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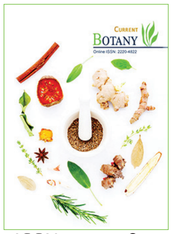

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IAA production and maize crop growth promoting potential of endophyte *Aspergillus niger* (AO11) under salt stress

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ABSTRACT

Maize is cultivated under a broad range of soil conditions and environments. Maize is slightly vulnerable to salt stress and therefore it is seriously affected by soil salinity all over the world. Recognizing the responses of maize to salt stress and making a good strategy to overcome this problem could aid to develop solutions in saline areas to improve maize productivity. We investigated in this research the impacts, tolerance and salt stress management in corn. Many endophytic fungi can produce the Indole-3-acetic acid (IAA) is known for their role in plant growth and development both with and without salt stress conditions. The current study was focused on the production of IAA by endophytic fungi (*Aspergillus niger*) and maize seeds germination and promotion of seedling growth and vigor. In order to evaluate the defense response of maize plant, in relation to A. *niger*, an experiment was designed with three replications of treatments (control, salt stressed, salt stressed inoculated with A. *niger*, and only A. *niger* inoculated plants. It was determined that A. *niger* has the ability to produce the IAA in NaCl and KCl stress peaking 53 μg/ml and was not significantly by alternating the nitrogen and carbon sources in the nutrient broth but increasing the tryptophan concentration raised its production level. High concentration stress of sodium chloride and potassium chloride decrease maize plant seeds germination percentage, shoot and root length also affected the fresh and dry weight of maize. A. *niger* improves salt resistance in maize and also increased the germination percentage up to 30%, also improved the chlorophyll level and it was proved an effective approach for improving maize germination and growth under salt stress.

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KEYWORDS: Phytohormones, auxins, salt stress, symbiotic association, growth promotion

INTRODUCTION

Soils with dissolved residual salts are known as salt affected soils. Salt stress has now turn a severe danger to crops development in arid and semi-arid areas of the globe due to excess evaporation and poor-rainfall,combined with piteous land and water governance methods [1]. The generic view was that salinizations happen mostly in all areas of the world [2]. Internationally, more than 800 million hectares of soil are impacted by alkalinity (397 million hectares) or sodium toxicity (434 million hectares) [3]. Salt concentrations more than 0.25 M affects corn crop and can inhibit development and trigger serious wilting [4]. Sodium was the primary hazardous ion which disrupts with potassium activity and therefore infuriates stomatal simulations exploit to severe evaporation and necrosis in maize [5]. Ultra-osmotic stress as well as harmful impacts of sodium and chloride ions on seed germination in a salty area could prevent and limited germination [6] also the corn plants under saline stress noted with decrease chlorophyll a and b and carotenoids with a decrease in total photosynthetic activity [7]. Endophytes are described as microorganisms completely resident in cells of stem,roots or leafs of plants. Over one million fungal species are estimated to be found in distinct crops genera reflecting the hypervariety of endophytic fungi [8]. Endophytic fungi are one of the greatest suppliers of natural bioactive compounds that can be used in various fields, such as agriculture, healthcare and food technology [9, 10]. A number of endophytes were investigated in order to develop growth-promoting metabolites comparable to those generated by their host crops, but in greater amounts [11]. Many fungal-endophytes are noted to enhance crop production in several ecosystems, [12]. Many endophytes were studied to ascertain their capacity for producing bioactive products comparable to those generated by their host crops [13]. In several plants [14], symbiosotic endophytic pathogens have been observed to enhance plant growth. Auxins (IAA) and gibberellins (GA3, GA4 and GA7) were recorded to be produced by various types of endophytic fungi [15]. Plant species and microbes such as bacteria, algae and fungi can generate IAA which enhance plant growth [16]. The function of microbial IAA in relationship between plant and microbes has currently obtained greater focus [17]. Fungi-based IAA can lead to the formation and growth of root and root hairs leads to improved absorption of nutrients by the corresponding plants [18]. The role of fungal produced IAA in different plant-fungal interaction systems recommends that fungi can be associated to plant for pathogenesis or symbiotic approach using IAA and related compounds leading to plant growth promotion and changes in the basal defense mechanisms of the plant [19]. Endophytic fungi colonization and symbiosis improve salt resistance in maize due to better nutrient absorption, regulate potassium/ sodium ratios in plant tissues, better osmotic adjustments and also improved photosynthetic and water usage efficiencies under salt stress [20]. Production by microbes, plant hormones and biochemicals relies on factors such as pH, temperature, incubation period, microbe types, development mechanisms and inner physiology [21]. Selection of optimal growth conditions is necessary to outline the strategies for production of GAs and other bioactive metabolites [22]. In current research IAA production of enophytic fungi (*Aspergillus niger*) under different salt stress conditions was determined and studied with combination of endophyte-maize plant growth promotion activities under salts stress conditions.

METHODS AND MATERIALS

Obtained from the Department of Botany, Abdul Wali Khan, University, Mardan, Pakistan, the endophyte *Aspergillus niger* strain (AO11) was cultured and purified on PDA media plates, containing (g L-1) potatoes, 200 gram; dextrose, 20 g; agar 15.0 g per 1,000 mL of distilled water; 80ppm streptomycin; pH 5.6±0.2 [23]. Selected endophyte was grown with 100, 500 and 1000 μg L-tryptophan in synthetic Czapek-Dox broth, incubated for 7 days at 30 °C on rotary shaker (120 rpm) [23]. The IAA was measured by spectrophotometer (PerkinElmer Lambda 25) in the *Aspergillus niger* culture filtrate at 540 nm using Salkowski reagent (150 mL of concentrated H_2SO_4, 250.50 mL of distilled water, 7.5 mL 0.5 M $FeCl_3.6H_2O$) and quantified by IAA standard graph [24]. *Aspergillus niger* was cultured in czapek medium supplied with 100, 500 and 1000 mg / L of NaCl and KCl and effect of carbon sources (sugar and fructose) and nitrogen sources (beef and yeast extract) in czapek medium were also determined on its IAA production. The maize seeds (GAUHER) were received from the National Agriculture Research Center (NARC) Islamabad in order to determine the growth promoting ability of the selected fungal strain under influence of salt stress. The experiment was designed as Control (Ctrl), *Aspergillus niger* treated seeds (ANTS), NaCl 50, 100 and 150 mM stressed seeds (N50, N100 and N150), NaCl 50, 100 and 150 mM stressed seeds in combination with *Aspergillus niger* (N50ANTS, N100ANTS and N150ANTS), KCl 50,100 and 150 mM stressed seeds (K50, K100 and K150), KCl 50,100 and 150 mM stressed seeds in combination with *Aspergillus niger* (K50ANTS, K100ANTS and K150ANTS). Maize seeds (*Zea maize* L.) were surface disinfected for 5 min with 0.1% $HgCl_2$, depth in 70% ethyl alcohol for 2 min and washed five times with sterile distilled water. Surface disinfected maize seeds have been seeded at a distance of 5 cm in 500 g sterilized soil in plastic container supplied with 50 g of selected fungal biomass. Seedlings were watered frequently with distilled water and each container got the weekly nutrient of 300 ml half strength sterile Hoagland solution [25]. The percentage of seed germination was calculated by GP = Nf * 100 / N. Where N is the total seeds count and Nf is the germinating seeds count [26]. According to Akhtar et al., (2015), chlorophyll content was calculated. Fresh 0.5 g leaves specimen from every treatment was crushed and dissolved in 80% acetone (v/v). The absorbance of chlorophyll a and b by the spectrometer was taken at 663 and 645 nm respectively [27], and calculated as:

Chl 'a' (mg/g fresh weight) = [12.7 (O.D 663) – 2.69 (O.D 645) × V/1000 × W]

Chl 'b' (mg/g fresh weight) = [22.9 (O.D 645) – 4.68 (O.D 663) × V/1000 × W]

Where V is the volume of sample, W is weight of fresh tissue, O.D 480 + 0.114 (O.D 663) – 0.638 (O.D 645). The Vigor Index of seeds (SV) was determined with VI= SL × GP, where the germination percentage is (GP) and the length of the radicle plus plumule is (SL) [28]. After three weeks of growing seedlings, the radicle, plumule and complete seedling length were calculated using the measuring scale in centimeters

RESULTS AND DISCUSSION

The *Apsrgillus niger* was first grown on PDA plates where the colony of selected strain shown thousands of gray-green conidia along with the blue green mycelia (Figure 1). By colorimetric test using Salkowski reagent, the IAA was detected in the *Aspergillus niger* culture exrtacts. Many researchers revealed IAA production by many fungal-endophytes [29] the *Aspergillus niger* fungus gives IAA by (85 μg/mL) and *T. Harzianum* (68 μg/mL) as well as *Penicillium citrinum* produced (52 μg/mL) during incubation for three days of 30 °C. The very same investigation on the development of IAA by *Aspergillus niger* has been examined for 5-16 days with highest total increased production of 1.28 to 6.8 μg/mL was noted at 6 days of incubation in the Czapek-Dox broth with 0.1% tryptophan [30]. It was also reported that IAA production at 28 °C was maximum [31, 32]. IAA

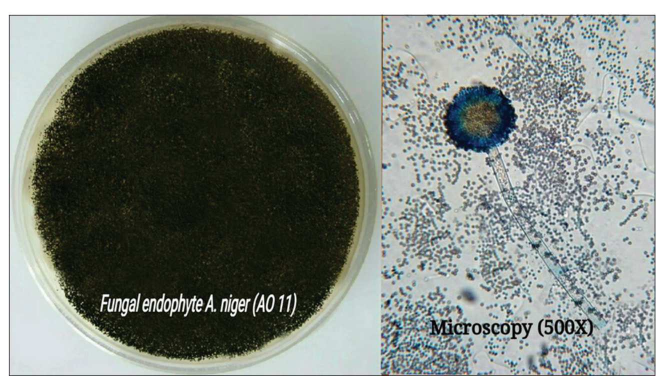

Figure 1: *Aspergillus niger* on PDA, and its microscopy at 500X showing spores and conidia along with mycelia

production was not significantly influenced by replacing the carbon and nitrogen resources in broth, while enhancing the concentration of L-tryptophan from 500 to 1000 µg/mL considerably increased the quantity of IAA discharged. Assessed in nutrient broth under saline conditions the variability of IAA production with NaCl stress was shown by *Aspergillus niger*, with production threshold reaching 20 µg/mL at 150 mM NaCl and gave 23 µg/mL in KCl 150 mM stress (Figure 2). In the existence of 200 µg tryptophan, tryptamine and tryptophol (6.2, 9.8 and 38.55 µg/mL) *Trichoderma atroviride* generates the larger quantity of IAA [33]. In sterile culture, majority of fungi can yield auxinic compounds [34]. Indole-3-acétic acid (IAA) is primarily manufactured with tryptophan by most of the species via the indole-3-Pyruvate and the tryptamines pathway, tryptophan often improved IAA biosynthesis by 2.7-folds, which depend on its accessibility [35]. The symbiotic association of *Aspergillus niger* with the maize crops under salinity was assessed in nursery studies due to the IAA production under different salt stress circumstances by this strain. When maize seeds were exposed to 150mM NaCl stress, up to 50 percent less germination was recorded, but in the same case this was 30 percent in *Aspergillus niger* symbiotic association, raised germination percentage by 30%. Our results are consistent with Minaxi et al., 2013, that also concluded that wheat inoculation with *G. etunicatum* enhanced its germination by 13.7% [36]. Compared with the control, all salt treatments have decreased the germination of maize seed, and the symbiotic association of the selected endophyte with maize in stress conditions remains significant, but in normal growth conditions this symbiotic association was highly significant (Figure 3). The concentration of chlorophyll in endophyte-free control plants was considerably greater, but it was more influential in the plants grown in normal growing conditions inoculated with *Aspergillus niger*. Our results are in compliance with the studies previously observed by Mathur and Vyas (2000) that show that AM root colonization has enhanced chlorophyll synthesis, with greater net CO_2 absorption and plant growth [37]. Nursery treatments with *A. niger* isolates under NaCl improved ($P \leq 0.05$) the leaf chlorophyll a and total chlorophyll of maize plants also same trend was noted for the KCl stress seedlings in symbiotic-association with this endophyte (Figure 4). Recent research shows that *G. intraradices* and *T. intraradicesis* being applied by Colla et al., (2015) considerably enhanced the quality of chlorophyll [38]. The present findings demonstrated a significant difference ($P<0.05$) between treatments for shoot and root length. Endophyte-treated maize crops have greater shoot and root length compared to untreated stressed crops. The results of the inoculation of wheat with *G. fasciculatum* as well as *G. etunicatum* have been

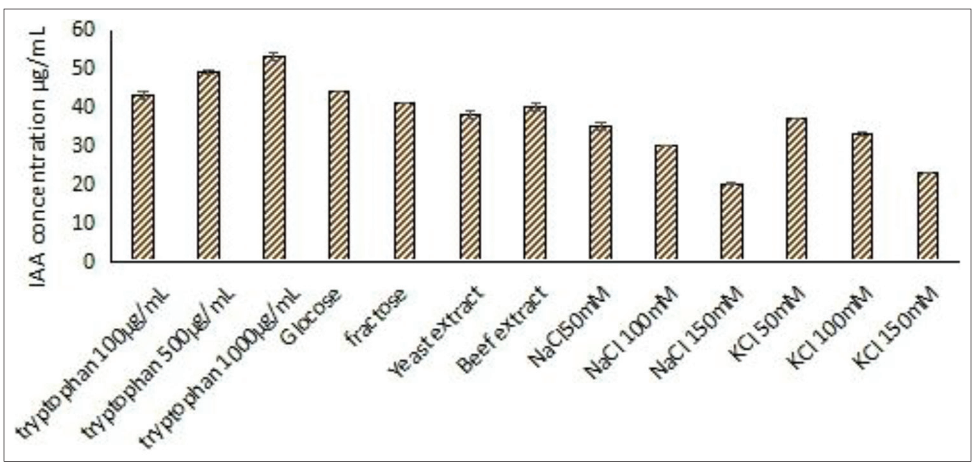

Figure 2: Effect of tryptophan, glucose, fructose, yeast extract, beef extract, NaCl and KCl 50, 100 and 150mM stress in nutrient broth on production of IAA by endophytic fungi *Aspergillus niger*

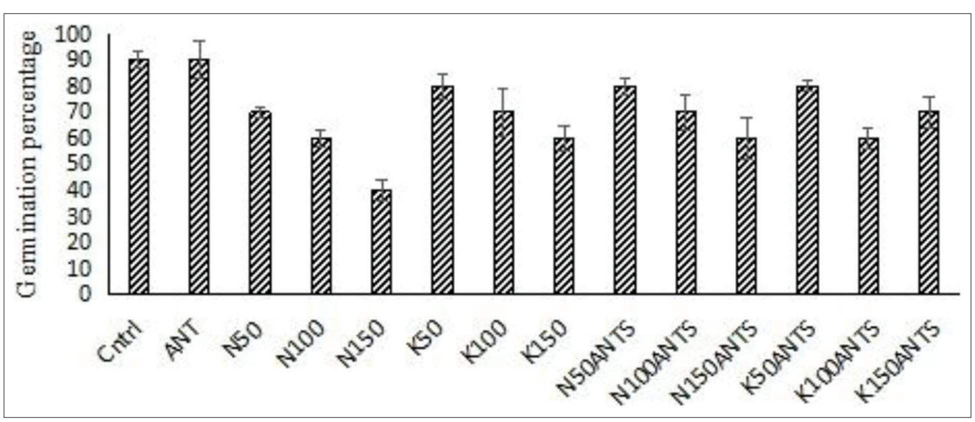

Figure 3: Germination percentage of maize seeds

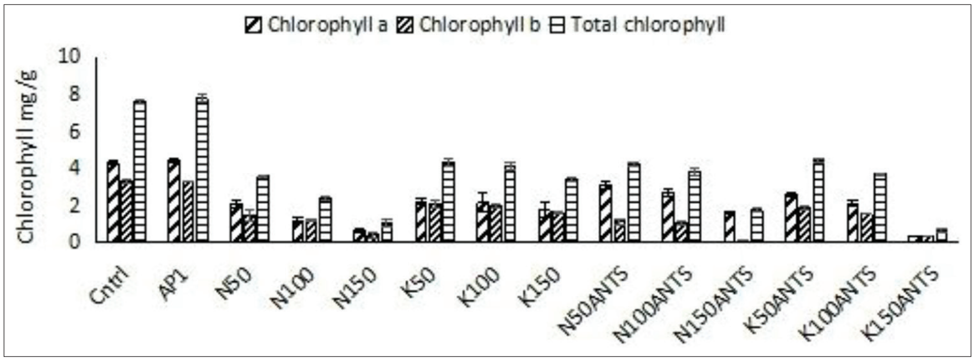

Figure 4: Chlorophyll content of maize seedlings

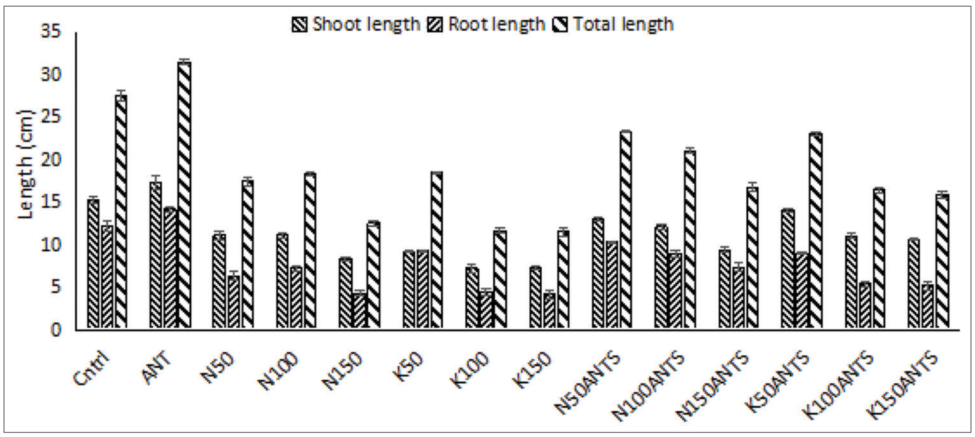

Figure 5: Root and shoot length of maize seedlings

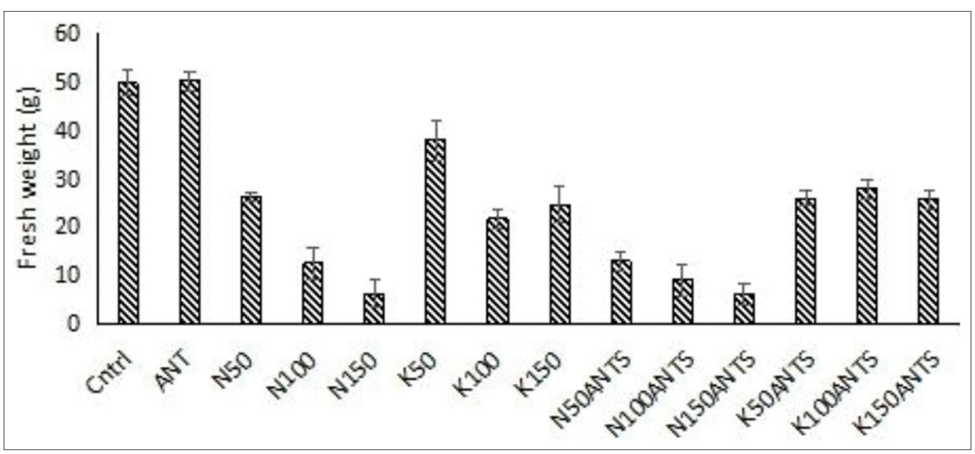

Figure 6: Fresh weight of maize plants

similarly observed for enhanced shoot and root dry weight, production of seeds and average height [39, 36]. Endophyte considerably improves shoot length when contrasted to salinity stressed and the endophyte-free control treatments (Figure 5). All endophyte treatments have improved the fresh weight in both normal and salt stress conditions. A*spergillus niger's* combination with maize has led to improved effects for fresh weight. There's always a mutualistic relationship in natural environments among crops and endophytic fungi [40]. Endophytic fungus manufactures some of the most important organic bioactive-compounds utilised in crops, healthcare as well as the food industry [41]. Endophytic fungi play an key part in crop defense and in developing crops perfect to tolerate biotic and abiotic stress, also help the crops in drought stress and increased biomass [42, 43]. Fresh weight values attained with endophyte cured crops significantly higher than those measured in salts stressed and control plants. The highest fresh weight was recorded from the ANT treatment, followed by control. Endophytic fungi could also improve crop growth, mainly because of development of endophyte-produced

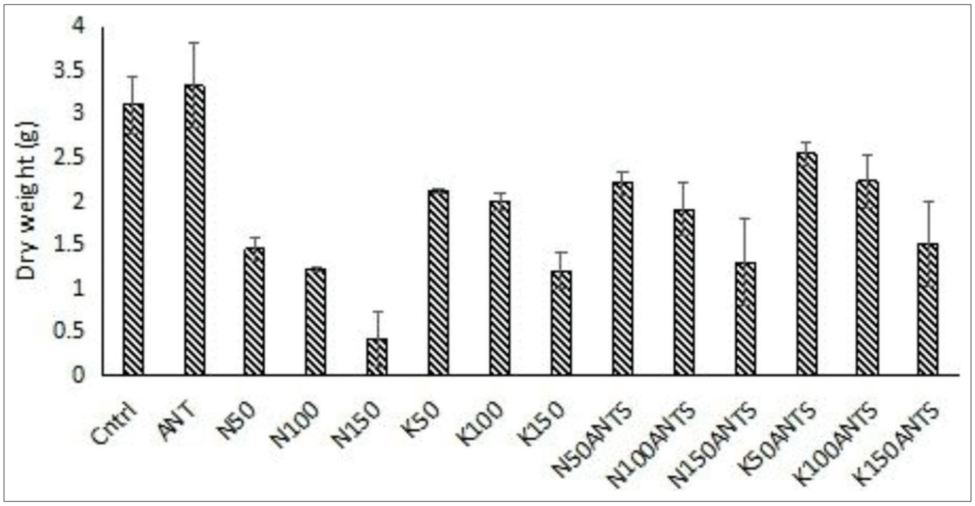

Figure 7: Dry weight of maize plants

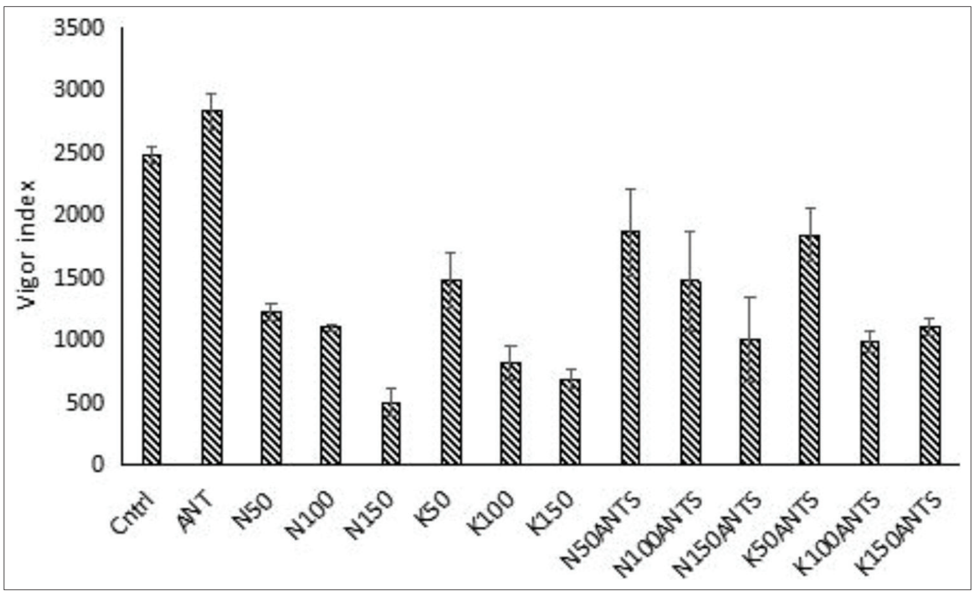

Figure 8: Vigor index of maize plants. Cntrl: control, ANT: *Aspergillus niger* treated plants N50, N100 and N150: NaCl 50,100 and 150 mM treated plants, K50, K100 and K150: KCl 50,100 and 150 mM treated plants, N50, N100ANTS, N150ANTS: *Aspergillus niger* with NaCl 50,100 and 150 mM treated plants, K50, K100ANTS, K150ANTS: *Aspergillus niger* with KCl 50, 100 and 150 mM treated plants. Data are mean of three replicates with standard error bars (Duncan test; $p<0.05$)

phytohormones, such as IAA and GA, resulting to enhanced root development and enhanced nutritional uptake [44]. For plant dry biomass, the same phenomenon has been observed (Figures 6 and 7). Plants treated with A. *Niger* isolates had a significantly greater Vigor index value compared to stressed, control and endophyte treated plants in stress conditions. Treatment ANT indicated the high vigor index of maize plants with an increase of 12.37 times compared to control plants similarly the association of A*spergillus niger* also improved the plant vigor index in salt stress compared to untreated stress plants (Figure 8). The endophyte has been used to secrete bioactive-compounds in its growing zone while the hormone shows significant impacts on plant growth both with and without stress circumstances [45]. A comparable behavior of an endophyte generating IAA was also determined, [46] where endophytic fungi manufacturing IAA increased the development of rice plants under the influence of salinity, cold and heat stress [47,48]. Hence our results are heavily supported by prior investigations.

CONCLUSION

In high salt stress circumstances A*spergillus niger* was able to produce IAA in significant amount. Salt stress had a major impact on Maize crop. The germination of maize crop was drastically decreased by the NaCl and KCl stress as well as the development of its seedlings were noted reduced. The content of chlorophyll amount in the salt stress and without salt stress situations treated with A*spergillus niger* was noted higher. However, it was greater in without salt stress treatments. Overall the *Aspergillus niger* has significantly improved the germination and growth of maize and helps to tolerate the severe salt stress conditions.

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