Assessment of foliar spray of iron and salicylic acid under artificial magnetism on Various Metabolites of *Pisum sativum*

ABSTRACT

The appraisal of foliar treatment of iron (Fe) and salicylic acid (SA) on plant under artificial magnetism is very crucial in understanding its impact on growth and development of plants. The present study was designed to document the potential role of Fe and SA on pea (*Pisum sativum* L.) mature variety exposed to different magnetism treatments (geomagnetism and artificial magnetism). Thus, a pot experiment was conducted using Completely Randomized Design under factorial with three replicates. Various artificial magnetic treatments were applied in pots prior to sowing. Further, 15 days germinated pea-seedlings were foliar supplemented with 250 ppm Fe and 250µM SA, moreover after 20 days of foliar fertilization plants were harvested to analyze and record various physiological attributes.

Data elucidate significant variations in pea plants among different treatments. Artificial magnetism treatments in combination with foliar application of Fe and SA significantly improved various contents of pea plants. It was observed that artificial magnetism with addition of Fe and SA foliar spray improved the tannin content, ascorbic acid as well as phytic acid in pea plants. Consequently, the present research interestingly highlights progressive role of Fe and SA foliar treatment on pea plants under artificial magnetism. Thus, foliar supplementation may be suggested for better growth and development of plants combined with magnetic treatments.

Introduction

Field pea or Green pea (*Pisum sativum* L.) belongs to family Fabaceae (legumes), one of the most important families of angiosperms. It is an annual crop grown in cold season. [1]. Family contains annual and pre-annual herbaceous plants, important both nutritionally as well as economically. Most members of this family serve as major sources of protein for both animals and humans by playing an important role in increasing soil fertility with nitrogen-fixing bacteria [2]. Germination of *Pisum sativum*

seeds can be enhanced by giving pre-sowing treatments bringing change in chemical and physical attributes by the process of breaking dormancy that help the seeds fight against abiotic, biotic stresses, pests and diseases, that gives uniformity in the healthy growth of crops [3]. Hettiarachchi *et al.* [4] found that TOP2 expression (Topoisomerase II) in green peas gets stimulated under different abiotic stresses such as cold and salinity stresses.

The changes that magnetic field is essential by playing an important role in the living environment. The effects of magnetic field bring changes in development and growth of living organisms. Plants are indeed the best examples to evaluate the biological changes of magnetic fields [5]. Magnetic field treatment effects on various plants have been reported in various studies with amazing results particularly under different abiotic sufferings such as salinity [3], drought [6] and temperature [7]. Electromagnetic field stimulates seed germination below an optimum treatment [8]. Electromagnetic field increases energy of seed and plays important role in energy dispersion to biomolecules and stimulates the metabolism hence, increasing germination [8]. Magnetic and electromagnetic treatments lead to enhance vigor of seed, enhance germination of seed and growth by vitalizing the protein activities, and by enhancing antioxidant enzymes [9–11]. Khade and Mancharkar [12] reported that results of different researches have shown beneficial effects of magnetic fields on growth and germination of selected varieties of beans observing enhancement in growth parameters and excessive yield. Mahajan and Pandey [13] studied that pre-sowing treatment of seeds with static magnetic field of 226 mT (millitesla) for 100 min enhanced germination rate and lowered the MDA, hydrogen peroxide (H_2O_2), and superoxide (O_2-) in *Vigna radiata* [3] also studied that seed priming at 200 mT enhanced α-trocopherol and ascorbic acid contents decreased H_2O_2 in soybean. Moreover, magnetic treatment before sowing of seed allows to decrease the cost of planting as growth rates enhanced [13]. Moon and Chung [14] studied that magnetic field significantly effects the germination of seed of tomatoes. In another study, Zia-Ul-Haq *et al.* [15] noted that physical before sowing treatments such as sowing seeds with magnetic treatment are much more environmental friendly, secure, and much more feasible than chemicals. It also increases the free movement of ions and radicals in the soil without causing any damage to the soil

organic matter and also has no harmful effects on seed profile thus resulting in healthy and uniform crop stands. There are many studies that treatment of water with magnetic field increases the overall physiological attributes by increasing amount of vitamin c in many plants and also reducing the farm operations. [16]. Teixeira and Dobránszki [17] noted that magnetic field can alter plant growth and development by stimulating the growth parameters by increasing photosynthetic pigments and endogenous activity of ascorbic acid, also increasing rate of cell regulation activity, metabolic activity by increased activity of tannins [18]. Magnetic field treatment increases the phytic acid transportation in plants thus resulting in increased growth [19].

Plant growth regulators are known to be positively affect the seed growth of different plant species. Many crops such as soybeans, peas, corn, and small grains have very less dormancy period after seed maturity. Gibberellins stimulate the seed dormancy [20]. Gibberellic acid plays important role in many biological functions of plants such as height of plant, cell division, expansion and elongation of leaves, photosynthesis, rate of transpiration rate and flowering [21]. Change in rate of metabolism of some plants like peas with plant growth regulators to control the diseases has shown contrasting results. Salicylic acid in recent times has become the keen interest in phytohormone response as it designates to be endogenous regulatory signals activating defense response to various abiotic stresses; heat [22], extreme drought [23], osmotic stress [24], heavy metals [25], and coolness [26]. Moreover, it plays key role in local and systematic responses under abiotic stress [27]. Salicylic acid (SA), also known as 2-hydroxy benzoic acid, is the most diverse group of phenolic compounds consisting of an aromatic ring having a hydroxyl group as a functional group that is biosynthesized by plants. SA induces tolerance in plants by mediating signal transduction in plants against abiotic stress [28, 29]. SA when applied foliarly enhancing contents of catalase (CAT) peroxidase (POD), relative water content (RWC), soluble sugars, superoxide dismutase (SOD), glycine betaine, protein, root and leaf K^+ while decreased Na^+, H_2O_2, O_2^-, and MDA contents [30]. SA priming increases total proline, soluble sugars, and indole acetic acid while decreases abscisic acid, total soluble protein and gibberellins [31]. Moreover, Yadav *et al*. [32] reported an increase in grain yield of wheat and height of plant and with better RWC%, total chlorophyll contents, and fluorescence variable/ fluorescence maximum with SA foliar

supplement under drought and salinity stress; however, membrane injury and lipid peroxidation gets hampered by validating the defensive role of SA. Moreover, SA negatively and positively parallel with other plants regulators to protect plants against pathogen attack [33]. Iron (Fe) is 4th most rich element on the earth and mostly found in the crystal lattices of most minerals [34]. Fe in plants is found mostly in Fe (III) form and very less in Fe (II) form and about 90% found in chloroplast and particularly in chloroplasts of those leaves which undergo rapid growth [35]. Quality of soil is very important for growth of plant and development. Deficiency of Fe leads to calcareous soil conditions, whereas higher amount of Fe in soil leads to waterlogging soil conditions [36]. Due to deficiency of Fe in plants chlorosis occurs in leaves that results in decreased translocation of photosynthates [37], limited accumulation of total reduced growth of root and shoot, biomass and area of leaf [38]. However, when there is a higher amount of Fe in plants, it can cause brown spots on leaf, reduced activity of photosystem II and decreased rates of photosynthesis [39]. Literature documented that exogenous supplement of Fe enhances the growth and productivity of crops [40–42]. Though vast literature regarding magnetic treatment and impact of foliar supplementation of SA and Fe has been documented but no research has yet been reported on the combined effects of the two factors (magnetism and foliar supplementation). Present study was conducted to evaluate the influence of geo and artificial magnetism on seedling growth of *Pisum sativum* L. and to determine the possible role of foliarly applied SA and Fe in improving seedling growth in response to magnetism.

Materials and methods

Study area: Study area of this research is Quetta which is the capital city of Balochistan province of Pakistan. Quetta is situated at 1679 meters above sea level. Whereas, the climate of the Quetta is quite cold and semi-arid. The temperature of Quetta is very diverse between summers to winter. The average rainfall is less than 250 mm per year. While June and July are the warmest months of the year where the temperature ranges from 35˚C to 40˚C. January is the coolest month of the year when the temperature ranges from 11˚C to -7˚C or less. For the experiment, seeds of mature variety of *Pisum sativum* were taken from the Balochistan Agriculture Research and Development Centre (BARDC). The experiment

was conducted in the Botanical Garden University of Balochistan, Quetta.

Experiment layout: A pot experiment was conducted in the prevailing environmental conditions of Quetta. The experimental design was completely randomized under factorial with three replicates. Pots of 32 cm diameter were filled with 8 kg of soil from Agriculture Department Quetta. The soil ratio was taken as 60:20:20. i.e., soil, sand, and manure, respectively. Seeds were sown at 1-inch depth. Five seeds were sown in each pot. Different treatments of artificial magnetism were applied prior to the sowing of the seeds in comparison to geo magnetism treatment (no external magnet placed). Treatment includes Geo Magnetism; Artificial Magnetism that includes; magnet South (S) root, magnet North (N) root, S root/ N shoot, N root/ S shoot, N/S root and S/N shoot, S/N root and N/S shoot under complete set of; no foliar spray (Control conditions/ control set/ control), Fe 250 ppm and SA 250 µM spray. Thus, a total 63 pots were used. The foliar concentration was selected on preliminary trials and one of the most suit able level was selected for the present research. After 15 days of seed germination, selected level of SA and Fe were foliarly applied to both the sets (i.e., geo and artificial magnetism treated plants). After 20 days of foliar spray, plants were harvested, and data was collected regarding various physiological attributes.

Tannins Analysis: Samples (root and shoot) were taken and mixed with 2 mL of diethyl ether and left overnight, and then decanted the solution, and 1 mL of 70% acetone was added and kept overnight. From each sample, 50 µL of the extract was taken in each test tube, and the volume was made up to 1 ml with distilled water. After dilution, 0.5 mL of folin phenol reagent was added vortexed, and then added 2.5 ml of 20% Na_2CO_3 solution mixed very well and kept for 40 min at room temperature. The absorbance was recorded at 725 nm using 70% acetone as a blank.

Ascorbic Acid (Vitamin C) Analysis: Ascorbic acid (Vitamin C) was analyzed with the titrant 2, 6-Dichlorophenolindophenol according to AOAC [43].

Principle: 2, 6-Dichlorophenolindophenol (DCPIP) is reduced to a colorless form by ascorbic acid. The dye is blue in alkaline solution and red in acid.

Preparation of Reagents: 2, 6- Dichlorophenolindophenol solution (0,001 mol/L): 0.05 g of DCPIP was dissolved in distilled water, diluted to 100 ml, and filtered. The

DCPIP solution was kept in a refrigerator.

Ascorbic Acid Solution: 0.05 g of pure ascorbic acid was dissolved in 20 mL of 10% oxalic acid and diluted with distilled water to exactly 250 mL in a volumetric flask.

Standardization of the 2, 6- Dichlorophenolindophenol: 10 mL of standard ascorbic acid solution was pipetted into a small flask and titrated with 2, 6-Dichlorophenolindophenol solution until a faint pink color persisted for 15 seconds. The concentration was expressed as mg ascorbic acid equivalent to 1 mL of DCPIP solution.

Titration: 5 g of green peas were weighed exactly into a breaker. 40 mL of oxalic acid was added and stirred for 5 minutes, then filtrated into a 100 mL volumetric flask and diluted to volume with distilled water. 10 mL was pipetted into a small flask and 2.5 mL acetone added. The solution was titrated with DCPIP until a faint pink color persisted for 15 seconds. Repeated with the blank sample.

Calculation: Vitamin (C % mg) = (a - b) * f * V1 * 100 / W * V2

a: mL for test solution titration

b: mL for test blank titration

f: mg ascorbic acid equivalent to one mL DPIP standard solution

V1: volume initial test solution

V2: volume test solution titrated W: weight of sample

W: weight of the sample

Pythic Acid Analysis: The barium phytate (Ba4 Phy) standard was prepared by dissolving Commercial phytin in 3% trichloroacetic acid (TCA) and filtered. Ferric phytate (Fe4 Phy) was precipitated from the filtrate by adding concentrated FeCl, solution. The precipitate was washed with 3% TCA, slurred in water, and converted to sodium phytate and Fe (OH), by addition of NaOH. The $Fe(OH)_3$ was filtered out, the sodium phytate solution adjusted to about pH 6 with HCl, and Ba4 Phy precipitated by addition of $BaCl_3$. The Ba4 Phy was dissolved in HCl and the double precipitation cycle repeated twice; the final Ba Phy was washed thoroughly with water followed by methanol, and dried in vacuum at 80°C.

Method: Weighed a finely ground sample (40 mesh) estimated to contain 5 to 30 mg.

phytate P into a 1 25-ml. Erlenmeyer flask.

Extracted with 50 ml. 3% TCA for 30 min, with mechanical shaking or 45 min. with occasional swirling by hand.

Centrifuged the suspension and transferred a 10-ml. Aliquot of the supernatant into a 40-ml. conical centrifuge tube.

Heated the tube and contents in a boiling-water bath for 45 min.

Centrifuged (10 to 15 min.) and carefully decant clear supernatant.

Washed precipitate twice by dispersing well in 20 to 25 ml. 3% TCA, heating in boiling-water bath 5 to 10 min., and centrifuging.

Repeated wash once with water.

Dispersed the precipitate in a few ml. water and added 3 ml. 1.5N NaOH with mixing.

Brought the volume to approximately 30 ml. with water and heated in boiling-water bath for 30 min.

Filtered hot (quantitatively) through a moderately retentive paper.

Washed precipitate with 60 to 70 ml. hot water and discarded filtrate

Dissolved the precipitate from the paper with 40 ml. hot 3.2N HNO_3 into a 100-ml. volumetric flask.

Washed paper with several portions of water, collecting the washings in the same flask.

Cooled flask and contents to room temperature and diluted to volume with water.

Transferred a 5-ml. aliquot to another 100-ml. volumetric flask and diluted to approximately 70 ml.

Added 20 ml. of 1.5M KSCN, diluted to volume, and read color immediately (within 1 min.) at 480 rim.

Ran a reagent blank with each set of samples.

Calculated iron content from a $Fe(NO_3)_3$ standard run at the same time or read from a previously prepared standard curve.

Calculated the phytate phosphorus from the iron results assuming a 4:6 iron: phosphorus molecular ratio [44].

Statistical Analysis: Statistical analysis was performed using "STATISTIX 8.1". Graph, mean and standard deviation calculated using MS. EXCEL.

Results:

Root Tannins: Data regarding tannin contents in roots of *Pisum sativum* revealed significant differences ($P < 0.05$) among different treatments. The highest tannin content was observed in plants which were given S/N root N/S shoot to root under Fe foliar spray while least tannin content was found when plants were subjected to S root / N shoot treatment under Fe foliar spray (Fig. 1)

The trend observed for root tannins contents of pea plant under artificial/ geo magnetism treatments and different foliar spray is as follow: S/N root & N/S shoot + Fe foliar spray > N/S root & S/N shoot + foliar spray > Geo magnetism + foliar spray > S root & N shoot + Control > S/N root & N/S shoot + control > S root + Control > N/S root & S/N shoot + SA foliar spray > N root & S shoot + Fe foliar spray > Geo magnetism + control > N root + Control > N root + SA foliar spray > S root + foliar spray > Geo magnetism + SA foliar spray > N/S root & S/N shoot + control > N root & S shoot + control > N root + foliar spray > N root & S shoot + SA spray > S root + SA foliar spray > S root & N shoot + SA foliar spray > S/N root & N/S shoot + SA foliar spray > S root & N shoot + foliar spray **(Fig. 1)**.

In a nutshell, the overall results of root tannins of *Pisum sativum* revealed that the level of tannin in roots increased under different artificial magnetism treatments and Fe foliar supplementation (Fig. 1).

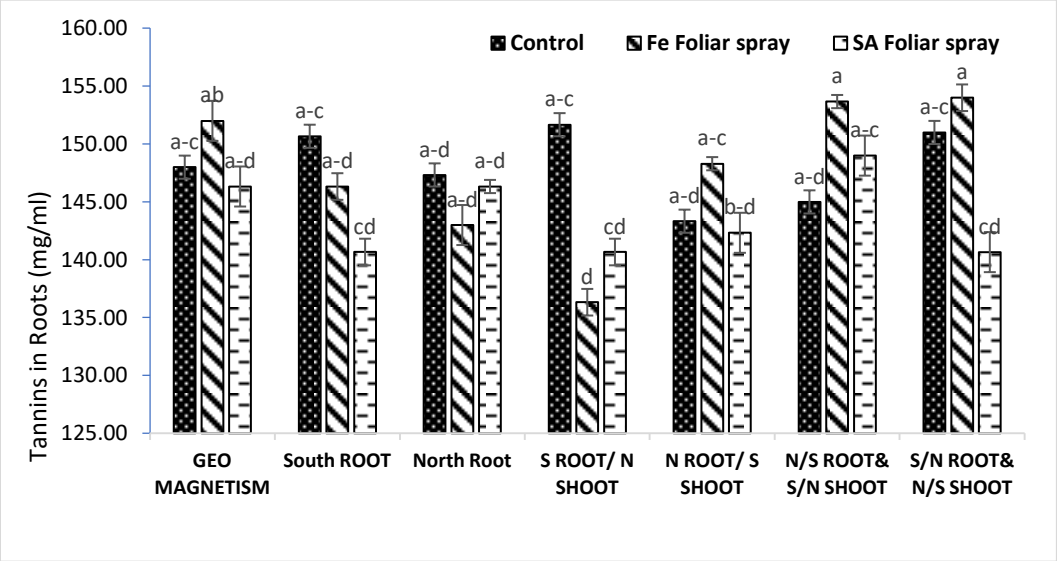

Figure (1): Effect of different magnetic treatments on the root tannin contents of *Pisum sativum* in response to Fe and SA Foliar Spray.

Shoot Tannins: Data recorded for the shoot tannin contents of Pea plant showed statistically significant ($P < 0.05$) results. The highest tannin content was recorded under S root & N shoot with SA foliar spray; however, minimum tannin content was observed under S/N root & S/N shoot treatment under control condition (Fig. 2).

The order of changes observed for shoot tannin contents of pea plant under artificial / geo magnetism treatments and different foliar spray are as follows: S root & N shoot + SA foliar spray > N root & S shoot + control > S root + Fe foliar spray > S root + control > S root & N shoot + control > N root & S shoot + Fe foliar spray > Geo magnetism + control > S root + SA foliar spray > N root + Fe foliar spray > N root + control > S root & N shoot + Fe foliar spray > Geo magnetism + Fe foliar spray > S/N root & N/S shoot + SA foliar spray > N root & S shoot + SA foliar spray > N root + SA foliar spray > Geo magnetism + SA > N/S root & S/N shoot + Fe foliar spray > N/S root & S/N shoot + control > S/N root & N/S shoot + Fe foliar spray > S/N root & S/N shoot control **(Fig. 2)**.

Considering the results of tannin in shoots of *Pisum sativum*, revealed that the level of tannin in shoots increased under different artificial magnetism treatments and Fe/SA foliar supplementation **(Fig. 2).**

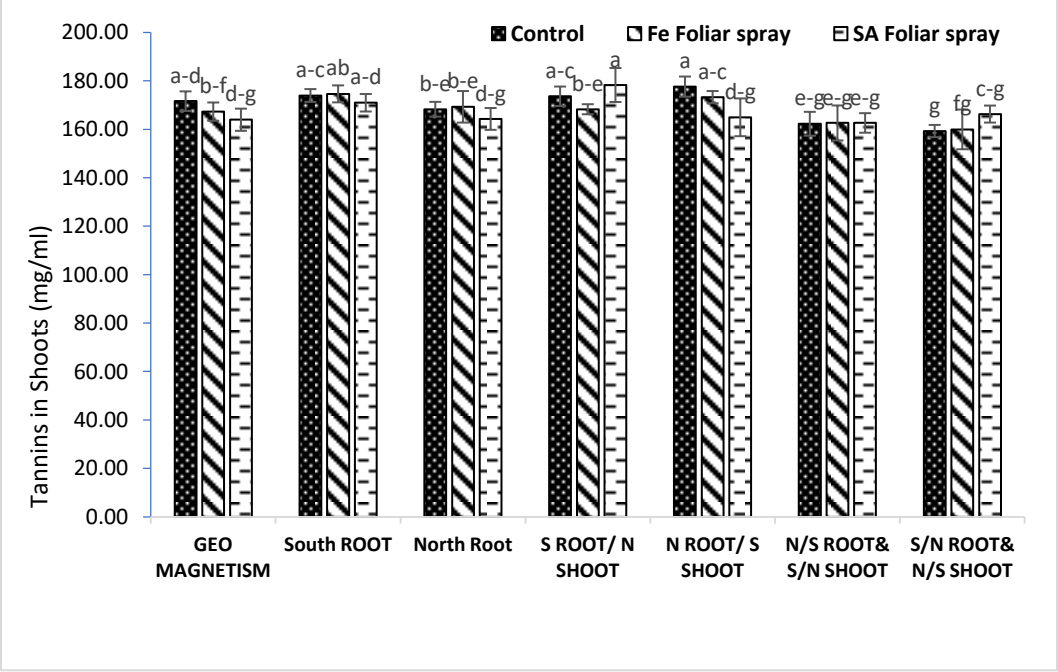

Figure. 2. Effect of different magnetic treatments on the shoot tannin contents of *Pisum sativum* in response to Fe and SA Foliar Spray.

Root Ascorbic acid: Results obtained for roots ascorbic acid contents of *Pisum sativum* showed statistically significant ($P < 0.05$) results by revealing that the maximum ascorbic acid accumulation was observed in roots of those plants which were grown under S root / N shoot magnetism + control condition while minimum ascorbic acid content was observed under S root under control condition (Fig. 3).

Data regarding ascorbic acid content in roots of *Pisum sativum* showed the following trend under artificial / geo magnetism with Fe and SA foliar spray: N root & S shoot + control > S root & N shoot + SA foliar spray > N root & S shoot + SA foliar spray > S/N root & N/S shoot + control > Geo magnetism + Fe foliar spray > N root + SA foliar spray > N/S root & S/N shoot + Fe foliar spray > S root & S shoot + Fe foliar spray > N/S root & S/N shoot + control > N root & S shoot + Fe foliar spray > N root + Fe foliar spray > S root + Fe foliar spray > Geo magnetism + SA foliar spray > S root + control **(Fig.** 3**)**.

Hence, from the overall variations of root ascorbic acid contents, it has been determined that ascorbic acid accumulation in roots increased when given the artificial magnetism treatments under control conditions and least nitrate contents was observed under S root magnetism and control condition **(Fig. 3)**.

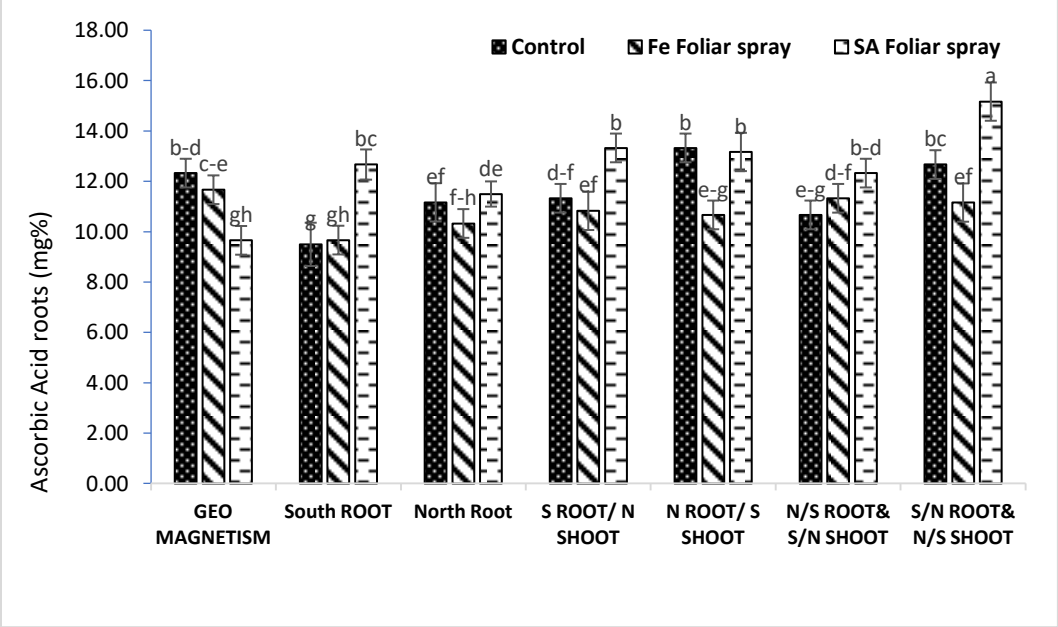

Figure (3): Effect of different magnetic treatments on the root Ascorbic acid contents of *Pisum sativum* in response to Fe and SA Foliar Spray.

Shoot Ascorbic Acid: Results recorded for Ascorbic acid in shoots of *Pisum sativum* showed statistically significance ($P < 0.05$) results among different treatments. The maximum Ascorbic acid content was observed in shoots of those *Pisum sativum* which were grown under S root and N shoot magnetism under control condition; however, minimum Ascorbic acid was observed under S/N root & N/S shoot with control condition (Fig. 4).

Hence, the order of changes observed for ascorbic acid content of shoot are given as: S root & N shoot + control > S root + SA foliar spray > S root & N shoot + SA foliar spray > Geo magnetism + SA foliar spray > N root & S shoot + control > N root + control > N root + Fe foliar spray > N/S shoot & S/N shoot + control > S root & N shoot + Fe foliar spray > N root & S shoot + SA foliar spray > S root + control > S root + Fe foliar spray > Geo magnetism + control > S/N root & N/S shoot + Fe foliar spray > N/S root & S/N shoot + SA foliar spray > Geo magnetism + Fe foliar spray > N/S root & S/N shoot + Fe foliar spray > N root + SA foliar spray > S/N root & N/S shoot + control **(Fig. 4)**.

Considering the results of ascorbic acid in shoots of *Pisum sativum* under foliar spray and artificial magnetism treatments, it was observed that SA and Fe foliar applications helped in improving the amount of ascorbic acid under different magnetism treatments **(Fig. 4)**.

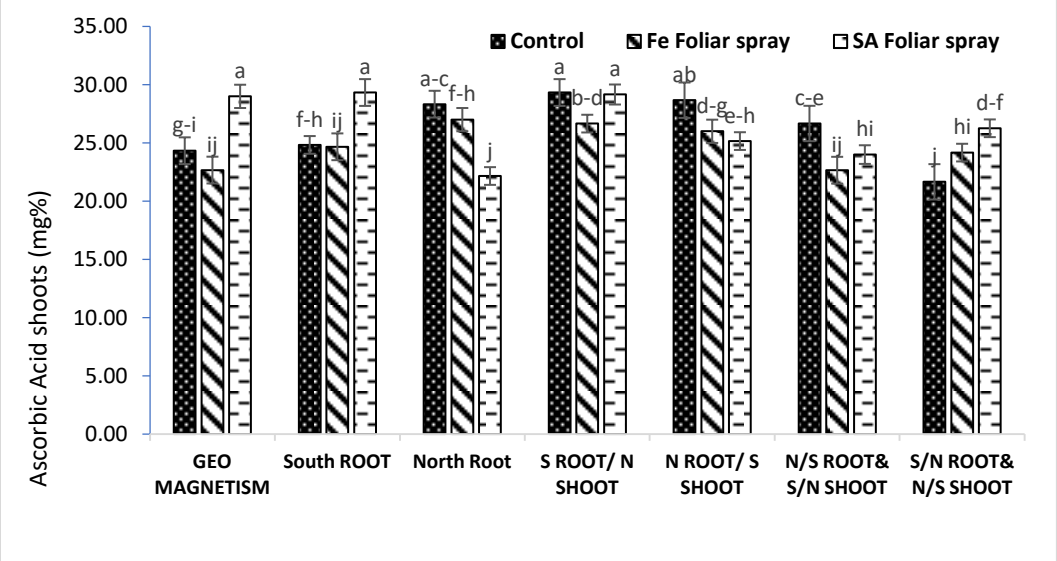

Figure (4): Effect of different magnetic treatments on the shoot Ascorbic acid contents of *Pisum sativum* in response to Fe and SA Foliar Spray.

Root Phytic Acid: The results obtained for phytic acid content in roots of pea plant showed statistically significant ($P < 0.05$) variations. Maximum phytic acid was observed in roots of S/N root & N/S shoot treatment plants under Fe foliar spray, whereas least phytic acid was observed in roots of S root / N shoot magnetism under control conditions **(Fig. 5)**.

The trend observed for root phytic acid content under artificial / geo magnetism with Fe and SA foliar spray is observed as : S/N root & N/S shoot + Fe foliar spray > N root + control > N/S root & S/N shoot + Fe foliar spray > N root & S shoot + Fe foliar spray > S root & N shoot + Fe foliar spray > S/N root & N/S shoot + control > S/N root & N/S shoot + SA foliar spray > S root + Fe foliar spray > N root + Fe foliar spray > S root + control > Geo magnetism + SA foliar spray > N root & S shoot + control > N root & S shoot + SA foliar spray > S root + SA foliar spray > Geo magnetism + Fe foliar spray > Geo magnetism + control > N/S root & S/N shoot + SA foliar spray > N/S root & S/N shoot + control > N root + SA foliar spray > S root & N shoot + SA foliar spray > S root & N shoot + control **(Fig. 5)**.

In a nutshell, overall results of phytic acid accumulation in roots of *Pisum sativum*, revealed that although the levels of phytic acid in roots increased under different artificial magnetism treatments and Fe foliar supplementation as compared to control treatments but still the levels were quite low occurring at 1-2 mg/ml percent when bio-chemically analyzed **(Fig. 5)**.

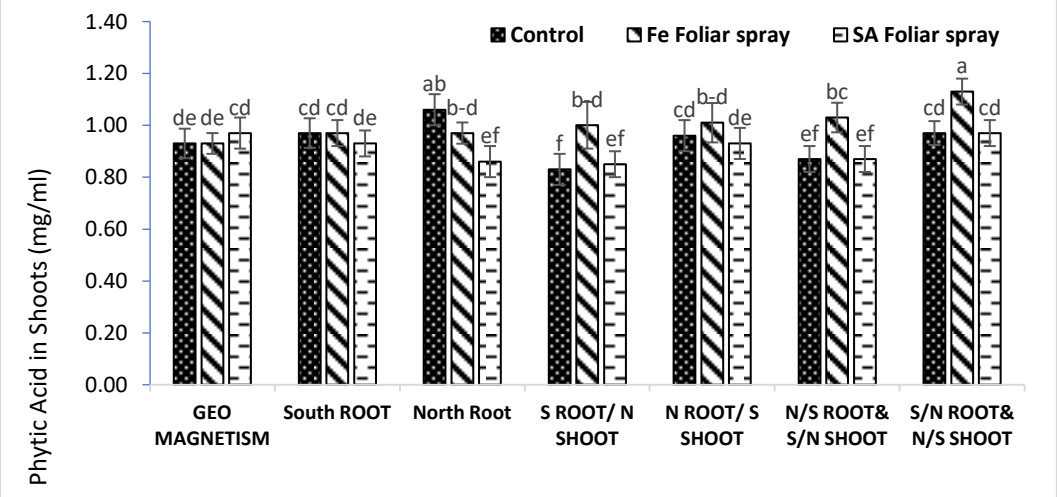

Figure (5): Effect of different magnetic treatments on the root Phytic acid contents of *Pisum sativum* in response to Fe and SA Foliar Spray.

Shoot Phytic Acid: Data obtained for Phytic acid accumulation in shoot of pea plant showed statistically non-significant ($P > 0.05$) results. However, the highest Phytic acid content was observed in the shoots of those pea plants grown under S root magnetism control condition whereas lowest Phytic acid content was observed in the shoots of pea plants under S root and N shoot magnetic treatment with Fe foliar application **(Fig. 6)**.

Hence, the results for Phytic acid content in shoots of pea plant revealed the following trend: S root + control > N root + Fe foliar spray > Geo magnetism + Fe foliar spray > N/S root & S/N shoot + SA foliar spray > Geo magnetism + control > S/N root + N/S shoot + SA foliar spray > N root & S shoot + SA foliar spray > N root + control > S/N root + N/S shoot + control > S root + Fe foliar spray > S root & N shoot + control > N/S root & S/N shoot + Fe foliar spray > S root + SA foliar spray > N root & S shoot + Fe foliar spray > S/N root & N/S shoot + Fe foliar spray > N/S root & S/N shoot + control > N root + SA foliar spray > S root & N shoot + SA foliar spray > N root & S shoot + control > Geo magnetism + SA foliar spray > S root & N shoot + Fe foliar spray **(Fig. 6)**.

Considering the results of phytic acid in shoots of *Pisum sativum* under foliar spray and artificial magnetism treatments, it was observed that there was very low level of phytic acid observed when plants were grown under geo/artificial geo magnetism in addition with SA and Fe foliar applications **(Fig. 6)**.

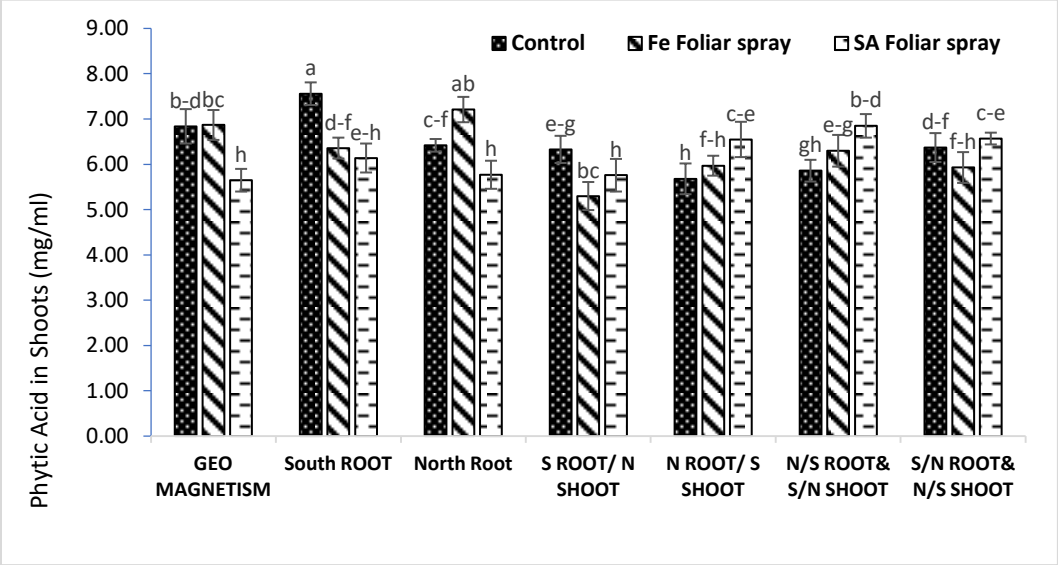

Figure. 6. Effect of different magnetic treatments on the Phytic acid shoot content of *Pisum sativum* in response to Fe and SA Foliar Spray.

Discussion

Tannins are found almost in all plants and in all climates throughout the world. The name tannin has been taken from the French word tannin and is used for a range of natural polyphenols. Plants such as algae, fungi and mosses do not contain much tannin. Whereas tannins are present in significant proportions in some plants, many others have too little. Tannins are usually found in large quantities in the bark of trees where they play an important protective role against micro-organisms by protecting the trees. Tannins are astringent, bitter plant polyphenols that either bind or shrink proteins. The term is applied to any large polyphenolic compound containing hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Alonso-Amelot *et al.*, [45] observed that an induced chemical adaptive response in non-adapted plants grown at high altitudes. On comparing sun exposed and self shaded fronds in *Pteridium arachnoideum* (fern), an accumulation of higher amounts of phenols and tannins was noted, with the increase in altitude. Dry season water-stress also caused an increase in phenols and tannins, thereby the conclusion that UV-B radiation and water availability are important factors in non-adapted plant acclimation response to stress at altitudinal gradients. Maheshwari and Grewal [16] reported about the studies which illustrated that magnetic treatment give several benefits in agriculture such as increasing biosynthesis of tannin content, reducing plant diseases, consequently improving quality of crop, increasing efficiency of fertilizers and reducing cost of farm operations, which is quite evident in the present findings, considering the results of tannin in roots and shoots of *Pisum sativum* revealed that the level of tannin in roots increased under different artificial magnetism treatments and Fe/SA foliar supplementation. Furthermore, it was observed that when pea plants were given the magnetic treatments with Fe/SA foliar spray there was overall increased production in tannin content in roots of *Pisum sativum* as compared to those plants which were not given the magnetic treatments and were not applied with Fe/SA foliar spray **(Fig. 1, 2)**. As present result showed that when pea plants were applied with foliar spray of Fe/SA, consequently increased the tannins accumulation in both roots and shoots of the pea plants which is quite consistent to those results of Borsani *et al.*, [46] which reported that salicylic acid has been observed to produce tannin content which help in a protective effect in plants

under stress factors of different abiotic nature, such as, heat, chilling, osmotic and salt stress. Spraying salicylic acid (200 ppm) in sunflower and ascorbic acid (100 ppm) in wheat caused the reduction of adverse effects of drought stress [47].Tannins are also present in the bark, fruits, leaves, fruits and seeds of many plants. The main function of these compounds is to provide protection against harmful insects, microbial pathogens and other herbivores. The storage of proantho-cyanidins in the endothelial layer of the seed coat in many species may be seen as a classic example of a pre-formed protective barrier[48].

Ascorbic acid is one of the most important compounds in plants that plays essential role in antioxidant activities in plants. It also plays an important role of modulating the plant development through hormones signaling. Ascorbic acid also acts as coenzyme in mobalizing the fats, protein and carbohydrates [49]. Hossain *et al*., [50] reported that under different adverse conditions reactive oxygen species (ROS) production in plants is common. In such conditions, plants counter these problems by their ability to produce ROS neutralizing substances, which include both non-enzymatic and enzymatic antioxidants. In this respect, ascorbic acid (AA) is one of the universal non-enzymatic antioxidants having substantial potential of not only scavenging ROS, but also modulating a number of fundamental functions in plants both under stress and non- stress conditions. Rosales *et al*., [51] reported that ascorbic acid increase the cell division and causes increasing dry and fresh weight of leaf on plants and also with antioxidant property decreases the damage from oxygen radicals, which are product by drought stress. Pastori *et al.,* [49] noted that the ascorbic acid has the ability of catching the free radicals or the active oxygen that produced during photosynthesis and respiration processes. Also, ascorbic acid (vitamin C) is one of the most important water soluble antioxidants in plants, which acts as a modulator of plant development by hormone signaling and as co-enzyme in reactions through which fats, carbohydrates and proteins are metabolized. External electric and magnetic fields have been reported to influence the stimulation of ions, vitamins and polarization of dipoles in living cells. Consequently, increasing the growth parameters [14]. It was noted in the present study that when plants were applied with magnetic field treatment in addition with Fe and SA it increased the ascorbic acid in both roots and shoots of the pea plants. It was observed

that when plants were given N root & S shoot treatment it increased the significant amount of ascorbic acid in both roots and shoots of the plants **(Fig. 3, 4)**. It was also documented by Hanan *et al.*, [52] that the antioxidant activity of herb increased by using treatment of magnetic and gave values of 933.50, 975.17, 1016.0 and 974.07µg of ascorbic acid/mg ext. in the two cuts during two seasons, respectively. Ascorbic acid as an abundant component in plant functions as an antioxidant and an enzyme cofactor. It takes part in a number of processes; include cell wall growth, photosynthesis, and cell expansion, resistance to stress and synthesis of gibberellins, ethylene, hydroxyl proline and anthocyanin [53].

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) also known as Inositol-6-phosphate or phytate in its salt form is found in concentrations of 1–3% dry matter in most cereals and legumes. Phytic acid is also found in some fruits and vegetables [54]. Phytic acid is an undesirable dietary agent which binds certain minerals such as Calcium (Ca"), Magnesium (Mg'"), [55] Iron (Fe"•) and Zine (Zn"). and renders them unavailable for their physiological functions by forming insoluble compounds [56]. Mate & Radomir, [57] found that during germination, phytase activity increases which is responsible for the reduction of phytic acid in plant seeds. It was also observed in the present study that when plants were bio-chemically analyzed there were very minute levels of phytic acid, and when plants were applied with the geo/artificial magnetism with addition of Fe and SA foliar spray. It was observed that when plants were given S/N root & N/S shoots in addition with Fe foliar spray phytic acid level was 1.13 mg/ml at its highest in roots. Whereas, the level was at its lowest 0.83 mg/ml when plants were grown with S root / N shoot treatment under control conditions. Whereas, highest level of phytic acid was observed when plants were grown with S root treatment under control conditions which was 7.56 mg/ml in the shoots of pea plants? Whereas, the level was at its lowest 5.97 mg/ml when plants were grown with S root / N shoot with Fe foliar spray **(Fig. 5, 6)**. Phytate is a normal constituent of almost all cereals constituting 1-3% by weight. About 30-90% of total phosphorus is in the form of phytate [58]. Rusydi & Azrina, [59] reported that phytate or phytic acid is formed when plant seeds are mature and it represents 60-90% of total phosphate in dormant seeds. Germination affects the content phytic acid in seed. Phytic

acid is stored as phosphate in seed of plants and often gets gathered in plant seed vacoule after biosynthesis. McDonald *et al.*, [60] Phytic acid provides phosphorus in young growing seedlings and during the seed germination,

Conclusion

Seeds treated with a magnetic field before sowing improves the growth and productivity of crops. Exposing *Pisum sativum* to artificial magnetism treatments in response to Fe and SA foliar application showed encouraging effects on plant growth as it improved several protective and growth contents by increased production of tannins, ascorbic acid and phytic acid of *Pisum sativum* in comparison to geomagnetism. Results also showed positive effects of artificial magnetism along with foliar supplementation of Fe and SA that enhanced tannin contents, amount of ascorbic acid as well as phytic acid in pea plant thus, suggesting the positive interaction of foliar spray with magnetism. In general, using different kinds of chemicals for better crop growth and yield, it has been a successful practice, but it is quite detrimental to the environment and soil. Using magnetic fields as a pre-sowing treatment with plant growth regulators can be safe and eco-friendly agricultural practices as com- pared to using chemicals. Moreover, understanding the positive and negative effects of different magnetic fields will help the researchers to understand the evolutionary changes in plants due to magnetic fields for future exploration. Furthermore, detailed proteomic and genomic analyses of plants treated with various magnetic treatments would further help to understand and explore the effects of magnetic fields on various plants.

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