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Assessment of Foliar Spray of Iron and Salicylic Acid under Artificial Magnetism on Various Metabolites of *Pisum sativum*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Evaluating the impact of foliar treatment of (SA) salicylic acid and iron (Fe) on plants with the treatment of artificial magnetism is very essential to understand its effects on germination and growth of *Pisum sativum*. The current research was designed to document the primary role of SA and Fe on matore variety of garden pea. Selected specie was given various geo and artificial magnetic treatments. Hence, a pot experimentation was designed using Completely Randomized Design under factorial with three replicates. Seeds were sown in pots having different geo and artificial magnetism treatments. Later, fifteen days of germination, pea plants were foliarly sprayed with 250 µM salicylic acid and 250 ppm iron. Furthermore, later twenty days of foliar supplementation seedlings were uprooted/harvested and taken to lab to analyze different bio-

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chemical attributes. Data annotate diverse variations in Matore variety among various magnetism treatments. Artificial magnetic treatments combined with foliarly supplementation of salicylic acid and iron significantly improved tannin content, ascorbic acid as well as phytic acid in pea plants. Thus, foliar supplementation based on this current research findings may be proposed for improved development and growth of plants in combination with magnetism.

Keywords: Foliar treatment; iron; salicylic acid; artificial magnetism.

1. INTRODUCTION

Pisum sativum commonly known as green pea or field pea, a member of Fabaceae (legumes) family, one of the most vital family of spermatophytes. It is an angiospermic crop, grown once a year in the cold season [1]. The family contains annual and bi-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 fertility of the soil by fixing nitrogen with the bacteria in their root nodules [2]. Hettiarachchi et al. [3] found that expression of TOP 2(Topo isomerase II) gene in green peas got stimulated by applying magnetic field treatment against abiotic stress such as cold. Growth of the seeds of *Pisum sativum* could be enhanced by applying magnetism treatments that bring changes in physiological and bio-chemical features by the process of breaking dormancy that help the seed to counter the abiotic, biotic stresses, ultimately helping in healthy growth of plants [4].

Magnetism plays key role in bringing changes in the living environment. Magnetism positively modifies the living cells of organisms helping in better growth and development. In this connection, plants are leading examples to evaluate chemical and physiological changes of magnetism [5]. Scientists have noted beneficiary effects of magnetism on various crops in their research with amazing results particularly against different abiotic sufferings such as salinity [3], drought [6] and temperature [7]. An electromagnetic field stimulates seed germination below an optimum treatment [8]. An electromagnetic field increases the efficiency of seed and plays important role in the dispersion of energy to activate bio molecules, stimulating the process of metabolism, hence, enhancing the germination [8]. Electromagnetic and magnetic treatments leads to enhance vigor of seed, enhance the germination of seeds and growth by vitalizing the protein activities [9–11]. Magnetism enhanced the growth and germination of the different variety of bean by improving the growth attributes [12]. Pre-sowing treatment of seeds with stationary magnetism of 226 millitesla for 90 minutes enhanced growth rate and lowered the superoxide (O_2^-), hydrogen peroxide (H_2O_2), and MDA in mung beans [3]. Moon and Chung [13] studied that magnetism treatment significantly effects the germination of seed of tomatoes. Priming of seed at 220 mT enhanced ascorbic acid and α-trocopherol contents lowered the H_2O_2 in soybean. Moreover, magnetic treatment before sowing of seed allows to decrease the expenditure of planting as growth rates enhanced [14]. Sowing seeds with magnetic treatment are secure, beneficial to environmental and very feasible as compare to applying chemicals [15]. Magnetism treatments speed up radicals and ions movement in the soil without causing any damage to the fertility of soil. Results of many studies are quite evident 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 positively modifies seedlings growth and crop yield by stimulating the growth parameters by speeding up the rate of process of photosynthesis 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 found to be positively affecting the growth and development of seeds of different plant species. Gibberellins activates the process of dormancy of vegetables like soya beans, corn, small grains and peas later seed maturity [20]. Gibberellic acid plays very essential role in many bio-physiological activities of seedlings including cell division, expansion, length of shoot, increase in leaf area, photosynthetic rate, flowering and rate of transpiration [21]. Change in metabolism rate of some plants such as pea and bean using plant growth regulators to counter the diseases have displayed contrasting results. In recent times salicylic acid has become the keen interest in phytohormone response to endogenously regulate signals activating immune response to counter abiotic stresses: extreme drought [22], heat [23], heavy metals [24], osmotic stress [25], and coolness [26]. Moreover, it plays key role in systematic responses against abiotic stress [27-29]. Salicylic acid when foliarly sprayed, it decreases Na^+, H_2O_2, O_2^-, and MDA (Malondialdehyde) whereas, enhances contents of protein, root and leaf K^+, peroxidase (POD), catalase (CAT), relative water content (RWC), soluble sugars, superoxide dismutase (SOD), glycine and betaine [30]. SA priming increases proline, indole acetic acid and soluble sugars while decreases soluble protein, abscisic acid and gibberellins [31]. Moreover, SA positively and negatively parallel with other plant regulators to safeguard plant against attack of pathogens [32] Furthermore, Yadav et al. [33] noted increased yield of wheat, height of plant, better root weight content and increase in amount of photosynthetic pigments. Furthermore, when salicylic acid foliarly sprayed, membrane injury and lipid peroxidation gets restricted by activating the protective role of salicylic acid.

Iron (Fe) is 4th highest element on the earth and mostly found in the crystal lattices of minerals [34]. Fe is also a very vital element for the plants. Most of the Fe (90%) is found in chloroplasts of the plants where it is mostly in the form of Fe^{+3} and very less in the Fe^{+2} form [35]. Quality and condition of the soil is a most essential feature that plays key role in development and growth of the plant. Decreased amount of iron in the soil lowers the quality of the soil consequently, leads to higher amount of accumulation of calcium carbonate in the soil, while increased level of iron in the soil results in poor drainage and flooding condition in the soil. In addition to this, decreased level of iron also leads to chlorosis of leaves [36,37]. Limited accumulation of iron reduces the growth of root and shoot, fresh biomass and area of leaf of plants [38]. However, when there is a higher amount of iron in plants, it causes spotting on leaf and lowers the rate of photosynthesis by hampering the activities of photosystem II [39]. Various studies have documented that foliar supplementations of iron tends to enhance the growth parameters and yield [40–42]. Although, findings regarding applying magnetism and impact of foliar supplementations of salicylic acid and iron have been documented however, no research has yet been documented on the combined effects of magnetic field treatments and salicylic acid/iron foliar applications. Current research was aimed to appraise the impacts of geo and artificial magnetism on seedling growth of *Pisum sativum* L. and to evaluate the potential role of foliar supplementations of salicylic acid and iron in elaborating the growth of seedlings in response to magnetic field treatments.

2. METHODS AND MATERIALS

2.1 Study Area

The present study was conducted during November, 2019 to December, 2019 at Botanical garden, University of Balochistan, Quetta, which is capital of Balochistan, province in Pakistan. The climate of Quetta fluctuates greatly between summer and winter. As far as average rainfall is concerned, it is quite low, records at 250mm or less every year. Whereas, temperature is at it's highest between June and July ranges between 35 ˚C and 40 ˚C. While temperature is at its lowest in the month of January, where the climate fluctuates between 11˚C and -7˚C. For the research, matore variety of *Pisum sativum* was selected, seeds of which were obtained from the Agriculture Research and Development Centre, Quetta.

2.2 Experiment Layout

A pot experiment was conducted in the prevailing environmental conditions of Quetta. The experimental design was completely randomized design under factorial treatments with three replicates. Pots of 32cm diameter were filled with 8 kg of soil from Agriculture and Research Department, Quetta. The soil ratio was taken as 20: 20: 60. i.e., manure, sand and soil respectively. Seeds were sown at 1 inch deep. 5 seeds were sown in each pot. Various 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 considering physiological attributes.

2.3 Tannins Analysis

For the analysis of tannin content in both (root and shoot) method developed by [43] was used. 0.1g of samples from both (root and shoot) were obtained and mixed with 2ml of diethyl ether, then 1ml of 70% acetone mixed and kept overnight. After that 50µL of the extract obtained from each sample in test tube, followed by adding distilled water for dilution and making volume up to 1 milliliter. Followed by mixing of 0.5 milliliter of Folin–Ciocâlteu reagent vortexed, and added 2.5 milliliter of 20% sodium carbonate solution and stored at room temperature for 40 minutes. The absorbance was taken at 725 nanometer using spectrophotometer. Acetone was used as a blank.

2.4 Ascorbic Acid Analysis

For the analyses of Ascorbic acid titrant 2, 6-Dichlorophenolindophenol (Quinone imine) determined according to AOAC [44].

2.4.1 Preparation of reagents

For this, first ascorbic acid was used to reduce the Quinone imine to colorless form which was red in acid solution and blue in alkaline solution. Followed by dissolution of Quinone imine solution (0,001 mol/L) in distilled water. Diluted the solution until final volume of 100 milliliter was obtained and filtered. The diluted Quinone imine solution was stored in a freezer for further process.

2.4.2 Ascorbic acid solution

To prepare ascorbic acid solution 20 milliliter of 10% ethanedioic acid was used and mixed with 0.05g of pure ascorbic acid, followed by dissolution of mixture using distilled water until final volume of 250 milliliter was obtained in a flask.

2.4.3 Standardization of Quinone imine

For this, titration of 10mL of standard ascorbic acid with 2, Quinone imine solution was obtained and pipetted into a small flask until appearance of pink color for 15 seconds.

2.4.4 Titration

For the process of titration samples of both roots and shoots weighted 5g was put into a breaker. Followed by addition of oxalic acid and stirred for at least 10 minutes. After that filtrated the solution into volumetric flask and diluted the solution using distilled water until 100mL of volume was obtained. From the 100 mL of solution, 10 mL of solution pipetted into beaker, followed by addition of 2.5 milliliter acetone.

At the end mixture was titrated using Quinone imine until appearance of pink color for 15 seconds. Repeated the process using the sample as blank.

Calculation of Ascorbic acid = (a - b) * f * V1 * 100 / W * V2.

W = Sample weight
a = Titration test of solution in milliliter.
b = Blank test for titration in milliliter.
f = Ascorbic acid equal to 1 milliliter (standard solution for Quinone imine).
V1= Initial solution for volume test.
V2 = Titrated solution for volume test.

2.5 Pythic Acid Analysis

For the determination of Phytic acid in roots and shoots of *Pisum sativum* barium phytate (Ba_4Phy) was prepared by adding phytin in 3% of tri chloro acetic acid (TCA) and filtered. Followed by addition of FeCl solution, making Ferric phytate (Fe_4Phy) as final product which was precipitated later. Followed by using of 3% trichloro acetic acid (TCA) to wash the precipitate and slurred in water, to convert the solution to Fe $(OH)_3$ and sodium phytate by adding NaOH. Then HCL was added to the sodium phytate solution to maintain its pH at 6. The Fe $(OH)_3$ was filtered out, and Ba_4 Phy precipitated by addition of $BaCl_3$. Followed by dissolution of Ba_4Phy by adding HCl. This cycle of double precipitation repeated twice. The end product of Ba_4Phy was rinsed thoroughly with water and later with methanol, and dried in vacuum at 80° C.

2.6 Methods

Fresh samples of both roots and shoots (40 mesh) were taken containing approximately 5 to 30mg phytate P and put into a 125 ml Erlenmeyer flask. Followed by addition of 50ml of 3% TCA with mechanical shaking and occasionally swirling by hand for 45 minutes. Followed by centrifuging the suspension and transferring of a 10 milliliter aliquot supernatant into a 40 milliliter centrifuge tube. Then heated the contents of tube for 45 minutes.

After that, centrifuged the mixture for 15 minutes and carefully. Then rinsed the precipitate two times by using 25 milliliter of 3% of tri chloro acetic acid followed by heating the precipitate for 10 minutes and centrifuging. Repeated the process of rinse once with water. Then dissolved the precipitate in 10ml of water followed by addition of 3ml of 1.5N NaOH. Then added few ml of water to make the final volume to 30ml and heated the solution in water bath for 30 minutes. Filtered the solution using a moderately retentive paper. Then rinsed the precipitate with 70ml hot water. Followed by dissolving the precipitate from the paper using 40 milliliter hot 3.2N nitric acid into a 100 milliliter volumetric flask. Then rinsed paper using various portions of water and collected the washings in the same volumetric flask. Allowed the content of flask to cool down at room temperature and diluted the content of flask with water. Followed by transfer of 5 milliliter aliquot to another 100 milliliter flask to dilute to exactly 70 milliliter. Then added 20 milliliter of 1.5M potassium thiocyanate to dilute the volume, and read the color after 1 minute at 480 rim. Followed by running a reagent blank with each set of samples. Then calculated iron content from a $Fe\ (NO_3)_3$ standard run at the same time. Followed by calculating the phytic acid and phosphorus from the iron results assuming P: Fe in a molecular ratio of 4:6 [45].

2.7 Statistical Analysis

For the Statistical analysis "STATISTIX 8.1" software was used. Whereas, MS. EXCEL, was used to calculation the mean, graphs, and standard deviation. Furthermore, a post hoc test, i.e., the LSD (least significant difference) was used, and the alphabets were added to graph bars of statistically significant attributes ($P<0.05$).

3. RESULTS

3.1 Root Tannins

Results of the tannin contents in roots of garden pea disclosed significant variations ($P < 0.05$) under various treatments. The maximum tannin content was noted in seedlings, those were applied with North/South shoot & South/North root to root under iron foliar supplementations while least tannin content was found when plants were subjected to South root / North shoot treatment under iron foliar applications (Fig. 1).

The tendency observed for root tannins contents of *Pisum sativum* under geo/artificial magnetism and different foliar supplementations is as follow: South/North root & North/South shoot + iron foliar supplementation > North/South root & South/North shoot + iron foliar supplementation > Geomagnetism + iron foliar supplementation >South root & North shoot + normal conditions > South/North root & North/South shoot + normal conditions > South root + normal conditions > North/South root & South/North shoot + salicylic acid foliar supplementation >North root & South shoot + iron foliar supplementation >Geomagnetism + normal conditions >North root + normal conditions > North root + salicylic acid foliar supplementation > South root + iron foliar supplementation > Geomagnetism + salicylic acid foliar supplementation >North/South root & South/North shoot + control > North root & South shoot + normal conditions >North root + iron foliar supplementation >North root & South shoot + salicylic acid foliar supplementation >South root + salicylic acid foliar supplementation >South root & North shoot + salicylic acid foliar supplementation >South/North root & North/South shoot + salicylic acid foliar supplementation >South root & North shoot + iron foliar supplementation (Fig. 1).

Considering, the results of tannin content in roots of garden pea, it showed that the amount of tannin content in roots enhanced under various artificial magnetic field treatments and iron foliar applications (Fig. 1).

3.2 Shoot Tannins

Results of the shoot tannin contents of Pea plant showed that the results were significantly different when observed statistically ($P<0.05$). The maximum amount of tannin content was noted under South root & North shoot with salicylic acid foliar applications; however, minimum tannin content was observed under South/North root & South/North shoot treatment under natural conditions (Fig. 2).

The order of changes observed for shoot tannin contents of *Pisum sativum* different foliar spray under and under geo / artificial magnetic treatments are as follows: South root & North shoot + salicylic acid foliar applications >North root & South shoot + natural condition >South root + iron foliar applications >South root + natural condition >South root & North shoot + natural condition >North root & South shoot + iron foliar applications >Geo magnetism + natural condition >South root + salicylic acid foliar applications >North root + iron foliar applications > North root + natural condition >South root & North shoot + iron foliar applications > Geomagnetism + iron foliar applications >South/North root & North/South shoot + salicylic acid foliar applications >North root & South shoot + salicylic acid foliar applications >North root + salicylic acid foliar applications >Geomagnetism + salicylic acid foliar applications >North/South root & South/North shoot + iron foliar applications >North/South root & South/North shoot + control >South/North root & North/South shoot + iron foliar applications >South/North root & South/North shoot + natural condition (Fig. 2).

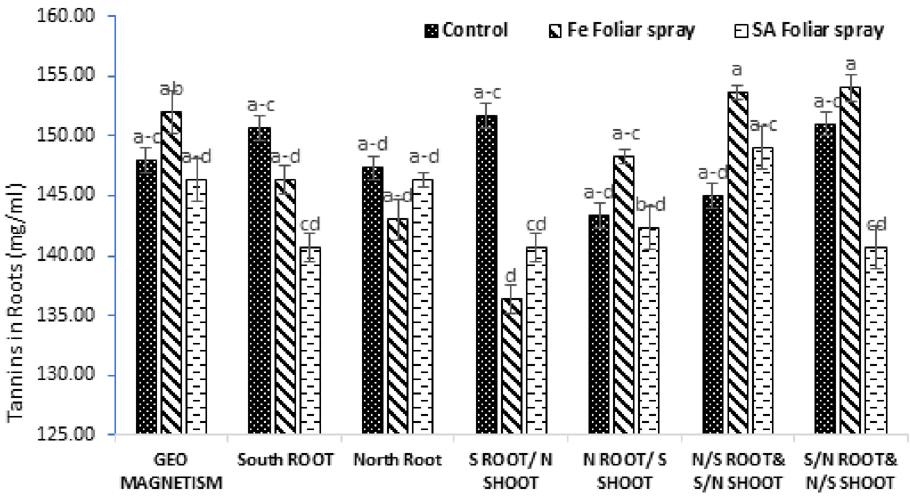

Fig. 1. Graph representing effect of various magnetism treatments on the Root Tannin content of Matore variety of *Pisum sativum* in response to iron/salicylic acid foliar supplementations (P < 0.05)

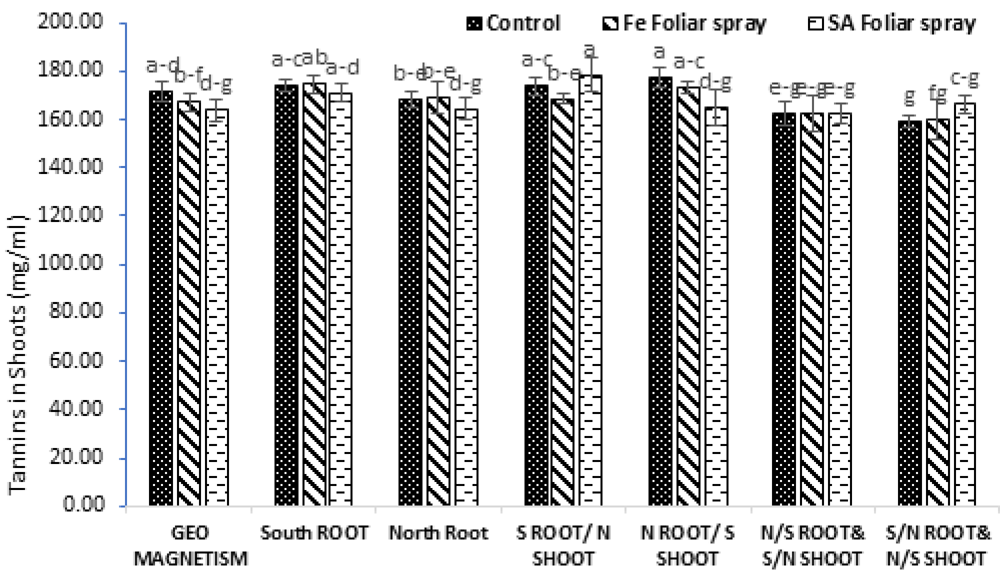

Fig. 2. Graph representing effect of various magnetism treatments on the Shoot Tannin content of Matore variety of *Pisum sativum* in response to iron/salicylic acid foliar supplementations (P< 0.05)

In the nutshell, results of tannin content in shoots of garden pea manifested, the level of tannin content in shoots increased when seeds were sworn using various artificial magnetism treatments and iron and salicylic acid foliar applications (Fig. 2).

3.3 Root Ascorbic Acid

Results obtained for roots ascorbic acid contents of *Pisum sativum* presented statistically significant (P<0.05) results by revealing that the highest level of ascorbic acid accumulation was recorded in roots of plants which were given South root / North shoot magnetism + natural condition while least amount of ascorbic acid content was recorded under South root under natural condition (Fig. 3).

Findings regarding level of ascorbic acid in roots of pea plants recorded the following tendency under geo / artificial magnetic treatments with iron and salicylic acid foliar supplementation: South/North root & North/South shoot + salicylic acid foliar applications >North root & South shoot + natural condition >South root & North shoot + salicylic acid foliar applications >North root & South shoot + salicylic acid foliar applications >South/North root & North/South shoot + natural condition >South root + salicylic acid foliar applications >Geomagnetism + natural condition >North/South root & South/North shoot + salicylic acid foliar applications >Geo magnetism + iron foliar applications >North root + salicylic acid foliar applications >South root & North shoot + natural condition > North/South root & South/North shoot + iron foliar applications >North root + natural condition >South/North root & North/South shoot + iron foliar applications >South root & North shoot + iron foliar applications >North root & South shoot + iron foliar applications > North/South root & South/North shoot + natural condition >North root + iron foliar applications >South root + iron foliar applications >Geo magnetism + salicylic acid foliar applications >South root + natural condition (Fig. 3).

Hence, from the overall variations of root ascorbic acid contents, it has been determined that ascorbic acid accumulation in roots enhanced when seeds were exposed to artificial magnetic treatments under control conditions and minimum amount of ascorbic acid was recorded under S root magnetism and control condition (Fig. 3).

3.4 Shoot Ascorbic Acid

Results recorded for Ascorbic acid in shoots of garden pea revealed results which were statistically significance (P<0.05) among different treatments. The maximum Ascorbic acid was recorded those plants, shoots of which were applied with South root and North shoot magnetic treatments under natural condition; however, least amount of Ascorbic acid was noted in shoots of those plants which were given South/North root & North/South shoot with natural condition (Fig. 4).

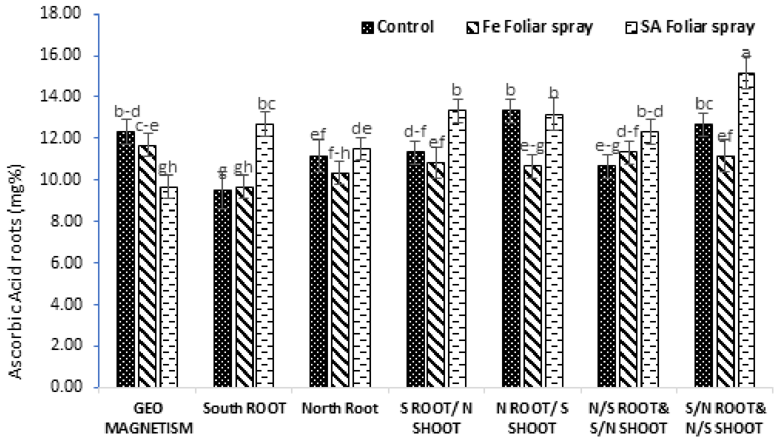

Fig. 3. Graph representing effect of various magnetism treatments on the Root Ascorbic Acid content of Matore variety of *Pisum sativum* in response to iron/salicylic acid foliar supplementations (P<0.05)

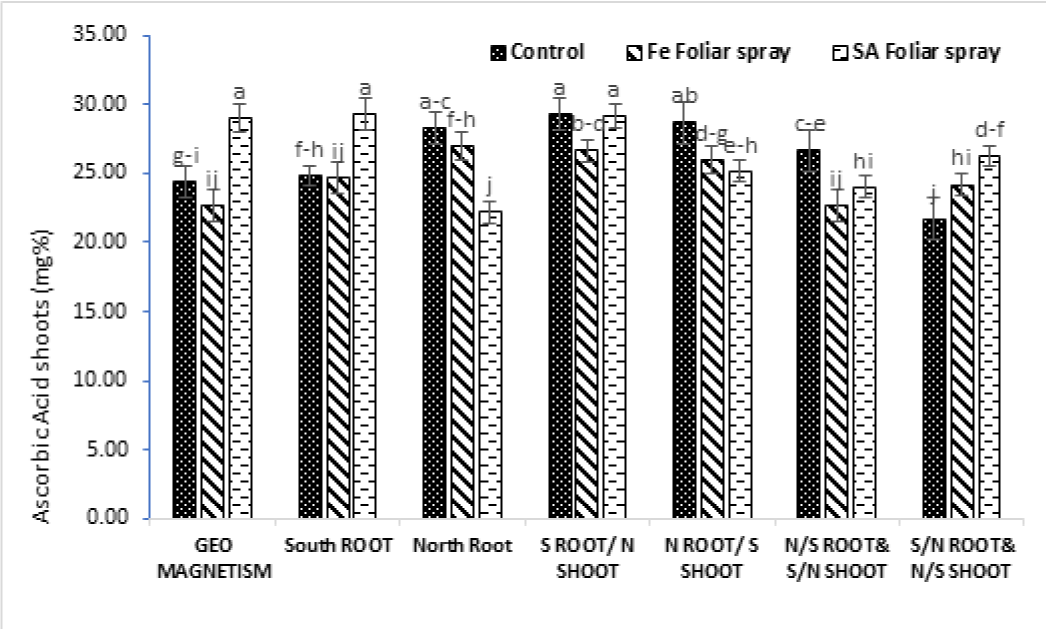

Fig. 4. Graph representing effects of various magnetism treatments on the Shoot Ascorbic Acid content of Matore variety of *Pisum sativum* in response to iron/salicylic acid foliar supplementations (P<0.05)

Hence, the order of changes observed for ascorbic acid content of shoot are given as: South root & North shoot + natural condition >South root + salicylic acid foliar supplementations >South root & North shoot + salicylic acid foliar supplementations >Geomagnetism + salicylic acid foliar supplementations >North root & South shoot + natural condition >North root + natural condition > North root + iron foliar supplementations >North/South shoot & South/North shoot + natural condition >South root & North shoot + iron foliar supplementations >South/North root & North/South shoot + salicylic acid foliar supplementations >North root & South shoot + iron foliar supplementations >North root & South shoot + salicylic acid foliar supplementations >South root + natural condition >South root + iron foliar supplementations > Geo magnetism + natural condition >South/North root & North/South shoot + iron foliar supplementations >North/South root & South/North shoot + salicylic acid foliar supplementations >Geomagnetism + iron foliar supplementations >North/South root & South/North shoot + iron foliar supplementations >North root + SA foliar spray >South/North root & North/South shoot + natural condition (Fig. 4).

In the nutshell, considering the results of ascorbic acid in shoots of pea plant, it was recorded that when plants were given iron and salicylic acid foliar supplementations under different artificial magnetism treatments, it helped in improving the level of ascorbic acid (Fig. 4).

3.5 Root Phytic Acid

As far results of level of phytic acid in roots are concerned, results were significant statistically (P<0.05). Maximum amount of phytic acid was recorded in roots of South/North root & North/South shoot treatment plants under iron foliar spray, whereas minimum amount of phytic acid was recorded in roots of South root/North shoot magnetic field treatments under natural conditions (Fig. 5).

The trend observed for root phytic acid under geo/artificial magnetic field treatments with iron and salicylic acid foliar supplementations is observed as : South/North root & North/South shoot + salicylic acid foliar supplementations >North root + natural conditions >North/South root & South/North shoot + iron foliar supplementations >North root & South shoot + iron foliar supplementations >South root & North shoot + iron foliar supplementations >South/North root & North/South shoot + natural conditions >South/North root & North/South shoot + salicylic acid foliar supplementations >South root + iron foliar supplementations >North root + iron foliar supplementations

>South root + natural conditions > Geomagnetism + salicylic acid foliar supplementations >North root & South shoot + natural conditions >North root & South shoot + salicylic acid foliar supplementations >South root + salicylic acid foliar supplementations >Geomagnetism + iron foliar supplementations >Geomagnetism + natural conditions >North/South root & South/North shoot + salicylic acid foliar supplementations >North/South root & South/North shoot + natural conditions >North root + salicylic acid foliar supplementations >South root & North shoot + salicylic acid foliar supplementations >South root & North shoot + natural conditions (Fig. 5).

In a nutshell, results of phytic acid accumulation in roots of pea plants, showed that the levels of phytic acid in roots improved among different artificial magnetic fields and iron foliar applications 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).

3.6 Shoot Phytic Acid

Data obtained for Phytic acid accumulation in shoot of garden pea revealed statistically significant (P<0.05) results. However, the highest Phytic acid content was noted in the shoots of plants which were given South root magnetism under natural conditions while least level of phytic acid was recorded in the shoots of garden peas under South root and North shoot magnetic treatment with iron foliar supplementation (Fig. 6).

Hence, the results for Phytic acid content in shoots of pea plant revealed the following trend: South root + natural conditions >North root + iron foliar supplementations >Geomagnetism + iron foliar supplementations >North/South root & South/North shoot + salicylic acid foliar supplementations >Geomagnetism + natural conditions >South/North root + North/South shoot + salicylic acid foliar supplementations >North root & South shoot + salicylic acid foliar supplementations >North root + natural conditions >South/North root + North/South shoot + natural conditions >South root + iron foliar supplementations >South root & North shoot + natural conditions >North/South root & South/North shoot + iron foliar supplementations >South root + salicylic acid foliar supplementations >North root & South shoot + iron foliar supplementations >South/North root & North/South shoot + iron foliar supplementations >North/South root & South/North shoot + natural conditions > North root + salicylic acid foliar

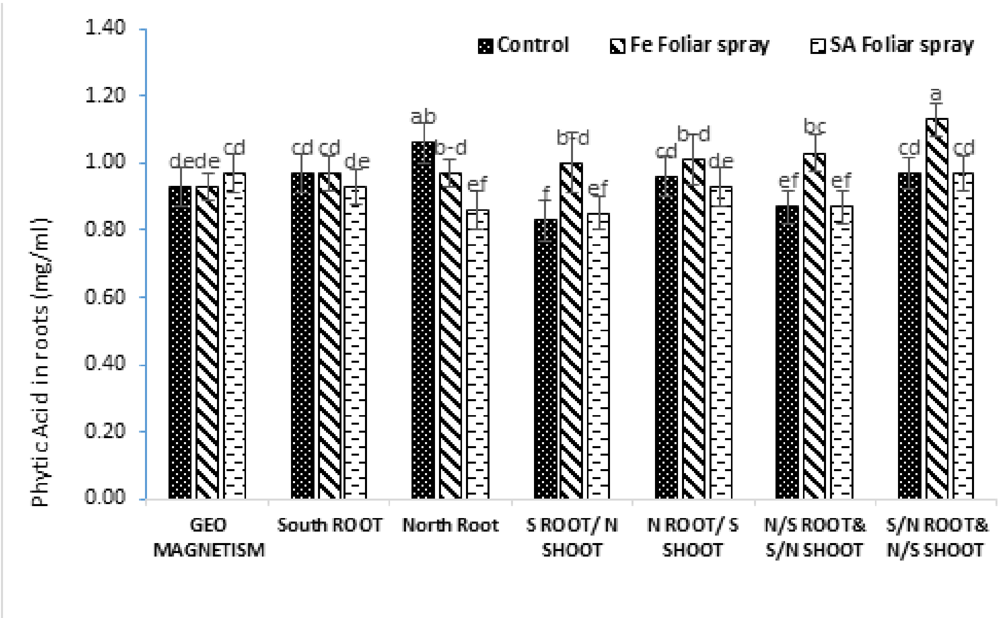

Fig. 5. Graph representing effects of various magnetism treatments on the Root Phytic Acid content of Matore variety of *Pisum sativum* in response to iron/salicylic acid foliar supplementations (P<0.05)

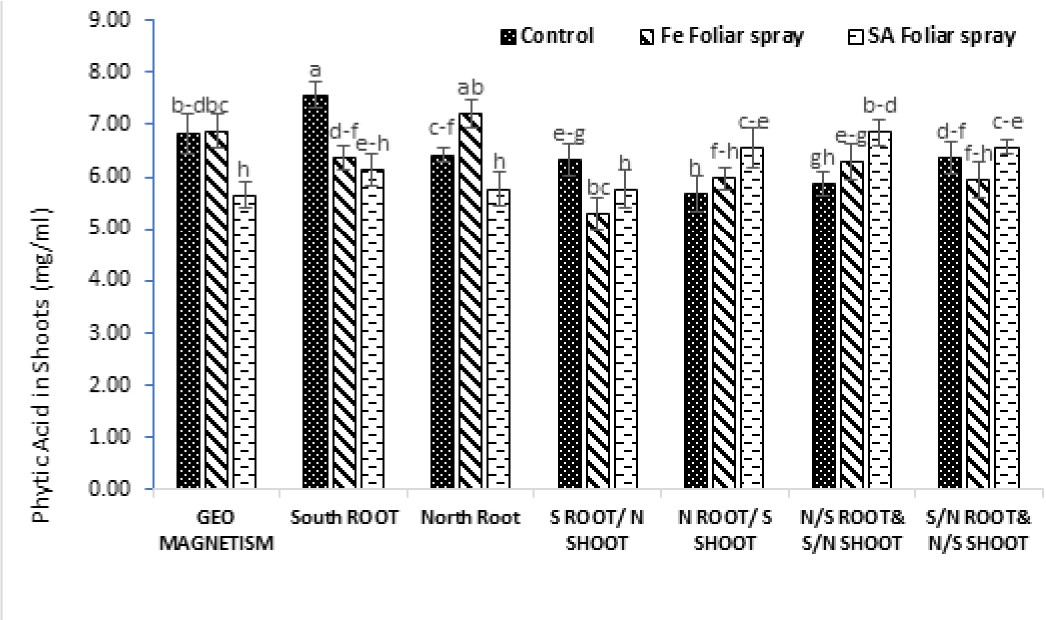

Fig. 6. Graph representing effects of various magnetism treatments on the Shoot Phytic Acid content of Matore variety of *Pisum sativum* in response to iron/salicylic acid foliar supplementations (P<0.05)

supplementations >South root & North shoot + salicylic acid foliar supplementations >North root & South shoot + natural conditions >Geomagnetism + salicylic acid foliar supplementations >South root & North shoot + iron foliar supplementations (Fig. 6).

Regarding the results of phytic acid in shoots of field pea, it was noted that there was very low level of phytic acid observed when plants were given geo/artificial geomagnetism in addition with salicylic acid and iron foliar applications (Fig. 6).

4. DISCUSSION

Tannin is present in every plant and almost in every climate throughout the world. The name tannin is obtained from the French word tannin and is used for a range of natural polyphenols. But Plants including mosses, fungi and algae do not have much tannin in their cells. While, some plants contain a significant proportion of tannin in their cells. Majority of tannin contents are present in the bark of trees, playing defensive role and protecting the trees against microorganisms. Tannin plays role of astringent, and polyphenols in bitter plants that helps in either binding or shrinking of proteins. The word “tannin” is used to define a large group of polyphenolic compounds that contain carboxyls and other vital groups (such as hydroxyls) to form strong complex compounds combined with proteins and other macro-molecules. There was an improved chemical stimulating response in those plants which were non-adopted and were grown at higher altitude. *Pteridium arachnoideum* (fern) when exposed to sun, there was high amount of accumulation of tannin and phenols noted in comparison with those plants which were grown self-shaded, with the increase in altitude. Water stress conditions also play an important role in increase of phenols and tannin contents, hence, it may be concluded that UV-B radiation and water stress conditions are key elements in non-adapted plants acclimation response to stress at altitude gradients [46]. 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, as per results of tannin in root and shoot of *Pisum sativum* showed that the amount of tannin in roots enhanced under different artificial magnetism treatments and iron/salicylic acid foliar applications. Furthermore, it was observed that when pea plants were given the magnetic treatments with iron/ salicylic foliar applications, there was overall increased production in amount of tannin 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 (Figs. 1, 2). As present result showed that when pea plants were applied with foliar spray of Fe/SA, consequently higher amount of tannins accumulated in both roots and shoots of the pea plants which is quite consistent to those results of Borsani et al. [47] which reported that salicylic acid has been observed to produce tannin content which help in a activating the defensive role in plants under stress factors of various abiotic stresses including chilling, heat, salt and osmotic stress. Applying 200 ppm SA in sunflower and 100 ppm ascorbic acid in wheat helped in reducing the negative effects of drought [48]. Tannin is found in large quantity the bark, leaves, seeds and fruits. The potential role of these chemicals is to protect the plants against herbivores and microbial pathogens. In many plant species proanthocyanidins is stored in the endogenous layer of the seed coat which is considered to be a classic example of a defensive barrier [49].

Vitamin C chemically known as (ascorbic acid) compound in plants, that plays essential role of antioxidant activities in plants. It also plays key function of modulating the plant development through hormones signaling. Ascorbic acid also acts as coenzyme in mobilizing the fats, protein and carbohydrates [50]. Under different adverse circumstances production of reactive oxygen species in plants is common. In such conditions, plants counter these problems by plant ability to activate compounds that neutralizes reactive oxygen species, which include both non-enzymatic and enzymatic antioxidant compounds. In this regard, ascorbic acid is one of the best worldly known non enzymatic antioxidant compound that not only have the ability to neutralize ROS, but also plays role in activating defensive mechanisms in plants against both non stress and stress conditions [51]. Rosales et al. [52] documented that vitamin C potentially have the ability to increase the rate of cell division and by increasing the both fresh and dry weight of leaves of plant and plays a role antioxidant compound to counter damaging effects against the oxygen radicals, which are produced in response of drought stress. Pastori et al. [50] noted that the vitamin C potentially quenches reactive oxygen in access amount during the processes of photosynthesis and respiration. Also, ascorbic acid antioxidant compound, plays the role of modulator in development of plant by hormone signaling and as co-enzyme in metabolizing reactions of proteins fats, and carbohydrates. Magnetic field treatments have been reported to influence the stimulation of ions and vitamins in living cells. Consequently, increasing the growth parameters [14]. It was observed in current research 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 applied with S shoot & N root treatment, it increased the significant amount of ascorbic acid in both roots and shoots of the plants (Figs. 3, 4). It was also documented by Hanan et al. [53] that the antioxidant activity of herb increased by using treatment of magnetic and gave values of 933.50, 975.17, 1016.0 and 974.7 ug of ascorbic acid/mg ext. in the two cuts during two seasons, respectively. Ascorbic acid is a compound plays role of defensive compound and an enzymatic co-factor. It takes part in a number of mechanisms, include growth of cell wall, photosynthesis, resistance to stress and synthesis of ethylene, hydroxyl proline and anthocyanine [54].

Phytic acid also known as 6-phosphate Inositol or phytate is found in concentrations of 1 – 3% dry mass in most cereals and legumes. Phytic acid is also present in fruits and vegetables of many plant species [55]. Phytic acid is an undesirable dietary agent which binds certain minerals such as Calcium (Ca''), Magnesium (Mg'''), [56] Iron (Fe''•) and Zine (Zn'') and renders them unavailable for their physiological functions by forming insoluble compounds [57]. Phytate is a normal constituent of almost all cereals constituting 1-3% by weight. 30 to 90 % of the phosphorus is found in the form of phytate [59]. Rusydi & Azrina, [60] reported that phytate or phytic acid is formed when plant seeds are mature and it constitutes about 60 to 90 % of total phosphate in dormant seeds. Phytic acid is stored as phosphate in seed of plants and often get gathered in plant seed vacoule after biosynthesis. Process of germination in seeds plays key role in the production of phytic acid. Phytic acid provides phosphorus in young growing seedlings and during the seed germination [61]. Mate & Radomir, [58] found that activity of phytase during germination, plays important role in hampering the production of phytic acid in seeds of many plants. It was also observed in the present study that when plants were bio-chemically analyzed there was very minute levels of phytic acid, and when plants were applied with the geo / artificial magnetism as well as SA & Fe foliar supplementations. It was also observed that plants, when applied with South/ North root & North / South shoot in combination with Fe foliar supplementation 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 seeds were sworn with South root/North shoot treatment under natural condition [62]. Whereas, highest level of phytic acid was observed when plants were grown with South root treatment under natural 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 seeds were sworn with South root/North shoot in combination with iron foliar supplementation (Figs. 5, 6). In a nutshell it can be proclaimed that the foliar supplementation of SA and Fe in combination to artificial magnetism can be recommended for the better growth and development of plants.

5. CONCLUSION

Seeds sworn under magnetism improves the yield and growth of plants. Exposing *Pisum sativum* to artificial magnetic treatments in response to iron and salicylic acid foliar supplementations revealed promising effects on growth of plants as it enhanced various protective and growth contents by increased production of tannins, ascorbic acid and phytic acid of *Pisum sativum* in comparison to geo magnetism. Results of present research are quite evident in displaying the positive role of artificial magnetism in combination with salicylic acid and iron foliar supplementations that increased the tannin contents, amount of ascorbic acid as well as phytic acid in *Pisum sativum* thus, indicating the conclusive interaction of iron and salicylic acid foliar spray with magnetic treatments. In general, applying different kinds of chemicals have been quite beneficiary for better crop development, production and being practiced successfully throughout the world, but, has been quite inimical to the soil and environment at the same time. Using magnetism treatments as a pre-sowing treatments along with plant growth regulators can be safe and eco-friendly agricultural exercise in comparison with chemicals. Furthermore, knowing the economically important roles of various magnetism treatments would be beneficiary for the scientists in understanding the evolutionary mutations in plants in response to magnetism for future exploration. Moreover, comprehensive genomic and proteomic investigations of plants grown with various magnetism treatments will be helpful in understanding and exploring the roles of magnetic field treatments on different plants in the future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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