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Comparative efficacy of different plant extracts on mycelial growth of *Curvularia lunata* (Wakker) Boedijn causing rice grain discoloration *in vitro*

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Abstract
In the present investigation, efforts were made to test the bio-efficacy of different plant extracts *viz;* Garlic, Neem, Datura, Calotropis, Turmeric and Asafoetida on the mycelial growth of predominantly occurred pathogen on grain discoloration, *Curvularia lunata* using poisoned food technique. Observations on radial growth were recorded after control plate fully occupied by the pathogen. Percent inhibition over control was calculated. Among all the plant extracts Garlic extract was highly effective resulted complete inhibition (100%) at all the concentrations (2%, 3%, 4%, 5%) followed by Neem extract resulted complete (100%) inhibition at 15 per cent and 20 per cent concentrations over the mycelial growth of the pathogen compared with other plant extracts and control.

Keywords: plant, mycelial growth, *Curvularia lunata* rice grain discoloration

Introduction
Rice (*Oryza sativa* L.) is the primary staple food in many countries. In India it is cultivated in an area of 44 M ha with 105 M T of production and 2386 kg ha-1 of productivity. In Andhra Pradesh, the area under cultivation of rice is approximately 1.79 M ha with 5.54 M T of production and 2381 kg ha-1 of productivity (Govt. of India, Ministry of Agriculture, Dept. of Agriculture & Cooperation, Directorate of Economics & Statistics, 2016).

Seed (or) grain discoloration is an early indication of poor seed or grain quality which is generally associated with micro-organisms and sometimes insect pests. Such grains are of poor market value and low consumption quality due to degradation in nutritional value. Grain discoloration of rice is a complex disease occurred, due to infection by certain microorganisms on glumes, kernels or both. The disease is causing both qualitative and quantitative losses of grain yield and also results in seedling mortality, reduction in germination and seedling vigour. Except for other factors several microorganisms especially fungi play a major role in the development of this disease. Under humid conditions, the fungal growth may be prominently seen. Two groups of fungi are associated in grain discoloration of rice (Ou, 1985) [12]. One group is field fungi, more or less parasitic and infects grain before harvest like *Drechslera oryzae, Pyricularia oryzae, Alternaria padwikii, Fusarium moniliforme, Curvularia geniculata, Sarocladium oryzae* etc. Other groups are storage molds, saprophytes *viz.*, *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp. etc.

The *C. lunata* (*Cochliobolus lunata*) found responsible for eye shaped spots. Besides, *F. equiseti, F. oxysporum (Gibberella zeae), F. moniliforme (Gibberella fujikuroi)* found responsible for pink discoloration and *S. oryzae* is responsible for light brown discoloration on the seed coat, endosperm and embryo of discolored seed (Sachan and Agrawal, 1994) [8].

Management of disease by application of different plant extracts at different stages of flowering had been reported by different researchers. Presently so many chemical measures are in practice for the management of grain discoloration instead of plant extracts.

Grain discoloration is the complex disease, many fungi responsible for this disease so, Integrated Disease Management strategies including chemical and plant extracts application necessary to check the disease.

The uses of fungicides (chemicals) to control the disease have been effective. However, the excessive use of these synthetic chemicals has caused environmental pollution and toxicity to living organisms. It has also increased costs to growers (West *et al.* 2003) [19] and their repeated use over decades has disrupted natural biological systems, and sometimes resulted in development of fungal resistance along with producing undesirable effects on non-target

organisms, fostered environmental and human health concerns (Yoon *et al*. 2013) [20]. Therefore, merit attention of all concerned to look into the potential of integrating in the management of economically important diseases, the products prepared from green plants should be preferred as they are environmentally nonpollutive and non- hazardous in preparation and use (Rout and Tewari, 2012) [7]

The secondary components of some plants contain medicinally active fractions of plant tissue that are toxic to pathogens (Gurjar *et al*. 2012) [4] and thus can be utilized in plant disease management programme. The effective control of rice diseases using plant extracts (Sena *et al*. 2013) [9]

Materials and Methods

In order to study the antifungal effect of certain plant extracts in the management of rice grain discoloration, an experiment was conducted by following poisoned food technique (Nene and Thapliyal, 1993) [11]. Different plant extracts and their concentrations used for the study mentioned in Table 2.

Preparation of plant extracts: Fresh test material from the above mentioned individual plant species was collected and washed thoroughly in tap water followed by washing in distilled water and air dried. Later, the test material was grounded with sterile water as 1:1 ratio using pestle and mortar. The macerate was filtered through muslin cloth to get the crude extract. Then the crude extract was filtered through Whatman filter paper No. 1 and finally, crude extract was filtered through the bacterial proof filter under vacuum to get rid of bacterial contamination. Then the different concentrations of plant extracts were prepared by adding the appropriate quantity of sterilized distilled water.

Poisoned food Technique: The effect of different plant extracts mentioned in the above table were evaluated against the predominant grain discoloration fungal pathogen *C. lunata* by poisoned food technique as described by Nene and Thapliyal (1993) [11].

Different concentrations of plant extracts were prepared separately. The experiment was laid out in Completely Randomized Design (CRD) with seven treatments and three replications. For each treatment, 60 ml of PDA medium transferred to 100 ml conical flask and sterilized in autoclave. To this medium, required concentration of plant extracts were separately added, mixed thoroughly and then poured into Petri plates, finally allowed to solidify. From seven-day old culture of pathogen, a five mm disc cut from outer margin with the sterilized cork borer and was transferred to the center of the plates containing the medium amended with test compound. Appropriate control was maintained by placing fungal discs in unamended plates and incubated at 25±1oC. The whole procedure was carried out under aseptic conditions.

The growth of fungal colony was measured after observing the full plate growth in control. The per cent inhibition was calculated by following the formula given by Vincent (1927) [18].

$$I = (C - T)/C \times 100$$

Where I= Per cent Inhibition
C= Radius of the colony of fungus in control
T= Radius of the colony of fungus in treatment.

Results and Discussion:

In order to assess the effect of different plant extracts *viz*; Garlic, Neem, Datura, Calotropis, Turmeric and Asafoetida on the mycelial growth of grain discoloration causing predominant pathogen *C. lunata*, poisoned food technique was used. Observations on radial growth were recorded after control plate fully occupied by pathogen. Percent inhibition over control was calculated and results were presented in the Table 1, Figure 1a to 1c.

While, Garlic extract was highly effective in inhibiting the mycelia growth of the pathogen. All the four concentration (2, 3, 4 and 5 per cent) of garlic extract showed significantly complete inhibition (100%) in the mycelial growth of the pathogen.

However, neem leaf extract at 15 and 20 per cent concentration showed complete inhibition (100%) in the mycelial growth and at 5 and 10 per cent concentrations it was showed 63.77and 83.33 per cent inhibition respectively.

Whereas, Datura leaf extract showed 28.89 and 40.74 per cent inhibition at 5 per cent and 10 per cent respectively. At 15 and 20 per cent it showed 50.37 and 60.74 per cent inhibition respectively.

While, Turmeric exhibits significantly higher inhibition (90.74%) at 20 per cent followed by 84.07 per cent at 15 per cent and 72.96 per cent at 10 per cent. Lowest inhibition per cent (61.11%) was observed at 5 per cent.

However, Calotropis showed significantly higher inhibition (57.41%) at 20 per cent and has efficacy was decreased with decrease in concentration i.e., 36.30 per cent at 15 per cent and 24.44 per cent at 10 per cent. Lowest inhibition (10.37%) was recorded at 5 per cent.

Whereas, Asafoetida showed the maximum of 42.59 per cent at 20 per cent followed by 35.19 per cent at its 15 per cent concentrations. The per cent inhibition of mycelia radial growth is proportionally to concentration of the plant extract amended. Asafoetida at its 5 and 10 per cent concentrations resulted very less inhibition *i.e.*, 7.04 and 19.63 per cent respectively.

All the concentrations of six plant extracts were significantly differed with control.

Among all the plant extracts tested, Garlic proved to be completely inhibiting the growth of the pathogen at all the four concentrations imposed. Neem at its two higher concentrations

Table 1: Evaluation of bio efficacy of plant extracts against *C. lunata* using poisoned food technique

S. N	Treatments	Concentrations	Radial Growth (cm) **	Per cent Inhibition
1	Garlic	2%	0.00	100.00 (90.00)
		3%	0.00	100.00 (90.00)
		4%	0.00	100.00 (90.00)
		5%	0.00	100.00 (90.00)
2	Neem	5%	3.27	63.70 (52.93)
		10%	1.50	83.33 (65.88)
		15%	0.00	100.00 (90.00)
		20%	0.00	100.00 (90.00)

3	Datura	5%	6.40	28.89 (32.50)
		10%	5.33	40.74 (39.65)
		15%	4.47	50.37 (45.19)
		20%	3.53	60.74 (51.18)
4	Calotropis	5%	8.07	10.37 (18.74)
		10%	6.80	24.44 (29.62)
		15%	5.73	36.30(37.03)
		20%	3.83	57.41 (49.24)
5	Turmeric	5%	3.50	61.11 (51.40)
		10%	2.43	72.96 (58.65)
		15%	1.43	84.07 (66.49)
		20%	0.83	90.74 (72.26)
6	Asafoetida	5%	8.37	7.04 (15.34)
		10%	7.23	19.63 (26.29)
		15%	5.83	35.19 (36.37)
		20%	5.17	42.59 (40.72)
7	Control		9.00	0.00 (0.00)
	C.D(P=0.05)		0.15	1.66
	SEm(±)		0.05	0.58
	SE(d)		0.07	0.83
	C.V(%)		2.45	1.72

*Figures in parenthesis are angular transformed values **values are means of these replications

Table 2: Different plant extracts and their concentrations used

S. No.	Plant Extracts	Concentrations used Percent (%)
1	Garlic	2, 3, 4, 5
2	Neem	5, 10, 15, 20
3	Datura	5, 10, 15, 20
4	Calotropis	5, 10, 15, 20
5	Turmeric	5, 10, 15, 20
6	Asafoetida	5, 10, 15, 20

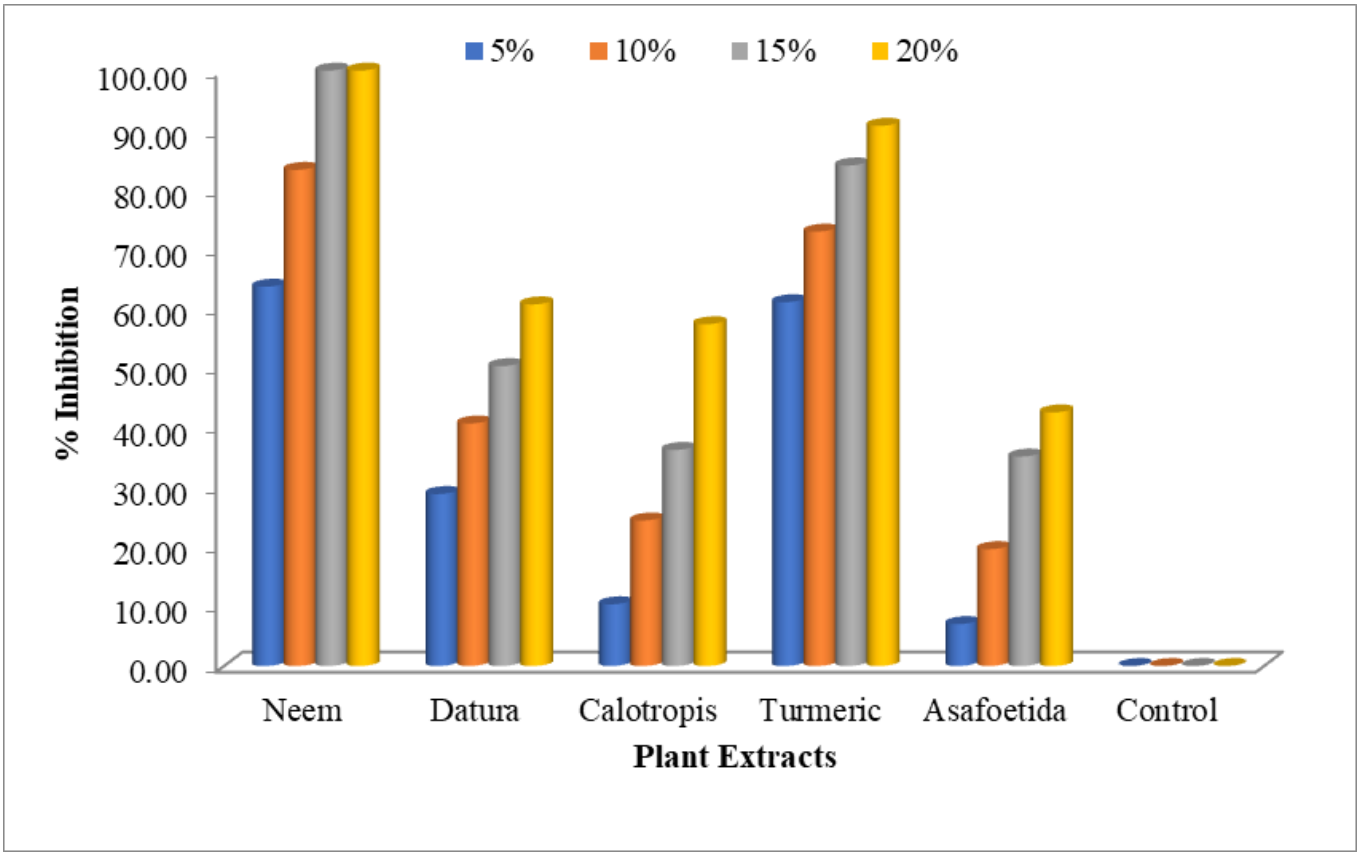

Fig 1a: Evaluation of bio efficacy of plant extracts (Neem, Datura, Calotropis, Turmeric and Asafoetida) against *C. lunata* using poisoned food technique

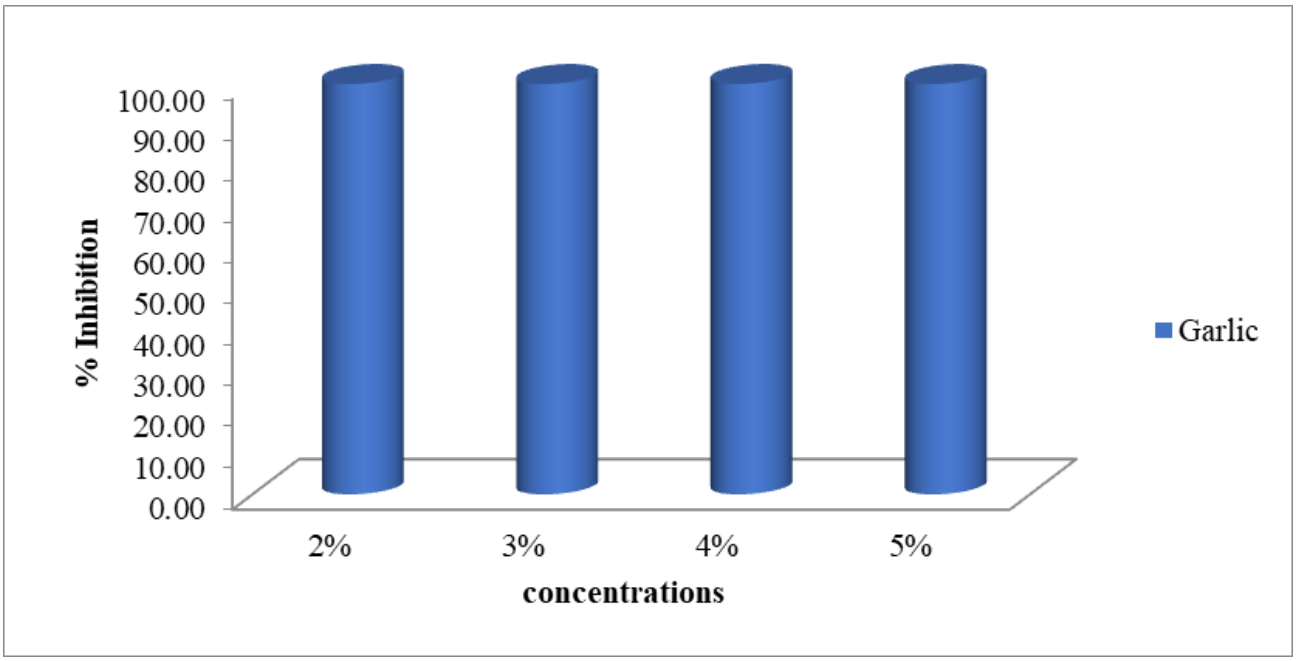

Fig 1b: Evaluation of bio efficacy of plant extracts (Garlic) WG against *C. lunata* using poisoned food technique

showed the complete inhibition. Based on the above results, these two plant extracts were found to be effective and used for further field studies.

These results were in agreement with the work done by Hajano *et al.* (2012) [5] tested the extract of garlic, neem and Calotropis with three different doses against the causal fungus M. oryzae and reported that, garlic extract performed better and significantly inhibited the mycelia growth of *M. oryzae* at its all three concentrations.

Hajano, results also showed that the neem extract failed to suppress the mycelial growth of the test fungus at its lower concentrations. However, the higher concentration (8 ml) of neem extract was highly effective in checking the mycelial growth of *M. oryzae* (20.58 mm). The extract of Calotropis at all concentrations failed to inhibit the colony growth of *M. oryzae* in comparision with garlic and neem.

This study summarised and concluded as follow:

Totally six plant extracts viz; Garlic, Neem, Datura, Calotropis, Turmeric and Asafoetida were evaluated against the mycelial growth of predominantly occured pathogen *C. lunata*. Among all the plant extracts tested, Garlic proved to be completely effective in inhibiting the growth of the pathogen at even very low concentrations i.e., 2, 3, 4 and 5 per cent. Whereas, Neem leaf extract showed completely effective in inhibiting the growth of the pathogen at higher concentrations (15 and 20%) compared with other plant extracts. Based on the above results, garlic clove extract was found to be effective followed by neem leaf extract and used for further field studies.

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