Pak. J. Agri. Sci., Vol. 58(2), 501-507; 2021
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/21.630
http://www.pakjas.com.pk

EFFECTS OF DIFFERENT BIO-LIQUIDS (EARTHWORM WASH) ON MORPHO-PHYSIOLOGICAL CHARACTERISTICS OF MAIZE

Iqbal, N[1,*]., A. Tanveer[1], M. Tahir[1], H.M. Akram[3] and M. Yaseen[2]

[1]Department of Agronomy, University of Agriculture Faisalabad, Pakistan; [2]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan; [3]Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan
***Corresponding author's e-mail: naeemiqbalveh@gmail.com**

Maize (*Zea mays* L.) is an important cereal, which is affected adversely by nutrients deficiency. With the objective to combat nutrients deficiency in maize through bio-liquid (earthworm wash), experiments were conducted at University of Agriculture, Faisalabad, Pakistan. First experiment was executed for the characterization of bio-liquids from three different plant sources. In the second experiment, the concentrations of extracted bio-liquids were optimized on morpho-physiological characteristics of hybrid maize (P-1543). The experiment was conducted under Completely Randomized Design (CRD) with factorial arrangement replicating four times. The treatments comprised of three sources of bio-liquids i.e. alligator weed (*Alternanthera philoxeroides*), alfalfa (*Medicago sativa*) and rice (*Oryza sativa*) straw in first experiment and four concentrations of bio-liquids (Zero, 10%, 15% and 20%) were tested on maize in second experiment. Foliar spray of bio-liquids was applied at 10, 15 and 20 days after the crop emergence. The evaluation was done 45 days after emergence on the basis of various morphological (plant height, number of leaf per plant, fresh weight of shoot, fresh weight of leaf) and physiological attributes (water potential, osmotic potential, chlorophyll fluorescence, cell membrane stability index and relative water contents). The results of first experiment showed that bio-liquid extracted from alligator weed proved to be best source of micro and macro nutrients as compared to alfalfa and rice straw bio-liquids. The results of second experiment indicated that foliar spray of bio-liquids from all sources improved plant height (16.55 cm), number of leaves per plant (7.80), leaf area per plant (187.41 cm^{-2}), total fresh weight per plant (5.74 g), water potential (-0.79 MPa), osmotic potential (-0.17MPa), chlorophyll fluorescence (0.84), cell membrane stability (40.27) and relative water content (91.80%) as compared to control. It can be concluded that growth of maize plants was improved when bio-liquids were sprayed on plants. Foliar application of alligator weed bio-liquid @ 15% showed maximum morpho-physiological performance of maize.
Keywords: Earthworm, vermicompost, bio-liquids, foliar spray, maize.

INTRODUCTION

Current agriculture is usually focused by excessive use of chemical fertilizers, however, the organic fertilizers application is minimum (Gill and Garg, 2014). The excessive reliance on synthetic fertilizers has caused diverse negative impact on the water and soil environment (Ju *et al.,* 2009), food contamination (Li *et al.,* 2007), decline in quality of soil (Ju *et al.,* 2009) and diminishing biodiversity (Gill and Garg, 2014). It is, therefore, need of today is to resolve such issues by turning to more viable and sustainable agriculture. As compared with fertilizer, organic culture is beneficial in managing environmental sustainability and biodiversity (Leite *et al*., 2010). Owing to higher ecological and economic outcomes, organic culture has been steadily adopted in developed countries (Lobley *et al.,* 2009; Sarwar *et al*., 2020). Earthworms are one of the old species present on earth regarding evolutionary view point and because of their ability to tolerate various climatic conditions. They have a vital role in mineralization and organic matter decomposition (Brown *et al*., 2004) and hence boost the nutrients availability and recover the soils biological, physical and chemical properties (Abdul Rida and Bouché, 1997). Earthworms ingest a large amount of dead organic matter, however, only a small amount of materials is assimilated in the body of worms and remaining part is excreted in the form of vermicasting that are enrich with NPK, micronutrients and also beneficial microbes (Bhat and Khambata, 1994). Gut of worms contains bacteria that break down organic wastes and also destroy dangerous chemicals. Auxin in present in worm's casting that has a role in stimulation of roots to grow deeper and faster. The castings produced by earthworm are normally more stable as compared to soil aggregates from parent and contains microorganisms, enzymes, hormones organic and inorganic materials that are formed when the soil is passed down through the gut of earthworm (Tersic and Gosar, 2012). Bio-liquids is a brown colored liquid extract of vermicomposts, normally the earthworms wash, the medium collected after the falling down water through the various layers of unit of vermiculture due to increased moisture content (Jayabhaye and Bhalerao, 2015). It is an extract of worm coelomic fluid containing different enzymes, growth hormones (gibberellic acid, Indole acetic acid and cytokinin), vitamins, micro and macro nutrients (Bucker field *et al.*, 1999) along with excretory substances and mucus secretion of earthworms (Ansari and Sukhraj, 2010), humic acid from organic waste materials and soil that can be absorbed easily by plant tissues (Nath and Singh, 2012). Nitrogen from dead earthworm's tissue releases in form of 45% ammonia, 25% nitrates, 3% soluble organic compound and 27% uncalculated material that improves the quality of bio-liquids. Nitrogen in bio-liquids is present in the form of enzymes, muscus, excretory nitrogenous substances of worms and plant growth hormones (Tripathi and Bhardwaj, 2004). Bio-liquids is rich in various enzymes, urease, amylase and phosphatase, N fixing bacteria i.e. *Rhizobium* sp., *Azotobacter* sp. and *Agrobacterium* sp. and phosphate solubilising bacteria (Zambare *et al.*, 2008).

Bio-liquid is a miracle tonic from farmer's friends for enhancing the growth and yield plant. Nath *et al.* (2009) reported that bio-liquid formed from animal wastes, kitchen/agro wastes increased flowering, plant growth and finally productivity. Application of 100% RDF (recommended dose of fertilizer) + bio-liquid @100 L ha^{-1} increased growth, flowering and corm yield of gladiolus (*Gladioious dalenii*) (Kumar *et al*., 2012). Studies reported the rise in radish (*Daucus carota*) yield by 7.3 % on application of bio-liquid weekly (Giraddi, 2003). Significant increase in growth of paddy (*O. sativa*) by applying bio-liquid foliar spray has also been reported (Thangavel *et al.*, 2003). Due to its significant addition in GDP, there is dire need to protect maize from adverse effects of nutrition stress by applying bio-liquids that is miracle tonic, farmer's friend, enhance plant growth and yield; eco-friendly and also economically viable.

Keeping this in view, the experiments were conducted with the objectives to characterize the nutrient concentration in alligator weed, alfalfa and rice straw bio-liquids and concentration of these bio-liquids was optimized on morpho-physiological parameters of maize.

MATERIALS AND METHODS

Experiment No.1. Extraction and biochemical characterization of three sources of bio-liquids (alligator weed, alfalfa and rice straw)

Standard method for extraction of three bio-liquids (alligator weed, alfalfa and rice straw): Bio-liquids (earthworm wash) were prepared following the standard method proposed by Ismail (1997) with slight modification. Three different sources (alligator weed, alfalfa and rice straw) were used after pre-decomposition for the preparation of bio-liquids. A plastic drum of dimension 120 cm length and 22 cm diameter fitted with water valve at the bottom was used for the collection of drainage. It was filled with gravel (2-4" size) to a height of 15 cm, above which a layer of course sand (15 cm) was placed and then two kilogram partially decomposed organic materials (alligator weed, alfalfa and rice straw separately for each species bio-liquid) and six kilogram well decomposed powdery cow dung was added. To this, 200 adult earthworms of local species were added. Well rotten cattle manure was taken from dairy farm and earthworms of local species (*Allolobophora caliginosa*) were collected from water channel at Agronomy Farm, University of Agriculture, Faisalabad, Pakistan. The compost material was kept moist with water by dropping water from the bottle hanged on top of the drum. The water was percolated slowly through the vermicompost and drilospheres, dissolving the nutrients from casting and also the washing from the drilospheres through the filter unit. After twenty five days, bio-liquid was collected through water valve at the bottom of drum.

***Chemical characterization of bio-liquids*:** Chemical characterization of bio-liquids regarding pH, EC and concentration of total N, P, K, Zn, Mg, Ca and Cu was done by following the standard procedures.

Experiment No.2. Optimizing the concentration of bio-liquids on morpho-physiological characteristics of maize under glass house

***Experimental design and treatment detail*:** The study was conducted in Glass House during spring season 2019 at University of Agriculture, Faisalabad (altitude 184.4 m, latitude 31.40º N, longitude 73.05º E). The treatments comprised of three sources of bio-liquids (alligator weed, alfalfa and rice straw) and four concentrations of bio-liquids (Zero, 10%, 15% and 20%). Bio-liquids extracted in first experiment were further diluted to various concentrations and preserved in plastic bottles. Five seeds of hybrid maize (P-1543) were sown in each plastic pot filled with river sand (500 g pot^{-1}) in glass house. The seed were further thinned to two per pot after complete emergence. The design used was completely randomize (CRD) having factorial arrangement with four replications. Foliar spray of each bio-liquid was applied at 10, 15 and 20 days after emergence to maize seedling separately. The plants were harvested 45 days after emergence and the evaluation was done on the basis of various morphological (plant height, number of leaves per plant, fresh weight of shoot, fresh weight of leaf) and physiological attributes (water potential, osmotic potential, chlorophyll fluorescence, cell membrane stability index and relative water contents).

***Procedure for recording data*:** Plant height was measured by taking 2 plants from each pot 45 days after sowing with the help of meter rod. Measurements were averaged to get the mean plant height.

Leaves per plant were recorded by counting number of leaves from two plants from each pot and then average was calculated. Total fresh weight per plant was recorded by weighing two plants at 45 days after emergence and then an average was calculated to get mean weight per plant.

Leaf water potential was determined with water potential equipment (Model 600), a method followed by Scholander *et al*. (1964). A third leaf from top of each plant was sealed in the chamber. Pressure was applied to the leaf and carefully observe the xylem sap comes out at the cut surface using nitrogen gas. The leaves from each treatment were placed in the chamber as soon as feasible after cutting from plant.

The leaf used for water potential measurement was further kept at -20 ºC. The leaf was thawed, cell sap was taken and collected in eppendorf tubes. A small drop of sap was used in cryoscopic osmometer (Osmomat 030-D) for measurement of osmotic potential.

Chlorophyll fluorescence was determined by using chlorophyll fluorometer (model OS30 p^+). Membrane stability was estimated by measuring the leachates conductivity from damaged membrane, the standard method of Shanahan *et al*. (1990). One gram leaf pieces (10 × 10 mm) were kept in 10 mL distilled water in glass tube for 24 h at 10^0C. Conductivity (C_1) was measured at 25^0C with the help of conductivity meter. For measuring the conductivity (C_2), samples were heated for 10 minute and cooled at 25^0C. Membrane stability index (MSI) was estimated as:

$$MSI = [1 - (C_1/C_2)] \times 100$$

Relative water content was determined by following the method of Smart and Bingham (1974). The fresh weight from each plant was determined. The fresh leaves were floating on distilled water for 4 hours and recorded the turgid weight. The leaf tissues were further dried at 65^0C for 24 hrs and dry weight was measured. Relative leaf water content was estimated from the formula proposed by Turner (1986).

$$\text{RWC (\%)} = (\text{Fresh Weight} - \text{Dry Weight}) / (\text{Turgid Weight} - \text{Dry Weight}) \times 100$$

***Statistical Analysis*:** The experimental data were subjected to analysis using statistical package Statistix 8.1 (Analytical Software, USA). Tuckey's Honest Significant Difference (HSD) test at 5% probability level was used for comparing the difference among treatments' means. (Steel *et al.,* 1997).

RESULTS

It is evident from Table 1 that appreciable amount of bio-available micro and macro nutrients were found in bio-liquids from all sources. Minimum pH and higher electrical conductivity values were noted from alligator weed bio-liquid (8.16 and 1.80, respectively) as compared to alfalfa and rice straw bio-liquids. Regarding total N content, maximum amount (0.54%) was obtained from alligator weed bio-liquid as compared to alfalfa and rice straw bio-liquids (0.51% and 0.43%, respectively). Phosphorous and K contents (6.044 ppm and 0.418 ppm, respectively) were again found to be higher in alligator weed bio-liquids as compared to alfalfa and rice straw bio-liquids. Similarly, the zinc, magnesium and calcium contents were also found to be higher in alligator weed bio-liquid (1.89 ppm, 35 ppm, 240 ppm, respectively). There was found to be no difference in copper content from all the sources.

Table 1. **pH, EC, N, P, K, Zn, Mg, Ca and Cu concentration in alligator weed, alfalfa and rice straw bio-liquids.**

Ingredients	Alligator weed bio-liquid	Alfalfa bio-liquid	Rice straw bio-liquid
pH	8.16	8.48	8.21
EC (ds m^{-1})	1.80	1.48	1.49
Total N (%)	0.54	0.51	0.43
P (ppm)	6.04	5.49	2.75
K (%)	0.42	0.37	0.22
Zn (ppm)	1.89	0.00	0.02
Mg (ppm)	35.00	28.00	31.00
Ca (ppm)	240.00	36.50	5.75
Cu (ppm)	0.09	0.09	0.09

Growth and physiological attributes of maize like plant height, number of leaves, fresh weight of plant, water potential, osmotic potential, chlorophyll fluorescence, relative water content and cell membrane stability were found to be positively affected by foliar spray of bio-liquids in the present study.

Plant height was positively affected when plants received foliar spray of bio-liquids. It is evident from the Table 2 that highest plant height (16.55 cm) was observed by applying foliar spray of alligator weed bio-liquid @15% followed by 20% alligator weed bio-liquid (16.50 cm). However, minimum plant height (12.93 cm) was found under control. Foliar spray of bio-liquids obtained from all sources considerably increased this growth attribute.

Foliar spray of bio-liquids resulted in an improved number of leaves as compared to control. It is evident from the Table 2 that higher number of leaves (7.80) was observed by applying foliar spray of alligator weed bio-liquid followed by alfalfa bio-liquid (7.50) both applied @15%. Minimum number of leaves (6.30) per plant was obtained under control conditions. An increase of 23.8% in number of leaves was observed by applying foliar spray of 15% alligator weed bio-liquid as compared to control.

Data concerning leaf area per plant presented in Table 2 indicated that leaf area per plant was improved by the application of bio-liquids. Maximum leaf area per plant (187.41) was recorded by the foliar application of alligator weed bio-liquid @15% followed (185.47) by the application of alfalfa bio-liquid @15%, however, minimum leaf area per plant (162.20) was recorded under control. There was an increase of 10% in leaf area per plant by the application of alligator weed bio-liquid

Data presented in Table 2 indicated that significantly higher fresh weight per plant (5.74 g) was reported by applying foliar

Table 2. Interactive impact of various sources and concentrations of bio-liquids on plant height, number of leaves per plant, leaf area per plant and total fresh weight per plant of maize.

Sources of bio-liquids	Concentration of bio-liquids (%)	Plant height (cm)	No. of leaves plant^{-1}	Leaf area plant^{-1} (cm^{-2})	Total fresh weight plant^{-1} (g)
Alligator weed	Control	13.62 de	6.30 g	162.20 f	3.75 c
	10	14.80 bc	7.27 bcd	180.32 b	4.39 bc
	15	16.55 a	7.80 a	187.41 a	5.74 a
	20	16.50 a	7.42 bc	183.19 ab	5.61 ab
Alfalfa	Control	12.93 e	6.37 fg	168.64 de	3.93 c
	10	13.50 de	6.97 de	183.29 ab	4.48 bc
	15	15.60 ab	7.50ab	185.47 ab	5.34 ab
	20	15.23 b	7.23 bcd	183.29 ab	5.61 ab
Rice straw	Control	12.97 e	6.30 g	163.97 ef	3.31 c
	10	13.27 e	6.70 ef	174.29 cd	3.96 c
	15	14.83 b	7.40 bc	183.07 ab	4.41 bc
	20	14.07 cd	7.07 cd	180.29 bc	4.63 abc
	HSD value	1.544	0.3380	6.2351	1.3243

Values sharing same letter do not differ at P = 0.05 according to HSD test

Table 3. Interactive impact of various sources and concentration of bio-liquids on water potential, osmotic potential, chlorophyll fluorescence, cell membrane stability and relative water content of maize.

Sources of bio-liquids	Conc. of bio-liquids (%)	Water potential (-MPa)	Osmotic potential (-MPa)	Chlorophyll fluorescence (fv/fm)	Cell membrane stability	Relative water Content (%)
Alligator weed	Control	0.82 abc	0.15 c	0.79 c	33.44 e	79.87 g
	10	0.80 d	0.16 bc	0.83 ab	38.07 b	86.93 bc
	15	0.80 a-d	0.16 bc	0.84 a	40.27 a	91.80 a
	20	0.80 d	0.17 abc	0.82 b	39.17 ab	86.37 cd
Alfalfa	Control	0.82 a	0.15c	0.80 c	33.80 de	81.30 fg
	10	0.80 bcd	0.16 bc	0.83 ab	35.73 c	86.63 c
	15	0.79 d	0.16 bc	0.82 ab	39.00 ab	89.53 ab
	20	0.79 d	0.18 a	0.82 b	39.17 ab	82.87 ef
Rice straw	Control	0.82 ab	0.15 c	0.80 c	31.20 f	79.33 g
	10	0.80 cd	0.15 c	0.82 b	35.67 cd	83.87def
	15	0.80 cd	0.16 bc	0.82 ab	37.07 bc	89.57 ab
	20	0.79 d	0.17ab	0.83 ab	35.57 cd	85.23 cde
	HSD value	0.0183	0.0171	0.0194	1.5446	2.7251

Values sharing same letter do not differ at P = 0.05 according to HSD test

spray of alligator weed bio-liquid @15% followed by application of alligator weed and alfalfa bio-liquids @20% (5.61 g). However, minimum fresh weight per plant (3.31) was observed under control. An increase of 69.75% in fresh weight per plant was recorded by applying foliar spray of 15% alligator weed bio-liquid as compared to control.

Water potential of leaves in the bio-liquid applied treatment was higher as compared to control. Water potential was improved by the application of bio-liquids. Analyzed data regarding leaf water potential is presented in Table 3 which showed that maximum water potential towards less negative (-0.79 MPa) was attained by the foliar application of alfalfa and rice straw bio-liquids @15% and 20%, respectively. However, minimum water potential towards more negative (-0.82 MPa) was recorded under control (Table 3).

Leaf osmotic potential was affected significantly by various sources and concentrations of bio-liquids. Analyzed data pertaining to leaf osmotic potential are presented in Table 3 and showed that maximum osmotic potential (-0.15 MPa) was recorded under control. Minimum osmotic potential (-0.18 MPa) was observed by applying alfalfa bio-liquid @ 20%.

There were significant differences among treatments regarding investigated chlorophyll fluorescence parameters. By estimating the chlorophyll fluorescence, information about variation in photochemistry and the heat dissipation rate can be acquired. Table 3 showed that maximum chlorophyll fluorescence (0.84) was observed by the foliar spray of alligator weed bio-liquid @15%. Minimum value of chlorophyll fluorescence (0.80) was observed under control. An increase of 5.9% in chlorophyll fluorescence was observed by the foliar spray of alligator weed bio-liquid as compared to control.

Cell membrane stability was improved by the foliar application of bio-liquids. Table 3 depicts that maximum value of cell membrane stability (40.27) was observed by the foliar spray of alligator weed bio-liquid @15% followed by alligator weed and alfalfa bio-liquid each @20% (39.17). The minimum value of cell membrane stability was observed under control (33.44).

The value of relative water content represents the water status in leaves. Maximum value of relative water content (91.80%) was recorded by the foliar application of alligator weed bio-liquid @15% followed by rice straw and alfalfa weed bio-liquid @ 15% (89.57% and 89.53%, respectively). Minimum relative water content (79.33%) was observed under control (Table 3).

DISCUSSION

Increase in pH of bio-liquid might be due to role of micro organisms in the decomposition of organic waste depicting metabolism under aerobic conditions. The increase in EC of the bio-liquid could be due to the reduction of organic matter and discharge of various salts in accessible forms such as potassium, ammonium and phosphate. Our results are in accordance with studies described by Bai and Vijayalakshmi (2000). They concluded that the EC was enhanced in bio-liquid after the earthworm *E. eugeniae* inoculation. Rise in N in the bio-liquid could be because the earthworms boosted the N cycle, which refer to the enhanced N in bio-liquid. Tripathi and Bhardwaj (2004) concluded that the reduction of organic carbon could be subjected to adding N in the form of excretory nitrogenous substances, mucus, stimulatory growth hormones released from gut of earthworms. The amount of P in bio-liquid was also higher. Studies by Lee (1985) reported that the increased P level in bio-liquid might be due to mineralization of P at the time of vermicomposting. The discharge of P in the soluble form is due to different enzymes in the gut of earthworm especially phosphatases and discharge of P could be due to presence of P-solubilizing micro-organisms in bio-liquid reported by Varghese and Prabha (2014). The rise in K content in bio-liquid might be due to distribution and changes of K forms between exchangeable and non exchangeable forms as mentioned by Varghese and Prabha (2014). Our results are in confirmatory with studies described by Suthar (2007) who find out that waste material processed by earthworm contains greater amount of exchangeable K, because of increased activity of micro-organism at the time of vermicomposting, that enhanced the process of mineralization. The results showed that the amount of Zn in bio-liquid was higher. The results of the current study are in confirmatory with Suthar (2010) who find out that content of Zn was higher in the bio-liquid. Calcium carbonate is produced in the earthworms' gut due to presence of calciferous glands that play a role in the production of Ca availability in the bio-liquid. Garg *et al.* (2006) concluded that the enhanced level of Ca might be due to the process associated with Ca metabolism in the gut that is responsible for increased Ca content in worm cast. Higher amount of Cu was seen in bio-liquid due to Cu containing enzymes. These results are in confirmatory with those reported by Ansari and Sukhraj (2010). They concluded that the vermiwash contains higher Cu content as compared to vermicompost.

Organic farming is an integrated approach for acquiring sustainable agriculture as it is environmental friendly and economically viable. Earthworms, vermicompost and bio-liquid (earthworm wash) are a miracle plant growth promoter, nutritionally much better than traditional compost and synthetic fertilizers. Earthworm casts contain nutrients which are quickly available to plants (Taylor *et al*., 2003). Foliar spray of bio-liquids improved the seedling development of maize, however, foliar spray of 15% alligator weed bio-liquid showed growth promoting impacts regarding morpho-physiological characters i.e., plant height, no. of leaves plant^{-1}, total fresh weight plant^{-1}, water potential, osmotic potential, cell membrane stability and relative water content.

It could be attributed that humic acid and other nutrients are produced at the time of vermicomposting (Manivannan, 2004). These results are in accordance with Nath and Singh (2012) and Rajan and Murugesan (2012). They have reported the growth enhancing impacts by using bio-liquids. Bio-liquid application after 30 days on *Capsicum frutescens* exhibited increase in number of leaves and shoot length than the control plants (Varghese and Prabha, 2014). Samadhiya *et al*. (2013) reported that bio-liquid sprayed on the tomato plants enhanced shoot length and number of leaves.

Plant height is essential parameter towards economic yield of maize. Increase in plant height due to use of bio enhancers was also reported by Bhalla *et al.* (2006) and Kumar *et al.* (2010). Number of leaves is linked to intercept photo active radiation and thus ultimately enhanced photosynthesis. Hemant *et al*. (2013) concluded that bio-liquid when sprayed on the tomato plants, improved the shoot length and number of leaves. Ghasem *et al.* (2014) observed 22.6% increases in plant height by the application of organic fertilizer on cucumber. Study also reported that 15% bio-liquid showed growth promoting impacts on *Abelmoschus esculentus* (Elumalai *et al.*, 2013). Leaf area represents the photosynthetic potential of plant. More the leaf area, more will be the dry matter accumulation. Elumalai *et al.* (2013) reported that bio-liquid application on *A. esculantus* showed growth promoting effects on plant height, number of leaves and leaf area per plant. The increase in said attributes might be due to improved micro and macro nutrients in plants through foliar application of bio-liquid. Lalitha *et al.* (2000) reported that application of natural fertilizers had pronounced effect on the growth of plant, indicating a better performance of okra. The positive impact of bio-liquids spray on crop growth was reported by Thangavel *et al.* (2003) and Samuthiravelu *et al.* (2012). Fatima and Seker (2014) reported the growth enhancing effect of bio-liquid on vegetable crops. It was concluded that bio-liqiuid at higher dilution enhanced seedling growth of crops.

Application of bio-liquids positively affected leaf water potential. The current investigation showed that application of bio-liquids resulted in lowering leaf water potential. The reduction in water potential of leaf might be because of more hydraulic resistance (Hattori *et al*., 2005). More negative values of osmotic potential showed a higher degree of osmoregulation and a greater capacity of leaf tissues for retaining water. Similarly, foliar spray of bio-liquids was effective in reducing the leaf osmotic potential.

Chlorophyll fluorescence is the re-emitted light by chlorophyll molecule on return from excited to non-excited states. Application of bio-liquids from all sources improved chlorophyll fluorescence values. At 20% concentrations maximum leaf chlorophyll content 2.9% was observed (Narkhede *et al.,* 2011). Venkataramana *et al.* (2009) reported that the foliar sprays of bio-liquids increased yield that might be because of increasing chlorophyll and nitrogen contents in the leaf. Results related to the current investigation were also reported by Khairnar and Gunjal (2012) on greengram and Dhok (2013).

Cell membrane stability is an effective for evaluating the plants against stress tolerance (Sairam *et al*., 2005). Foliar spray of bio-liquids from all sources significantly improved the cell membrane stability. Relative water content (RWC) is a measure of water status in plant and predicting the metabolic activities in leaves. Application of bio-liquids significantly improved the relative water content.

***Conclusion*:** The present investigation concluded that the foliar spray of bio-liquids obtained from different sources and concentrations has better growth promoting effect regarding morpho-physiological traits as compared to control. Bio-liquid extracted from alligator weed proved to be the best source of nutrients as compared to alfalfa and rice straw bio-liquids.

REFERENCES

Abdul Rida, A.M. and M.B. Bouche. 1997. Earthworm toxicology: from acute to chronic tests. Soil Biol. Biochem. 29:699-703.

Ansari, A.A. and K. Sukhraj. 2010. Effect of vermiwash and vermicompost on soil parameters and productivity of okra (*Abelmoschus esculentus*) in Guyana. Afr. J. Agri. Res. 5:1794-1798.

Bai, L.L. and G.S. Vijayalakshmi. 2000. Vermicomposting of sugar factory filter pressmud using an African earthworm species *Eudrilus eugeniae* (Kinberg) with a note on its physico-chemical features, Pollution Res. 19:481-483.

Bhalla, R, P. Kanwar, S.R. Dhiman and R. Jain. 2006. Effect of biofertilizers and biostimulants on growth and flowering in gladiolus. J. Ornamental Horti. 9:248-252.

Bhat, J.V. and P. Khambata. 1994. Role of earthworms in agriculture. Ind. Counc. Agri. Res. 22:36.

Brown, G.G., N.P.Benito, A. Pasini, K.D. Sautter, M. Guimaraes and E. Torres. 2004. No-tillage greatly increases earthworm populations in Parana State, Brazil, Pedobiol. 47:764-771.

Bucker field, J.C., T. Flavel, K.E. Lee and K.A. Web. 1999. Vermicompost soil and liquid form as plant growth promoter. Pedobiologia 42:753-759.

Dhok Swati, P. 2013. Role of vermicompost and vermiwash as a biotic indicators for enhancement of soil health in sustainable agriculture. Int. J. Agric. Sci. 9:388-391.

Elumalai, D., P.K. Kaleena, M. Fathima and M. Hemavathi. 2013. Influence of vermiwash and plant growth regulators on the exomorphological characters of *Abelmoschus esculentus* (Linn.) Moench. Afr. J. Basic Appl. Sci. 5:82-90.

Fatima, M. and M. Sekar. 2014. Studies on growth on promoting effect of vermiwash on the germination of Vegetable Crops. Int. J. Curr. Microbiol. Appl. Sci. 3:564-570.

Garg, V.K., Y.K. Yadav, A.S. Sheoran, P. Chand and Kaushik. 2006. Live stocks excreta management through vermicomposting using an epigeic earthworm *Eisenia foetida*. Environmentalis 26:269-276.

Ghasem, S., A.S. Morteza and T. Maryam. 2014. Effect of organic fertilizer on cucumber (*Cucumis sativus*) yield. Int. J. Agri. Crop Sci. 7:808-814.

Gill, H.K. and H. Garg. 2014. Pesticide: environmental impacts and management strategies. Pesticides toxic effects. Intech. Rijeka, Croatia. pp. 187-230.

Giraddi, R.S. 2003. Method of extraction of earthworm wash: A plant promoter substance. 7th National Symposium on Soil Biology and Ecology, Bangalore.

Hattori, T., S. Inanaga, H. Araki, P. An, S. Morita, M. Luxova and A. Lux. 2005. Application of silicon enhanced drought tolerance in *Sorghum bicolor*. Physiol. Plant. 123:459-466.

Hemant, S., P. Dandotiya, J. Chaturvedi and O.P. Agrawal. 2013. Effect of vermiwash on the growth and development of leaves and Stem of tomato plants. Int. J. Current Res. 5:3020-3023.

Ismail, S.A. 1997. Vermicology: The Biology of Earthworms. Orient longman Press, Hyderabad, pp. 92.

Jayabhaye, M.M. and S.A. Bhalerao. 2015. Influence of vermiwash on germination and growth parameters of seedlings of green gram (*Vigna radiata* L.) and black gram (*Vigna mungo* L.). Int. J. Curr. Microbiol. Appl. Sci. 4:635-643.

Ju, X.T., G.X. Xing, X/P. Chen, S.L. Zhang, L.J. Zhang, X.J. Liu, Z.L. Cui, B. Yin, P. Christie and Z.L. Zhu. 2009. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. PNAS. 106:3041-3046.

Khairnar, A.V. and B.S. Gunjal. 2012. Effect of potash fertilization and foliar spray of vermiwash on growth and yield of green gram (*Vigna radiata* L.). Int. J. Agric. Sci. 8:307-308.

Kumar, R., B. Deka and C. Roy. 2010. Effect of bioregulators on vegetative growth, flowering and corm production in gladiolus cv. Candyman. J. Ornamental Hort. 13:35-40.

Lalitha, R., K. Fatima and S.A. Ismail. 2000. The impact of biopesticide and microbial fertilizers on productivity and growth of *Abelmoschus esculentus*. Vasundara Earth. 1:4-9

Leite, L.F.C., F.C. Oliveira, A.S.F. Araújo, S.R.S. Galvao, J.O. Lemos and E.F.L. Silva. 2010. Soil organic carbon and biological indicators in an acrisol under tillage systems and organic management in Northeastern Brazil. Aust. J. Soil Res. 48:258-265.

Li, M.L., J. Gu, H. Gao, Q.J. Qin and M.J. Liu. 2007. Effects of different organic fertilizer on plant character, quality and yield of soybean. J. Northwest A & F Univ. 35:67-72.

Lobley, M., A. Butler and M. Reed. 2009. The contribution of organic farming to rural development: an exploration of the socio-economic linkages of organic and non-organic farms in England. Land Use Policy. 26:723-735.

Manivannan, S. 2004. Standardization and nutrient analysis of vermicomposting sugarcane wastes, pressmud-trash-bagasse by *Lampito mauriti* (kingberg) and *Perionyx exacavatus* (perrier) and crop productivity. Ph.D. Thesis, Annamalai University, India.

Narkhede, S.D., S.B. Attarde and S.T. Ingle. 2011. Study of effect of chemical fertilizer and vermicompost on growth of chilli pepper plant (*Capsicum annum*). J. Appl. Sci. Environ. Sanitation 6:327-332.

Nath, G. and K. Singh. 2012. Combination of vermiwash and biopesticides against aphid (*Lipaphis erysimi*) infestation and their effect on growth and yield of mustard (*Brassica compestris*). Dyn. Soil Dyn. Plant. 6:96-102.

Nath, G., K. Singh and D.K. Singh. 2009. Chemical analysis of vermicomposts/ vermiwash of different combinations of animal, agro and kitchen wastes. Aust. J. Basic Appl. Sci. 3:3671-3676.

Rajan, M.R. and P. Murugesan. 2012. Influence of vermiwash on germination and growth of cow pea (*Vigna ungiculata*) and rice (*Oryza sativa*). IOSR J. Pharmacy. 2:31-34.

Sairam, R.K., G.C. Srivastava, S. Agarwal and R.C. Meena. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. Biol. Plant 49:85-91.

Samadhiya, H., P. Dandotiya, J. Chaturvedi and O.P. Agrawal. 2013. Effect of vermiwash on the growth and development of leaves and stem of tomato plants. Int. J. Current Res. 5:3020-3023.

Samuthiravelu, P., B. Sangeetha, N. Sakthivel, J. Ravikumar, L. Isaiarasu, R. Balakrishna and S.M.H. Qadri. 2012. Impact of organic nutrients on the incidence of major pests, leaf productivity in mulberry and food consumption and utilization of *Bombyx mori* L. management of pests using organic nutrients. J. Biopest. 5:228-232.

Sarwar, N., K. Mubeen, A. Wasaya, A.U. Rehman, O. Farooq and M. Shehzad. 2020. Response of hybrid maize to multiple soil organic amendments under sufficient or deficient soil zinc situation. Asian J. Agric. Biol. 8:38-43.

Scholander, P.L., H.T. Hammel, E.D. Bradstreet and E.A. Hemminsoln. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Natl. Acad. Sci. 52:119-125.

Shanahan, J.F., I.B. Edwards, J.S. Quick and J.R. Fenwick. 1990. Membrane thermostability and heat tolerance of spring wheat. Crop Sci. 30:247-251.

Steel, R. G. D., J. H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics, A Biometrical Approach, 3rd Ed., McGraw Hill, Inc. Book Co. N.Y. pp. 172-177.

Suthar, S. 2007. Nutrients changes and biodynamic of epigeic earthworms Perionyx excavates during recycling of some agricultural waste. Bioresource Tech. 1:315-320.

Suthar, S. 2010. Evidence of plant hormone like substances in vermiwash: an ecologically safe option of synthetic chemicals for sustainable farming. Ecol. Eng. 36:1089-1092.

Taylor, M., W.P. Clarke and P.F. Greenfield. 2003. The treatment of domestic waste water using small scale vermicompost filter beds. Ecol. Eng. 21:197-203.

Tersic, T. and M. Gosar. 2012. Comparison of elemental contents in earthworm cast and soil from a mercury-contaminated site. Sci. Total Environ. 430:28-33.

Thangavel, P, R. Balagurunathan, J. Divakaran and J. Prabhakaran. 2003. Effect of vermiwash and vermicast extract on soil nutrient status, growth and yield of paddy. Adv. Plant Sci. 16: 187-190.

Tripathi and P. Bhardwaj. 2004. Earthworm diversity and habitat preferences in arid regions of rajasthan. Zoos' Print J. 19:1515-1519.

Turner, N.C. 1986. Crop water deficit: a decade of progress. Adv. Agron. 39:1-51.

Varghese, S.M. and M.L. Prabha. 2014. Biochemical Characterization of Vermiwash and its Effect on Growth of *Capsicum frutescens*. Malaya J. Biosciences. 1:86-91.

Venkataramana, P., B. Narasimhamurthy, J.V. Krishnarao and C.K. Kamble. 2009. Efficacy of foliar spray of verminwash and cow dung wash on biochemical and yield attributes and yield of mulberry (*Morus alba* L.). Karnataka J. Agric. Sci. 22:921-923.

Zambare, V.P., M.V. Padul, A.A. Yadav and T.B. Shete. 2008. Vermiwash: Biochemical and biological approach as eco-friendly soil conditioner. ARPN J. Agri. Biol. Sci. 3:28-37.

[Received 2 Jun 2020; Accepted 11 Aug 2020; Published (online) 18 April 2021]