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EFFICACY OF PRIMING TREATMENT ON GERMINATION, DEVELOPMENT AND ENZYME ACTIVITY OF *ALLIUM CEPA* L. AND *BRASSICA OLERACEA* VAR CAPITATA

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ABSTRACT: The aim of this study was evaluation of the effect of aging germination and activity of antioxidant enzymes in seeds of *Allium cepa* L. and *Brassica oleracea* var capitata with seed priming treatment. In the present paper the different seaweed extract from *Ulva lactuca* L. (G1), *U. reticulata* forsskal (G2), *Padina pavonica* L. (B3), *Sargassum johnstonii* Setchell & Gardner (B4), *Kappaphycus alvarezii* (R5) and *Gracillaria corticata* J. Ag. (R6) was applied as seed priming and performed prior to accelerated ageing treatment with the investigation of activities of catalase (CAT) and peroxidase (POD) during accelerated aging. Our result indicates that to enhance germination characteristics in aged seeds with priming treatment also reveals positive effect of seed priming on the germination percentage, vigour index, seedling length and antioxidant activity of enzyme. The highest germination percentage, vigour index, seedling length and enzyme activity were achieved in given priming treatment with aging (12 day of aging) as compared to control condition (0 day of aging).

KEYWORDS: Priming treatment, Seaweed Liquid Fertilizer, % germination, Catalase and Peroxidase

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1. INTRODUCTION

Bioactive substances extracted from marine algae are used in agricultural and horticultural crops as bio-fertilizers to improve their yield and quality and to reduce the negative environmental impact

[1]. Seaweeds have been reported to stimulate the growth and yield of crops, enhance antioxidant properties, and develop tolerance to drought stress [2]. Priming is a pre-sowing treatment. It is controlled- hydrations in which seeds are unguarded to an external water potential sufficiently low to prevent radicle protrusion but stimulating physiological and biochemical activities [3] and improve radicle emergence, germination rate, germination vigour, seedling establishment and yield by making changes in metabolic activities in the seeds of many crops [4, 5, 6]. Seed priming technique is controlled hydration and drying that result in more rapid germination when the seed is reimbibed [7]. This process may improve speed and uniformity of germination and percentage germination especially in typical conditions such as high and low temperature, salinity and stressful conditions [8, 9, 10, 11, 12]. During priming, the germination process is induced by soaking seeds in water or in solutions containing exogenous molecules such as salts [13], metals [14] or hormones [15], but then halted by seeds drying. Priming treatment has been suggested that the strategy activates a series of physiological processes that improve plant growth under stressful conditions [16], including the induction of antioxidant systems [17]. The effect of hydropriming and biopriming (seaweed extract as a primer) treatment enhance the speed of seed germination, seedling growth and seed vigour index of brinjal, tomato and chilli [18]. Plant growth promoters containing from plant growth regulators or plant extracts is found to be effective in increasing crops germination and seedling establishment [19, 20, 21, 22, 23] and production of peroxidase in barley aleurone [24].The reactive oxygen species such as O_2 and H_2O_2 are produced during reactions of photosynthesis, photorespiration, respiration, flowering and other reactions of cellular metabolism in the plants [25, 26]. Plant possesses a protective system composed of antioxidant such as peroxidase and catalase. Catalase is primary H_2O_2 scavenger in the peroxisomes and the mitochondria [27]. Catalase play a role of specific peroxidase and their function is to protect cells from toxic effects of hydrogen peroxide. Peroxidase is involved in a large number of biochemical and physiological processes and may change quantitatively and qualitatively during plant growth and development [28].Biopriming treatment in combination with hydropriming has become a viable treatment for increasing seed germination percentage and seedling vigour index. The main objective of this study was to evaluate the effects of different concentration of different seaweed extract as a primer by presoaking treatment on seed germination by paper towel and pot methods and also determined the enzyme activity of *Allium cepa* L. and *Brassica oleracea* var capitata.

2. MATERIALSAND METHODS

Preparation of seaweed extract

Green seaweeds of *Ulva lactuca* L. (G1)&*Ulva reticulata* Forsskal (G2), brown seaweeds of *Padina pavonica* L. (B3) & *Sargassum johnstonii* Setchell & Gardner (B4) and red seaweeds of *Kappaphycus alverazii* (R5) *& Gracillaria corticata* J. Ag. (R6) were collected from Okha and Beyt-Dwarka, Gujarat, India. First stage of the experiment: seaweeds were washed 3-4 times with tap

water to remove all epiphytes, sand particles and salinity. Second stage is: Seaweed was dried under sunlight and to grind for powdered. After these stages, seaweed powdered and water was mixed in precaution of 1:20 (W/V) and boiled up to 1 hour. After boiling, the prepared mixture was filtered with muslin cloth [29] and prepared the standard solution. Different dosages of seaweeds extract like 1%, 2%, 3%, 4% & 5% were prepared as per experimental condition.

Priming treatment

Seeds of *Allium cepa* L. and *Brassica oleracea* var capitata were collected from Anand Agriculture University, Anand. Before experiment run the seeds were sterilized up to 2-5 minutes in 0.1% $HgCl_2$ and washed several times with water. Taken 50 sterilized seeds and immersed in water (as a control), and different dosages of selected seaweeds extract up to 48 hours at room temperature. After immersing the seeds were removed and dried on filter paper for one minute and reached up to their original weight of moisture content [30]. The primed seeds were transferred on tissue paper and fold two times and put into zip locked bag carefully for maintaining the moisture content. The paper towel experiment was run up to 12 days and pot method up to 20 days and measured percentage germination [31] formula:

$$\text{Germination percentage (GP)} = \frac{n}{N} \times 100 \quad (1)$$

Whereas, n= number of seeds that were germinated, N: total number of seed in each experiment

At the final day of germination normal seedling counted which determine percentage germination. These root, shoot and seedling length was measured with scale. Seedling vigour index (SVI) was calculated formula were [32] respectively:

$$\text{Seed vigour index} = \text{Seedling length (cm)} \times \text{\% germination} \quad (2)$$

Whereas, Seedling length= Root length+ Shoot length (cm)

Enzyme activity

In pot method, after germination 21 days of aging plantlets material with control and different treatments were ground with a mortar-pestle in 100mM HEPES-NaOH (pH-7.5), 5mM $MgCl_2$ and 1mM dithiothretol. The ratio of buffer to plant material was 3:1. The prepared extract was filtered through two layers of muslin cloth and clarified by centrifugation at 15,000g for 15min. The supernatant was used for catalase and peroxidase activity.

Peroxidase assay:

Peroxidase activity (EC. 1.11.1.7) was determined and in assay contained 1ml phosphate buffer 0.1M, 0.5ml H_2O_2 and 1ml O-dianisidine and 0.2ml of enzyme extract. After 5min stop the reaction by adding 1ml of 2N H_2SO_4 and measured at 430nm [33].

Catalase assay:

Catalase activity (EC. 1.11.1.6) was measured as 3ml phosphate buffer, 2ml 0.005M H_2O_2 and 1ml

of enzyme extract added and incubate at 20^0C for 1min and add 10ml of 0.7N H_2SO_4 and titrate with 0.01N KMnO4 and a faint purple colour persists for at least 15sec [34].

Statistical analysis

The results are reported as mean values of the three replicates along with standard error mean and figures are draw in excel sheet.

3. RESULTS AND DISCUSSION

Results revealed that the effect of bio-priming up to 48 hours on percentage germination of *Allium cepa* L. and *Brassica oleracea* var capitata was significant. After 12 days, % germination, root length, shoot length, seedling length and seed vigour index (SVI) was measured in paper towel method of *Allium cepa* L. and *Brassica oleracea* var capitata seeds. The short periods of priming resulted in early seedling length compared to the control seeds. In *Allium cepa* L., 100% germination was found at 4% concentration of G2 and R6 treatment and in control, it is found 40% germination (Fig-1). 100% germination was recorded at 4% concentration in all treatment of *Brassica oleracea* var capitata (Fig-2). The pansy seeds primed with 0.3, 0.5 and 0.7% KNO_3 solution for 6-24 hours and the results was recorded to reduce their percentage, especially at the highest concentration [35]. The early emergence and its effect on early maturity of seed priming treatment may be as a result of advancement in seed metabolic activities [36, 37]. The result was recorded that in many water melons coated seeds, germination and subsequent seedling growth can be inhibited by mechanical restriction exerted by the seed coat [38]. The observation was reported seedling emergence in 48 hours primed seeds but 24 hours primed Bambara ground nuts (*Vigna subterranea* (L.) Verdc) seeds are in conformity and the primed duration affected days to seedling emergence and final percentage seedling establishment [39].The positive effect of priming on the early stages of germination process by mediation of cell division in germinating seeds [40].

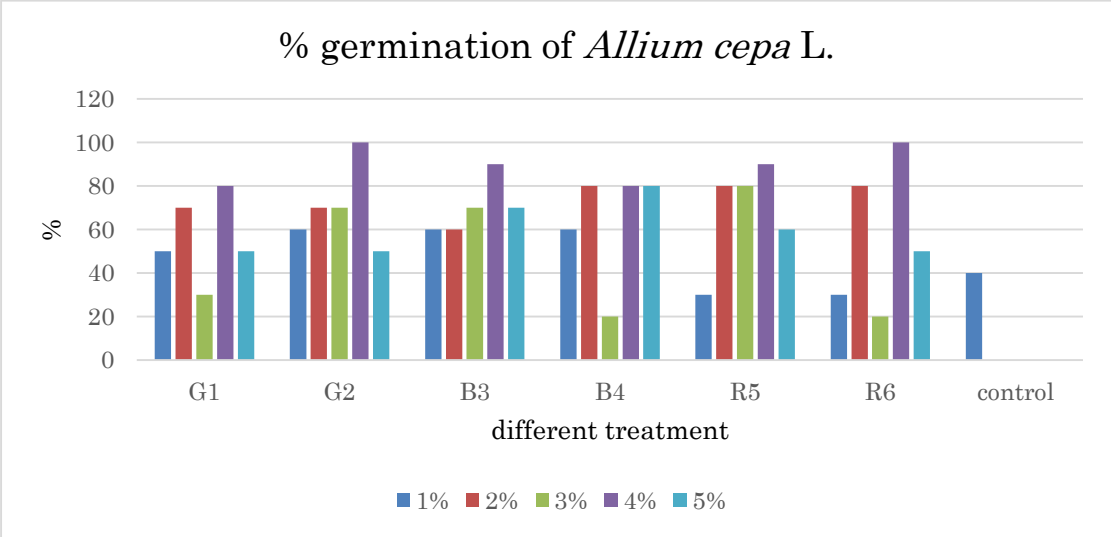

Fig-1: Effect of bio-priming on percentage germination of *Allium cepa* L. by paper towel method.

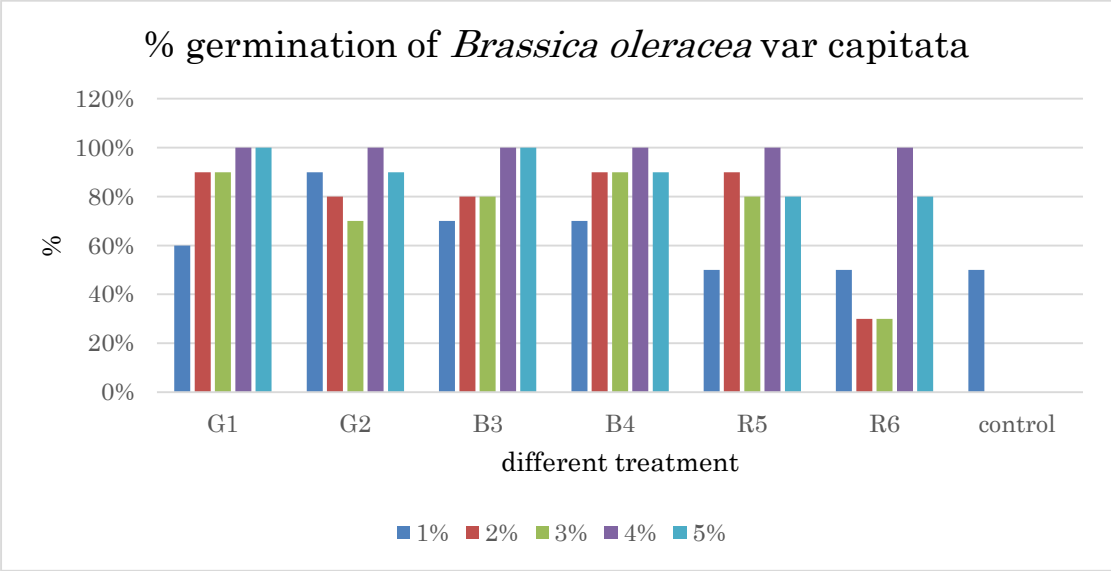

Fig-2: Effect of bio-priming on percentage germination of *Brassica oleracea* var capitata by paper towel method.

In paper towel method, root length (cm) and shoot length (cm) following bio-priming treatment was highest in all treatment at 4% concentration but, at 5% concentration it is decreased in both target seeds of *Allium cepa* L. and *Brassica oleracea* var capitata that was found of root length 4.64±0 cm in *Ulva lactuca* L. (G1), 3.22±0 cm in *U. reticulata forsskal* (G2), 5.33±0.01 cm in *Padina pavonica* L. (B3), 6.36±0.02 cm in *Sargassum johnstonii* Setchell & Gardner (B4), 3.4±0.4 cm in *Kappaphycus alvarezii* (R5), 4.98±0 cm in *Gracillaria corticata* J. Ag. (R6) in *Brassica oleracea* var capitata and 2.68±0.04 cm in *Ulva lactuca* L. (G1), 2.5±0 cm in *U. reticulata* forsskal (G2), 0.98±0.02 cm in *Padina pavonica* L. (B3), 0.94±0 cm in *Sargassum johnstonii* Setchell & Gardner (B4), 0.81±0.01 cm in *Kappaphycus alvarezii* (R5) , 1.39±0.01 cm in *Gracillaria corticata* J. Ag. (R6) in *Allium cepa* L. (Fig-3,4).

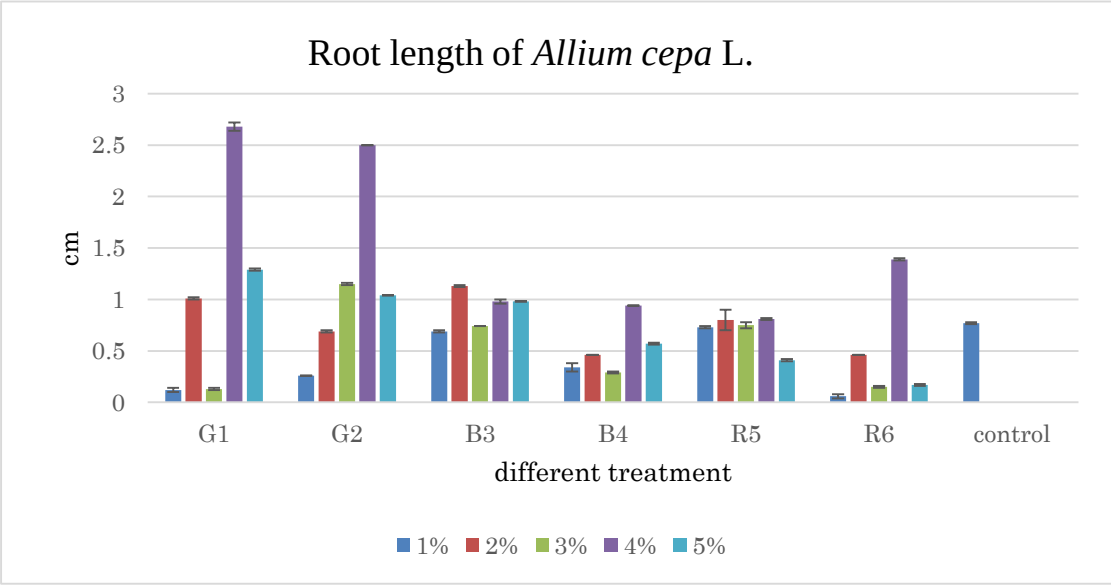

Fig-3: Effect of bio-priming on root length of *Allium cepa* L. by paper towel method.

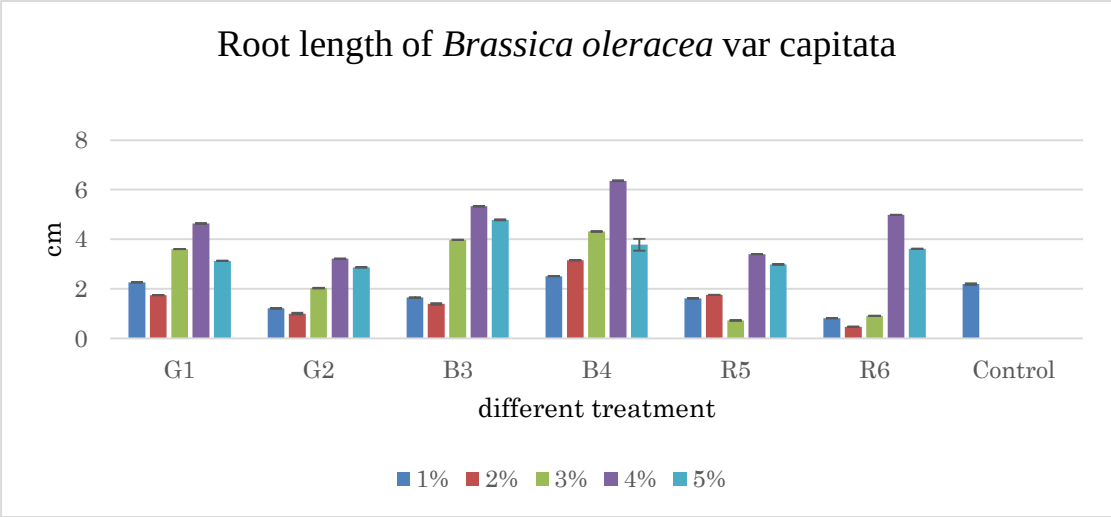

Fig-4: Effect of bio-priming on root length of *Brassica oleracea* var capitata by paper towel method.

At 1% concentration, shoot length result was recorded 1.69±0.01 cm in *Ulva lactuca* L. (G1), 1.1±0 cm in *U. reticulata* forsskal (G2), 1.76±0 cm in *Padina pavonica* L. (B3), 1.4±0.02 cm in *Sargassum johnstonii* Setchell & Gardner (B4), 0.98±0 cm in *Kappaphycus alvarezii* (R5), 0.78±0 cm in *Gracillaria corticata* J. Ag. (R6) in *Brassica oleracea* var capitata and 1.06±0.02 cm in *Ulva lactuca* L. (G1), 0.56±0 cm in *U. reticulata* forsskal (G2), 0.75±0.01 cm in *Padina pavonica* L. (B3), 1.68±0 cm in *Sargassum johnstonii* Setchell & Gardner (B4), 0.02±0.02 cm in *Kappaphycus alvarezii* (R5) , 0.47±0.01 cm in *Gracillaria corticata* J. Ag. (R6) in *Allium cepa* L. (Fig-5, 6). The treatment of milk thistle seeds with salicylic acid (SA) could decrease the plumule and radicle length under low salinity stress conditions [41] and the same results was observed in pepper seeds under salinity and non-salinity stress conditions [42]. The highest seedling length was observed 10.85±0.03 cm in B4 treatment and lowest observation of 6.52±0 cm in R6 treatment at 4% concentration in *Brassica oleracea* var capitata and maximum length was recorded 7.8±0.04 cm at 4% in G1 treatment in *Allium cepa* L. The improvement of seed vigour index was highest with

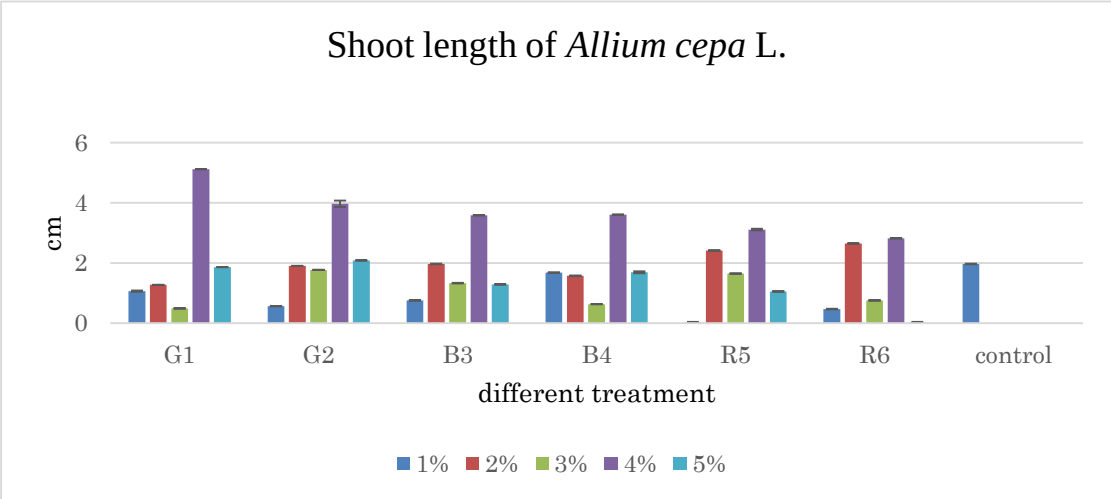

Fig-5: Effect of bio-priming on shoot length of *Allium cepa* L. by paper towel method.

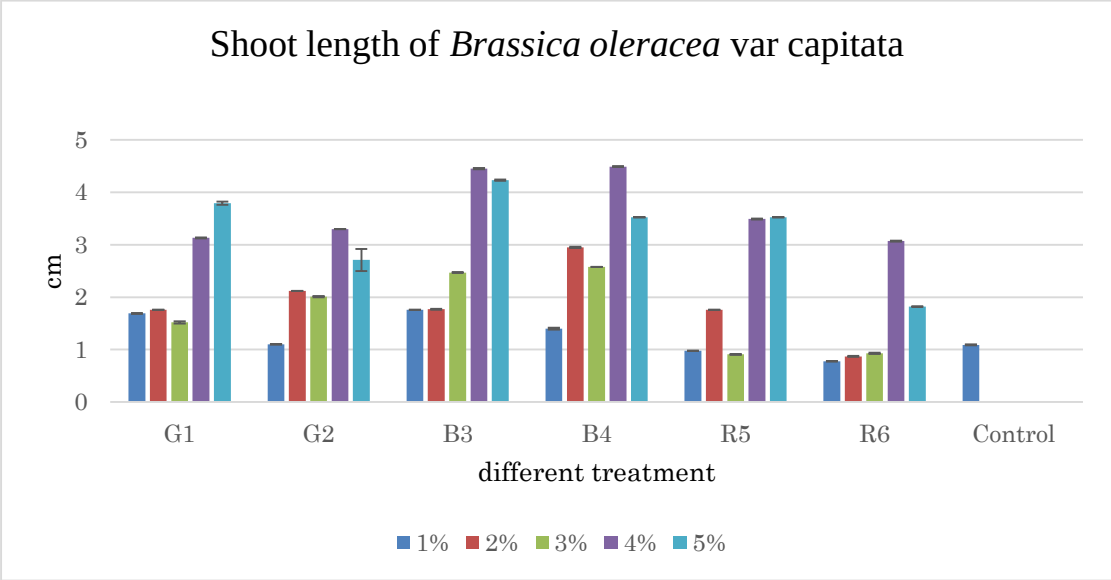

Fig-6: Effect of bio-priming on shoot length of *Brassica oleracea* var capitata by paper towel method.

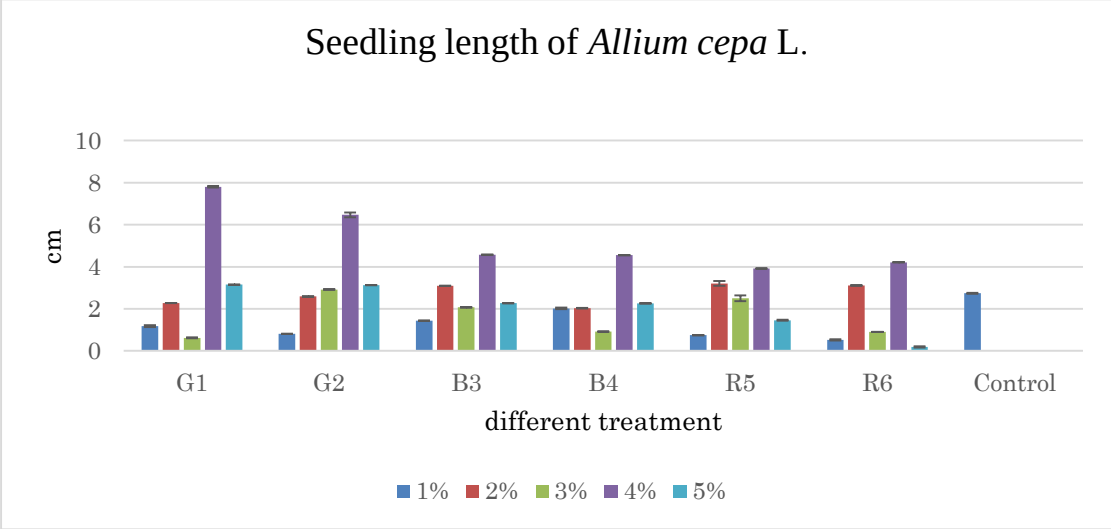

Fig-7: Effect of bio-priming on seedling length of *Allium cepa* L. by paper towel method.

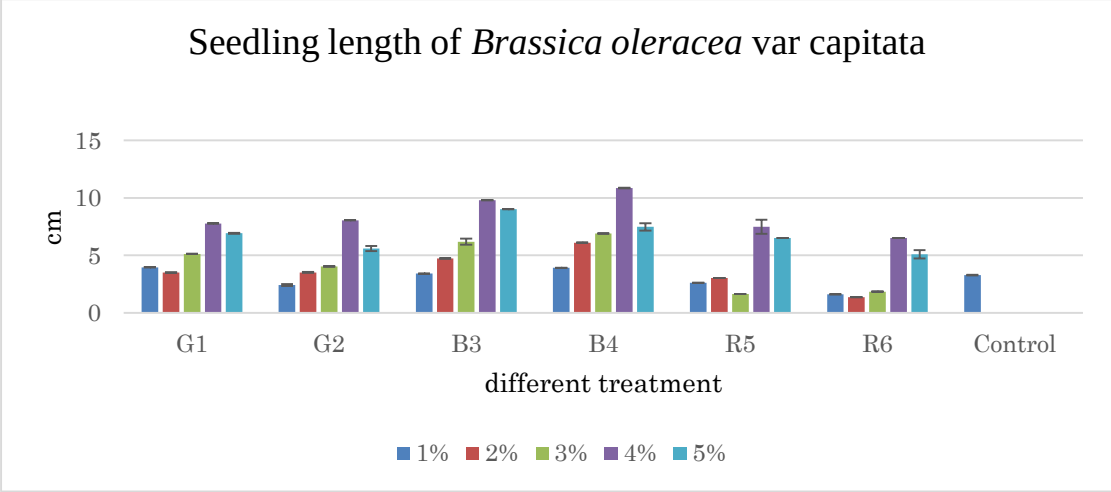

Fig-8: Effect of bio-priming on seedling length of *Brassica oleracea* var capitata by paper towel method.

Sargassum johnstonii Setchell & Gardner (B4) (1085±3) at 4% followed by 978±0, 805±1, 777±1, 749±61 and 652±0 in B3, G2, G1, R5 & R6 respectively in *Brassica oleracea* var capitata . In *Allium cepa* L. at 4% concentration treatment of all seaweed extract seed vigour index was highest but after 4% it was decreased. The effect of priming treatment improves tomato seedling length [43] and osmopriming improves radicle and plumule length in tomato seeds [44]. In pot method, 100% of seed germination was found in *Brassica oleracea* var capitata and *Allium cepa* L. In both target plant experiment B4 treatment impact was highest on growth parameters as compared to control (Table-1, 2). Seed vigour index was presented 1863 in *Sargassum johnstonii* Setchell & Gardner (B4) and 1161 (control) and 1536 in *Sargassum johnstonii* Setchell & Gardner (B4) and 1058 (control) in *Brassica oleracea* var capitata and *Allium cepa* L. respectively. When the survival ability is reduced, seedling cannot make a powerful root system and therefore, root growth can be considered as a screening scale for seed vigour [45]. In osmopriming and hydropriming treatment shoot and root length was increased in primed seeds may be due to its involvement in cell elongation or cell division and the meristematic growth [46]. Seed vigour index of germinating seeds have profound influence on the establishment and yield of crops [47]. The relationship between peroxidase and catalase activities can be very interesting for the explanation of physiological and biochemical effects of these enzymes in plants germinating under contrasting light conditions. Higher peroxidase activities can be related with high concentration of SLF of B4 treatments in *Allium cepa* L. and *Brassica oleracea* var capitata. All treatments resulted in an increase in peroxidase and catalase activities were 1.561 µkat/g fresh weightand 26.7 µkat/g fresh weightand 1.659 µkat/g fresh weightand 30 µkat/g fresh weightin B4 respectively in *Brassica oleracea* var capitata and *Allium cepa* L. relative to the control (Table-1, 2). However, the R5 treatment of priming resulted in a noticeable decrease in the activities of both peroxidase and catalase. Higher peroxidase and catalase activity on the base of more intensive production of toxic H_2O_2 could be expected in light grown seedlings. Seaweed extract of *Sargassum* or *Ulva* antagonizes the oxidative damaging effect of abiotic stress not only directly through activating the antioxidative system such as catalase and peroxidase [48]. Peroxidase activity developed prior to radicle protrusion and increased notably afterwards in tomato seeds [49]. During germination of radish seeds the result have shown peroxidase activity appear at the time of radicle emergence and increased in the period of the seedling development [50].

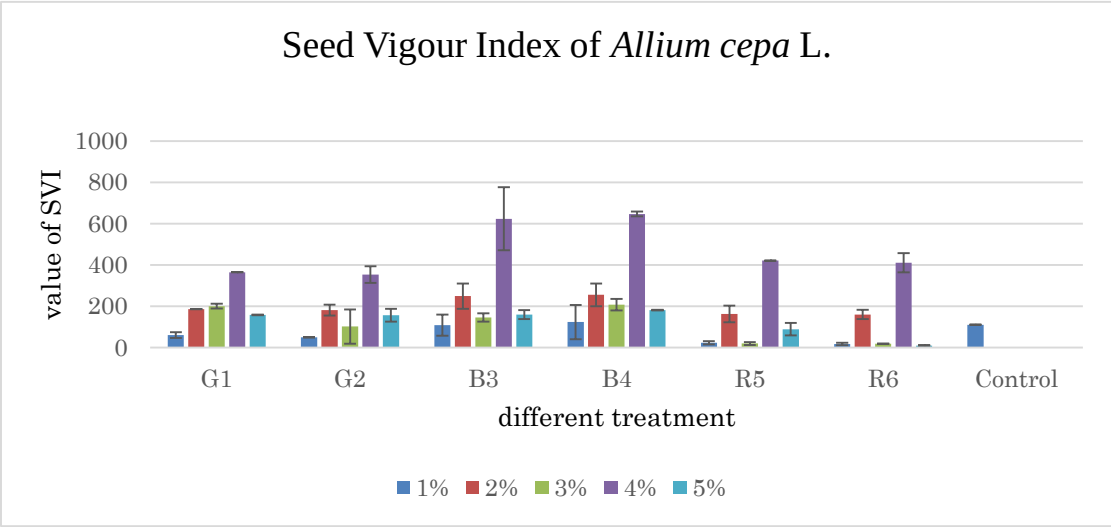

Fig-9: Effect of bio-priming on seed vigour index of *Allium cepa* L. by paper towel method.

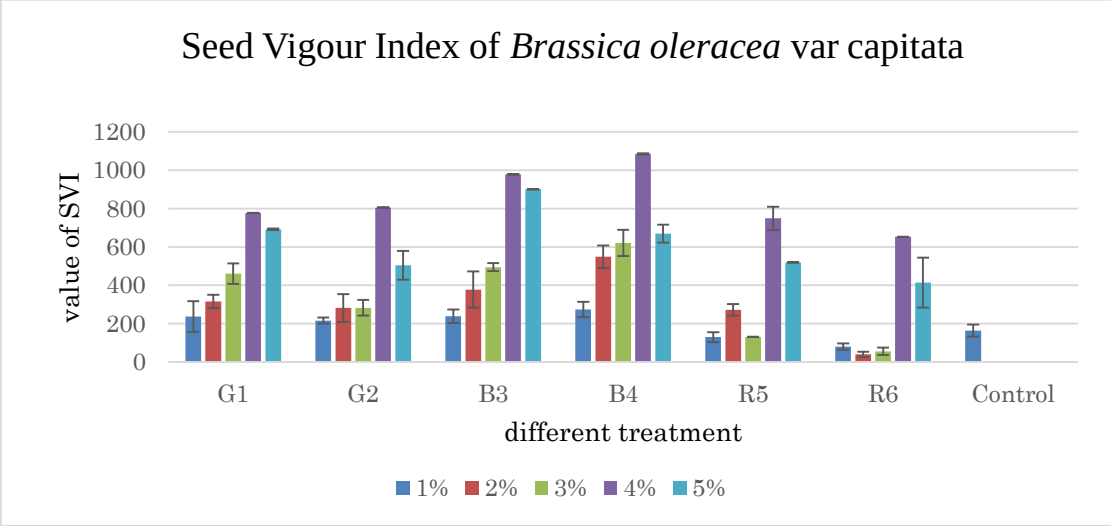

Fig-10: Effect of bio-priming on seed vigour index of *Brassica oleracea* var capitata by paper towel method.

Table-1: Effect of bio-priming on growth parameters and enzyme activity of *Allium cepa* L. by pot method.

***Allium cepa* L.**	**growth parameters**					**Enzyme activity µkat/g fresh weight**	
Different treatment	**% germination**	**root length (cm)**	**shoot length (cm)**	**seedling length (cm)**	**seed vigour index**	**catalase**	**peroxidase**
G1	100%	5.81±0.01	7.99±0.01	13.8±0.02	1380	26.3	1.436
G2	100%	5.81±0.01	7.54±0.44	13.35±0.45	1335	25.8	1.428
B3	100%	6.39±0.05	8.73±0.01	15.12±0.04	1512	29.7	1.643
B4	100%	6.47±0.03	8.89±0.03	15.36±0.06	1536	30	1.659
R5	100%	5.02±0	5.85±0.01	10.87±0.01	1087	21.3	0.661
R6	100%	5.09±0.01	5.79±0.01	10.88±0.02	1088	21.9	0.678
Control	100%	4.71±0.01	5.87±0.03	10.58±0.02	1058	20.8	0.619

Table-2: Effect of bio-priming on growth parameters and enzyme activity of *Brassica oleracea* var capitata by pot method.

***Brassica oleracea* var capitata**	**growth parameters**					**Enzyme activity µkat/g fresh weight**	
Different treatment	**% germination**	**root length (cm)**	**shoot length (cm)**	**seedling length (cm)**	**seed vigour index**	**catalase**	**peroxidase**
G1	100%	8.64±0.02	9.65±0.01	18.29±0.01	1829	25.5	1.216
G2	100%	8.75±0.01	9.38±0.02	18.13±0.03	1813	25.1	1.211
B3	100%	8.89±0.01	9.71±0.03	18.6±0.02	1860	26.5	1.548
B4	100%	8.86±0.02	9.77±0.03	18.63±0.05	1863	26.7	1.561
R5	100%	6.82±0.02	7.81±0.01	14.63±0.03	1463	23.8	0.925
R6	100%	6.84±0.02	7.78±0.02	14.62±0	1462	24.3	0.933
Control	100%	5.41±0.01	6.2±0.02	11.61±0.03	1161	20.4	0.857

4. CONCLUSION

It is well established that seed priming is a new method for improvement of healthy seed germination. Especially, seaweed extract use as a bio- primer material in bio- priming treatment that is very cheap, organic, eco-friendly and easily available in Okha, Gujarat. Our finding also showed that seed priming especially bio- priming by seaweed extract improve seed germination, growth parameters such as root length, shoot length, seedling length and seed vigour index and enzyme activity of catalase and peroxidase that were investigated highest results found at 4% concentration of all treatments as compare to control. Moreover from results of this study in pot method, we conclude that germination of *Allium cepa* L. and *Brassica oleracea* var capitata seeds with treatment of bio-primer materials represented as a: brown seaweed extract> green seaweed extract> red seaweed extract.

5. ACKNOWLEDGEMENT

We would like to thank the experts for support and assistance. Also the authors wish to thank Anand Agricultural University, Anand, Gujarat for supporting this work.

6. CONFLICT OF INTEREST

No. conflict of interest

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