

## Antifungal activity of some Indigenous plants

D. Sai Kumari<sup>1</sup> & B. Thirupathi<sup>2</sup> & Dr. Neeti saxena<sup>3</sup>

<sup>1</sup>Ph.D Scholar, <sup>2</sup>Ph.d Scholar and <sup>3</sup>Asst.Professor

Mycology and Plant Pathology Lab, Department of Botany, University College  
For women, Koti, Osmania University, Hyderabad, Telangana, India -500095.

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**ABSTRACT:** Seeds of horse gram were treated with 4 plant extracts they were locally available indigenous plants namely Neem (*Azadirachta indica*), Betel (*Piper betel*), Papaya (*Carica papaya*) and Tulasi (*Ocimum sanctum*) were evaluated. Seeds were collected from Karimnagar district, Telangana, India. The main theme is the antifungal activities of leaf extracts with Water and Methanol as solvents on the most frequently occurring horse gram pathogens of the horse gram (*Aspergillus*, *Curvularia*, *Fusarium*, *Phoma*, *Alternaria* species.) at Karimnagar district. Among all those treated horse gram seeds the Maximum inhibition of seed borne fungi was found in seeds were treated with "water" plant extracts at 5.0, 7.0, 10% solutions in respective plant extracts. Minimum inhibition of mycelium on seeds were treated with solvents "Methanol" at 5.0, 7.0, 10% plant extracts of solutions in respective plant extractions. Highest fungal inhibition was recorded in Neem (53:34%) and betel (46:26%), and normal range of inhibition in Papaya (34:20%) and lowest inhibition in Tulasi (21:15%). The leaf extracts were helped to reduce the pathogenicity in the development of seed germination. In this study we demonstrated the effects and control of leaf extracts against seed borne fungi of horse gram.

**Key Words:** Seed germination, plant extract, Karimnagar, Horse gram.

### I. Introduction

*Macrotyloma uniflorum* Lam is a nutritious food legume, belongs to family fabaceae (Naik 2000). It is cultivated for its seed and mostly eaten as dal. It is rich in protein iron, calcium and Polyphenols. Green plant of horse gram is valuable manure, the fodder being rich in protein, it is widely used as a feed to horses and animals (B. G. Prakash et al. 2008).

Horsegram has excellent therapeutic properties and traditionally used to cure kidney stones, asthma, bronchitis, leukoderma, urinary discharges, heart diseases, piles, it also possess anti-diabetic, anti-ulcer activity weight loss, aids in digestion, it regulates blood sugar levels in diabetics, promoting sperm count in men, regulate the menstrual cycle by boosting haemoglobin, it relieves the constipation, it prevent the formation stones in kidney, it is natural remedy for common cold, fever, and horse gram widely used in Ayurveda medicine to treat number of health disorders and also helps in dietary management of obesity due to the presence of beneficial bioactive compounds.

Plant disease caused by a variety of fungi may cause significant losses on agricultural crops. All plants are attacked by some kinds of fungi, and each of parasitic fungi can attack one or many kinds of plants. More than 10,000 species of fungi can cause disease in plants (Agarios, 2005). Fungicides use is arguably the most important component of pest and disease management crop. They destroy the agricultural crops reducing them unfeasible. To eradicate the fungal spores and species (Brown, 1968) is the best practice to implement preventive agrochemical spray programmes (McConnell et al, 2003).

In many countries to avoid this type of harmful effects of fungicides through the use of indigenous plant products (Khattach and Hameed 1986) and biocontrol agents (Metcalfe 1975) to protect grains. Using of locally available plant materials are widely used to protect stored products against damage by insect infestation (Giloob and Webley, 1980). Seeds of horse gram were treated with 4 local available plants namely Neem (*Azadirachta indica*), Betel (*Piper betel*), Papaya (*Carica papaya*), Tulasi (*Ocimum sanctum*) were they grown by farmers with minimum cost (or) some of them were grown naturally.

In the present study was undertaken to determine the effect of these botanicals on the inhibition of seed borne fungi of horse gram seed.

### 2. Materials and Methods

**Plant Material:** The present study was conducted in mycology laboratory, Department of Botany, Osmania University, Hyderabad. During the period of March 2016 to September 2016. Horse gram seeds of field variety were collected from district Karimnagar, Telangana, India.

**Horse gram seeds:** Field variety of horse gram seeds were collected from the formers of Indurthi village, Karimnagar district, Telangana, India, during the month of December 2016. The Present study was conducted in Mycology and Plant Pathology Lab, Department of Botany, University College for Women, Koti, Hyderabad, and Telangana, India.

#### Preparation of Plant extracts:

The fresh leaves of Neem, Betel, Papaya, Tulasi were collected from the surroundings of Osmania university, Hyderabad, Telangana. The leaves were washed in running water, and kept in shade for air drying and then they were dried in oven at 45°C to gain constant weight. The powder was prepared by pulverizing the dried leaves with the help of a grinder. Then the powder was passed through a 25 mesh diameter sieve to obtain fine and uniform dint. The powder was preserved in airtight condition at 20°C in polythene bags till their use in extracts preparation. Water soluble extracts were prepared as described by Rivillasacevedo and Soriano – Garica(2007), with some modification.

The prepared leaf powders were used for preparation of plant extraction. Each plant sample 15gm was taken in a 500ml beaker and mixed separately with 100ml of different solvents like distilled water, Methanol. Then the mixture was stirred for 30mins by a magnetic stirrer at 5000rpm and left to stand for next 24 hrs. The mixture was then filtered through a fine cloth and again through filter paper (Whatmann no.1). The filtered materials were taken in a round bottom flask and condensed by evaporation of solvent in a both at 60°C, 50°C temperature for distilled water and Methanol, respectively. Evaporation was done to make the volume 10ml. After the evaporation of solvent, the condensed extracts were preserved in tightly corked labelled bottles and stored in a refrigerator until their use. Different concentrations of plant extracts were prepared by dissolving the stock solutions were in the respective solvents. The resulting aqueous solutions were used for the fungal growth inhibition assay.

The seeds were treated with different plant extracts at 5.0, 7.0 and 10% of respective plant extracts. The treated seeds were then dried under shade and kept for 3 months in a plastic container to prevent infestation. The seeds were then taken to test their seed borne fungi by using agar plate method ( ). 100 seeds from each treatment were placed on the Petri dish containing agar medium. Plates without extracts served as control. Each treatment was replicate thrice.

#### Staining procedure:

After 7 days of treatment seed borne mycelium were grown on the seeds, cultured them on a separate test tubes for pure cultures, on the 4<sup>th</sup> day a small piece of mycelium was taken from the pure culture placed on a clean slide add safranin and methyl blue to the mycelium fragment. Placed a coverslip on the fragment without air bubbles then observed for identification of mycelium under electron microscope.

### III. Results

The present study tested the antifungal activity of leaf extracts and their respective dilutions from the plant extracts belonging to the 4 plant families against *Aspergillus*, *Curvularia*, *Fusarium*, *Phoma*, *Alternariaspecies*. The extracts were chosen based on their traditional usage and antimicrobial properties (or) previous studies that have demonstrated the antifungal properties using different kinds of extracts (Guo et.al.1997, Wilson et.al, 1997, Zhu et.al.2005).

We have taken 100 seeds of Horse gram for the studies were tested the antifungal activity of plant extracts and their effects of respective dilutions from the plant extracts at 5.0, 7.0 and 10% of solutions in respective leaf extracts.

#### 1. The table showing seed borne mycelium of horse gram which are treated with water extracts of different plants.

S. No	Name of the plant	5.0%	7.0%	10%	Control	Inhibition %
1.	Neem ( <i>Azadirachta indica</i> )	38	46	50	285	53%
2.	Betel ( <i>Piper betle</i> )	46	52	55	285	46%
3.	Papaya ( <i>Carica papaya</i> )	59	62	67	285	34%
4.	Tulasi ( <i>Ocimum sanctum</i> )	72	80	90	285	21%

## 2. The table showing seed borne mycelium of horse gram which are treated with the methanol extracts of different plants.

S. No	Name of the plant	5.0%	7.0%	10%	Control	Inhibition %
1.	Neem ( <i>Azadirachta indica</i> )	46	52	55	285	34%
2.	Betel ( <i>Piper betle</i> )	68	70	73	285	26%
3.	Papaya ( <i>Carica papaya</i> )	68	76	84	285	20%
4.	Tulasi ( <i>Ocimum sanctum</i> )	72	80	90	285	15%

Results on the effect of different plant and plant parts had significant effects on the seed borne fungi of horse gram seeds. The plant extracts and its level shows remarkable effect on the mycelium were recorded in leaf extracts. Among the extracts the highest antifungal activity was recorded in the seeds were treated with water extracts of all plants. The seeds treated with the "water" extracts were shown maximum activity followed by "methanol" extracts had showed the minimum activity against seed borne fungi. The rate of antifungal activity was increased with the increasing the dose of plant extract. Among all those plants Neem (53:34%) showed maximum activity then betel (46:26%) followed by papaya (34:20%) and Tulasi (21:15%) shown minimum activity.

### IV. Discussion:

In this study among all those treated seeds the highest antifungal activity found in Neem (*Azadirachta indica*) with Water and Methanol extracts. And observed the same value of activity observed in Betel (*Piper betle*). The minimum activity observed in Papaya (*Carica papaya*). Normal range of activity had shown by Tulasi (*Ocimum sanctum*) with the respect of different solvents with different plant extracts. The antifungal activity increased with the increasing the dose levels of plant extracts. Farmers may use these plant extracts in their storage structure, management of store grain pest. And these plants available in worldwide.

### V. Conclusion

The study has shown that 4 locally available indigenous plants namely Neem (*Azadirachta indica*), Betel (*Piper betle*), Papaya (*Carica papaya*), Tulasi (*Ocimum sanctum*) are very effective in inhibiting the fungal growth of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Phoma*, *Alternaria alternate*, *Fusarium* species. Therefore the plant extracts can successively use for controlling seed borne fungal pathogens of horse gram.

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