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Effect of Nutripriming with Boric Acid, Zinc Sulphate Heptahydrate and its Combination on Germination Physiology of Lentil (*Lens culinaris* L.) cv. HUL 57

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ABSTRACT

Background: In rainfed agriculture, lentil is considered an important legume crop. But in rainfed conditions adequate soil moisture condition is a major obstacle that leads to reduced germination percent, slow down growth of the seedlings and finally reduction in yield. Seed priming is an alternative which can improve the germination percentage and seedling establishment of lentil.

Methods: The present work, deals with the lentil (*Lens culinaris* L.) cv. HUL 57 where seeds were primed with different concentrations/ combinations of boric acid (H_3BO_3) and zinc sulphate heptahydrate ($ZnSO_4.7H_2O$). The parameters taken into consideration was germination percentage (ranging from 18 to 96 h), radicle and plumule length, fresh and dry weights of radicle and plumule at different time intervals (ranging from 48 to 96 h) during both the consecutive years *i.e.,* 2014-15 and 2015-16.

Result: Among all the priming treatments, priming with 4 mM boric acid (B_2), 2 mM zinc sulphate heptahydrate (Zn_1) and 2 mM of zinc sulphate + 4 mM of boric acid (T_1) significantly improved the germination percentage, plumule and radicle lengths, fresh and dry weights of lentil seedlings over other treatments in both the consecutive years.

Key words: Boric acid, Lentil, Seed priming, Zinc sulphate heptahydrate.

INTRODUCTION

Lentil (*Lens culinaris*) belongs to the Leguminaceae (Fabaceae) family, nutritious amongst the *rabi* pulses, used as a cheap source of protein and cultivated in rainfed areas. Due to changing climatic conditions plants are suffering from various environmental stresses. Seed priming is an alternating way out which have the ability to activate the pre-germination metabolism without radicle protrusion and improves the germination rate, seedling establishment and overall growth and productivity of crop plants (Mondal and Bose, 2019). Moreover, Ghassemi-Golezani *et al*. (2013) noted that hydro-priming of lentil seeds increased the height of the plant, pods and seeds number per plant, biological and grain yield and harvest index in comparison to the non-primed one. For instance, Toklu (2015) observed that $ZnSO_4$, GA_3 and PEG-6000 priming treatments having a positive effects of germination rate, germination percentage, yield component and grain yield of lentil plants. However, Kumar *et al*. (2019) reported that seed priming having the potential to increase the growth, yield and seed quality characteristics of lentil which enhance the income of farming communities. In addition, Bhateshwar *et al*. (2020) noted that the maximum germination percentage, number of branches per plant, seeds per pod, 1000 seeds weight, yield per plant and yield per ha were increased when seeds were primed with neem leaf extracts. Scientific reports related to seed priming depicted that it is an important contributory factor for the improvement of synchrony and speed of germination and also establish the seedlings for better growth near future (Ghassemi-Golezani *et al*., 2008, 2013; Bhateshwar *et al*., 2020) in lentil.

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Based on this documentation, the present study aimed to investigate the effects of different micronutrients like boric acid, zinc sulphate and its combination in a various concentrations range for the purpose of seed priming, during the time of seed germination, emergence, seedling establishment and growth of lentil (cv. HUL 57).

MATERIALS AND METHODS

The whole experiment was carried out in the Seed Physiology Laboratory, Department of Crop Physiology, Visva-Bharati in the year 2014-15 and 2015-16. Healthy and bold seeds were surface sterilized with 0.01% $HgCl_2$ solution. For priming, the sterilized lentil seeds were soaked in different concentrations/ combinations of boric acid (H_3BO_3) and zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) solution

(Treatment details were as follows: (Unit: in mM) [Boric acid: B_0 = Control, B_1 = 2, B_2 = 4, B_3 = 6, B_4 = 8, B_5 = 10, B_6 = 15, B_7 = 20, B_8 = 30]; [Zinc sulphate heptahydrate: Zn_0 = Control, Zn_1 = 2, Zn_2 = 4, Zn_3 = 6, Zn_4 = 8, Zn_5 = 10, Zn_6 = 15, Zn_7 = 20, Zn_8 = 30]; [Combination: T_0 = Control; T_1= 2 mM $ZnSO_4.7H_2O$ + 4mM H_3BO_3; T_2 = 2mM $ZnSO_4.7H_2O$ + 6 mM H_3BO_3; T_3= 4 mM $ZnSO_4.7H_2O$ + 4 mM H_3BO_3 and T_4= 4 mM $ZnSO_4.7H_2O$ + 6 mM H_3BO_3]) for 12 h. The seeds without any treatment referred as control (non-primed). Then 50 dried seeds of each treatment along with the control one were taken and placed in different petridishes. The petridishes of 3.0-inch diameter were used to conduct germination physiology of lentil under normal light and room temperature (25±2°C) conditions. The parameters considered for the experiment was germination percentage (ranging from 18 to 96h), seedling growth in terms of radicle and plumule lengths, fresh and dry weights of radicle and plumule at different time intervals (ranging from 48 to 96 h) during both the consecutive years *i.e.*, 2014-15 and 2015-16 (Mondal *et al*., 2011, Kumar *et al*., 2016). The experiment was arranged in 3 replications and the data were analyzed statistically by following completely randomized design.

RESULTS AND DISCUSSION

Table 1 depicted the germination percentages of boric acid primed and non-primed lentil seeds at different studied hours (18, 24, 36, 48, 72 and 96 hours) during both 2014-15 and 2015-16. The results showed that the germination percentage was increased with increasing duration and varied significantly among the different priming treatments. The highest germination percentage was recorded after 18 h of sowing in treatment B_2 *i.e.*, 4 mM H_3BO_3 (91% and 89%). The germination percentage was increased gradually upto 48h and thereafter it did not change much.

Table 2 denoted the germination percentages of lentil seeds primed with Zinc sulphate heptahydrate at different

Table 1: Effect of boric acid on germination percentage at different study hours.

Boric acid (mM)	Germination percentage											
	18 hours		24 hours		36 hours		48 hours		72 hours		96 hours	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
B_0	85.0	85.0	86.0	86.0	89.3	90.3	91.0	91.0	92.3	92.0	93.0	94.0
B_1	87.0	87.0	88.3	88.0	94.0	94.0	96.0	97.0	97.0	98.0	98.0	98.7
B_2	91.0	89.0	92.0	92.0	98.0	97.0	100.0	99.0	100.0	99.0	100.0	99.7
B_3	88.0	87.0	90.0	92.0	95.0	96.0	97.0	96.0	98.0	96.7	99.0	97.0
B_4	81.0	84.0	83.0	90.0	92.0	93.0	94.0	95.0	95.3	96.7	96.3	97.0
B_5	83.0	82.0	83.0	87.0	89.0	91.0	94.0	97.0	94.3	96.7	94.3	96.7
B_6	83.0	83.0	85.0	83.0	93.0	95.0	94.0	95.0	94.0	95.0	94.0	95.0
B_7	81.0	83.0	85.0	85.0	87.0	87.0	88.0	89.0	89.0	89.0	89.0	89.0
B_8	71.0	77.0	74.0	79.0	78.0	81.0	81.0	83.0	81.0	84.0	81.0	84.7
SEm (±)	2.44	2.1	3.58	2.5	2.8	2.7	2.17	2.3	1.80	2.0	1.74	1.9
CD at 1%	9.94	8.5	14.57	10.3	11.6	11.28	8.83	9.5	7.30	8.3	7.08	7.9
CD at 5%	7.3	6.2	10.6	7.5	8.4	8.2	6.4	6.9	5.3	6.1	5.2	5.8

*B_0= Control; B_1= 2 mM; T_2= 4 mM; B_3 = 6 mM; B_4 = 8 mM; B_5 = 10 mM; B_6= 15 mM; B_7 = 20 mM; B_8 = 30 mM.

Table 2: Effect of zinc sulphate heptahydrate on germination percentage at different study hours.

Zinc sulphate (mM)	Germination percentage											
	18 hours		24 hours		36 hours		48 hours		72 hours		96 hours	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Zn_0	85.0	95.0	88.0	88.0	88.0	88.0	93.0	91.7	94.3	92.0	95.0	93.0
Zn_1	89.0	95.0	96.0	95.0	97.0	97.0	99.0	100.0	99.0	100.0	99.7	100.0
Zn_2	83.0	93.0	92.0	95.0	95.0	95.0	98.0	97.0	98.7	98.0	99.3	99.0
Zn_3	87.0	89.0	92.0	93.0	94.0	94.0	94.0	95.0	96.0	96.3	96.7	97.7
Zn_4	79.0	81.0	87.0	88.0	89.0	90.0	93.0	93.0	94.0	93.0	95.3	94.0
Zn_5	76.0	77.0	83.0	87.0	87.0	91.0	94.0	91.0	94.7	91.7	95.3	92.3
Zn_6	34.0	34.0	48.0	54.0	67.0	68.0	80.0	73.0	81.0	75.0	81.0	75.0
Zn_7	14.0	15.0	30.0	32.0	46.0	44.0	60.0	52.0	61.0	53.0	61.0	53.0
Zn_8	3.0	2.0	5.0	4.0	12.7	7.0	14.3	8.0	14.3	10.0	14.3	10.0
SEm (±)	2.89	2.6	2.24	3.3	2.9	2.8	2.2	2.8	1.7	2.4	1.6	2.2
CD at 1%	11.77	10.6	9.10	13.5	11.9	11.4	8.8	11.5	7.1	9.6	6.7	9.0
CD at 5%	8.60	7.8	6.64	9.8	8.7	8.3	6.4	8.4	5.2	7.0	4.9	6.6

*Zn_0= Control; Zn_1= 2 mM; Zn_2= 4 mM; Zn_3= 6 mM; Zn_4= 8 mM; Zn_5 = 10 mM; Zn_6= 15 mM; Zn_7 = 20 mM; Zn_8 = 30 mM.

intervals of time during both 2014-15 and 2015-16 Seed priming with 2mM $ZnSO_4$, $7H_2O$ (Zn_1) recorded the highest germination percentage (89.0-100%) in all the studied hours during both the years; followed by Zn_2 (83.0 -99.7%) and Zn_3 (87.0-97.0%) and showed statistically at par results.

In Table 3, the highest germination percentage (88.1-99.7%) was obtained by T_1 (2mM $ZnSO_4.7H_20$ + 4mM H_3BO_3) in all the studied hours followed by other treatment combinations and recorded significantly greater germination percentage than that of T_0 (87.3-94%) and T_4 (86.3-95.3%) in both years. The results showed a decreasing tendency of germination percentage due to increasing the concentration of priming treatments.

Fig 1 revealed the fresh weight of plumule and radicle obtained from H_3BO_3 primed and non-primed seeds at 48, 72 and 96h after sowing. Treatment B_2 recorded the highest fresh weights of plumule (4.4, 7.2 and 10.9 mg in 2014-15 and 8.5, 11.1 and 13.1 mg in 2015-16 at 48, 72 and 96 h respectively). Whereas, fresh weight of radicle showed similar trends to that of fresh weight of its plumule.

Fig 2 noted the data regarding $ZnSO_4.7H_2O$ primed and non-primed plumules and radicles fresh weight, where Zn_1 showed the highest value of fresh weights of plumule (5.0, 11.8 and 13.8 mg in 2014-15 and 5.6, 9.4 and 12.1 mg in 2015-16 at 48, 72 and 96 h respectively) and radicle (11.4, 15.3 and 17.5 mg in 2014-15 and 13.8, 15.3 and 16.8 mg in 2015-16 respectively) which was significantly greater than that of all other priming treatments.

The treatment T_1 showed the highest value of fresh weight of plumule (7.18, 13.33 and 17.75 mg in 2014-15 and 10.17, 14.55 and 17.74 mg in 2015-16 at 48, 72 and 96 h, respectively) and radicle (13.75, 17.75 and 20.50 mg in 2014-15 and 14.30, 17.93 and 19.72 mg in 2015-16 at 48, 72 and 96 h, respectively), followed by T_2 at all the stages. The control (T_0) treatment recorded the lowest value of fresh weight of plumule and radicle (Fig 3).

In Fig 4, the highest dry weights of plumule (1.90, 3.91 and 4.30 mg in 2014-15 and 1.35, 3.41 and 4.24 mg in 2015-16 at 48, 72 and 96 h respectively) and radicles (3.77, 5.72 and 7.02 mg in 2014-15 and 2.86, 2.27 and 6.40 mg in 2015-16 at 48, 72 and 96 h respectively) were recorded in B_2 followed by other treatments.

Fig 5 recorded that the seeds primed with Zn_1 (2mM $ZnSO_4$. $7H_2O$) recorded the maximum plumule (1.15, 2.42 and 3.02 mg in 2014-15 and 0.97, 2.15 and 2.74 mg in 2015-16 at 48, 72 and 96 h respectively) and radicle dry weights (*i.e.*, 2.99, 3.52 and 4.23 mg in 2014-15 and 2.85, 3.39 and 3.94 mg in 2015-16 at 48, 72 and 96 h respectively) which

Table 3: Effect of zinc sulphate heptahydrate and boric acid combination on Germination percentage at different study hours.

Treatments	Germination percentage											
	18 hours		24 hours		36 hours		48 hours		72 hours		96 hours	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
T_0	83.1	83.0	84.7	86.3	88.3	87.3	91.3	92.7	92.7	93.7	93.0	94.0
T_1	88.1	87.7	91.7	90.0	94.7	94.0	99.0	96.3	99.3	97.3	99.7	99.7
T_2	87.3	82.7	89.7	88.0	92.7	91.0	95.0	95.3	96.7	98.0	97.0	99.0
T_3	87.0	83.3	89.3	86.0	90.3	90.3	93.0	94.0	95.7	95.7	95.7	97.0
T_4	83.1	82.0	85.0	85.0	86.3	88.0	92.7	92.7	94.7	94.0	95.3	94.3
SEm (±)	2.51	4.03	3.65	4.41	2.8	3.5	1.5	1.5	1.5	1.5	1.4	1.0
CD at 1%	NS	NS	NS	NS	8.7	11.0	4.7	4.6	4.6	4.9	4.5	3.2
CD at 5%	NS	NS	NS	NS	5.3	6.7	2.8	2.7	2.6	2.8	2.5	1.8

*T_0= Control; T_1 = 2 mM $ZnSO_4$. 7 H_2O + 4 mM H_3BO_3; T_2 = 2 mM $ZnSO_4$. 7 H_2O + 6 mM H_3BO_3; T_3 = 4 mM $ZnSO_4$. 7 H_2O + 4 mM H_3BO_3 and T_4= 4 mM $ZnSO_4$. 7 H_2O + 6 mM H_3BO_3.

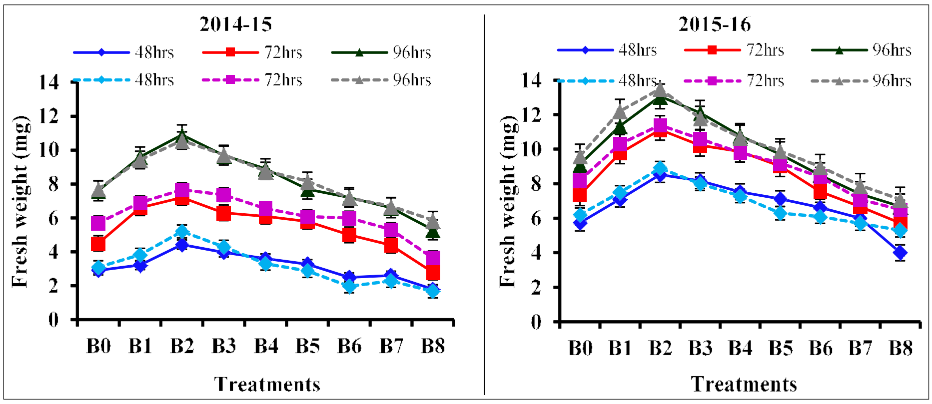

Fig 1: Effect of boric acid treatment on plumule and radicle fresh weights.
The solid lines in sub-figures indicate the shoot plumule weight; whereas, the dotted lines indicate the radicle fresh weight.

was significantly greater than treatments at all the stages during both the years. The treatment Zn_8 was recorded the lowest value of dry weight. Dry weight of plumule and radicle showed similar pattern just like fresh weight obtained from 2 mM $ZnSO_4.7H_2O$ + 4 mM H_3BO_3 at 48, 72 and 96 h, which showed statistically significant results among the other treatments presented in Fig 6.

In Fig 7, the graphs showed that the length of plumule decreased gradually with the increasing concentrations of H_3BO_3. It was observed that treatment B_2 showed the highest plumule length (6.20, 7.46 and 8.57 mm in 2014-15 and 5.41, 6.48 and 7.3 mm in 2015-16 at 48, 72 and 96 h respectively) and radicle length (9.42, 11.52 and 12.62 mm in 2014-15 and 9.42, 11.60 and 12.63 mm in 2015-16 at 48,

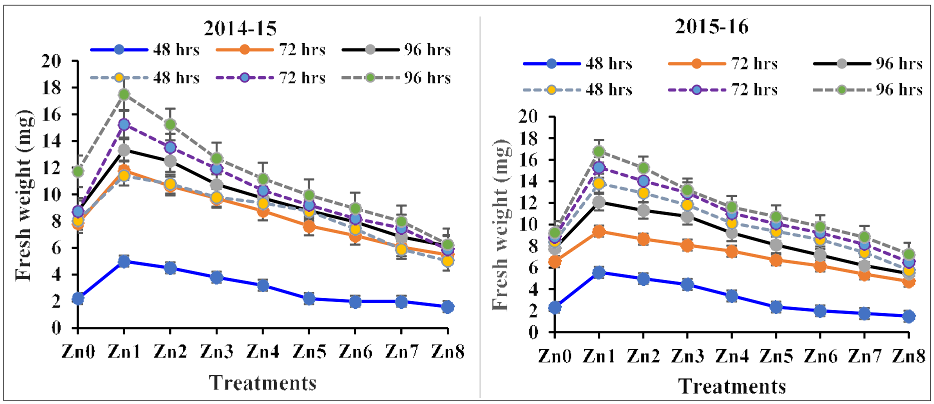

Fig 2: Effect of zinc sulphate heptahydrate treatment on plumule and radicle fresh weights.
The solid lines in sub-figures indicate the plumule fresh weight; whereas, the dotted lines indicate the radicle fresh weight.

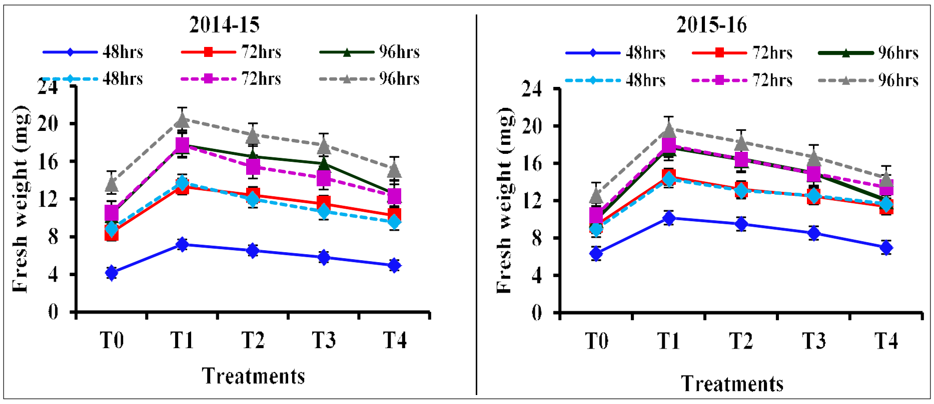

Fig 3: Combine effect of zinc sulphate heptahydrate and boric acid on plumule and radicle fresh weights.
The solid lines in sub-figures indicate the plumule fresh weight; whereas, the dotted lines indicate the radicle fresh weight.

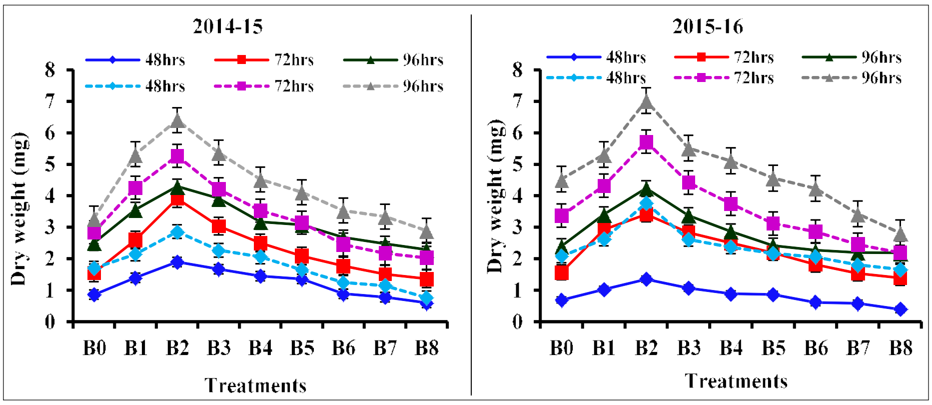

Fig 4: Effect of boric acid treatment on plumule and radicle dry weights.
The solid lines in sub-figures indicate the plumule dry weight; whereas, the dotted lines indicate the radicle dry weight.

72 and 96 h respectively) which was significantly greater than that of all other nutripriming treatments.

Fig 8 showed the plumule and radicle length and the result showed that Zn_1 (2 mM $ZnSO_4 \cdot 7H_2O$) obtained highest plumule length (4.6, 6.0 and 7.6 mm in 2014-15 and 5.0, 6.5 and 7.7 mm in 2015-16 at 48, 72 and 96 h) and radicle length (11.72, 16.93 and 19.63 mm in 2014-15 and 12.90, 18.68 and 21.60 mm in 2015-16 at 48, 72 and 96 h respectively) it was significantly greater than that of all other priming treatments in all the studied hours.

In Fig 9 treatment T_1 recorded the highest plumule (5.17, 6.25 and 7.08 mm in 2014-15 and 5.50, 6.72 and 7.41 mm in 2015-16 at 48, 72 and 96 h, respectively) and radicle lengths (12.48, 15.25 and 17.96 mg in 2014-15 and 13.68,

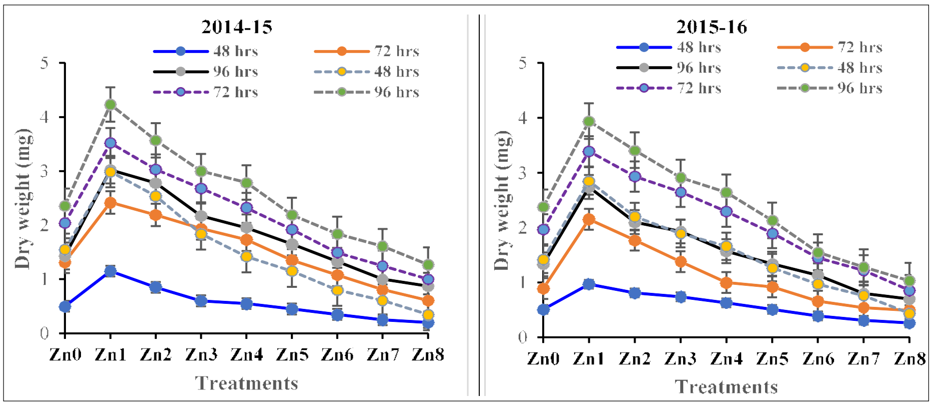

Fig 5: Effect of zinc sulphate heptahydrate treatment on plumule and radicle dry weights.
The solid lines in sub-figures indicate the plumule dry weight; whereas, the dotted lines indicate the radicle fresh weight.

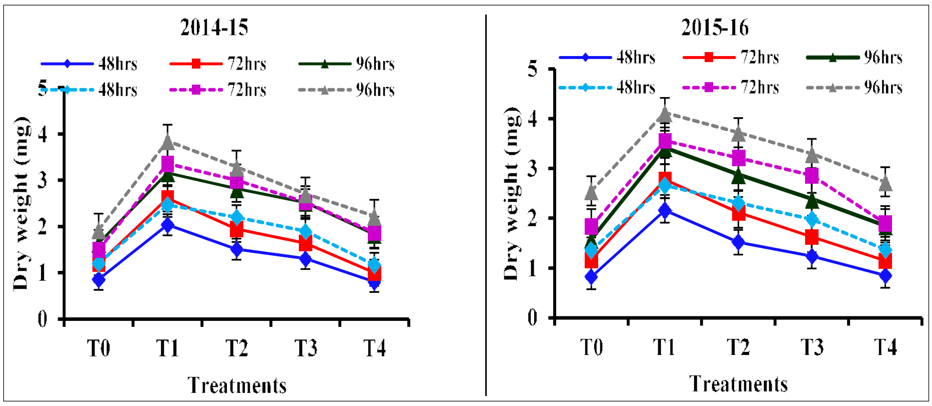

Fig 6: Combine effect of zinc sulphate heptahydrate and boric acid on plumule and radicle dry weights.
The solid lines in sub-figures indicate the plumule dry weight; whereas, the dotted lines indicate the radicle dry weight.

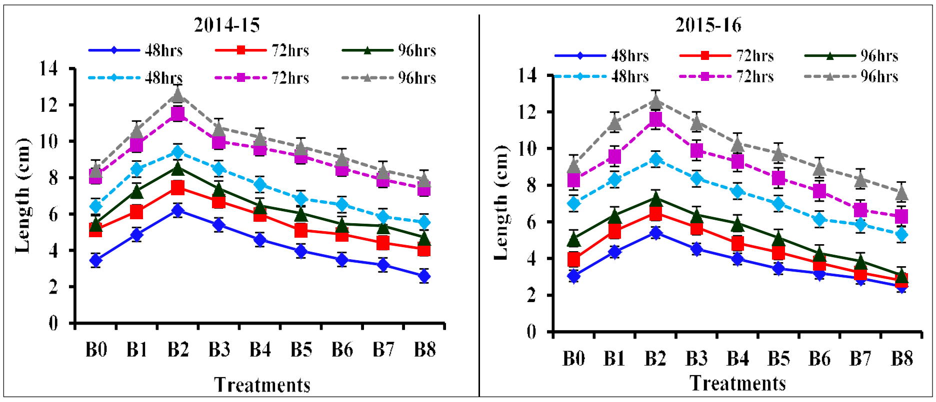

Fig 7: Effect of boric acid treatment on plumule and radicle lengths.

16.22 and 18.70 mg in 2015-16 at 48, 72 and 96 h, respectively) and which were statistically significant than that of treatment T_0 and T_4.

Erratic rainfall patterns and temperature extremes create a stressful situation specially for germinating seeds and young seedlings as a result there is slow and non-uniform germination with a compromised vigour was observed in lentil (*Lens culinaris* Medik.) and finally yield potential is also affected (Ghasemi-Golezani *et al*., 2013).

In present investigation with lentil Var. HUL 57, while primed with alone/ different combinations of zinc sulphate heptahydrate and boric acid were found to improve the germination percentage as compared to control. Whereas, the lower dose of zinc sulphate heptahydrate and boric acid were found effective for priming treatment of lentil seeds in comparison to higher doses. In this context, the lower doses of $ZnSO_4 \cdot 7H_2O$ and H_3BO_3 were taken into consideration and apply it in combination as priming agent to get the best possible result in terms of early growth phase of lentil. The variety HUL 57 showed improved germination percentage, better seedling establishment and seedling vigour while primed with these micronutrients. Likely, an increase in germination percentage/ velocity/ rate in micronutrient primed seeds were recorded by various scientists' time to time with a number of field crops, vegetable and ornamental plants as reported by Mondal and Bose (2019). The same has been observed in the present investigation where in respect to control all primed sets enhanced the plumule and radicle lengths, fresh and dry weights of growing seedlings. The priming technology offered the synchronized germination and improved seedling vigour which has been noticed by a number of workers (Farooq *et al.,* 2006; Yuan-Yuan *et al.,* 2010; Ella *et al.,* 2011; Mondal *et al.,* 2011). The critical scrutinization of the results for all the above-mentioned parameters showed that the application of zinc sulphate heptahydrate and boric acid both have an optimum concentration where the maximum attainments of the values were observed in present case. Similarly, Mondal and Bose (2022) reported that in plant system has the capacity to act as per the concentration of the chemicals, which are beneficial for their growth, otherwise the level more than that may be either toxic or inhibitory. In another study, Farooq *et al.* (2006) noted that the optimum concentration of zinc sulphate heptahydrate and boric acid either used separately or in combined form during the time of seed priming may improve the homeostasis by maintaining the suitable criteria

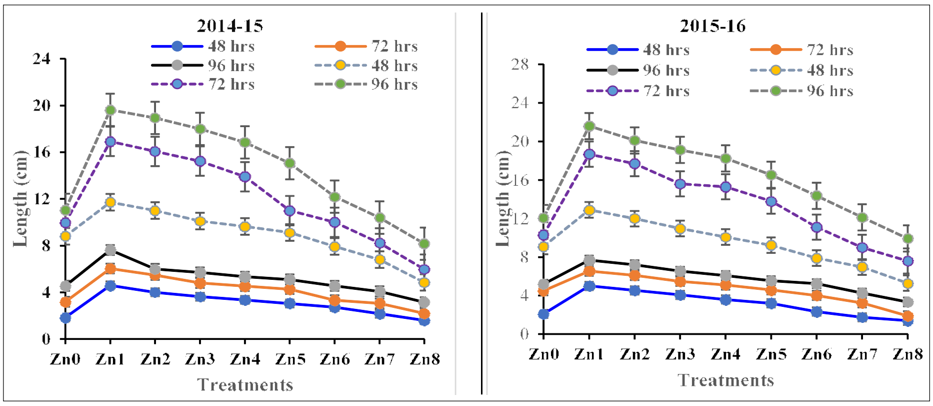

Fig 8: Effect of zinc sulphate heptahydrate treatment on plumule and radicle lengths.
The solid lines in sub-figures indicate the plumule length; whereas, the dotted lines indicate the radicle length.

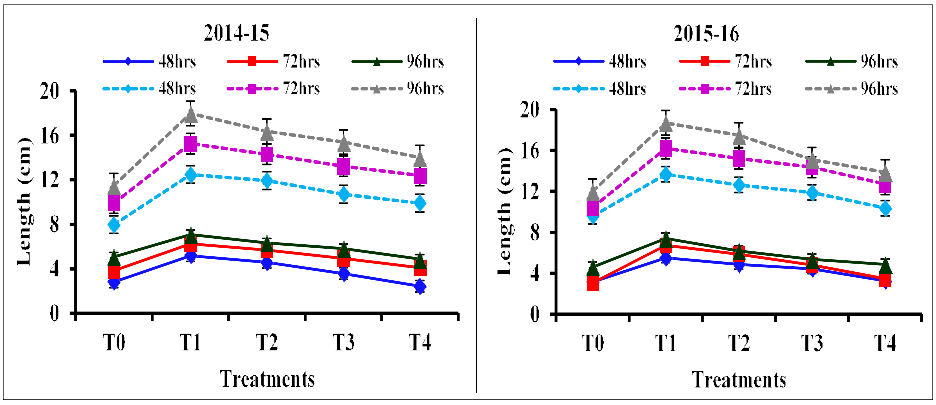

Fig 9: Combine effect of zinc sulphate heptahydrate and boric acid on plumule and radicle lengths.
The solid lines in sub-figures indicate the plumule length; whereas, the dotted lines indicate the radicle length.

for the germinating seeds and seedling. In addition, Ghassemi-Golezani *et al*. (2008) revealed that a useful and simplified technique *i.e.*, hydropriming is responsible for enhancing the seedling emergence rate and percentage of lentil. Whereas, Farooq *et al*. (2019) observed that under normal and water deficit condition, osmopriming improved the lentil performance by improving early and synchronized emergence, better accumulation of sugar and Calcium which have the potential to reduce the oxidative damage and resulting better seedling growth and biomass production. In context to this, Toklu *et al*. (2015) provide conclusive evidence that in terms of germination properties, various plant characteristics, components of grain yield and grain yield in lentil; seed priming with GA_3, PEG and $ZnSO_4$ should be considered as farmers recommendation and further research is needed in this respect.

Moreover, different experiment was done by various scientist in support of lentil seed priming; where Singh *et al*. (2017) indicates a possibility of enhancing productivity in lentil by using PGR (Cycocel, GA_3 and IAA) as priming agent for the improvement of parameters related to growth and yield. Whereas, Bhateshwar *et al*. (2020) reported that by using botanical extracts for seed priming like neem leaf extract, castor oil, ginger extract and onion were responsible for the betterment of the growth and yield in lentil.

CONCLUSION

Seed germination and seedling establishment of lentil can be inhibited by multifarious environmental conditions and the responses are varied according to the species and cultivars. In a nutshell we can conclude that seed priming with 4mM boric acid, 2 mM zinc sulphate heptahydrate and zinc sulphate heptahydrate (2 mM) + boric acid (4 mM) as single or in combination may be used to improve the germination physiology as well as seedling growth of lentil, in the field conditions during erratic climatic conditions. However, in respect to environment and farmers seed priming with micronutrients is a safe, eco-friendly, cost effective and sustainable approach.

Conflict of interest: None.

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