Assessment of *Amaranthus viridis* L. Leaves on Growth, Antifungal and Antioxidant Activity of *Cicer arientinum* L.

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***Abstract*—The bioactivity of *Amaranthus viridis* L. leaves was carried out on *Cicer arientinum* L (Chick pea) seeds. Leaves were collected to prepare extract, leachate and dried paste and *Cicer* seeds were treated for two hours. After 2 h treated *Cicer* seeds were transferred to individual pots and kept for germination. The vegetative parameters were calculated after twenty-one days. The study of these vegetative parameters indicated that there is positive plant to plant interaction. Treated *Cicer* seeds showed significant increase in root-shoot length, number of leaflets, fresh and dry weight as compared to plant grown with distilled water. The effect of anti-oxidant enzymes showed significant rise in *Cicer* leaves treated with leachate as compared to distilled water. The anti-oxidant property, crude yield of alkaloids, tannins, saponins and glycosides of fresh and dried leaves of *Amaranthus* showered positive effect on growth of *Cicer* plant. Further anti-fungal analysis of the *Amaranthus* fresh leaf extract on *Fusarium* species proved effective and the phytochemical extracted can be used to control fungal growth. GC-MS analysis showed presence of Artemisyl acetate, an inhibitory fungus antimicrobial compound.**

***Index Terms*—*Amaranthus viridis*, amylase, catalase, *Cicer arientinum*, dehydrogenase**

I. Introduction

A. viridis is sometimes eaten as cooked vegetable, fodder for cattle and green manure; the leaves are diuretic and purgative and are used for treating inflammations, boils and abscesses, gonorrhoea, orchitis and haemorrhoids [1].

Important secondary metabolites identified as allelochemicals are phenolics, alkaloids, flavonoids, terpenoids, momilactone, hydroxamic acids, Brassinosteroids, Jasmonates, salicylates, glucosinolates, carbohydrates and amino acids [2]-[4]. The presence of allelochemicals may play an important role for the competitive advantage of *Amaranthus*, when growing either as a weed or as a food crop and competing with other plants for the same resources [5].

The oxidative defence systems include several anti-oxidant enzymes such as catalase, dehydrogenase, protease and amylase [6]. There are numerous reports that catalase, dehydrogenase, protease and amylase seem to play a vital role during germination and growth [7].

Cicer (desi chana) is an important source of cheap protein with high energy and nutritive value [8]. Soil borne diseases such as fusarium wilt dry root rot, collar root and black root rot are the major limiting factor in chick pea production [9].

The aim of the present study was to analyse the bioactivity of *Amaranthus* fresh and dried leaves on germination and growth of *Cicer* seeds. The Bioassays was carried out to study the effect on *Cicer* leaves and compared with control. The anti-fungal activity of *Amaranthus* leaves was also analysed against *Fusarium*.

II. Materials and Methods

A. Plant Extracts

Disease free and fresh *Amaranthus viridis* L. plant was collected from Changa campus. The leaves were separated and washed under tap water. The fresh leaves were used to prepare 4% fresh leaf extract and leachate. 4% fresh leaf extract was prepared by crushing 4 g fresh leaves in distilled water. For Leachate preparation 250 g of Fresh *Amaranthus* leaves were soaked for 24 h in 500 ml distilled water. Similarly, 10 g of leaves were oven dried at 55-60º C for 24 h and used to make dried leaf paste (1g) with required quantity of water (10ml).

B Pot Culture

Cicer seeds were purchased from D Mart store, Ahmedabad. Ten non-sterilized *Cicer* seeds each were soaked in 4% *Amaranthus* fresh leaf extract, *Amaranthus* fresh leaf leachate and coated with *Amaranthus* dried leaf paste for 2 h [10]. After 2 h the treated *Cicer* seeds was shifted to pots and kept for germination under natural conditions (25-29º C). A total of three replications of

Manuscript received January 6, 2020; revised March 28, 2020.

doi: 10.18178/ijpmbs.9.2.70-74

treated *Cicer* seeds were kept undisturbed and watered at regular intervals. *Cicer* seeds with distilled water were kept as control. The readings for vegetative parameters of *Cicer* plant like germination percentage, root length, shoot length, number of lateral roots and leaflets, fresh and dry weight was taken after 21 days regularly.

C. Enzymes

1) Dehydrogenase

2 ml sodium succinate, 1ml phosphate buffer, 1ml TTC and 2ml enzyme extract (*Cicer* leaves) is taken and incubated in water bath at 30ºC. Add 7ml acetone and centrifuge at 2000rpm. The supernatant was measured at 460nm by Shimadzu UV visible Spectrophotometer [11].

2) Catalase

The rate of decomposition of H_2O_2 was followed by decrease in absorbance at 240nm by Shimadzu UV visible Spectrophotometer in a reaction mixture containing 1.5ml phosphate buffer, 1.2ml H_2O_2 and 300µl of enzyme extract [12].

3) Amylase

One g *Cicer* leaves was homogenized with 10ml of 0.1M Phosphate buffer (pH=6.5). The homogenate was centrifuged at 5000rpm for 15 min. The supernatant was taken as the crude source of the enzyme. One ml of the enzyme solution was mixed with an equal volume of 0.1% starch solution in 0.1N sodium acetate buffer, pH 5.0 and incubated at 37ºC for 10 min. The reaction was stopped with 3ml iodine-HCl solution (600mg KI and 60 mg I_2 in 100ml of 0.05N HCl). The blank was after inactivating the enzyme with 3ml iodine-HCl solution prior to addition of starch. The intensity of blue colour was measured at 620nm by Shimadzu UV visible Spectrophotometer [13].

4) Protease

Protease activity was measured by incubating the reaction mixture consisting of one ml enzyme extract, 0.1ml 0.1M $MgSO_4.7H_2O$ and one ml BSA (0.5mg/ml dissolved in DW) for 1h at 37 ºC followed by adding 1ml 50% trichloroacetic acid (TCA) and subsequent analysis of residual protein by Folin-phenol reagent. The intensity of blue colour was measured at 650nm by Shimadzu UV visible Spectrophotometer [13].

D. Phytochemical Constituents

1) Total phenolic content

The total phenolic content was estimated by Folin Ciocalteu method. 1 ml leaf extract was mixed with 5 ml distilled water, 1 ml sodium carbonate (20 %) and 1 ml Folin Ciocalteu reagent. The mixture was allowed to stand in water bath at 40 ºC for 30 min. The absorbance was measured at 765 nm using Shimadzu UV visible spectrophotometer. Standard graph was prepared by using different concentrations of phenol [14], [15].

2) Total Flavonoid content

The Flavonoid content was determined by Aluminium Chloride method using Catechin as the reference compound. A volume of 125µl of extract is added to 75 µl of 5% $NaNO_2$ solution. The mixture is allowed to stand for 6 min, then 150 µl of Aluminium chloride was added and incubated for 5 min, followed by addition of 750µl of NaOH (1M). The final volume was adjusted to 2500 µl with distilled water. After 15 min the mixture turned to pink and the absorbance was measured at 510 nm using Shimadzu UV visible spectrophotometer [14], [15]

3) Gas chromatography and mass spectroscopy analysis

Analysis by GC-MS was performed using Thermo GC- Trace Ultra Ver: 5.0. Pyrolysis auto sampler interfaced to a Perkin Elmer Turbo mass Gold equipped with a fused silica capillary column. The fraction was pyrolyzed at 610 ºC and then introduced to the GC column. Helium was employed as carrier gas (1ml / min). Qualitative identification of the different constituents was performed by composition of the relative retention times and mass spectra with those of authentic reference compounds by retention indices (RI) and mass spectra. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) [14].

E. Fungicidal Assay

Petri dish with Potato dextrose Agar (PDA) was prepared by dissolving 6.72 g Potato dextrose broth (PDB) and 8.5 g Agar (Bacteriological) in 250ml distilled water. Agar well diffusion method was used where a drop of culture suspension was placed in the centre of nutrient agar plate and spread all over the plate with sterile spreader. Three wells were made on the Potato dextrose agar medium containing plate with sterile cork borer and wells were filled with 100 µl of plant extract. Plates were observed for zone of inhibition by measuring the colony diameter [14]. Sterile water was used as control. Further 100 ml Potato Dextrose Broth (9.6 g PDB was dissolved in 400ml distilled water) was prepared in conical flask and it was inoculated with *Fusarium oxysporum* f. sp. *ciceri*, f. sp *Chlamydosporum*,f. sp. *Pallidoroseum* and f.sp. *vasinfectum* and tested against 10 ml *Amaranthus* fresh leaf extract (aqueous).

F. Statistical Analysis

The data for germination percentage, root length, shoot length, number of lateral roots, number of leaflets and dry weight were calculated using SPSS/PC software. One-way analysis of variance (ANOVA) test was used to analyse data and mean was compared at 5% and 10% level of significance [16].

III. Results and Discussion

A. Leaf Extracts

Cicer (Chick pea) seeds were treated with *Amaranthus* fresh leaves, fresh leaf leachate and dried leaves. Aqueous 4% extract of *Amaranthus* fresh leaf, *Amaranthus* leachate and *Amaranthus* dried leaf paste significantly increased the germination percentage, root and shoot length, number of leaflets, fresh as well as dry weight of treated *Cicer* plant as compared to control in readings taken after 21 days (Table I). The low amount of weed powder increased the amount of organic matter in soil, resulting in the better growth than the control [17].

The extracts of *Parthenium hysterophorus* root, *Datura stramonium* root and stem and *Argemone mexicana* leaf stimulated elongation of Wheat seedlings due to induction of growth promoting hormones [18]. Allelochemicals can stimulate or inhibit plant growth depending on their concentration [19].

TABLE IA. EFFECTS OF AMARANTHUS 4% FRESH LEAF EXTRACT, LEACHATE AND DRY LEAF PASTE ON SEEDS GERMINATION AND SEEDLINGS GROWTH OF CHICKPEA AFTER 21 DAYS

Chickpea seeds treatment	Gemination (%)	Root Length (cms)	Shoot Length (cms)
Control	30	2.7±1.5	27.3±4.3
4 % Amaranthus fresh leaf extract	80	4.7±1.3	34.8±1.5
Amaranthus fresh leaf leachates (1:2 w/v)	70	3.5±4.1	35.1±1.8
Amaranthus dry leaf paste (1:10 w/v)	90	4.6±1.6	31.9±0.3

TABLE IB. EFFECTS OF AMARANTHUS 4% FRESH LEAF EXTRACT, LEACHATE AND DRY LEAF PASTE ON SEEDS GERMINATION AND SEEDLINGS GROWTH OF CHICKPEA AFTER 21 DAYS

Chickpea seeds treatment	Number of Lateral roots	Number of Leaflets
Control	3±0.6*	38.7±1.2
4 % Amaranthus fresh leaf extract	1.7±0.9*	50.7±4.5
Amaranthus fresh leaf leachates (1:2 w/v)	1.7±0.9*	57.7±0.2
Amaranthus dry leaf paste (1:10 w/v)	2±0.6*	55.3±0.2

*: Significant at 5%

TABLE IC. EFFECTS OF AMARANTHUS 4% FRESH LEAF EXTRACT, LEACHATE AND DRY LEAF PASTE ON SEEDS GERMINATION AND SEEDLINGS GROWTH OF CHICKPEA AFTER 21 DAYS

Chickpea seeds treatment	Fresh wt./ mg Plant	Dry wt./ mg Plant
Control	630±0.2	100±0.01*
4 % Amaranthus fresh leaf extract	679±0.03	90±0.01*
Amaranthus fresh leaf leachates (1:2 w/v)	775±0.1	110±0.01*
Amaranthus dry leaf paste (1:10 w/v)	652±0.1	120±0.01*

*: Significant at 5%

B. Anti-oxidant Enzymes

Maximum increase in dehydrogenase, catalase, amylase and protease activity was recorded in *Cicer* leaves treated with *Amaranthus* fresh leaf leachate in contrast to control (Table II). Dehydrogenase enzymes catalyse the reversible reduction of pyruvate to lactate with $NADH_2$ as the co enzyme [20]. Anti-oxidant catalase enzyme increases when plants are treated with phenolic compounds at low level [21]. Amylase activity increases slowly during initial days of germination and convert starch to soluble sugars needed for growth [22]. Proteases activate different signalling processes by carrying out controlled Proteolysis [23].

TABLE IIA. EFFECTS OF *AMARANTHUS* 4% FRESH LEAF EXTRACT, LEACHATE AND DRIED LEAF PASTE ON ANTI-OXIDANT ACTIVITY OF *CICER ARIENTINUM* LEAVES

Chickpea leaves treatment	Dehydrogenase enzyme (U/ml)	Catalase enzyme (U/ml)
Control	0.24±0.02*	0.66±0.04*
4 % Amaranthus fresh leaf extract	0.34±0.04*	1.05±0.2*
Amaranthus fresh leaf leachates (1:2 w/v)	0.38±0.04*	1.08±0.1*
Amaranthus dry leaf paste (1:10 w/v)	0.23±0.03*	0.78±0.04*

*: Significant at 5%

TABLE IIB. EFFECTS OF *AMARANTHUS* 4% FRESH LEAF EXTRACT, LEACHATE AND DRIED LEAF PASTE ON ANTI-OXIDANT ACTIVITY OF *CICER ARIENTINUM* LEAVES

Chickpea leaves treatment	Amylase enzyme (U/ml)	Protease enzyme (U/ml)
Control	1.19±0.2*	2.33±0.4*
4 % Amaranthus fresh leaf extract	0.82±0.2*	1.76±0.4*
Amaranthus fresh leaf leachates (1:2 w/v)	1.61±0.2*	2.44±0.4*
Amaranthus dry leaf paste (1:10 w/v)	0.48±0.1*	1.6±0.4*

*: Significant at 5%

C. Anti-fungal Activity

The quantitative analysis of *Amaranthus* 4% fresh leaf extract showed the presence of Phenolics and Flavonoids (Table III). The aqueous *Amaranthus* 4% fresh leaf extract was tested against *Fusarium oxysporum*, f. sp. *ciceri,* f. sp. *chlamydosporum*, f. sp. *pallidoroseum* and f. sp. *vasinfectum* and it was found to be effective. The zone of inhibition was highest in *Fusarium oxysporum* f. sp. *vasinfectum* (Table IV, Fig. 1). Further analysis by GC-MS isolated 'Artemisyl acetate' a phytochemical (Flavonoid) (Table V, Fig. 2). Artemisyl acetate was also fungistatic against *Tiarosporella phaseolina* (1000µl/L), *Fusarium moniliforme* (750µl/L) and *F.solani* (750µl/L) [24]. Fungal growth in potato dextrose broth was 7.22g/100ml and in flask with potato dextrose broth having extract was 0.13g/100ml (Table VI).

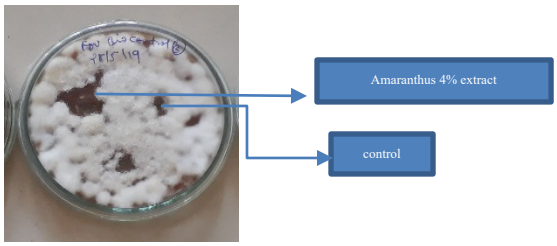

Figure 1. Antifungal activity of *Amaranthus viridis* 4% fresh leaf extracts (aqueous) on *Fusarium oxysporum* (f. sp. *vasinfectum*) (FOV).

TABLE III. QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN *AMARANTHUS VIRIDIS* 4% FRESH LEAF EXTRACTS (AQUEOUS)

Type	Total Phenolic content (mg/ml)	Total Flavonoid content (mg/ml)
Amaranthus 4%fresh leaf extract (mg / ml)	2.2±0.3*	1.3±0.3**

**: Significant at 10%

TABLE IVA. EFFECT OF *AMARANTHUS VIRIDIS* 4% FRESH LEAF EXTRACTS (AQUEOUS) ON *FUSARIUM* SPECIES (ZONE OF INHIBITION)

Type	*Fusarium oxysporum* (f. sp. *ciceri,* (FOC) mm)	*Fusarium oxysporum* (f. sp. *chlamydosporum*, (FOCh) (mm)
Amaranthus 4%fresh leaf extract (mg / ml)	0.2±0.1**	0.4±0.1**

**: Significant at 10%

TABLE IVB. EFFECT OF *AMARANTHUS VIRIDIS* 4% FRESH LEAF EXTRACTS (AQUEOUS) ON *FUSARIUM* SPECIES (ZONE OF INHIBITION)

Type	*Fusarium oxysporum* (f.sp. *pallidoroseum*, (FOP) (mm)	*Fusarium oxysporum* (f.sp. *vasinfectum*, (FOV) (mm)
Amaranthus 4%fresh leaf extract (mg / ml)	0.4±0.03**	0.8±0.1**

**: Significant at 10%

TABLE V. IMPORTANT COMPOUND IDENTIFIED IN THE GC-MS ANALYSIS OF *AMARANTHUS VIRIDIS* FRESH LEAF EXTRACTS

1	19.98	2.23	Artemisyl acetate	196

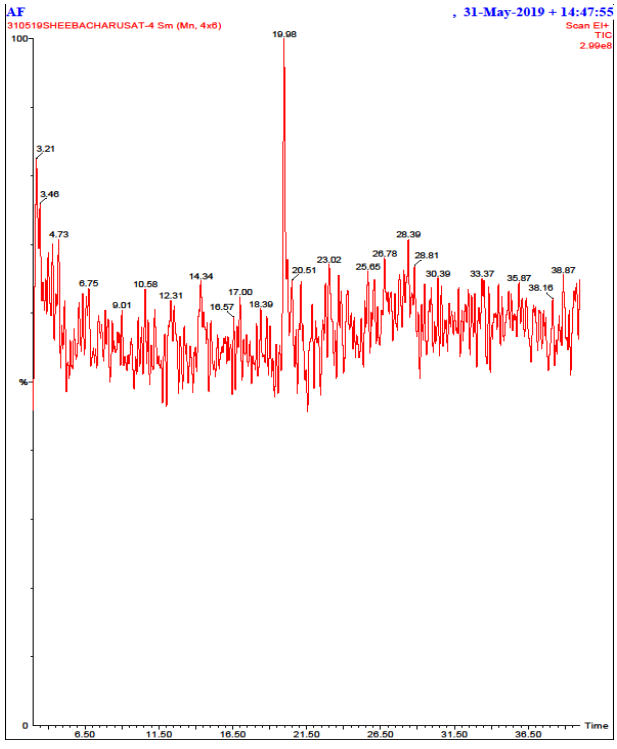

Figure 2. GC-MS analysis of *Amaranthus viridis* fresh leaf extracts.

TABLE VI. GROWTH INHIBITION OF *FUSARIUM OXYSPORUM* F.SP. *VASINFECTUM* (FOV)

Type	FOV growth (control) (g / 100 ml)	FOV growth + 4%fresh leaf extract (g / 100 ml)
Amaranthus 4%fresh leaf extract (mg / ml)	7.22±0.1*	0.13±0.01*

*: Significant at 5%

IV. CONCLUSION

Amaranthus fresh and dried leaves enhanced the vegetative parameters and anti-oxidant enzymes in treatd Cicer seeds. The anti – fungal property of *Amaranthus* leaf extracts can also be used to control Fusarium species – pathogenic to plants.

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Sheeba Menon did the laboratory and field trials. It is her PhD work. Janki Thakker explained the methodology and guided for research paper and scientific writing.

ACKNOWLEDGEMENT

We thank University Grants Commission for sanctioning fellowship under 'Faculty development program'.

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