118 were procured from Himachal Pradesh Agricultural University (Palampur), India. Before treatment, the seeds were surface sterilized using 0.01% mercuric chloride for 1 min. followed by 8-10 times washing with distilled water. Then, seeds were dried in the folds of filter paper and presoaked. To avoid background phthalates contamination the glassware washed and dried at 150^{o}C for 25 min. The stock solution of DBP (1600 mg/L) was prepared according to the method of Kaur et al., 2017 [19]. The working concentrations viz. 25, 50, 100, 200, 400, 800, 1600 mg/L were prepared through serial dilution. Petri plates were lined by autoclaved double layer of Whatman filter paper no. 1. The seeds were treated with different concentrations of DBP periodically and were kept in seed germinator at 25±0.5^{o}C and photoperiod of 16 h for 7 days. The seedlings were observed daily for germination and morphological indices.

Study of germination indices of barley

The effect of DBP on barley seedlings were analyzed for following germination indices:

Germination percent was the first parameter studied to know the impact of DBP on viability of barley seeds. The indices like percent germination (G%), germination speed (GS), peak value (PV), mean daily germination (MDG) and germination value (GV) were calculated according to the method given by Czabator et al., 1962 [20]. The mean germination time (MGT) determined using the method of Ellis and Robertis, 1981 [21]. Seed vigour index (SVI), phytotoxicity index (PI), germination rate index (GRI) and seed mortality (SM) were determined according to the method of Orchard et al., 1977; Mekki et al., 2007, Wang et al., 2004 and Osman, 2004 respectively [22, 23, 24, 25].

The formulae for each index are as followed:

G%=(No. of germinated seeds/total number seeds)× 100(1)

GS = n1/d1 + n2/d2 + n3/d3 + ... (2)

Where, n is number of germinated seeds and d is number of days.

PV=Final germination percentage/Number of days (3)

MGT = n1×d1+n2×d2+n3×d3+....../No. of observation days (4)

GV = PV × MDG (5)

Where, PV is the peak value and MDG is the mean daily germination

MDG = Total germinated seeds / No. of observation days (6)

$$GRI = \sum Gt / Tt \quad (7)$$

Where, Gt is germination percentage at tthday and Tt is the days of germination test

SVI = SL × G% (8)

Where, SL is seedling length (cm) and G% is germination percentage

SM = (Number of non-germinated seeds / Number of observation days) × 100 (9)

$$PI = \frac{R_{LC} - R_{LT}}{R_{LT}} \quad (10)$$

Where, R_{LC} is the root length of control and R_{LT} is the root length of treatment.

The value of PI ranged between 0 and 1 and the higher value indicates the toxic effects and lower one indicates the stimulatory/positive effects [26].

Study of early growth indices of barley

The barley seedlings (30) were randomly selected from each treatment and length of shoot and root was measured. The root and shoot inhibition percentage was determined by comparing the length of control to treatment [24]. The fresh weight and dry weight were measured using the method of Lin et al., 2012 [27]. The root weight ratio, shoot weight ratio, shoot/root weight ratio and root/shoot weight ratio were recorded according to the method given by Rogers et al., 1992 [28]. Net primary productivity (NPP) was calculated as per the method given by Malik, 2009 [29].

Statistical analysis

The results were analyzed for mean, standard error, one and two-way analysis of variance (ANOVA). The differences ($p \leq 0.05$) among means were compared by honestly significant difference (HSD) using Tukey's test [30] and the results were expressed in Mean±S.E. All the experiments were performed in triplicate.

RESULTS AND DISCUSSIONS:

Effects of DBP on seed germination

The germination consequences under the exposure of DBP are summarized in **Table-1** and **Figure-1**. The %G was decreased greatly at higher concentrations and the percent decrease with respect to control ranged 37.37-62.63%. Here, the decrease in %G can be considered as indicator of stress induced by DBP. Germination speed (GS) decreased with the increase in concentration and the percent decrease ranged 19.20 to 54.44% when compared to control. Peak value (PV) was significantly decreased with the increase in concentration and followed the similar trends that of %G. The mean daily germination (MDG) and mean germination time (MGT) significantly decreased and the percent decrease ranged 37.37-62.63% and 31.25-59.56% respectively. The maximum percent decrease was 84.56% (at 1600 mg/L) for germination value (GV) and the seed vigour index (SVI) of the treated seedlings of barley was reduced greatly than control. The percent decrease in SVI was 42.77%, 49.22%, 53.38%, 63.43%, 71.44%, 78.25% and 83.79% at 25, 50, 100, 200, 400, 800 and 1600 mg/L of DBP respectively. Germination vigor index (GRI) was also decreased significantly with the increase in concentration of DBP as compared to control and the maximum percent decrease was 54.44% (at 1600 mg/L). The toxicity potential of DBP was measured in the terms of PI and seed mortality (SM). Both were increased as the concentration increased. As the germination is a metabolically active stage of an inert quiescent seed and starts with the adsorption of water [31] and controlled by both endogenous and exogenous factors [32]. The endogenous factors like the plant growth regulators (PGRs) play an important role in germination and early growth. The decline in %G might be due to the disturbance of PGRs functioning and the enzymatic activities of barley seedlings under DBP stress. Moreover, the germination is supposed to be the most vulnerable and sensitive stage to the abiotic stresses [33]. The stress may lead to the imbalance in the osmotic potential and may result into various morphological and physiological perturbations in plants [34].

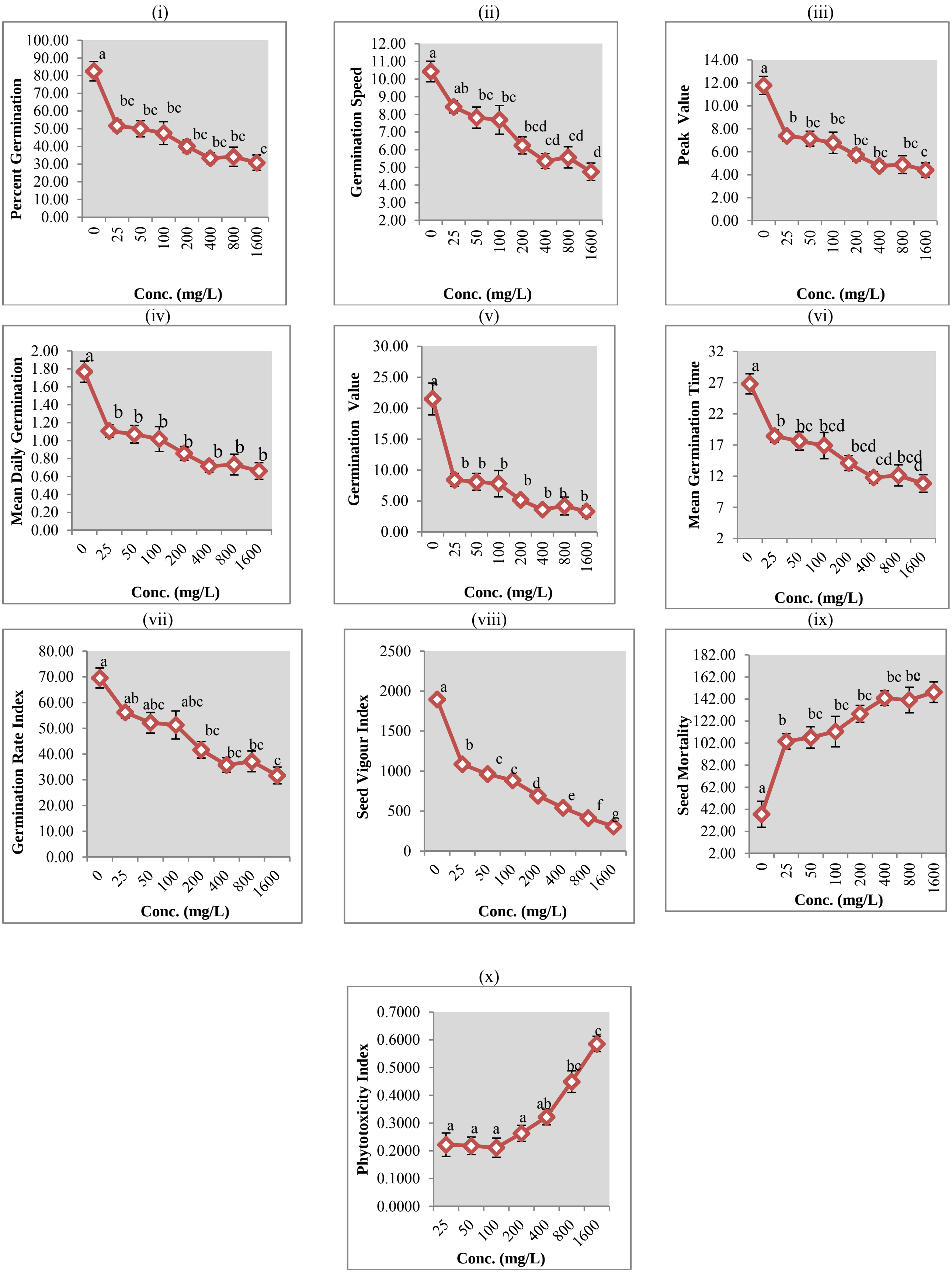

Figure-1 Effects of DBP on germination indices of barley seedlings (i) Germination percentage (ii) Germination speed (iii) Peak value (iv) Mean daily germination (v) Mean germination time (vi) Germination value (vii) Germination rate index (viii) Seed vigour index (ix) Seed mortality (x) Phytotoxicity index. Different letters indicate a significant difference for treatment. Results are presented in Mean±S.E.

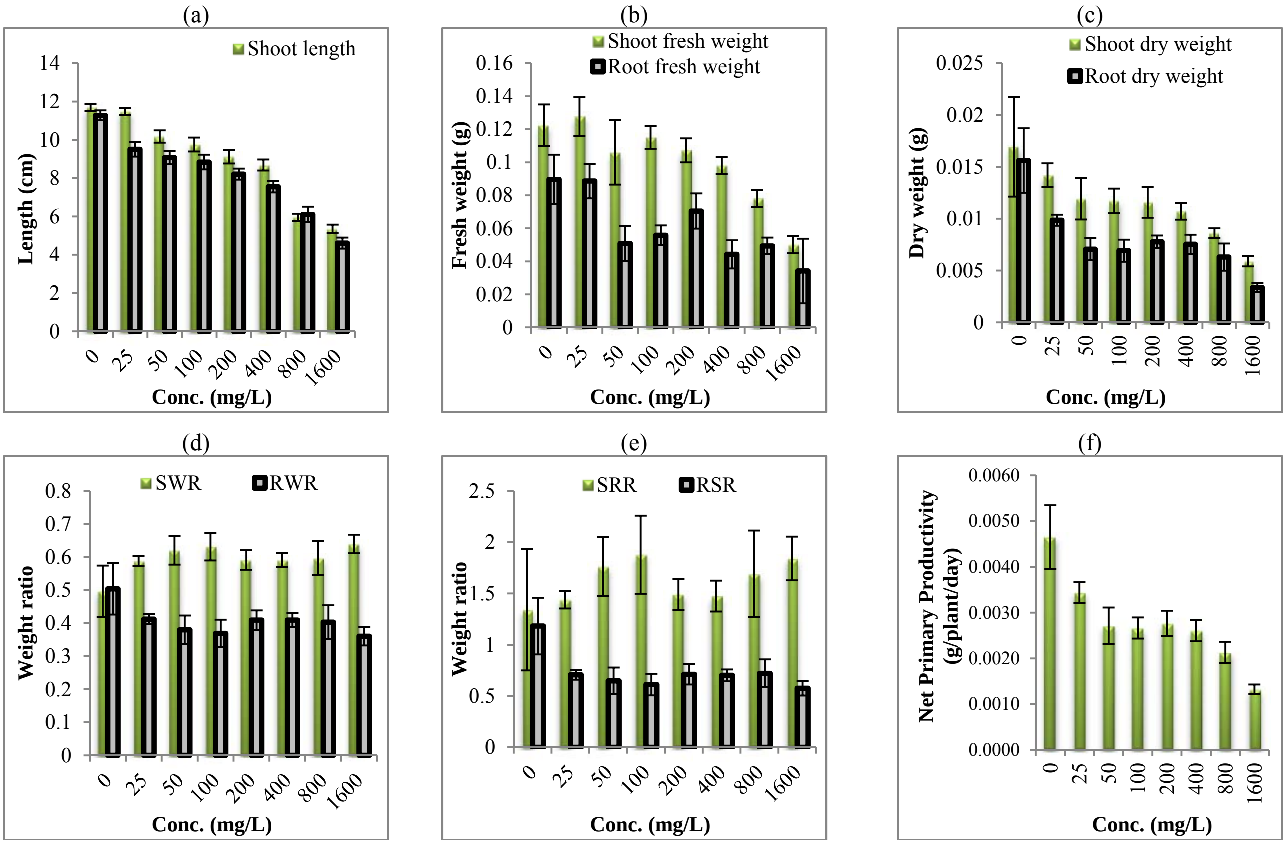

Figure-2 Effects of DBP on growth indices of barley seedlings (a) Shoot-root length (b) Shoot-root fresh weight (c) Shoot-root dry weight (d) Shoot weight ratio, Root weight ratio (SWR-RWR) (e) Shoot root ratio, root shoot ratio (SRR-RSR) (f) Net primary productivity.

Table-1 Germination indices of barley seedlings under DBP exposure

Conc. (mg/L)	%G	GS	PV	MDG	GV	MGT	GRI	SVI	SM	PI
0	82.50±5.48	10.43±0.58	11.79±0.78	1.77±0.12	21.48±2.57	26.80±1.62	69.54±3.88	1894.75±29.99	37.50±11.75	-
25	51.67±3.27	8.43±0.33	7.38±0.47	1.11±0.07	8.40±1.05	18.43±0.99	56.19±2.23	1084.38±21.89	103.57±7.01	0.22±0.04
50	50.00±4.54	7.82±0.60	7.14±0.65	1.07±0.10	8.10±1.35	17.64±1.46	52.14±3.99	962.17±26.94	107.14±9.73	0.22±0.03
100	47.50±6.48	7.69±0.81	6.79±0.93	1.02±0.14	7.81±2.13	16.91±2.10	51.29±5.42	883.34±29.26	112.50±13.88	0.21±0.04
200	40.00±3.56	6.24±0.48	5.71±0.51	0.86±0.08	5.17±0.80	14.11±1.19	41.63±3.21	692.93±21.47	128.57±7.64	0.26±0.03
400	33.33±3.09	5.36±0.42	4.76±0.44	0.71±0.07	3.61±0.63	11.79±0.92	35.73±2.81	541.06±13.92	142.86±6.61	0.32±0.03
800	34.17±5.41	5.57±0.60	4.88±0.77	0.73±0.12	4.20±1.44	12.12±1.69	37.73±4.04	412.09±15.02	141.07±11.59	0.45±0.04
1600	30.83±4.35	4.75±0.49	4.40±0.62	0.66±0.09	3.32±0.91	10.84±1.42	31.68±3.27	307.17±11.77	148.21±9.33	0.59±0.03
One-way ANOVA summary										
F ratio	F-ratio $_{(7, 56)}$ 12.81**	F-ratio $_{(7, 56)}$ 11.46**	F-ratio $_{(7, 56)}$ 12.81**	F-ratio $_{(7, 56)}$ 12.81**	F-ratio $_{(7, 56)}$ 15.55**	F-ratio $_{(7, 56)}$ 12.52**	F-ratio $_{(7, 56)}$ 5.33**	F-ratio $_{(7, 232)}$ 507.39**	F-ratio $_{(7, 56)}$ 12.81**	F-ratio $_{(6, 203)}$ 18.04**
HSD	20.77	2.48	2.97	0.45	6.69	6.54	21.22	95.68	44.51	0.14

%G, Germination percentage; GS, Germination speed; PV, Peak value; MDG, Mean daily germination; MGT, Mean germination time; GV, Germination value; GRI, Germination rate index; SVI, seed vigour index; SM, Seed mortality; PI, Phytotoxicity index.
Results are presented in Mean±S.E. ** Significant at $p<0.01$ * significant at $p<0.05$.

Table-2 Effect of DBP on growth parameters of barley seedlings

Conc. (mg/L)	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)	SWR	RWR	SRR	RSR	NPP (g/plant/day)
0	11.69 ±0.18	11.28 ±0.26	0.1223 ±0.0126	0.0896 ±0.0150	0.0169 ±0.0048	0.0156 ±0.0031	0.496 ±0.078	0.504 ±0.078	1.342 ±0.592	1.182 ±0.276	0.0047 ±0.0007
25	10.77 ±0.30	8.68 ±0.44	0.1278 ±0.0117	0.0886 ±0.0104	0.0142 ±0.0011	0.0099 ±0.0005	0.588 ±0.015	0.412 ±0.015	1.438 ±0.084	0.707 ±0.048	0.0034 ±0.0002
50	10.12 ±0.31	8.74 ±0.33	0.1060 ±0.0195	0.0507 ±0.0105	0.0119 ±0.0020	0.0071 ±0.0011	0.620 ±0.043	0.380 ±0.043	1.763 ±0.287	0.648 ±0.129	0.0027 ±0.0004
100	9.69 ±0.35	8.82 ±0.39	0.1151 ±0.0069	0.0558 ±0.0059	0.0117 ±0.0012	0.0069 ±0.0011	0.631 ±0.041	0.369 ±0.041	1.877 ±0.381	0.612 ±0.105	0.0027 ±0.0002
200	9.12 ±0.35	8.21 ±0.29	0.1072 ±0.0073	0.0705 ±0.0106	0.0116 ±0.0015	0.0078 ±0.0006	0.591 ±0.030	0.409 ±0.030	1.489 ±0.152	0.712 ±0.135	0.0028 ±0.0003
400	8.68 ±0.29	7.55 ±0.30	0.0981 ±0.0051	0.0443 ±0.0085	0.0107 ±0.0008	0.0075 ±0.0009	0.591 ±0.022	0.409 ±0.022	1.475 ±0.151	0.701 ±0.057	0.0026 ±0.0002
800	5.96 ±0.19	6.10 ±0.40	0.0781 ±0.0052	0.0494 ±0.0050	0.0086 ±0.0005	0.0063 ±0.0013	0.597 ±0.051	0.403 ±0.051	1.693 ±0.421	0.721 ±0.135	0.0021 ±0.0002
1600	5.35 ±0.22	4.61 ±0.29	0.0501 ±0.0052	0.0342 ±0.0196	0.0059 ±0.0005	0.0034 ±0.0004	0.640 ±0.028	0.360 ±0.028	1.842 ±0.213	0.576 ±0.072	0.0013 ±0.0001

Two-way ANOVA Summary						One-way ANOVA summary
F-ratio	SL × RL	SFW × RFW	SDW × RDW	SWR×RWR	SRR × RSR	NPP
Treatment	$F_{ratio\ (1, 464)}$ 34.74^{**}	$F_{ratio\ (1, 64)}$ 53.55^{**}	$F_{ratio\ (1, 64)}$ 15.19^{**}	$F_{ratio\ (1, 64)}$ 77.90^{**}	$F_{ratio\ (1, 464)}$ 50.01^{**}	F ratio $_{(1, 32)}$ 7.91**
Dose	F ratio $_{(7, 464)}$ 88.86^{**}	$F_{ratio\ (7, 64)}$ 7.94^{**}	$F_{ratio\ (7, 64)}$ 7.53^{**}	$F_{ratio\ (7, 64)}$ 0.000008	$F_{ratio\ (7, 64)}$ 0.18	
Treatment × Dose	F ratio $_{(7, 464)}$ 2.24	$F_{ratio\ (7, 64)}$ 0.93	$F_{ratio\ (7, 64)}$ 0.27	$F_{ratio\ (7, 64)}$ 2.16^{*}	$F_{ratio\ (7, 64)}$ 1.05	
HSD	1.52	0.56	0.0087	0.30	1.26	0.0015

SL, Shoot length; RL, Root length; SFW, Shoot fresh weight; RFW, Root fresh weight; SDW, Shoot dry weight; RDW, Root dry weight; SWR, Shoot weight ratio; RWR, Root weight ratio; SRR, Shoot root ratio; RSR, Root shoot ratio; NPP, Net primary productivity. Results are presented in Mean±S.E. ** Significant at $p<0.01$ * significant at $p<0.05$.

Table-3 Shoot, root and seedling elongation inhibition

Results are presented in Mean±S.E.

Conc. (mg/L)	Shoot inhibition ratio	Root inhibition ratio	Seedling inhibition ratio
25	1.14±2.12	15.17±3.50	8.15±2.09
50	12.64±2.77	18.93±3.18	15.89±2.39
100	16.21±3.09	20.98±3.42	18.72±2.69
200	21.51±3.31	26.29±2.88	23.93±2.77
400	25.79±2.20	32.19±2.88	29.06±1.81
800	48.58±1.87	44.93±3.90	47.10±2.11
1600	54.18±1.80	58.53±2.75	56.42±1.67

Effect on early growth indices of barley seedlings

The effects of DBP on the growth indices of barley are shown in **Table-2** and **Figure-2**. Both shoot and root length were decreased with the increase in concentration of DBP. The inhibition ratios **(Table-3)** showed that the roots were more vulnerable to DBP exposure at higher concentration than shoots. Similar trends of inhibition in root elongation were recorded in mung bean seedlings [35]. Here, the responsible factor may be the direct contact of roots to DBP. The shoot and root fresh weight, shoot and root dry weight were observed to decline under the exposure of different concentrations of DBP and at higher concentration the percent decline was more than 50% as compare to control. The decline was more prominent in case of root fresh weight, root dry weight (61.88%, 78.49% respectively) than shoot fresh and dry weight (59.09% and 65.17% respectively). The similar observations were made by Ma et al., 2013; Ma et al., 2014 [16, 35]. The information regarding the dry matter allocation in shoot and root play an important role in different agro-ecosystems. Therefore, the seedlings analyzed for different weight ratios like shoot weight ratio (SWR), root weight ratio (RWR), shoot/root ratio (SRR) and root/shoot ratio (RSR). RWR and RSR were decreased with the increase in concentrations of DBP. In case of trees under normal conditions RSR ranged 1:5 to 1:6 [36, 37] and this ratio is the indicator of dry matter distribution in different parts of a plant [38]. As all the growth indices related to roots like root elongation, root fresh and dry weight, RWR and RSR showed similar trends of inhibition which can be attributed to the more sensitivity of roots to DBP stress. The study is also supported by the work of Dueck et al., 2003 who studied the effect of DBP on the morphology and physiology of six plants and revealed that the roots of *Phaseolus* and *Plantago* were more vulnerable to the DBP stress than shoots [39]. NPP was decreased and the percent decrease was 54.19%, 59.14%, 59.0%, 61.88%, 65.17%, 78.48%, 28.45%, 51.28% and 71.56% at 25, 50, 100, 200, 400, 800 and 1600 mg/L of DBP respectively when compared to control. According to Muller and Kordel, 1993 the uptake of DBP from treatment media took place via the cuticle of roots and the accumulated DBP might led to impairment into the metabolic processes related to the normal growth of seedlings [40]. The increase in SWR and SRR was noticed at similar conditions and percent decrease was 28.85% and 37.25% respectively and possible reason might be the hormone like acting behavior of phthalates as reported by Gao et al., 2016 [41].

CONCLUSIONS:

The present study elucidated that the exposure of DBP under controlled conditions significantly affected the germination and early growth indices of barley seedlings. Thus, the DBP induced the significant detrimental consequences to the barley seedlings. Moreover, the roots of seedlings showed more sensitivity to DBP stress than shoots. However, the further studies are required to understand the responsible mechanisms for the DBP induced stress consequences to barley seedlings.

ACKNOWLEDGEMENTS:

The authors are highly thankful to University Grants Commission (India) for financial assistance provided under National Fellowship for Higher Education (NFHE) scheme (vide letter no. F1-17.1/2015-16/NFST-2015-17-ST-HIM-1038/(SA-111/Website)) and Guru Nanak Dev University, Amritsar for providing necessary facilities.

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