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Elicitation Strategies For Improving Secondary Metabolites In Medicinal Plants

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Abstract:

A Nutraceutical is any substance or ingredient that is food or part of food that provides medical or health benefits including prevention and treatment of disease. Earlier studies have shown that Tulsi, Fenugreek, Dill, Coriander, Moong, Spinach, Lemongrass, Wheatgrass, Curry leaves, and Mint have medicinal and nutraceutical values. These values are due to the presence of secondary metabolites and the dose/servings which depend on the concentration of secondary metabolites. The concentration of secondary metabolites is limited in these plants and the treatment with elicitors derived from chemicals can be an effective strategy to increase the yield of metabolites obtained from the plant cell. So, in the present study, we have implemented the elicitation method for improving the secondary metabolites.

In this study, we have used two elicitor's salicylic acid and silver nitrate at concentrations of 0.5 ppm and 1 ppm. The results show that at the concentration of 0.5 ppm of both salicylic acid and silver nitrate, the improvement in yields of alkaloids was seen in wheatgrass, lemongrass, dill, and improvement of flavonoids in Fenugreek, Spinach, Tulsi, Coriander, Mint, and Wheatgrass. The total phenolic contents were improved in Fenugreek, Spinach, and Mint. So, it may be concluded that these elicitors are suitable for improvement in the Alkaloid, Flavonoids, and Phenolic content of the above plants at 0.5 ppm concentration. However, at the higher concentration of 1 ppm of the elicitors, the improvement in the alkaloid and flavonoid contents was not seen in all plants. In some cases, the concentration of phytoconstituents is decreased at the specific concentration of elicitor so it may also conclude the biosynthesis of phytoconstituents is decreased at that concentration so that concentration is not suitable for improvement of phytoconstituents.

Keywords: Elicitors, salicylic acid, silver nitrate, alkaloids, nutraceuticals.

INTRODUCTION: [1]

A nutraceutical is defined as any substance that is a food or part of a food and provides medical or health benefits, including preventing and treating disease. Nutraceuticals are classified as traditional or natural nutraceuticals (e.g., nutrients, herbals, phytochemicals, probiotic microorganisms, nutraceutical enzymes) and non-traditional or artificial nutraceuticals (e.g., fortified and recombinant nutraceuticals). Classification of Nutraceutical is mentioned in the Table no. 1. Whereas the information of plant used in this research are mentioned in Table no.2.

Table 1: Classification of Nutraceuticals

Classification of Nutraceuticals				
Traditional			**Non-Traditional**	
Chemical Constituents	**Probiotic Microorganism**	**Nutraceutical Enzyme**	**Fortified Nutraceutical**	**Recombinant Nutraceutical**
a) Nutrients such as vitamins, and amino acids with nutritional functions b) herbal such as Willow bark (salix nigra), lavender (Lavendula Angustifoliaa) c) Phytochemicals such as carotenoids, Flavonoids, Non-Flavonoid polyphenolics, and Phenolic Acids.	Transforms the toxic flora of the intestine into a host-friendly colony of Bacillus bulgaricus. Example Lactose intolerance prevented by β-galactosidase enzyme	a) Hemicellulase enzyme (microorganism and mushrooms) b) Pancreolipase (Pancreatic Juice)	Fortified food obtained from the agricultural breeding or added nutrients/ ingredients e.g., orange juice fortified with calcium cereals, or added vitamins folic acid	Foods achieved through biotechnology or genetic engineering such as bread, alcohol, vinegar, etc.

Table 2: Plants Used in Research

Sr.no.	**Name of Plant**	**Synonym**	**Biological Source**	**Family**	**Chemical Constituents**	**Nutraceutical Values**

1.	Fenugreek[2]	Fenugreek seed, Greek clover, Trigonella foenumgraecum.	Leaves of *Trigonella foenum graecum*	Leguminosae	Alkaloids, Amino acids, Saponins, Steroidal sapinogens,	Antioxidant, Antidiabetic, Immunomodulatory, Anti-inflammatory
2.	Dill[3,4]	Fructus anethi, Anethum, European dill	Dried ripe fruits of *Anethum graveolens Linn*	Umbelliferae	Terpenes, Anethofuran, Nutmeg, Phenylpropene, Alkaloids, Polyphenols, Flavonoids	Hypoglycaemic effect, Antioxidant, Cardiotonic
3.	Spinach[5]	Kale	Leaves of *Spinacia oleracea*	Amaranthaceae	Oxalic acid, Flavonoids, Alkaloids, Polyphenols, Vitamins, Fatty acids, Amino acids	Antioxidant, Anti-osteoarthritis, Antidiabetic, Anti-bacterial, Anti-inflammatory
4.	Mung[6]	Mung-beans	Seeds of *Vigna radiata*	Leguminosae	Flavonoids, Phenolic acid, Tannins	Antidiabetic, Anti-inflammatory Hyperlipidemic, Antihypertensive, Anticancer
5.	Tulsi[7,8]	Kali tulsi	Fresh and dried leaves of *Ocimum sanctum*	Labiatae	Terpenoids, Polyphenols, Flavonoids, Alkaloids	Antibacterial, Anti-cancer, Antidiabetic, Anti-inflammatory
6.	Coriander[9]	Dhaniya	Dried ripe fruit of the plant *Coriandrum sativum*	Umbelliferae	Alcohols, Hydrocarbons, Ketones, Ethers, Alkaloids, Flavonoids, Polyphenols	Antifungal, Antidiabetic, Antioxidant, Benefit heart health
7.	Lemon Grass[10]	Lemongrass oil	Leaves and aerial parts of the	Poaceae	Terpenoids, Flavonoids, Alkaloids, Tannins, Phenols,	Astringent, Antimicrobial, Anti-inflammatory, Antioxidant

			plants of *Cymbopogon citratus*		Resins, Glycosides	
8.	Wheat grass[11]	Crested wheatgrass	Freshly sprouted leaves of the	Poaceae	Phenols, Cinnamic acid, Alkaloids,	Antioxidant, Immunomodulatory
9.	Mentha[12]	Pulegium Mill	Fresh flowering tops of *Mentha piperita*	Labiatae	Terpenoids, Alkaloids, Flavonoids, Phenols	Antioxidant, Antibacterial, Antifungal
10.	Curry leaves[13,14]	Kari Patta	Fresh leaves of *Bergera koenigii*	Rutaceae	Terpenoids, Alkaloids, Flavonoids, Phenols	Antimicrobial, Antipyretic, Anti-inflammatory, Cytotoxic Antioxidant

Elicitors: [15-19]

Elicitors are compounds stimulating any type of plant defense and responsible for increasing the production of secondary metabolites (Phytoconstituents) in plants. The types of elicitors are mentioned in Figure no. 1.

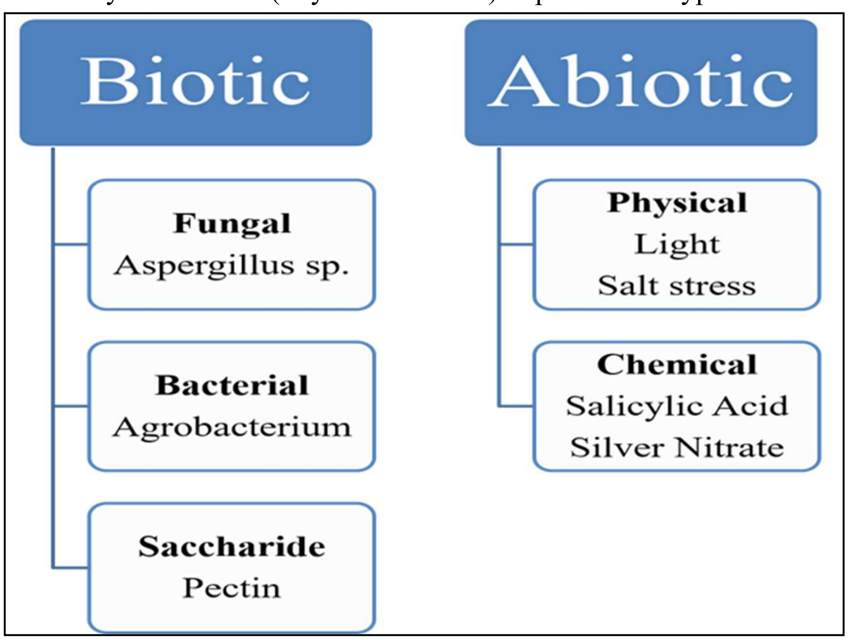

Figure 1: Elicitor types

MATERIALS AND METHODS:

MATERIAL:

Collection of Plants/ Seeds:

The plants as well as seeds of *Occimum sanctum* L. (Tulsi), *Coriandrum sativum* L. (Coriander), *Mentha Piperita* (Mentha), *Murrya koeniggi* (Curry leaves), *Trigonella foenum* (Fenugreek), *Anethum graveolens*

(Dill), *Cymbopogon* (Lemongrass*), Pascopyrum* (Wheatgrass), *Spinacia oleracea* (Spinach) and *Vigna radiata* (Moong), etc. collected from local seed store and nursery. The seeds were sowed and plants cultivated in house as mentioned below.

Chemicals Used:

Aqueous Sodium carbonate solution (10%), Folin-Ciocalteu reagent, Gallic Acid, Aqueous Aluminum Chloride solution (1M), Potassium acetate, Quercetin, Bromocresol green indicator, Sodium phosphate solution (2M), Citric acid, Atropine sulfate.

Preparation of soil beds :

The small beds were prepared in boxes using soil, collected from the local farm.

Cultivation/Propagation of Plant/Seeds:

The seeds were propagated on soil beds and the seedlings were cultivated in pots. Each group contained three beds/pots for each elicitor concentration.

Preparation and Implementation of Elicitors Sample: Two elicitor samples were prepared with two different concentrations as per the following table no. 3. The elicitors were spread on a plant (5ml) at two days intervals using a spraying bottle up to twenty days.

Table 3: Elicitors Concentration

Sr. no.	Name of Elicitor	Concentration
1	Salicylic acid (SA)	0.5 ppm/ 1 ppm
2	Silver nitrate ($AGNO_3$)	0.5 ppm/ 1 ppm

Harvesting of Plants and Extraction: The seedlings were harvested using the uprooting method, dry it in the oven and extract according to the process mentioned in the determination of phytoconstituents (Total Alkaloid content, Total Phenolic content and total flavonoid content).

Methods of Determination of Phytoconstituents by Colorimetric Analysis:

1. **Determination of Total Alkaloids:** [20]

The plant materials (1g) were ground and then extracted with methanol. The extract was filtered and methanol was evaporated to dryness. A part of this residue was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 10 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. Also prepared the calibration curve using atropine sulfate and determine the concentration.

2. **Determination of Total Phenolic Contents:** [21]

About 1 mL (1mg/ml) of hydroalcoholic extract of crude drug and 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally, the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined using a colorimeter at 700 nm wavelength. The calibration curve was generated by preparing Gallic acid at different concentrations (5, 10, 15, 20, and 25 µg/mL). The reaction mixture without a sample was used as blank.

3. **Determination of Total Flavonoid Contents:** [22]

About 1 ml (diluted) of extract of the crude drug was taken in a test tube and add 100 µl Aluminum chloride (1M) solution from the sidewall. Then 100 µl of potassium acetate was added. Volume was made up to 4 ml by adding 2.8 ml of solvent (ethanol). Allowed to stand for 30 minutes at room temperature and total Flavonoid content was determined using a colorimeter at 517 nm wavelength. Quercetin was used as standard.

RESULTS AND DISCUSSION:

The following table 4 contains a percent change in the concentration of Alkaloids. The graphical representation of the same is mentioned in figure no. 2. The elicitor Salicylic acid and silver nitrate having a concentration of 0.5 ppm show the increase in the percentage of alkaloids in the Dill. Also, the silver nitrate elicitor at 0.5 ppm increases the concentration of alkaloids in the lemongrass.

Table 4: Alkaloid contents (All the values are in %)

Plant Name	**Salicylic Acid**		**Silver Nitrate**		**Control**
	0.5 ppm	**1 ppm**	**0.5 ppm**	**1 ppm**	
Fenugreek	98.9507947	91.98399	95.1221889	93.72046	100
Spinach	99.6516596	95.81259	96.8586604	95.81259	100
Tulsi	99.6516596	95.81259	96.8586604	95.81259	100
Coriander	99.0316004	96.44888	98.3856779	95.48048	100
Mint	97.8856822	94.71421	98.2451162	92.59989	100
Wheatgrass	100.322406	99.03471	100	98.06943	100
Mung	99.357119	98.06943	99.357119	98.06943	100
Dill	103.835928	97.21432	105.230336	96.16826	100
Curry Leaves	98.5263901	96.32697	98.5263901	97.42668	100
Lemongrass	96.4442801	94.31511	101.770408	95.37969	100

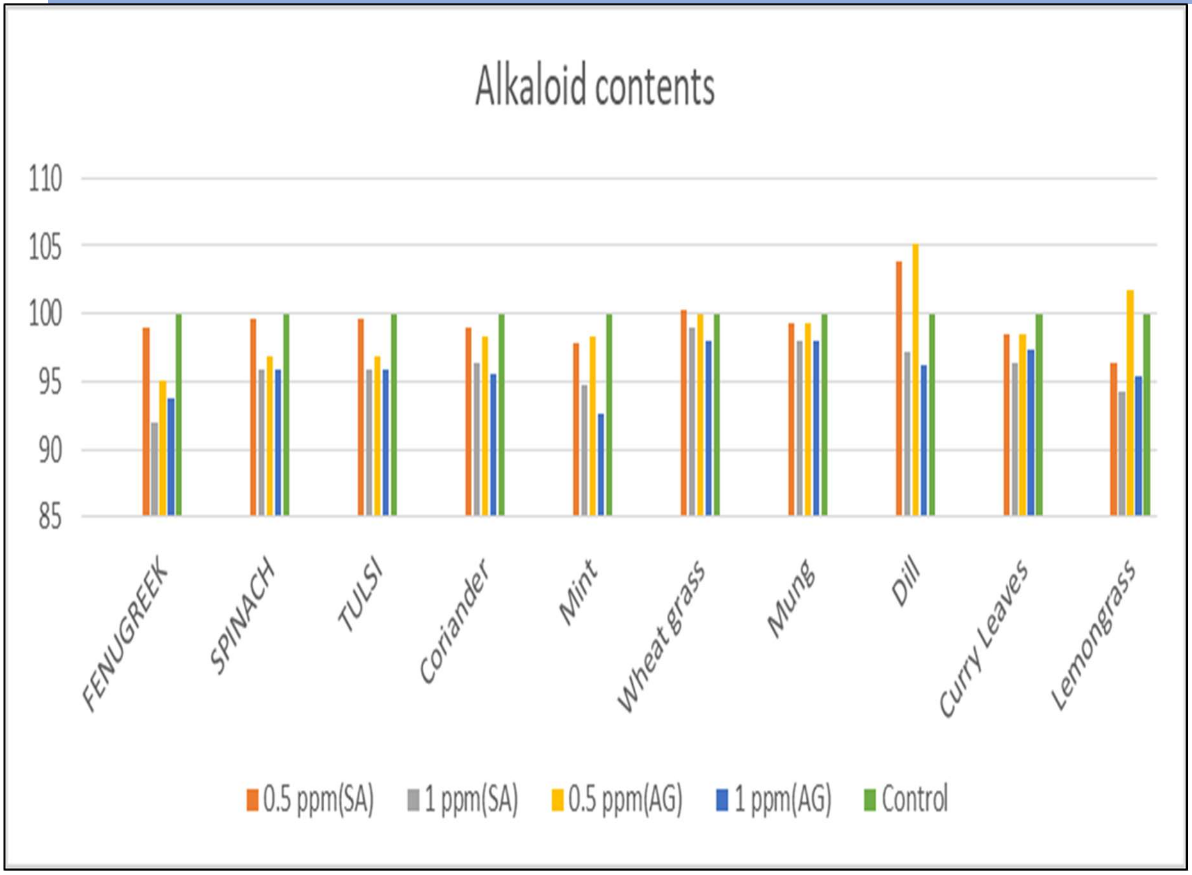

Figure 2: Alkaloid Content

The following table no. 5 contains a percent change in the concentration of Flavonoids. The graphical representation of the same is mentioned in figure no. 3. The elicitor Salicylic acid and silver nitrate having a concentration of 1 ppm show a decrease in the percentage of Flavonoids in the plants as compared to the 0.5 ppm concentration

Table 5: Flavonoid Content (All the values are in %)

Plant Name	Salicylic Acid		Silver Nitrate		Control
	0.5 ppm	1 ppm	0.5 ppm	1 ppm	
Fenugreek	100.014783	99.86682	100	99.92599	100
Spinach	100.01483	99.92603	100.01483	99.94081	100
Tulsi	100	99.92599	100.044421	99.92599	100
Coriander	100.01483	99.9556	100	99.92603	100
Mint	100.014788	99.92597	99.9703799	99.92597	100
Wheatgrass	100.01483	99.92603	100.01483	99.9112	100
Mung	100	99.94078	99.9851721	99.94078	100

Dill	100	99.955 61	100	99.940 78	100
Curry Leaves	99.95559 86	99.911 2	99.92602 72	99.911 2	100
Lemongr ass	100	99.925 99	100	99.940 78	100

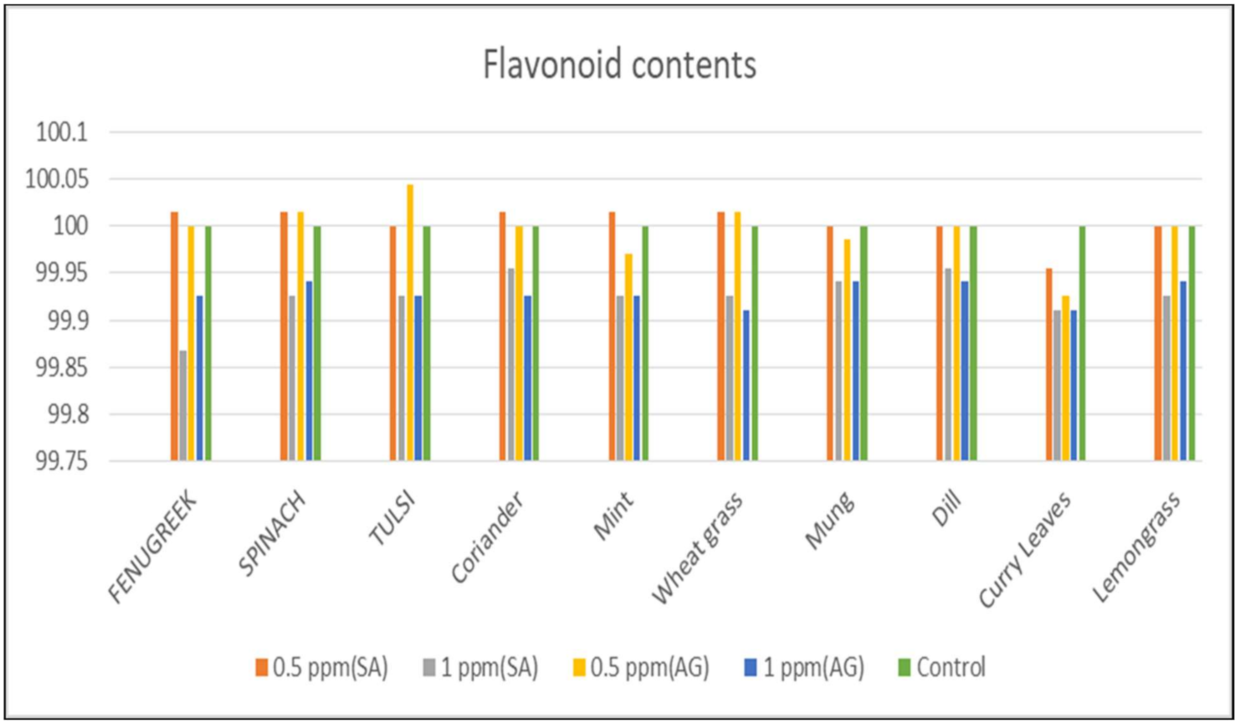

Figure 3: Flavonoid content

The following table no. 6 contains a percent change in the concentration of Phenolics. The graphical representation of the same is mentioned in figure no. 4. The elicitor Salicylic acid and silver nitrate having a concentration of 1 ppm show an increase in the percentage of total phenolic contents in spinach leaves, Dill, and Curry leaves.

Table 6: Phenolic Content (All the values are in %)

Plant Name	**Salicylic Acid**		**Silver Nitrate**		**Cont rol**
	0.5 ppm	**1 ppm**	**0.5 ppm**	**1 ppm**	
Fenugree k	100.1989 07	100.49 71	100.3975 149	100.59 64	100
Spinach	102.8844 75	103.38 3	102.9859 672	103.18 3	100
Tulsi	99.90049 79	100.39 86	100	100.29 88	100
Coriande r	99.70355 73	100.19 77	99.90128 458	100.59 29	100
Mint	100.5970 14	100.99 49	100.0994 029	100.99 49	100
Wheatgra	99.90029	100.29	99.90029	100.19	100

ss	86	85	861	88	
Mung	99.80148 83	100.59 52	100.0991 07	100.29 76	100
Dill	98.63935 99	100.77 64	98.73644 438	101.06 79	100
Curry Leaves	100.0974 71	101.55 75	100.3901 755	102.14 11	100
Lemongr ass	100	100.69 74	100	100.39 86	100

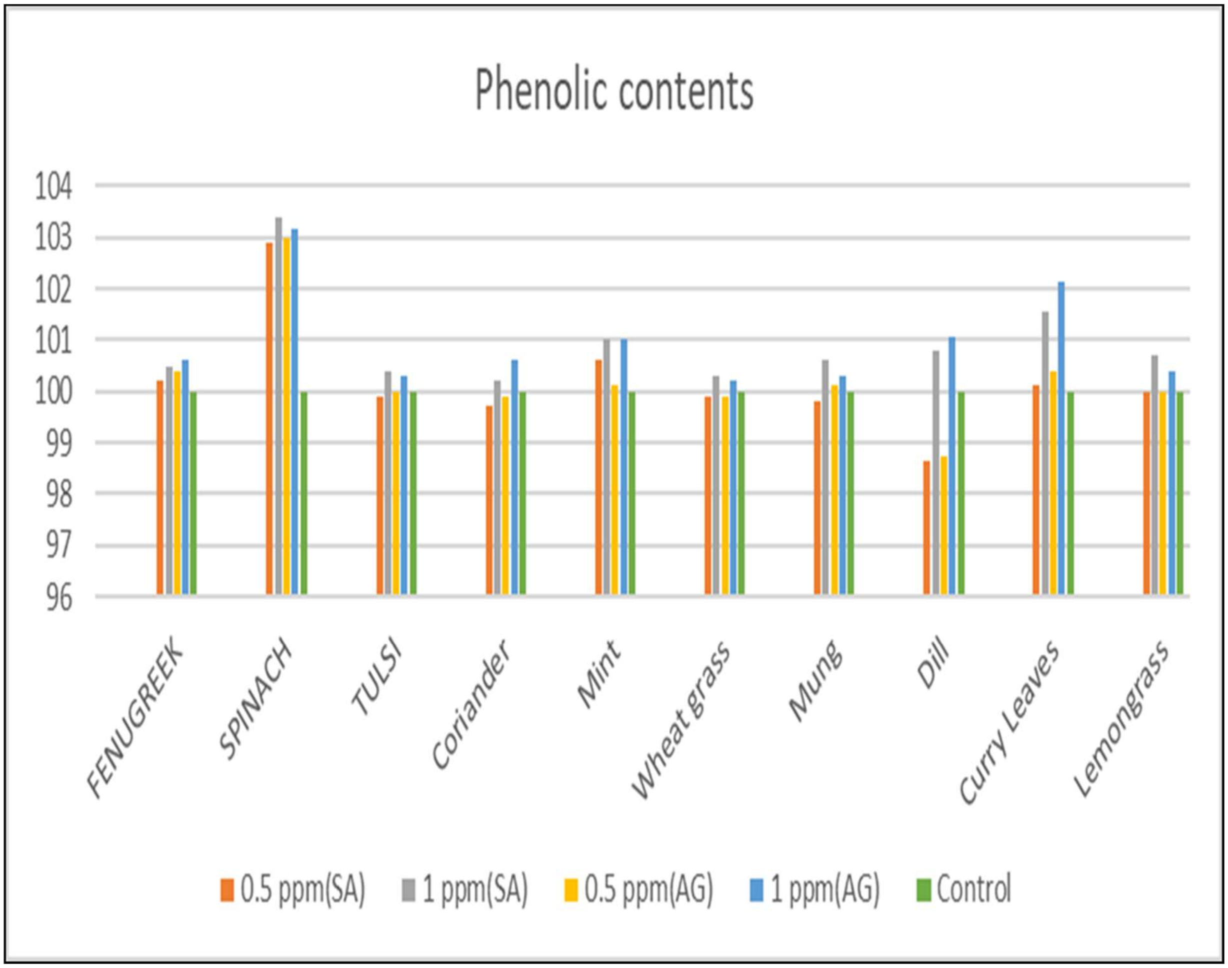

Figure 4: Phenolic content

CONCLUSION:

The present investigation clearly demonstrates that the application of elicitors significantly influences the production of key secondary metabolites in plants of nutraceutical importance. Plants naturally synthesize a variety of secondary metabolites as a defense mechanism against biotic stressors such as microbial pathogens and insect attacks. This study reaffirms that the strategic application of chemical elicitors, such as salicylic acid and silver nitrate, can effectively stimulate and enhance the biosynthesis of specific phytoconstituents even under controlled conditions.

In this research, the elicitors were applied at two different concentrations (0.5 ppm and 1 ppm) to a range of nutraceutically valuable plants. It was observed that at a 0.5 ppm concentration of salicylic acid and silver nitrate, there was a significant enhancement in the alkaloid content of wheatgrass, lemongrass, and dill. This finding suggests that these elicitors, at an optimized lower concentration, can effectively stimulate alkaloid biosynthesis in these plants.

Similarly, flavonoid content was found to increase substantially in fenugreek, spinach, tulsi, coriander, mint, and wheatgrass following treatment with the elicitors at 0.5 ppm. This indicates that low-dose elicitor treatment is beneficial not only for alkaloid production but also for the improvement of flavonoid profiles across a broader range of plant species.

Moreover, the study also recorded a marked improvement in total phenolic content in fenugreek, spinach, tulsi, coriander, mint, wheatgrass, dill, curry leaves, and lemongrass at both 0.5 ppm and 1 ppm concentrations. This demonstrates the broad-spectrum effectiveness of salicylic acid and silver nitrate in enhancing phenolic compound production, which is crucial for the antioxidant potential and therapeutic value of these plants.

However, it was also observed that at higher elicitor concentrations, the production of secondary metabolites either plateaued or decreased. This suggests that there is a threshold beyond which elicitor treatment becomes counterproductive, likely due to metabolic imbalances or stress-induced inhibitory effects on biosynthetic pathways. Thus, precise optimization of elicitor dosage is critical to maximize metabolite yield without compromising plant health or biosynthetic efficiency.

The insights derived from this study provide a strong foundation for future applications of elicitor technology in the agricultural and nutraceutical industries. By combining the knowledge of elicitor responses with high-throughput screening and biotechnological tools, it may be possible to engineer plant cells or culture systems capable of producing valuable phytoconstituents at a commercial scale. This approach could greatly enhance the sustainable production of bioactive compounds, offering significant advantages for the development of functional foods, dietary supplements, and herbal therapeutics.

In conclusion, the use of salicylic acid and silver nitrate as elicitors presents a promising strategy for improving the qualitative and quantitative profile of nutraceutical plants, contributing not only to enhanced crop value but also to the advancement of plant-based health products in the future.

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