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Studies on analysis on biochemical characters of leaf over liquid organic inputs (Panchagavya and Jeevamruth) on maize (*Zea mays* L.)

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Abstract

Organic farming is based on the system-oriented approach and the use of organic liquid product like Panchagavya resulted in higher growth, yield and quality of crops and hence there had been an increasing interest in the use of liquid formulations. The present study was carried out to validate the shelf life of panchagavya and jeevamruth by screening scientifically under *in vivo* condition using maize (*Zea mays*). The liquid organic formulations like jeevamruth, panchagavya and the panchagavya formulations with groundnut oil cake and sesame oil as a substitute to ghee were freshly prepared and used for further studies.The maize plants in treatment T_1 and T_3 increased the quantity of photosynthetic pigments and root oxidation activity than in the control plants. The soluble protein (0.87 mg/g) and total sugar content (11.20 µg/g) improved in T_3 and T_5 treated plants.

Keywords: *Phyllosphere microflora*, absorbance, root oxidation activity

Introduction

Organic farming is based on the system-oriented approach and can be a promising option for sustainable agricultural intensification in the tropics. Organic farmers rely on crop rotation, green manures, compost, biological pest control and mechanical cultivation to maintain the soil productivity and for controlling the pests. It may offer several potential benefits such as a greater yield stability especially in risk-prone tropical ecosystems, higher incomes in traditional farming systems, an improved soil fertility and long-term sustainability of farming systems, a reduced dependence of farmers on external inputs, the restoration of degraded or abandoned land, the access to attractive markets through certified products and new partnerships within the whole value chain, self-confidence and autonomy of farmers (Mendez *et al*., 2010) [6].

The liquid formulations such as panchagavya, jeevamruth and beejamruth were ecofriendly organic preparations made from products of cow. Among these, panchagavya is one of the widely used traditional liquid organic formulations, which is a fermented product made from five ingredients obtained from cow, such as milk, urine, dung, curd and clarified butter (Amalraj *et al*., 2013). In panchagavya, more number of beneficial microorganisms was found to be high under higher acidity. They have not only enhanced the microbes in the environment but also acted as a catalysts with a synergistic effect to promote all the useful microbes of the environment and these microorganisms secrete proteins, organic acids and antioxidants in the presence of organic matter and converted them into energy thereby the soil micro flora and fauna changed a disease inducing soil to a disease suppressive soil (Somasundaram *et al.*, 2003) [8].

Materials and Methods

Field experiment to assess the impact of Liquid organic formulations on Maize

The trials were laid out at the Eastern block farms at the fields were the trails of Department of Sustainable Organic Agriculture, carried out. The experiment was conducted using maize var. COH(M)5 (*Zea mays*) as test crop to assess the different doses of liquid organic formulations on crop. The land was ploughed, levelled and divided into 20 plots. The diameter of each plot was 30 x 4.5 m^2, leaving 15 m^2 for irrigation channels. The treatments were allocated to each respective plot by following randomized block design to minimize the experimental error. The individual plot was ploughed and formed with ridges and furrows by adopting a spacing of 60 cm between the two ridges. Maize seeds were sown in the side of the ridges by adopting 25 cm spacing apart. All the cultural practices including gap filling, thinning, weeding and plant protection measures were carried out as per the TNAU recommendations.

During the rain and irrigation periods, utmost care was taken while randomizing the treatments in the layout of the field experiment (Fig 1).

Treatment Details

The following treatments were adopted for field study,

T_1 - TNAU Panchagavya @ 3% spray

T_2 - Jeevamruth @ 3% spray

T_3 - Panchagavya (Groundnut cake instead of ghee) @ 3% spray

T_4 - Panchagavya (Castor oil instead of ghee) @ 3% spray

T_5 - Recommended dose of NPK

As per the treatment details given for maize, calculated quantity of Liquid formulations of 3 per cent were uniformly applied as a foliar spray in each plot for the treatments T_1 to T_4 for three times at different periodical growth intervals. The first spraying was initially given during the 30 days after sowing (DAS) (Vegetative stage), the second spraying was given during 60 days after sowing (DAS) (Flowering stage) and the third spraying was given during 90 days after sowing (DAS) (Harvesting stage).

Analysis on biochemical characters of leaf over liquid organic formulations on maize

Total chlorophyll

The leaf chlorophyll content was estimated using SPAD meter – Minolta SPAD-502. It is a simple and non- destructive method of chlorophyll estimation. It can be used in the laboratory as well as in the field of standing crops. Chlorophyll meter is a light weight portable instrument to measure the relative content of chlorophyll of leaves without causing any damage to the plants. SPAD (Soil Plan Analytical Development) is a simple diagnostic tool to measure the chlorophyll content in terms of SPAD values. It is based upon a unique linear relationship between SPAD values and leaf area based nitrogen concentration in plants. Measurements are taken simply by inserting the leaves and closing the measuring head. Same leaf can be measured throughout the growing process. It possesses small measuring area of 2 x 3 mm allowing measurements of small leaves and compactable and light weight in nature (225g) for portability. It has high measuring accuracy (Sivakumar, 2015) [7].

Total carotenoid

Total carotenoids were estimated by the method described by Zakaria *et al.*, (1979) [7]. The sample (0.5g) was homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide in a water bath at 60°C for 30 minutes. The extract was transferred to a separating funnel containing 10 to 15ml of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the extract was noted. The absorbance was read in a spectrophotometer at 450 nm using ether as blank.

Soluble protein

The leaf protein was estimated as per the method described by Lowrey *et al*. (1951) [5]. The true protein content of the sample was expressed as 100 g^{-1}. The procedure followed is given below:

About 250 mg of leaf sample was weighed and macerated with 10 ml of phosphate buffer solution. The contents were centrifuged for 10 minutes at 3000 rpm and the supernatant is collected and made up to 25 ml.1ml of supernatant is pipetted out to a test tube. Five ml of ACT and 0.5 ml of Folin's reagent is added. The mixture is kept undisturbed for 30 minutes for colour development and then the optical density is measured at 660 nm in spectrophotometer

Total sugars

The amount of total soluble sugars could be estimated using either anthrone or phenol-sulphuric acid method colorimetrically. Plant sample of 100mg of the sample was taken into boiling tube and was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100ml and centrifuged. The supernatant was collected and 0.5 and 1ml was taken in aliquots for analysis. The volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water. Then add 4ml of anthrone reagent was added and heated for 8 min in a boiling water bath and then cooled rapidly and the green to dark green colour was read at 630 nm (Hedge and Hofreiter *et al.*, 1962) [3].

Root oxidation activity

Measurement of root activity was done using α-Naphthylamine following Ando *et al.* (1983). After taking out the plants from each CW, roots were washed thoroughly with tap water on a sieve and sponged to remove excess water before weighing. Approximately 1g of root sample was taken for analysis from each plant and immersed in a flask containing 10ml of 20 mg/l α-naphthylamine (α- NA) and 10ml of phosphate buffer (pH 7). The flask containing the roots was air plugged and kept at room temperature in total dark condition, since α-naphthylamine is light sensitive. After 10 min., 2 ml of solution was taken out and initial concentration (A1) of α-NA solution was determined after adding 1ml of 1% sulphanilic acid and 1ml of 100mg/l $NaNo_2$ to the sample aliquot and absorbance was read at 530 nm using spectrophotometer. After an incubation period of 2 hour at room temperature, the final concentration (A2) of α-NA was determined. The root activity was calculated as the amount of α -NA oxidized by the roots using the equation given by Chen (2011).

Root oxidation activity (mg g^{-1} dry weight $2h^{-1}$) = A1-A2-A0

Where,

A1 are the initial and of α-NA,

A2 are final values of α-NA,

A0 is the difference in the initial and final values of α-NA sample without root that served as control

Results

Analysis of biochemical characters on maize leaves

The photosynthetic pigments are one of the most important physiological parameter which effectively influences the plant growth and yield. The photosynthetic pigments like chlorophyll and carotenoids were analysed at various stages of maize (vegetative, flowering and harvesting stage) in all the treatments respectively.

Total chlorophyll

The analysis on chlorophyll content of leaf samples in each fixed treatments showed a decreased trend with respect to progressive growth stages of maize. The highest chlorophyll content was recorded under T_1 (41.68 to 28.85 spad units) followed by T_3 (40.6 to 28.73 spad units) which did not differ significantly. The lowest chlorophyll content was recorded under T_5 (32.0 to 20.25 spad units) during vegetative, flowering and harvesting stages (Table 1).

Total carotenoids

The analysis on carotenoid content in leaf samples of each fixed treatments revealed a decreased nature of carotenoid content with respect to progressive growth stages of maize. The highest carotenoid content was recorded under T_1 (0.32 mg/g to 0.24 mg/g) followed by T_3 (0.29 mg/g to 0.23 mg/g) from vegetative to harvesting stage. The lowest carotenoid content was recorded under T_5 (0.24 mg/g to 0.14 mg/g) from vegetative to harvesting stage. There was a decrease in the carotenoid content from initial to final stage of maize (Table 1).

Soluble protein

At all the three stages, the maximum soluble protein content was documented under T_3 (0.87 mg/g to 0.20 mg/g) from vegetative to harvesting stage. The lowermost soluble protein was documented under T_5 (0.42 mg/g to 0.12 mg/g) during vegetative to harvesting stage. The overall analysis of soluble protein content exhibited a decreased drift with reverence to progressive growth stages of maize (Table 1).

Total sugars

Total sugar content in maize is an indicator of drought tolerant. In case of induction of stress conditions the induction of total sugar content production might be high. Among the treatments, the highest total sugar was recorded in T_5 (11.20 µg/g), followed by T_2 (7.12 µg/g), T_4 (6.98 µg/g), T_3 (4.65 µg/g) and T_1 (4.25 µg/g) (Table 2).

Root oxidation activity of maize

The highest root oxidation activity was documented in T_3 (4.55 mg g^{-1} dry weight 2 h^{-1}) and T_4 (4.43 mg g^{-1} dry weight 2 h^{-1}). The minimum root oxidation activity was recorded in T_5 (2.75 mg g^{-1} dry weight 2 h^{-1}) during the flowering stage of maize (Table 2).

Table 1: Analysis of biochemical characters on total chlorophyll (SPAD units), total carotenoids (mg/g) and soluble protein (mg/g) of maize

		Vegetative stage			Flowering stage			Harvesting stage	
Treatments	Chl. (spad units)	Carotene (mg/g)	Soluble protein (mg/g)	Chl. (spad units)	Carotene (mg/g)	Soluble protein (mg/g)	Chl. (spad units)	Carotene (mg/g)	Soluble protein (mg/g)
T_1	41.68	0.32	0.87	33.75	0.27	0.47	28.85	0.24	0.20
T_2	37.53	0.28	0.76	27.50	0.24	0.42	23.88	0.21	0.18
T_3	40.60	0.29	0.85	33.68	0.26	0.45	28.73	0.23	0.20
T_4	38.50	0.22	0.67	30.25	0.19	0.32	26.09	0.16	0.18
T_5	32.0	0.24	0.42	24.65	0.19	0.21	20.25	0.14	0.12
SEd	0.66	0.004	0.29	0.96	0.01	0.27	0.47	0.01	0.14
CD (0.05)	1.43	0.10	0.61	2.10	0.02	0.59	1.10	0.02	0.30

Table 2: Analysis of biochemical characters on total sugars (µ/g) and root oxidation activity (mg g^{-1}dry weight $2h^{-1}$) of maize

Treatments	Flowering stage	
		Root oxidation activity
	Total sugars (µ/g)	(mg g^{-1}dry weight $2h^{-1}$)
T_1	4.25	3.39
T_2	7.12	3.28
T_3	4.65	4.55
T_4	6.98	4.43
T_5	11.20	2.75
SEd	0.30	0.05
CD (0.05)	0.65	0.12

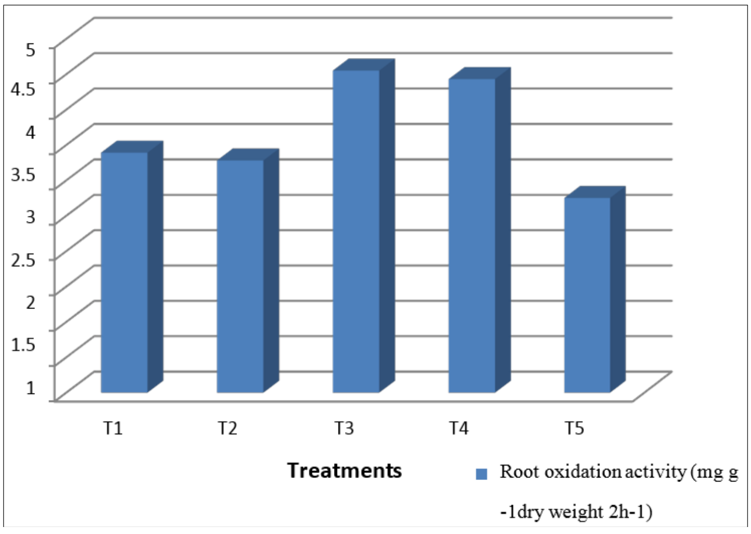

Fig 1: Stimulation of biochemical character of root oxidation activity

Fig 2: Overview of experimental field

Discussion

Analysis on biochemical characters of leaf over liquid organic formulations on maize

The quality of the maize plants was determined by the biochemical parameters. Among the leaf sample of five fixed treatments, the assessment on photosynthestic pigments such as chlorophyll and carotenoids were found to decrease from vegetative to harvesting stage of maize. The highest chlorophyll and carotenoid contents were recorded under T_1 and T_3 (28.85 to 41.68 spad units and 0.23 to 0.32 mg/g). Similarly, the short- term plant growth test with *Cajanus cajan* seeds treated with panchagavya showed higher chlorophyll content (23 spad units) and photosynthetic activity (18.8 µ mol /m^2/s) after 15 days of sowing (Leo *et al.*, 2013). Correspondingly, the treatment T_3 recorded the highest soluble protein (0.20 to 0.87 mg/g) and the total sugar content was observed to be high in T_5 (7.12 µg/g). Likewise, Hannah *et al.* (2005) observed that the panchagavya solution sprayed at the rate of three per cent in tomato crop resulted in improvement in quality of soluble protein, total sugars and also increased number of leaves that could positively indicate the photosynthetic activity that was due to increased chlorophyll content of the plant, since the leaf numbers was a growth index of enhanced the crop growth and yield.

The total sugar content was exhibited to be higher among the control recommended dose of fertilizer treatment. These results were in accordance with the study of Houman and Victoria (2014) [4] that the sugars played an important role in Osmotic Adjustment (OA) in corn. The soluble sugar concentration increased in the treatments that undergone stress conditions. Total sugar content in maize was an indicator of drought tolerant. Sugars played an important role in the osmotic balance. In case of induction of stress conditions the induction of total sugar content would be more. But the crop treated with liquid organic formulations did not experience any condition relevant to stress or tolerance. The treatments of panchagavya formulations showed enhanced root oxidation activity than the control recommended dose of NPK plot. The highest root oxidation activity was documented in T_1 (4.55 mg g $^{-1}$ dry weight 2 h^{-1}) and T_3 (4.43 mg g $^{-1}$ dry weight 2 h^{-1}) plots. The rhizospheric characteristics favors the oxidizing environment in the root zone to facilitate good plant growth and thereby a cumulative development of oxidizing activity in roots were reported (Aparna *et al.*, 2015) [1].

The treatments with the application of liquid organic formulations indicated an improved root oxidation activity than the treatment plot applied with recommended dose of NPK and this cumulative development in root characteristic was favored by oxidizing environment of the rhizosphere which enhanced the nutrient absorption and eventually in the overall efficiency of maize growth.

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