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Effect of Seaweed and Zinc on Growth, Leaf Proline, Chlorophyll Content and Soil Attributes of *Manilkara hexandra* (Roxb.) cv. Thar Rituraj

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ABSTRACT

Background: The aim of the work is to evaluate the influence of different doses of seaweed extract and zinc sulphate on the growth and development of the khirni plants.

Methods: A field experiment was conducted during the year 2021-22 at the Instructional Farm, Department of Fruit Science, College of Horticulture and Forestry, Jhalawar. The experiment consisted of 16 treatments laid out in Randomized Block Design with three replications.

Result: Among different doses of seaweed and zinc, combined application of Seaweed 6 ml/l + zinc sulphate 600 mg/l showed significantly superior over all other treatments. From the experiment, it may be summarized that T15 - SWE (6 ml/L) + ZS (600 mg/L) was better in improving growth parameters vis-vis stress parameters in khirni.

Key words: Foliar spray, Growth, Khirni, Seaweed, Stress, Zinc.

INTRODUCTION

Manilkara hexandra (Roxb.) belonging to the family Sapotaceae, is a socio-economically important underutilized fruit species of Western-Central India. It is locally known as '*Khirni*', '*Raina*' and '*Rayan*' by tribal people of different states of India. It is believed to be originated in India (Stewart and Brandis, 1992). The tree is found as wild in the South, North and Central India mostly in the states of Rajasthan, Gujarat, Madhya Pradesh and Maharashtra (Malik *et al.,* 2010). It is a commercially and medicinally important tropical tree species and is a significant source of livelihood and nutritional support for local tribal population (Mishra and Pareek, 2014). In India, this species is occasionally cultivated in backyards, homestead gardens, public parks as avenue tree and in farmer's fields near villages due to its economic importance as fruit tree having nutritional and medicinal properties. Fruits of khirni have high economic value as mature fresh fruits which are sweet and a good source of minerals, sugars, protein, carbohydrate and vitamin A (Pareek *et al.*, 1998). Its bark and fruits are also used for several medicinal purposes like the treatment of ulcers, dyspepsia, opacity of the cornea, bronchitis, urethrorrhea, leprosy, *etc*. (Anonymous, 1962; Warrier *et al.*, 1995; Pareek *et al.*, 1998; Hoareau, 1999; Raju and Reddy, 2005; Chanda and Parekh, 2010). The seeds contain approximately 25% oil, which is used for cooking purposes (Xian-zi, 1996). The bark also contains 10% tannin, which is used for the treatment of fever and may be utilized for tanning purposes (Anonymous, 1962).

The benefits of seaweed as a source of organic matter and fertilizer nutrients have led to their use as a soil conditioner for centuries. They are used as food for humans, fodder for cattle, substitutes for chemical fertilizers and as a source of various fine chemicals. Besides this, it is used for obtaining many industrial products such as agar and alginate (Khan *et al.*, 2009). Seaweed extracts are marketed as liquid fertilizers and bio-stimulants because they contain multiple growth regulators such as cytokinins (Durand *et al.*, 2003), auxins (Sahoo, 2000), gibberellins (Strik and Staden, 1997) and macronutrients such as nitrogen, phosphorus, potassium and micronutrients

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such as calcium, magnesium and sulfur optimally necessary for plant growth and development. Moreover, it helps in promoting and sustaining the growth of beneficial soil microorganisms (Khan *et al.*, 2009), developing tolerance to environmental stress, increasing nutrient uptake from soil (Turan and Kose, 2004). Seaweed extracts are eco-friendly and help in mitigating environmental pollution and play a biologically active role in intrinsic plant systems. The foliar spray induces faster growth and increased yield in cereals, vegetables, fruit plants and horticultural crops (Mahima *et al.,* 2018).

Additionally, thought to be essential for fruit growth and development is Zinc. It is one among the components needed to synthesise chlorophyll, which makes it helpful for photosynthetic activity (Chaudhuri *et al.,* 2025). Zinc is a pivotal micronutrient for auxin enhancement, protein synthesis, seed production and fruit maturity. It plays an important role in a wide range of processes such as growth hormone productions, especially IAA and elongation of internode. The zinc sulphate is an important source of zinc for plants. Zinc acts as a co-factor of numbers of enzymes and is also involved in the production of growth regulator and chloroplast development (Hambidge *et al.*, 2000). Foliar application of zinc is the most effective in controlling zinc deficiency and improvement of vegetative growth attributes, fruit morphological attributes and internal fruit quality attributes like total soluble solids and ascorbic acid contents. Physiological, morphological and biochemical abnormalities are usually initiated by Zn deficiency resulting in declined growth of plants (Nadeem *et al.,* 2020). Zinc promotes enhancement of chlorophyll in leaves which is an important parameter of carboxylation efficiency. It is essential for water uptake (Disante *et al.*, 2010). Zinc supports healthy root growth thereby leading to more conversion of assimilates for increased Vigor of plant system. Therefore, the effect of seaweed and zinc was studied on various growth parameters of khirni.

MATERIALS AND METHODS

A field experiment was carried out at the Instructional Farm, Department of Fruit Science, College of Horticulture and Forestry, Jhalawar in khirni block during the years 2021-22 using plants having age of 2 years and planted at distance of 6m× 6m. The treatments under study were T_0- Control, T_1- Seaweed extract 2 ml/l, T_2- Seaweed extract 4ml/l, T_3 - Seaweed extract 6 ml/l, T_4 -Zinc sulphate 200 mg/l, T_5- Zinc sulphate 400 mg/l, T_6- Zinc sulphate 600 mg/l, T_7- Seaweed 2 ml/l+ Zinc sulphate 200 mg/l, T_8-Seaweed 2 ml/l+Zinc sulphate 400 mg/l, T_9- Seaweed 2 ml/l+Zinc sulphate 600 mg/l, T_{10}- Seaweed 4 ml/l+Zinc sulphate 200 mg/l, T_{11}- Seaweed 4ml/l+Zinc sulphate 400 mg/l, T_{12}- Seaweed 4 ml/l + Zinc sulphate 600 mg/l, T_{13}- Seaweed 6 ml/l+Zinc sulphate 200 mg/l, T_{14}- Seaweed 6 ml/l+Zinc sulphate 400 mg/l, T_{15}- Seaweed 6 ml/l + Zinc sulphate 600 mg/l. The application of treatments were mode in the form of foliar spray. The spray was done during 1st week of July 2021 after recording initial (base) growth parameters of plants.

The plant growth parameters recorded during the period of experimentation were height of plants (cm), rootstock girth (mm), scion girth (mm), number of nodes per plant, number of shoots per branch, number of leaves per plant, leaf length, leaf width, leaf area, leaf perimeter and leaf area index which were measured initially in July and later at every two-month interval till March. Chlorophyll content and proline content were measured initially and at the end of the experiment.

Plant height was measured from the base of plant to the highest tip of the plant with the help of a measuring scale. The girth of rootstock and scion was measured separately by using digital vernier caliper, number of nodes per plant, number of shoots per branch and number of leaves per plant were counted manually at every two months interval from starting of experiment till the end of experiment.

The average increase in leaf length and leaf width was calculated on the basis of cumulative increase in initial value. The leaf area was calculated using formula as suggested by Montgomery (1911) for this purpose. The leaf area of five tagged plants was recorded using graph paper. For measuring leaf perimeter, selected tagged leaves under various treatments were measured by encircling the thread and thread length was ultimately recorded with the help of measuring scale. Leaf area index was computed by the formula as suggested by Watson (1947). Stress related parameter; the proline content was measured as per the method given by Bates *et al.* (1973), besides the chlorophyll content as per the method suggested by Sadasivam and Manickam (1997).

RESULTS AND DISCUSSION

Effect on growth parameters

It was demonstrated from the Table 1 that the treatment T_{15} (Seaweed 6 ml/L+Zinc Sulphate 600 mg/L) had maximal plant height (28.47%) rootstock girth (32.39%) and scion girth (20.08%). It was shown in Table 2 that topmost number of nodes/plant (33.62%), No. of shoots/branch (3.74%), No. of leaves/plant (27.91%) and Leaf perimeter (18.80%), Table 3 illustrated that the highest Leaf length (27.89%), Leaf width (13.65%), Leaf area (24.40%) and Leaf area index (0.40). Among the different treatments, T_{15} (Seaweed 6 ml/L+Zinc sulphate 600 mg/L) was noted superior as compared to rest of the treatments and including control. This improvement can be attributed to the beneficial impact of seaweed extract, which contains essential macro elements (N, P, K and Mg) and microelements (Fe, Zn, Cu and Mn). These elements fulfill the plant's mineral needs for processes such as photosynthesis, respiration, cell division and elongation. The improved growth of plants may be attributed to the increased availability of microelements such as Fe, Zn, Cu and Mn. These microelements are essential for protein synthesis, chlorophyll formation, as well as the production of the growth

hormone IAA and cell division. These processes collectively contribute to the regulation of cell respiration, photosynthesis, protein synthesis and the uptake of nutrients and water, ultimately promoting enhanced plant growth. The growth attributes of khirni plants strongly align with those obtained by Rishitha and colleagues (2023) in strawberry, Harhash *et al.* (2021) in grapevines cv. Flame Seedless, Al-Hchami, (2013) in peach trees, Al- Hadethi *et al.* (2015 and 2013) in apricot trees, Alam *et al.* (2013) in strawberry, along with beneficial effect in orange and mandarin as obtained by Rayorath *et al.* (2008), Sharaf *et al.* (2011), Abbas *et al.* (2012), Abdel Hamied-Sheven (2014)

Table 1: Effect of seaweed and zinc on plant height, rootstock girth, scion girth of khirni.

Treatments	Height of the plant (cm)		Rootstock girth (mm)		Scion girth (mm)	
	Initial value	Final Value	Initial value	Final Value	Initial value	Final Value
T_0	218.87	227.33 (3.86)	25.33	27.77 (9.61)	23.80	25.15 (5.67)
T_1	216.54	243.23 (12.32)	24.50	28.87 (17.83)	23.92	26.31 (9.99)
T_2	214.89	243.23 (13.18)	24.15	28.50 (17.99)	23.30	25.89 (11.11)
T_3	219.80	251.32 (14.34)	27.33	33.00 (20.73)	24.87	27.83 (11.90)
T_4	223.54	244.54 (9.39)	27.67	32.65 (18.01)	25.67	28.30 (10.24)
T_5	231.28	256.00 (10.68)	27.50	32.45 (18.01)	25.66	28.36 (10.53)
T_6	226.35	253.67 (12.06)	29.33	35.10 (19.65)	26.25	29.02 (10.55)
T_7	210.32	243.66 (15.85)	25.83	32.19 (24.60)	24.08	27.03 (12.25)
T_8	211.97	246.43 (16.25)	27.70	34.55 (24.72)	24.87	28.00 (12.58)
T_9	219.87	258.00 (17.34)	27.33	34.53 (26.32)	25.77	29.10 (12.90)
T_{10}	220.12	260.43 (18.31)	26.00	32.77 (26.03)	24.75	28.00 (13.13)
T_{11}	210.30	253.20 (20.39)	25.05	31.67 (26.41)	24.25	27.53 (13.52)
T_{12}	221.34	270.00 (21.98)	24.67	32.34 (31.10)	23.07	27.16 (17.74)
T_{13}	213.33	271.2 (27.12)	24.00	31.65 (31.87)	23.16	27.50 (18.73)
T_{14}	213.00	273.00 (28.16)	24.17	31.95 (32.20)	23.10	27.67 (19.78)
T_{15}	212.50	273.00 (28.47)	25.33	33.54 (32.39)	23.33	28.02 (20.08)
CD (5%)	-	0.90	-	0.66	-	0.53
SEm (±)	-	0.31	-	0.23	-	0.18

Table 2: Effect of seaweed and zinc on number of nodes/plant, number of shoots/branch, number of leaves/plant and leaf perimeter of khirni.

Treatments	No. of nodes/plant		No. of shoots/branch		No. of leaves/plant		Leaf perimeter (cm)	
	Initial value	Final value	Initial value	Final value	Initial value	Final value	Initial value	Final value
T_0	131.33	140.72 (7.14)	16.67	16.79 (0.75)	111.67	121.20(8.53)	13.00	14.16(8.97)
T_1	136.44	154.25 (13.05)	16.33	16.53 (1.20)	105.00	124.33 (18.40)	12.96	14.23(9.76)
T_2	129.69	145.67 (12.32)	17.00	17.23 (1.35)	104.67	124.17 (18.62)	13.20	14.53(10.11)
T_3	132.50	156.36 (18.00)	17.67	17.93 (1.49)	106.83	127.83 (19.65)	12.63	14.13(11.87)
T_4	133.67	153.67 (14.96)	17.33	17.55 (1.25)	107.00	123.17 (15.11)	14.03	15.63(11.39)
T_5	129.59	148.78 (14.81)	17.67	17.90 (1.32)	104.67	121.33 (15.91)	13.00	14.53(11.79)
T_6	127.87	147.80 (15.58)	17.33	17.56 (1.30)	107.00	125.00 (16.82)	13.83	15.23(10.14)
T_7	137.16	163.65 (19.31)	18.00	18.31 (1.75)	103.00	123.67 (20.06)	15.76	17.40(10.37)
T_8	127.99	151.61 (18.45)	17.00	17.29 (1.74)	106.33	128.17 (20.53)	15.50	17.36(12.03)
T_9	133.13	159.66 (19.93)	18.00	18.35 (1.94)	106.17	129.00 (21.50)	16.53	18.63(12.69)
T_{10}	125.87	153.89 (22.26)	17.33	17.65 (1.86)	104.67	127.83 (22.12)	16.93	19.26(13.77)
T_{11}	126.83	160.34 (26.41)	17.76	18.16 (2.25)	106.00	131.50 (24.05)	13.60	15.50(13.98)
T_{12}	133.09	170.44 (28.06)	16.67	17.09 (2.54)	105.33	131.00 (24.37)	16.76	19.33(15.30)
T_{13}	133.00	172.19 (29.46)	16.67	17.12 (2.72)	102.50	129.33 (26.17)	16.93	19.63(15.95)
T_{14}	133.87	177.00 (32.22)	15.33	15.84 (3.30)	105.00	133.67 (27.30)	16.83	19.62(16.53)
T_{15}	132.94	177.65 (33.62)	16.03	16.63 (3.74)	108.67	139.00 (27.91)	16.66	19.80(18.80)
CD (5%)	-	0.84	-	0.07	-	0.89	-	0.76
SEm (±)	-	0.29	-	0.03	-	0.30	-	0.26

and Al Hayani *et al.* (2016); and that of zinc with those as reported by Razzaq *et al.* (2013) and Dawood *et al.* (2001) in Kinnow mandarin.

Moreover, the maximum numbers of secondary branches (7.49 and 6.58) were observed in the treatment of 75 ppm Zn+0 ppm GA3 in the first and second seasons, respectively. The spraying of Zn may compensate for the lack of Zn and avoid deficiency of Zn in plants especially in poor micro-elements sandy soils. So, by increasing the level of Zn, the vegetative growth parameter will be improved

Table 3: Effect of seaweed and zinc on leaf length, leaf width, leaf area and leaf area index of khirni.

Treatments	Leaf length (cm)		Leaf width (cm)		Leaf area (cm^2)		Leaf area index	
	Initial value	Final value	Initial value	Final value	Initial value	Final value	Initial value	Final value
T_0	7.50	8.03 (7.06)	5.30	5.45 (2.89)	37.80	39.38 (4.18)	0.22	0.26
T_1	7.80	8.75 (12.17)	5.31	5.72 (7.72)	36.43	40.87 (12.18)	0.24	0.29
T_2	7.71	8.65 (12.19)	4.32	4.71 (9.02)	31.00	35.14 (13.35)	0.28	0.34
T_3	7.00	7.87 (12.42)	4.79	5.23 (9.18)	34.00	39.10 (15.00)	0.27	0.32
T_4	7.13	7.80 (9.39)	5.63	5.97 (5.97)	40.25	43.90 (9.06)	0.28	0.34
T_5	6.90	7.62 (10.43)	5.10	5.42 (6.29)	38.50	42.43 (10.20)	0.25	0.32
T_6	7.10	7.87 (10.84)	5.51	5.89 (6.96)	41.67	46.36 (11.26)	0.30	0.35
T_7	7.33	8.27 (12.77)	4.44	4.83 (8.78)	38.88	45.42 (16.81)	0.25	0.32
T_8	6.45	7.46 (15.65)	4.93	5.37 (9.06)	46.20	54.32 (17.57)	0.27	0.31
T_9	6.93	8.05 (16.10)	4.66	5.18 (11.15)	41.15	48.43 (17.69)	0.26	0.32
T_{10}	6.33	7.63 (20.52)	4.60	5.13 (11.52)	44.35	52.52 (18.42)	0.27	0.33
T_{11}	6.93	8.47 (22.16)	5.20	5.82 (11.92)	44.30	54.23 (22.41)	0.29	0.35
T_{12}	7.00	8.83 (26.19)	4.87	5.48 (12.52)	46.67	56.34 (20.71)	0.26	0.33
T_{13}	7.60	9.63 (26.75)	4.93	5.58 (13.10)	38.13	46.76 (22.62)	0.28	0.35
T_{14}	7.95	10.13 (27.42)	5.00	5.67 (13.40)	35.00	43.23 (23.51)	0.29	0.37
T_{15}	7.78	9.95 (27.89)	5.20	5.91 (13.65)	35.00	43.54 (24.40)	0.30	0.40
CD (5%)	-	0.86	-	0.25	-	1.04	-	0.04
SEm (±)	-	0.30		0.09		0.35	-	0.01

Note: 1. Data in parentheses indicates a per cent increment.
2. CD has been calculated based on percentage value.

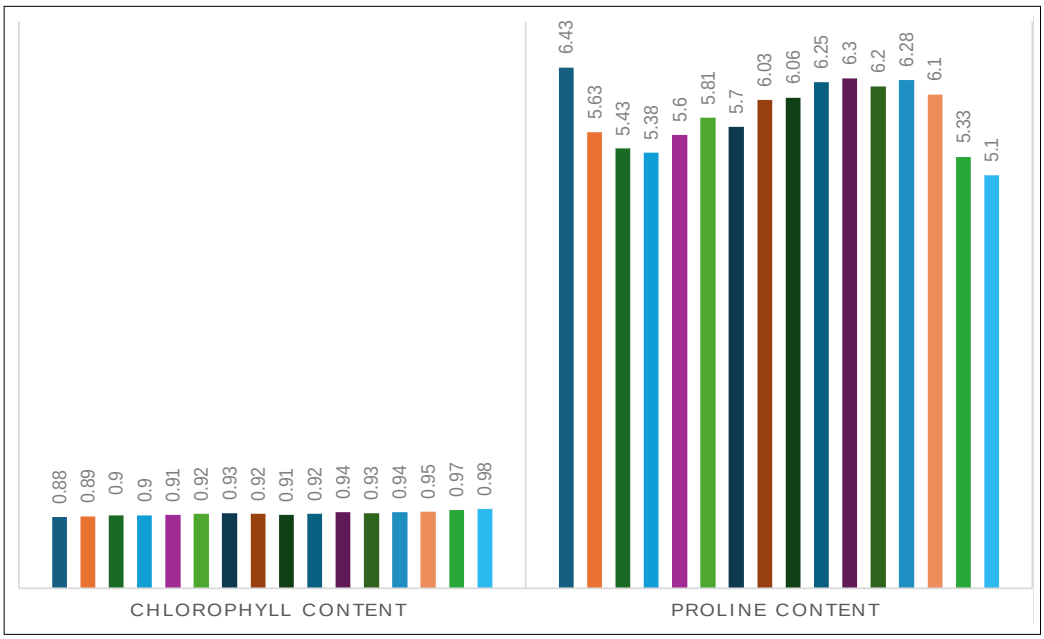

Fig 1: Effect of foliar spray of seaweed and zinc on chlorophyll content and proline content of khirni.

Table 4: Effect of foliar spray of seaweed and zinc on soil parameters of *Khirni* (*Manilkara hexandra* L.) cv. Thar Rituraj.

Treatments	Soil parameters						
	pH	EC (dS m^{-1})	OC (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	WHC (%)
Initial values	7.59	0.42	0.46	275	25.00	326.00	37.00
T_0	7.59	0.42	0.46	275.00	25.00	326.00	37.00
T_1	7.58	0.41	0.48	273.00	24.00	324.00	36.00
T_2	7.57	0.40	0.49	274.00	23.00	325.00	35.00
T_3	7.56	0.40	0.49	275.00	24.00	326.00	37.00
T_4	7.55	0.39	0.50	276.00	25.00	326.33	38.00
T_5	7.54	0.38	0.50	277.00	24.33	324.67	39.00
T_6	7.54	0.39	0.51	276.33	24.67	325.00	41.00
T_7	7.53	0.38	0.51	278.33	25.00	325.33	42.00
T_8	7.52	0.39	0.52	279.33	25.67	325.67	43.00
T_9	7.51	0.37	0.52	279.67	26.00	326.00	44.00
T_{10}	7.49	0.38	0.53	280.00	26.33	326.67	45.00
T_{11}	7.48	0.38	0.54	280.67	26.00	327.00	46.00
T_{12}	7.46	0.37	0.54	281.00	26.67	327.67	47.50
T_{13}	7.45	0.36	0.55	281.33	27.00	328.00	48.00
T_{14}	7.44	0.35	0.56	282.00	27.67	329.00	49.50
T_{15}	7.42	0.34	0.57	283.00	28.67	330.33	50.60
SEm (±)	-	-	-	-	-	-	-
CD (5%)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

(Atteya *et al.*, 2018). In the case of zinc application, the plots which received 30 kg/ha of $ZnSO_4$ recorded the tallest plant height which were 10.56, 3.64, 4.71 and 4.72% taller than 20 kg/ha of $ZnSO_4$. However, it is found to be comparable with 40 kg/ha of $ZnSO_4$. Such kind of increment due to zinc application is a result of higher nitrogen uptake and enhanced enzymatic activity. (Bhattacharjee *et al.,* 2022).

The results of study presented regarding beneficial effect of seaweed and zinc are congruous to the findings as reported by Ali and Mohamed (2016), Goma and Ibrahim (2020) in banana, Abdulrahman (2013) in Almond seedlings, Al- Rawi *et al.* (2016) in peach plants, Chhonkar and Singh (1981) in Guava, Banik and Sen (1997) in mango, Sarolia *et al.* (2007) in guava and Waskela *et al.* (2013) in guava.

Effect on proline and chlorophyll content

The data concerning chlorophyll content (0.98 mg/g) and proline content (5.10 µmol./g) are presented in Fig 1. Data reveals that there were significant variations among the various treatments and it was obvious that maximum increase in chlorophyll content and minimum increase in proline content was found in T_{15} (Seaweed 6ml/l+Zinc sulphate 600mg/l).

The better increase in growth parameters, chlorophyll and reduction in proline content under T_{15} (Seaweed 6 ml/l + Zinc sulphate 600 mg/g) may have association with the combined effect of seaweed and zinc. The effect may be explained in the light of the fact that auxins content in the seaweed extracts have an effective role in cell division and enlargement. This perhaps led to an increase in the shoot growth, leaves area and plant dry weight (Gollan and Wright, 2006). Seaweed extracts contain cytokinins which induce physiological activities like activating enzymes that are involved in photosynthesis and increase in total chlorophyll in the plant and via these ways it positively influenced shoots characteristics (Thomas, 1996). Besides, the favorable influence of applied zinc through foliar feeding might also play augmentory role in stimulating the stimulatory effect on most of the physiological and metabolic process governing chlorophyll synthesis, auxin enhancement, protein synthesis and acceleration of synergy in the plants.

Effect on soil parameters

The data showcased in Table 4 illustrates the influence of foliar application of seaweed extract and zinc sulphate in individual as well as well combination effect during experimental season 2021-22 in the Khirni cv. Thar Rituraj. The findings disclose that there were no discernible differences in soil parameters *viz*; soil EC, soil pH, organic carbon (%), available N (kg/ha.), P (kg/ha), K (kg/ha) and water holding capacity (%). However, statistically T_{15} (Seaweed 6 ml/l+Zinc sulphate 600 mg/l) was the most effective treatment from the remaining treatments. It is due to that the seaweed and Zinc was applied as a foliar spray (directly on leaves), the nutrients have not reached the soil in significant amount. In other view, the applied substances have not leached into the soil or interacted with soil microorganisms and particles.

Zinc plays a pivotal role in the fundamental process involved in the cellular mechanism and respiration. The

presence of sufficient available zinc in chloroplasts and whole plant tissue system may also be considered a possible reason for increase in growth attributes of the plants. Zinc is an essential component of enzymes responsible for nitrogen, thereby resulting in an increase in uptake of nitrogen by the plant. Along with, involvement of zinc in synthesis of tryptophan which is a precursor of indole acetic acid synthesis regulates tissue growth and development (Alloway, 2008) and thereby improvement of shoot parameters. From the result of investigation, it may be concluded that the application of T15 (Seaweed 6 ml/l + Zinc sulphate 600 mg/l) emerged better in its effectiveness on growth and development of *Khirni* (*Manilkara hexandra* L.) cv. Thar Rituraj. All the plant growth and development parameters were found significantly superior over all other individuals as well as combined treatments.

CONCLUSION

From the result of investigation, it is concluded that T15 (Seaweed 6ml/l + Zinc sulphate 600 mg/l) emerged better in its effectiveness on growth and development of Khirni (Manilkara hexandra L.) cv. Thar Rituraj. All the plant growth and development parameters were found significantly superior in T15 (Seaweed 6ml/l + Zinc sulphate 600mg/l) over all other individual as well as combined treatments. In this treatment (T15) the per cent increase in parameters like height of the plant (28.47%), rootstock girth (32.39%), scion girth (20.08%), No. of nodes/plant (33.62%), No. of shoots/branch (3.74%), No. of leaves/plant (27.91%) and leaf parameters (18.80%) like Leaf perimeter (18.80 cm), Leaf length (27.89cm), Leaf width (13.65cm) leaf area (24.40%). Leaf area index (0.40) and physiological parameters like proline content (5.10 µmol. /g) and chlorophyll content (0.98) were significant at the time of final observation during March.

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Conflict of interest

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