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Original Research Article

Effect of Natural Growth Enhancer on Growth, Physiological and Biochemical Attributes in Black Pepper (*Piper nigrum* L.)

Alagupalamuthirsolai Muthalagu*, S. J. Ankegowda, Mohammed Faisal Peeran, Hosahalli Jagannath Gowda Akshitha, Balaji Rajkumar and Narendra Chaudhary

ICAR-Indian Institute of Spices Research, Regional Station, Appangala, Madikeri, Karnataka–571 201, India

*Corresponding author

A B S T R A C T

Black pepper (*Piper nigrum* L) popularly known as king of spices and it is a very important commercial crops grown in the Western Ghats. The production of healthy planting materials plays a crucial role in cultivation of black pepper. The experiment was conducted to study the efficacy of natural extract on growth and development of black pepper cuttings under glass house condition with an aim to produce healthy planting materials. The experiment was laid out in CRBD comprising five treatments viz., Untreated check (water), Panchagavya (3%), *Vetiveria zizanioides* extract (3%), *Moringa oleifera* Leaves Extract (3%); *M. oleifera* Leaves Extract 3% + *V. zizanioides* Root extract 3%. All the treatments were imposed as drenching and foliar spray at monthly interval starting from April to June. The results obtained revealed that all treatments were very effective in stimulating growth parameters *viz.*, shoot and root length, number of leaves per plant, shoot and root dry weight per plant, total chlorophyll, carotenoids, soluble protein, photosynthetic rate, transpiration rate, water use efficiency and chlorophyll fluorescence. Maximum stimulation of growth and physiological parameters were recorded in plants treated with Moringa Leaves Extract 3% + Vetiver Root extract 3% compared to other treatments.

Keywords

Piper nigrum L, *Moringa oleifera* leaves extract, *Vetiveria zizanioides* root extract, Panchagavya, growth, photosynthesis, water use efficiency

Introduction

Black pepper (*Piper nigrum* L.), the 'King of Spices' belongs to the family Piperaceae and it is cultivated for its valuable green and dried fruits, owing to high international market prices, black pepper production area is expanding over a decade both in traditional and non-traditional areas. However, the availability of quality planting material of high yielding varieties is one of the major constraints in cultivation of black pepper. The conventional propagation methods have certain limitations due to low success rate, high mortality, poor rooting and poor survival of transplanted cuttings. Hence, there is a need for improvement in conventional method of propagation with an input management technology to boost the production of quality planting material.

The dependency of the farmers on the use of inorganic fertilizers as a source of plant nutrients levy high cost and also leads to degradation of soil and environment (Phiri, 2010). Thus, there is a need to search continuously for alternatives which are safe, natural source of plant nutrients, growth enhancers and even ward off pest and diseases. One such alternative is Moringa, (*Moringa oleifera* Lam) (family: Moringaceaea relatively drought-tolerant tree produces plenty of biomass and the leaf extracts possess growth-enhancing capabilities. Moringa is rich in secondary metabolites such as ascorbic acid and total phenols, antioxidant activity, zeatin, Ca, K, Mg which makes it a natural plant growth enhancer.(Yasmeen *et al.*, 2012a, b, 2013; Nouman *et al.*, 2012 a, b, c). Ca and K play essential role in crop growth and development through osmoregulation, enzyme activation, photosynthesis (Hasegawa *et al.*, 2000; Epstein and Bloom 2005).

Another medicinal and aromatic herb, Vetiver (*Vetiveria zizanioides* Linn.) is a deep rooted perennial grass known for its natural antioxidants property which inhibits free radical by preventing the generation of reactive oxygen species (ROS) in the biological systems. It is grown in a wide range of weather conditions (15^{o}C to 55^{o}C) and survives in soil p^H 3 to 10, with an annual rainfall of 300 mm to 5000 mm. It is used in the form of oil or root extract which are rich in antioxidants and polyamines that might induce growth of plants, Zhou and Yu (2010) and Kim *et al.*, (2005). In addition, being a deep rooted grass it used to reduce soil erosion.

Panchagavya, an organic product reported as a potential source for promoting growth and immunity in plant system. Panchagavya is a bio promoter prepared by the combination of five products obtained from the cow (*Bos taurus* L.) *viz*., dung, urine, milk, curd and ghee. It contains N, P, K and micronutrients necessary for plant and growth hormones like Indole Acetic Acid (IAA) and Gibberellic acid (GA) required for the growth as well as predominance of fermentative microorganisms like yeast, azotobacter, phoshobacteria and lactobacillus (Selvaraj, 2003). Padmapriya *et al.*, (2007) reported that, Panchagavya (3%) increases the total chlorophyll, total phenol content and increases the yield in turmeric crop.

The natural extracts derived from *M. oleifera*, *V. zizianoide* and Panchagavya, were investigated to ascertain their effects on growth of shoot and root of black pepper cuttings under nursery condition with the aim to find out possible supplement or substitute to inorganic fertilizer.

Materials and Methods

Plant materials

Pot culture experiment was conducted during 2016 under glasshouse conditions at ICAR-Indian Institute of Spices Research, Regional Station, Appangala, Madikeri, India. The potting mixture (soil, sand and farm yard manure in 2:1:1 ratio) was filled in polybags of 20 cm×10 cm size, which had perforations at the bottom for drainage. The pH of the potting mixture was 5.8. Healthy rooted single node cuttings of black pepper var. Panniyur-1 obtained from serpentine method of multiplication was used for planting in polythene bags. The experiment was arranged in a completely randomized design (CRD) with six replicates.

Preparation and application of natural extracts

An aqueous extract of moringa leaves at 3% concentration (MLE) was prepared by blending 30 g of young moringa leaves with 675 ml of 80 % ethanol as suggested by Makkar and Becker (1996). The obtained suspension homogenized and filtered by wringing using a mutton cloth. Finally, the solution re-filtered using No. 2 Whatman filter paper and rose to one liter with water (Fuglie 2000). The 3% vetiver root extract (VRE) was also prepared as prescribed following the same procedure as described for MLE

Preparation of Panchagavya requires mainly five products of cow along with certain other ingredients as listed below (Natarajan, 2002); (1) Fresh cow dung - 7 kg; (2) Cow urine - 3 l; (3) Cow milk - 2 l; (4) Cow curd - 1 kg (5) Cow ghee - 1 kg; (6) Sugarcane juice - 3 l or 500 g jaggery; (7) Tender coconut water – 3 l; (8) Ripe banana – 12 Nos.; (9) 100 g yeast + 100 g jaggery dissolved in 2 l of warm water. Cow dung and ghee were mixed in an 80 L plastic container and stirred thoroughly both in morning and evening and kept aside for 2 days. After 2 days cow urine and water added to the mixture and kept for 7 days mixing twice every day. After 7 days, rest of the materials were added. The mixture is incubated for four weeks under room temperature and was used after proper sieving through a fine cloth.

Treatments and data analysis

The treatment consist of T_1-Control (water), T_2- 3% Panchagavya (spraying and drenching); T_3-3% Vetiver Root extract (spraying and drenching); T_4- 3% Moringa Leaves Extract (spraying and drenching); T_5-3% Moringa Leaves Extract + 3% Vetiver Root extract (spraying and drenching). The above mentioned treatments were given 1st and 2nd month after planting under nursery.

Plants were allowed to grow upto 15 days after 2nd treatment, the following observations on root and shoot length, dry biomass of shoot and root and numbers of leaves per plant were recorded. Net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), intercellular CO_2 concentration (ci) and leaf temperature (Tleaf) were measured in fully expanded active leaves of plants in each treatment under control saturating photosynthetic photon flux (900 mmol $m^{-2}s^{-1}$) using an infrared gas analyzer (LCpro-SD Advanced Photosynthesis Measurement System, England). The measurements were recorded when both Pn and gs were stable. Average was taken from ten readings spaced evenly 120 s. Chlorophyll fluorescence was recorded by using chlorophyll flurometer (Os-30p) in 10-15 minutes dark adapted leaves. Total chlorophyll and carotenoid content were estimated by adopting the method of Arnon (1949) and expressed as mg g^{-1} of fresh weight. Soluble protein content was estimated with TCA extract of leaves sample following the method of Lowry *et al.,* (1951) and expressed in mg g^{-1} fresh weight. Data was subjected to analysis of variance and Duncan's multiple range tests was used to differentiate means as described by Duncan (1955). Values were considered at a significance level of 95% ($p < 0.05$). Statistical analyses were performed using WASP-Web Agri Stat Package 2.0.

Results and Discussion

Growth characteristics

Growth characteristics (*i.e.,* number of leaves per plant, shoot and root length, shoot and root dry weight per plant) of black pepper plants were significantly and positively affected by MLE (3%) + VRE (3%), used as spray and drench compared to control as shown in (Fig. 1A, 1B and 1C). Combined MLE (3%) + VRE (3%), treatment found to be the best for increasing number of leaves per plant, shoot and root length, shoot and root dry weight per plant by 44.7%, 83.4%, 43.7%, 62.1% and 26.4%

respectively compared to the control. This may be due to that MLE contains essential macro- and micro-nutrients such as Ca, Mg, K, P, Fe, Mn, Cu and Zn. The MLE also contains antioxidants, various phenolics, β carotene and ascorbic acid coupled with amino acids including proline, total soluble sugars and K as osmoprotectants. It is also rich in phytohormones such as indole-3-acetic acid (IAA), gibberellins (GAs) and zeatin as a cytokinin. Many researches highlighted this diverse composition of MLE can play a vital role in recuperating the growth in different crops (Yasmeen *et al.*, 2012, Rady *et al.*, 2013, Rehman *et al.*, 2014) and also VRE contains rich source of antioxidants and polyamines it might induce growth of plants (Zhou and Yu, 2010 and Kim *et al.,* 2005). The results of this study showed that the combined treatment of MLE (3%) + VRE (3%) exhibited significant increase in vegetative growth than control. Furthermore, Azra, (2011) found that spraying wheat, peas and tomato with *M. oleifera* extract at 3.5% increased all growth parameters.

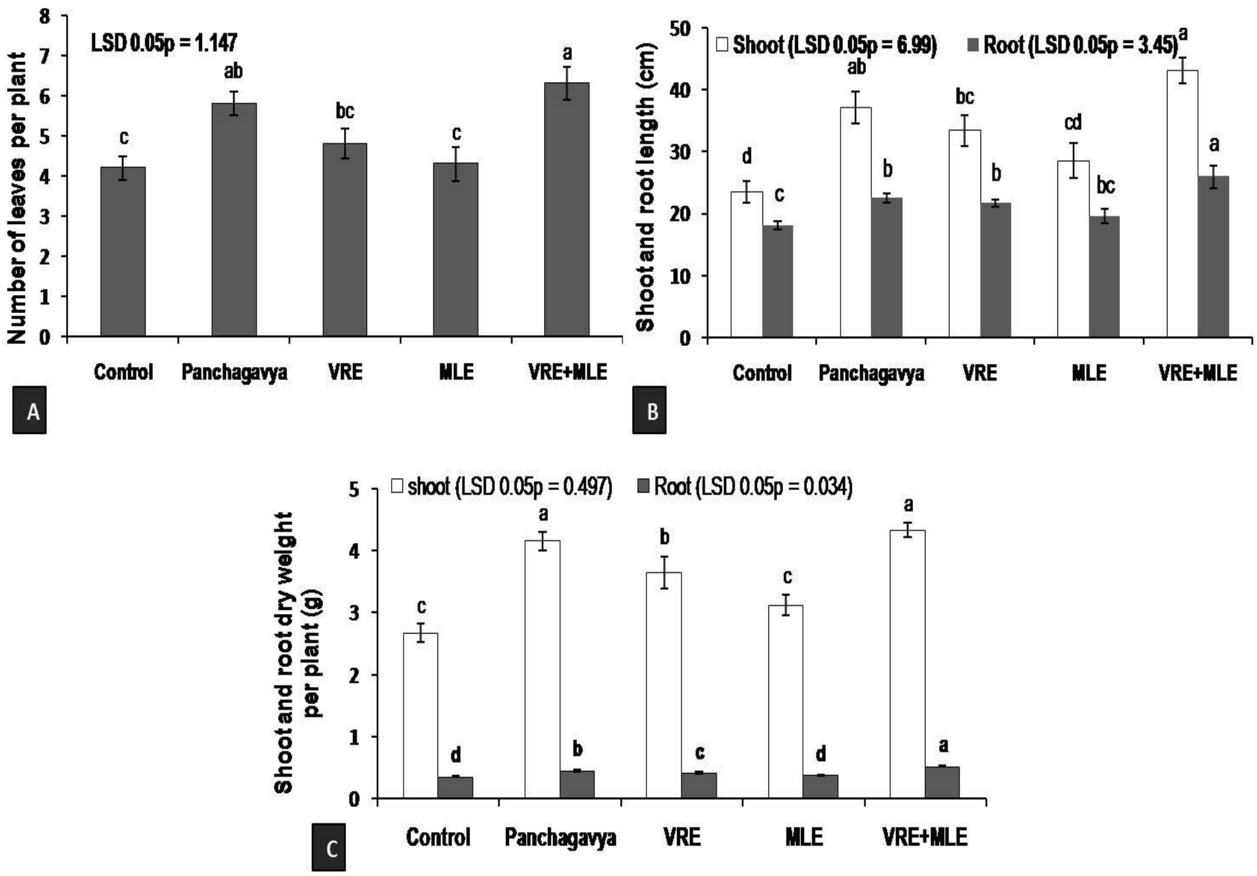
Photosynthetic pigments

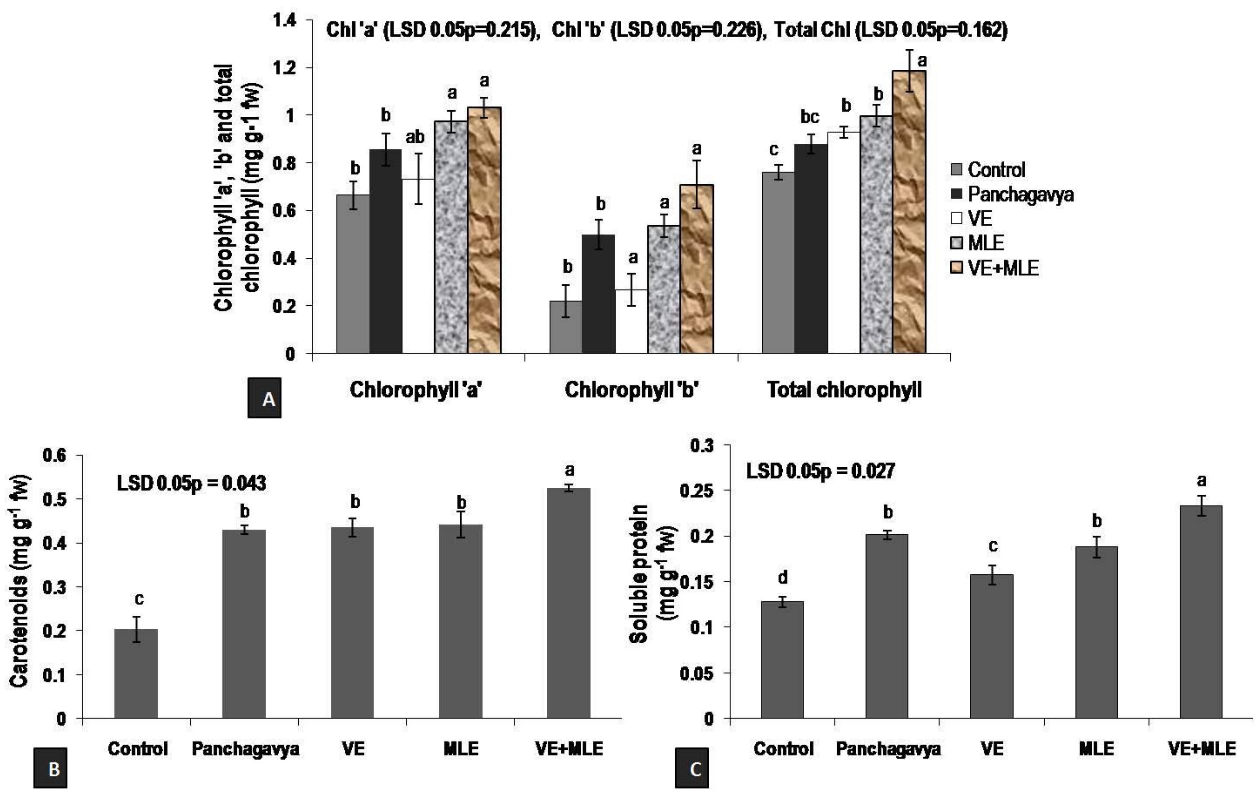
It has been reported that availability of nutrients in soil significantly affects the photosynthetic rate (Pn), chlorophyll (Chl) and transpiration in pepper vine (Shangguan *et al.*, 2000; Thakur *et al.*, 2009). Data presented in (Fig. 2A) showed that foliar spraying and soil drenching with T5, significantly increased the concentrations of chlorophyll 'a', 'b' and total chlorophyll contents by 55.1%, 122% and 55.9% respectively than control. The carotenoid content of the leaves showed a similar response in case of Chlorophyll content (increased 159% than control) in response to T5 (Fig. 2B). Augumentation of chlorophyll and carotenoid content may be due to that MLE contains essential macro- and micro-nutrients such as Ca, Mg, K, P, Fe, Mn, Cu and Zn. Sheren *et al.,* (2015) found that foliar spraying with the aqueous extracts of leaves of *M. oleifera* on Pear trees increased total chlorophyll content in leaves. In addition, Mona (2013) observed that fertilization of rocket (*Eruca vesicaria* subsp. sativa) plants with *M. oleifera* at rates 2% extracts potentially increased the content of, N, P and K in leaves.

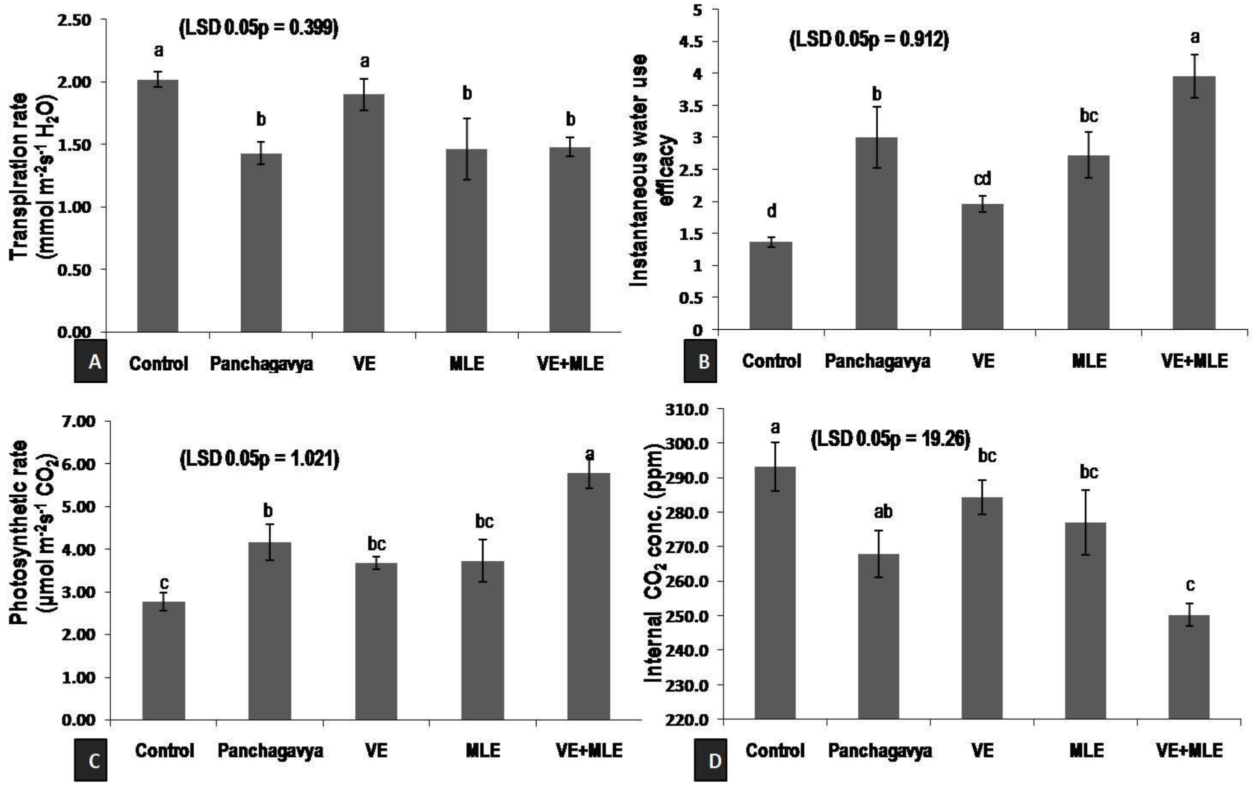
Furthermore, Okali and Owusu (1975) reported that the concentration of chlorophyll contents was highest in plants that were maintained at the highest nutrient level. Furthermore, *M. oleifera* leaves extract enriched with a source of zeatin (a natural derivative of cytokinin), exogenously-applied cytokinin (found in MLE) can delay the senescence process (Tetley, 1974), possibly through activation of cytokinin dependant isopentenyl transferase (ipt) biosynthesis, increasing chlorophyll and carotenoid concentrations (Safi-naz S. Zaki *et al.,* 2015).

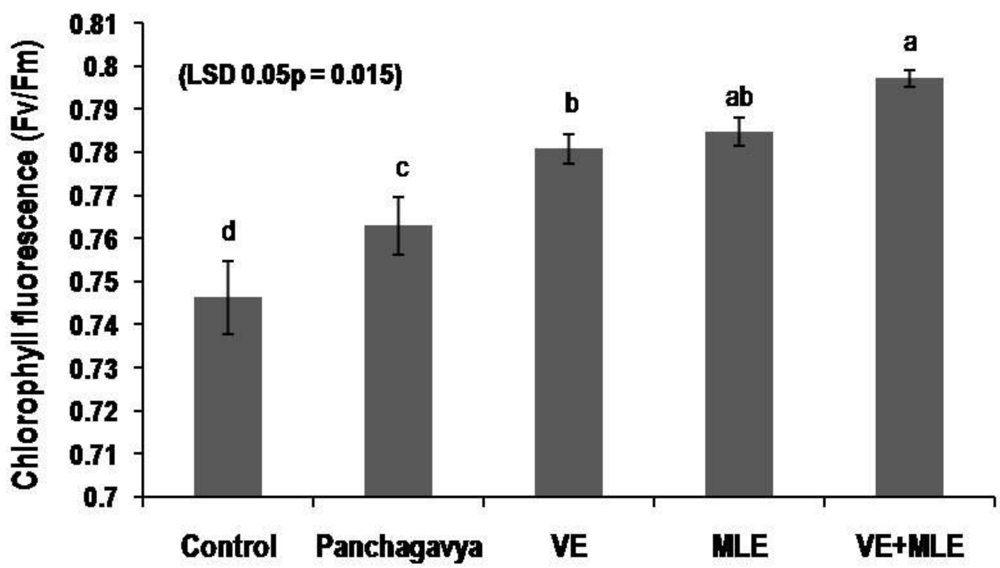
Soluble protein

Proteins are essential for the formation of protoplasm, while growth hormones favoured rapid cell division, cell multiplication and cell enlargement. The enriched content of MLE of crude proteins and growth promoting hormones, that is, auxins and cytokinins increased the possible reason for this acceleration of growth in *Phaseolus vulgaris* L. (Makkar *et al.*, 1996 and Moyo *et al.*, 2011). Foliar application of MLE increases leaves total soluble proteins in tomato (Yasmeen *et al.*, 2014). Concurrently, our analysis also showed that the black pepper crop grown under spraying and soil drenching with MLE (3%) + VRE (3%) had increases the concentration of soluble protein content upto 82%, than control (Fig. 2C)


LSD 0.05p = 1.147
Number of leaves per plant
Control
Panchagavya
VRE
MLE
VRE+MLE
A
Shoot (LSD 0.05p = 6.99)
Root (LSD 0.05p = 3.45)
Shoot and root length (cm)
B
shoot (LSD 0.05p = 0.497)
Root (LSD 0.05p = 0.034)
Shoot and root dry weight per plant (g)
C


Chl 'a' (LSD 0.05p=0.215), Chl 'b' (LSD 0.05p=0.226), Total Chl (LSD 0.05p=0.162)
Chlorophyll 'a', 'b' and total chlorophyll (mg g-1 fw)
Control
Panchagavya
VE
MLE
VE+MLE
Chlorophyll 'a'
Chlorophyll 'b'
Total chlorophyll
A
LSD 0.05p = 0.043
Carotenoids (mg g-1 fw)
B
LSD 0.05p = 0.027
Soluble protein (mg g-1 fw)
C





Further, Moringa leaves have been reported as source of strong enzymatic antioxidant system (Anwar *et al.*, 2007; Nouman *et al.*, 2012a). It has been reported that activation of self defence system by exogenous application of MLE is also associated with higher mineral contents present in moringa leaves making it excellent plant growth enhancer (Yasmeen *et al.*, 2013).

Photosynthesis and water use efficiency

Chlorophyll content and soluble protein are as indicator of photosynthesis activity and strong linear relationship between nutrient availability and chlorophyll content and it take part in the process of organogenesis (Sabo *et al.,* 2002, Chernyad'ev and Monakhova, 2003, Bojovic and Stojanovic, 2005 and Tranavicience *et al.,* 2008). This study also showed that untreated control (T1) has higher transpiration rate than other treatment (Fig. 3A).

The ratio of photosynthesis to transpiration (instantaneous water use efficacy) was higher in T5 treatment; with the loss of one mmol of water, 1.3, 3.0, 1.96, 2.72 and 3.96 µmol of CO_2 was fixed in pepper vine grown under, T1, T2, T3, T4 and T5 respectively (Fig. 3B). A high photosynthetic rate with lower CO_2 concentration inside the sub-stomata cavity in T5 suggested a more efficient carboxylation system (Fig. 3B and 3C).

The instantaneous water use efficacy of the leaves (represented by the ratio of photosynthesis to transpiration) is a measurement of carbon gained through photosynthesis with per unit of water transpired. A higher photosynthetic rate with lower transpiration in T5 indicates that water used more efficiently than in other treatments. Higher photosynthetic rate is mainly due to greater chlorophyll and soluble protein contents as well as the availability of nutrients to plants.

Activity of photosystem II (Fv/Fm)

Photosystem I and II complexes are both Fe containing proteins, Fe in PSII is important for water splitting (Hulsebosch *et al.,* 1996), thus Fe deficiency may lead to decreased PSII activity. The Fv/Fm is a measurement of the light energy transfer in dark adapted samples or the photochemical quantum yield of open PSII centers (DeEll and Toivonen, 2003).The activity of PSII was investigated in the present study by using chlorophyll fluorescence technology. In the present study the highest values of Fv/Fm in leaves (increased by 6.84% than control) was found with foliar spray and drenching with MLE (3%) + VRE (3%) (Fig. 4), which has attributed to higher chlorophyll a content in these plants and this result was supported by Redondo-GoLmez *et al.,* (2007) in *Atriplex portulacoides*.

In addition, Fe induced an increase in maximum potential PSII efficiency (Fv/Fm) of peach leaves (Eichert *et al.,* 2010). Thus, it is concluded that in the current study, highest Fv/Fm values of black pepper leaves in T5 plants might be due to the higher Fe concentration in these plants, and hence foliar spray and drenching of Fe (found in MLE) can meet the plants requirement of this element.

It can be concluded from the afore mentioned results, that *M. oleifera* leaves extract 3%+ Vetiver root extract 3% in combination was the most effective treatments in providing Black pepper plants improved leaf growth and physiology. Generally, it is concluded that *M. oleifera* leaves extract with Vetiver root extract can be recommended as natural growth stimulant to be used effectively by farmers for various crops due to its high potentiality, high nutritive value, antioxidant effect, easy preparation and environmentally friendly nature.

Authors' Contributions

AM conducted the experiment, work, analyzed the results, and drafted the manuscript. MFP, HJGA, BR and NC designed and made interpretation the results with the research group and ASJ supervised certain scientific approaches and advised the research.

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