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PRELIMINARY PHYTOCHEMICAL SCREENING OF TWO VEGETABLE CROPS *ABELMOSCHUS ESCULENTUS* (L.) MOENCH AND *AMARANTHUS TRICOLOR* L. TREATED WITH ORGANIC FERTILIZERS

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ABSTRACT
Preliminary phytochemical screening was done using water, petroleum ether, chloroform, acetone and ethanol for lady's finger on the 60th day and amaranth on the 45th day of growth. Quinone was completely absent in water extract of lady's finger. Phenol and phlobatannin were absent in petroleum ether extract. Most of the secondary metabolites tested were present in all the solvent extracts used. In amaranth, alkaloids and flavonoids were absent in water extract, quinone in chloroform and acetone extract. Ethanol extract showed the presence of most of the secondary metabolites tested.

KEY WORDS: *Abelmoschus*, *Amaranthus*, secondary metabolites.

INTRODUCTION

Phytochemicals are bioactive compounds obtained from plants. They are widely applied in herbal medicines. Two types of metabolites are produced by plants and they are primary metabolite that is utilized for the growth and development of plants and secondary metabolites synthesized by the plant parts that are widely used for preparation of traditional herbal medicines (Bansode and Salalkar, 2015). Fertilizers are commonly used for growing all crops, with application rates depending on the soil fertility. Fertilizers are applied to crops both as solids as well as liquid. In general, organic fertilizers release nutrients over an extended period of time. They act much like the slow-release fertilizers. Organic fertilizers can describe those fertilizers with an organic - biologic -origin *i.e*., fertilizers derived from living or formerly living materials. Organic fertilizers can also describe commercially available and frequently packaged products that strive to follow the expectations and restrictions adopted by "organic agriculture". The "organic fertilizer" products typically contain both organic materials as well as acceptable additives such as nutritive rock powders, ground sea shells (crab, oyster, *etc*.), other prepared products such as seed meal or kelp and cultivated microorganisms and derivatives. Green leafy vegetables are the cheapest of all the vegetables within the reach of poor men, being richest in nutritional value. Green leafy vegetables represent an important proportion of foods with medicinal value. Organic agriculture encourages vigorous management of soil and atmosphere avoiding chemical pollution by chemical fertilizers. It decreases the entry of toxic residues in to the soil by promoting production of fresh, quality foods.

MATERIALS AND METHODS

The plant materials taken for the present study were *Abelmoschusesculentus* (L.) Moench and *Amaranthus tricolor* (L.) belonging to the family Malvaceae and Amaranthaceae respectively. A pot study was carried out to analyse the presence of secondary metabolites under different organic fertilizer treatments.

Collection of seeds

Seeds of both *Abelmoschus esculentus* (L.) Moench and *Amaranthus tricolor* (L.) were obtained from Tamil Nadu Agricultural University, Coimbatore.

Collection of Fertilizers

The bio-fertilizers such as *Azospirillum,* VAM and Phosphobacteria were collected from TNAU, Coimbatore. The dosages used were as per the TNAU Agriportal.

Methods

Pot culture experiment was conducted with the two test plants. The size of the experimental pot was 30cm ×24cm × 30cm. Triplicates were maintained for each treatment. The soil was cleaned by removing stones and other unwanted materials. The red soil and sand soil were mixed in the ratio of 1:1 and filled in pots of 7 kg capacity. A study was undertaken to assess the effect of different organic fertilizers on the presence of various secondary metabolites in both the plants.

The seeds were soaked in different organic fertilizers for 12 hours. The organic fertilizers used for the study were *Azospirillum,* Phosphobacteria and Vesicular Arbuscular Mycorrhizal (VAM) fungi. In the growing stages of the plants, the bio-fertilizers were sprayed on the plants and phytochemical screening was carried out on the 60th day of the plant growth for *Abelmoschus esculentus* (L.) Moench and on 45th day of growth for *Amaranthus tricolor* (L.).

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The phytochemical study was carried out in the two vegetable crops taken for the study following the method of Harborne (1984).

Preparation of plant extracts

The leaves of both the plants taken for the present study were collected, cleaned and air dried under shade for

almost three weeks. After drying, the leaves were then blended using a household electric blender. This fine powder was analysed for phytochemical constituents present in it. The plant sample was soaked in water, acetone, petroleum ether, ethanol and chloroform for overnight extraction and filtered through whatman No.1. Filter paper. The following qualitative tests were conducted on the leaf extracts of the two test plants.

Test for Alkaloids

Mayer's test

To 1 ml of extract, 2 ml of Mayer's reagent was added. Appearance of dull white precipitate indicates the presence of alkaloids.

Test for Tannins

To 1 ml of extract, 2 ml of 0.1% Ferric chloride was added. Brownish green or blue black colouration indicates the presence of tannins.

Test for Flavonoids

To 1 ml of extract, 1 ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids**.**

Test for Quinones

A small amount of the extract was treated with conc. HCL and observed for the formation of yellow precipitate.

Test of phlobatannins

To 1 ml of extract, few drops of 1% aqueous hydrochloric acid were added. A red precipitate formed indicates the presence of phlobatannins**.**

Test for Phenol

To 1 ml of extract, lead acetate solution was added and the precipitate formation indicates the presence of phenolic compounds**.**

Test for Carbohydrates

Molisch's Test

Two drops of Molisch's reagent was added to an aqueous or hydrochloric acid solution of the extract and 2 ml of concentrated sulphuric acid was added by the side of the test tube. The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrates.

Test for Steroids

Liebermann – Burchard Test

The extract was dissolved in 2 ml of chloroform to which 10 drops of acetic acid and 5 drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

Test for Terpenoids

To 5 ml of filtrate, 2 ml of chloroform was added and 3 ml of concentrated sulphuric acid was added carefully. An interface with reddish brown colorations indicates the presence of terpenoids.

Test for fixed oil and fat

To 1 ml extract, a few drops of Sudan III solution were added. A shining orange colour showed the presence of fixed oil and fat.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the 60th day in *Abelmoschus esculentus* (L.) Moench and on the 45th day in *Amaranthus tricolor* (L.). The solvents used for extraction were water, petroleum ether, chloroform, acetone and ethanol. The phytochemical screening was carried out to analyse the presence of various secondary metabolites such as alkaloids, tannins, flavonoids, quinones, phlobatannins, phenol, carbohydrates, steroids, terpenoids and fats & oil.

***1. Abelmoschusesculentus* (L.) Moench**

The phytochemical tests were carried out for all the treatments on the 60th day in lady's finger. In the extract using water, it was observed that the quinones were completely absent in the plant. Phlobatannins was present only in the control plant. All the other secondary metabolites were observed in the plant (Table 1).

In the leaf extract using petroleum ether, phenol and phlobatannins were absent in the organic fertilizer treated plants. Other secondary metabolites such as alkaloids, tannins, flavonoids, carbohydrates, fats & oil, steroids and terpenoids were present in all the treated plant on the 60th day (Table 2).

In the leaves extracted with chloroform, the alkaloids, tannins, carbohydrates, flavonoids, steroid, terpenoids and fats & oil were present in all the organic fertilizer treated plants. Quinones, phenol and phlobatannins were completely absent in the fertilizer treated plants (Table 3).

When the dried leaves were extracted with acetone, the following secondary metabolites were observed– alkaloids, flavonoids, tannins, phenol, carbohydrates, steroids, terpenoids and fats &oil. Quinones and phlobatannins were completely absent in the plants on the 60th day (Table 4).

The dried leaf powder extract of lady's finger in ethanol showed the presence of alkaloids, tannins, flavonoids, phenol, steroids, terpenoids and fats & oil. Carbohydrates and phlobatannins were seen only in the control plants in acetone extract (Table 5).

Studies carried out by Shad *et al*. (2014) have shown that the fruits of the medicinal plants examined were a valuable source of nutraceuticals and exhibited good level of antimicrobial activity.

TABLE 1: Preliminary phytochemical Analysis of *Abelmoschus esculentus* (L.) Moench in water extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	+	+	+	+
2	Tannins	-	+	-	+	+
3	Flavonoids	+	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	-	-	-	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

TABLE 2: Preliminary phytochemical Analysis of*Abelmoschusesculentus* (L.) Moench in petroleum ether extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	+	+	+
3	Flavonoids	+	+	+	+	+
4	Quinones	+	-	-	-	-
5	Phlobatannins	-	-	-	-	-
6	Phenol	+	-	-	-	-
7	Carbohydrates	+	+	+	+	+
8	Steroids	-	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

TABLE 3: Preliminary phytochemical Analysis of *Abelmoschusesculentus* (L.) Moench in chloroform extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	-	+	+
3	Flavonoids	-	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	-	-	-	-	-
6	Phenol	+	-	-	-	-
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

TABLE 4: Preliminary phytochemical Analysis of *Abelmoschusesculentus* (L.) Moench in acetone extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	-	+	+	+	+
3	Flavonoids	-	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	-	-	-	-	-
6	Phenol	-	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

***2. Amaranthustricolor* (L.)**

The phytochemical tests carried out in *Amaranthus tricolor* (L.) using different solvent extracts showed the following results. In the leaf powder extracted with water, alkaloid and flavonoids were completely absent. The other secondary metabolites such as tannins, quinones, phlobatannins, phenol, carbohydrates, steroids, terpenoids and fats & oil were present in the leaves of amaranth on the 45th day (Table 6).

In the petroleum ether extract of the leafy vegetable, alkaloid, phlobatannins and terpenoids were completely absent. All the other secondary metabolites were present in the leaves of amaranth (Table 7). In the chloroform extract of the dried leaf powder, the quinones were completely absent in control plants as well as the organic fertilizer

treated plants. The alkaloids, flavonoids, phlobatannins, phenol, carbohydrates, steroids, terpenoids and fats & oil were present in the green leafy vegetable on the 45th day (Table 8). The acetone extract of the leafy vegetable showed the presence of phenol, carbohydrates, steroids and terpenoids in control as well as in the treated plants. Flavonoids and quinones were completely absent in the acetone extract. Alkaloids, tannins and fats & oil were present in few of the treated plants (Table 9). The ethanol extract of the leafy vegetable on the 45th day showed the presence of almost all the secondary metabolites except, alkaloid and phlobatannin (Table 10). Studies on the phytochemical constituents of *Amaranthus tricolor* (L.) have shown the presence of carbohydrates, proteins, amino acids, steroids, cardiac glycosides, alkaloids, tannins and flavonoids (Pulipati *et al*., 2017).

TABLE 5: Preliminary phytochemical Analysis of*Abelmoschusesculentus* (L.) Moench in ethanol extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	+	+	+
3	Flavonoids	-	+	+	+	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	-	-	-	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	-	-	-	-
8	Steroids	+	+	+	+	+
9	Terpenoids	-	+	+	+	+
10	Fats and oil	+	+	+	+	+

Earlier studies have shown that medicinal plants respond best to organic source of nutrients which are also environment friendly (Menon and Potty, 1998 and Kurian *et al*., 2000). Pulipati *et al*. (2017) have carried out research on the total phenol, tannin and flavonoid content of *Amaranthustricolor* (L.). Studies on the phytochemical constituents of *Amaranthus tricolor* Linn. leaf by Tharun *et al.* (2012) have shown the presence of carbohydrates, tannins and flavonoids in ethyl acetate fraction and steroids in petroleum fraction. Traditionally, the boiled leaves of *Amaranthus* are used as laxative, diuretic, anti-gonorrheal, expectorant, to relieve breathing in acute bronchitis. The leaves, shoots, tender stems and grains are eaten as pot herb in sauces or soups, cooked with other vegetables with a main dish or by itself. They are highly nutritious and contain vitamins and minerals. Amaranth is a multipurpose crop supplying high nutritional quality grains and leafy vegetables for food and animal feed. *Amaranthus*, green leafy vegetable is used due to its antioxidant property. The presence of various phytochemical is responsible for its high antioxidant activity.

TABLE 6: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in water extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	+	+	+
3	Flavonoids	-	-	-	-	-
4	Quinones	+	+	+	+	+
5	Phlobatannins	-	+	+	+	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	Fats and oil	+	+	+	+	+

TABLE 7: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in petroleum ether extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	+	+	+
3	Flavonoids	+	+	+	+	+
4	Quinones	+	+	+	+	+
5	Phlobatannins	-	-	-	-	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	-	-	-	-	-
10	Fats and oil	+	+	+	+	+

TABLE 8: Preliminary phytochemical Analysis of *Amaranthus tricolor* (L.) in chloroform extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	+	+	+	+
2	Tannins	-	+	+	-	+
3	Flavonoids	+	+	+	-	+
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	+	+	+	+
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	-	+	+	+
10	Fats and oil	+	+	+	+	+

TABLE 9: Preliminary phytochemical Analysis of*Amaranthus tricolor* (L.) in acetone extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	+	-	-	-	-
2	Tannins	-	+	+	-	+
3	Flavonoids	-	-	-	-	-
4	Quinones	-	-	-	-	-
5	Phlobatannins	+	+	+	+	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	Fats and oil	-	-	-	+	-

TABLE 10: Preliminary phytochemical Analysis of*Amaranthus tricolor* (L.) ethanol extract

S.No	Treatment	T_0	T_1	T_2	T_3	T_4
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	+	+	+
3	Flavonoids	-	-	-	-	-
4	Quinones	+	+	+	+	+
5	Phlobatannins	-	+	+	+	-
6	Phenol	+	+	+	+	+
7	Carbohydrates	+	+	+	+	+
8	Steroids	+	+	+	+	+
9	Terpenoids	+	+	+	+	+
10	Fats and oil	+	+	+	+	+

Use of bio-fertilizer is needed as an alternative source to bring forth the eco-friendly methods of farming. The extent of benefit from the microorganisms depends on their number and their efficiency, which however, is governed by soil and environmental factors. The uses of organic sources enhance the absorption and release of macro as well as micronutrients and thus ensure their availability to the plant throughout its growing season. Through bio-fertilizers, fertilizer application can be reduced by 50%.

CONCLUSION

The study on the phytochemical constituents of lady's finger on the 60^{th} day and amaranth on the 45^{th} day showed the presence of a number of secondary metabolites that could be utilized in pharmacology. Further studies are to be carried out to isolate the compound from the plants, so that it could be used in pharmacy.

Conflict of Interest:

Conflict of interest declared none.

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