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Studies on production and total phenol content (TPC) of leaf biomass of the selected medicinal plants

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Abstract - Wild medicinal plants waste biomass going waste every year, it may be utilize for the welfare of agricultural purpose. Such studies are meager in India. By using chemical fertilizer, weedicides and pesticides causes pollution which decreases soil fertility, kills ecofriendly soil micro flora and many other hazardous effects. We are unaware of biomass produced by wild medicinal plants going waste every year. If it is utilized for various purposes in agriculture, it improves crop yield ecofriendly. Because of most of the plants consist of antimicrobial activity in them. It may be utilized as a source of insecticide, fungicide, bactericides and herbicides.

In the present study plant leaf biomass in the form of dry weight, total phenol content was carried out of selected ten wild medicinal plants. Plant waste biomass in the form of leaf extract was used against seed mycoflora, seedling emergence, spore germination, sporulation and dry mycelial weight were carried out for *Aspergillus niger.* It was also found that all the test medicinal plants leaf extract was found more or less inhibitory effect on all above mentioned factors.

KEY WORDS: TPC; seed mycoflor; growth of seed borne fungi; spore germination; leaf biomass; Cauliflower

I. INTRODUCTION

Indian plant wealth is about 45,000 species of plants. Every year huge medicinal plants biomass were going waste. It may be utilized for agricultural purpose because all most all plants having antimicrobial activity. If we utilize plant based product which does not causes any harmful effect on biodiversity. There is a great boom to use herbal medicine to treat almost all afflictions of human beings. Herbal plants play an important role in the health care of the country. Due to its effectiveness and no side effect, people prefer herbal medicines now days. But herbal medicinal plants may be used for welfare of agriculture, such studies are meager in our country.

In the present studies *Sapindus laurifolius* produced more leaf biomass (39 gm) and *Helicteres isora* produced very less leaf biomass (25 gm) as compared to the other test medicinal plants. Leaf biomass of *Semecarpus anacardium* showed more TPC (2.1 mg) and leaf biomass of *Dioscorea bulbifera* showed very less TPC (0.8 mg) as compared to the other test medicinal plants. Seeds of cauliflower were soaked in the

5% aqueous leaf extract of selected medicinal plants found to be inhibitory for the incident of seed mycoflora and stimulatory for seed germination of cauliflower. Leaf extract of *Vitex negundo* showed 72% of seed mycoflora and *Semecarpus anacardium* showed less seed mycoflora (40%) of cauliflower seeds. Seeds of cauliflower observed 85% of germination with greater elongation of root and shoot in the leaf extract of *Semecarpus anacardium.* Spore germination and dry mycelial weight of *Aspergillus niger* found stimulatory in the leaf extract of *Balanites aegyptiaca* (65%) and leaf biomass *Solanum xanthocarpum* showed inhibitory effect on spore germination (25%) and dry mycelial weight (18 mg)

II. MATERIALS AND METHODS

1. Studies on biomass production of the medicinal plants:

During the present studies ten very common and easily available ten medicinal plants were selected. The fresh leaves of the selected medicinal plants were collected. The collected plant material was washed and cleaned separately. 100gm of fresh plant material was weighed (Arnoud, 1988). The weighed plant material was dried in the hot air oven. The dried plant material was reweighed .The dry biomass of the medicinal plants was determined by subtracting the dry weight from the fresh weight. The resulted weight in gm/100gm was treated as the biomass produced by the selected medicinal plants (Guy,1981)

2. **Studies on total phenol content (TPC) of the biomass of the medicinal plants:**

Total phenol content (TPC) of the biomass of the test medicinal plants was estimated by using Folin-Ciocalteu method as described by Mahadeven and Sridhar in 1996. For this 1ml of the alcoholic extract of biomass of the test medicinal plants was taken in a graduated test tube. 1ml of Folin–Ciocalteu reagent and 2ml of sodium carbonate (Na_2Co_3) solution was added to the test tube. The test tube was well shaken and heated in a boiling water bath for exactly one minute. The test tube was cooled under running tap water. The blue colored solution in the test tube was diluted to 25ml with distilled water and the absorbance was measured at 650nm in a colorimeter. The unknown were read from a standard curve made from different concentrations of catechol. A blank containing all the reagents minus alcoholic extract of biomass of the test medicinal plants was used to adjust the absorbance to zero. Yubedee (1998) studied phenolic content of different species of Dioscorea and similar study in sunflower carried out by Pathak and Srivastva (2000). Pandey et al (2004) studied changes in TPC of leafy vegetables.

3. Preparation of aqueous extract of Biomass of medicinal plants:

During the present study leaf extract of ten test medicinal plants was prepared separately. Leaves of selected plants were dried and grind into fine power. 5 gm leaf powder of each medicinal plant dissolved separately into 100 ml of sterile water. This used in further experiment as 5% leaf extract tested for the study of seed mycoflora, root and shoot length and growth of seed borne fungi of cauliflower. Seeds of cauliflower were soaked in 5% leaf extract of each plant throughout the night. Such treated seeds used further. These treated seeds studied by the method suggested by Neergaard (1973).

4. Detection of seed mycoflora:

a. Collection of Seed Samples:

Seed samples of the test vegetables were collected from field, store houses and market places common test vegetable selected for further study. A composite seed sample of individual test

vegetable was prepared by mixing the individual samples together, preserved in gunny bags at room temperature during the studies.

b. Moist blotter plate method:

The isolation is made by blotter test method as described by International Seed Testing Association (ISTA, 1966), De Tempe (1970), Neergaard (1973) and Agarwal and Sarbhoy (1978).

Ten treated seeds of cauliflower were placed at equal distance on the moist blotting paper in the petridishes. Four hundred seeds were tested for each treatment. The plates were incubated at room temperature for seven days. On the seventh day the seeds were examined under compound microscope for the determination fungal species. Identification and further confirmation of twelve different species of fungi occurred on the seeds was made by preparing slides of the fungal growth and observing under compound microscope. Out of these *Aspergillus niger* was very common and maintained on PDA slants in the form of pure culture for further studies.

c. Identification of seed borne fungi:

The seed borne fungi were identified on the basis of sporulating characters like asexual spores or fruiting structures. Detailed examination of fungal characters was done under compound microscope and their identification was confirmed with the help of latest manuals (Subramanian, 1971; Jha, 1993 and Mukadam, 1997). Pure culture of the identified fungi were prepared and maintained on PDA (Potato Dextrose Agar) slants.

d. Preparation of spore suspension:

For this 10ml sterile distilled water was poured in to the sporulating pure cultures of the seed borne fungi maintained on PDA slants for seven days at room temperature. The slants were shaken and the content was filtered through muslin cloth. The filtrate was used as spore suspension.

e. Study of spore germination:

During the present studies, 25ml of GN medium supplemented separately with 2ml of 5% plant extract was poured in 100ml borosil conical flasks. The flasks were autoclaved and inoculated separately with 2ml spore suspension of the test seed borne fungi which were maintained on PDA slants for seven days. The flasks were incubated at room temperature for twenty four hours. After incubation the spore germination was studied by preparing slides of the incubated solution and observing under the compound microscope. The germ tube lengths of the germinating spores were measured in microns (µ) with the help of calibrated microscope. The flasks poured with 25ml of GN medium without the supplementation of 2ml of 5% plant extract inoculated separately with spore suspensions of test fungi were served as control.

5. Study of growth and sporulation of seed borne fungi:

During the present studies dominant seed borne fungus of cauliflower vegetable like *Aspergillus niger* was grown in GN medium supplemented separately with 2ml of five percent plant extracts of medicinal plant biomass for seven days at room temperature. After incubation contents of the flasks were filtered through pre-weighed Whatman filter paper No. 1. The filter papers with mycelial mat were oven dried for twenty four hours at sixty degree centigrade and reweighed. Growth of the seed borne fungus in terms of dry mycelial weight was measured by subtracting the initial weight of the filter paper from the final

weight of filter paper with mycelial mat. The seed borne fungus grown in GN medium without supplementation of medicinal plant biomass extract were served as control. The sporulation was studied by preparing slides of the seed borne fungus before filtration.

Table- 1: Studies on production and total phenol content (TPC) of leaf biomass of the medicinal plants.

Sr. No.	Botanical Name	Leaf biomass (gm/100gm)	TPC (mg/gm)
01.	*Abrus precatorius* L.	26	2.0
02.	*Aegle marmelos* (L.) Corr.	38	1.9
03.	*Balanites aegyptiaca* Delile.	29	1.9
04.	*Datura metel* L.	31	2.0
05.	*Dioscorea bulbifera* L.	30	0.8
06.	*Helicteres isora* L.	25	1.9
07.	*Sapindus laurifolius* Vahl.	39	1.6
08.	*Semecarpus anacardium* L.	32	2.1
09.	*Solanum xanthocarpum* Schra.	27	2.0
10.	*Vitex negundo* L.	29	0.2

Table- 2: Effect of leaf biomass of medicinal plants on seed mycoflora and seed germination of cauliflower.

Sr. No.	Extract of leaf biomass (5%)	Incidence of seed mycoflora (%)	Seed germination		
			%	RL (mm)	SL (mm)
01.	*Abrus precatorius* L.	55	85	42	39
02.	*Aegle marmelos* (L.) Corr.	55	80	43	31
03.	*Balanites aegyptiaca* Delile.	50	82	30	26

04.	*Datura metel* L.	85	70	39	30
05.	*Dioscorea bulbifera* L.	75	65	40	42
06.	*Helicteres isora* L.	80	62	33	45
07.	*Sapindus laurifolius* Vahl.	45	80	40	35
08.	*Semecarpus anacardium* L.	40	86	43	34
09.	*Solanum xanthocarpum* Schra.	70	80	39	35
10.	*Vitex negundo* L.	72	50	32	30
	Control	90	62	43	39

RL-Root length SL- Stem length

Table No.3: Effect of leaf biomass of selected medicinal plants on spore germination, growth and sporulation of *Aspergillus niger*

Sr. No.	Extract of leaf biomass	*Aspergillus niger*		
		Spore germination (%)	Dry mycelium Wt. (mg)	Sporulation
01	*Abrus precatorius* L.	30	25	++
02	*Aegle marmelos* (L.) Corr.	58	38	+++
03	*Balanites aegyptiaca Delile*.	65	45	+++
04	*Datura metel* L.	35	30	++
05	*Dioscorea bulbifera* L	35	30	+++
06	*Helicteres isora* L.	40	28	++
07	*Sapindus laurifolius* Vahl.	34	31	+++

08	*Semecarpus anacardium* L.	32	29	++
09	*Solanum xanthocarpum* Schra.	25	18	++
10	*Vitex negundo* L.	45	40	+++
	Control	80	50	+++

III. RESULT AND DISCUSSION

It is evident from the results presented in table-1 that the *Semecarpus anacardium* (39 gm/ 100gm) and *Aegle marmelos* (38gm/ 100gm) were found to produce more leaf biomass and *Helicteres isora* produce very less leaf biomass (25gm/100gm) as compared to the other test medicinal plants. It is clear from the results that the leaf biomass of *Semecarpus anacardium* (2.1mg/ gm) and *Solanum xanthocarpum* (2mg/gm) showed more TPC while the leaf biomass of *Vitex negundo* showed very less TPC (0.2mg/gm) as compared to the leaf biomass of the other test medicinal plants. Similar study carried out by Bhadra et al (2000) in bael fruit TPC and found that decreased TPC with growth of bael fruit. Srivastava (2000) studied that TPC in sunflower and observed that it maximum after treatment with benomyl powder. Similarly Lavanya (2007) carried out in sunflower necrosis.

It is clear from the results presented in table-2 that leaf extracts of all the test medicinal plants were found to be inhibitory for the incidence of seed mycoflora. The leaf extract of *Semecarpus anacardium* was found to be inhibitorier and leaf extract of *Datura metel* was found to be very less inhibitory for the incidence seed mycoflora of Bhendi as compared to the leaf extracts of the other test medicinal plants. In all leaf extracts of the test medicinal plants found to be inhibitory for seed germination. Same results were observed in shoot length and root length in Bhendi stimulatory for elongation of root and elongation of shoot of Bhendi. Except in *Dioscorea bulbifera and Helicteres isora* it was stimulatory for elongation of root and shoot of Bhendi. Chouksey and Srivastava (2001) studied root extract of *Terminalia arjuna*, found antimicrobial effect against *Aspergillus niger*, *Candida* and *Bacillus oryzae*, Similarly Raja et al found that leaf extract was inhibitory for incidence of seed mycoflora and similar study carried out by Bodke et al (2005), Dhekle (2007), Mashooda Begum and Lokesh (2008) and Musiyimi (2008).

It is clear from the results presented in table-3 that, the leaf biomass of all test medicinal plants were found to be found inhibitory for spore germination, growth and sporulation of *Aspergillus niger*. It is also evident from the results that the leaf biomass powder of *Solanum xanthocarpum* was found to be more inhibitory and leaf biomass powder of *Balanites aegyptica* was found to be more stimulatory for the spore germination, growth and sporulation of *Aspergillus niger* as compared to leaf biomass of other test medicinal plants. Danai (1994) carried out comparative study on the species of *Aspergillus* occurring different plant seeds. Similar result observed by Sharma and Suchdev (1984).

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