean. Compost tea as a foliar spray (F) and as a soil drench (SD), significantly decreased disease severity, while increased it by seed soaking (SS). Soil drenching, significantly increased yield weight, number of leaves, pods and seeds and weight of seeds. Soil microorganisms were enhanced significantly with the foliar spray treatment. Peroxidase enzyme (PO) increased by 200% by all methods. Polyphenol oxidase enzyme (PPO) reached 1411% by integrated treatment with F, SD and SS at 50% compost tea concentration. Total phenols reached 120% by SD at 25%. It can be concluded that compost tea after brewing for six days, can control chocolate leaf spot disease in broad bean. In addition, it improves almost all yield parameters, plant disease resistance against the fungal pathogen and enhanced soil microbes.

Keywords: chocolate leaf spot, compost tea, fungal pathogen, *Vicia faba*, soil microorganisms

Synthetic fungicides are widely used in agriculture to control fungal plant diseases. Most of them are highly toxic (Zaker, 2016), sometimes carcinogenic for humans and wild animals, nonbiodegradable, and long-term environmental pollutants. They are lack prolonged efficacy because of the development of resistance in plant pathogens (Daoubi et al., 2005). Efforts are going on to use organic products as alternatives to agrochemicals for plant protection.

Compost tea is an organic extract produced from composted organic matter and containing a diversity of naturally occurring microbes and soluble macronutrients. Previous studies have mentioned that compost extracts helped to enhance plant health quality, yield and nutritional traits (Kim et al.,

2015). Compost tea is a possible alternative to synthetic chemical fungicides because of its antimicrobial activities (Siddiqui et al., 2009 and Seddigh and Kiani, 2018). Microorganisms in compost teas may act through one or more biological control mechanisms, competing for nutrients and/or space (Al-Mughrabi et al., 2008). Bacterial extracts, for bacteria isolated from compost tea, i.e. *Brevibacterium linens* and *Bacillus subtilis* contain antifungal compounds, indicating that antibiosis is the main mechanism of action (On et al., 2015). Compost teas are safe for health and the environment, relatively cheap and advantageous for agronomy.

Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt as a source of food for both humans and animals and it improves soil fertility through nitrogen fixation. It is attacked by many foliar diseases such as chocolate spot (*Botrytis fabae* and *Botrytis cinerea*), which causes considerable losses in the yield and its component. Chocolate spot disease is a major problem for faba bean plants, because it infects and destroys the entire canopy of plants, limiting photosynthetic activity and affecting the productivity (Eisa et al., 2006).

The present work was designed to investigate the potential of compost tea to reduce chocolate leaf spot disease in broad bean caused by *B. fabae*, its possibility to induce resistance, and at the same time estimate the effect of compost tea on soil microorganisms and yield parameters of faba bean plants.

MATERIALS AND METHODS

1. Compost Source

Commercial compost from El-Arabiya-for-Organic-Fertilizer Factory was used. Compost was produced from a mixture of agricultural and animal wastes by aerobic method.

2. Chemical Analysis of Compost

Commercial compost was analyzed (Table 1) by unit of soil fertility, Department of Soil fertility and Microbiology, Desert Research Center.

Table (1). Elemental analysis of compost.

C/N	Major elements						
	%		mg/g				
	N	P	K	Fe	Mn	Zn	Cu
16.8	1.46	0.78	1.12	0.325	0.146	0.0785	0.0354

3. Source of Pathogen

Isolate of *B. fabae* was obtained from Department of Legume and Forage Crop Diseases, A.R.C, Giza, Egypt. The growing colonies were

transferred to new plates of faba bean dextrose agar (FDA) and incubated at 18-20°C.

4. Compost Tea Preparation

Aerated CT was produced by mixing mature commercial compost with distilled water at a ratio of 1:5 (w/v) and supplemented with 2% molasses for microbial growth stimulation. The entire contents were continuously aerated at room temperature with a fish tank bubbling pump. For further application, CT was poured through cheesecloth then cotton in lab experiments, sterilized by passing through 0.2 µm filter and stored in freezer until use.

5. Antifungal Activity of Compost Tea

Assay of antifungal activity by CT depended on the growth retardation level of the pathogen in growth medium containing CT. Double strength potato dextrose agar (PDA) medium was supplemented with faba bean leaf extract for growing tested pathogen (*B. fabae*). While still warm, medium was combined with an equal volume of filter sterilized CT, and after mixing, was poured into plates. Tested pathogen was inoculated in plate's central points. At the same time, it was inoculated in the same manner in single strength, CT- free medium plates. Plates were incubated at 26°C. Incubation was over when mycelium growth reached the substantial size either in the control or in the test plates, whichever came first, and the diameter of the colonies were measured. Antifungal activity in percent was determined against untreated control (Ibrahim, 2008).

6. Plant Materials

Faba been seeds (cv. Giza 429) were obtained from Legume Crops Research Department, Agricultural Research Center, Giza, Egypt, were used to investigate the effect of compost tea against *B. fabae* with different application methods.

7. Application of Compost Tea on Faba Bean Infected Plants

CT after brewing for six days (as an effective age for disease suppression from lab experiments results), and plastic bags (30 cm diameter, 40 cm height), each filled with 11 kg of un-sterilized clay soil were used in this experiment. Seven treatments of CT applied as follows:

Foliar spray (F)	Foliar spray + soil drench
Soil drench (SD)	Foliar spray + seed soaking
Seed soaking (SS)	Soil drench + seed soaking
Foliar spray + soil drench + seed soaking	

Three concentrations (25%, 50% and 75%) of CT were used with all tested application methods.

Faba beans (cv. Giza 429) were planted, mid-November 2013, at a rate of 5 seeds/bag. Twenty days after seeding, the plants were thinned to 3 seedlings/bag. Treatments of CT were applied as F at 40 days from sowing and one day before the artificial inoculation with *B. fabae* spore suspension. The suspension prepared by, flooded one single colony of *B. faba* in plate by 5 ml sterilized distilled water, scraping the surface of the colony to release the spores, took up the water containing the released spores from the plate and transferred to a 5 mL sterilized 0.85% NaCl solution containing 0.05% Tween 80, vortexed the spore suspension for one minute to break any mycelium fragment, then gently with a pipette tip took the suspension to perform spore count by count chamber (2.5×10^5 conidia/ml supplemented with 0.05% Tween 20). Bags inoculated with only *B. faba* spore suspension served as control treatment. For SD treatments CT was added directly, before seed sowing, to soil at a rate of 100 ml/bag. Seed treatments were done by soaking faba bean seeds in CT concentrations for 24 h before seeding. All inoculated bags were covered with hyaline polyethylene sacs for 48 h as moist chambers (Mahmoud et al., 2012). The trial was designed as randomized complete block design (RCBD) with three triplicates for each treatment.

8. Disease Severity

Assay of disease severity was done according to the scale of Bernier et al. (1993). After fifteen-days post inoculation, assay was done as follows: 1 = no disease symptoms or very small specks (highly resistant); 3 = few small discrete lesions (resistant); 5 = some coalesced lesion with some defoliation (moderately resistant); 7 = large coalesced sporulating lesions, 50% defoliation and some dead plant (susceptible); 9 = extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (highly susceptible). Chocolate spot disease severity % was assessed according to the following formula,

$$\text{Disease severity \%} = \frac{\sum (n \times v)}{9 N} \times 100$$

Where, (n) = Number of plants in each category; (v) = Numerical values of symptoms category; (N) = Total number of plants; (9) = Maximum numerical value of symptom category.

9. Yield and Growth Parameters

Yield weight (g), number of leaves, number of pods and dry seeds weight (g) and number of seeds per plant were recorded after fifteen-day post inoculation with *B. fabae* and after 55 days from starting.

10. Oxidative Enzymes Activities

Peroxidase enzyme activity was measured as described by Chance and Maehly (1955). Polyphenol oxidase activity was assayed according to

the method of Taneja and Sachar (1974). Enzyme activity was expressed as $\Delta 420$ and $\Delta 430/\text{min/g}$ fresh weight, respectively. All crude leaf enzyme extract was prepared according to Patra and Mishra (1979).

The percentage of increase in PO and PPO activities were calculated using the following formula:

Increase (%) =

Value of treatment – value of control / value of control $\times 100\%$

11. Total Phenols Assay

From one of three replicates for each treatment, the tenth plant leaf was taken and used in extraction process according to the method of Simons and Ross (1971). Total phenols were calculated for each treatment as milligrams of catechol per one-gram fresh weight according to standard curve of catechol. The increase in total phenol content as percentage was calculated using the following formula:

Increase (%) =

Value of treatment – value of control / value of control $\times 100\%$

12. Total Microbial Count

For all microbial investigations in soil, 1 g of soil was suspended in 10 ml of sterilized water and stirred for half an hour. Then, serial dilutions were prepared, cultured, and enumerated by total plate counts in nutrient plate agar.

Media

- Potato dextrose agar

Glucose	20.0 g	Agar	15.0 g
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*Leaf extract	100 ml	**Potato infusion	1.0 L
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*Freshly plucked leaves of faba bean were washed and dried in the shade (3-5 days). Following washing and drying, leaves were ground in a domestic blende, then 5 g from the powder was added to 100 ml of sterile distilled water, and boiled for 10 min in conical flasks. The extract filtered through Watman No.1. The final volume was 100 ml. **A portion of 300 g potatoes was peeled and diced. Five hundred ml distilled water was added, the mixture was boiled for 30 min, filtered through cheesecloth and the final volume was adjusted to one liter.

- Nutrient agar

Glucose	10.0 g	Peptone	10.0 g
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Yeast extract	5.0 g	Agar	15.0 g
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13. Statistical Analysis

Data were analyzed statistically in triplicates for the least significant difference (LSD). The LSD technique was used to test the differences between means at probability < 0.05 .

RESULTS AND DISCUSSION

1. Elemental Analysis of Compost

The elemental analysis of compost showed that C/N ratio was 16.8; N, P and K % were 1.46, 0.78 and 1.12, respectively, and Fe, Mn, Zn and Cu mg/g were 0.325, 0.146, 0.0785 and 0.0354, respectively (Table 1).

2. Antifungal Activity of Compost Tea

Aerated CT was brewed for seven days. It was tested daily, *in vitro*, against fungus pathogen *B. fabae*. It showed antifungal activity towards the fungus after three days, and reached 100% inhibition after six days (Fig. 1 and 2). In these experiments, the antifungal activity increased with brewing time. Accumulation of soluble nutrients from compost to CT during brewing time enhanced beneficial microorganisms in tea (Ingham, 2005), which produce antimicrobial compounds. These microbial inhibitory substances accumulated by time and make the suppression effect of tea (Weltzien, 1991).

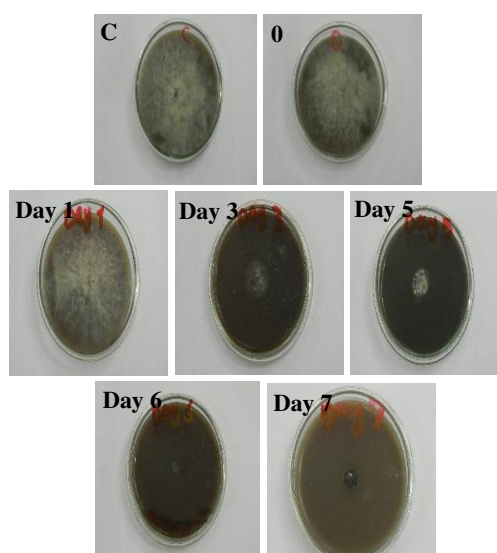


Fig. (1). Antifungal activity of aerated compost against *Botrytis fabae* in PDA containing CT, at zero day (0), one day (Day 1), three days (Day 3), five days (Day 5), six days (Day 6), seven days (Day 7) and PDA without CT as a control (C).

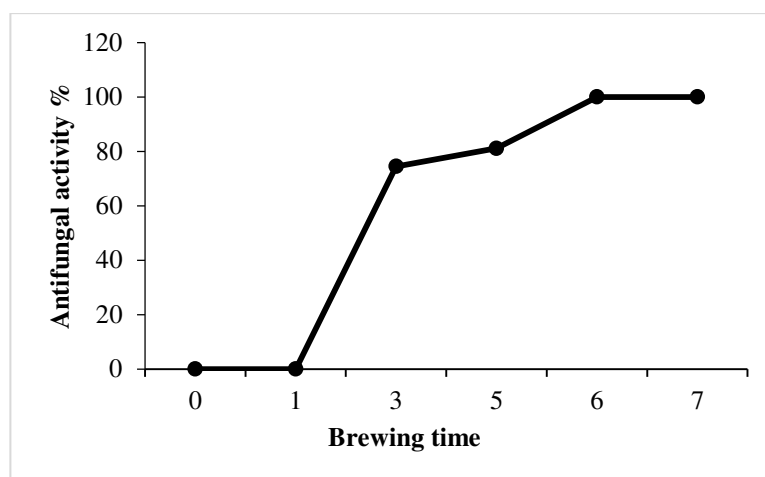


Fig. (2). Antifungal activity % of compost tea during brewing time.

3. Effect of Compost Tea on Disease Severity

After fifteen days of inoculation with *B. fabae*, F decreased disease severity the most (Table 2 and Fig. 3). Compost Tea at 50 and 75% concentrations, significantly decreased the chocolate spot disease severity by 59 and 50%, respectively compared with control. In addition, SD treatment at 25 and 50% CT, significantly decreased disease severity. An integrated treatment with both methods, F and SD at 50% CT decreased the disease severity, significantly, about 40% (Fig. 3). In contrary, SS increased disease severity. The application of CT to soils either can be categorized as induced suppression (Baker and Cook, 1974), by making conditions more favorable for the development of resident antagonists, or can be categorized as introduced suppression (Hornby, 1983) by adding antagonists to the soil.

The efficiency of CT in controlling disease severities was reported by several authors. In this respect, Al-Mughrabi (2007) revealed that, CT reduced severity by 29 and 27%, when applied foliarly against late blight (*Phytophthora infestans*) of potato (*Solanum tuberosum*). In addition, CT as a SD significantly suppressed the severity of late blight foliar disease (*Phytophthora infestans*) in tomatoes and potatoes (Islam et al., 2013). Moreover, dollar spot disease (*Sclerotinia homoeocarpa*) can be controlled by CT (Hsiang and Tian, 2007). The authors suggested that, suppression by CT depend on having beneficial effects on soil microorganisms, and increase both of their diversity and number in the soil. On other hand, diseases, powdery mildew, Septoria leaf spot, and bacterial speck, incidence and severity, were insignificantly reduced by CT (McGrath, 2007). Possible reasons might be including that CT was ineffective including lack of appropriate organisms in compost, unsuitable recipe or brewing conditions, applications made at the wrong time of day and treatments needed earlier in crop development or perhaps included a seed treatment.

Table (2). Effect of compost tea treatments and concentrations on disease severity % (DS %).

Treatments		DS%	SD
F	25% CT	50.62	8.56
	50% CT	11.11	0.00
	75% CT	13.58	4.28
	Mean	25.10	22.13
SD	25% CT	20.99	11.31
	50% CT	13.58	4.28
	75% CT	29.63	6.41
	Mean	21.40	8.03
SS	25% CT	48.15	12.83
	50% CT	53.09	3.55
	75% CT	30.86	4.27
	Mean	44.03	11.67
F+SD	25% CT	25.93	4.35
	50% CT	16.05	4.28
	75% CT	34.57	18.32
	Mean	25.52	9.27
F+SS	25% CT	29.63	6.41
	50% CT	40.74	12.83
	75% CT	33.33	0.00
	Mean	34.57	5.66
SD+SS	25% CT	33.33	0.00
	50% CT	33.33	0.00
	75% CT	25.93	7.41
	Mean	30.86	4.27
F+SD+SS	25% CT	40.74	12.83
	50% CT	25.92	12.83
	75% CT	33.33	0.00
	Mean	33.33	7.41
Control		27.16	5.66
L.S.D. (5%)	App. methods	2.64	
	Concentrations	1.51	
	Interaction	610.50	

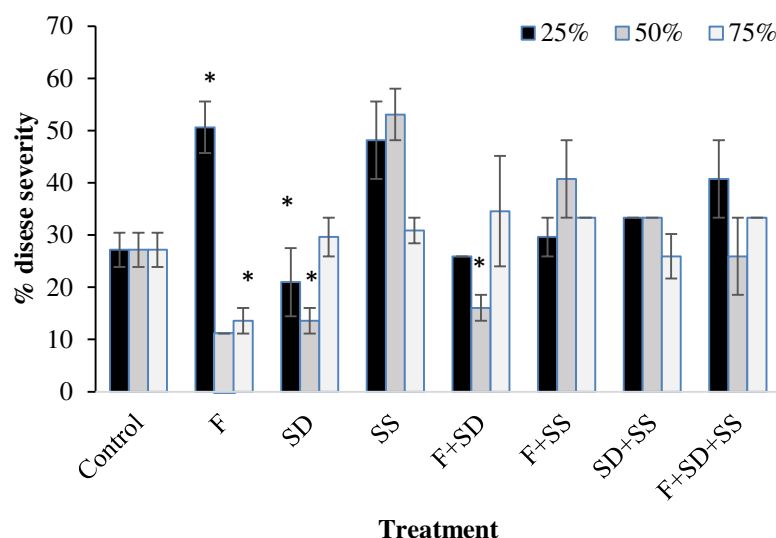


Fig. (3). Effect of compost tea treatments on disease severity % (DS %).

(F) foliar spray, (SD) soil drench, (SS) seed soaking.

* Significant ($p < 0.05$)

4. Effect of Compost Tea on Yield Weight

Compost tea has a role as a liquid biofertilizer, because it has a high nutrient value and rapid availability for plants. It has been found that CT contains nitrogen mainly as inorganic form like ammonia (Price and Duddles 1984 and Gross et al., 2007) and can provide nutrients instantly to the plants much like the chemical fertilizers. It is also boost the plant and soil-enhancing activity of soil life, because it is rich in microorganisms that recycle organic matter. In this study, adding CT as a SD increased yield weight significantly; the increment was almost 200% at 25% CT concentration, and more than 100% at 50 and 75% CT concentrations (Table 3 and Fig. 4). These results are in agreement with El Hanafi (2005), who revealed that the application of CT to root zone showed a remarkable yield, and increased root growth significantly compared with control. In addition, Essah et al. (2013) mentioned that tuber yield in potato production was an optimum, with reduced or no fungicide application.

Yield weight increased significantly with some other concentrations within treatments, with 50% CT as foliar, with 75% CT as SS treatment, with 50% CT as integrated foliar and SD treatment, with 75% CT as integrated foliar and SS treatment, and with 75% CT as integrated foliar, SD and SS treatment.

Table (3). Effect of compost tea treatments and concentrations on yield weight.

Treatments		Yield weight	SD
F	25% CT	19.69	0.63
	50% CT	25.12	0.16
	75% CT	19.82	0.81
	Mean	21.54	3.29
SD	25% CT	64.27	1.75
	50% CT	26.47	0.89
	75% CT	63.63	0.59
	Mean	51.46	21.65
SS	25% CT	6.25	0.46
	50% CT	16.31	0.38
	75% CT	25.62	0.67
	Mean	16.06	9.69
F+SD	25% CT	16.18	1.22
	50% CT	25.20	0.81
	75% CT	21.04	1.40
	Mean	20.81	4.51
F+SS	25% CT	19.16	1.11
	50% CT	12.77	0.89
	75% CT	26.88	1.50
	Mean	19.60	7.06
SD+SS	25% CT	20.96	3.09
	50% CT	20.78	0.67
	75% CT	20.67	0.78
	Mean	20.80	0.15
F+SD+SS	25% CT	16.75	0.75
	50% CT	13.86	0.97
	75% CT	28.13	1.04
	Mean	19.58	7.54
Control		21.63	0.00
L.S.D. (5%)	App. methods	1.66	
	Concentrations	0.95	
	Interaction	6.65	

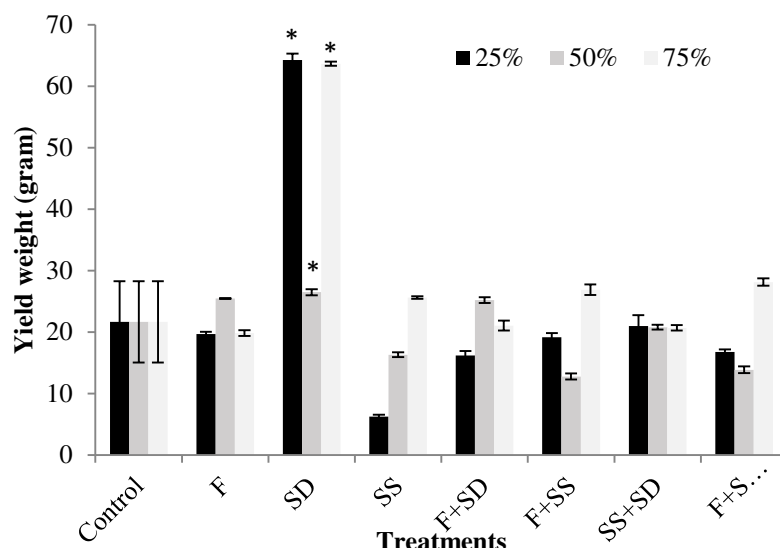


Fig. (4). Effect of compost tea treatments on yield weight of faba bean. (F) foliar spray, (SD) soil drench, (SS) seed soaking.

* Significant ($p < 0.05$)

5. Effect of Compost Tea on the Number of Leaves

Foliar spray and SD treatments with CT at 25 and 75% concentrations, significantly increased number of leaves, comparing with control (Table 4 and Fig. 5). Makkar et al. (2017) stated that there was an indication of high photosynthetic efficiency combined with foliar application of CT. El-Sherbeny et al. (2012) mentioned that, medium CT level did not significantly increase the number of leaves of *Brassica rapa* (turnip) plants, compared with control. Decrement of the number of leaves with the increase of CT concentration (Fig. 5) might be due to the age of applied CT (six days), which contains several microbial metabolites beside nutrients. Some of these metabolites may act against increasing the number of leaves at high concentrations of CT. These results are in agreement with Kim et al. (2015), who revealed that, the red leaf lettuce treated with F spray and with higher CT concentration was less effective in increasing the number of leaves than in the lower CT concentration. Integrated treatment of SD with SS at 25% CT significantly increased the number of leaves. Several authors reported similar results. Hegazi and Algharib (2014) recorded that the number of plant leaves, improved as plants received 25% of mineral NPK + 75% of CT as a SD, but Suganthi and Jayanandhan (2015) mentioned that the SS with CT increased the number of leaves by around 43% compared with water SS as a control. Meanwhile, foliar application of CT, did not significantly affect the number of leaves per plant in tomatoes infected by powdery mildew (Segarra et al., 2009). At 15 cm³/seedling of CT, as SD, with or without

mineral NPK, for "Aggizi" olive seedlings under greenhouse condition, all vegetative growth, plant height increment %, number of leaves/seedling and dry weight of leaves %, all increased compared with control (Haggag et al., 2014). In contrast, Mohd Din et al. (2017) reported that the effect of compost extract on the shoots length was not significant.

Table (4). Effect of compost tea treatments and concentrations on leaves number.

Treatments		Leaves number	S.D.
F	25% CT	44.89	0.70
	50% CT	28.10	0.58
	75% CT	32.44	0.77
	Mean	35.44	8.36
S.D	25% CT	36.56	0.20
	50% CT	24.11	2.83
	75% CT	33.22	1.26
	Mean	31.30	6.44
S.S.	25% CT	15.56	1.17
	50% CT	18.67	0.66
	75% CT	17.89	2.22
	Mean	17.37	1.62
F+S.D	25% CT	27.11	1.34
	50% CT	17.56	2.22
	75% CT	17.10	1.53
	Mean	20.89	5.39
F+S.S.	25% CT	23.00	1.46
	50% CT	28.56	0.20
	75% CT	23.44	1.02
	Mean	25.00	3.09
S.D+S.S.	25% CT	32.22	0.77
	50% CT	19.56	0.84
	75% CT	24.67	1.33
	Mean	25.48	6.37
F+S.D+S.S.	25% CT	25.22	2.17
	50% CT	25.89	1.54
	75% CT	17.22	1.07
	Mean	22.78	4.82
Control		31.337	0
L.S.D. (5%)	App. methods	0.52	
	Concentrations	0.30	
	Interaction	2.09	

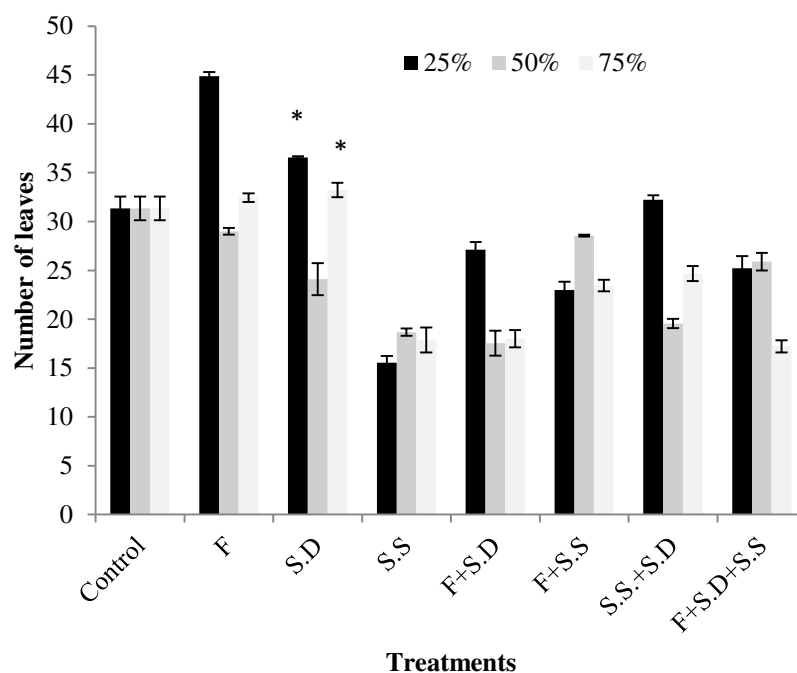


Fig. (5). Effect of compost tea treatments on leaves number of faba bean.
(F) foliar spray, (SD) soil drench, (SS) seed soaking.
* Significant ($p < 0.05$)

6. Effect of Compost Tea on the Number of Pods

Adding CT as a SD enhanced number of pods, significantly, compared with control (Table 5 and Fig. 6). It was about 127% at 25% SD concentration. These results are in agreement with Ali (2015), who mentioned that applying CT to faba bean enhanced the number of pods per plant. In addition, integrated treatment of F, SD and SS increased the number of pods significantly, compared with control at 75% concentration.

Table (5). Effect of compost tea treatments and concentrations on pods number.

Treatments		Pods number	SD
F	25% CT	3.220	0.19
	50% CT	3.330	0.00
	75% CT	3.110	0.19
	Mean	3.220	0.11
SD	25% CT	8.333	0.33
	50% CT	3.670	0.00
	75% CT	8.333	0.20
	Mean	6.779	2.48
SS	25% CT	1.447	0.39
	50% CT	2.667	0.36
	75% CT	3.443	0.20
	Mean	2.519	1.00
F+SD	25% CT	3.223	0.39
	50% CT	3.557	0.51
	75% CT	3.223	0.39
	Mean	3.334	0.19
F+SS	25% CT	3.557	0.20
	50% CT	2.553	0.69
	75% CT	3.667	0.34
	Mean	3.259	0.53
SD+SS	25% CT	2.890	0.19
	50% CT	2.780	0.19
	75% CT	2.777	0.51
	Mean	2.816	0.06
F+SD+SS	25% CT	3.333	0.34
	50% CT	1.890	0.38
	75% CT	4.443	0.20
	Mean	3.222	1.28
Control		3.667	3.14
L.S.D. (5%)	App. methods	0.110	
	Concentrations	0.060	
	Interaction	0.450	

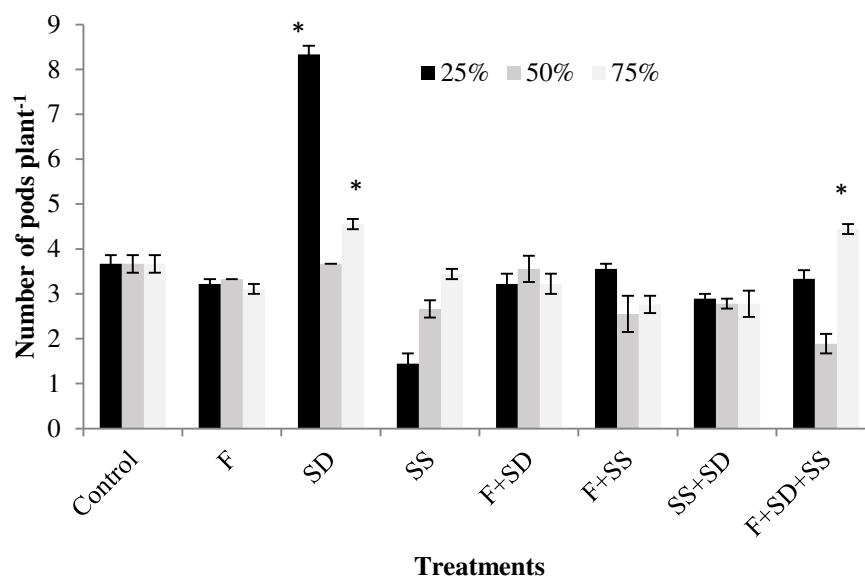


Fig. (6). Effect of compost tea treatments on the number of pods.

(F) foliar spray, (SD) soil drench, (SS) seed soaking.

* Significant ($p < 0.05$)

7. Effect of Compost Tea on the Number and Weight of Seeds

Application of compost as SD increased the number of seeds and weight of seeds, significantly, compared with control, (Table 5, Fig. 7 and 8). Seed soaking, alone or integrated with F or SD treatments, at 75% concentration, significantly increased the weight of seeds. Besides that, F when integrated with SD at 50% concentration CT also increased the weight of seeds. The enhancement is usually due to the stimulation of growth by directly improving the nutrient availability, or indirectly by promoting the cation exchange capacity of plants (Ingham, 2005). According to Hegazi and Algharib (2014), application of CT as SD, was better than as a foliar. It has an effective role on the enhancement of plant yield components (100-seed weight) after it was added during the irrigation by SD method (Ali, 2015). SS with CT, usually, gets a healthy bunch of soil microbes coating the seed.

Table (6). Effect of compost tea treatments and concentrations on seeds number/plant and seeds weight.

Treatments		Seeds number	SD
F	25% CT	5.667	0.34
	50% CT	7.670	1.09
	75% CT	5.333	0.34
	Mean	6.223	1.26
SD	25% CT	21.443	1.17
	50% CT	8.000	0.33
	75% CT	19.780	1.17
	Mean	16.408	7.33
SS	25% CT	1.667	0.34
	50% CT	5.110	0.38
	75% CT	7.670	1.09
	Mean	4.816	3.01
F+SD	25% CT	3.997	0.58
	50% CT	8.223	0.51
	75% CT	6.443	0.84
	Mean	6.221	2.12
F+SS	25% CT	3.223	0.51
	50% CT	3.443	0.51
	75% CT	6.220	0.84
	Mean	4.296	0.16
SD+SS	25% CT	6.110	0.38
	50% CT	5.110	0.19
	75% CT	6.000	0.88
	Mean	5.740	0.55
F+SD+SS	25% CT	3.333	0.36
	50% CT	2.777	0.51
	75% CT	6.887	0.51
	Mean	4.332	2.23
Control		8.220	0.00
L.S.D. (5%)	App. methods	0.180	
	Concentrations	0.100	
	Interaction	0.710	

Treatments		Seeds weight	SD
F	25% CT	6.880	0.23
	50% CT	6.710	0.86
	75% CT	7.400	0.15
	Mean	6.997	0.36
SD	25% CT	22.950	0.52
	50% CT	9.667	0.25
	75% CT	22.453	0.66
	Mean	18.357	7.53
SS	25% CT	2.767	0.42
	50% CT	6.797	0.34
	75% CT	10.583	1.51
	Mean	6.716	3.91
F+SD	25% CT	6.480	0.44
	50% CT	11.293	0.67
	75% CT	7.703	0.12
	Mean	8.492	2.50
F+SS	25% CT	4.120	0.58
	50% CT	5.117	0.31
	75% CT	10.317	1.46
	Mean	6.518	3.33
SD+SS	25% CT	7.580	0.32
	50% CT	7.343	0.66
	75% CT	9.947	0.05
	Mean	8.290	1.44
F+SD+SS	25% CT	5.083	0.31
	50% CT	4.980	1.16
	75% CT	7.857	0.77
	Mean	5.973	1.36
Control		9.520	0.00
L.S.D. (5%)	App. methods	0.240	
	Concentrations	0.130	
	Interaction	0.940	

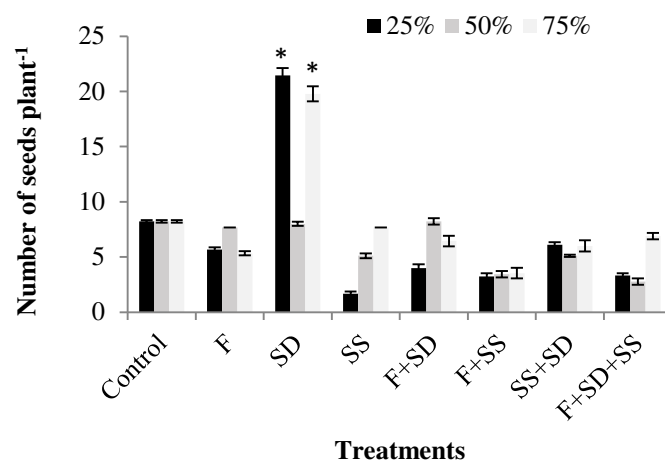


Fig. (7). Effect of compost tea on the number of seeds.

(F) foliar spray, (SD) soil drench, (SS) seed soaking.

* Significant ($p < 0.05$)

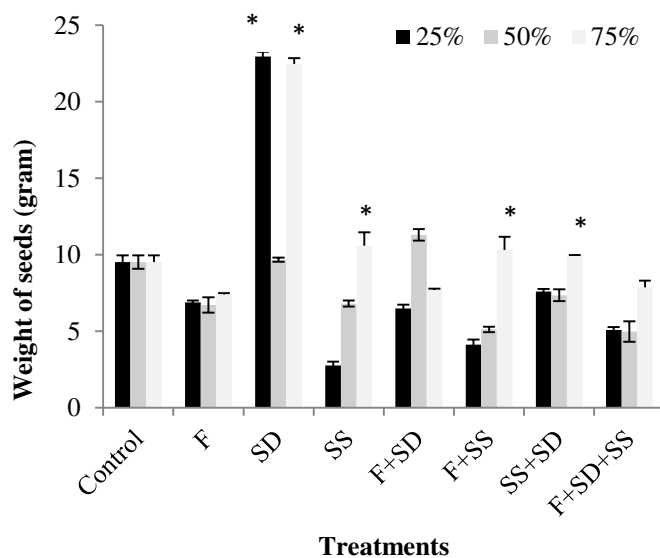


Fig. (8). Effect of compost tea treatments on the weight of seeds

(F) foliar spray, (SD) soil drench, (SS) seed soaking.

* Significant ($p < 0.05$)

8. Effect of Compost Tea Treatments on Soil Microorganisms

Adding CT significantly increased soil microorganisms in all application methods compared with the control (Table 7 and Fig. 9). Foliar spray was the most significantly effective on soil microorganisms compared with other methods. This approach reveals that CT contains long chain carbon molecules that provide carbon and oxygen for soil microorganisms (Grobe, 1997). Compost tea naturally occurring microorganisms might have the opportunity to become a part of the soil and rhizosphere microbial community (Bess, 2000). The total microbial count was 676.7×10^3 , with 25% CT (Fig. 9). Foliar spray with CT allows nutrients to be absorbed by the plants directly through stomata on their leaf surfaces. In our experiments CT age (six days), which was mainly effective for disease suppression, might be, was not loaded enough with nutrients as normally used fresh CT, because of the microbial growth rates and nutrients consumed by microorganisms. Therefore, direct absorption by leaf surface was more effective in root zone and microbial community in rhizosphere. Spraying of CT led to a positive effect on N, P and K concentrations uptakes, (Abd El-Hameed, 2008). Microorganisms' different populations were greater in the treatments of CT, and increased with time as well (Taha et al., 2016). These factors affecting soil microorganisms with consideration of CT age (six days old), concentration and application method. On the other hand, the highest values coming from foliar application compared with other application methods may refer to the age of CT that inhibit the pathogen and decrease disease severity the most by foliar application as mentioned before.

9. Effect of Compost Tea on Oxidative Enzymes Activities

All plants possess resistant mechanisms. Oxidative enzymes have a role in the defensive reactions of infected plants. Enzymes responsible for induction of resistance in plants include (PO) and (PPO). Thus, attention has been given to them. In this study, PO increased by around 200%, compared with control, with all CT treatments, with almost no difference among treatments (Fig. 10). Polyphenol oxidase enzyme in faba bean leaves increased by 1411% (Fig. 11) by integrated treatment with F, SD and SS at 50% CT concentration. While increased 811% with SS treatment at 75% CT concentration. Moreover, 760% and 714% with F and SS and foliar treatments respectively at 25% CT concentration. Soil drench treatment decreased the enzyme activity when applied alone or integrated with F or F and SS as shown in Fig. (11).

Table (7). Effect of compost tea treatments and concentrations on soil microorganisms.

Treatments		CFU*1000	SD
F	25% CT	676.67	228.98
	50% CT	84.67	16.17
	75% CT	170.00	26.46
	Mean	310.44	320.01
SD	25% CT	46.67	10.41
	50% CT	323.33	158.85
	75% CT	5.67	2.89
	Mean	125.22	172.79
SS	25% CT	59.33	9.24
	50% CT	114.33	7.77
	75% CT	108.67	10.26
	Mean	94.11	30.25
F+SD	25% CT	357.33	40.50
	50% CT	143.00	18.36
	75% CT	41.00	12.29
	Mean	180.44	161.46
F+SS	25% CT	352.33	11.37
	50% CT	91.00	18.33
	75% CT	242.67	46.69
	Mean	228.67	131.23
SD+SS	25% CT	226.67	31.56
	50% CT	181.33	17.24
	75% CT	114.67	25.32
	Mean	174.22	56.34
F+SD+SS	25% CT	281.67	118.93
	50% CT	76.67	13.58
	75% CT	143.67	30.66
	Mean	167.33	104.53
Control		40.30	0.00
L.S.D. (5%)	App. methods	30.04	
	Concentrations	17.17	
	Interaction	120.15	

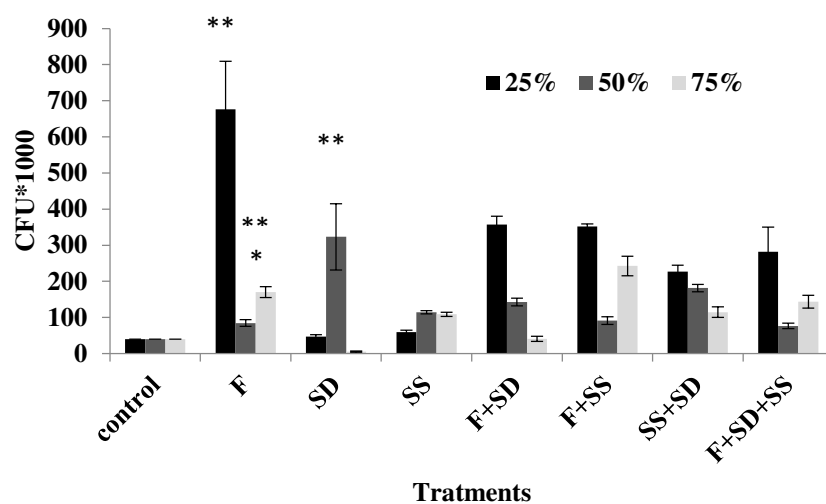


Fig. (9). Effect of compost tea treatments on the total microbial count of soil (F) foliar spray, (SD) soil drench, (SS) seed soaking.
**Most significant ($p < 0.01$)

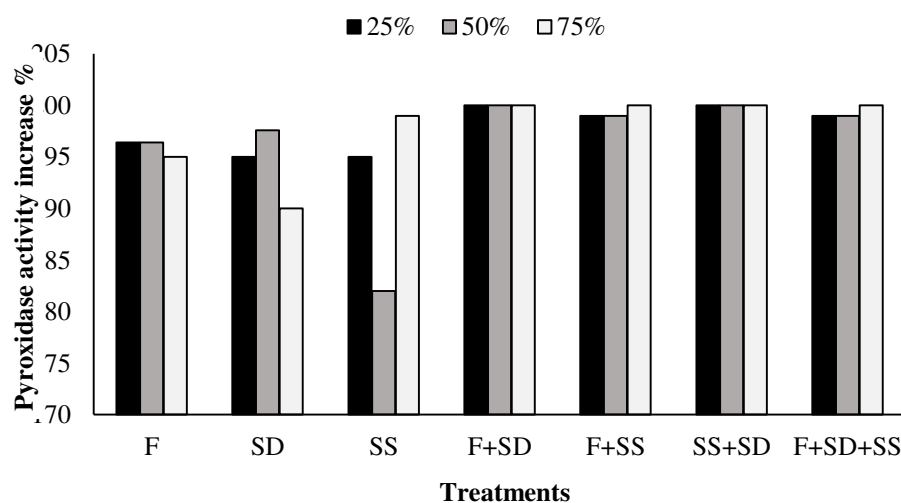


Fig. (10). Effect of compost tea treatments on peroxidase enzyme activity. (F) foliar spray, (SD) soil drench, (SS) seed soaking.

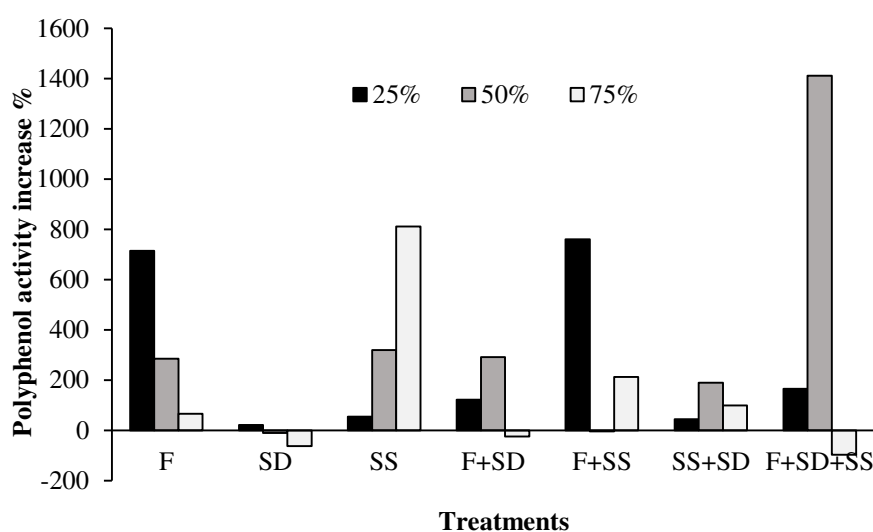


Fig. (11). Effect of compost tea treatments on polyphenol oxidase enzyme activity.

(F) foliar spray, (SD) soil drench, (SS) seed soaking.

Goldstein (1998) reported that compost extracts activate disease-resistant genes in plants, in response to the presence of pathogen; they mobilize chemical defense against the pathogen invasion. Activity of inducible enzymes such as PO and PPO were increased in infected okra plants pretreated with CT. The induction increments were observed in the early phase of infection (Siddiqui et al., 2009). Both of PPO and PO enzymes are responsible for phenolic compounds oxidation into anti-microbial quinones. In plant cells infected by phytopathogens, these conferring disease resistance during incompatibility reactions (Chittoor et al., 1999). Moreover, Kazan et al. (1998) added that, PO induction was a consequence of the biological and chemical activities of the CT. The importance of PO activity in disease resistance might be due to its ability to oxidize phenolic compounds to quinones, which are more toxic to pathogen than the original phenolics. On other hand, Segarra et al. (2009) reported that PO was not activated when CT was applied to foliage. He regarded this lack of response in the plant due to a priming effect, since plant defenses would only be activated by the further presence of the pathogen. In this case, the effects of CT could be comparable with the effects of beneficial microorganisms in the induction of plant resistance (ISR) by priming.

10. Effect of Compost Tea on Total Phenols

Phenolic compounds are produced by plants and microorganisms, with variation among and within species. Organisms synthesize phenolic compounds as a response to ecological pressures such as pathogen and insect attack, UV radiation and wounding. Secondary metabolite antioxidants such as phenolics, in plants may provide protection against a number of diseases (Gülçin, 2012). Total phenols were enhanced by about 120%, by CT as SD at 25% concentration. It reached about 61% with SD treatment integrated with F and SS treatments at 50% concentration, compared with control, as revealed by Fig. (12). These results could be discussed in light of the findings of Estiarte et al. (1994), who suggested that, defensive properties including phenolics were formed in a greater concentration at low nitrogen content (nutrient stress). Synthesis of high phenolic compounds initiated by the key enzyme, phenylalanine ammonia lyase (PAL) correlated to these stress biotic factors (Naoumkina et al., 2010 and Sarma et al., 2015). These properties increased when the root absorbed more organic nutrients, as a result of the root-rhizosphere induction (Shen et al., 2013). By 10% CT treatment as SD, total phenols increased over 62% greater than control in pepino fruit (Javanmardi and Hasanshahian, 2014). The rest of results can be discussed by the findings of Pant et al. (2009), who revealed that CT-treated pak cho plants had lower total phenolics compared with control plants. The significant positive effect on total phenolics compared with the control was under chemical fertilization. Many reports have demonstrated the beneficial effect of compost extracts, but the influence on phenolic content and antioxidant properties is still lacking. (Mohd Din et al., 2017).

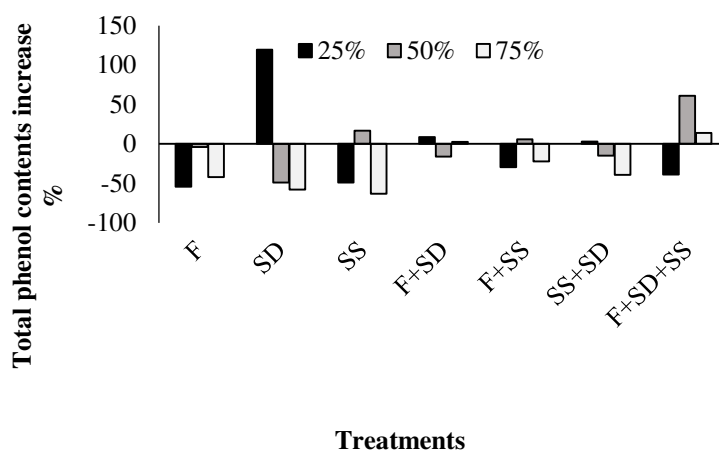


Fig. (12). Effect of compost tea treatments on total phenols. (F) foliar spray, (SD) soil drench, (SS) seed soaking.

CONCLUSION

This study clearly demonstrated that the application of compost tea on chocolate leaf spot disease in broad bean (*V. faba*), decreased diseases severity significantly by almost 60% with foliar application, and 50% by soil drenching, while SS increased disease severity. El-Sayed (2017) revealed that using commercial fungicides (Fungicop, Tridex and Oxydor), as a foliar before inoculation, in pots, against chocolate spot caused *B. fabae* decreased diseases severity by 89.2, 90.4 and 96%, respectively. The number of pods increased by about 26 to 37% (soil drench with compost tea increased number of pods by about 127% with only 25% compost tea concentration). At the same time, compost tea improved growth parameters, oxidative enzymes, phenol contents and soil fertility. Soil drenching significantly increased yield weight, number of leaves, number of pods, weight of seeds and number of seeds. Soil microorganisms were enhanced significantly with foliar spray treatment. Peroxidase enzyme increased by 200% by all methods. Polyphenol oxidase enzymes reached 1411% with foliar application at 50%. Total phenols reached 120% by soil drenching at 25%.

From the practical point of view, compost tea could be suggested as an eco-friendly strategy that could control leaf spot disease in broad bean and reduced the chemical-based fungicide dependency with no negative effect on soil microbes.

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أثر استخدام شاي الكمبوست في مقاومة مرض التبقع الشيكولاتي وكثافة ميكروبات التربة لنبات الفول البلدي

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تم إجراء الدراسة بغرض دراسة تأثير شاي الكمبوست كمبيد حيوي بديل في مكافحة مرض التبقع الشيكولاتي في الفول، مع دراسة تأثير طرق المعاملة المختلفة على مكافحة المرض، بالإضافة إلى تأثيره على كل من ميكروبات التربة وإنتاجية النبات وعوامل نموه المختلفة. وقد أوضحت الدراسة أن إضافة شاي الكمبوست بالرش على النبات أو بإضافته للتربة له قدرة ملحوظة على خفض درجة الإصابة بالمرض، بعكس معاملة البذور بشاي الكمبوست التي أدت إلى زيادة الإصابة بالمرض. وقد أوضحت أيضًا أن معاملة التربة بشاي الكمبوست أدى إلى زيادة ملحوظة في كل من وزن المحصول وعدد الأوراق وعدد القرون وكذلك وزن وعدد البذور. كما أوضحت النتائج أن المعاملة بالرش الورقي لشاي الكمبوست أدت إلى زيادة أعداد ميكروبات التربة بدرجة عالية. وكذلك زيادة نسبة إنزيمات الأوكسيداز ٢٠٪ وإنزيمات البيروكسيداز ١٤١٪. كما أدت معاملة التربة بشاي الكمبوست بنسبة ٢٥٪ إلى زيادة الفينولات الكلية بنسبة ١٢٠٪. وعليه فإنه يمكن من الدراسة إستخلاص أن شاي الكمبوست بعد تخميره لمدة ٦ أيام يمكنه مكافحة مرض التبقع الشيكولاتي في الفول ويزيد من مقاومة النبات للفطر الممرض بالإضافة إلى تحسينه لخواص المحصول بالإضافة إلى زيادة خصوبة التربة.