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Biochemical changes in sunflower plants infected by *Alternaria* leaf blight

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Abstract
Alternaria leaf blight of sunflower has been considered as a potentially destructive disease. Due to cons of using chemical pesticides, in this current study we are using eco-friendly approach such as compost tea and seaweed formulation for the control of *Alternaria* disease. Compost tea is a liquid extract made from compost that contains a variety of nutrients, growth compounds and beneficial microorganisms. The microrganisms present in the compost tea produce biomolecules with antibiosis potential which contributes to disease suppression. Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mL^{-1} at 60 DAS showed increased defense enzymes such as superoxide dismutase (39.47 µg/50 per cent inhibition) and peroxidase (16.86 µg/min/mg protein) when compared to superoxide dismutase (59.37 µg/50 per cent inhibition) and peroxidase (10.76 µg/min/mg protein) in control plants. Secondary metabolites such as phenolics (67.92 mg/g FW) and flavonoids (60.01 mg/g FW) was maximum in foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mL^{-1} at 60 DAS when compared to phenolics (57.48 mg/g FW) and flavonoids (41.87 mg/g FW) in control plants. This study demonstrated that foliar spray of compost tea and propiconazole can be used effectively for the management of *Alternaria* blight in fields as it exhibited more defense enzyme activity and increased growth and yield parameters.

Keywords: *Alternaria*, compost tea, peroxidase, superoxide dismutase, sunflower

Introduction
Sunflower (*Helianthus annuus* L.) a member of family Asteraceae (Compositae) is an important edible oilseed crop. In the world, sunflower ranks fourth in area after soybean, rapeseed and groundnut. North America is the native of sunflower and since ancient times it was grown as an ornamental plant. In 1969, it was introduced into India for the first time as oilseed crop. Presently in India, the sunflower crop occupies an area about 3.81 lakh ha with a production of 2.51 lakh tonnes and productivity of 660 Kg ha^{-1}. Sunflower is being grown in India across Karnataka, Maharashtra and Andhra Pradesh. Among these, Karnataka occupies first position accounting to 2.20 lakh ha with a production of 0.98 lakh tonnes and productivity of 445 kg ha^{-1}.

Among the biotic stresses, the diseases are the major constraints in the successful production of sunflower. Gulya and Masirevic (1991) recorded eighty pathogens occurring on sunflower. The major diseases of sunflower in Karnataka are necrosis virus, *Alternaria* blight, rust, powdery mildew, downy mildew and collar rot. Among these *Alternaria* blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara has been considered as a potentially destructive disease in many parts of countries growing sunflower (Allen *et al*., 1983) [1] and in north Karnataka (Shankergoud *et al*., 2006) [30].

Balasubramanyam and Kolte (1980a) [7] reported that the *Alternaria* blight significantly reduced average flower size, number of seeds per head, seed weight, seed yield per plant and also oil content. Depending on the extent of infection, the loss in the yield varied from 11.30 to 73.33 per cent (Reddy and Gupta, 1977) [27]. *Alternaria* leaf spot in northern Karnataka is known to cause more than 80 per cent yield loss under epiphytotic conditions (Hiremath *et al*., 1990; Balasubramanyam and Kolte, 1980b; Amaresh, 1997) [21, 8, 3]. According to Allen *et al*. (1981) [2], the nature of reduction in the yield is determined by the stage of plant growth when disease epidemic develops.

Several effective pesticides for the control of *Alternaria* leaf blight have been recommended but they are not regarded to be long term solutions, due to fungicide residue, exposure to heal the risk, concerns of expense and other environmental hazards.

A variety of biological controls are available for use, but further development and effective adoption should be required for understanding of the complex interactions between plants and pathogens.

Compost tea is a liquid extract made from compost that contains a variety of nutrients, growth compounds and beneficial microorganisms. Organism present in compost tea consumes the foods that plants secrete out such as plant exudates, both from roots and leaves. The disease-suppressive bacteria and fungi that occur in aerobic tea compete out the disease causing organisms in the phyllosphere and rhizosphere. The microbial composition of compost tea prepared in an aerated and non-aerated manner and isolated bacterial and fungal isolates that showed significant inhibition of either *Phytophthora infestans* or *Magnoporthe griseae*. The microorganisms in compost tea may potentially produce biomolecules with antibiosis potential, contributing to disease suppression (Anil *et al*., 2017) [28].

Seaweed extract is one of the biostimulants which can be applied as a foliar spray and enhance plant growth, tolerance to abiotic stress, photosynthetic activity and resistance to diseases, improving the yield and productivity of many crops (Sharma *et al.*, 2014) [31]. Therefore in view of the importance of the crop and disease management, present investigation on *Alternaria* blight of sunflower was conducted to determine the biochemical changes induced by compost tea and seaweed formulation spray.

2. Material and Methods

The present investigation was undertaken during 2018-19 at Zonal Agricultural Research Station, University of Agricultural Sciences, GKVK, Bengaluru. The experimental site is located in the eastern tract (Zone 5) of Karnataka at 12° 58' N latitude and 77° 35' E longitude and at an altitude of 930 m above mean sea level. The biochemical evaluation and experiments were carried out at the Department of Plant Biotechnology, GKVK, Bengaluru.

2.1 To determine the biochemical changes induced by compost tea and seaweed formulation spray

Assessment of biochemical changes (peroxidase activity, super oxide dismutase activity, phenolics and flavonoids) induced by compost tea and seaweed formulation was done by taking 60 days old leaf sample and analysed under lab condition.

2.1.1 Estimation of protein concentration by Lowry's method (Lowry *et al*., 1951) [24]

The Lowry's method is based on the Biurate reaction of protein with alkaline cupric tartarate. The product formed was Cu^{2+}protein complex. Folin- Ciocalteu reagent is added to the Cu^{2+} protein complex, blue color is formed due to the reduction of phosphomolybdate and phosphotungstate by the aromatic amino acids (tyrosine and tryptophan) present in the protein. The intensity of the color formed depends on the amount of these aromatic amino acids present in the protein and will vary from protein to protein (Lowry *et al*., 1951) [24].

2.1.2 Soluble protein extraction from leaf samples

Leaves from different treatments were frozen in liquid nitrogen to prevent proteolytic activity and homogenized using a pestle and mortar. The homogenate was then suspended in extraction buffer [Phosphate buffer 0.1 M, pH 7.8, 1 mM PMSF (protease inhibitor) and 0.1 per cent of polyvinyl pyrrolidone (PVP)] and held on ice for15 min. The crude protein extracts were centrifuged at 14,000 rpm at 4°C for 30 min. The pellet was discarded and the supernatant containing the soluble proteins was used for further experiments. Protein concentration was determined by the method of Lowry *et al*. (1951) [24] using bovine serum albumin (BSA) as standard.

2.1.2.1 Denaturing discontinuous SDS-PAGE gel (Laemmli, 1970)

The SDS-PAGE of soluble protein extract was performed in gel slabs by preparing 10 per cent resolving gel and 5 per cent stacking gel. The method followed was based on the procedure described by Laemmli (1970).

2.2. Guaiacol Peroxidase enzyme assay (Castillo *et al*., 1984)

The method proposed by Castillo *et al*. (1984) with slight modification was adopted for assaying the activity of peroxidase in the protein extract. The reaction catalyzed by peroxidase is given below:

$$RH_2 + H_2O_2 \longrightarrow 2H_2O + R$$

2.2.1 Spectrophotometric assay of Guaiacol Peroxidase (POX) activity

Peroxidase activity is assayed as an increase in optical density due to the oxidation of guaiacol to tetra-guaiacol. Absorbance due to the formation of tetra-guaiacol was measured at a time interval of 30 sec up to 1 min at 470 nm.

2.2.1.1 Native page analysis for peroxidase isoenzyme analysis (Davis, 1964)

Native PAGE was performed as the method described by Davis (1964) for peroxidase isoenzyme activity by using 10 per cent resolving gels and 5 per cent stacking gel. Protein extract, 25 µg, of all genotypes and treatments are loaded in each gel track. Electrophoresis is performed initially at 80 volts and when the protein had entered the resolving gel the voltage was increased to 120 volts. Electrophoresis was conducted at 40ºC for about 3 h. Later the gel was stained for peroxidase isoenzymes.

For staining, the gel was incubated in a solution containing 0.1 M Potassium phosphate buffer (pH 6.1), 20 mM Guaiacol and 5.55 mM 30 per cent H_2O_2 for 10-20 min until the bands appeared. Then the gel was then washed with 7.5 per cent acetic acid and 1 per cent glycerol to stop the reaction. The isoenzyme bands appeared in brick red colour, which were stable for 24 h and the pattern was photographed.

2.3. Super oxide dismutase (SOD) enzyme assay (Dhindsa *et al*., 1981)

The enzyme SOD is a metallo protein, which catalyzes the dismutation of superoxide radical to H_2O_2 and molecular oxygen. It is considered to be a key antioxidant in aerobic cells and constitutes the first line of defense against reactive oxygen species (ROS). SOD catalyzes the dismutation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2).

2.3.1 Spectrophotometric assay of superoxide dismutase (SOD) activity

The SOD activity was measured by the method described by Dhindsa *et al*., (1981) with slight modifications. SOD activity

in the supernatant was assayed by its ability to inhibit photochemical reduction of nitro blue tetrazolium chloride. The absorbance of samples along with the blank was recorded at 560 nm wavelength.

2.3.1.3 Native page analysis for SOD isoenzyme activity

Native PAGE was performed according to the method described by Davis (1964) for superoxide dismutase isoenzyme activity and the procedure is same as above mentioned for peroxidase activity. The gel was incubated in a staining solution containing 100 per cent NBT (w/v), 0.2 M EDTA (w/v), 0.1 M sodium phosphate buffer (pH 7.5), commercial grade TEMED and 5 per cent riboflavin (w/v) for 30 min until the bands appeared. The isoenzyme bands appeared as white or colorless in a dark blue background and the isoenzyme pattern was photographed.

2.4. Total Phenolics (Bay and Thorpe, 1954)

Phenolics, the aromatic compounds with hydroxyl groups, are widespread in the plant kingdom. They include an array of compounds like tannin and flavanols and occur in all parts of the plants where they offer resistance to diseases and pests. Total phenols estimation was carried out with Folin-Ciocalteu reagent (FCR).

2.4.2 Extraction of Phenolics

One gram of sample was homogenized in 10 ml of 80 per cent ethanol in a pestle and mortar. The homogenate was centrifuged at 10000 rpm for 20 min, the supernatant was collected and the residue re-extracted with five times the volume of 80 per cent ethanol and re-centrifuged. After this, the supernatant was collected and evaporated to dryness and dissolved the residue in 2ml of distilled water.

2.4.2.1 Estimation of phenolics

Phenol extract (0.2 ml) was diluted with 3ml distilled water and 0.5 ml of diluted Folin- ciocalteau reagent was added. The contents were mixed thoroughly. Exactly after 3 min, 2 ml of saturated sodium carbonate was added. The content were allowed to stand for 1 min in boiling water bath, cooled and absorbance was measured at 650 nm against the reagent blank. By using rutine used as a standard phenolic, the standard graph was constructed.

The phenolic concentration present in the test samples were calculated using rutine standard and the concentration is expressed as mg phenols/ g fresh weight.

2.5 Estimation of Toatal flavonoids (Woisky and Salatino, 1998)

2.5.1 Estimation of flavonoids (mg rutine equivalents/ g FW): Spectrophotometric method: Absorbance at 510 nm

2.5.2 Method

Five grams of sunflower leaves was homogenized in a pestle and mortar with 20 ml of methanol (80%) for 2-3 times. The extracts were pooled and the volume was made up to 50 ml. 1.0 ml of extract was taken in a test tube, 0.3 ml of 5 per cent $NaNO_2$ was added and 0.3 ml of 10 per cent $AlCl_3$ was added for 2 minutes. 3.4 ml of 4 N NaOH was added after another 2 min. The mixture was incubated for 10 minutes at room temperature. The intensity of the brick red colour against blank was measured at 510 nm. By using rutine as standard, the standard curve for flavonoids was prepared.

2.5.3 Calculation

$$\text{Total flavonoid content (mg rutin equivalents/100g)} = \frac{OD_{510nm} \times \text{Standard value (mg/OD)} \times \text{Total Volume of extract}}{\text{Assay volume} \times \text{Weight of sample (g)}} \times 100$$

2.6 Statistical analysis

The field experimental data was analysed statistically by Fischer´s method of analysis of variance by Panse and Sukhatme (1967). The level of significance used in the F test was P= 0.05. The critical difference was worked out wherever F-test was significant.

3. Results

Experiments were conducted on various aspects of *Alternaria* blight of sunflower with reference to biochemical studies of biological controls at GKVK, Bengaluru, during 2018-19.

3.1 To determine the biochemical changes induced by compost tea and seaweed formulation spray

3.1.1 Defense enzyme response in sunflower

Soluble proteins were extracted from the sunflower leaves grown under field condition, where they were subjected to different treatments as described earlier. The protein level was estimated by Lowry's method and the concentration of protein was calculated using BSA standard curve. The protein concentration was normalized by taking an equal amount of soluble protein across the treatments to evaluate the defense enzyme activity, POX and SOD.

3.1.1.1 Peroxidase (POX) defense enzyme activity in sunflower leaves

- **In gel POX activity**

The peroxidase isoenzyme activity was analysed in-gel by running native PAGE. The staining of gel was done as given in Materials and Method using guaiacol as a substrate. The peroxidase isoenzyme bands were stained brick red colour by oxidation of guaiacol to tetraguaicol. The banding pattern of peroxidase isozymes varied among treatments and peroxidase isoenzyme band intensity increased in protein samples extracted from leaves. Among the different treatments, foliar spray of compost tea with propiconazole showed higher POX enzyme activity. Control treatment from the field showed less enzyme activity (Plate 1).

T_1	Foliar spray of compost tea (1:10) @ 30, 45 and 60 DAS
T_2	Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 and 60 DAS
T_3	Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS
T_4	Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS
T_5	Foliar spray of propiconazole @ 1 mlL^{-1} at 45 and 60 DAS
T_6	Foliar spray of mancozeb @ 3.0 gL^{-1} at 45 and 60 DAS
T_7	Control

Ø Spectrophotometric POX assay

Soluble protein extracts derived from sunflower leaves were assayed for POX defense enzyme activity spectrophotometrically and the data are presented in (Table 1). All the treatments were significantly superior over control with respect to peroxidase activity. Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS recorded significantly higher peroxidase activity of

16.86 µg/min/mg protein followed by T4 (Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS) 15.38 µg/min/mg protein which was on par with T5 (Foliar spray of propiconazole @ $1mlL^{-1}$ at 45 and 60 DAS). The least peroxidase activity was seen in control (T7) 10.76 µg/min/mg protein.

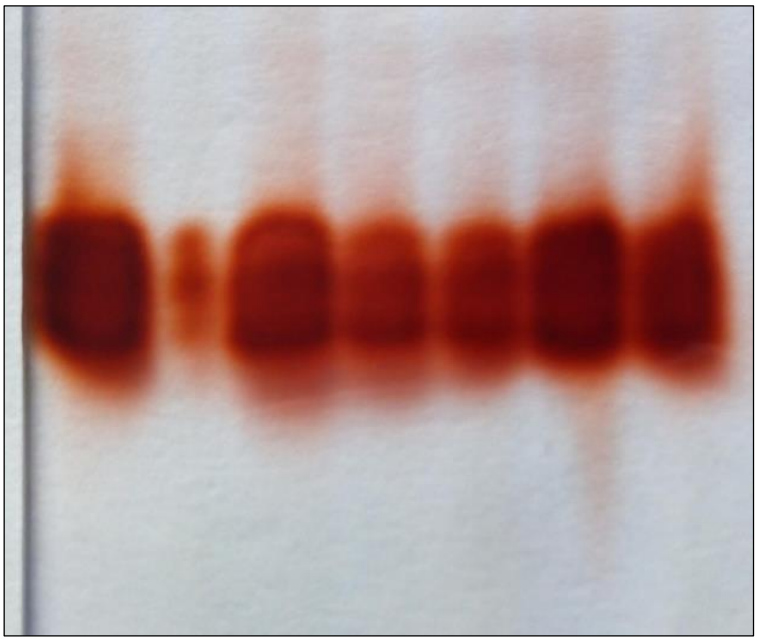

Plate 1: POX enzyme activity in treated leaf samples of sunflower

Table 1: Effect of different treatments on peroxidase in sunflower leaves

Treatment	Peroxidase (µg/min/mg protein)
T_1: Foliar spray of compost tea (1:10) @ 30, 45 and 60 DAS	12.80
T_2: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 and 60 DAS	14.00
T_3: Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	16.86
T_4: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	15.38
T_5: Foliar spray of propiconazole @ $1mlL^{-1}$ at 45 and 60 DAS	15.23
T_6: Foliar spray of mancozeb @ 3.0 Gl^{-1} at 45 and 60 DAS	12.25
T_7: Control	10.76
S.Em±	0.05
CD at 5%	0.15

3.1.1.2 Superoxide dismutase (SOD) defence enzyme activity in sunflower leaves

Ø In-Gel Superoxide dismutase (SOD) defense enzyme activity

Superoxide dismutase isoenzyme defense activity was analyzed in gel by running native PAGE. The gel was stained using Nitro-Blue Tetrazolium chloride (NBT) as substrate as mentioned in Materials and Method. The SOD isoenzymes bands were colourless by inhibiting the blue colour formed by NBT. The rest of the gel stained dark blue. The activity of SOD was more in leaf proteins extracted from foliar spray of compost tea @ 30, 45 DAS and propiconazole @ 60 DAS followed by other treatments (Plate 2).

T_1	Foliar spray of compost tea (1:10) @ 30, 45 and 60 DAS
T_2	Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 and 60 DAS
T_3	Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS
T_4	Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS
T_5	Foliar spray of propiconazole @ 1 mlL^{-1} at 45 and 60 DAS
T_6	Foliar spray of mancozeb @ 3.0 gL^{-1} at 45 and 60 DAS
T_7	Control

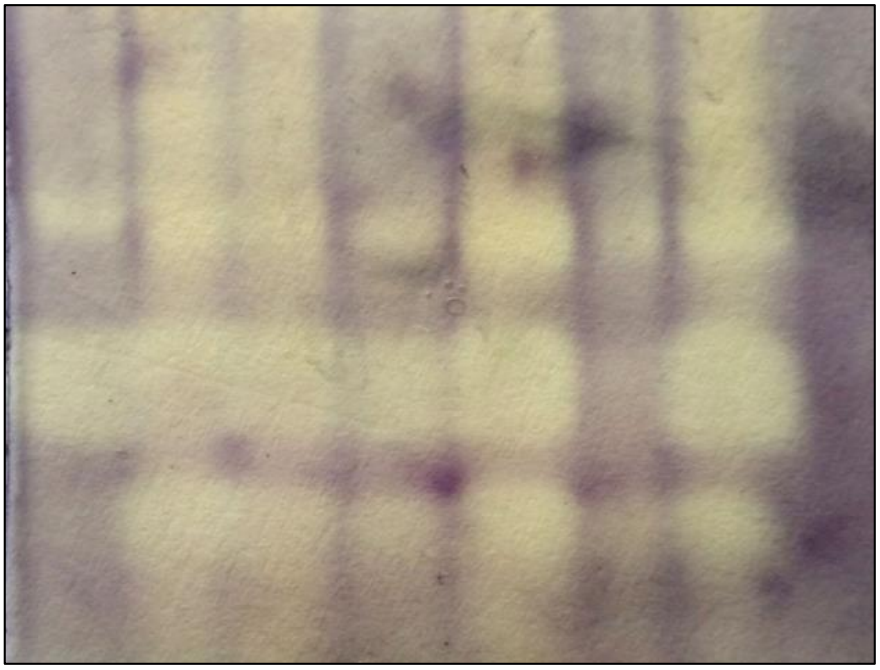

Plate 2: SOD enzyme activity in treated leaf samples of sunflower

Ø Spectrophotometric Superoxide dismutase (SOD) assay

The leaves derived protein extracts from sunflower were assayed for SOD defense enzyme activity spectrophotometrically and the data are presented in (Table 2). All the treatments differed significantly superior over control. Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS recorded significantly higher super oxide dismutase (SOD) activity of 39.47 µg/min/mg protein which is followed by T4 (45.72 µg/min/mg protein). The least SOD activity was seen in control (T7) 59.370 µg/min/mg protein.

From the above details it can be inferred that in sunflower plants, foliar spray of compost tea @ 30, 45 DAS and propiconazole @ 60 DAS and foliar spray of seaweed formulation (LBD-1) @ 30, 45 DAS and propiconazole @ 60 DAS treatments exhibited a high level of defense activity as seen by enhanced POX and SOD activity. Therefore, compost tea induced defense priming response has been seen in this study. This response can mimic ISR type of a defense response thus help in managing *Alternaria* blight disease.

Table 2: Effect of different treatments on superoxide dismutase in sunflower leaves

Treatment	Superoxide dismutase (µg/50 per cent inhibition)
T1: Foliar spray of compost tea (1:10) @ 30, 45 and 60 DAS	44.84
T2: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 and 60 DAS	53.04
T3: Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	39.47
T4: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	45.72
T5: Foliar spray of propiconazole @ 1mlL^{-1} at 45 and 60 DAS	47.11
T6: Foliar spray of mancozeb @ 3.0 gL^{-1} at 45 and 60 DAS	52.26
T7: Control	59.37
S.Em±	0.35
CD at 5%	0.63

Note: Lower values for SOD indicates higher activity.

The overall results reveal that POX and SOD defense enzyme activities varied between the treatments and increased in the case of compost tea treatments as compared to control. Thus, in compost tea treated plants exhibits increased defense-like responses as seen by enhanced POX and SOD activities, and thus is likely to contribute to management of *Alternaria* blight disease in the field.

3.1.2 Effect of different treatments on secondary metabolites in sunflower:

3.1.2.1 Total Phenolics in leaf samples of sunflower

Total phenolics were extracted from the leaves of sunflower by a procedure explained in Materials and Methods. Results reveal that phenolic concentration increased in compost tea treated plants in sunflower. All the treatments differed significantly superior over control. Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS recorded high concentration of phenolics 67.92 mg per 1g Fresh weight which is on par with T1 (Foliar spray of compost tea @ 30, 45 and 60 DAS) and T6 (Foliar spray of mancozeb @ 3.0 gL^{-1} at 45 and 60 DAS). The least phenolics content was observed in control (T7) 57.48 mg per 1g Fresh weight (Table 3, Fig. 1).

Table 3: Effect of different treatments on phenolics concentration in sunflower leaves

Treatment	Phenolics (mg/g FW)
T1: Foliar spray of compost tea (1:10) @ 30, 45 and 60 DAS	66.88
T2: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 and 60 DAS	63.58
T3: Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	67.92
T4: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	61.08
T5: Foliar spray of propiconazole @ 1mlL^{-1} at 45 and 60 DAS	62.40
T6: Foliar spray of mancozeb @ 3.0 gL^{-1} at 45 and 60 DAS	65.36
T7: Control	57.48
S.Em±	1.02
CD at 5%	1.82

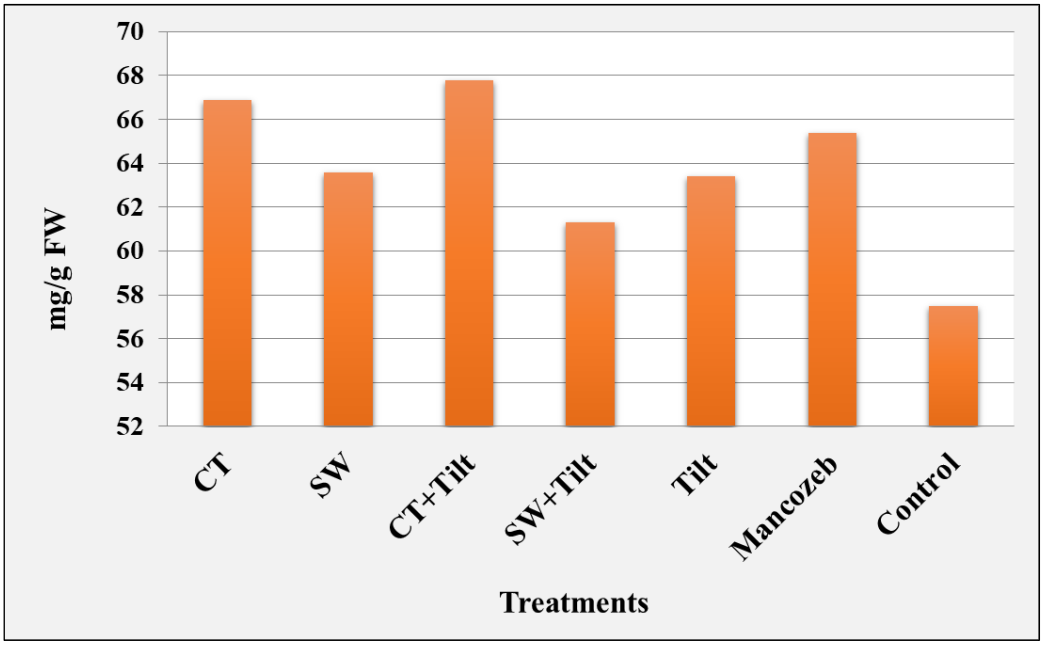

Fig 1: Phenolics content in treated and untreated sunflower plants (CT- Compost tea, SW- Seaweed formulation)

3.1.2.2 Estimation of total flavonoids in leaf samples of sunflower

Total flavonoids were extracted from treated sunflower leaf samples and levels estimated by using Rutin based standard curve. All the treatments differed significantly superior over control. Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS showed highest flavanoid content with 60.01 mg/g fresh weight which is on par with T2 (Foliar spray of seaweed formulation (LBD-1) @ 30, 45 and 60 DAS) and T1 (Foliar spray of compost tea @ 30, 45 and 60 DAS). The least flavonoids content was observed in control (T7) 37.86 mg/g fresh weight (Table 4, Fig. 2).

Table 4: Effect of different treatments on flavonoids concentration in sunflower leaves

Treatment	Flavonoids (mg/g FW)
T_1: Foliar spray of compost tea (1;10) @ 30, 45 and 60 DAS	55.73
T_2: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 and 60 DAS	55.74
T3: Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	60.01
T_4: Foliar spray of seaweed formulation (LBD-1) @ 2 mlL^{-1} at 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS	44.90
T_5: Foliar spray of propiconazole @ 1mlL^{-1} at 45 and 60 DAS	45.77
T_6: Foliar spray of mancozeb @ 3.0 gL^{-1} at 45 and 60 DAS	53.33
T_7: Control	37.86
S. Em±	1.45
CD at 5%	2.58

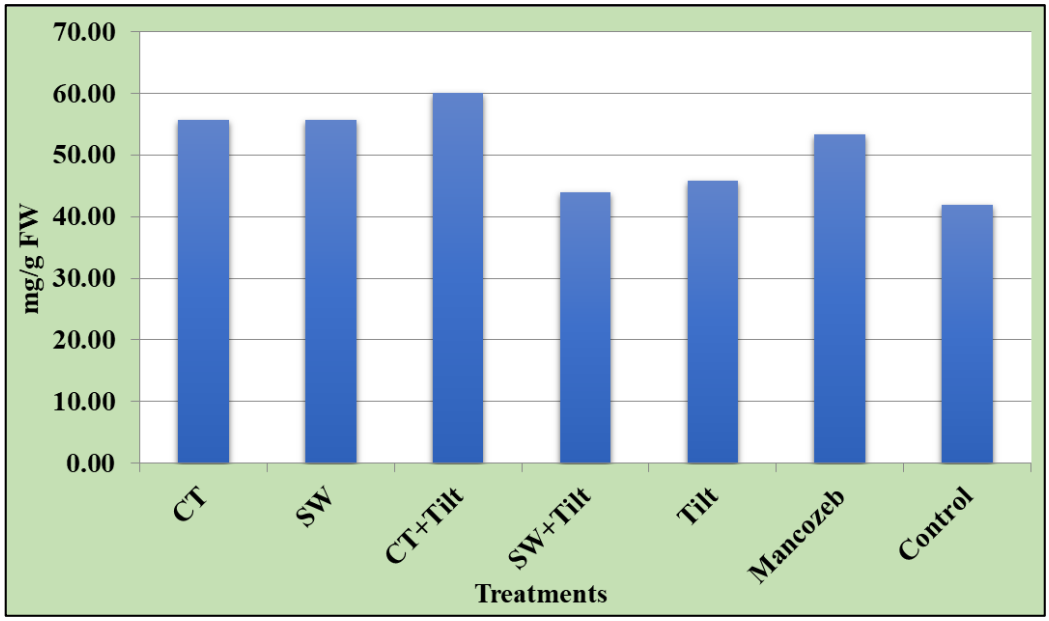

Fig 2: Flavonoids content in treated and untreated sunflower plants (CT- Compost tea, SW- Seaweed formulation)

By the above results, we can conclude that compost tea treated sunflower plants increased the secondary metabolite concentrations in leaves. The secondary metabolites phenolics and flavonoids play important roles in defense response in plants.

4. Discussion

In the current study, POX and SOD play a very important role in plant defense and abiotic stress responses. The correlations between intensification of diseases and enzymes activity are reported to be significant (Silva *et al*., 2004; Jetiyanon, 2007) [32, 22]. A significant increase in POX and SOD activities were detected both in gel assays as well as in spectrophotometric assays in leaves. This clearly showed the enhanced activity of isozymes in infected plants of sunflower crops when treated with compost tea (Plate 1, 2). This enhanced activity of isoenzymes induces the ISR-like resistance in plants.

Sang and Kim (2011) investigated the direct and indirect effect of compost watery extracts (CWEs) from different regions of Korea on control of anthracnose disease on pepper and cucumber and found increased Peroxidase enzyme and hydrogen peroxide accumulation compared with controls. The authors suggest that compost tea induces systemic defense in plants. Earlier work in our group also recorded enhanced POX and SOD activities in leaves of potato with exposure to different compost tea treatments (Anil *et al.*, 2014; 2017) [4, 5].

Non-pathogenic microorganisms are reported to induce defense via the JA pathway in plants (Taiz and Zeigher, 2010) [33]. The increased concentration of PO, PPO and PAL activities in plants treated with compost tea indicated that the protective effect of compost tea is due, at least in part, to the induction of resistance in plants (Weltzien, 1991) [35].

The current study demonstrated that increased POX and SOD defense enzyme activity in sunflower when challenged with the pathogen of *Alternaria* blight disease. The activity of these defense enzymes is further enhanced with pathogen infection. These results suggested that microorganisms in compost tea induce systemic resistance, enhancing the defense preparedness of the sunflower plants against the disease.

The compost tea treated sunflower plants increased the secondary metabolite such as phenolics and flavonoids concentrations in leaves (Fig. 1 and 2). Comparative analysis of the soyabean genotypes and *P. colocasiae* infection showed that the phenol content was highest in the resistant genotypes than the susceptible genotypes. In infected plants, phenolics are substrates for the synthesis of compounds involved in disease resistance, like pterocarpan, phytoalexins and hydroxyl cinnamic acid esters (Dixon and Lamb, 1990) [18]. Volatile metabolites produced by microorganisms during the fermentation of compost tea induces enzymes such as PPO and PO, these are responsible for the oxidation of phenolic compounds into anti-microbial quinones in plant cells infected by phytopathogens and thus conferring disease resistance during incompatibility reactions by means of antibiosis (Chittoor *et al.*, 1997) [13]. These quinones are highly toxic to the pathogen than the original phenols. Chemicals such as salicylic acid (SA) can induce resistance in plants (De Meyer *et al.*, 1998) [16]. Phenolics production such as of bioresistant phenylpropanoid polymers (lignins and suberins) at or near the infection site which act to scar over the wound and as a barrier to the penetration or the propagation of the pathogen (Ebel and Grisebach, 1988; Davin and Lewis, 1992) [19, 14].

Flavonoid too play an important role in the defense of the plant and influences the resistance of plants to adverse environmental conditions, its reducing power being directly proportional to its concentration (Raza and Murthy, 1988) [26]. Flavonoids also act as signalling molecules in interactions between plants and microorganisms (Cheynier *et al*., 2013) [12]. Flavonoids are incorporated into the cell walls of necrotic and adjacent cells (Beckman, 2000) [10] and they play role in tightening of the plant structures and tissues by modulating auxin (IAA) activity, which can lead to the differentiation of tissues, promotion of callose and tylose formation and closure of the vascular system to prevent pathogen infection. They also inhibit the cell wall degrading enzymes of pathogens, by chelating metals required for their activity (Treutter, 2005) [34]. The antifungal activity is often based on the inhibition of spore development and mycelium hyphae elongation. Flavonoid anti-pathogenic activity can also be more specific.

It is suggested that the mechanism of flavonoid antibacterial activity is based on their ability to inactivate microbial adhesion.

Thus flavanoids and phenolics contribute to resistance in multiple important ways in different plant species. In potato plants our earlier study shows enhancement of flavonoids and phenolics with *P. infestans* challenge (Anil, *et al.*, 2014, Anil *et al*., 2017; Roopashree *et al*., 2017) [4, 5, 28].

5. Conclusion

The present investigations was conducted to know the biochemical changes induced by compost tea and seaweed formulation at ZARS, UAS, GKVK, Bengaluru, during 2018-19 and the results obtained thereon are summarized below.

Foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS showed increased defense enzymes such as superoxide dismutase (39.47 µg/50 per cent inhibition) and peroxidase (16.86 µg/min/mg protein) when compared to superoxide dismutase (59.37 µg/50 per cent inhibition) and peroxidase (10.76 µg/min/mg protein) in control plants.

Secondary metabolites such as phenolics (67.92 mg/g FW) and flavonoids (60.01 mg/g FW) was maximum in foliar spray of compost tea (1:10) @ 30, 45 DAS and propiconazole @ 1 mlL^{-1} at 60 DAS when compared to phenolics (57.48 mg/g FW) and flavonoids (41.87 mg/g FW) in control plants.

This study demonstrated that foliar spray of compost tea and propiconazole can be used effectively for the management of *Alternaria* blight in fields as it exhibited more defense enzyme activity and increased growth and yield parameters.

6. References

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