Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 962-970
Received: 08-05-2020
Accepted: 10-06-2020

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Effect of salicylic acid, mycorrhiza and trichoderma in pearl millet by mitigating fluoride toxicity

Lalit and Prasann Kumar

Abstract
The research work entitled on *"*Ameliorative Effect of Salicylic acid, Trichoderma and Mycorrhiza in Pearl Millets (*Pennisetum glaucum* L.) Under Fluoride Toxicity*"***.** The present research work was carried out during *Kharif* season in the Department of Agronomy on the terrace of Block-25 School of Agriculture, Lovely Professional University, Jalandhar, Punjab with one variety of pearl millets Moti Bajra. The result indicates that average plant height was significantly reduced with 9.3%, 18.58%, 3.09% and 18.81% when exposed to fluoride stress (T1) as compared to control (T0). The stem girth of the plant was significantly increased by 4.6% at 30DAS, 8.6% at 60DAS, 2.7% at 90DAS and 36.8% at 120DAS of interval in controlled treatment (T0) as compared to treatment where fluoride toxicity was created artificially in (T1). The chlorophyll content was reduced with 39.4% at 30DAS, 6.6% at 60DAS, 24.3% at 90DAS and 46% at 120DAS when plant exposed to fluoride stress as compared to control treatment. In the application of mycorrhiza and salicylic acid in the presence of fluoride toxicity, the content of chlorophyll was increased compared to fluoride stress. The application of mycorrhiza helps to mitigate the stress of fluoride and provide sufficient nutrients to the plant that increases the growth, morphological and biochemical parameters of the plant.

Keywords: Chlorophyll, fluoride, mycorrhiza, plant height, salicylic acid, stem girth, toxicity

Introduction
Pearl millet (*Pennisetum glaucum* L.) is commonly cultivated type of millet. The species of pearl millet is P typhoideum and americicanum. In India, the local name of the Pearl millet is Bajra. At first pearl millet was cultivated about 6000 to 8000 years ago in dryland areas of western Africa. Some evidence of archaeologist indicates that the primary origin of the pearl millet is North Africa then spreading to India and another country. Similarly, in West Africa, a huge amount of genetic biodiversity recorded. Carbonized grains of pearl millet is found at archaeological sites in North-Western India indicates India has also had pearl millet origin. Pearl millet is also known as Bajra in Hindi and the name of pearl millet is cumbu in Tamil, Sajjalu in Telugu and Bajiri in Maharashtra Gujarat and Rajasthan. In Australia, pearl millet is known as bulrush millet, Cattail Millet in the USA.
Pearl millet (*Pennisetum glaucum* L.) is the most important food and millet crop in the world. The nutritive value is enormous and grains quality is superior over sorghum. Grains of pearl millets cooked like as rice and mostly savoured by the population of villages living in the semiarid region of Tamil Nadu. In North India, the flour of pearl millets grains used to make chapatti and roti similar to chapatti prepared from sorghum and maize flour. Pearl millets grains constitute the important ingredients used for the preparation of poultry and animal feed. Nutritive value of pearl millet straw is low as compared to maize and sorghum.
Moti Bajra variety widely used for its grains quality as well as fodder purpose. Height of this hybrid is 230cm tall, having 2-3 productive tillers. Grains of this variety are slate in colour and medium bold in size. This variety is resistant to many diseases like downy mildew, smut and ergot. 90 to 95 days are required for its maturation stage. As compare to Sudan grass and sorghum 3 to 5 ton/ac dry matter produced by pearl millet. The dry matter also used for the mulching purpose (Schonbeck *et al*., 2006) [21]. Crop residue of pearl millet plant remains in soil surface consist of 60 to 80% potassium. The 100gm raw grains of pearl millet contains 1582 kJ energy, 11% protein, 4.2% fat, 73% carbohydrates, 8.5% fibre and 285mg phosphorus. In cooked food, nutritional value influenced by the cooking method (Rosolem *et al*., 2005) [20].

Fluoride
Fluoride considered as one of 14th essential nutrient physiologically required for growth and development of human beings.

In Earth, crust fluorine is 17th most generous element. Fluorine never found a free state in nature. It mostly presents in gaseous form. Fluorine is found pale yellow in gaseous form, liquid it is bright yellow in colour. Constituents of these compounds are in minerals having minerals fluorspar (fluorite CaF_2), Fluorapatite ($3Ca_3 (PO4)_2$ Ca FCL_2) and Cryolite (Na_3 AIF_6) in soil. The small amount of airborne fluoride concentration is present in the atmosphere. Source of the fluoride in the air is due to dust particles, coal burning, production of phosphate fertilizer in an industrial area and volcanic activities (Murray, 1986) [11]. Drinking water is a usually greater contributor to regular consumption of fluoride. Seawater usually contains around one mg per litre whereas rivers and lakes fluoride concentration are below 0.5 mg l^{-1}. In groundwater, the acceptable limit of fluoride is 1.5mg l^{-1}. Above that limit, it is affecting and causing several diseases to a human being. The 80% of the world's illness caused by drinking or poor water and 65% of world's bacterial fluorosis induced by fluoride pollution of drinking water (Felsenfeld *et al*., 1991) [3]. Natural fluoride source in the soil is fluorite. Fluoride content in the soil ranges from 200 to 300ppm (ATSDR 2003). Scientist observed that the content of fluoride also increased with the depth of soil (WHO 2001). The Intensive irrigation with fertilized application develops the Cl^-, SO_4^{2-}, F^- and NO_3^- in underground water. Due to alkalization, a large amount of fluoride concentration measured in irrigated lands with groundwater. Normally fluoride absorbed by the soil surface and minimal amount of fluoride is uptake by the plant. Fluoride harms plant shown as by leaf necrosis and chlorosis decreases in photosynthetic pigment, reduction in biomass and growth rate of a plant (Yadu *et al*., 2018) [26].

Mycorrhiza

Mycorrhiza is the symbiotic association between soil-borne fungi and roots of higher plant. Activities of agricultural development improved by the use of Vesicular arbuscular mycorrhizal (VAM) (Johansson *et al*., 2004) [7]. The interaction with the contact of plant root its underground part known as mycelium and does not cause any harm to the plants. There are two forms of mycorrhiza known as ecto and endomycorrhiza. The ectomycorrhiza in the root cortex distinguished by an extracellular fungal growth, whereas endomycorrhiza forms intercellular and intracellular fungal structures, called vesicles and arbuscles. Except for the zone of nutrient deficiency, the network of extracellular hyphal rapidly expand and enhance the development of inorganic nutrient production (Smith *et al*., 2010) [24]. Mycorrhizal fungi association improved nutrient uptake, substance-promoting growth development, drought resistance, salinity and controls plant defence mechanism. Mycorrhiza improves the nutrient uptake efficiency of a plant through roots (Hause *et al*., 2007) [6].

Trichoderma

Trichoderma is a fungal species in the Hypocreaceae family that found in all soils, this fungus is commonly culturable in soil (Harman *et al*., 2004) [5]. Trichoderma has free-living species of fungi that are highly interacting with soil, root and foliar environment. These fungi fungus used as a bioagent to control the diseases caused by plant pathogens (Kubicek *et al*., 2011) [8]. Species of Trichoderma has a major role in interaction with the pathogens and plants. It involves the development of antibiotics and degrading enzymes of the cell wall, the fulfilment of essential nutrients and improves the plant protection mechanism (Lu et. al., 2004) [9]. This type of fungi also have major participation in decomposition and mineralization of waste of plant residue in the field and helps in improving the plant growth by improving germination percentage, the height of the plant and its dry weight (Brotman *et al*., 2013) [1]. At the time of seed sowing, it colonizes the surface of seed and protects the plant from harmful soil-borne microbes (Contreras-Cornejo *et al*., 2009) [21].

Salicylic acid

Another name of salicylic acid is ortho- hydroxybenzoic acid. This type of chemicals refers to a varied group of phenolic plants. Salicylic acid used as a plant hormone by plants. It used as medicine in ancient time (WHO, 2019). It is soluble in water and highly soluble in an organic solvent. It vigorously transported, metabolized and easily transferred from its primary application place to all tissues of plant (Raskin *et al*., 1990) [19]. The phenolic compound has a crucial role in managing the various physiological process like the uptake of ions, growth of plant and photosynthesis process (Lynn *et al*., 1990) [10]. Salicylic acid known for managing signals and decreases the plant response in abiotic situations drought, chilling effect and resistance to heavy metals at some point (Yang *et al*., 2003) [27]. Salicylic acid also helps in the germination of seeds, the formation of flowers, respiration and for vegetative growth of a plant. Another role of salicylic acid to tolerance pathogens by producing the protein-related pathogenesis (Slaymaker *et al*., 2002) [23].

Methodology

The pot experiment was conducted in the field of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab with one variety of pearl millets Moti Bajra. Pearl millets variety is taken from agriculture seed market Phagwara, The size of pot used in the experiment was of 30cm in diameter and 25 cm height 25cm and area of pot were 0.0706 m^2. Each pot filled with 10kg soil with small holes in the bottom of pots. According to the plan of research work Fluoride, stresses created in a plant by exogenous application of fluoride in the soil. One best concentration after initial screening within the range of 1-100 ppm of fluoride finally selected after screening. Fluoride (100ppm) concentration applied in the soil for creating stress in the pearl millets plant. Salicylic acid (1ppm) applied through foliar application after 30 days of sowing. Trichoderma and Mycorrhiza applied at the rate of the recommended dose in soil. For Trichoderma recommended dose is 20-25 gm/100m^2 and for 0.0706$m^{2,}$ it is 17.6 mg. For mycorrhiza, the recommended dose is 10 kg/ha and for 0.0706 m^2 it is 70mg. The various measurements were taken at three stages such as 30, 60, 90 and 120 DAS.

Treatments Detail

T0- Control; T1- Fluoride (100ppm/pot); T2-Trichoderma (*T.viride*:17.6 mg/pot); T3-Salicylic acid : 1ppm); T4-Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot); T5-Fluoride (100 ppm/pot) + Trichoderma (*T.viride*:17.6mg/pot); T6- Fluoride (100ppm/pot) + Salicylic acid (1ppm/pot); T7- Fluoride (100 ppm/pot)+ Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot): T8- Fluoride (100 ppm/pot) + Trichoderma (*T.viride*:17.6mg/pot) + Salicylic acid (1ppm/pot); T9-Fluoride (100 ppm/pot) + Trichoderma (*T.viride*:17.6mg/pot)+ Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot); T10-Fluoride (100

ppm/pot)+Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot) + Salicylic acid (1ppm/pot); T11-Fluoride (100 ppm/pot) + Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot) +Trichoderma (*T.viride*:19mg/pot) + Salicylic acid (1ppm/pot); T12- Endomycorrhizal fungi (AMF, *Glomus* species: 70 mg/pot)+Trichoderma (*T.viride*:17.6 mg/pot) + Salicylic acid (1ppm/pot).

Observation Recorded

Plant Height

Plant height measured at 30, 60, 90 and 120 days after sowing. Measuring scale used to measure the height of the plant from the surface of to the topmost leaf of the plant. Height of plant observed in centimetres (cm) (Fig. 1).

Fig 1: Measuring the height of a plant

Stem Girth

The stem girth recorded from base of the plant to the tip of the stem of the plant at 30, 60 and 90 and 120 days of time interval by using a digital Vernier calliper. Mean stem girth was calculated and expressed in the cm (fig 2).

Fig 2: Measuring stem girth of a plant at 30 DAS

Chlorophyll content (mg g^{-1} fresh weight)

The chlorophyll content in the leaf of Pearl Millet was estimated by the method of Arnon DI. (1949).

Principle

Chlorophyll was extracted in 80% acetone and the absorbance is measured at 645nm and 663nm. The amount of chlorophyll calculated using the absorbance coefficient

Reagent

- Acetone (80%, pre-chilled)

Procedure

Chlorophyll extracted from 100mg of the leaf sample using 20ml of 80% acetone. The supernatant transferred to a volumetric flask after centrifugation at 5000 rpm for 10 minutes. The extraction repeated until the residue became colourless. The volume in the flask made up to 100ml with 80% acetone. The absorbance of the extract read in a spectrophotometer at 645nm and 663nm against 80% acetone blank. The amount of the chlorophyll content calculated by using the formula as given below.

Chlorophyll 'a' (mg/g Fresh Weight) = $12.25(A663)-2.79(A645) \times \frac{V}{1000 \times W}$

Chlorophyll 'b' (mg/g Fresh Weight) = $21.50(A645)-5.10(A663) \times \frac{V}{1000 \times W}$

Total chlorophyll (mg/g Fresh Weight) = $20.2(A645)+8.02(A663) \times \frac{V}{1000 \times W}$

where, V= Final volume of the extract, W= Fresh weight of the leaves, A= Absorbance at the specific wavelength, the value expressed as the mg/g fresh weight.

Fig 3: Sample for observation

Results and Discussion

Plant Height (cm)

The individual and combined effect of Salicylic Acid, Trichoderma and mycorrhiza on plant height (cm) was studied in pearl millet variety Moti Bajra, under the fluoride stress. Data of plant height was recorded at 30, 60, 90 and 120 days after sowing (DAS) (Table1, Fig. 3). The average plant height was significantly reduced with 9.3%, 18.58%, 3.09% and 18.81% when exposed to fluoride stress (T1) as compared to control (T0) on the dates of 30, 60, 90 and 120 DAS of

interval. Similarly, when another comparison we observe that plant height decreased in (T2) and (T3) but there is a slight increase in treatment (T4) when compared to Control (T0) the plant height increased significantly with 4.69%, 5.85%, 7.0% at 30, 60, 90 DAS and get decreased with 14.8% at 120 DAS of interval. From the result, it was indicated that average plant height was increased in treatment (T5) by 10.52% at 30DAS, 24.59 at 60 DAS, 6.84% at 90 DAS and 6.70 at 120 DAS of interval and (T7) by 18.34% at 30 DAS, 12.4% at 60DAS, 17.64% at 90DAS and 6.23% at 120 DAS of the interval as compared to treatment where fluoride toxicity was used artificially in treatment (T1). The treatment of soil by mycorrhiza and trichoderma helped mitigate the induced stress of fluoride toxicity and improving the growth and height of the plant. The combined application of mycorrhiza, trichoderma and salicylic acid with fluoride toxicity in (T11) plant height was significantly increased by 9.38%, 6.25%, 0.96% and 11.1% at 30, 60, 90 and 120 DAS of an interval as compared to treatment (T1). Similarly in the treatment (T12) without fluoride toxicity combination of two microbes and salicylic acid significantly increases the height of the plant by 26.6% at 60DAS, 15.6% at 9 DAS and 7.34 % at 120DAS of an interval as compared to treatment (T0). The availability of mycorrhiza and trichoderma increase the uptake of nutrient. A (Garg et. al., 2017) [4] studied about mitigating the stress caused by heavy metals by symbiotic association of AM fungi with roots of a plant. It reported that increasing the nutrient by inducing AM fungi helps to rise growth, morpho-physiological parameters of plant and reduces the concentrations of metals in the tissues of a plant. In soil toxic metals were immobilised by mycorrhizal fungi by binding the ions of toxic component into the components of the cell wall and a huge amount of organic acid in the rhizosphere were secreted as glycoprotein, oxalic acid, glomalin and citric, sometimes act as a chelating agent and decreases uptake of the toxic metals in the plant that helps to increase the height and growth of a plant (Kumar and Dwivedi, 2018b, c) [15, 16]. The survival of plants is crucial in stages of growing the seedlings and reasonable germination of seeds. When fluoride enters the metabolism of the plant then the cell partition rate is decreased. The stress of fluoride is induced in the beginning development of seedling and the germination rate was decreases. Aleurone of gibberellic acid degenerated when the stress of fluoride is induced and slightly decreases the germination of seeds (Kumar and Dwivedi, 2014) [18]. Due to this unusual metabolism in saccharides endosperm. Seedling having inappropriate development and uptake of the nutrient is unbalanced when fluorine is intervention and it decreases the length of root and shoot, fresh and dry masses and also lower down the vigours index of *Abelmoschus esculentus*, *Triticum aestivum*, *Oryza sativa*, *Cicer arietinum* and *Citrullus lanatus*.

Table 1: Plant Height (cm) of Pearl millet during *Kharif*

Treatments	30DAS	60DAS	90DAS	120DAS
T0	10.16^{a}±.57	37.66^{ab}±14.15	70.00^{cde}±2.00	134.66^{b}±2.51
T1	9.66^{a}±1.75	30.66^{b}±4.50	68.00^{de}±2.00	109.33^{gh}±3.21
T2	9.50^{a}±.50	33.33^{b}±4.93	70.00^{cde}±2.64	105.33^{gh}±2.51
T3	10.16^{a}±1.60	33.00^{b}±10.00	53.00^{f}±2.00	102.33^{h}±2.51
T4	10.66^{a}±1.15	40.00^{ab}±12.49	75.33^{b}±3.05	114.66^{de}±2.51
T5	11.16^{a}±2.46	40.66^{ab}±7.57	73.00^{bc}±2.00	116.66^{d}±4.16
T6	10.16^{a}±1.25	31.33^{b}±4.16	72.00^{bcd}±2.00	110.33^{ef}±1.52
T7	11.83^{a}±2.75	35.00^{ab}±1.00	80.00^{a}±2.00	116.6^{d}±2.51
T8	9.50^{a}±1.32	29.33^{b}±6.35	67.00^{e}±2.00	110.33^{ef}±1.52
T9	10.16^{a}±.28	51.33^{a}±13.50	71.33^{bcd}±1.52	115.33^{d}±2.51
T10	11.16^{a}±1.04	37.33^{ab}±12.70	66.80^{e}±1.57	105.66^{fgh}±2.51
T11	10.66^{a}±.76	32.66^{b}±2.51	68.66^{de}±3.51	123.00^{c}±2.00
T12	10.16^{a}±1.25	51.33^{a}±8.50	83.33^{a}±1.52	145.33^{a}±2.51

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Fluoride; T2-Trichoderma (*T.viride)*: T3-Salicylic acid ; T4-Endomycorrhizal fungi (AMF); T5-Fluoride + Trichoderma (*T.viride*:); T6- Fluoride + Salicylic acid T7- Fluoride + Endomycorrhizal fungi (AMF): T8- Fluoride + Trichoderma (*T.viride*) + Salicylic acid; T9-Fluoride + Trichoderma (*T.viride*)+ Endomycorrhizal fungi (AMF); T10-Fluoride + Endomycorrhizal fungi (AMF) + Salicylic acid; T11-Fluoride + Endomycorrhizal fungi (AMF)+Trichoderma (*T.viride*) + Salicylic acid; T12- Endomycorrhizal fungi (AMF)+Trichoderma (*T.viride*) + Salicylic acid.

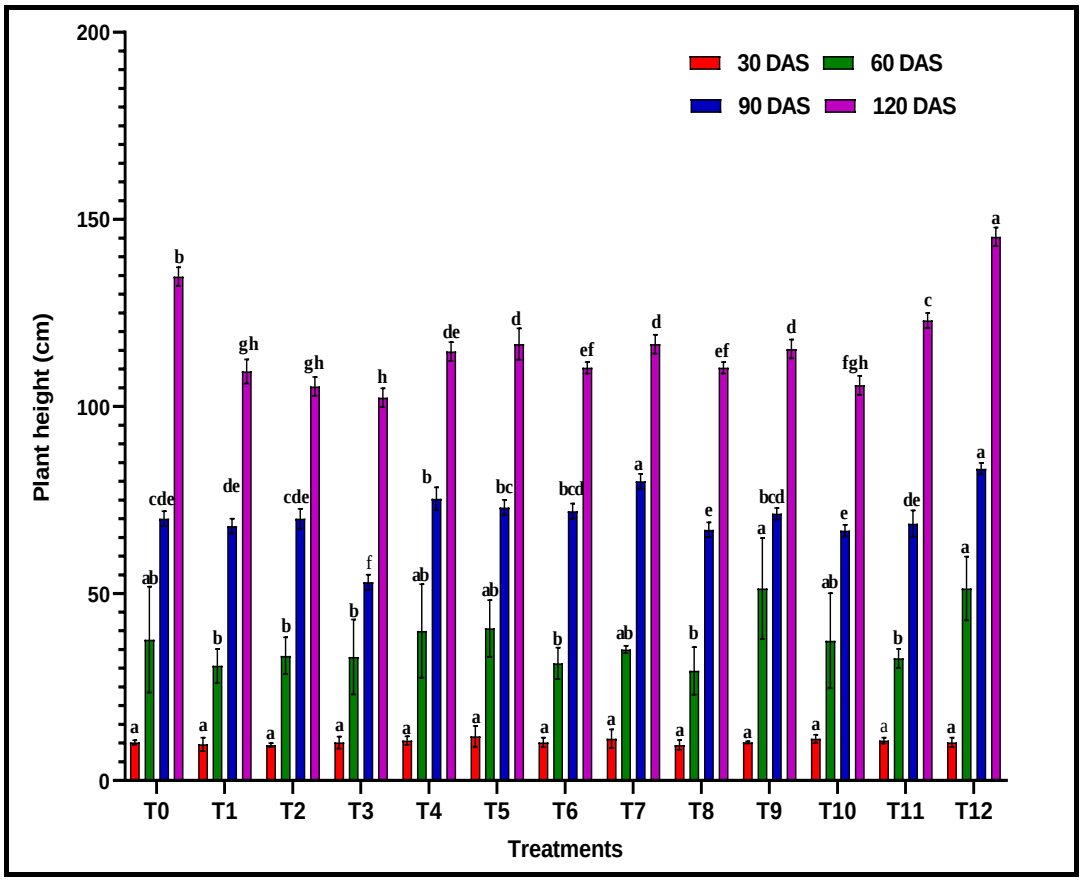

Fig 3: Plant Height (cm) of Pearl Millet during *Kharif*

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Fluoride; T2-Trichoderma (*T. viride)*: T3-Salicylic acid ; T4-Endomycorrhizal fungi (AMF); T5-Fluoride + Trichoderma (*T. viride*:); T6- Fluoride + Salicylic acid T7- Fluoride + Endomycorrhizal fungi (AMF): T8- Fluoride + Trichoderma (*T. viride*) + Salicylic acid; T9-Fluoride + Trichoderma (*T. viride*)+ Endomycorrhizal fungi (AMF); T10-Fluoride + Endomycorrhizal fungi (AMF) + Salicylic acid; T11-Fluoride + Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid; T12-Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid.

Stem girth (cm)

Effect of salicylic acid and biofertilizers (trichoderma and mycorrhiza) and their combined application on stem girth was studied in Moti Bjara variety of Pearl millet under fluoride stress. Data were recorded at 30, 60, 90 and 120DAS (days after sowing) (Table 2, fig 4). From the result, it was indicated that average stem girth of the plant was significantly increased by 4.6% at 30DAS, 8.6% at 60DAS, 2.7% at 90DAS and 36.8% at 120DAS of interval in controlled treatment (T0) as compared to treatment where fluoride toxicity was created artificially in (T1). Similarly, the stem girth of a plant in the treatment (T2) was reduced by 6.2% at 30DAS, 4.8% at 60DAS, 22.7% at 90DAS and 37.4% at 120DAS (days after sowing) as compared to the controlled treatment (T0). The exogenous application of mycorrhiza in the soil treatment (T4) was increased the average stem girth of a plant by 4.7% at 60DAS, 8.8% at 90DAS and reduced in the initial stage by 23.1% at 30DAS and 13.2% at 120 DAS of an interval as compared to controlled treatment (T0). The combined application of mycorrhiza with fluoride toxicity in the treatment (T7) the average stem girth of the plant was significantly increased by 18.7% at 30DAS, 10% at 60DAS, 17.1% at 90DAS and 17.9% at 120 intervals as compared to treatment (T1) where fluoride toxicity was created artificially. Similarly, the combined form of mycorrhiza and trichoderma was increased in treatment (T9) the average stem girth of a plant by 5.8% at 30DAS, 24.2% at 60DAS and 15.7% at 90DAS interval and slightly decreased by 10.9% at 120DAS as compare to treatment (T1). The inoculation of mycorrhiza increased the uptake of nutrient in the soil and also helping the plant to mitigate the stress induced by fluoride toxicity. The application of salicylic acid and mycorrhiza under fluoride toxicity in treatment (T10) stem girth of the plant increased by 9.2%, 1.5 %, 23% and 25% at 30, 60, 90 and 120 DAS of the interval as compared to (T1). The foliar application of salicylic acid and exogenous application of mycorrhiza, trichoderma with fluoride toxicity increased the average stem girth of a plant by 8.7% at 30DAS, 3.7% at 60DAS and decreased in 90 DAS by 19.1% then slightly increased by 30.1% at 120 DAS in (T11) compare to the treatment (T1). Singh *et al*., 2019 was conducted a pot experiment by using different species of fungus such as *Glomus aggregation, Funneliformis mosseae, Rhizophagus intraradices* and *R. fasciculatus* used for sowing in the maize plant. The treatment of AMF influenced significantly the potential of phytoremediation and growth of a plant. Maize plant treated with *F. mosseae* increases the length of roots and weight of shoot by 49 and 113% as compared with control *F. mosseae* behaves as bio-filter in plant roots and also harmonize direct translocation of toxic metals such as cadmium (Cd), nickel (Ni) and bioaccumulation factors i.e. soil to shoot micronutrients uptake and then the root to shoot translocation. The result indicates that arbuscular mycorrhizal fungi help for growth and morphological parameters of the maize plant.

Parameters of biochemical play an important role in determining the sensitivity of plant against specific stress. More than 50% of respiration is suppressed by the stress of fluoride and preventing the various activities of glycolytic and mitochondrial enzymes i.e. dehydrogenase of succinate, enolase, and maltose. In chloroplast and mitochondria having the acidic pH within organelles of cells that accumulate the fluoride and stop the activities of ATPase in the membrane. Transportation of phosphorous is inhibited due to an analogue of phosphate ions complexes with aluminium fluoride (AlF^{-4}) ions. The fluoride content is exposed to the protein present in the membrane of potato inhibiting the transportation of phosphorus. The various process is prohibited like glycolysis, signalling, and synthesis of nucleotide due to phosphorylation inhibition. In various four varieties of rice leakage of ions in root and lipid peroxidation (H_2O_2) having due to the rise in the amount of fluoride content. The fluoride stress is induced in plant affected the metabolism of total N and amino acids. The fluoride is induced in tea plant leaves soluble protein is degraded due to increases in the content of amino acid. Large numbers of content having aspartic, glutamic acid, serine and asparagine are accumulated in the leaves (Kumar *et al*., 2016a, b) [13, 14]. Those plants which are sensitive and tolerant to fluoride a correlation will find in between the fluoride stress and metabolism of nitrogen has detected. In the manner of specific-species total nitrogen present in the biomass of shoots accumulated the stress of fluoride. Possibly developing the strategies to preventing the degradation of protein and the plant of maize being tolerant of fluoride.

Table 2: Stem Girth of Pearl Millet during *Kharif*

Treatments	30DAS	60DAS	90DAS	120DAS
T0	4.73^{a}±1.44	10.72^{a}±2.00	12.40^{ef}±.52	9.66^{a}±.48
T1	4.51^{a}±.90	9.76^{a}±1.51	12.06^{f}±.15	6.10^{d}±.26
T2	4.45^{a}±.87	10.25^{a}±1.66	10.10^{h}±.26	7.03^{c}±.15
T3	3.81^{a}±.86	9.79^{a}±.53	9.53^{i}±.41	7.46^{c}±.20
T4	3.84^{a}±2.32	11.26^{a}±1.71	13.60^{b}±.20	8.53^{b}±.35
T5	5.55^{a}±.55	12.18^{a}±2.03	14.11^{a}±.19	7.50^{c}±.36
T6	4.86^{a}±.80	11.03^{a}±2.59	12.55^{de}±.48	7.53^{c}±.25
T7	5.08^{a}±1.33	10.95^{a}±2.65	14.56^{a}±.07	7.43^{c}±.30
T8	5.10^{a}±1.02	8.62^{a}±4.08	12.93^{cd}±.04	6.50^{d}±.30
T9	4.79^{a}±1.08	12.89^{a}±3.52	14.32^{a}±03	5.50^{e}±.30
T10	4.97^{a}±1.01	11.41^{a}±2.59	13.18^{bc}±.05	9.43^{a}±.35
T11	4.94^{a}±.59	10.14^{a}±2.13	10.12^{h}±.21	8.73^{b}±.20
T12	5.24^{a}±.58	10.55^{a}±1.01	11.31^{g}±.04	7.36^{c}±.20

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Fluoride; T2-Trichoderma (*T. viride)*: T3-Salicylic acid ; T4-Endomycorrhizal fungi (AMF); T5-Fluoride + Trichoderma (*T. viride*); T6- Fluoride + Salicylic acid T7- Fluoride + Endomycorrhizal fungi (AMF): T8- Fluoride + Trichoderma (*T. viride*) + Salicylic acid; T9-Fluoride + Trichoderma (*T. viride*)+ Endomycorrhizal fungi (AMF); T10-Fluoride + Endomycorrhizal fungi (AMF) + Salicylic acid; T11-Fluoride + Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid; T12-Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid.

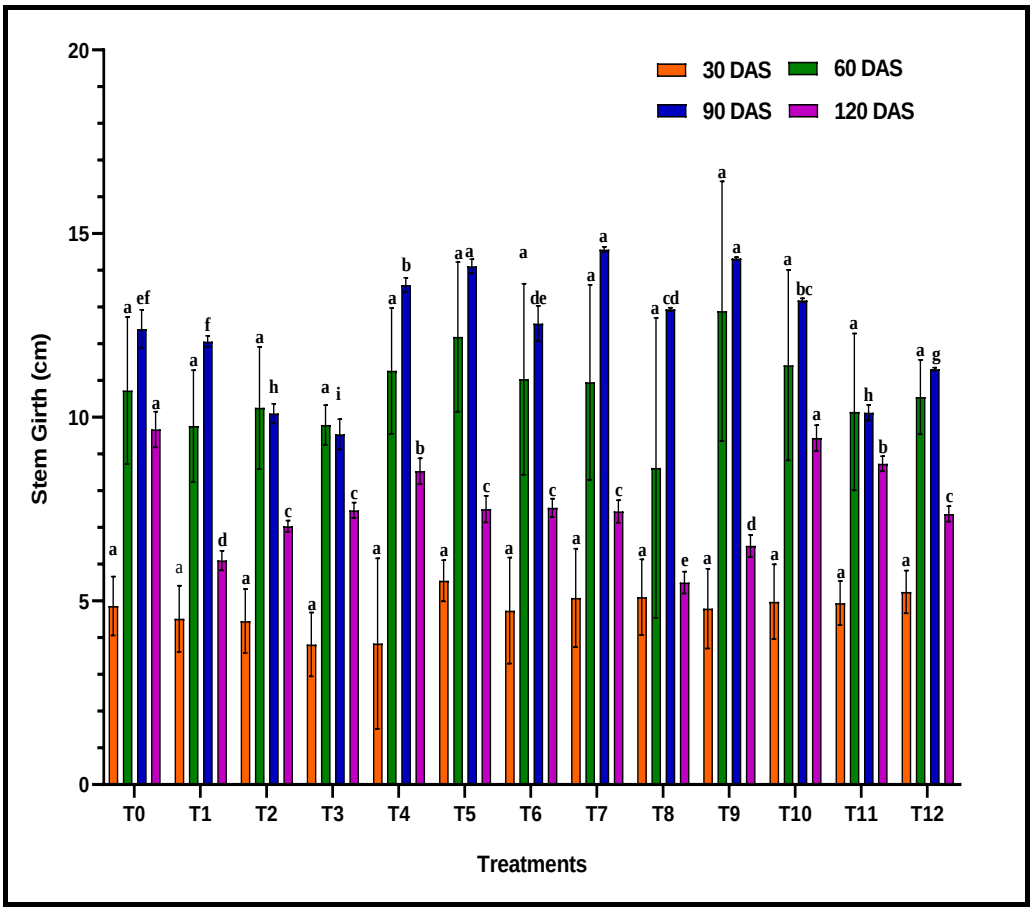

Fig 4: Stem Girth of Pearl Millet during *Kharif*

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Fluoride; T2-Trichoderma (*T. viride)*: T3-Salicylic acid; T4-Endomycorrhizal fungi (AMF); T5-Fluoride + Trichoderma (*T. viride*:); T6- Fluoride + Salicylic acid T7- Fluoride + Endomycorrhizal fungi (AMF): T8- Fluoride + Trichoderma (*T. viride*) + Salicylic acid; T9-

Fluoride + Trichoderma (*T. viride*)+ Endomycorrhizal fungi (AMF); T10-Fluoride + Endomycorrhizal fungi (AMF) + Salicylic acid; T11-Fluoride + Endomycorrhizal fungi (AMF) +Trichoderma (*T. viride*) + Salicylic acid; T12-Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid.

Chlorophyll "a" Content (mg g^{-1} FW)

The individual and combined effect of salicylic acid, trichoderma and mycorrhiza on chlorophyll 'a' were studied in pearl millet variety Moti Bajra, under the fluoride stress. Data of chlorophyll was recorded at 30, 60, 90 and 120 days after sowing (DAS) (Table 3, Fig. 5). It is evident that the average chlorophyll content was significantly reduced with 39.4% at 30DAS, 6.6% at 60DAS, 24.3% at 90DAS and 46% at 120DAS when plant exposed to fluoride stress (T1) as compared to control (T0). Similarly, when the exogenous application of Mycorrhiza applied in the soil of treatment (T4) than the content of chlorophyll was raised by 36.3%, 5.1%, 20%, and 66.7% at 30, 60, 90 and 120 DAS of an interval as compared to controlled treatment (T0). From the result when trichoderma is applied with fluoride toxicity in treatment (T5) it was indicated that average content of chlorophyll was reduced by 13% at 30DAS and then increased by 8.7% at 60DAS, 46.14 at 90DAS and 47.7% at 120 DAS of an interval as compared to the treatment where fluoride toxicity was created artificially in treatment (T1). Similarly, when the plant exposed to fluoride toxicity with combined application of mycorrhiza than average content of chlorophyll was reduced by 1.9% at 30DAS and increased by 20.1% at 60DAS, 51.3% at 90DAS and 4.4% at 120DAS of an interval as compared to treatment (T1). In the application of biofertilizer and salicylic acid in presence of fluoride toxicity the content of chlorophyll was increased by 9.2%, 33.7%, 18.3% and 24.3% at 30, 60, 90 and 120DAS of an interval as compared to the treatment (T1). The application of mycorrhiza helps to mitigate the stress of fluoride and provide sufficient nutrients to the plant that increases the growth, morphological and biochemical parameters of the plant. Deeptimayee Panigrahy *et al*., 2019 [12] conducted a pot experiment to determine the *Rhizophagus irregularis* fungi effect by immunizing in *Eleusine coracana* cultivate under various Zinc concentration. Parameters of plant growth as the length of shoot, biomass, length of root, other biochemical parameters as carbohydrates, total protein, the content of proline, sugar reduction, total chlorophyll, activities of catalase were determined. The result shows that 100 ppm concentration of zinc increases the growth of a plant and the concentration of zinc goes above 200 ppm causes stress in the plant. The activities of enzymes antioxidation were increases and stress induced by the different concentration of zinc alleviated by inoculation of arbuscular mycorrhiza.

Table 3: Chlorophyll "a" Content (mg g^{-1} FW) Pearl Millet during *Kharif*

Treatments	30DAS	60DAS	90DAS	120DAS
T0	15.20^{a}±.47	21.57^{fg}±.08	18.57^{bcd}±3.73	16.04^{b}±.46
T1	9.21^{de}±.16	20.14^{i}±.06	14.05^{d}±1.26	8.66^{h}±.02
T2	14.90^{a}±1.26	20.99^{gh}±.39	12.34^{d}±4.14	8.67^{h}±.02
T3	9.99^{c}±.19	21.42^{g}±.02	14.84^{d}±3.67	9.71^{ef}±.04
T4	9.67^{cd}±.04	20.45^{hi}±.03	20.05^{abcd}±8.25	9.67^{f}±.02
T5	8.15^{f}±.03	22.08^{f}±1.10	26.09^{abc}±6.21	16.59^{a}±.02
T6	5.95^{h}±.34	20.25^{i}±.04	27.43^{ab}±2.75	5.81^{j}±.02
T7	9.03^{de}±.06	25.21^{c}±.04	28.86^{a}±8.28	9.06^{g}±.03
T8	8.71^{ef}±.08	30.04^{b}±.15	16.25^{cd}±5.73	10.26^{d}±.02
T9	9.72^{cd}±.24	24.61^{d}±.08	14.18^{d}±5.85	9.92^{e}±.03
T10	10.15^{c}±.10	30.40^{b}±.04	17.21^{bcd}±5.71	11.44^{c}±.01
T11	11.47^{b}±.12	31.15^{a}±.04	17.72^{bcd}±5.34	9.60^{f}±.02
T12	7.16^{g}±.01	22.74^{e}±.04	12.77^{d}±5.31	7.11^{i}±.02

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Fluoride; T2-Trichoderma (*T. viride)*: T3-Salicylic acid ; T4-Endomycorrhizal fungi (AMF); T5-Fluoride + Trichoderma (*T. viride*:); T6- Fluoride + Salicylic acid T7- Fluoride + Endomycorrhizal fungi (AMF): T8- Fluoride + Trichoderma (*T. viride*) + Salicylic acid; T9-Fluoride + Trichoderma (*T. viride*)+ Endomycorrhizal fungi (AMF); T10-Fluoride + Endomycorrhizal fungi (AMF) + Salicylic acid; T11-Fluoride + Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid; T12-Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid.

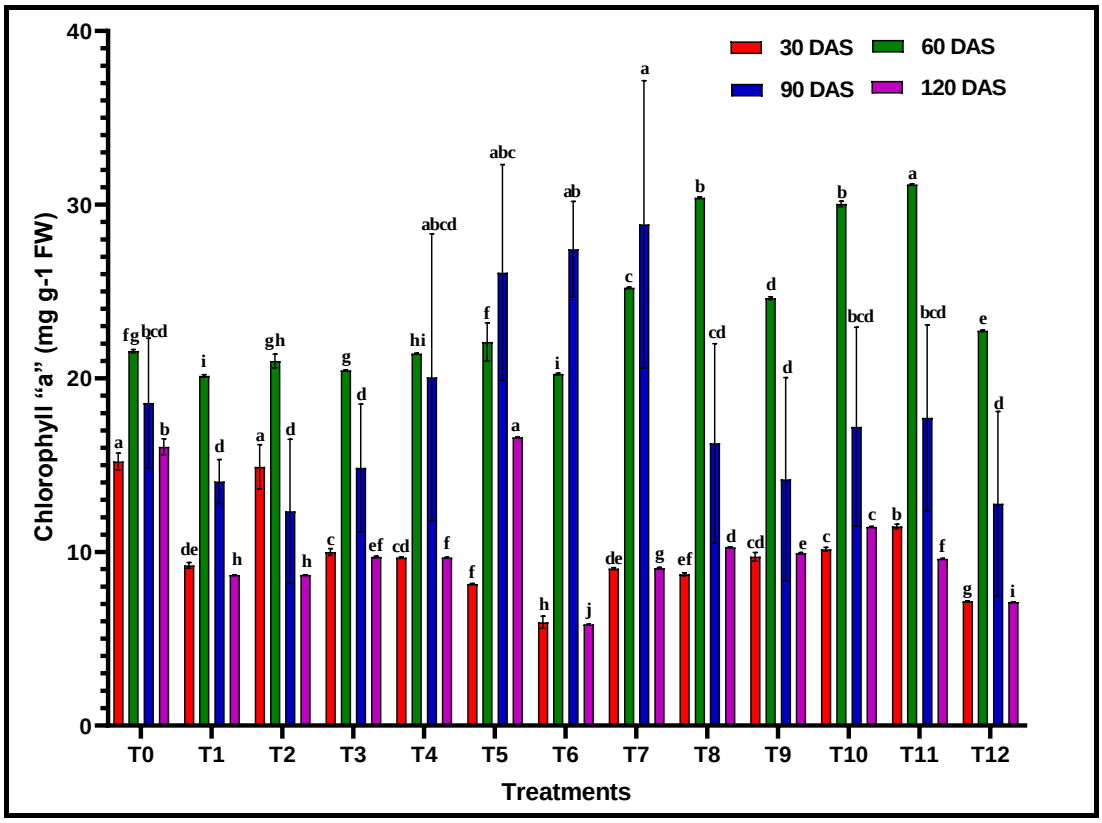

Fig 5: Chlorophyll "a" Content (mg g^{-1} FW) Pearl Millet during *Kharif*

where, DAS; Days After Sowing, Data in form of Mean±SD at $p<0.05$, T0- Control; T1- Fluoride; T2-Trichoderma (*T. viride)*: T3-Salicylic acid ; T4-Endomycorrhizal fungi (AMF); T5-Fluoride + Trichoderma (*T. viride*:); T6- Fluoride + Salicylic acid T7- Fluoride + Endomycorrhizal fungi (AMF): T8- Fluoride + Trichoderma (*T. viride*) + Salicylic acid; T9-Fluoride + Trichoderma (*T. viride*)+ Endomycorrhizal fungi (AMF); T10-Fluoride + Endomycorrhizal fungi (AMF) + Salicylic acid; T11-Fluoride + Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid; T12-Endomycorrhizal fungi (AMF)+Trichoderma (*T. viride*) + Salicylic acid.

Conclusion

From the result, it concluded that plant height reduced when exposed to the fluoride toxicity as compared to control. Exogenous application of mycorrhiza with fluoride toxicity was able to mitigate the fluoride toxicity by enhancing the height of the plant as compared to fluoride stress. The average stem girth of the plant was significantly increased in controlled treatment as compared to treatment were artificially created fluoride toxicity. The chlorophyll content was reduced when plant exposed to fluoride stress as compared to control treatment. In the application of mycorrhiza and salicylic acid in the presence of fluoride toxicity, the content of chlorophyll was increased compared to fluoride stress. The application of mycorrhiza helps to mitigate the stress of fluoride and provide sufficient nutrients to the plant that increases the growth, morphological and biochemical parameters of the plant.

Acknowledgements

All the authors gratefully acknowledge the support provided by Lovely Professional University, Punjab.

Author Contributions

All the authors equally contributed to writing the manuscript.

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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