SUMMARY

Salinity is a major abiotic stress problem distributed in arid and semi arid areas of the world agricultural land. It is a global problem, about 900 million hectares of land throughout the world, is estimated to be affected by salinity and is increasing every year across the globe. Seventy eight percent of the soil in India is affected by salt out of which 22% land is in the central region (Madhya Pradesh and Maharashtra).

High salinity affects the plant growth in several ways- water stress, ion toxicity, nutritional disorders, oxidative stress all these factors affects the growth, development and survival of plants. Salinity has been reported to affects all the morphological, biochemical and molecular parameters of plant growth that includes photosynthesis, protein synthesis, carbohydrate, proline accumulation and lipid peroxidation. It has been predicted to be a major problem in coming decades, which significantly affects the agricultural land and agricultural production worldwide.

During exposure to salinity, plants initially face water stress, which reduces expansion of leaves. After salt application the osmotic effects of salinity stress can be observed immediately in the form of inhibition of cell expansion and division as well as stomatal closure. The ionic stress arises in plants on exposure to salt stress for long term, which leads to premature senescence of leaves and reduce the photosynthesis process. High sodium concentration leads to disruption of protein synthesis and enzyme activity. Excessive accumulation of sodium in the cells of plants leads to osmotic stress and ultimately causes cell death.

The most sensitive stage in plant growth to salinity is the seedling stage. Seedling growth parameters such as percentage of germination, shoot and root length, fresh and dry weight and chlorophyll content was reported to decrease in various crop plants.
including Vigna, Cotton, Sorghum, Chick pea, Faba bean, Fenugreek, Dry bean and Cowpea.

Salinity known to generate lipid peroxidation in plants which in turn responsible for induction of oxidative damage in plants which occur as reactive oxygen species (ROS) in cells and starts ageing of the plants ultimately leading to the death of the plants life.

Rhizobacteria present in the rhizosphere promote nitrogen fixation and regulates metabolic activities of plants and reduce the formation of ROS and promote its removal to avoid harmful effects of salinity and increases the productivity of plants. Rhizobacteria enhanced the growth and productivity of plants by increasing plant height, leaf size, root area, nutrient content, chlorophyll content, increased nodulation, also the bacteria produced plant growth hormones for the promotion of plant growth and productivity. Rhizobacteria known to increase the synthesis of osmolyte proline to maintain osmotic balance in plants subjected with salinity and water stress.

The symbiotic system of Cowpea and Rhizobacteria can positively affects the soil quality and fertility. Plant productivity may improve under salt stress conditions with the right combination of salt tolerant rhizobia with host plant. Very few reports are available on conferring tolerance to salt stress of plant with rhizobia. Tolerance against salinity is important in establishment of Cowpea in saline stressed conditions; therefore this work has been done for investigation of the role of Rhizobacteria on the growth of Cowpea plant Variety-Pusa Sukomal and RC101 under different level of salt stressed condition.

Cowpea is a widely cultivated crop in arid and semi arid regions where the salinity problem is especially acute and vulnerable. Because of the high level of protein
present in this crop it is used as maintaining the balance of nutrition of low income people. Cowpea associate with symbiotic Rhizobacteria which increases the soil fertility makes it important. Cowpea is cultivated by commercial and subsistence farmers in India. Cowpea can be used at all stages of growth as a vegetable crop.

For this study we have taken the following objectives these are collection and growing of plants in controlled conditions, growth parameters measurement, photosynthetic pigments, protein extraction, lipid peroxidation, proline and carbohydrate estimation in control as well as salinity stressed plants.

Firstly we have collected the Cowpea plant variety-Pusa Sukomal and Rhizobacterial strains BR2 and BR3 from IARI Pusa, New Delhi, RC101 variety from Agriculture University, Gwalior. Rhizobacteria were maintained on YEMA medium.

Growth and biochemical parameters such as germination percentage, shoot and root lengths, fresh and dry weight, quantitative analysis of Carbohydrate, Proline, Lipid peroxidation (MDA), Protein and qualitative analysis of proteins were studied at 7 and 15 days old seedlings. Agronomic parameters such as fresh and dry weights were recorded at 30 and 90 day old field grown plants.

Growth pattern of Rhizobacterial cultures BR2 and BR3, on YEM broth were assessed (Figure 1).

Inoculum from 14 days old cultures of Rhizobacterial strains was transferred to YEM broth with different NaCl salt (0, 25, 50 and 75mM) levels. The Rhizobacterial strain BR3 has shown increased growth under NaCl saline stress compared to BR2 suggesting its more salinity resistance compared with BR2 strain.
The role of *Rhizobacterium* on Cowpea seed germination in saline conditions was studied at 7 and 15 day old seedlings, both by analyzing physico and biochemical parameters such as % germination (at 7th day), shoot and root length, fresh and dry weight of the seedlings. In biochemical studies we analyzed qualitatively carbohydrates, chlorophyll pigments content-Chlorophyll a (*Chl* a), chlorophyll b (*Chl* b), carotenoids (*caro*), total Chlorophyll (*t Chl*), proline and MDA (lipid peroxidation) and protein content. All experiments were carried out using Cowpea variety Pusa Sukomal. The percentage germination in absence of *Rhizobacterium* has reduced from 96.67% (25mM) to 89.33% with increase in salt concentration (Table 1). When the seeds were inoculated with *Rhizobacterium* BR2 and BR3 before exposing NaCl solution, 100% germination was recorded in control as well as in 25mM NaCl water, which has reduced to 90.67% (BR2) and 94.67% (BR3) when the NaCl concentration was increased 75mM (Table 1 and Figure 4).

The shoot length of 7 day seedlings in control recorded 22.64cms and when the seeds were exposed to different concentrations of NaCl salinity they recorded 21.12±1.07cms (25mM), 15.52±1.18 (50mM) and 12.40±1.28cms (75mM) shoot length. When the seeds were inoculated with *Rhizobacterium* BR2 strain 7 day old seedlings gained 27.48±1.33cms, 23.52±0.71cms, 18.16±1.39cms and 12.44±1.03cms of shoot length and with BR3 strain inoculation seedlings of 7 days gained 29.76±1.37cms, 25.28±0.94cms, 21.50±0.65cms and 12.30±0.54cms shoot length at 0 (control), 25, 50 and 75mM NaCl concentrations respectively (Table 2). While 15 day old seedlings recorded 28.20±1.23, 24.10±1.15, 22.50±1.48 and 18.90±1.13cms shoot length in the absence of *Rhizobacterium*. On application of *Rhizobacterium* strain BR2, 15 days old seedlings of Pusa Sukomal measured 31.20±1.65, 27.50±1.55, 28.30±1.89, and
23.50±1.35 cms shoot length and on BR3 strain inoculation 33.70±1.18, 30.30±1.41, 28.50±1.44 and 24.80±0.63 cms shoot length respectively under 0, 25, 50 and 75mM NaCl salinity (Table 2).

Root length results of 7 day old seedlings of Pusa Sukomal (compiled in Table 3 and Figure 6) without *Rhizobacterium* inoculation was 11.36±0.53, 9.70 ± 0.49, 7.04 ± 0.43 and 5.24± 0.44 cms respectively. With *Rhizobacterium* BR2, root length was 12.20±0.40, 10.44±0.74, 9.12±0.74 and 8.72±0.85 cms and with BR3 the 7 day seedlings recorded 13.08±0.40, 12.70±0.30, 10.92±0.65 and 9.68 ± 0.80 cms root length under 0, 25, 50 and 75mM NaCl saline conditions.

Roots in 15 day old seedlings germinated without *Rhizobacterium* inoculation in non saline water (0mM) gained 14.80 cms length and on exposure to NaCl salinity, length of roots was 12.80, 12.30 and 11.80 cms. When the seeds were applied with *Rhizobacterium* before germination, seedlings showed 15.10, 13.20, 13.10 and 11.20 cms with BR2 strain and 16.40, 14.60, 13.30 and 11.20 cms with BR3 in 0, 25, 50 and 75mM NaCl salinity waters respectively (Table 3 & Figure 8).

Fresh and dry weight of 7 and 15 day old seedlings of Cowpea var. Pusa Sukomal was measured after exposure to NaCl, with or without *Rhizobacterium* (BR2 and BR3) inoculation. Control seedlings, under non saline conditions without *Rhizobacterium*, gained 7.58g in 7 days and 11.56g in 15 days. When the seeds were grown in NaCl salinity, 6.50g (25mM), 4.96g (50mM), 2.54g (75mM) fresh weight. The 15 day seedlings recorded 9.39g (25mM), 8.11g (50mM) and 5.92g (75mM) fresh weight (Table 4).

With BR2 *Rhizobacterium*, 7 day old seedling showed 10.14g, 8.12g, 5.59g and 4.09g fresh weight and 15 days old seedlings showed 13.59g, 12.15g, 10.17g, and 9.57g
fresh weight at 0 (control), 25, 50 and 75mM NaCl concentrations respectively (Table 4). Similarly in BR3 Rhizobacterium presence, 7 day old seedlings under 0, 25, 50 and 75mM concentration recorded 11.05g, 9.18g, 6.25g and 4.51g fresh weight respectively. While 15 day old seedling attained 14.33g (0mM), 13.17g (25mM), 11.39g (50mM) and 9.36g (75mM) fresh weight (Table 4 and Figure 9).

Pusa Sukomal seedlings of 7 days old in the absence of Rhizobacterium, showed 0.92g, 0.82g, 0.72g and 0.39g dry weight respectively under control (0 NaCl) and at 25, 50 and 75mM NaCl concentrations. With BR2 the dry weight was 1.28g (control) 1.06g (25mM), 0.99g (50mM) and 0.81g (75mM). On application of Rhizobacterium strain BR3, the dry weight was 1.46g (control), 1.25g (25mM), 1.09g (50mM) and 0.86g (75mM) (Table 5 and Figure 10).

Seedlings of 15 day old gained 1.37g dry weight under 0mM NaCl salinity. At 25mM NaCl concentration seedlings recorded 1.25g at 25mM, 1.07mM at 50M and 0.86g at 75mM concentration (Table 5).

On application of Rhizobacterium BR2, the dry weight of the 15 day old seedlings was 2.01 g, in control, 1.49g in 25mM, 1.33g in 50M and 1.18g in 75mM NaCl salinity. When BR3 Rhizobacterium was applied 15 day old seedlings under 0, 25, 50, and 75M salinity levels gained 2.08g, 1.55g, 1.43g and 1.16g dry weight respectively.
Biochemical Parameters

The carbohydrate content in control, without rhizobacterial inoculation was 0.799 mg/gfw in 7 day and 1.019 mg/gfw in 15 day old seedlings. In the presence of rhizobacterial strains BR2 and BR3, the carbohydrate content was 0.942 mg/g and 1.166mg/g respectively in 7 days seedlings and 2.424 mg/g (BR2) and 3.509 mg/g (BR3) respectively (Table 6). Without *Rhizobacterium* inoculation, the carbohydrate concentration was 2.211mg/g, 2.838mg/gfw and 6.256mg/gfw in 7 days and 5.640mg/g, 6.748mg/g and 7.761mg/gfw in 15 days old seedlings under 25, 50 and 75 mM NaCl salinity.

With *Rhizobacterium* strain BR2 and BR3 treatment, the estimated carbohydrate content in 7 days old seedlings was 2.270mg/gfw and 2.647mg/gfw (25mM), 3.047mg/gfw (BR2) and 3.480mg/gfw (BR3) (50mM), 6.256mg/gfw and 8.260mg/gfw in 75mM NaCl salinity conditions. Similarly, the content of carbohydrate in 15 days old seedlings was 5.640mg/gfw and 6.642mg/gfw under 25mM salinity, 7.555mg/gfw (BR2) and 7.826mg/gfw (BR3) in 50mM salinity, 7.808mg/gfw and 10.452mg/gfw in 75mM salinity conditions respectively (Table 6).

Our results show that the carbohydrate content has increased with increasing concentration of NaCl. Further, the Rhizobacterial inoculation has enhanced the carbohydrate level as compared to control. Strain BR3 has shown significant increase in carbohydrate content compared with BR2 and control. This effect is most significant at 75mM NaCl concentration.
Photosynthetic pigments- Chlorophyll a (Chl a), Chlorophyll b (Chl b), total chlorophyll (t Chl) and carotenoids (caro) content in 7 and 15 days old seedlings was analyzed in presence and absence of Rhizobacterium under different concentrations of NaCl salinity stress. The data has been presented in the Table 7.

Control 7 day seedlings contained 192.330µg/gfw (Chl a), 89.272µg/gfw (Chl b), 56.062µg/g fw (Caro) and 311.914µg/g fw (t Chl). Whereas the 15 day seedlings contained 313.095 (Chl a), 281.018µg/gfw (Chl b), 641.944µg/gfw (t Chl) and 64.644µg/gfw (Caro). On exposure to salinity, the amount of Chl a in 7 days old seedlings was 155.030µg/gfw, 143.602µg/gfw and 80.603µg/gfw, the Chl b was 68.459µg/gfw, 52.355µg/gfw and 55.655µg/gfw, total chlorophyll content was 247.367µg/gfw, 216.324µg/gfw and 151.851µg/gfw and the carotenoids concentration was 51.330µg/gfw, 35.544µg/gfw and 24.723µg/gfw in 7 day old seedling raised in 25, 50 and 75mM NaCl salinity levels (Table 7). Similarly the carotenoids concentration was 58.319µg/gfw, 40.612µg/gfw and 37.648µg/gfw in seedlings raised in 25, 50 and 75mM NaCl salinity levels at 15 day.

The co culture of BR2 has shown increase in the levels of Chl a (241.438 / 354.859µg/gfw), Chl b (116.422 / 250.984µg/gfw), total chlorophyll (396.601 / 641.525µg/gfw) and carotenoids (72.335/ 79.722µg/gfw) in 7 days and 15 days old seedlings respectively compared to control under non saline conditions (Table 7). On exposure to 25mM salinity stress, the pigments levels were 178.312µg/gfw and 297.960µg/gfw (Chl a), 93.468µg/gfw and 147.968µg/gfw (Chl b), 301.577µg/gfw and 458.669µg/g (t Chl) and 50.108µg/gfw and 68.404µg/gfw (Caro) in 7 and 15 days old seedlings respectively (Table 7).
At 50mM NaCl salinity, the BR2 inoculation resulted 131.898µg/gfw (Chl a), 89.371µg/gfw (Chl b), 246.516µg/gfw (t Chl) and 35.393µg/gfw (Caro) in 7 days and 275.695µg/g (Chl a), 190.018µg/gfw (Chl b), 492.138µg/gfw (t Chl) and 53.608µg/gfw (Caro) in 15 days old seedlings. Similarly, at 75mM NaCl exhibited 129.840µg/gfw and 252.495µg/gfw of Chl a, 95.674µg/gfw and 130.959µg/gfw of Chl b, 251.586µg/gfw and 395.777µg/g fw of t Chl and 33.231µg/gfw and 38.657µg/gfw of Caro in 7 days and 15 days old seedlings respectively.

The co culture of Rhizobacterium strain BR3 recorded Chl a content was 237.289µg/gfw, 229.176µg/gfw, 216.793µg/gfw and 213.141µg/gfw in 7 days and 421.994µg/gfw, 392.701µg/gfw, 348.330µg/gfw and 324.543µg/gfw in 15 days old seedlings under 0, 25, 50, 75mM NaCl salinity stress. The Chl b content was 122.908µg/gfw, 114.388µg/gfw, 102.708µg/gfw and 92.464µg/gfw in 7 days old seedlings and 359.519µg/gfw, 312.454µg/gfw, 252.992µg/gfw and 154.183µg/gfw in 15 days old seedlings irrigated with 0, 25, 50 and 75mM NaCl solution respectively (Table 7). The total chlorophyll content in 7 days seedlings was 399.617µg/gfw, 380.952µg/gfw, 353.998µg/gfw and 338.172µg/gfw respectively and in 15 days old seedlings it was 840.642µg/gfw, 754.140µg/gfw, 638.143µg/gfw and 490.688µg/gfw respectively in 7 days and 15 days old seedlings grown under 0, 25, 50 and 75 mM NaCl stress. The amount of carotenoids was 89.521µg/gfw and 92.701µg/gfw (0mM), 70.044µg/gfw and 69.661µg/gfw (25mM), 61.979µg/gfw and 67.627µg/gfw (50mM) and 54.773µg/gfw and 76.595µg/gfw (75mM) respectively (Table 7).

The mean proline content in 7 days old seedlings without Rhizobacterial treatment was 177.495µg/gfw, 345.683µg/gfw, 475.974µg/gfw and 599.358µg/gfw in 0, 25, 50 and 75M NaCl salinity. In 15 days old seedlings the proline content was
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168.380µg/gfw, 231.847µg/gfw, 362.665µg/gfw and 566.305µg/gfw under 0, 25, 50 and 75mM NaCl salinity concentrations (Table 8).

With inoculation of BR2 strains the proline content increased to 286.007µg/gfw, 553.209µg/gfw and 613.365µg/gfw in 0, 50 and 75mM NaCl concentration in 7 day old seedlings. The 15 days seedlings contained 204.503µg/gfw, 562.563µg/gfw and 581.848µg/gfw in control, 50 and 75mM NaCl salinity conditions.

The levels of proline in presence of *Rhizobacterium* recorded as 553.209µg/gfw (BR2) and 564.722µg/gfw of plant (BR3) in 7 days seedlings and 562.563µg/gfw (BR2) and 566.305µg/gfw (BR3) in 15 days seedlings growing in 50mM NaCl salinity stress conditions. Similarly at 75mM NaCl salinity stress the concentration of proline was 613.365µg/gfw (BR2) 613.557µg/gfw (BR3) in 7 days seedlings and 581.848µg/gfw (BR2), 609.336µg/gfw (BR3) in 15 days old seedling (Table 8).

The level of MDA in 0mM salinity at 7days old seedlings without Rhizobacterial treatment was 117.887µg/gfw, 124.524µg/gfw, 159.606µg/gfw and 200.377µg/gfw in 0, 25, 50 and 75mM NaCl Salinity. In 15 days old seedlings the MDA content was 115.675µg/gfw in 0mM NaCl Salinity (Table 9). At 25mM, 50mM and 75mM the level of MDA was 137.482µg/gf, 184.574µg/gf and 306.887µg/g respectively (Table 9).

With inoculation of Rhizobacterial strains- BR2 and BR3, the MDA content was reduced to 106.509µg/gfw and 91.339µg/gfw at 7 day of germination and 113.146µg/gfw and 104.929µg/gfw at 15th day of germination respectively (Table 9). At 25mM, 50mM and 75mM NaCl concentration with BR2 the MDA content was 113.146µg/gfw, 130.529µg/gfw and 195.636µg/gfw in 7 days and 127.369µg/gfw, 187.103µg/gfw and 298.985µg/gfw in 15 days old seedlings inoculated with BR2. With BR3 strain the MDA levels were 107.141µg/gfw (25mM), 117.571µg/gfw (50mM) and
192.16µg/gfw (75mM) recorded in 7 days and in 15 days old seedlings the level MDA were recorded 118.835µg/gfw (25mM), 176.989µg/gfw (50mM) and 220.604µg/gfw (75mM) respectively (Table 9).

The mean protein content in 7 days old seedlings without Rhizobacterial treatment was 116.22µg/gfw, 152.00µg/gfw, 168.22µg/gfw and 122.89µg/gfw in 0, 25, 50 and 75mM NaCl salinity. In 15 days old seedlings the protein content was 149.78µg/gfw, 127.11µg/gfw, 174.22µg/gfw and 131.56µg/gfw under 0, 25, 50 and 75mM NaCl salinity concentrations (Table 10).

The protein content with inoculation of BR2 strains recorded 126.22µg/gfw, 136.67µg/gfw and 139.78µg/gfw in 0, 25mM, 50mM and 75mM NaCl concentration in 7 day old seedlings. The 15 days seedlings contained 122.67µg/gfw, 137.11µg/gfw and 165.11µg/gfw and 107.56µg/gfw in 0mM, 25mM, 50mM and 75mM NaCl salinity conditions.

The levels of protein in presence of Rhizobacterium BR3 strains 136.00µg/gfw (0mM) 112.00µg/gfw (25mM), 117.56µg/gfw (50mM) and 141.18µg/gfw (75mM) in 7 days seedlings. Similarly with inoculation of BR3 strain at 15 days old seedlings protein content were recorded 95.11µg/gfw (0mM), 126.00µg/gfw (25mM), 150.67µg/gfw (50mM) and 118.89µg/gfw (75mM) respectively (Table 10).

Seed germination experiment was done with the prior coating of the Cowpea seeds with Rhizobacterial stains culture and allowed to grow for 7 and 15 days. At 7 day the seed germination and at 7 and 15 day the plant height-shoot, root length, fresh and dry weight were recorded. Two sets were prepared for this experiment one was inoculated and another was non-inoculated with Rhizobacterial strains. In biochemical studies we analysed the photosynthetic pigments, protein extraction and determination.
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Biochemical parameters at 7 and 15 days old seedlings- carbohydrate has increased with increasing salinity. The Rhizobacterial strain enhanced the carbohydrate content in control as well as salinity stressed plants. Chlorophyll content recorded significant progressive decline with increasing concentration of NaCl stress while Rhizobacterial inoculated seeds shown to increased in chlorophyll content. Proline content was increased with increasing concentration of NaCl, with inoculation of Rhizobacterial the further increment was shown in proline content in control as well as salt stressed plants at both stages of seedlings. Proline serves as osmoprotectant in saline stressed conditions. Salt stress known to result in extensive lipid peroxidation. In the process of lipid peroxidation the MDA content level can be considered as an indicator of oxidative damage of plants. MDA content was increased with increasing concentration of NaCl but when inoculated with Rhizobacterial strains it reduced the level of MDA at both stages of seedlings. In plant tissue MDA level is being extensively used as efficient criteria to discriminate crop cultivars in respect to salt stress tolerance. Protein content increased up to 50mM NaCl and thereafter it decreased at 75mM NaCl. The level of protein high in the Rhizobacterial strain BR2 inoculated seedlings followed by control and BR2. Specific protein have been induced and suppressed under salt stress conditions reported earlier. Our results of SDS-PAGE shown that some proteins (50, 48, 32 and 27kDa) were found to be highly expressed and some proteins were found to be suppressed (~65kDa and ~40kDa) at high salt concentrations. The Rhizobacterial strains shown to significantly decreased the level of these salt induced proteins when compare to
control. The BR3 strain was shown to lower the expression of the salt induced proteins much efficiently compare to control and BR3 strain of Rhizobacteria.

Agronomic parameters

Cowpea plants were cultivated for the measurement of the agronomic growth parameters in soil characteristics-sand 84.2%, slit 12.9%, clay 2.9%, pH 7.8, EC 0.5dsm and organic matter 11.2%. Three plots were prepared one as non-inoculated and another two as inoculated with both the Rhizobacterial culture for the plantation and cultivation of Cowpea seeds in the field conditions and the field were irrigated with saline water (0, 25, 50 and 75mM). Data of growth parameters-plant height shoot, root length number of root nodules, fresh and dry weight were recorded at 30 and 90 days of sowing of the plants in the field. The plant height shoot length and root length for 30 and 90 days old plants were recorded. The height of the 30 days old control plants measured 35.00cms in Pusa Sukomal and 29.67cms in RC101 variety. On exposure to salinity in absence of Rhizobacterium the plants recorded 38.33cms (25mM) 30.00cms (50mM) and 15.00cms (75mM) shoot length at 25, 50 and 75mM NaCl salinity in Pusa Sukomal. While in RC101 24.67cms (25mM) 22.67cms (50mM) and 13.00cms (75mM) respectively.

With BR2 Rhizobacterium, the plants height recorded as 44.33cms and 45.24cms height in 30 days old plants of Pusa Sukomal and RC101 varieties respectively under non saline conditions. While plants with BR2 shoot length recorded 44.67cms (25mM) and 34.00cms (50mM) 29.67cms (75mM) and 34.00cms (25mM), 31.33cms (50mM) and 26.21cms (75mM) recorded in Pusa Sukomal and RC101 respectively at 30 days old plants (Table 11).

With BR3 inoculation the plant height shoot length were recorded as 49.00cms (25mM) and 34.08cms (50mM) 29.67cms (75mM) and 42.67cms (25mM), 33.69cms
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(50mM) and 26.54cms (75mM) recorded in Pusa Sukomal and RC101 respectively at 30 days (Table 11).

The mean shoot length of 90 days old control plants was 45.33cms in Pusa Sukomal and 36.67cms in RC101. While the height of plants irrigated with different concentration of sodium chloride 25mM (57.33cms), 50mM (42.67cms) and 75mM (25.00cms) recorded in Pusa Sukomal while in RC101 shoot length were recorded 25mM (40.00cms), 50mM (34.87cms), 75mM (20.00cms) in 90 days old plants (Table 12).

The application of BR2 strain of *Rhizobacterium* showed 62.67cm and 50.00cm of shoot length in 90 days old control plants of Pusa Sukomal and RC101. On increase in concentration 25mM, 50mM and 75mM the shoot length were recorded as 65.00±2.89cms, 44.33±2.60cms and 38.67±1.76cms in Pusa Sukomal while in RC101 the shoot length were 47.00±1.15cms, 38.43±1.71cms cms and 36.53±1.29cms in 90 days old plants respectively (Table 12).

Similarly, with BR3 *Rhizobacterium* inoculation, 0mM NaCl salinity the plant height shoot length recorded as 64.33±2.40cms and 53.67±2.73cms shoot length respectively in Pusa Sukomal and RC101. When NaCl salinity was applied, the shoot length reached 60.67±2.33cms (25mM) and 38.00±1.73cms (50mM) and 37.33cms (75mM) in Pusa Sukomal and 52.67±3.18cms (25mM), 47.33±2.33 (50mM) and 38.67±2.03cms (75mM) in RC101 variety respectively (Table 12).

The plant height Root length of Pusa Sukomal plants and RC101 plants of 30 days age without inoculation of Rhizobacteria was 10.00cms. With 25, 50 and 75mM NaCl water root length recorded 9.00cms, 8.67cms, and 5.00cms of root length in Pusa
Sukomal variety and 8.67cms, 5.33cms and 5.00cms of root length in RC101 respectively.

With inoculation of *Rhizobacterium* BR2 recorded 17.00cms, 16.00cms, 15.67cms and 10.67cms root length were recorded respectively on irrigation with 0, 25, 50 and 75mM NaCl water (Table 13). Similarly, variety RC101 with BR2 under 0, 25, 50 and 75mM salinity irrigation 11.33cms, 11.33cms, 8.67cms and 5.67cms long. On BR3 inoculation variety RC101 were recorded as 12.33cms, 11.67cms, 11.00cms and 10.00cms in 0, 25, 50 and 75mM NaCl concentration of salinity respectively (Table 13).

The mean root length of 90 days old control plants was recorded as 24.67cms, 21.67cms, 14.67cms and 9.33cms in Pusa Sukomal and 12.33cms, 9.67cms, 12.00cms and 11.00cms in RC101 (Table 14). The application of BR2 strain of *Rhizobacterium* under 0, 25, 50 and 75mM NaCl salinity stress recorded 19.33cms, 24.33cms, 16.33 and 15.00cms root length in Pusa Sukomal and 13.67cms, 13.33cms, 15.00cms, and 11.67cms root length respectively in RC101 variety in 90 days of growth (Table 14). With *Rhizobacterium* BR3 inoculation, under 0, 25, 50, and 75mM NaCl salinity the root length were recorded 25.00cms, 22.67cms, 17.33cms and 15.67cms in Pusa Sukomal and 13.67cms, 15.67cms, 15.17cms and 16.33cms in RC101 variety respectively (Table 14).

In our study, the nodules were relatively small and colourless to black in colour (Figure 14). The average numbers of nodules 8.53±1.73, 4.27, 4.17 and 2.47 and 9.67±0.88, 9.57, 9.33 and 2.67 were observed in 0, 25, 50 and 75mM NaCl salinity in control without inoculation of *Rhizobacterium* in RC101 and Pusa Sukomal variety. With BR2 strain inoculation the number of root nodules were 17.33, 15.84, 9.16, 4.32 and with BR3 strain inoculation the root nodules were recorded as 30.42, 25.67, 8.87,
3.73 in 0, 25, 50 and 75mM NaCl salinity in RC101 variety plants at 30 days. On inoculation of BR2 strain the number of root nodules were 27.00 (0mM), 8.67 (25mM), 16.67(50mM), 4.00 (75mM) and with BR3 inoculation the number of root nodules were recorded as 22.33 (0mM), 10.00 (25mM), 10.00 (50mM) and 12.33 (75mM) at 30 days old Pusa sukomal plants (Table 15).

At 90 days the number of nodules recorded in control plants (without *Rhizobacterium* inoculation) was 18.33±1.67 and 15.00±2.89 Pusa Sukomal and RC101 varieties. On increase in concentration of NaCl 25, 50 and 75mM the number of root nodules were 16.67±1.67, 14.67±1.76 and 8.67±0.33 in Pusa Sukomal and 9.67±0.88, 9.00±0.00 and 6.00±1.15 in RC101 plants. While inoculation of BR2 and BR3 strains of *Rhizobacteria* number of nodules were recorded 31.33±2.03, 26.33±1.76, 19.67±1.20, 9.00±1.00 and 31.67±2.03, 21.33±2.03, 13.67±3.48, 6.00±1.15 and 35.33±2.33, 30.00±2.89, 15.67±2.03, 8.67±0.88 root nodules were recorded in 90 days field plants of RC101 variety (Table 16).

The fresh weight in control (without inoculation of Rhizobacteria) under non-saline conditions the fresh weight of 30 day old Pusa Sukomal plants and RC101 plants 16.10g and 13.52g respectively. On irrigation with 25, 50 and 75mM NaCl water fresh weight of Pusa Sukomal and RC101 variety at 30 days was 17.75±1.73g, 14.04±0.58g, 9.29±0.24 and 14.36±0.46g, 11.18±0.53g, 8.40±0.49g respectively (Table 17). With inoculation of BR2 and BR3 strains the fresh weight was 16.71±0.89g, 18.43±0.49g, 14.18±0.60g and 11.01±0.57g and 18.76±0.87g, 20.04±0.44g, 14.19±0.39g and 12.55±0.29g of fresh weight under 0, 25, 50 and 75mM NaCl salinity in Pusa Sukomal variety (Table 17).
The variety of Cowpea RC101 with BR2 and BR3 strain of *Rhizobacterium* inoculation fresh weight were recorded as 15.24±0.94g, 14.04±0.45g, 11.84±0.86g, 9.12±0.35g and 15.29±1.00, 14.86±0.62g, 12.29±0.86g and 9.16±0.26g respectively under 0, 25, 50 and 75mM NaCl salinity conditions in 30 days (Table 17).

The 90 days old Pusa Sukomal and RC101 field plants attained 34.83g and 26.19g fresh weight. On irrigation with NaCl water 25, 50 and 75mM recorded 31.92±2.70g, 27.26±1.09g, 19.55±1.07g and 20.37±1.71, 22.61±2.51g, 17.92±0.75g fresh weight in Pusa Sukomal and RC101 variety (Table 18). In the presence of BR2 38.49±2.38g (0mM), 36.27±0.44g (25mM), 35.24±1.80g (50mM) and 21.82±1.67g (75mM) and with BR3 51.72±1.07g (0mM), 49.16±1.20 (25mM), 33.85±1.36 (50mM) and 23.16±0.26g (75mM) fresh weight were recorded. The fresh weight with variety RC101 of Cowpea attained 40.74±1.55 and 25.54±0.93g, 35.01±1.37 and 23.17±1.53g and 41.23±1.18g, 36.96±1.45g, 37.96±1.45g, 21.87±1.38g g with inoculation of BR2 and BR3 strain in 90 days under saline field conditions.

Dry weight of 30 and 90 days old field grown plants of Cowpea varieties (Pusa Sukomal and RC101) with or without Rhizobacterial inoculation is given in the Table 19 and Table 20. Dry weight of Cowpea varieties Pusa Sukomal and RC101 plants without Rhizobacterial treatment gained 2.73g and 1.66g dry weight. With NaCl salinity 25, 50 and 75mM saline conditions the dry weight recorded as 2.93g, 1.94g, 0.99g and 1.97g, 1.08g, 0.93g respectively in Pusa Sukomal and RC101 varieties of plants at 30 days (Table 19).

With inoculation of Rhizobacterial strain BR2 2.29±0.21g, 1.87g, 1.24 and 0.82g while with BR3 strains 2.31±0.18g, 1.99g, 1.34g and 0.87g of dry weight were recorded respectively in RC101 variety of Cowpea. With inoculation BR2 2.59g (0mM),
2.99g (25mM), 1.24g (50mM) and 0.91g (75mM). With BR3 2.71g (0mM), 3.41g (25mM), 1.96g (50mM) and 1.09g (75mM) dry weight were recorded respectively in Pusa Sukomal in 30 days field plants (Table 19). At 90 days old Cowpea variety Pusa Sukomal without Rhizobacterial treatment recorded 7.44g, 6.81g, 7.29g and 3.07g dry weight were recorded in 0, 25, 50 and 75mM NaCl salinity (Table 20). Pusa Sukomal with inoculation of BR2 the dry weight recorded 8.72g, 8.35g, 7.29g and 3.42g and with BR3 11.16g, 10.52g, 7.29g and 4.77g under 0, 25, 50 and 75mM NaCl salinity conditions at 90 days respectively.

The dry weight of RC101 at 90 days recorded to 8.57g (0mM), 6.28g (25mM), 5.17g(50mM) and 2.77g (75mM) respectively. On inoculation of Rhizobacterial strain BR2 the dry weight 10.13, 7.81, 7.63 and 2.97g while with BR3 Rhizobacterial strain 10.32, 8.59g, 8.67g and 3.47g dry weight were recorded respectively under 0, 25, 50 and 75mM NaCl salinity conditions at 90 days (Table 20).

Agronomic parameters of Cowpea plant variety – Pusa Sukomal and RC101 grown in the field conditions shown that increased in the growth parameters plant height shoot, root length, number of root nodules, fresh and dry weight with 25mM NaCl saline condition as compare to control and thereafter the growth reduced with increase in the salinity level. The Seeds treated with Rhizobacterial culture prior to sowing in the field shown to increase in the growth parameters at 30 day of sowing. While at 90 days Rhizobacterial strains treated seeds accompanied by sodium chloride salinity increased growth parameters compared to seeds irrigated with sodium chloride alone. The overall Pusa Sukomal with BR3 strains shown to better combination compare with other treatments. In the presence of Rhizobacterium the seeds were found to healthy than control seeds.
Overall results shows that the application of Rhizobacterial strains culture on Cowpea seeds-Pusa Sukomal and RC101 has enhanced the saline toxicity tolerance. The BR3 strain show more effectively reduces the harmful effects of salt stress compare with control and BR2. The Pusa Sukomal with BR3 combination was found to be better than other combinations used, indicate that the plant variety and *Rhizobacterium* strains (Salt tolerant) can be of better choice in cultivation of Cowpea with prior inoculation of Rhizobacterial culture on seeds in saline conditions when used in the correct combinations.