

Effect Of Fertilizer Treatment On Primary Metabolites Of Boerhaavia Diffusa Linn

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Abstract

Metabolites are very important products for plants because they are directly involved in the all activity like reproduction, growth and their development and play a very significant role in their life. They produced from metabolism and catalyzed by different types of enzymes. Estimation of primary metabolites have made in cutting of B. diffusa treated with different fertilizer doses as cow dung 10 ton/ha N:P:K 60:40:20 kg/ha, cow dung 10 ton/ha N:P:K 45:30:15 kg/ha, cow dung 10 ton/ha+ N:P:K 30:20:10 kg ha, and cow dung 5 ton/ha + N:P:K 60:40:20 kg/ha, cow dung 5 ton/ha+ NPK 45.30:15 kg/ha and cow dung 5 ton/ha+ N:P:K 30:20:10 kg/ha.

Maximum amount of total sugar, was found with in treated cutting was found with root, leaf and stem respectively in cow dung 10 ton/ha + N:P:K 45:30:15 kg/ha. Maximum amount of total soluble protein was found with root and stem respectively of cow dung 10 tan/ha+ N:P:K 45:30:15 kg/ha but in leaf it was maximum in cow dung 10 tanha N:P:K 30:20:10 kg/ha treated cutting and the maximum amount of total soluble protein was found with root and stem respectively of cow dung 10 tan/ha+ N:P:K 45:30:15 kg/ha but in leaf it was maximum in cow dung 10 tanha N:P:K 30:20:10 kg/ha treated cutting. Maximum amount of TCA precipitated protein was leaf in cow dung 10 ton/ha N:P:K 45:30:15 kg/ha treated cutting but in stem it was maximum in cow dung 10 ton/ha NPK 60:40:20 kg/ha treated cutting as compared to control.

INTRODUCTION

Boerhaavia diffusa, common name punarnava is an important medicinal herb, belonging to the is family Nyctaginaceae (four o'clock) family. The parts of plant such as leaves, stem, and root have medicinal properties and use by native and tribal people in India. It has many ethnobotanical uses. Stem prostrate, branches cylindrical, thickened at nodes. Leaves simple, alternate, ovate, 1-3 cm long, oblong or suborbicular, green and glabrous above with rounded or sub cordate base and sub undulate margins. Petiole pink. Flowers are pink, small or minute and non-involucrate and are born in small umbels which are arranged in auxiliary and terminal panicles. Only few plants establish in to perennial from and most of new plants die during the dry season. Vegetative growth is fast during rainy season, latter on it declines during dry season. All parts of the plants are medicinally useful but roots are more important. The leaves are eaten as pot- herb. Few local people collect entire plants for sale to herbal dealers and practitioners.

MATERIALS AND METHODS

Experiments were conducted to determine the best response of nitrogen, phosphorus and potash fertilization on, production of primary metabolites (i.e.; carbohydrates & proteins of Boerhaavia diffusa).

Land Preparation:

A Field experiment was conducted with six treatment combinations replicated three times to evaluate the effect of fertilizer combination on primary metabolites (carbohydrates and proteins),. The Plot area was (2m ×2m) for each plot for the species. The Soil was pretreated with 10%BHC and Bavistine 0.1% w/w to avoid insect attack and fungal infection. Following fertilizer combinations were applied in plot area:

N: P: K	Cow dung
60:40:20	10 ton/ha

45:30:15	10 ton/ha
30:20:10	10 ton/ha
60:40:20	5 ton/ha
45:30:15	5 ton/ha
30:20:10	5 ton/ha

Nitrogen was applied in the form of urea (46% N), phosphorus, in the form of the single super phosphate (16% P₂O₅) and basal dose of potash in the form of murate on potash (60% K₂O). Control, without N, P and K and biofertilizer as form of cow dung treatments were also mentioned simultaneously.

Planting Boerhaavia diffusa:

Collected shoot in plant of Boerhaavia diffusa in the month of July from campus of Pt. Ravishankar Shukla University, Raipur. The cuttings were treated with 0.1% Bavistin for 15 minutes. These treated cuttings were planted in to the experimental plot area at a spacing of 20 x 20cm row to row.

Estimation of Primary Metabolites:

Primary metabolites were estimated in plant extract. Plant extract was prepared by homogenizing 1 gm of plant tissue with 5 ml of sodium-phosphate buffer (pH-7.2) 1 with mortar and pestle. It was then centrifuged at 15,000 rpm for 20 min at 4°C. Finally, supernatant only was used for the estimation of total sugars, reducing sugars. Soluble proteins and TCA precipitated proteins.

Estimation of Total Sugars:

Total sugar of plant tissue was estimated according to Sipro (1996). For this 50µl of aliquot was diluted to 1 ml with distilled water and then 4ml of anthrone reagent was added, gently. The whole mixture was vortexed and boiled for 10 min. in water bath and cooled in dark. Finally, absorbance was measured at 630nm by using UV-Vis spectrophotometer (Jasco-Japan). The final concentration of reducing sugar in filtrate was determined by preparing a calibration curve of standard Glucose solution and expressed in terms of total Sagar mg/g plant tissue.

Estimation of Reducing Sugar:

Reducing sugar of plant tissue was estimated according to Wood et al (1988). For which 200 µl aliquot was diluted to 5ml. To this was added 1ml of Somogyi reagent and was boiled in water bath for 20 min. After cooling. 1ml of arsenomolybdate reagent was added to it and vortexed to complete the mixing. After 15 min, absorbance was measured with the help of an UV-Vis spectrophotometer Casco-Japan) at 500nm. The final concentration of total sugar was determined with the help of a calibration prepared from standard protein and expressed mg/gm plant tissue.

Estimation of Total Water-Soluble Protein:

Total water-soluble protein was estimated cording to Lowery etal1951) for which, 50 µl aliquot was diluted up to 1ml with distilled water. To this was added 5ml alkaline copper agent and was vortexed for complete mixing. Then 0.5 ml of Folinicocaltue reagent was added vortexed and allow to develop colour for 20 min at room temperature: The absorbance was measured at 750nm with the help of an UV Vis spectrophotometer (Jasko-Japan). Protein concentration was determined with the help of a calibration curve prepared from standard protein and expressed mg/gm plant tissue.

Estimation of TCA precipitated proteins:

Protein was precipitated by adding 1ml of 10% TCA to 1ml of aliquot. The sample was centrifuged for 15 min. at 3000 rpm and pellet was obtained containing proteins. It was washed with ethanol: ether (1:1) and recentrifuged for 15 min. at 3000 rpm. Again, pellet was collected and dissolved in 2.5 ml of 1N NaOH. This was used for the estimation of TCA precipitated protein following the method of Lowry et al. (1951).

RESULT

Estimation of Primary Metabolites:

Estimation of primary metabolites was made in cutting of B. diffusa treated with different fertilizer doses as cow dung 10 ton/ha N:P:K 60:40:20 kg/ha, cow dung 10 ton/ha N:P-K 45:30:15 kg/ha, cow dung 10 ton/ha+ N:P:K 30:20:10 kg ha, and cow dung 5 ton/ha + N:P:K 60:40:20 kg/ha, cow dung 5 ton/ha+ NPK 45.30:15 kg/ha and cow dung 5 ton/ha+ N:P:K 30:20:10 kg/ha,

Estimation of total sugar in cuttings of Boerhaavia diffusa treated with different combination of fertilizer:

Maximum amount of total sugar with 15.46±0.088 mg/g, 14.2±0.174 mg/g and 13.74±0.369 mg/g in treated cutting was found with root, leaf and stem respectively in cow dung 10 ton/ha + N:P:K 45:3±0:15 kg/ha. This was followed by cow dung 5 ton/ha NPK 60:40:20 kg/ha treated cutting, 14.56±0.086 mg/g 13.93 ±0.384 mg/g and 13.16±1.92 mg/g, in root leaf and stem respectively. Control had values 11.13±0,066 mg/g, 10.33±0.143 mg/g and 10.661±0.142 mg/g in root, leaf and stem respectively.

Estimation of reducing sugar in cuttings of Boerhaavia diffusa treated with different combination of fertilizer:

Maximum amount of reducing sugar 2.7±40.1 mg/g was found in root with cow dung 10 ton/ha+ NPK 60:40:20 kg/ha treated but in leaf and stem 1.8±0.057 mg/g and 1.86± 0.183 mg/g it was maximum in cow dung 10 tan/ha+ NPK 45:30:15 kg/ha treated cuttings followed by 2.5±0.057 mg/g, 1.56±0,202 mg/g and 1.731±0,120 mg/g in root, leaf and stem of cow dung 5 tan/ha+N.PK 60:40: 20 kg/ha respectively as compared to 1.73±10.120 mg/g, 1.0±0.152 mg/g and 1.26±-0.03 mg/g in root, leaf and stem of control.

Estimation of total soluble protein in cuttings of Boerhaavia diffusa treated with different combinations of fertilizer:

The amount of total soluble protein in root was lower as compared to control to cow dung 10 ton/ha NPK: 45:30:15 kg/ha treated cutting but decreased to cow dung 10 tan/ha N:P:K 60:40:20 kg/ha treated cutting Maximum amount of total soluble protein 31.23±0.66 mg/g and 14.80±.404 mg/g was found with root and stem respectively of cow dung 10 tan/ha+ N:P:K 45:30:15 kg/ha but in leaf 18.0±0.14 mg/g it was maximum in cow dung 10 tanha N:P:K 30:20:10 kg/ha treated cutting followed by 26.5± 1.65 mg/g. 15.36± 0.425 mg/g and 11.9±1.132 mg/g in root, leaf and stem of cow dung 5 tan/ha N:P:K45:30:15 kg/ha treated cuttings control had value 22.63±0.319 mg/g. 10.36 ±0.721 mg/g and 5.5 ±0.233 mg/g in root. Leaf and stem respectively.

Estimation of TCA precipitated protein in cuttings of Boerhaavia diffusa treated with different combinations of fertilizer:

Maximum amount of TCA precipitated protein was 5.13± 0.506 mg/g in root and 3.66± 0.589 mg/g and leaf in cow dung 10 ton/ha N:P:K 45:30:15 kg/ha treated cutting but in stem with 3.93±0.589 mg/g it was maximum in cow dung 10 ton/ha NPK 60:40:20 kg/ha treated cutting followed by 4.26±10.20 mg/g, 2.09± 0.063 mg/g. 3.86 ± 0.290 mg/g in root, leaf and stem respectively in cow dung 5 ton/ha NPK 60:40:20 kg/ha treated cuttings as compared to 3.86±2.90 mg/g. 1.86± 0.520 mg/g and 2.06±10.133 mg/g in root, leaf and stem of control.

Table: 1 Estimation of total sugar in cuttings of B. diffusa treated with different combinations of fertilizer

Cow dung/ha	N:P:K kg/ha	Root (mg/g)	Leaf(mg/g)	Stem (mg/g)
0 ton/ha	0:0:0	11.13±0.06	10.33±0.143	10.66±0.142
5 ton/ha	60:40:20	14.56±0.086	13.93±0.384	13.16±1.92
	45:30:15	13.5±0.68	13.56±0.578	12.10±1.927
	30:20:10	13.79±0.05	13.60±0.1	11.0±0.152
10 ton/ha	60:40:20	15.40±0.28	14.1±0.305	13.6±1.123
	45:30:15	15.46±0.08	14.2±0.174	13.7±0.369
	30:20:10	14.73±0.12	13.7±0.384	13.6±0.185

Table: 2 Estimation of reducing sugar in cuttings of B.diffusa treated with different combinations of fertilizer.

Cow dung/ha	N:P:K kg/ha	Root (mg/g)	Leaf(mg/g)	Stem (mg/g)
0 ton/ha	0:0:0	1.73±0.120	1.0±0.152	1.26±0.03
5 ton/ha	60:40:20	2.5±0.057	1.56±0.202	1.73±0.120
	45:30:15	2.23±0.120	1.3±0.11	1.26±0.120
	30:20:10	1.86±0.088	1.26±0.03	1.33±1.33
10 ton/ha	60:40:20	2.7±0.1	1.56±0.207	1.29±0.03
	45:30:15	2.66±0.12	1.8±0.057	1.86±0.183
	30:20:10	2.43±0.08	1.7±0.102	1.36±0.185

Table: 3 Estimation of total soluble protein in cuttings of B. diffusa treated with different combination of fertilizer

Cow dung/ha	N:P:K kg/ha	Root (mg/g)	Leaf(mg/g)	Stem (mg/g)
0 ton/ha	0:0:0	22.63±0.319	10.36±0.72	5.5±0.233
5 ton/ha	60:40:20	26.5±1.65	15.36±0.42	11.9±1.132
	45:30:15	25.2±0.688	15.63±0.31	11.8±0.351
	30:20:10	23.66±0.808	14.8±0.404	10.33±0.233
10 ton/ha	60:40:20	24.9±0.371	12.03±1.29	11.93±0.176
	45:30:15	31.23±0.66	14.76±0.40	14.8±0.404
	30:20:10	28.0±0.585	18.0±0.95	12.2±0.378

Table: 4 Estimation of TCA precipitated protein in cuttings of B. diffusa treated with different combinations of fertilizer

Cow dung/ha	N:P: K kg/ha	Root (mg/g)	Leaf(mg/g)	Stem (mg/g)
0 ton/ha	0:0:0	3.86±0.29	1.86±0.520	2.06±0.133
5 ton/ha	60:40:20	4.26±0.20	2.09±0.063	3.86±0.290
	45:30:15	4.1±0.14	2.4±0.746	2.36±1.29
	30:20:10	4.06±0.3	2.0±0.052	2.4±0.746
10 ton/ha	60:40:20	3.93±0.38	2.23±1.29	3.4±0.30
	45:30:15	5.13±0.50	3.66±0.589	3.06±0.308
	30:20:10	4.13±0.20	3.06±0.088	3.93±0.589

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