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Exogeneous application of *Moringa oleifera* leaf extract and Salicylic acid on modulation of Salinity Stress effects in Pea (*Pisum sativum*) plants

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ABSTRACT

Soil salinity, a scourge and severe impediment that have negative effects in agriculture production, is a serious and environmental global problem. The present world population is 7.7 billion and is expected to cross 9.6 billion by the end of year 2050, this increase put enormous pressure in future on agriculture sector to get sustainable development. In the present study, the negative effect of salinization is ameliorated with to treatment of MLE (Moringa leaf extract) and Salicylic acid (SA), mitigating the deleterious effects of abiotic stresses. The synergistic effect of SA and MLE spray is studied on different parameters(seed germination, shoot/root length and fresh/dry weight) in Pea plants.The maximum increase is recorded in MLE (100%) and SA(100µM). Nearly 80% seeds germinated and the weight parameter shows excellent results with 20 fold increase in comparison to control.

Key words : *Exogeneous, Moringa oleifera, Stress effects*

Introduction

Agriculture is facing most remarkable global challenge in finding enough water to support the world's food needs. There is an ever-increasing consciousness for using sea water, an alternative, to irrigate crops. However, application of sea water cause salinity oxidative stress which negatively mitigate growth the 7% of global area land (Desoky *et al*., 2019; Zorb *et al*., 2019). *Moringa oleifera,* a natural foliar bio-stimulants, is rich in secondary metabolites (ascorbic acid, phenols), zeatin (natural cytokinin), vitamin A, B_2 and C, with antioxidant activity and osmo-protectant,which alleviate the endogeneous level of PGRs (plant growth regulators), making it a natural plant growth enhancer with positive effect on photosynthetic pigments and photochemical activity to overcame the osmotic stress salinity condition and significantly affected the prominent parameters (germination, plant height, fresh weight, dry weight and plays an essential role in crop growth and development (Makkar and Becker, 1996; Basra *et al*. 2011; Rady *et al*., 2013; Yasmeen *et al*., 2013; Amirigbal *et al*., 2014; Howladar, 2014; Osman and Abohassan, 2015; Rady and Mohamed, 2015 and Aluko 2016; Rehman *et al*., 2017; Muthalagu *et al*., 2018; Bagues *et al*., 2019; Bulgari *et al*., 2019; Desoky *et al*., 2019; Hernández, 2019). Besides *Moringa* extract, Salicylic acid (SA),a phenolic type endogeneous regulator,procured from *Salix* (willow) bark, modify and alleviate adverse effects of salinity stress in pea, barley, wheat, rice and sunflower (Khan *et al*., 2007; Arfan *et al*., 2007; Babar *et al*., 2014) and alleviates the inhibitory effect of salinity stress. Salicylic acid (SA), a phenolic growth regulator and a non-enzymatic

antioxidant,plays an important role in ion uptakeand membrane permeability (Simaei *et al*., 2011, 2012). The fresh water scarcity ,a serious global challenges, requires an alternative source for agriculture irrigation.Due to population increase from 7.2 to 9.6 billion by year 2050, the problem compounded and in order to cope with it to get sustainable development ,there is increasing awareness to utilize diluted sea water for crop irrigation to raise saline stress crops.Keeping this in view, the present studies is focused to ameliorate the adverse effects of salinity in Pea (*Pisum sativum*), a third most prominent legume vegetable, a rich source of proteins, sugars and vitamin supplements of man diet globally (Hussein *et al*., 2006) with the standardizing the concentration of *Moringa* leaf extract (MLE) and Salicylic acid (SA) to neutralize and nullify the sea water salinity effect.

Material and Methods

Plant Material

The present studies is conducted at Department of Botany, Post Graduate Govt. College for Girls, Sector-11, Chandigarh, to study the effect of Salicylic acid (SA) and *Moringa* leaf extract (MLE) to counteract the stress conditions during germination and subsequent development. The plant material used in the present studies is *Pisum sativum* (Fabaceae),an annual plant, is seeds which are uniformily(equal size)procured in morning hours from Sec-26, Grain market, Chandigarh and transferred to the physiological laboratory of the department. The geographic coordinate of the experimental area is 30.7333° N latitude and 76.7794° E 40° 20' 07" N longitude.

Sterilization Method

The procured uniform size seeds are first washed in running tap water with 5% teepol for 15 minutes which is followed by surface sterilization with solutions of Streptomycin (0.1%,20min), Sodium hypochlorite (4%, 15min) and dip in ethanol (70%, 3sec) before rinsing with sterilized double distilled water & inoculated on the Whatman filter paper 1 in falcon petri-dish irrigated with tap water. The sterilized seeds are washed with distilled water and air dried seeds are sown, after their soaking in distilled water.

Media and culture conditions

To study the effect of osmotic stress on germination and subsequent growth of various parameters in *Pisum sativum,* the effects of different combination of MLE and Salicylic acid alone and /or in combination with sodium chloride (1-3%). T_1-Control; T_2-1% NaCl; T_3-2% NaCl; T_4-3% NaCl; T_5-3%NaCl +10µM SA; T_6-3% NaCl+ 50%MLE; T_7- 3% NaCl+100µM SA; T_8-3% NaCl+10%MLE; T_9-3%NaCl+50µM SA; T_{10}-3%NaCl+100% MLE +100µM SA. The prefertigation medium pH was adjusted at 5.6. Sixteen replicates for each treatment and the experiments are repeated twice. All experimental manipulations are done under aseptic conditions and the cultures incubated at 25±2 ^{0}C under 12 hr photoperiod with 3500 lux light intensity.

Data recording

The petriplates inoculated with seeds are observed every week and their response to different combinations (T_1-T_{1o}) is recorded. For analyzing the time taken in weeks for study of growth and subsequent development of plant is studied under following parameters : seed germination, first leaf, shoot length, root length, dry weight and fresh weight of plant. The data is recorded on second day and subsequently on 7, 15, 30, 60 days. The data recorded is correlated and effects of the treatment are assessed. The other parameters are calculated as follows:

1. **Germination percentage** (GP %) = (Number of germinated seeds/Total number of seeds) × 100, according to (Scott *et al.,* 1984).

2**. Fresh and dry weight determination**: Sample of fresh weight of herb was measured and then ovendried at 70 °C for 48 h till constant weight to determine the dry weight. It has been expressed as (g).

Preparation of *Moringa* Leaf Extract (MLE)

Moringa oleifera, foliar extract is prepared from fresh leaves collected from the mature plant growing on the B-road, Sector-26, Chandigarh. The fresh leaves are air-dried, grinded and extracted. For extraction, the young leaves are grounded in ratio of (1:10) with one litre water and 10 kg fresh foliar material and subsequently sieved through whatman no.1 filter paper and diluted with distilled water to obtain a 50-90% concentration (v/v). The pea seeds are soaked in tap water and MLE using ratio of 1:5 (seed weight to solution volume) for 2hrs at 25°C

temperature followed with distilled water washing and re-dried overnight at room temperature. Before inoculation on petriplate in early morning, the treated seeds underwent foliar spray of MLE ,to facilitate foliar extract penetration, 0.1% Tween-20 surfactant is added .

Preparation of Salicylic acid(SA)

The concentration of salicylic acid used in the present study ar e10µM, 50µM and 100µM. The concentration of salicylic acid is prepared in the physiological laboratory in the standard conditions by dissolving 0.0585 mg of salicylic acid in 1 mL of distilled water and concentration results in 10µM. Likewise the other concentrations of 50 µM and 100µM are prepared respectively by dissolving 0.2925mg of salicylic acid in 1 ml of distilled water and 5.85mg of salicylic acid in 1ml of distilled water.

Salinity and SA treatment

The pea (*Pisum sativum*) plants are fortnightly subjected to osmotic salt stress with different salinity levels (NaCl; 1-3%). The weekly (7 days) periodical cycle is maintained subjected to saline irrigation water, however, control plants are irrigated with 0.5 L tap water in same cycle.

Results

(A) Effect of osmotic salt stress on % seed germination, shoot /root length and Fresh and dry weight

The salinity related osmotic stress hampered the growth characteristics(i.e seed germination,shoot/root length and Dry/fresh organic matter) of *Pisum sativum* in comparison to control(T_1), the salt treatment 1%, 2%, 3% NaCl significantly reduced the germination percentage by 20%, 30%, 40% respectively. The data reveal that the cumulative yield decreased by increasing the soil salinity level. However, the seeds maintained in non-salt treated (control) ,shows 100% germination with 7cm and 3.5 cm shoot and root length respectively (Fig. 1). The fertigation of pea seeds respectively with 1%, 2% and 3% NaCl salt treatment reduced the dry weight by 20%, 30% and 40%.

B. Effect of Moringa leaf Extract (MLE) alone and primed with Salicylic acid (SA) on% seed germination, shoot/root length and Fresh and dry weight

Presently, the beneficial effect of foliar application of Moringa leaf extract (MLE) significantly increased all growth parameters(seed germination percentage, shoot /root length and dry/fresh weight of pea plant) compared to contol(T_1).The synergistic effect of MLE is observed when primed with different concentrations of salicylic acid and 3% salt stress(NaCl), 80% seeds sprouted under combined treatment with SA(100 µM), however, when used alone, MLE shows an decrease in rate of germination was recorded as 60%. The highest increase is recorded at 100 µM and 100% concentration respectively of SA and MLE. However, when Salicylic acid is used at lower concentration (10µM, 50µM), the seeds shows wrinkled appearance with chlorophyll degradation. Average length of shoot of salt treated control plants is 1.7cm which increased

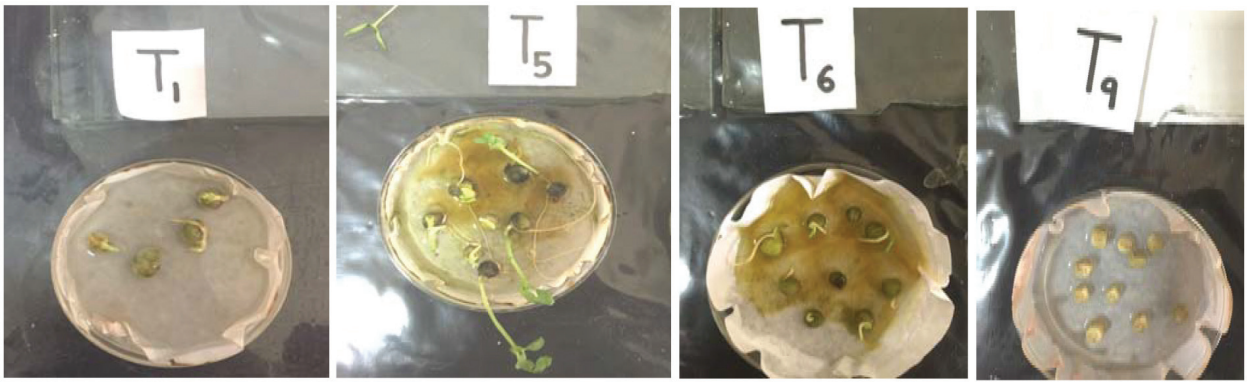


Fig. 1. Depicts the germination in control treatments
Fig. 2. Seedling development in NaCl(3%) + SA(10µM)
Fig. 3. Seed germination in T_6-3%NaCl+ 50%MLE Alleniating salinity stress
Fig. 4. Seed wrinkles,discolouration in NaCl(3%)+ SA(50µM)

to 5.7cm when 100µM SA treatment was applied. The shoot length and root length is respectively at 3.78, 4.23, 7.00 cm and 6cm, 8cm, 10cm with MLE at 10%, 50%, 100% concentrations (Fig. 3). The weight parameters in the combined treatment (100%MLE; 100µM SA) shows excellent results and recorded increased cumulative fresh weight (1.6gms) and dry weight (0.2gms) after 21 days, showing approximately 20 fold increase in comparison to the control.

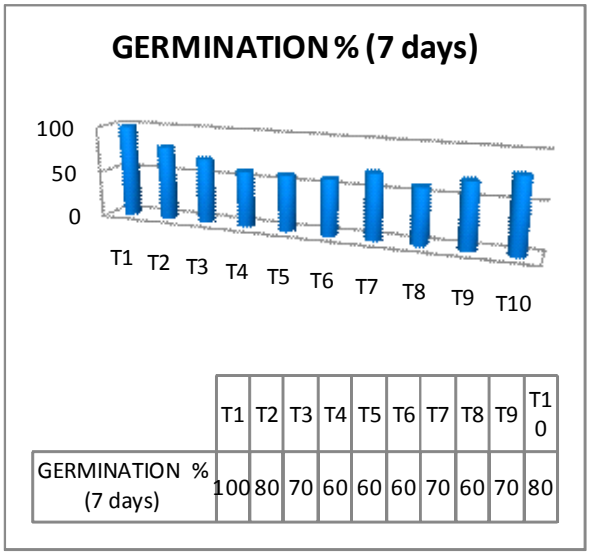
Effect of Salicylic acid (SA) on % seed germination, shoot /root length and Fresh &dry weight

The effect of salicylic acid is observed on rate of germination percentage when seeds are primed with different concentrations of salicylic acid (10µM, 50µM, 100µM) with salt(NaCl;1-3%) concentration. The SA mitigated, abridged and have alleviates the inhibitory effect of salinity, significantly increase germination response. A dynamic increase in shoot length is seen after the use of Salicylic acid (Fig.2), however when Salicylic acid (50µM) is used in the solution enriched with 3% NaCl, results in the appearance of wrinkles an discolouration of seeds which results in the hampering of germination frequency (Fig. 4). The result showed that pea seedlings are severely influenced by salt stress, with decrease in shoot length 7 cm to 0.8cm under control conditions. The antagonistic impacts of salinity were decreased by salicylic acid. The shoot lengths recorded were 2.1, 3.1 and 5.7 cm with 10µM, 50µM and 100µM concentration of Salicylic acid, respectively. In comparison to shoot, the most prominent increase in root length (5.7 cm) is seen at 100µM salicylic acid concentration under saline conditions, however, root lengths are recorded 4.5 cm for 10µM 4.8cm for 50µM and 100µM when compared with 1.7cm (3% NaCl).

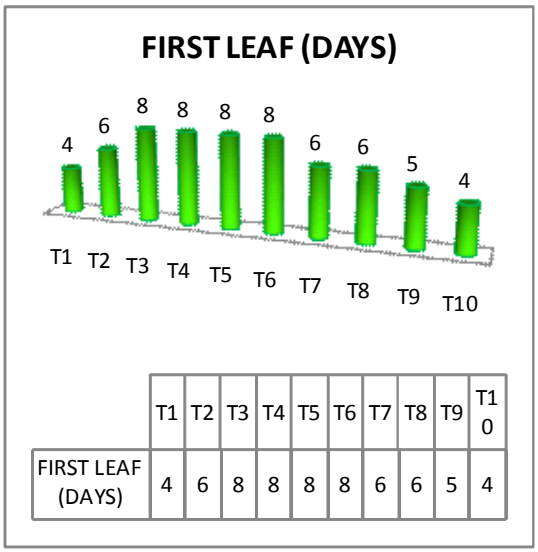
In the combination containing 100µm SA in 3% NaCl solution, results in 0.88 gms and 0.11 grams fresh and dry weight respectively after 21 days.

Discussion

]In the present study, the negative effect of salt salinity stress in reduction of growth and productivity of pea plants is attributed to increase specific ion toxicity, osmotic stress and nutritional imbalance, which causes the increase of growth inhibitors (Abscisic acid), which lead to stomatal closure, accumulation of toxic ions (Rady *et al*., 2015). The negative effect is ameliorated with the integrated application of MLE (Moringa Leaf Extract) and Salicylic acid (SA). The alleviated response in recuperating the growth characteristics, is attributed to the protection of photosynthetic pigments from salt stress related oxidative damage due to their antioxidant characteristics and enhanced parameters (total carbohydrate, soluble sugars, proline), which protect plants against membrane injury and ameliorate the adverse effects of sea water irrigation created soil salnity. Furthermore, the *Moringa* leaf extract (MLE), is a rich source of natural cytokinin (zeatin) which a scavenge free radical preventing purine breakdown and enhanced mobilization of germination related metabolites/inorganic solutes such as zeatin, ascorbic acid, Ca and K to the growing plu-

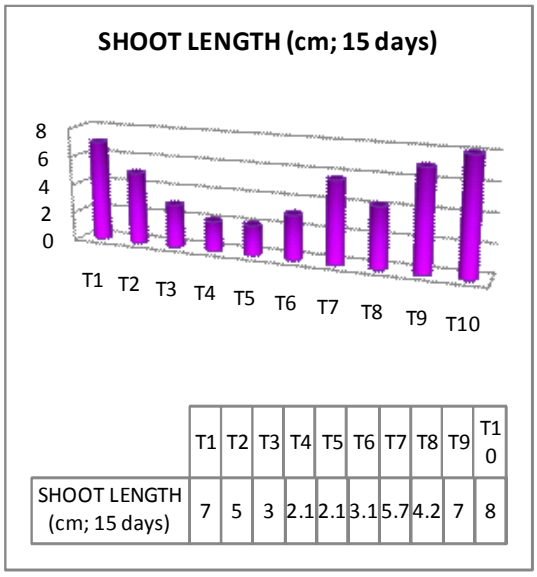


	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
GERMINATION % (7 days)	100	80	70	60	60	60	70	60	70	80

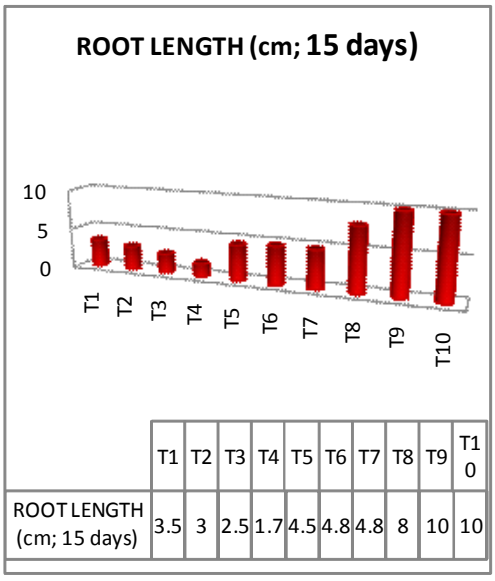


	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
FIRST LEAF (DAYS)	4	6	8	8	8	8	6	6	5	4

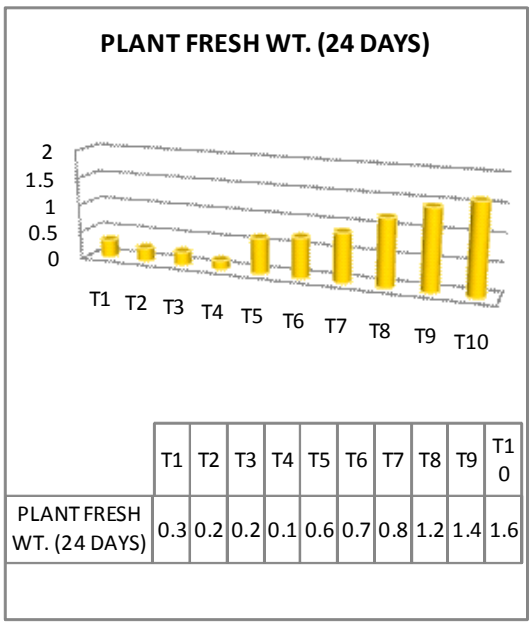
mule and amylase activity contributing to early vigor and preventing pre-mature leaf senescence, hence its effectiveness in mitigating the salinity stress (Afzal *et al*., 2012; Howladar, 2014; Rady *et al*., 2015; Merwad, 2017; El-Sayed *et al*., 2017; Bulgari *et al*., 2019; Desoky *et al*., 2019) with increased germination percentage in pea plants in present studies, in compliance with earlier studies in beans, cowpea, *Eryngium foetidum* Maize, pepper, tomato (Azooz *et al*., 2004; Iftikhar, 2009; Phiri and Mbewe, 2010; Wahid and Farooq, 2012; Mozumder and Hossain, 2013; Bashir *et al* 2014; Muhammad, 2014; Aluko, 2016; Rehman *et al*., 2017; Abusuwar and Abohassan, 2017; Hala and Nabila, 2017). In osmotic stress, it is observed that seeds are discoloured due to due to degradation of chlorophyll and its biosynthesis reduction under salinity stress condition. On the other hand, Salicylic acid (SA) act as important signal molecule for modulating plant responses, mitigating the deleterious effects of some environmental stresses in pea plants,in compliance to earlier studies in Maize (Misra and Saxena, 2009; Parida and Das, 2005; Hassan *et al*., 2017). Pre-treating pea plants with *Moringa oleifera* leaf extract



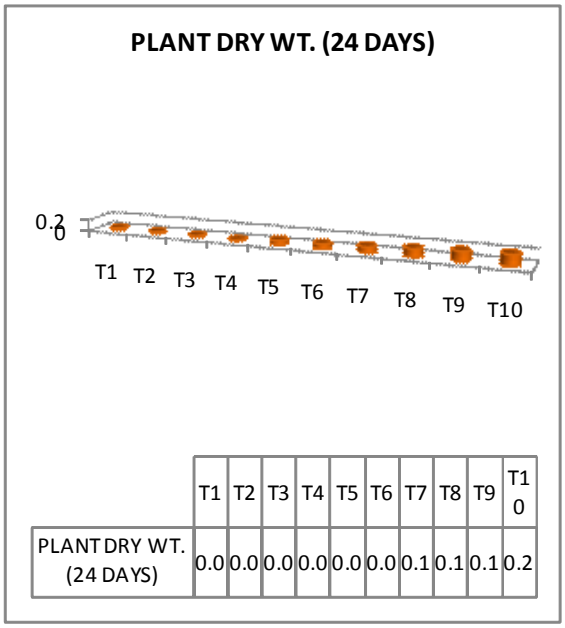
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
SHOOT LENGTH (cm; 15 days)	7	5	3	2.1	2.1	3.1	5.7	4.2	7	8



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
ROOT LENGTH (cm; 15 days)	3.5	3	2.5	1.7	4.5	4.8	4.8	8	10	10



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
PLANT FRESH WT. (24 DAYS)	0.3	0.2	0.2	0.1	0.6	0.7	0.8	1.2	1.4	1.6



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
PLANT DRY WT. (24 DAYS)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2

(MLE; 100%) and/or salicylic acid (SA; 50µM), used as seed soaking and/or foliar spray, significantly improved plant growth characteristics and plant productivity as well as physio-chemical attributes under the adverse conditions of the studied soil salinity. The increased chlorophyll concentration of SA-treated *Pisum sativum* leaves attributed to the influence of SA on endogenous cytokinin (zeatin) contents, which may increase further after natural zeatin-contained MLE application, results in enhanced chloroplast differentiation, chlorophyll biosynthesis, and prevented chlorophyll degradation. The perusal of literature reveals that the many fold increase in the weight parameters in cumulative treatment (MLE100; 100µM SA) is in compliance with earlier studies in eggplant, peanut, soya beans, sorghum and tomato, *Vicia faba* (Palada, 1996; El Tayeb, 2005; Horvath *et al*., 2007; Culver *et al*., 2012; Rady *et al*., 2013; Ozobia, 2014; Merwad, 2017; Rehman *et al*., 2017; Rady *et al*., 2017; Desoky *et al*., 2019). However a variable concentration (MLE 30) is required in Wheat, which depicts the optimum concentration of MLE varies with genera (Yasmeen *et al*., 2013).

Conclusion

The world population is increasing at an alarming rate and expected to reach about 9.6 billion by year 2050. In order to get sustainable development and cope with fresh water scarity, the present study investigated the effect of integrated application of natural growth stimulants(MLE and SA) used as foliar spray and/or seed soaking to neutralize and mitigate the sea water irrigation oxidative stress and to bridge the illusion that the sea water is used for irrigation.

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