ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 3, Issue, 11, pp.147-149, November, 2013

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF BLACK GRAM, *Vigna mungo* L.

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ARTICLE INFO

Article History:
Received 24th August, 2013
Received in revised form
16th September, 2013
Accepted 10th October, 2013
Published online 20th November, 2013

Key words:
Black gram,
Chemical fertilizer,
Leaf extracts,
Phytochemicals.

ABSTRACT

Black gram (*Vigna mungo* L.) is an important pulse crop occupying unique position in Indian agriculture. The study of phytochemicals, secondary metabolic substances in plants are known to provide protection against insect attacks and plant diseases. The current investigation is an attempt to study the preliminary phytochemicals that are present in black gram using different solvent extracts. An experiment was conducted to study the influence of chemical fertilizers (DAP and superphosphate) and leaf extracts (*Ocimum basilicum* and *Lantana camara* L.) on the presence of secondary metabolites. The results showed the presence of flavonoids, terpenoids, quinone, sterols, oil and fat by various tests.

INTRODUCTION

Plants are exclusive source of drugs for majority of the world population. Black gram originated in India, where it has been in cultivation from ancient times and is one of the most highly prized pulses of India. *Vigna mungo* L. is used in traditional Indian (Ayurveda) medicine. Phytochemical analysis mainly applies to the quality control of herbal medicine to determine the various chemical components, such as saponins, alkaloids, volatile oils, flavonoids and anthraquinones. Pharmacologically, extracts have demonstrated to possess immunostimulatory activity (Solanki and Jain, 2010). Ajayi *et al*. (2011) reported their studies on screening for phytochemical constituents of seven different plant seed species namely *Artocarpus communis, Artocarpus heterophyllus, Calophyllum inophyllum, Garcinia kola, Garcinia mangostana, Pentaclethra macrophylla* and *Treculia africana* using generally accepted laboratory technique for qualitative determinations. Their study indicated the presence of saponins, flavonoids and reducing sugars in all the aqueous extract of these plants. The results of experiment conducted by Xavier and Kavitha (2012) on the phytochemical and biochemical analysis of the plant extract of *Acacia concinna* (wild) have shown various colour changes when treated with different chemical reagents. Yadav and Agarwala (2011) investigated the presence of phytochemicals and determined the total phenolic and flavonoid contents of selected medicinal plants such as *Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia* and *Xanthium stramarium*. In this, the result showed that crude aqueous and organic solvent extract of these tested plants contain medicinally important bioactive compounds. The phytocomponents in black gram help in treating ailments like liver diseases, cancer, diabetes and kidney diseases by functioning as antioxidants. An investigation was carried out to determine the phytochemcials present in the root nodule of *V. mungo* grown under different treatments of organic manures (Anbuselvi *et al*., 2012). Al-othman *et al*. (2012) have investigated the phytochemical analysis of selected medicinal plants. The results showed various concentrations of the observed phytochemicals. The present investigation was undertaken to study the presence of various secondary metabolites in black gram.

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MATERIALS AND METHODS

A study was conducted to assess the effect of chemical fertilizers and leaf extracts on the phytochemical analysis of the plants at the time of yield.

Collection of Various Materials

Red sandy loam soil was collected from Kovaipudur Pirivu, Coimbatore.

Collection of Seeds

The seeds of black gram (*Vigna mungo* L.var.co.6) were procured from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore.

Collection of *Ocimum basilicum* and *Lantana camara*

Ocimum basilicum and *Lantana camara* leaves were collected from Kovaipudur Pirivu, Coimbatore. Leaf extracts were taken afresh by grinding the leaves in a mixie and filtering with the help of a muslin cloth.

Farm Yard Manure

Farm Yard Manure (FYM) is prepared basically using cow dung. It is highly useful and some of its properties are

- Farm yard manure is rich in nutrients.
- A small portion of nitrogen is directly available to the plants while a larger portion is made available as and when the FYM decomposes.
- Availability of potassium and phosphorus from farm yard manure is similar to that from inorganic sources.
- Application of farm yard manure improves soil fertility.

Herbal Extract

***Ocimum basilicum* L.**

The other names are basil, garden basil and sweet basil. The plant belongs to the family Lamiaceae. Basil is a low growing (30 – 100 cm) annual plant. It has slightly hairy stem and ovate, entire to slightly toothed leaves. The plant is generally used in treatments for problems concerning digestion and nervous system. Leaves are taken (fresh or dried) in case of fever, abdominal cramps, gastro-enteritis, nausea and poor digestion.

***Lantana camara* L.**

Lantana camara L. belongs to the family Verbenaceae. It grows as a perennial shrub. Leaves are opposite, ovate with very small rounded teeth, somewhat rough and hairy. Leaves are aromatic when crushed. Flowers are borne in dense clusters. Fruits are fleshy, greenish that changes to black and each fruit contain one seed. The methanolic extract of *Lantana camara* show healing of gastric ulcers and also prevents development of duodenal ulcers in rat.

Chemical Fertilizer

Di-Ammonium Phosphate

Di-Ammonium Phosphate (DAP) is used as a chemical fertilizer. When applied to plant, it temporarily increases the soil pH resulting in increased growth of the plant.

Super Phosphate

- It is a cost effective fertilizer for pasture development.
- Ideal for capital or maintenance applications.
- Readily available phosphorus and sulphur.
- Super phosphate sulphur is a readily available form of sulphate for plants to absorb.

Methods

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 also FYM is well mixed and filled in pots having 7 kg capacity. A study was conducted to assess the effect of leaf extracts (*Ocimum basilicum* and *Lantana camara*) and chemical fertilizer (di-ammonium phosphate and super phosphate) singly and in combination on phytochemical aspect. The treatments were given at every 10 days interval i.e., on 25th day, 35th day and 45th day after sowing the seeds.

Treatments

T_0 - Control
T_1 - Di-ammonium phosphate (DAP) – (1%)
T_2 - Super phosphate (SP) – (1%)
T_3 - Leaf extract of *Ocimum basilicum* (extract 1) – (1%)
T_4 - Leaf extract of *Lantana camara* (extract 2) – (1%)
T_5 - Combination of both the leaf extracts
T_6 - DAP + leaf extracts 1 and 2
T_7 - DAP + SP + leaf extracts 1 and 2

Preliminary Phytochemical Anlaysis

The preliminary phytochemical analyses of various primary and secondary plant metabolites for eight treatments were carried out using the method of Harborne (1984).

Preparation of Plant Extracts

At the time of yield the plants were taken and shade dried. The dried plant materials were ground coarsely for further use. The plant samples were soaked in water, chloroform and methanol for overnight extraction and later phytochemical analysis were carried out.

Test for Alkaloids

Mayer's Test

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

Test for Flavonoids

Sodium Hydroxide (NaOH) Test

A small amount of the extract was treated with aqueous NaOH and HCl. Later, the sample was observed for the formation of yellow orange colour.

Test for Terpenoids

Salkowski Test

Five ml of the extract was mixed with 2 ml of chloroform and conc. H_2SO_4 (3 ml) was carefully added to form a layer. The formation of a reddish brown colour indicates the presence of terpenoids.

Table 1. Influence of chemical fertilizers and leaf extracts on the preliminary phytochemical analysis of black gram (*Vigna mungo* L.)

Test for	Methanol								Chloroform								Water							
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	-	+	-	+	+	+	-	-	+	-	+	-	+	-	+	+	-	+	+	+	+	-
Terpenoids	+	-	-	-	+	+	-	+	-	+	-	+	-	+	+	+	+	-	+	-	+	-	+	-
Quinone	+	-	+	-	+	+	+	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	+
Sterols	-	-	+	-	-	-	-	-	+	-	+	+	+	-	+	+	-	-	+	+	+	-	+	+
Oil and Fat	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+

Test for Quinones

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

Fixed Oil and Fat

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

Test for Sterols

Liebermann – Burchard Test

Extract (1 ml) was treated with 10 drops of acetic acid and to this, 5 drops of H_2SO_4 was added. The sample was observed for the formation of pink colour.

RESULTS AND DISCUSSION

The experiments were conducted in black gram (*Vigna mungo* L.) with two different leaf extracts (*Ocimum basilicum* and *Lantana camara*) and chemical fertilizers such as di-ammonium phosphate and super phosphate and the phytochemicals were analysed and the results of the study is given below.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of different extracts (methanol, chloroform and water) of black gram showed the following results. In the present study, a phytochemical analysis was carried out to detect the active constituents such as alkaloids, flavonoids, sterols, terpenoids, quinone, oil and fat (Table 1). In methanol, chloroform and water extracts, the alkaloids were absent in all the treatments. In methanol extract, the flavonoids were present in T_0, T_3, T_5, T_6 and T_7. In chloroform extract, the flavonoids were present in T_2, T_4 and T_6. In water extract, the flavonoids were present in all the treatments except T_2 and T_7. Yadav and Agarwala (2011) investigated the presence of phyto chemicals and determined the total phenolic and flavonoid contents of selected medicinal plants. In this, the result showed that crude aqueous and organic solvent extract of the tested plants contain medicinally important bioactive compounds. The terpenoids were present in T_0, T_4, T_5 and T_7 of methanol extract. In chloroform extract, the terpenoids were present in T_1, T_3, T_5, T_6 and T_7 and in water extract; terpenoids were present in T_0, T_2, T_4 and T_6. The quinone was present in T_0, T_2, T_4, T_5 and T_6 of the methanol extract of the plant. In the chloroform extract, quinone was present in T_0 and T_3 only. In water extract, the quinone was present in T_0, T_5 and T_7. The sterols were present in T_2 of methanol extract and in T_0, T_2, T_3, T_4, T_6 and T_7 of chloroform extract. In water extract, sterols were present in T_2, T_3, T_4, T_6 and T_7. The oil and fat was present in T_2 of methanol extract. In chloroform extract, the oil and fat was present in T_0, T_3 and T_7. In water extract, oil and fat was present in T_2 and T_7. Kannan and Jagadeesan (2001) have reported the presence of flavonoids in *Maliquera longifolia*. In 54 Indian medicinal plants, the preliminary phytochemical analysis showed positive indication for alkaloids, flavonoids, steroids and terpenoids by Xavier and Kavitha (2012).

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