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ALLELOPATHIC EFFECTS OF PARTHENIUM HYSTEROPHORUS ON GERMINATION OF SORGHUM SEEDS

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International Allelopathy Society has redefined Allelopathy as any process involving secondary metabolities produced by plants, algae, bacteria, fungi and viruses that influences the growth and development of agricultural and biological system; a study of the functions of secondary metabolities, their significance in biological organization, their evolutionary origin and elucidation of the mechanisms involving plant-plant, plant-microorganisms, plant-virus, plant-insect, plant-soil-plant interactions.

Recently a new dimension has been added to the topic of allelopathy that can be employed in weed control in two ways a) selecting an appropriate crop cultivar or incorporating an allelopathic character into a desired cultivar, b) applying residues and straw mulches or growing an allelopathic cultivar into rotational sequence that allows residues to remain in the field. The chemicals that bring about allelopathic influences between the plants and other organisms are called allelochemicals.

Sorghum is the most important food and fodder crop of dryland agriculture. Among cereals it occupies fifth position in acreage and production in the world and it is grown on 47.8 million ha. With annual production of 64 million tonnes. The major *Sorghum* producing areas in the world are the great plains of North America, Sub-Saharan Africa, North Eastern China, India, Nigeria and Argentina. In the world the production of *Sorghum* varies from 500 to 1000 kg/ha in tropical countries and 1500 - 3000 kg/ha in temperate areas (House, 1982). It is as low as 600 kg/ha in Africa and as high as 3127 kg/ha in America (Peacock and Willson, 1984). In India this crop occupies third position among the cereals with the annual area under cultivation of 16 million hectares and annual crop yield about 11.90 million tonnes. The leading states in *Sorghum* production in India are Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Gujarat, Rajsthan, Uttar Pradesh (Pandy and Chadha, 1996). *Sorghum* is used as major source of human food in variety of forms. Green and dried fodder is utilized as cattle feed. Apart from this malted *Sorghum* is used in preparation of infant food. Sweet *Sorghum (S. vulgare* var. saccharatum) is used in the production of syrup.

Chemical Composition of *Sorghum:*

The investigation of biochemical composition of any cereal grain is important from the point of nutritional value. As *Sorghum* is a major food crop of dryland agriculture, several attempts are made to find out chemical composition of seeds of different varieties of *Sorghum.*

1. Carbohydrates : Like other cereals, the chief nutritional reserve in *Sorghum* seeds is starch. The starch content ranged from 67 to 70% in 17 samples of grain *Sorghum.* The seeds of fodder *Sorghum* varieties also contain appreciable level of starch. Arora and Luthra (1972) indicated that in seeds of 19 strains of fodder *Sorghum* the range of starch varied from57.4 to 70.6%. There is also variation in colour of starch. As testa is absorbed before maturity and pigments are absent, colourless starchesare produced but white *Sorghums* having coloured testa produce offcoloured straches. In waxy *Sorghum* Sagrain the colour of starch ispale blue-grey and in Leoti it is light tan (Mac Master and Hilbert,1944), The starch is deposited in the form of starch granules with average size between 16-20 pm. (Francis and Smith, 1916). Employing Scanning Electron Microscopy, Davis and Harbers (1974) pointed outthat in soft endosperm of C42Y yellow and ACCO 1023, smooth and spherical granules were present while in CP622 waxyendosperm starch granules were irregular in shape. The polymer starch is made of two structural components, amylose and amylopectin. On the basis of starch character, *Sorghum* is divided into 2 types, waxy and nonwaxy *Sorghum.* In waxy *Sorghum* starch is rich in amylopectin and in non waxy *Sorghum* starch contain more than 20% amylose. Waniska (1976) reported that waxy endosperm, heterowaxy and nonwaxy endosperm type *Sorghum* contained 2.2%, 12.3% and 6.4% amylose respectively. Amylopectin is always a dominant component of *Sorghum* starch and in one *Sorghum* cultivar (TX 615) as much as 100% amylopectin is present (Sullins and Rooney, 1974). In *Sorghum* glucose is the principal free sugar.

2. Proteins and Amino Acids : The crude protein content is similar for immature and mature *Sorghum* grain. On the basis of study of 522 lines of *Sorghum* from the world, Srinivasan (1972) grouped the *Sorghum* lines in two categories, Group-I - comprising 323 lines having 12% protein and above that. Group-II having 199 lines with protein content below 12%. Austin (1972) investigated this aspect in 96 Indian *Sorghum* varieties utilized in *Sorghum* Improvement Programme. They classified *Sorghum* varieties into four groups on the basis of lysine percentage of the grain and protein percentage. Group I had 9 lines with protein content less than 10%. Group II comprising of 24 lines having 10- 12% protein. Group III of

30 lines containing protein content above12%. Group IV of 33 lines having small seeded fodder types.

Based on solubility the seed proteins are classified into four different classes such as albumins, globulins, glutelins and prolamines. The proportion of these four classes in *Sorghum* proteins is albumin (6%), globulin (10%), glutelins (38%) and prolamine (46%).

3. Lipids : The lipid content in *Sorghum* seeds is in the range 2.4 to 6.5% (Hulse *et al*, 1980).Oleic and linoleic acid are the major unsaturated fatty lipid (80%) in *Sorghum* lipids while saturated fatty acids, palmitic and stearic acid are in less proportion (15%). Lipids in *Sorghum* contained 71.5% free fatty acid with palmitic acid. *Sorghum* seeds contain gluten, fiber lipids, 18% unsaponifiable matter and 20%free fatty acids.

4. Vitamin content : In dry grains of *Sorghum,* ascorbic acid (Vitamin C) is absent but as germination starts ascorbic acid starts to develop. Tocopherol (Vitamin E) and Vitamin K are also detected in the embryo. Yellow endosperm contain p carotene in higher quantity than white endosperm *Sorghum. It* contains comparatively higher amount of B group vitamins.

5. Crude fibre : The crude fibre content in the *Sorghum* seeds is in the range 1.2 to 3.5%. The studies of Bidwell *et al* (1992) revealed that the *Sorghum* bran contains far more crude fibre than other seed parts. Subramaniam *et al* (1983) noticed that the crude fibre content in the grain is reduced as the seeds mature.

Parthenium causes many skin diseases, allergic diseases Hke dermatitis, fever and asthama. Due to the allergenic properties present the weed *Parthenium* has become health hazard to human as well as animals. It is errect, much branched, hairy herbaceous plant attaininga height about half to two meter. Stems are hairy, rigid, angular andlongitudinally grooved. The leaves are 8-12 cm long, alternate,

pinnatifi and irregulary dissected. The upper and lower surface of leaf and stem is covered with 2-3 celled and 4-5 mm long trichomes. Inflorescence is axillary or terminal panicle which consists of capitula containing bracts in two whorls. Five female florets and two male florets are present in inner five bracts. Female florets which are also called ray florets are jug shaped, white in colour with cup shaped, indistinctly bilobed corolla surrounding style and bifurcated stigma. Male florets which are also called disc florets are yellowish in colour, 1.5 mm long with distinct and infundibuliform corolla . Parthenin is the typical organic compound present in different parts of *Parthenium* (Patil, 1978). Different phenolic compounds reported from *Parthenium* are caffeic, p-coumaric, p-hydroxybenzoic, anistic and

ferulic acid. Stem, leaf and seeds are rich in total lipid content. After its entry *Parthenium* has extended in all parts of India. It spreads over nearly five million hectares of land . Extensive range of habitats is occupied by this weed. It forms a very large, dense stands covering several hectares and attacked and destroyed several crops, vegetables, orchards, grazing lands. In some cases it may cause a complete failure of crop.

Methodology:

PROCUREMENT OF LEAF LITTER AND SEEDS

Leaf litter of *Parthenium hysterophorus* L was collected from agricultural. The leaf litter was preserved temporarily in polythene bags in dry conditions. Seeds of *Sorghum bicolor* (L.) Moench var. M. 35-1 which formed the major plant material in the present investigation were obtained from local market.

Leaf leachate preparation

Two hundred gram dried leaves were weighed, washed quickly with tap water to remove surface dust and soaked in one litre of sterilized distilled water for 24 hours. After 24 hours, leachate was filtered through Whatman No. 1 filter paper and filtrate was used for further studies.

pH

Leaf leachate pH was measured with the help of pH meter (Model LI-10T)

Nitrate and Nitrite content

For estimation of nitrate content from leaf leachates the method of Cataldo *et al.* (1975) was followed. 0.2 ml of leaf leachate was mixed thoroughly with 0.8 ml salicylic acid- H2S04 reagent (5% w/v) salicylic acid in concentrated H2S04). After 20 minutes 19 ml of 2 N NaOH was added slowly to raise the pH above 12. Then samples were cooled to room temperature and absorbance was read at 410 nm. Nitrate content was estimated with the help of standard curve and it was expressed as pg ml*1. Nitrite content from leaf leachates was estimated by the method of Nair *et al.* (1988). Three ml of leaf leachate was mixed with 3 ml of 1% sulfanilamide in 1 M HC1 and 3 ml of 0.02% NEEDA. After 15 minutes the absorbance was read at 540 nm. Nitrite content was estimated with the help of standard curve and it was **expressed as *u*g** ml^{-1}.

Inorganic constituents

The contents of Potassium, Calcium, Magnesium, Manganese, Iron, Zinc in the leaf leachates were determined with the help of Atomic Absorption Spectrophotometer (Perkin Elmer, 3030). The values were expressed as $\boldsymbol{u}$ g per ml leaf leachate.

Preparation of Extract

Ten gram dried leaves of each species were soaked in 50 ml of absolute alcohol for 24 hours. The leaf leachates were filtered through Whatman No. 1 filter paper. The filtrates were condensed to 5 ml on boiling waterbath and 5 ml distilled water was mixed in each 5 ml extract. Then the extracts were centrifuged at 5000 rpm for 10 minutes. Supernatants were used for further analysis. The Glass Plates (6 cm x 12 cm and 6 cm x 18 cm) were coated with a thin uniform layer of slurry of silica gel G (40 g silica gel G (with binder 13% CaSO**4**, Qualigens Fine Chemicals) with 80 ml distilled water). The plates were air dried and activated in oven at 100°C for 1 hr.

GERMINATION STUDIES

Healthy seeds of *Sorghum* were surface sterilized by treating with 0.1% Mercuric chloride for 5 minutes. Then seeds were rinsed with distilled water for 4-5 times. Twenty seeds were placed in sterilized petriplates with moistened filter paper.

Eight ml of leaf leachates were added in each petriplate. The petriplates supplied with distilled water served as control. Petriplates arranged in triplicates for each treatment. Seeds were allowed to germinate at 30°C in BOD incubator and used for further analysis.

Germination percentage and Seedling Growth

The germination percentage was recorded after 24 and 48 hours. The emergence of radicle was considered as a criterion for germination. Seedling growth analysis with respect to root length,

shoot length, vigour Index and fresh weight was performed at 72 hours stage.

METABOLIC STUDIES OF GERMINATING SEEDS

In order to study influence of leaf leachates on various metabolic aspects of germinating *Sorghum* seeds, the analysis was performed at 72 h germination stage.

Enzyme Polyphenol oxidase

Activity of enzyme polyphenol oxidase from germinating *Sorghum* seeds (72 h stage) was .One gram germinating seeds were crushed in 15 ml 0.1 M phosphate buffer (pH-6.1). The resultant homogenate was filtered through 4 layers of musline cloth. The filtrate was

centrifuged at 10,000 rpm for 20 minutes. The supernatant served as enzyme source. The assay mixture contained 4 ml 0.1 M phosphate buffer (pH-6.1), 1 ml 0.01 M Catechol prepared in 0.1 M phosphate buffer (pH-6.1), 0.5 ml enzyme and mixed well. The increase in OD at 30 seconds interval up to 180 seconds at 495 nm was recorded. The enzyme activity was expressed as AOD min'1 mg'1 protein. Five hundred miligram germinating *Sorghum* seeds (72 h stage) were homogenised in 10 ml ice-cold 0.2 M sodium borate buffer (pH-8.8) and filtered through 4 layered musline cloth. Filtrate was centrifuged at 10,000 rpm for 20 minutes. Supernatant served as enzyme source. Assay mixture containing 2.8 ml 0.2 M sodium borate buffer (pH-8.8), 0.2 ml enzyme, 0.2 ml 0.1 M Lphenylalanine was incubated at 36°C. Absorbance was read at 0 min, 10 min, 20 min and 30 min at 290 nm. The enzyme activity was expressed as AOD min'1 mg'1 protein.

Preparation of samples:

Two g germinating seeds of ***Sorghum*** (72 hours) from each treatment were extracted in 3 ml of 0.1 M Tris HC1 buffer (pH-7.5) containing 0.02 M EDTA, 0.05 M NaCl, 0.03 M mercaptoethanol. The samples were centrifuged at 10,000 rpm for 20 minutes and the supernatant was cooled and used as enzyme source.

Result and discussion:

Due to extracts of unburnt and burnt residues of *Parthenium hysterophorus*, seedling were inhibited and in this case extract of burnt residue of *Parthenium* was more toxic. Then it was concluded that this was because of highly alkaline nature of extract. As we will see latter among plants studied. *Parthenium* leaf leachate is found to be more inhibitory to seed germination and seedling growth of *Sorghum*. The higher pH of the leaf leachate may be one of the factors for such inhibitory effects. *Parthenium* leaf leachate have highest nitrite content which is highly reactive, potentially toxic ion. Potassium content is maximum in *Parthenium* leaf leachate. The role of potassium as activator of various enzymes is very well established.

Germination Percentage and Seedling Growth

Influence of leaf leachates of different plant species on germination percentage during early phases (24 and 48 h) of seed germination in four crop species namely *Sorghum* is recorded in Table-2. It is evident from the Table-6 that among all leaf leachates *Parthenium* leaf leachate is most inhibitory for seed germination during early phase (24 h) of germination in the crop.

Inhibitory effect does not prevail after 48 h in most cases and there is recovery in germination.The germination percentage in *Sorghum* seeds is marginally reduced due to treatment of all the leaf leachates .Influence of leaf on seedling growth with respect to root length and shoot length (at 72 h stage) of *Sorghum*, in Fig 1 and 2. From Fig. 1 and 2 it is clear that root length and shoot length in seedlings of *Sorghum* are reduced due to treatment of leaf. The leachates of all the plants increases root length and shoot length of seedlings of *Sorghum*. In *Sorghum* seedlings maximum reduction in root length and shoot length is caused by *Parthenium* leaf leachate . In *Sorghum* seedling maximum reduction in vigour index is caused due to *Partheniu* leaf .

Fresh weight of seedling is decreased due to leaf leachates of all the plant species in all the crops. In case of *Sorghum* fresh weight of seedling is reduced due to treatment of leaf leachates of all the plant in *Parthenium* .

Germination of the seed of the higher plant is considered as consecutive number of steps which causes a quiescent seed, with a lower water content to show a rise in its general metabolic activity and to initiate the formation of seedling from the embryo. In many seeds radicle (root) is the part of embryo which protrudes and in some seeds it is shoot. The protrusion of the part of the embryo through the seed coat is mainly because of cell division and cell enlargement.

The reduction in ascorbic acid content in leaf leachate treated *Sorghum* seeds in one of the main features responsible for suppression of seedling growth since it is very well documented in several studies that this compound is essential for cell division and cell expansion during germination. Further the reduction in this antioxidant may also cause increase in severity of oxidative stress in the germinating seeds.

CONCLUSION:

Sorghum popularly known as jo war is the most important food and fodder crop of dryland agriculture. *Sorghum* grain is used as human food in various forms and green and dried fodder is used for feeding cattle throughout the country. To investigate the allelopathic influence the plants selected are tree species *Eucalyptus globulus* Labill, *Moringa oleifera* Lamk., *Melia azadirachta* L. which are economically valuable farmside and roadside trees, a weed species *Parthenium hysterophorus* which is abnoxious weed species, widely spread, health hazardous weed.

Some of the significant findings of the study are as follows :

1. *Parthenium* leaf leachate have alkaline pH range.
2. *Parthenium* leaf leachate has highest nitrite content.
3. Maximum potassium content is present in *Parthenium* leaf leachate.
4. *Parthenium* leaf leachate phloroglucinol, protocatechuic acid andferulic acid are detected.
5. In case of *Sorghum*, root length and shoot length of seedlings are reduced due to treatment of leaf leachates of all the plants.
6. It is evident from the foregoing account that the treatment of leaf leachates of *Parthenium, Eucalyptus,* exert a marked influence on different facets of metabolism of germinating *Sorghum* seeds. All these metabolic disturbances are ultimately responsible for the unsatisfactory seed germination and an overall decline in seedling vigour and growth.

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Tables and Graphs

1. Influence of leaf leachates on germination percentage of Sorghum

	Sorghum	
Treatment	**24 h**	**48h**
Control	**96**	**96**
Parthenium leaf leachate	**78**	**78**

2.Phenoliccompounds detected in the leaf leachates

Parthenium leaf leachate
1.Floroglucinol
2.Protocatechuic acid
3.Ferulic acid
4.Unidentified

3. Effect of leaf leachates of different species on root length of seedlings(72 h stage) of Sorghum

Sorghum	
Treatment	**Average root length**
1. Control	**5.4 cm**
2.Parthenium leaf leachate	**1.6 cm**

4.Effect of leaf leachates of different species on shoot length of seedlings(72 h stage) of Sorghum

Sorghum	
Treatment	**Average root length**
1. Control	**3.3 cm**
2.Parthenium leaf leachate	**0.8 cm**