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EFFECT OF DIFFERENT ABIOTIC STRESSES ON ACETYLCHOLINESTERASE INHIBITION, ANTIOXIDANT ACTIVITY TOTAL PHENOL & FLAVONOID CONTENT OF SELECTED *ALLIUM SPECIES*: RESULTS FROM A 6-MONTH FIELD STUDY

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Keywords:

Abiotic stress, *Allium species*, Antioxidant activity, Acetylcholine sterase inhibition, Total phenol content, Total flavonoid content

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ABSTRACT: Environmental factors influence plant growth, morphology, as well as nature and quantity of secondary metabolites. Modification of abiotic factors during plant growth can impact the production of bioactive phytoconstituents. A six-month field study was carried out to assess the effect of different abiotic stresses on bioactive compounds (flavonoids, phenols, quercetin), acetylcholine sterase (AChE) inhibition and antioxidant capacity of two selected *Allium* species *i.e. Allium cepa* L. (AC) & *Allium sativum* L. (AS; family Amaryllidaceae). AC and AS plants were grown for six months (from November 2018 to April 2019) on three different soil types (*i.e.* black, red, clay-loamy) and various stresses (salinity, water deprivation, flooding, fertilizer, metal, shade) were applied on the plants. At the end of the season, plants were collected, dried and plant yield was determined. Hydro alcoholic extracts of all samples were prepared. Extract yield, total flavonoids (TFC), total phenols (TPC), AChE inhibition, and antioxidant activity were determined in all the extracts. The results show the varied response of AC & AS plants to different soil types and stresses. AS plants grown in red soil with salt stress and AC plants on red soil with fertilization give higher biomass yield. AC plants grown in black soil and AS in red soil under metal stress have the highest TFC, TPC, AChE inhibition, and antioxidant activity. Hence these conditions may be recommended for incorporation in cultivation practices of these valuable medicinal plants. This would ensure a commercial supply of plants with higher phenol and flavonoid content and better activity.

INTRODUCTION: Onions *Allium cepa* L. (AC) and garlic *Allium sativum* L. (AS) are highly valued members of the genus Allium (Family Amaryllidaceae) [1]. AC and AS are considered imperative dietary components due to their unique flavors and nutritional value. Both plants have been traditionally used as a functional food to enhance physical and mental health [2, 91]. These were used as popular remedies for many diseases like cold, flu, asthma, ophthalmic problems, inflammatory disorders, *etc.* [3, 4, 5, 92].

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Several commercial formulations (*i.e.*, extract, essential oil, macerate powder) have gained popularity due to their remarkable culinary and therapeutic properties [6, 7, 93]. Both plants have been extensively investigated phytochemically and pharmacologically. Both have shown diverse and significant biological activities like antioxidant, antibacterial, antiviral, anticancer, immuno-modulatory effects [95]. These reduce the risk of cardiovascular disorders and are strong neuroprotectants in various neurodegenerative disorders [8, 9, 10, 11]. These effects are attributed to their high organosulphur, phenolic, and flavonoid content [12, 13, 96]. These are also rich in anthocyanins, tannins, proteins, sterols, glycosides, saponins, and carbohydrates. Apart from these, phytoconstituents AC and AS are also rich sources of vitamins *i.e.*, vitamin B-6, vitamin B-12, vitamin C, Riboflavin, Niacin, Folate, Vitamin A, E, K, *etc.*, minerals *i.e.*, Potassium, Selenium, water, Calcium, Iron, Magnesium, Zinc, Copper, protein, carbohydrates, non-dietary fibers, *etc.* [14, 15, 16, 17].

Both plants were amongst the earliest to be cultivated 1. The cultivation practices for AC and AS are well documented [18, 19, 94] but the emphasis has been on plant growth and yield rather than bioactive constituents and related biological activities. Studies show that abiotic and biotic factors influence not only plant growth but also the nature and amount of phytoconstituents present in plants [20]. These factors can be modified in cultivation practices to enhance plant growth and production of valuable active constituents/markers and their activity. Considering the medicinal importance of both plants, *i.e.*, AC & AS, it was thought worthwhile to identify the environmental factors during cultivation that enhance biomarker production and augment the plants' biological activities. The objective of the present study was to understand the effect of abiotic factors (season, soil type, metal, salinity, flooding, drought, fertilizer, shade stress) on the production of a marker compound, total flavonoids content (TFC), total phenols content (TPC), acetylcholinesterase (AChE) inhibition and antioxidant activities of both selected *Allium* species.

MATERIAL AND METHODS:

Field Trial: The field experiment was conducted at the Medicinal Plant Garden of the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India. Onion seeds and garlic cloves were procured from the National Horticultural Research and Development Foundation (NHRDF), Bathinda, Punjab (151005), India in October, 2017.

The plants were authenticated by the National Horticultural Research and Development Foundation (NHRDF), Bathinda, Punjab (151005), (India) with Specimen number: NHRDF/SC/BTI/RD-2/2017-2018/515. Fresh plantlets of AC and AS were prepared before the start of the season. Based on literature reports, the plantlets were sown in the month of November under different conditions and cultivated for six months [21, 22, 23, 24]. **Fig. 1** summarizes the plan of work of this investigation.

Experimental Design: Season for field study: November 2018 to April 2019.

Propagation: Seven plots ($1 \times 1m^2$) were prepared for each soil type. Farmyard manure (FYM) was added 2.5 kg in each plot [25].

The plantlets (20 plantlets) of length 5 cm of both onion & garlic were directly planted in plots ($1 \times 1m^2$) at a distance of 10 cm in a row; each row was 10 cm apart and immediately irrigated.

Application of Abiotic Stresses:

Control Plants (No Stress): Plants were allowed to grow on different soils without any stress being applied. For normal growth of plants, irrigation was done at weekly intervals [26].

Fertilization: Plants of onion and garlic grown in different soils were applied with fertilizers (Urea 3 g/m^2, phosphorus 6 $g/m^{2,}$ and potash 3 g/m2) and (Urea 6 g/m^2, phosphorus 5 $g/m^{2,}$ and potash 6 g/m^2) respectively once in a month. According to ratio 30:60:30 and 60:50:60 for onion & garlic respectively [25, 27, 28].

Shade Stress: Plants were cultivated under full shade conditions to determine the effect of shade on growth and secondary metabolite production [29].

Salt Stress: Salt stress was applied by adding 3mM/L sodium chloride (NaCl) solution to each plot at monthly intervals [30].

Metal Stress: Metal stress was applied by adding 75 µM solution of copper sulphate ($CuSO_4$) to each selected plot at monthly intervals [31].

Water Stress: Two types of water stress were applied:

Drought: Plants were irrigated after 14 days and these plants were grown under water scarcity conditions.

Flooding: Plants were kept submerged underwater [32].

Collection of Plant: According to literature, the bulbs of both plants exposed to different conditions were collected after 6 months, shade dried and weighed [21, 22, 33, 34].

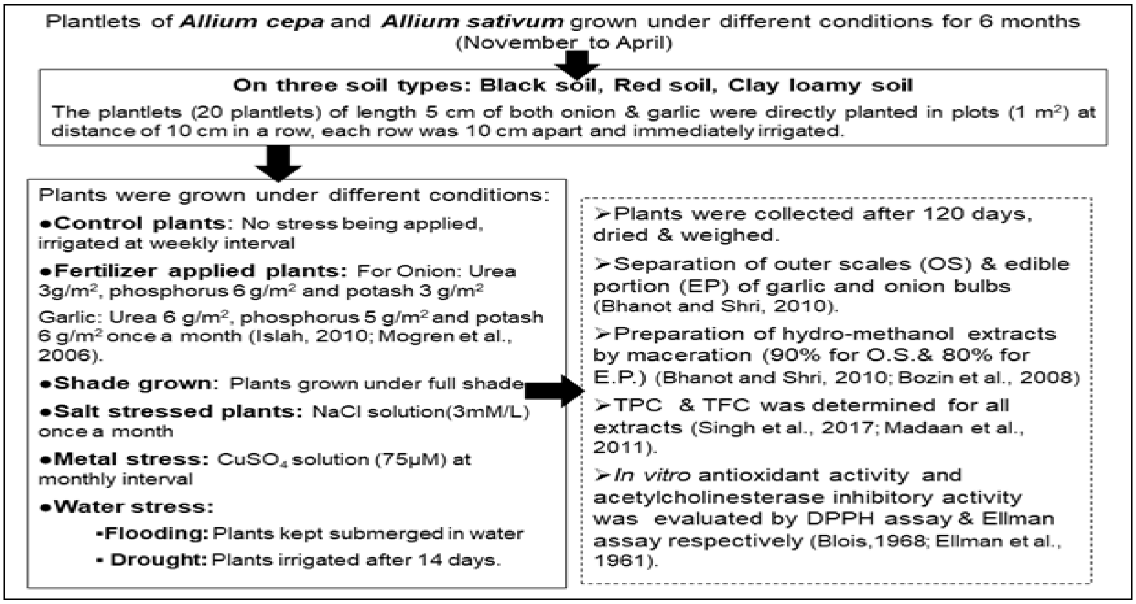

FIG. 1: PLAN OF WORK IN THE PRESENT INVESTIGATION

Preparation of Extracts: Onion and garlic bulbs were harvested at the maturity stage from all stress treated plots separately, dried, and weighed. Outer scales & edible portions were separated from both plants. The edible portion of AC & AS were ground with 80% methanol and filtered. The filtrate was collected, concentrated and dried to prepare extracts, and yield (% w/w, dry weight basis) was recorded for all plants [8, 35]. Outer scales of AC & AS were ground with 90% methanol and allowed to stand with solvent for 2-3 days for exhaustive maceration and filtered. The filtrate was collected, concentrated, and dried to prepare extracts, and yield (% w/w, dry weight basis) was recorded for all plants [8, 35, 36].

Phytochemical Screening: Phytochemical evaluation of prepared extracts was carried to determine the presence or absence of flavonoids, saponins, steroids, carbohydrates, triterpenoids, tannins and proteins as per standard tests [36, 37, 38, 39, 40].

Standardization of Extracts of AC and AS Affected by Different Stresses:

Estimation o Total Phenol Content: Total phenol content (TPC) analysis of hydro-methanol extracts was determined by Folin-Ciocalteu procedure using the standard plot of Gallic acid [36, 41].

Results were expressed as percentage w/w and calculated using the following formula:

$$\text{Total phenolic content (\% w/w)} = GAE \times V \times D \times 10^{-6} \times 100/W,$$

GAE - Gallic acid equivalent (µg/ml), V - Total volume of sample (ml), D - Dilution factor, W - Sample weight (g).

Estimation of Total Flavonoid Content: Total flavonoid content (TFC) of hydro-methanol extracts was determined by using the Aluminium chloride method using the standard plot of quercetin [36, 41].

Results were expressed as percentage w/w and calculated by using the following formula:

$$\text{Total flavonoid content (\% w/w)} = QE \times V \times D \times 10^{-6} \times 100/W,$$

QE - quercetin equivalent (µg/ml), V - total volume of sample (ml), D - dilution factor, W - sample weight (g).

***In-vitro* Evaluation of Biological Activities of the Extracts:**

***In-vitro* Antioxidant Activity:** The antioxidant activity of various plant extracts was determined by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. The DPPH scavenging activity was evaluated as described by Blois (1958), Kamboj and Rana, (2014), and Singh *et al.*, 2018 with slight modifications [42, 43, 44]. The IC_{50} value of each extract was calculated by using linear regression analysis and expressed in µg/ml. All readings were taken in triplicate.

***In-vitro* Acetylcholinesterase (Ache) Inhibitory Activity:** The AChE inhibitory effect of each test extract was evaluated using the method of Ellman *et al.*, (1961) [45]. The enzyme acetylcholine stress hydrolyzes the substrate acetylcholine, which results in the production of thiocholine. The latter reacts with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB), thereby producing 2-nitrobenzoate-5-mercaptothiocholine and %-thio-2-nitrobenzoate, which can be detected at 412 nm. All the readings were taken in triplicate [11, 45, 46].

RESULTS AND DISCUSSION:

Effect of Abiotic Stresses on Plant Growth: AC and AS are herbaceous, perennial, flowering plants, which are monocots in the order Asparagales. Both plants can be easily propagated by seeds, cloves, direct sowing or bulbs [47].

In this study, propagation was done by plantlets which spread fast and evenly. The plants grown under different conditions were collected at the end of the study period, shade dried, and weighed separately. **Fig. 2** and **3** summarizes the amount of plant collected per unit area expressed as g/m^2.

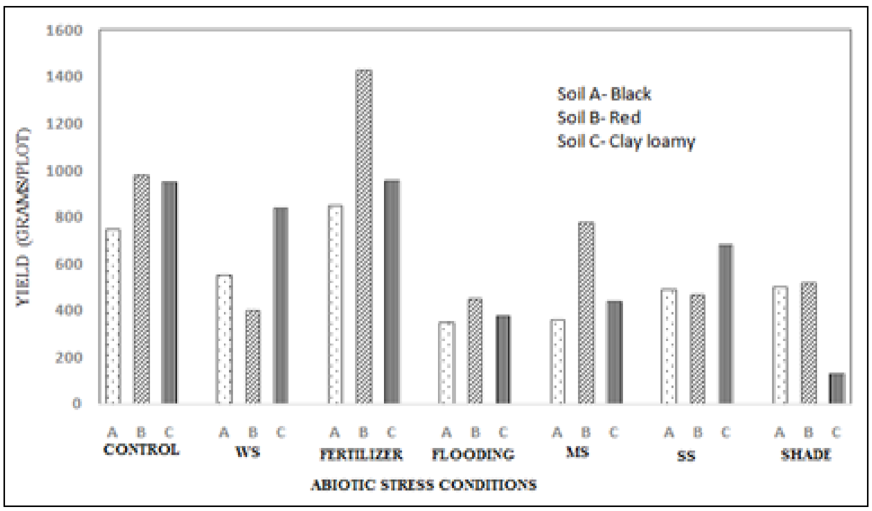

FIG. 2: PLANT YIELD OF *ALLIUM CEPA* GROWN UNDER DIFFERENT STRESS CONDITIONS WS- water scarcity; MS- metal stress; SS- salt stress.

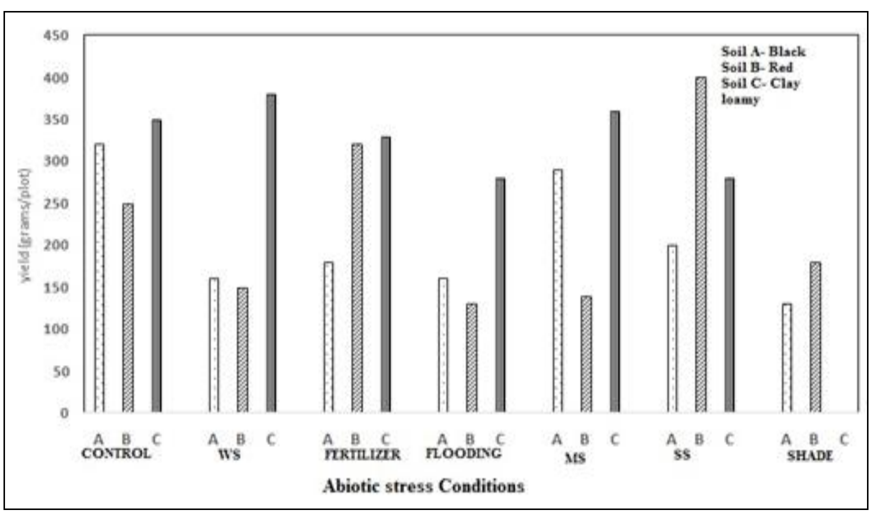

FIG. 3: PLANT YIELD OF *ALLIUM SATIVUM* GROWN UNDER DIFFERENT STRESS CONDITIONS WS- water scarcity; MS- metal stress; SS- salt stress

According to literature, for the cultivation of onion and garlic, plantlets or medium-sized bulbs can be used for sowing during two seasons in a year *i.e.* April to May and October to November [25, 48]. These grow well in pH range of 6-7 and in a mild season without extremes of heat and cold. The recommended soils for their cultivation are red soil, black soil, red loam soil, and clay loam soil with good drainage facilities. These plants require sufficient soil moisture during their growth period, but heavy rains during bulb germination and bulb formation affect the crop growth [48, 49, 50, 51]. The results found in our study are in consonance with the previous studies as the plantlets grew well in all three soil types with weekly irrigation (except for the water stress groups). Reports on crop yields show that yield of onion bulbs (25-40 ton/hectare) is generally higher than that of garlic (50-70 quintal / hectare) [52, 53, 54, 98]. In this investigation, also yield of AC was significantly higher than the yield of AS in all cultivation conditions. Nitrogen, phosphorus, and potassium are the primary nutrients necessary for plant growth. Nitrogen fertilizers improve plant vigor, water utilization, number of seeds, size of leaves and stems, number of roots [55]. It is well documented that the application of nitrogen fertilizer increases the bulb yield of onion and garlic significantly [18, 56, 97, 98].

In our study yield of AC was maximum with fertilizers irrespective of soil type.

Effect of Abiotic Stresses on AC and AS Extract Yield: By reviewing the literature, it was found that onion and garlic have been used in folk medicines since ancient times in different forms, *e.g.*, Juice, paste, poultice, powder, and in food items. Various methods such as maceration, Soxhlet extraction, Freeze-drying, Pressurized Liquid Extraction, Subcritical water extraction, *etc.*, are reported for the preparation of extracts from AC and AS [57, 58, 59, 60, 61, 62, 63]. Alcoholic or aqueous extracts of both plants are generally prepared since these contain higher quantities of flavonoids and phenols [36]. Thus based on literature, in our study, hydro-methanol extracts of AC and AS were prepared by maceration. Numerous studies show phytochemical variations in outer scales and edible portions of both plants; therefore, it results in changes in pharmacological activities as well [1, 64, 65, 66, 67, 68, 69, 70]. So, the edible portion and outer scales were separated, and their hydro-methanol extracts were prepared.

Table 1 summarizes the yield (% w/w, dry weight basis) of hydro-methanol extract prepared from plants grown under different conditions.

TABLE 1: YIELD OF METHANOL EXTRACTS OF AC & AS PLANTS GROWN UNDER DIFFERENT ABIOTIC STRESSES

Soil Type	Abiotic stress	Yield (% w/w, dry weight basis)			
		AC		AS	
		Edible portion	Outer scales	Edible portion	Outer scales
Black	Control	10.90	6.15	10.56	9.21
	Water stress	5.10	6.70	10.82	8.56
	Fertilization	19.85	7.17	9.15	4.12
	Flooding	11.12	5.45	3.68	3.25
	Metal stress	6.36	5.80	4.12	5.17
	Salt stress	9.10	11.15	9.53	5.65
	Shade	9.62	4.16	5.88	1.8
Red	Control	15.35	7.95	11.22	7.44
	Water stress	11.55	2.45	5.11	4.67
	Fertilization	21.77	6.90	6.25	4.35
	Flooding	12.01	4.46	4.54	6.71
	Metal stress	17.57	6.55	2.42	2.26
	Salt stress	8.73	9.29	11.59	6.14
	Shade	14.38	4.06	13.65	7.25
Clay loamy	Control	4.30	8.15	8.65	7.59
	Water stress	18.46	3.12	8.11	7.04
	Fertilization	13.24	3.85	6.76	3.95
	Flooding	5.11	5.07	10.12	6.95
	Metal stress	12.62	1.12	8.89	4.86
	Salt stress	8.78	2.04	7.42	8.21
	Shade	7.39	6.38	NA	NA

N.A - Not applicable

The edible portion of AC plants grown in red soil under fertilization gave the highest yield of methanol extract. It is emphasized here that red soil is porous and rich in iron content with a pH range of 6.6 to 8.0. Red soil is loose, aerated and responds well to fertilizers. It contains salt in a lower quantity. It has been reported that red soil increases the biomass yield of onion [71].

Phytochemical Screening of Extracts: The results of phytochemical screening revealed the presence of flavonoids, carbohydrates, tannins, steroids, saponins, and triterpenoids in both parts of the plants, while edible portions of the onion and garlic also contain proteins and amino acids. These results are in agreement with earlier reports [37, 38, 39, 40, 72].

Standardization of AC and AS extracts: Phytochemical screening, in accordance with the literature, revealed the presence of phenols and flavonoids in hydro-methanol extracts of onion and garlic. Therefore, all the prepared hydro-methanol extracts of AC and AS were standardized with respect to TPC and TFC.

Estimation of Total Phenol Content: Quantification of total phenol in hydro-methanol extract of all the stress affected plants was done on the basis of a standard curve of gallic acid. The standard curve of absorbance of gallic acid was prepared **Fig. 4. Table 2** presents the TPC of all the prepared extracts.

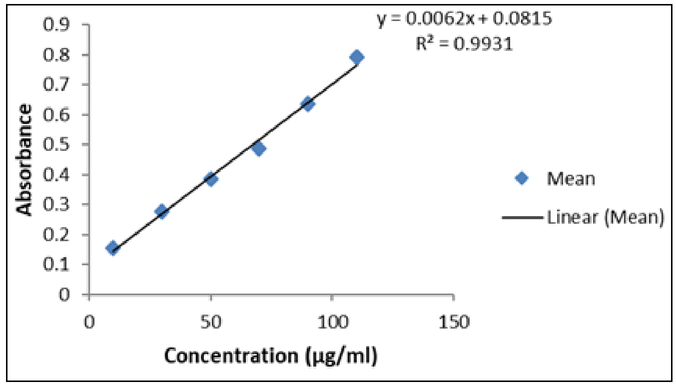

FIG. 4: STANDARD CURVE OF GALLIC ACID

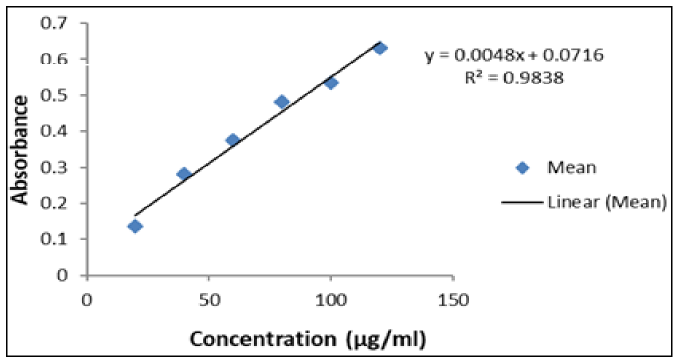

FIG. 5: STANDARD CURVE OF QUERCETIN

TABLE 2: TOTAL PHENOLIC CONTENT IN EXTRACTS OF AC AND AS

Soil	Sample	Total phenol content (% w/w)* (Mean^n ± S.D)			
		AC		AS	
		Edible portion	**Outer scales**	**Edible portion**	**Outer scales**
Black	Control	19.34 ± 0.34	34.37 ± 0.43	12.06 ± 0.84	06.25 ± 0.48
	Water stress	66.52 ± 0.31^{a,b,c}	57.32 ± 0.38^{p,q,r}	49.52 ± 0.49^{x,y,z}	17.35 ± 0.84$^{\$,\#,@}$
	Fertilization	44.74 ± 0.24^{a,b,c}	52.50 ± 0.38^{p,q,r}	44.60 ± 0.78^{x,y,z}	20.27 ± 0.48$^{\$,\#,@}$
	Flooding	23.65 ± 0.36^{a,b,c}	36.59 ± 0.28^{p,q,r}	48.86 ± 0.27^{x,y,z}	23.45 ± 0.34$^{\$,\#,@}$
	Metal stress	70.29 ± 0.40^{a,b,c}	71.16 ± 0.31^{p,q,r}	52.16 ± 0.18^{x,y,z}	11.03 ± 0.73$^{\$,\#,@}$
	Salt stress	31.35 ± 0.35^{a,b,c}	63.46 ± 0.33^{p,q,r}	41.28 ± 0.68^{x,y,z}	04.48 ± 0.61$^{\$,@}$
	Shade	51.53 ± 0.30^{a,b,c}	47.45 ± 0.41^{p,q,r}	18.12 ± 0.67^{x,y,z}	03.33 ± 0.38$^{\$,\#,@}$
Red	Control	17.51 ± 0.37^{a,c}	53.49 ± 0.38^{p,r}	27.37 ± 0.68^{x,z}	04.05 ± 0.73$^{\$,@}$
	Water stress	14.55 ± 0.31^{a,b,c}	56.46 ± 0.32^{p,q,r}	32.37 ± 0.58^{x,y,z}	07.31 ± 0.72$^{\$,\#,@}$
	Fertilization	28.41 ± 0.39^{a,b,c}	54.42 ± 0.28^{p,q,r}	43.06 ± 0.24^{x,y,z}	36.23 ± 0.45$^{\$,\#,@}$
	Flooding	13.24 ± 0.46^{a,b,c}	15.44 ± 0.29^{p,q,r}	38.08 ± 0.28^{x,y,z}	34.05 ± 0.82$^{\$,\#,@}$
	Metal stress	67.66 ± 0.28^{a,b,c}	68.36 ± 0.52^{p,q,r}	54.46 ± 0.45^{x,y,z}	47.27 ± 0.28$^{\$,\#,@}$
	Salt stress	41.27 ± 0.20^{a,b,c}	50.36 ± 0.46^{p,q,r}	39.11 ± 0.81^{x,y,z}	26.21 ± 0.62$^{\$,\#,@}$
	Shade	26.01 ± 0.66^{a,b,c}	62.39 ± 0.36^{p,q,r}	29.41 ± 0.65^{x,y,z}	05.38 ± 0.44$^{\$,\#,@}$
Clay loamy	Control	16.36 ± 0.36^{a,b}	38.53 ± 0.34^{p,q}	31.21 ± 0.73^{x,y}	13.03 ± 0.90$^{\$,\#}$
	Water stress	48.60 ± 0.32^{a,b,c}	29.46 ± 0.27^{p,q,r}	16.41 ± 0.61^{x,y,z}	08.31 ± 0.37$^{\$,\#,@}$
	Fertilization	49.32 ± 0.38^{a,b,c}	61.43 ± 0.44^{p,q,r}	42.48 ± 0.62^{x,y,z}	15.15 ± 0.52$^{\$,\#,@}$
	Flooding	39.50 ± 0.38^{a,b,c}	25.18 ± 0.21^{p,q,r}	37.47 ± 0.71^{x,y,z}	24.19 ± 0.23$^{\$,\#,@}$
	Metal stress	59.44 ± 0.31^{a,b,c}	69.45 ± 0.45^{p,q,r}	46.38 ± 0.47^{x,y,z}	21.67 ± 0.77$^{\$,\#,@}$
	Salt stress	43.46 ± 0.48^{a,b,c}	55.47 ± 0.33^{p,q,r}	35.03 ± 0.59^{x,y,z}	09.52 ± 0.53$^{\$,\#,@}$
	Shade	45.22 ± 0.20^{a,b,c}	65.47 ± 0.43^{p,q,r}	NA	NA

N.A - Not applicable, n=3,* on dry weight basis. a $p < 0.05$ vs. black soil control of AC edible portion; b $p < 0.05$ vs. red soil control of AC edible portion; c $p < 0.05$ vs. clay loamy soil control of AC edible portion. p $p < 0.05$ vs. red soil control of AC outer scales; q $p < 0.05$ vs. red soil control of AC outer scales r $p < 0.05$ vs. clay loamy soil control of AC outer scales. x $p < 0.05$ vs. black soil control of AS edible portion; y $p < 0.05$ vs. red soil control of AS edible portion; z $p < 0.05$ vs. clay loamy soil control of AS edible portion. $ $p < 0.05$ vs. black soil control of AS outer scales; # $p < 0.05$ vs. red soil control of AS outer scales; @ $p < 0.05$ vs. clay loamy soil control of AS outer scales.

The total phenol content of AC and AS plants grown under different abiotic stresses is shown in **Table 3.** The data is expressed as Mean ± S.D. (n=3) and analyzed by Two way ANOVA followed by Tukey's post-hoc analysis test.

TABLE 3: TOTAL FLAVONOID CONTENT OF THE EXTRACTS OF AC AND AS

Soil	Sample	Total flavonoid content (% w/w) * (Mean^n ± S.D)			
		AC		AS	
		Edible portion	Outer scales	Edible portion	Outer scales
Black	Control	17.36 ± 0.32	30.64 ± 0.22	11.02 ± 0.76	06.16 ± 0.44
	Water stress	58.37 ± 0.34 ^{a,b,c}	52.29 ± 0.54 ^{p,q,r}	40.27 ± 0.42 ^{x,y,z}	15.51 ± 0.26$^{\$,\#,@}$
	Fertilization	38.15 ± 0.33 ^{a,b,c}	46.47 ± 0.38 ^{p,q,r}	36.51 ± 0.43 ^{x,y,z}	17.06 ± 0.31$^{\$,\#,@}$
	Flooding	19.29 ± 0.45 ^{a,b,c}	32.12 ± 0.67 ^{p,q,r}	39.17 ± 0.93 ^{x,y,z}	19.71 ± 0.14$^{\$,\#,@}$
	Metal stress	62.97 ± 0.89 ^{a,b,c}	64.40 ± 0.99 ^{p,q,r}	42.74 ± 0.18 ^{x,y,z}	10.41 ± 0.54$^{\$,\#,@}$
	Salt stress	28.27 ± 0.28 ^{a,b,c}	56.52 ± 0.27 ^{p,q,r}	33.46 ± 0.31 ^{x,y,z}	04.74 ± 0.19$^{\$,\#,@}$
	Shade	45.63 ± 0.26 ^{a,b,c}	41.69 ± 0.42 ^{p,q,r}	16.64 ± 0.22 ^{x,y,z}	02.86 ± 0.36$^{\$,\#,@}$
Red	Control	15.42 ± 0.21 ^{a,c}	47.47 ± 0.27 ^{p,r}	23.54 ± 0.40 ^{x,z}	03.93 ± 0.21 $^{\$,@}$
	Water stress	10.84 ± 0.66 ^{a,b,c}	51.59 ± 0.22 ^{p,q,r}	26.59 ± 0.29 ^{x,y,z}	07.13 ± 0.61$^{\$,\#,@}$
	Fertilization	25.47 ± 0.51 ^{a,b,c}	48.37 ± 0.14 ^{p,q,r}	35.16 ± 0.86 ^{x,y,z}	29.46 ± 0.32$^{\$,\#,@}$
	Flooding	09.74 ± 0.23 ^{a,b,c}	12.99 ± 0.96 ^{p,q,r}	31.71 ± 0.26 ^{x,y,z}	27.28 ± 0.45$^{\$,\#,@}$
	Metal stress	59.46 ± 0.33 ^{a,b,c}	60.69 ± 0.23 ^{p,q,r}	43.29 ± 0.39 ^{x,y,z}	38.48 ± 0.34$^{\$,\#,@}$
	Salt stress	35.33 ± 0.52 ^{a,b,c}	44.37 ± 0.53 ^{p,q,r}	32.17 ± 0.34 ^{x,y,z}	22.44 ± 0.41$^{\$,\#,@}$
	Shade	24.53 ± 0.11 ^{a,b,c}	55.63 ± 0.34 ^{p,q,r}	24.30 ± 0.42 ^{x,y,z}	05.41 ± 0.38$^{\$,\#,@}$
Clay loamy	Control	13.65 ± 0.36 ^{a,b}	33.47 ± 0.28 ^{p,q}	25.49 ± 0.32 ^{x,y}	12.29 ± 0.26 $^{\$,\#}$
	Water stress	42.43 ± 0.58 ^{a,b,c}	26.51 ± 0.31 ^{p,q,r}	14.27 ± 0.72 ^{x,y,z}	08.28 ± 0.64$^{\$,\#,@}$
	Fertilization	43.58 ± 0.42 ^{a,b,c}	54.33 ± 0.41 ^{p,q,r}	34.09 ± 0.52 ^{x,y,z}	13.52 ± 0.29$^{\$,\#,@}$
	Flooding	34.46 ± 0.42 ^{a,b,c}	21.64 ± 0.65 ^{p,q,r}	30.49 ± 0.28 ^{x,y,z}	21.64 ± 0.17$^{\$,\#,@}$
	Metal stress	53.56 ± 0.57 ^{a,b,c}	61.67 ± 0.42 ^{p,q,r}	37.74 ± 0.31 ^{x,y,z}	18.51 ± 0.37$^{\$,\#,@}$
	Salt stress	37.42 ± 0.29 ^{a,b,c}	50.41 ± 0.44 ^{p,q,r}	28.48 ± 0.24 ^{x,y,z}	09.34 ± 0.62$^{\$,\#,@}$
	Shade	40.33 ± 0.40 ^{a,b,c}	57.51 ± 0.35 ^{p,q,r}	NA	NA

N.A - Not applicable, n=3,* on dry weight basis. a $p < 0.05$ vs. black soil control of AC edible portion; b $p < 0.05$ vs. red soil control of AC edible portion; c $p < 0.05$ vs. clay loamy soil control of AC edible portion. p $p < 0.05$ vs. red soil control of AC outer scales; q $p < 0.05$ vs. red soil control of AC outer scales r $p < 0.05$ vs. clay loamy soil control of AC outer scales. x $p < 0.05$ vs. black soil control of AS edible portion; y $p < 0.05$ vs. red soil control of AS edible portion; z $p < 0.05$ vs. clay loamy soil control of AS edible portion. $ $p < 0.05$ vs. black soil control of AS outer scales; # $p < 0.05$ vs. red soil control of AS outer scales; @ $p < 0.05$ vs. clay loamy soil control of AS outer scales.

Total flavonoid content of AC and AS plants grown under different abiotic stresses is shown in **Table 4.** The data is expressed as Mean ± S.D. (n=3) and analyzed by Two way ANOVA followed by Tukey's post-hoc analysis test. According to the literature, there is variation in the phytochemical distribution in bulb and outer scales of onion and garlic. It is well documented that the edible portion of AS contains a higher amount of TPC and TFC as compared to outer scales, and in contrast, outer scales of AC contains a higher amount of TPC and TFC as compare to the edible portion of the plant [65, 69, 37, 28, 63, 73].

From the results shown in **Tables 3 & 4,** it is evident that the outcomes of this study are consonant with previous findings. Secondary metabolites are biosynthesized to help plants to cope with any change or stress. It is evident from **Tables 3** & **4** that in response to different stresses applied in this study, the TPC and TFC increases. The most marked increase in TPC and TFC was observed in plants with mental stress. Literature shows that plants exposed to heavy metal stress show differential responses in synthesis and accumulation of pharmacologically active molecules. Usage of heavy metals in optimum concentration acts as abiotic elicitors that improve the biosynthesis of specific bioactive compounds [74, 99, 100]. It is reported that heavy metal treatment increases the biosynthesis of secondary metabolites in many plant species.

In red cabbage stress of copper metal increased the level of phenolic and flavonoid compounds as well as antioxidant activity [75]. In case of *Cajanus cajan* L. it was found that under the Zn and Ni treatment, amount of ascorbic acid has increased owing to which the antioxidant activity enhanced 76. When *Matricaria chamomilla* plants were exposed to Cd and Cu (60 µM to120 µM) during cultivation, the amount of ferulic acid, cinnamic acid, caffeic acid, p-coumaric increased significantly [77].

Similarly, in our study, it is found that metal stress showed the most marked effect on total phenol content and total flavonoid content of onion and garlic plants. Our results showed:

Metal Stress in Black Soil Resulted in Highest Total Phenol Content, Total Flavonoid Content in Onion Extracts:

Metal Stress in Red Soil Resulted in Highest Total Phenol Content, Total Flavonoid Content in Garlic Extracts:

***In-vitro* Evaluation of Bioactivities:**

Antioxidant Activity of Prepared Extracts: The antioxidant activity of extracts of different stress-affected plants of AC and AS (edible portion and outer scales) were evaluated using *in-vitro* DPPH assay. The IC_{50} values are reported in **Table 4.**

- Metal stress-affected AC plants grown in black soil have the highest antioxidant potential.
- Metal stress affected AS plants are grown in red soil have highest antioxidant potential.
- Higher TPC and TFC is strongly correlated with antioxidant activity [65, 78, 79, 80, 101, 102, 103].

  In this study also plants extracts with higher TPC and TFC had better antioxidant activity.

Acetylcholinesterase Inhibitory Activity: The IC_{50} values of the extracts in the Ellman assay are reported in **Table 5.**

TABLE 4: THE IC_{50} VALUES OF EXTRACTS OF AC AND AS IN DPPH ASSAY

Soil	Sample	IC_{50} value (µg/ml)* (Mean^n ± S.D)			
		AC		AS	
		Edible portion	Outer scales	Edible portion	Outer scales
Black	Control	28.68 ± 0.06	16.70 ± 0.44	31.65 ± 0.29	41.42 ± 0.22
	Water stress	23.31 ± 0.10 ^{a,b,c}	5.75 ± 0.04 ^{p,q,r}	12.91 ± 0.08 ^{x,y,z}	30.62 ± 0.24 $^{\$,\#,@}$
	Fertilization	29.75 ± 0.09 ^{a,b,c}	7.48 ± 0.04 ^{p,q,r}	17.55 ± 0.21 ^{x,y,z}	29.78 ± 0.13 $^{\$,\#,@}$
	Flooding	55.32 ± 0.38 ^{a,b,c}	26.96 ± 0.11 ^{p,q,r}	12.96 ± 0.03 ^{x,y,z}	28.44 ± 0.17 $^{\$,\#,@}$
	Metal stress	13.85 ± 0.12 ^{a,b,c}	4.70 ± 0.21 ^{p,q,r}	9.25 ± 0.08 ^{x,y,z}	32.29 ± 0.21 $^{\$,\#,@}$
	Salt stress	30.11 ± 0.15 ^{a,b,c}	17.40 ± 0.12 ^{p,q,r}	18.70 ± 0.25 ^{x,y,z}	42.11 ± 0.17 $^{\$,\#,@}$
	Shade	18.81 ± 0.03 ^{a,b,c}	23.93 ± 0.19 ^{p,q,r}	30.56 ± 0.26 ^{x,y,z}	44.56 ± 0.03 $^{\$,\#,@}$
Red	Control	36.90 ± 0.11 ^{a}	18.44 ± 0.05 ^{p}	26.36 ± 0.48 ^{x,z}	43.11 ± 0.16 $^{\$,@}$
	Water stress	41.37 ± 0.12 ^{a,b,c}	20.68 ± 0.05 ^{p,q,r}	24.90 ± 0.52 ^{x,y,z}	38.39 ± 0.12 $^{\$,\#,@}$
	Fertilization	33.76 ± 0.07 ^{a,b,c}	16.83 ± 0.01 ^{p,q,r}	17.56 ± 0.08 ^{x,y,z}	20.35 ± 0.12 $^{\$,\#,@}$
	Flooding	33.31 ± 0.13 ^{a,b,c}	16.59 ± 0.04 ^{p,q,r}	19.50 ± 0.08 ^{x,y,z}	24.49 ± 0.30 $^{\$,\#,@}$
	Metal stress	17.27 ± 0.21 ^{a,b,c}	8.63 ± 0.11 ^{p,q,r}	8.02 ± 0.01 ^{x,y,z}	15.32 ± 0.29 $^{\$,\#,@}$
	Salt stress	31.28 ± 0.23 ^{a,b,c}	15.63 ± 0.12 ^{p,q,r}	18.88 ± 0.09 ^{x,y,z}	26.85 ± 0.14 $^{\$,\#,@}$
	Shade	35.61 ± 0.15 ^{a,b,c}	17.80 ± 0.07 ^{p,q,r}	25.92 ± 0.10 ^{x,y,z}	41.59 ± 0.27 $^{\$,\#,@}$
Clay loamy	Control	36.88 ± 0.28 ^{a}	18.43 ± 0.14 ^{p}	25.88 ± 0.50 ^{x,y}	31.41 ± 0.11 $^{\$,\#}$
	Water stress	27.99 ± 0.08 ^{a,b,c}	13.99 ± 0.05 ^{p,q,r}	30.64 ± 0.32 ^{x,y,z}	35.65 ± 0.09 $^{\$,\#,@}$
	Fertilization	26.29 ± 0.17 ^{a,b,c}	12.92 ± 0.11 ^{p,q,r}	18.10 ± 0.16 ^{x,y,z}	31.35 ± 0.23 $^{\$,\#,@}$
	Flooding	32.23 ± 0.15 ^{a,b,c}	15.93 ± 0.07 ^{p,q,r}	19.59 ± 0.08 ^{x,y,z}	27.30 ± 0.31 $^{\$,\#,@}$
	Metal stress	20.32 ± 0.05 ^{a,b,c}	9.73 ± 0.10 ^{p,q,r}	16.12 ± 0.24 ^{x,y,z}	28.81 ± 0.12 $^{\$,\#,@}$
	Salt stress	43.19 ± 0.07 ^{a,b,c}	21.58 ± 0.05 ^{p,q,r}	24.06 ± 0.55 ^{x,y,z}	33.03 ± 0.11 $^{\$,\#,@}$
	Shade	27.21 ± 0.12 ^{a,b,c}	13.16 ± 0.09 ^{p,q,r}	NA	NA
Ascorbic acid (Standard)		4.63 ± 0.41			

NA - Not applicable, n=3, * on dry weight basis. a $p < 0.05$ vs. black soil control of AC edible portion; b $p < 0.05$ vs. red soil control of AC edible portion; c $p < 0.05$ vs. clay loamy soil control of AC edible portion. p $p < 0.05$ vs. red soil control of AC outer scales; q $p < 0.05$ vs. red soil control of AC outer scales r $p < 0.05$ vs. clay loamy soil control of AC outer scales. x $p < 0.05$ vs. black soil control of AS edible portion; y $p < 0.05$ vs. red soil control of AS edible portion; z $p < 0.05$ vs. clay loamy soil control of AS edible portion. $ $p < 0.05$ vs. black soil control of AS outer scales; # $p < 0.05$ vs. red soil control of AS outer scales; @ $p < 0.05$ vs. clay loamy soil control of AS outer scales.

Comparison of antioxidant activity *i.e.*, IC_{50} values of AC and AS of control plants grown in soil A, B, and C with plants grown under different abiotic stresses shown in **Table 5.**

The data is expressed as Mean ± S.D (n=3) and analyzed by Two way ANOVA followed by Tukey's posthoc analysis test.

TABLE 5: THE IC_{50} VALUE OF EXTRACTS OF AC AND AS IN ELLMAN ASSAY

Soil	Sample	IC_{50} value (µg/ml)* (Mean^n ± S.D)			
		AC		AS	
		Edible portion	Outer scales	Edible portion	Outer scales
Black	Control	110.00± 1.00	105.47 ± 0.48	96.41 ± 0.41	207.59 ± 1.20
	Water stress	53.83 ± 0.79 ^{a,b,c}	59.35 ± 0.10 ^{p,q,r}	51.64 ± 0.37 ^{x,y,z}	92.17 ± 0.98 $^{\$,\#,@}$
	Fertilization	85.20 ± 0.59 ^{a,b,c}	66.13 ± 0.17 ^{p,q,r}	56.72± 0.55 ^{x,y,z}	89.53 ± 0.53 $^{\$,\#,@}$
	Flooding	109.45 ± 0.41 ^{a,b,c}	104.10 ± 0.54 ^{p,q,r}	52.00 ± 0.15 ^{x,y,z}	85.63 ± 0.52 $^{\$,\#,@}$
	Metal stress	45.16 ± 0.39 ^{a,b,c}	36.99 ± 0.17 ^{p,q,r}	37.00 ± 0.35 ^{x,y,z}	97.00 ± 0.67 $^{\$,\#,@}$
	Salt stress	105.63 ± 0.86 ^{a,b,c}	55.27 ± 0.43 ^{p,q,r}	58.36 ± 0.32 ^{x,y,z}	305.9 ± 0.13 $^{\$,\#,@}$
	Shade	67.72 ± 0.12 ^{a,b,c}	81.51 ± 0.15 ^{p,q,r}	91.00 ± 0.80 ^{x,y,z}	447.48 ± 0.51 $^{\$,\#,@}$
Red	Control	110.8 ± 0.32	62.81 ± 0.21 ^{p,r}	73.72 ± 0.48 ^{x,z}	364.61 ± 0.67 $^{\$,@}$
	Water stress	124.18 ± 0.23 ^{a,b,c}	60.35 ± 0.25 ^{p,q,r}	66.00 ± 0.50 ^{x,y,z}	115.31 ± 0.41 $^{\$,\#,@}$
	Fertilization	106.6 ± 0.42 ^{a,b,c}	62.64 ± 0.28 ^{p,q,r}	57.10 ± 0.39 ^{x,y,z}	61.03 ± 0.36 $^{\$,\#,@}$
	Flooding	334.00 ± 0.15 ^{a,b,c}	117.84 ± 0.38 ^{p,q,r}	60.02 ± 0.28 ^{x,y,z}	63.98 ± 0.63 $^{\$,\#,@}$
	Metal stress	51.65 ± 0.26 ^{a,b,c}	50.68 ± 0.20 ^{p,q,r}	31.84 ± 0.28 ^{x,y,z}	53.00 ± 0.13 $^{\$,\#,@}$
	Salt stress	93.92 ± 0.70 ^{a,b,c}	68.64 ± 0.25 ^{p,q,r}	59.70 ± 0.17 ^{x,y,z}	77.00 ± 0.35 $^{\$,\#,@}$
	Shade	106.97 ± 0.48 ^{a,b,c}	56.09 ± 0.10 ^{p,q,r}	69.19 ± 0.13 ^{x,y,z}	302.00 ± 0.60 $^{\$,\#,@}$
Clay loamy	Control	110.8 ± 0.81^{a}	103.87 ± 1.14 ^{p,q}	67.22 ± 0.63 ^{x,y}	95.15 ± 0.19 $^{\$,\#}$
	Water stress	80.19 ± 0.26 ^{a,b,c}	106.4 ± 0.32 ^{p,q,r}	93.13 ± 0.11 ^{x,y,z}	107.11 ± 0.21$^{\$,\#,@}$
	Fertilization	79.68 ± 0.47 ^{a,b,c}	57.43 ± 0.55 ^{p,q,r}	58.01 ± 0.12 ^{x,y,z}	94.21 ± 0.83 $^{\$,\#,@}$
	Flooding	96.75 ± 0.46 ^{a,b,c}	108.79 ± 0.29 ^{p,q,r}	60.68 ± 0.14 ^{x,y,z}	81.30 ± 0.27 $^{\$,\#,@}$
	Metal stress	58.04 ± 0.31 ^{a,b,c}	48.21 ± 0.12 ^{p,q,r}	55.99 ± 0.43 ^{x,y,z}	86.60 ± 0.46 $^{\$,\#,@}$
	Salt stress	86.43 ± 0.10 ^{a,b,c}	60.50 ± 0.35 ^{p,q,r}	62.11 ± 0.17 ^{x,y,z}	103.37 ± 0.86$^{\$,\#,@}$
	Shade	81.67 ± 0.33 ^{a,b,c}	54.16 ± 0.27 ^{p,q,r}	NA	NA
Donepezil (standard)		6.00 ± 0.09			

NA - Not applicable, n=3, * on dry weight basis. a $p < 0.05$ vs. black soil control of AC edible portion; b $p < 0.05$ vs. red soil control of AC edible portion; c $p < 0.05$ vs. clay loamy soil control of AC edible portion. p $p < 0.05$ vs. red soil control of AC outer scales; q $p < 0.05$ vs. red soil control of AC outer scales r $p < 0.05$ vs. clay loamy soil control of AC outer scales. x $p < 0.05$ vs. black soil control of AS edible portion; y $p < 0.05$ vs. red soil control of AS edible portion; z $p < 0.05$ vs. clay loamy soil control of AS edible portion. $ $p < 0.05$ vs. black soil control of AS outer scales; # $p < 0.05$ vs. red soil control of AS outer scales; @ $p < 0.05$ vs. clay loamy soil control of AS outer scales.

Comparison of acetylcholinesterase inhibitory activity *i.e.* IC_{50} values of AC and AS of control plants grown in soil A, B and C with plants grown under different abiotic stresses shown in **Table 6.** The data is expressed as Mean ± S.D (n=3) and analyzed by Two way ANOVA followed by Tukey's post-hoc analysis test.

- The metal stress effected AC plants grown in black soil have the highest acetylcholine stress inhibitory potential among all the stress-affected plants.
- The metal stress effected AS plants has the highest acetylcholine stress inhibitory potential among all the stress-affected plants.

Phenols and flavonoids are well known for their antioxidant and neuroprotective potential [79, 81, 82, 83, 84, 85, 104, 105]. Therefore, the presence of the higher amount of phenol and flavonoid compounds in hydro-methanol extracts of metal stress treated plants might be the possible reason for the observed higher antioxidant and acetylcholine stress inhibitory potential [86, 87, 88, 89, 90, 106].

CONCLUSION: Environmental factors have a strong impact on the growth of plants as well as the biosynthesis of plant metabolites. Based on the results of this investigation, it may be recommended that for higher biomass yield, plants may be cultivated in red soil with fertilization for AC and salt treatment for AS. Secondary metabolites are produced by plants to protect them from any change or stress. In this field experiment, too, it is evident that as compared to control-group plants, the plants subjected to different stresses had higher TPC, TFC content. Consequently, the antioxidant activity and AChE inhibition were also more distinct. The impact was most prominent with mental stress as metal act as elicitors and enzyme inducers. Therefore for higher total phenol content, total flavonoid content and better antioxidant and acetylcholinesterase inhibitory activity AC and AS plants should be grown in black and red soil, respectively and with metal stress.

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