

Interactive Effect of NaCl Salinity and Zinc on Growth and Development of *Catharanthus Roseus* (L.)

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Abstract--- Salinity is a major abiotic stress that adversely affect plant growth. In the present investigation the effect of various concentration of NaCl salinity, Zn and their interactive effect on growth of *Catharanthus roseus* was investigated. The shoot length, root length, total length, number of leaves and leaf area per plant decreased with increasing NaCl concentration and increased with application of zinc. However in interactive effect, Zn mitigates the toxic effects of NaCl, especially with lower concentration of Zn i.e. 5 ppm and 10 ppm as compare to 25 ppm and 50 ppm Zn, which improves growth of *Catharanthus roseus*.

Keywords--- NaCl salinity, zinc, *Catharanthus roseus*, growth

I. INTRODUCTION

Salinity is major abiotic stress affecting plant growth especially in arid and semi-arid regions of the world (Davidson and Chevalier, 1987; Khajesh – Hossemi *et al.*, 2003). The number of earlier reports indicates that salinity disturbs water relation, create water imbalance in plant nutrition and affect biochemical and physiological processes of plant (Karim *et al.*, 1993), physiological processes like increased respiration, ion toxicity, decreased CO₂ assimilation rate (Hajlaoui *et al.*, 2006). Salinity modifies metabolic processes, causes ion toxicity, oxidative stress, osmotic stress and genotoxicity (Ibrahim *et al.*, 2012; Hussein *et al.*, 2017). High salt concentration hampers vital processes like seed germination, seedling growth and vigor, vegetative growth, flowering as well as fruit set and ultimately reduces crop yield and quality of produce was reported by Sairam and Tyagi (2004). Salinity affect seed germination, growth and development, at later stage which adversely affect crop yield (Wu *et al.*, 2015).[1]

Zn is an essential trace metal that despite having no redox activity, is involved in many vital physiological events in plants (Sagardoy *et al.*, 2009). Zinc is a constituent of various enzymes like oxidoreductases, transferases and hydrolyses (Mishra and Dubey, 2005). It is involved in many biochemical and physiological processes and plays a central role in detoxification of ROS in plants cells (Broadley *et al.*, 2007; Cakmak, 2000). Despite of Zn being essential element it's excess in plants can induce phytotoxic effects (Chaney, 1993). Supply of zinc could mitigate the adverse effects of NaCl was reported earlier by Parker *et al.*, (1992). According to Gunes *et al.* (1999)

zinc mitigate the toxicity of other heavy metals in plants and adversely affects the activities of free radicals.[2]

Catharanthus roseus (L.) G. Don is a diploid (2n=16), an annual or perennial herbaceous sub shrub emanating a pungent smell, belonging to family apocynaceae and cultivated mainly as an ornamental and medicinal plant. It is native of Madagascar and commonly known as Madagascar periwinkle.[3] The use of *Vinca* has been found in folk medicine literature of Europe as diuretic, antidiarrhetic, antihemorrhagic and wound healing. It is used as a remedy for diabetes, on muscle relaxation, circulatory diseases and on central nervous system. Leaf, stem, root and flower extracts are antifungal while leaf extract is antibacterial. Vincristine and vinblastine are used in chemotherapeutic treatments of blood cancer. Due to its anticancerous effect, The National Cancer Council of Malaysia uses the periwinkle logo as its symbol for cancer patients. The stress conditions are suitable for synthesis of secondary metabolites. *C. roseus* has been extensively studied for phytochemical aspects but physiology of the species under salinity has been rarely attempted so far. Therefore, in present study an attempt has been made to study the growth of this plant under NaCl salinity, zinc and in the combination of both, NaCl and zinc.[4]

II. MATERIAL AND METHOD:

Catharanthus roseus G. Don. (*Vinca rosea* L.) plants were raised in soil bed from the seeds in the botanical garden of Krishna Mahavidyalaya, Rethare Bk, Dist-Satara. The plants, after their establishment after a month were further grown in the sand media at various NaCl salinity levels (0.0, 25, 50, 100, 200 mM NaCl); zinc levels (0.0, 5, 10, 25, 50 ppm) and also in various

combination of NaCl and Zn for next two months. The salinity levels in the media were raised slowly beginning with 25 mM NaCl and 5 ppm Zn for the plants except control reaching the final respective levels slowly. Plants were treated twice a week with alternate watering with equal amount of tap water to check the loss of water due to evaporation from the culture containers and to maintain the salt and zinc concentration in the medium. After 2 months,

when effect of salt treatment was visually evident, the plants were used for further studies.[3]

III. RESULT AND DISCUSSION:

Effect of NaCl salinity on linear growth (shoot and root length, shoot / root ratio, total length), foliage production (number of leaves, leaf area) per plant of *Catharanthus roseus* (L.) is depicted in Table 1.

Table: 1. Effect of NaCl salinity on growth and development of *C. roseus* (L.)

Growth Parameter	NaCl Treatment (mM)				
	00 (Control)	25	50	100	200
Shoot length (cm) (S)	50.10 (±1.20)	48.06 (±1.36)	46.40 (±1.70)	44.50 (±1.40)	42.20 (±1.39)
Root length (cm) (R)	19.64 (±1.31)	16.94 (±1.20)	16.00 (±1.20)	14.70 (±1.20)	12.00 (±1.20)
Total length (cm) (S+R)	69.74 (±1.25)	65.54 (±1.27)	62.40 (±1.45)	59.20 (±1.32)	54.20 (±1.30)
S/R	2.55	02.83	02.90	03.03	03.52
No. of leaves Plant ⁻¹	33.00 (±2.0)	29.00 (±3.0)	32.00 (±3.0)	28.00 (±2.0)	26.00 (±1.0)
Leaf area (cm ²) Plant ⁻¹	310.76 (±1.20)	308.38 (±2.10)	306.38 (±2.30)	304.22 (±4.80)	198.12 (±3.50)

± S.D. Each value is mean of five determinations

It can be seen from the present investigation that salinity has reduced length of shoot and root, however, shoot: root ratio increases with increasing salinity levels. The number of leaves, leaf area, produced per plant also reduced as the salinity levels in the growth medium increase. This adverse effect of salinity is more significant at higher salinity levels (100 and 200 mM) as compared to lower salinity levels.[7]

Salt stress shows reduction in growth and development. Several workers have reported decrease in height of plants due to salinity. Minhas *et al.* (1990) in Indian mustard, Francois *et al.* (1992) in *Hibiscus cannabinus*, Khan and Rizvi, (1994) in *Atriplex griffithii*, Gouia *et al.*, (1994) in bean and cotton. According to Cruze *et al.* (1990), plant height can be regarded as a good indicator of the degree of salt tolerance in *Lycopersicon* species. According to He and Cramer (1993), growth analysis is fundamental to the characterization of a plant's response to and environmental stress.. The height of a plant is usually a very good indicator of its conditions or the success of species in various environmental conditions.[30] It is a very easily detectable growth characteristic of the plants growing in field conditions. According to Xiong and Zhu (2002) salt stress inhibits the efficiency of translocation and assimilation of photosynthates and might cause decrease in growth of shoot. It is reported by many workers that NaCl salinity decreased the length of plant (Memon *et al.*, 2010). According to Razzaque *et al.* (2011) reduction in

plant growth has been attributed to reduced water absorption due to osmotic effect, deficiency in nutrition due to ionic imbalance and decrease in metabolic activities Jamil *et al.* (2006) reported reduction in shoot and root growth in sugar beet, cabbage and pakchoi under NaCl salinity. According to Jaleel *et al.* (2008) plant height, root length, leaf area, growth rate, fresh wt., dry wt. of *Catharanthus roseus* was reduced under NaCl salinity stress. Similar results were found in *Withania somnifera* (Jaleel *et al.* 2008). Decrease in the root growth, leaf area, fresh weight and yield of tomato was reported by Latef and Chaoxing (2011). Recently Muhammad Ali *et al.* (2021) reported adverse effect of salinity on shoot and root length, biomass and leaf area of okra varieties.[9]

Change in shoot to root ratio is a common plastic response to stress (Begg and Turner, 1976) and is generally assumed to be of adaptive value (Taleisnik, 1987). Root growth has been shown to be a sensitive parameter for evaluating the salt tolerance (Hannon and Baradshaw, 1968). Nigwekar and Chavan (1987) noticed increase in shoot length at moderate salinity treatment and decrease at higher NaCl concentration in horsegram. According to Jaleel *et al.* (2007) shoot and root length of seedlings of *Catharanthus roseus* was inhibited by salinity.[10]

The measurement of leaf area determines the plant's productive investment (Causton and Venus, 1981). Leaf area in salinity was a good predictor of absolute NaCl tolerance (Rawson *et al.*, 1988). According to Meiri and

Poljakoff-Mayber (1970) total leaf area and the number of leaves of bean plants under saline conditions, were usually smaller than those on control plants. Such a reduction in linear growth and in the number of leaves per plant due to sea water salinity was noticed by Kurian, (1976) in safflower, Nukaya (1983) in tomato plant, Bhivare and Nimbalkar (1984) in french bean, He and Cramer (1993) in *Brassica napus* in the later stages of growth. Okusanya and Oyesiku (1994) correlated the drastic reduction in number of leaves and leaf area in *Vigna vexillata* under saline condition with high content of Na^+ and Cl^- in shoot which may have become toxic to the leaves turning them chlorotic and leading to severe leaf loss and reduced growth. According to Ratnakar and Rai (2013) salinity adversely affected growth parameters like shoot length, root length of *Trigonella* seedlings. Salt stress depress the height of plant, length of root, number of branches, fresh wt. and dry wt. of shoots and roots of moringa (Hussein and Baker, 2014). Chowdhury *et al.* (2018) reported decreased shoot length, root length, fresh and dry weights of root and shoot of sunflower.[12]

Results obtained in the present investigation follows the earlier observations made by different workers. The overall height of the *Catharanthus roseus* reduced with increase in salinity concentration. However the root length affect more as compare to shoot which is also responsible

for increase in S/R ratio may be due to reduction in water and mineral absorption as reported by Adu *et al.* (1994). The reduction in leaf number and even in leaf area reported in the present investigation also correlated with earlier investigation and this may be due to adverse effect on meristematic tissues during leaf growth.[13]

The effect of zinc on linear growth (shoot and root length, shoot: root ratio), foliage production (number of leaves, leaf area) per plant of *Catharanthus roseus* G. Don has been recorded in Table 2. It is evident from the present investigation that, the effect of different concentration of zinc varies with plant organs. The shoot length increased with Zn especially lower Zn concentration i.e. 5, 10 ppm. Lower Zn concentration increased root length but the higher Zn concentration decreased root length. Similarly S/R ratio increases against control, highest ratio is observed by 10 ppm Zn concentration. The leaf area and number of leaves per plant are also increased mostly by the 10 ppm Zn treatment but 50 ppm Zn conc. affects these growth parameters adversely.

Number of workers reported the adverse effect of Zn on the growth and development of plants, Vaillant *et al.* (2005) in *Datura*, Khudsar *et al.*, (2004) in *Artemisia* plants. Szopinski *et al.* (2019) reported reduction in root growth of *A. arenosa*. [15]

Table: 2. Effect of zinc on growth and development of *C. roseus* (L.)

Growth Parameter	Zinc Treatment (ppm)				
	00 (Control)	5	10	25	50
Shoot length (cm) (S)	50.10 (±0.30)	65.50 (±0.20)	75.70 (±1.50)	57.20 (±1.62)	53.10 (±1.90)
Root length (cm) (R)	19.64 (±0.25)	21.50 (±1.24)	19.80 (±2.05)	18.70 (±1.31)	16.00 (±1.35)
Total length (cm) (S+R)	69.74 (±0.27)	87.00 (±0.70)	95.50 (±1.75)	75.90 (±1.45)	69.10 (±1.60)
S/R	02.55	03.05	03.82	03.06	03.32
No. of leaves Plant ⁻¹	33.00 (±2.0)	39.00 (±3.0)	45.00 (±3.0)	33.00 (±2.0)	26.00 (±1.0)
Leaf area (cm ²) Plant ⁻¹	310.76 (±3.15)	444.74 (±3.80)	632.36 (±4.24)	357.02 (±1.30)	230.20 (±1.35)

± S.D. Each value is mean of five determinations

El-Mansi *et al.* (1990) reported that Zn increased all growth parameters of pea plants. According to Singh *et al.* (1992) application of Zn resulted in more vegetative growth on acid soil leading to higher dry wt. production. Sharma *et al.* (1994) showed that Zn promotes the growth of cabbage. Soil application of Zn increased growth of chickpea (Khan *et al.* 2000) Stimulatory effects of Zn on growth of plants have been reported by Marschner (2012). This is attributed to function of zinc in CO_2 assimilation, as a component of auxin production that promoted the elongation process. Application of Zn positively affects

auxin biosynthesis which can promote absorption of minerals, cell division and thus enhance the growth (Cakmak, 2008). Farahat *et al.* (2007) observed increase in plant height, stem diameter, length of root, fresh and dry weights of shoot and roots in *Cupressus* under Zn treatment. Basil plants treated with Zn performed superior as compared to non-treated plants (Said-Al Ahl and Mohmoud, 2010). Low concentration of Zn (1mM ZnCl_2) promoted root and hypocotyls growth in tomato (Kosesakal and Unal, 2012) while higher concentration showed adverse effect on root and hypocotyls growth (3-7

mM ZnCl₂). According to Vafa *et al.* (2015) growth parameters like height of plant, number of leaves and fresh wt. and dry wt. of savoy plant was improved by the application of nano-zinc. Zinc causes increase in height of mungbean (Samreen *et al.*,2017),in wheat (Alam and Shereen 2002). Yadav and Sharma (2018) observed that supply of Zn is more useful for growth of barley plant; plant height, flag leaf area, tillers and biomass of barley plant was significantly increased by the application of Zn. The positive effect of Zn on different plant organs reported by earlier work correlated with this present investigation.

But the higher doses of Zn i.e. 50 ppm adversely affect root growth, number of leaves and leaf area however it increases shoot growth and S/R ratio. The lower doses of Zn may be enhanced CO₂ assimilation and production of growth hormones.[16]

The Interactive effect of NaCl and zinc on linear growth (shoot and root length, shoot: root ratio), foliage production (number of leaves, leaf area) per plant of *Catharanthus roseus* G. Don has been recorded in Table 3 to 6.[17]

Table: 3. Effect of NaCl salinity and zinc (5 ppm) on growth and development of *C. roseus* (L.)

Growth Parameter	Treatment				
	00 (Control)	25 mM NaCl + 5 ppm Zn	50 mM NaCl+ 5 ppm Zn	100 mM NaCl+ 5 ppm Zn	200 mM NaCl+ 5 ppm Zn
Shoot length (cm) (S)	50.10 (±1.09)	56.00 (±1.25)	52.00 (±1.37)	52.00 (±1.30)	45.10 (±1.24)
Root length (cm) (R)	19.64 (±0.20)	24.20 (±0.57)	24.40 (±0.90)	18.40 (±0.80)	20.83 (±0.54)
Total length (cm) (S+R)	69.74 (±0.65)	80.20 (±0.80)	76.40 (±0.84)	70.40 (±0.98)	65.93 (±0.75)
S/R	02.55	02.31	02.13	02.83	02.16
No. of leaves Plant ⁻¹	33.00 (±2.0)	30.00 (±2.0)	33.00 (±3.0)	28.00 (±2.0)	19.00 (±3.0)
Leaf area (cm ²) Plant ⁻¹	310.76 (±3.31)	522.45 (±1.50)	485.30 (±3.35)	317.25 (±3.80)	200.08 (±3.60)

± S.D. Each value is mean of five determinations

Table: 4. Effect of NaCl salinity and zinc (10 ppm) on growth and development of *C. roseus* (L.)

Growth Parameter	Treatment				
	00 (Control)	25 mM NaCl + 10 ppm Zn	50 mM NaCl+ 10 ppm Zn	100 mM NaCl+ 10 ppm Zn	200 mM NaCl+ 10 ppm Zn
Shoot length (cm) (S)	50.10 (±1.50)	63.00 (±2.30)	61.00 (±2.54)	51.00 (±2.50)	40.20 (±2.12)
Root length (cm) (R)	19.64 (±0.25)	21.60 (±0.54)	21.80 (±1.09)	17.50 (±2.01)	19.50 (±1.15)
Total length (cm) (S+R)	69.74 (±0.85)	84.60 (±1.30)	82.80 (±1.52)	68.50 (±2.25)	59.70 (±1.66)
S/R	02.55	02.92	02.80	02.91	02.06
No. of leaves Plant ⁻¹	33.00 (±1.0)	40.00 (±3.0)	44.00 (±3.0)	29.00 (±2.0)	27.00 (±3.0)
Leaf area (cm ²) Plant ⁻¹	310.76 (±3.51)	614.04 (±5.31)	698.15 (±5.31)	322.22 (±3.37)	280.26 (±3.53)

± S.D. Each value is mean of five determination

Table: 5. Effect of NaCl salinity and zinc (25 ppm) on growth and development of *C. roseus* (L.)

Growth Parameter	Treatment				
	00 (Control)	25 mM NaCl + 25 ppm Zn	50 mM NaCl+ 25 ppm Zn	100 mM NaCl+ 25 ppm Zn	200 mM NaCl+ 25 ppm Zn
Shoot length (cm) (S)	50.10 (±1.85)	58.00 (±1.50)	56.00 (±0.95)	47.00 (±0.87)	38.10 (±0.90)
Root length (cm) (R)	19.64 (±0.50)	23.50 (±0.45)	22.75 (±0.80)	17.20 (±0.91)	15.60 (±1.25)
Total length (cm) (S+R)	69.74 (±1.20)	81.50 (±1.25)	78.75 (±0.87)	64.20 (±0.90)	53.70 (±0.96)
S/R	02.55	02.46	02.46	02.73	02.44
No. of leaves Plant ⁻¹	33.00 (±2.0)	38.00 (±2.0)	42.00 (±3.0)	24.00 (±2.0)	19.00 (±3.0)
Leaf area (cm ⁻²) Plant ⁻¹	310.76 (±1.33)	620.92 (±3.54)	599.59 (±3.40)	277.19 (±2.30)	170.59 (±1.54)

± S.D. Each value is mean of five determinations

Table: 6. Effect of NaCl salinity and zinc (50 ppm) on growth and development of *C. roseus* (L.)

Growth Parameter	Treatment				
	00 (Control)	25 mM NaCl + 50 ppm Zn	50 mM NaCl+ 50 ppm Zn	100 mM NaCl+ 50 ppm Zn	200 mM NaCl+ 50 ppm Zn
Shoot length (cm) (S)	50.10 (±1.23)	50.00 (±1.50)	49.92 (±1.10)	36.00 (±0.95)	30.20 (±1.30)
Root length (cm) (R)	19.64 (±0.57)	23.00 (±0.60)	20.20 (±0.70)	20.00 (±0.47)	12.60 (±0.58)
Total length (cm) (S+R)	69.74 (±0.94)	73.00 (±0.96)	70.12 (±1.00)	56.00 (±0.72)	42.80 (±0.93)
S/R	02.55	02.17	02.47	01.80	02.39
No. of leaves Plant ⁻¹	33.00 (±2.0)	30.00 (±2.0)	36.00 (±3.0)	18 (±3.0)	14.00 (±2.0)
Leaf area (cm ⁻²) Plant ⁻¹	310.76 (±2.40)	571.34 (±5.60)	529.15 (±3.47)	145.90 (±3.71)	98.08 (±3.10)

± S.D. Each value is mean of five determinations

Even though salinity adversely affect growth in *Catharanthus roseus*, the interactive effect of Zn mitigated this effect especially lower doses of Zn i.e.5, 10, 25 ppm Zn. The highest shoot length recorded with 10 ppm Zn with 25 mM NaCl and highest root length and total height of plant with 10 ppm Zn + 50 mM NaCl. The S/R ratio also noticed highest with 10 ppm Zn with 25 mM NaCl treatment. Similarly the number of leaves and leaf area per plant was noticed higher with all concentrations of Zn with 50 mM NaCl.[22]

Ramezani *et al.* (2012) have indicated a trend to alleviate effect of salinity by iron and zinc application. According to Hussein and Baker (2018) application of nano-Zn mitigate the adverse effect of salinity. Prasad and Saradhi (1995) found that Zn salts improve crop growth and ameliorate stress in *Brassica*. Zn and salinity shows synergic and positive effect on the root biomass of *Spartina* (Redondo-Gomez *et al.*, 2011). NaCl has decreased plant height and root length in mustard this

decrease is more in 200 mM NaCl concentration, however combined effect of NaCl and Zn has enhanced plant height (Ahmad *et al.*, 2017). Application of Zn mitigates the hazardous effect of salt and drought stress (Hezaveh *et al.*, 2019; Rostami *et al.*, 2019). Recently Muhammad Ali *et al.* (2021) noticed that exogenous application of Zn overcomes adverse effect of salinity and increased length, fresh wt. and dry wt. of shoot and root of okra. However the effect of various elements on growth performance of *Catharanthus roseus* is scanty. According to Sri Vasuki *et al.* (1980) all the micronutrients increased the height of *C. roseus*. However maximum growth in *C. roseus* was found in the combination of Zn + B. In the present investigation the interactive effect of NaCl and zinc enhance the growth parameters up to 50 mM NaCl+25 ppm Zn conc., while combination of higher conc. decreases the different growth parameters in *Catharanthus roseus*. Thus it is noticed that at lower conc. zinc mitigate the effect of NaCl salinity;

however it fails to do so at combination of higher concentrations.[25]

IV. SUMMARY AND CONCLUSION

The salinity has reduced length of shoot and root, the number of leaves and leaf area produced per plant. It is evident that NaCl salinity arrests the overall growth of *C. roseus*. This adverse effect of salinity is more significant at higher salinity levels (100 and 200 mM) as compared to lower salinity levels. However, shoot: root ratio increases with increasing salinity levels. It can be concluded that *C. roseus* gets adjusted to lower salinity regimes (up to 50 mM NaCl). However, higher salinity seems to be detrimental for this species.

The effect of different concentration of zinc varies with plant organs. The shoot length and root length increased with Zn especially lower Zn concentration i.e. 5, 10 ppm. Similarly S/R ratio increases against control, highest ratio is observed by 10 ppm Zn concentration. The leaf area and number of leaves per plant are also increased mostly by the 10 ppm Zn treatment but 50 ppm Zn conc. affects these growth parameters adversely.

The interactive effect of Zn mitigated the adverse effect of salinity especially lower doses of Zn i.e.5, 10, 25 ppm Zn. The highest shoot length recorded with 10 ppm Zn with 25 mM NaCl and highest root length and total height of plant with 10 ppm Zn + 50 mM NaCl. The S/R ratio also noticed highest with 10 ppm Zn with 25 mM NaCl treatment. Similarly the number of leaves and leaf area per plant was noticed higher with all concentrations of Zn with 50 mM NaCl.

In the present investigation the interactive effect of NaCl and zinc enhance the growth parameters up to 50 mM NaCl+25 ppm Zn conc., while combination of higher conc. decreases the different growth parameters in *Catharanthus roseus*. Thus it is concluded that at lower conc. zinc mitigate the effect of NaCl salinity; however it fails to do so at combination of higher concentrations.

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