

Estimation of Antioxidants from Pulses with treatment of various chemical fertilizers and biofertilizers.

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ABSTRACT

Food legumes are provide energy, essential minerals, vitamins and several compounds considered beneficial for good health. Pulses are a good source of antioxidants substances that prevent or delay some type of cell damage. Flavones, catechins, polyphenol and phytoestrogens are all type of antioxidants and phytochemicals found in pulses. In present investigation *Cajanuscajan*, *Glycine max*, *Vignaradiata* and *Cicerarietinum* are taken for study. On the basis of morphological study four samples from each plant treated with chemical fertilizers and biofertilizers were selected. The samples were named as *Cajanuscajan* (Cc)- Cc 19-1, Cc 19-2, Cc 19-3, Cc 19-4 ; *Glycine max* (Gm)- Gm 19-1, Gm 19-2, Gm 19-3 and Gm 19-4 ; *Vignaradiata* (Vr)- Vr 19-1, Vr 19-2, Vr 19-3 and Vr 19-4 ; *Cicerarietinum* (Ca)- Ca 19-1, Ca 19-2, Ca 19-3 and Ca 19-4. In present study DPPH method was used for estimation of antioxidants. From this investigation it is observed that all the samples showed good antioxidant activity ranges from IC₅₀ value 42 µg/ml to 62 µg/ml. It is found that the samples treated with chemical fertilizers showed low antioxidant properties while samples control or treated with only biofertilizers had high antioxidants properties. It is concluded that, application of different concentration of nutrients directly affects on antioxidant properties of pulses. Without application of any chemical fertilizers plant showed excellent antioxidant properties.

Keywords: Flavone, Catechin, Polyphenol, Phytoestrogen, Antioxidants, DPPH method, IC₅₀ value.

INTRODUCTION

Food legume belonging to family Fabaceae which are used directly or indirectly in the form of unripe pods, green grains and dry seeds as a source of food. The word 'Pulse' is derived from the latin word 'Puls' meaning pottage ie. Seeds boiled to make porridge or thick soup. In general, they are an indispensable source of supplementary protein to daily vegetarian diets [1]. They also provide energy, essential minerals, vitamins and several compounds considered beneficial for good health [2]. Their cultivation enriches soil by adding nitrogen and improves the physical, chemical and biological soil properties. Majority of legumes are grown for their green pods, green seeds or dried seeds [3].

Antioxidants are substances that may prevent or delay some types of cell damage. They work interactively inside and outside of the body's cells, protecting cell membranes from the damaging effect of highly reactive molecules called free radicals. If the body cannot remove free radicals efficiently, oxidative stress occurred and can harm cells and body function. Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, parkinson's disease and other inflammatory diseases [4]. An intake of antioxidants is believed to reduce these risks. Flavonoids, Flavones, Catechins, Polyphenol and Phytoestrogens are all types of antioxidants and phytonutrients, found in pulses. Natural antioxidants play an important role in health maintenance and prevention of the chronic and degenerative diseases, such as cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and aging [5].

METHODOLOGY

The experiments were conducted in the field of Sembal and Aashi Village at Tah- Warora, Chandrapur District, Maharashtra, during the years 2016 - 2018. Total seven treatments were given to both Kharif and Rabi crops. Following crop varieties were chosen to study the Kharif and Rabi crops.

Kharif crops-

- *Cajanuscajan* (variety - Daptarisupriya)
- *Glycine max* (variety - Pragati)
- *Vignaradiata* (variety - Mahabij)

Rabi crops-

- *Cicerarietinum* (variety - Mahabij)

The treatments control without any chemical fertilizer and inoculation of biofertilizers, other treatments were applied to seedling plants with inoculation of individuals and combination of biofertilizers, rhizobium and P.S.B. and treatments to seedling plants with different combination of chemical fertilizers N, P, K, Ca, Mg, Fe, Mn, Zn and Cu. The macro and micronutrients concentration of each selected crop as per recommendation of ICAR and PKV Akola.

The effect of total seven treatments of macro and micronutrients in combination of chemical fertilizers and biofertilizers were studied for growth, development and yield of both Kharif and Rabi crops as recommended dose of chemical fertilizers. Presence and absence of nutrients in soils were taken into consideration in the experimental study. The chemical fertilizers were used in different ratio along with combination of macro and micronutrients however the proportion of macro and micronutrients of soil were different in different plants. Biofertilizers were given to the plants by seed treatment method at the time of seed sowing.

Application of biofertilizers:

15-25 gm of biofertilizers were mixed with 20 ml solution of jaggery. The slurry was then poured over the 1kg of seeds spread on a cemented floor and mixed properly in such a way that a thin layer was formed around the seeds. The treated seeds were dried in the shade overnight and then they were ready to use.

Application of chemical fertilizers:

The individual element was given to field after 15 days from seed sowing and 10-15 days of interval in stepwise manner to study its effect on growth and development of crops.

Treatments-

T ₀	Control
T ₁	N + P + K
T ₂	T ₁ + Ca + Mg
T ₃	T ₁ + Rhizobium
T ₄	T ₃ + Ca + Mg
T ₅	Fe + Mn + Zn + Cu
T ₆	T ₅ + Rhizobium
T ₇	Rhizobium + P.S.B.

From above observation four samples were selected from each plant i.e. Control which is without any treatment, Plant of highest yield, Plant of lowest yield and plant treated with biofertilizers were collected and labelled for estimation of antioxidants. For *Cajanuscajan* (Cc) - it was named as Cc 19-1 (Control - T₀), Cc 19-2 (N, P, K, Ca, Mg, + *Rhizobium* - T₄P₆), Cc 19-3 (Fe, Mn, Zn, Cu - T₅P₇) and Cc 19-4 (*Rhizobium spp* + PSB - T₇) respectively. For *Glycine max* (Gm) - Gm 19-1 (Control - T₀), Gm 19-2 (N, P, K, Ca, Mg + *Rhizobium* - T₄P₃), Gm 19-3 (N, P, K - T₁P₆) and Gm 19-4 (*Rhizobium japonicum* + PSB - T₇) respectively. For *Vignaradiata*, (Vr) - Vr 19-1 (Control - T₀), Vr 19-2 (Fe, Mn, Zn, Cu + *Rhizobium* - T₆P₇), Vr 19-3 (N, P, K, + *Rhizobium* - T₃P₃) and Vr 19-4 (*Rhizobium fredii* + PSB - T₇) and For *Cicerarietinum*, (Ca) - Ca 19-1 (Control - T₀), Ca 19-2 (Fe, Mn, Zn, Cu + *Rhizobium* - T₆P₆), Ca 19-3 (Fe, Mn, Zn, Cu - T₅P₆) and Ca 19-4 (*Rhizobium ciceri* + PSB - T₇) respectively.

Estimation of Antioxidant Potential by DPPH Method :

DPPH radical scavenging activity of samples was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to the method of Blois [6]. Briefly, 3 ml of alcoholic solution of samples was added to 1 ml of DPPH(1,1-diphenyl 1-2-picrylhydrazyl) as the free radical source. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of compounds of each extract was determined at 517nm. The DPPH radical scavenging activity was calculated using the following formula.

Radical scavenging (%) =

The antioxidant activity of the hydro alcoholic extract was expressed as IC₅₀. Then % inhibitions were plotted against respective concentration of sample

used and from the graph IC₅₀ was calculated. Ascorbic acid was used as reference standard.

RESULTS AND DISCUSSION

DPPH method was used to determine the antioxidant activity. All the selected legume seeds extract showed good antioxidant activity ranges from 42 µg/ml to 62 µg/ml. The hydro- alcoholic extract of all the sample showed concentration dependent free radical scavenging activity compared with ascorbic acid, which was used as a standard. In sample Cc 19-1 the IC 50 value is 59 µg/ml, sample Cc 19-2 had IC 50 value 55 µg/ml, sample Cc 19-3 had IC 50 value 53 µg/ml and sample Cc 19-4 showed 47 µg/ml IC 50 value. Here IC 50 value decreases from sample cc 19-1 to Cc 19-4. So Cc 19-4 showed lowest IC 50 value ultimately it had high antioxidants properties.

In *Glycine max* also the IC 50 value decreases from sample Gm 19-1 to sample Gm 19-4. Gm 19-1 showed 49.1 µg/ml, Gm 19-2 showed 48 µg/ml, Gm 19-3 had 46.5 µg/ml and Gm 19-4 had 42 µg/ml IC 50 value. Like *Cajanuscajan*, sample Gm 19-4 showed good antioxidant properties as compared to other samples of *Glycine max*.

In *Vignaradiata* IC 50 was found in Vr 19-1 - 48 µg/ml, Vr 19-2 - 55 µg/ml, Vr 19-3 - 52 µg/ml and Vr 19-4 - 58 µg/ml. Control sample of *Vignaradiata* was rich in antioxidant properties.

In *Cicerarietinum* sample Ca 19-1 had low IC 50 value 53 µg/ml as compared to other three samples. It means Ca 19-1 also showed good antioxidant properties. IC 50 found Ca 19-2 - 56 µg/ml, Ca 19-3 - 54 µg/ml and Ca 19-4 had 62 µg/ml IC 50.

Flavonoids, isoflavonoids and tocopherol are present in pulses. All of them act as a good antioxidants. The roots, stem and seeds of *Cajanuscajan* contain isoflavonoids. The exception is flavanone, cajaflavanone which has been reported from the roots [7]. Studies on test tube have been found that antioxidants from mung beans can neutralized free radical damage linked to cancer growth in lung and stomach cells [8].

Table -a) % inhibition and IC 50 value of Ascorbic acid.

Sr. no.	Conc. Of Ascorbic acid	% inhibition	IC 50 value %
1	0	0	9 µg/ml
2	10	50.86	
3	20	58.13	
4	30	60.55	
5	40	64.01	
6	50	67.82	
7	60	76.81	
8	70	77.16	
9	80	76.47	
10	90	77.16	
11	100	77.16	

Table - b) % inhibition and IC 50 value of *Cajanuscajan*.

Sr. no.	Sample	IC 50 value µg/ml
1	% inhibition of Hydro-Alcoholic extract of Cc 19-1	59
2	% inhibition of Hydro-Alcoholic extract of Cc 19-2	55
3	% inhibition of Hydro-Alcoholic extract of Cc 19-3	53
4	% inhibition of Hydro-Alcoholic extract of Cc 19-4	47
5	% inhibition of Standard Ascorbic Acid	9

Table -c) % inhibition and IC 50 value of *Glycine max*.

Sr. no.	Sample	IC 50 value µg/ml
1	% inhibition of Hydro-Alcoholic extract of Gm 19-1	49.1
2	% inhibition of Hydro-Alcoholic extract of Gm 19-2	48
3	% inhibition of Hydro-Alcoholic extract of Gm 19-3	46.5
4	% inhibition of Hydro-Alcoholic extract of Gm 19-4	42
5	% inhibition of Standard Ascorbic Acid	9

Table -d) % inhibition of IC 50 value of *Vignaradiata*.

Sr. no.	Sample	IC 50 value µg/ml
1	% inhibition of Hydro-Alcoholic extract of Vr 19-1	48
2	% inhibition of Hydro-Alcoholic extract of Vr 19-2	55
3	% inhibition of Hydro-Alcoholic extract of Vr 19-3	52
4	% inhibition of Hydro-Alcoholic extract of Vr 19-4	58
5	% inhibition of Standard Ascorbic Acid	9

Table -e) % inhibition of IC 50 value of *Cicerarietinum*.

Sr. no.	Sample	IC 50 value µg/ml
1	% inhibition of Hydro-Alcoholic extract of Ca 19-1	53
2	% inhibition of Hydro-Alcoholic extract of Ca 19-2	56
3	% inhibition of Hydro-Alcoholic extract of Ca 19-3	54
4	% inhibition of Hydro-Alcoholic extract of Cc 19-4	62
5	% inhibition of Standard Ascorbic Acid	9

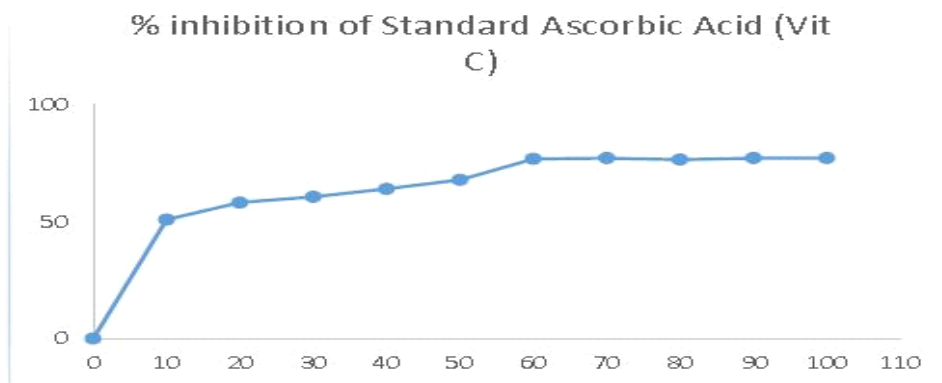


Fig. No. 1 % inhibition of Standard Ascorbic Acid (Vit C)

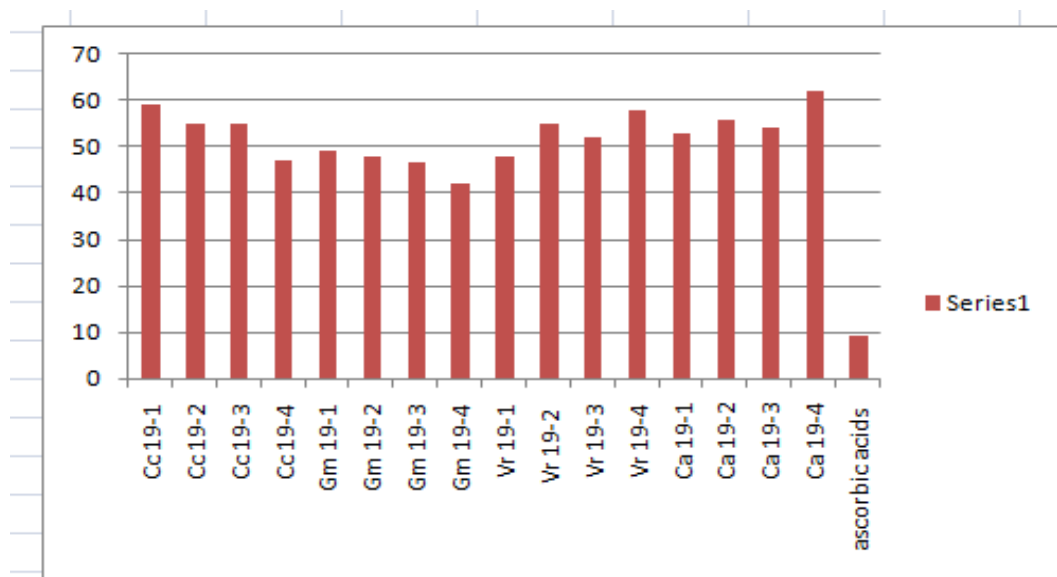


Fig- Antioxidant Content in *Cajanucajan*, *Glycine max*, *Vignaradiata* and *Cicerarietinum*.

CONCLUSION

From antioxidants investigation, it is concluded that, application of different concentration of chemical fertilizers directly affects on antioxidant properties of pulses. Without application of any chemical fertilizers plant showed excellent antioxidant properties.

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