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S Suresh Rao
Department of Plant Pathology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

M Reddi Kumar
Professor, Department of Plant Pathology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

P Madhusudhan
Scientist, Agricultural Research Station, Nellore, Andhra Pradesh, India

B Ravindra Reddy
Project officer, ITDA-Srisailam, Kurnool, Andhra Pradesh, India

Corresponding Author:
S Suresh Rao
Department of Plant Pathology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

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

S Suresh Rao, M Reddi Kumar, P Madhusudhan and B Ravindra Reddy

Abstract
In the present investigation, efforts were made to test the bio-efficacy of different natural preparations *viz;* Cow urine, Cow dung, modified Panchagavya, Panchagavya, Beejamrutha and Jeevamrutha on the mycelial growth of rice grain discoloration causing pathogen *Curvularia lunata* using poisoned food technique. Observations on radial growth were recorded after control plate fully occupied (7 days) by the pathogen. Percent inhibition over control was calculated. Among all the natural preparations, Jeevamrutha was highly effective resulted complete inhibition (100%) at all the concentrations (1%, 2%, 3%, 4%) followed by Modified Panchagavya showed significantly complete inhibition (100%) at 4, 3 and 2 percent concentrations over the mycelial growth of the pathogen compared with other natural preparations and control.

Keywords: Comparative, natural, preparations, mycelial, *Curvularia lunata*

Introduction
Rice (*Oryza sativa* L.) is the primary staple food in many countries. In India it is cultivated in an area of 437.8 L/ha (Lakh hectares) with 118.4 M T (Million Tonnes) of production and 2705 kg ha^{-1} of productivity. In Andhra Pradesh, the area under cultivation of rice is approximately 23.56 L/ha with 13.71 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, Press Information Bureau 2021).
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) [8]. 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) [12].
Management of disease by application of different natural preparations at different stages from seed, transplantation and 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 and natural preparations. Grain discoloration is the complex disease, many fungi responsible for this disease so, Integrated Disease Management strategies including chemicals, plant extracts and natural preparations application is 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) [24] 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) [25]. Therefore, merit attention of all concerned to look into the potential of integrating in the management of economically important diseases, the products prepared from desi cow and green plants should be preferred as they are environmentally non-pollutive and non-hazardous in preparation and use (Tiwari *et al*., 2011) [22].

The secondary components of some natural preparations contain medicinally active fractions that are toxic to pathogens and thus can be utilized in plant disease management programme. The effective control of rice diseases using natural preparations (Patil *et al.*, 2008) [9].

Materials and Methods

In order to study the antifungal effect of certain natural extracts in the management of rice grain discoloration, an experiment was conducted by following poisoned food technique (Nene and Thapliyal, 1993) [7]. Different natural preparations and their concentrations used for the study mentioned in Table 2.

Preparation of Cow urine: Fresh cow urine was brought to the laboratory and filtered through Whatman filter paper No. 1 and finally, in aseptic conditions, it was passed through the bacterial proof filter under vacuum to get rid of bacterial contamination.

Dung extract: Fresh cow dung was collected and brought to the laboratory, to which distilled water was added in 1:1 ratio on weight by volume (W/V) basis. Now the dung extract is passed through the muslin cloth to remove clumps and other wastes, later it was passed through the Whatman filter paper No.1. Now the fine filtered dung extract is vacuum filtered to avoid bacterial contamination.

Jeevamrutha: Jeevamrutha was prepared by using fresh cow dung (2.5 kg), cow urine (2.5 lit), jaggery (0.5 kg), pulse flour (Bengal gram 0.5 kg) and soil (0.25 kg). All the ingredients were mixed in 50 liters of water in a plastic drum and kept for 7 days under shade. The above solution was stirred thrice a day with a long stick.

Bheejamrutha: Fresh cow dung (1kg), cow urine (1 lit), water (4 lit) and lime (100 g) were used for the preparation of beejamrutha. Cow dung tied in a cloth was dipped in a bucket containing 4 litres of water for overnight. Next day morning, the tied dung was frequently squeezed after repeatedly dipping in the water. Later the required quantities of cow urine and lime were added to this extract. The above solution was stirred with a long stick before use.

Panchagavya consists of nine products *viz*; cow dung, cow urine, milk, curd, jaggery, ghee, banana, tender coconut water and water. Cow dung (7 kg), Cow ghee (1 kg) were mixed thoroughly both in morning and evening hours and kept it for 3 days. After 3 days, the cow urine (10 lit) and water (10 lit) were mixed and kept it for 15 days with regular mixing both in morning and evening hours. Later, after 15 days' cow milk (3 lit), cow curd (2 lit), tender coconut water (3 lit), jaggery (3 kg) and well ripened banana (12 nos.) were added to the previous mixture. Finally, panchagavya was ready to use after 30 days of ingredients mixing. All the above mentioned items were added to a wide mouthed mud pot as per the same order. The container was kept open under shade.

In Modified Panchagavya were the ghee content is reduced to half and curd is doubled as aforesaid in panchagavya.

All the prepared natural compounds were filtered through muslin cloth to get the crude extract. Then the crude extract was filtered through Whatman filter paper No. 1 and finally, again filtered through the bacterial proof filter under vacuum to get rid of bacterial contamination. Then the different concentrations of natural preparations were prepared by adding the appropriate quantity of sterilized distilled water.

Poisoned food Technique: The effect of different natural preparations 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) [7].

Different concentrations of natural preparations 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 natural preparations 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±1 ºC. 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 percent inhibition was calculated by following the formula given by Vincent (1927) [23].

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

Where,
I= Percent 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 natural preparations *viz*; Cow urine, Cow dung, Modified panchagavya, Panchagavya, Beejamrutha and Jeevamrutha on the mycelial growth of rice 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 1 to 3.

While, Jeevamrutha was highly effective in inhibiting the mycelia growth of the pathogen. All the concentrations of Jeevamrutha showed significantly complete inhibition (100%) of mycelial growth of the pathogen.

Whereas, modified Panhagavya showed significantly complete inhibition (100%) at 4, 3 and 2 percent. Lowest percent inhibition (83.70%) was recorded at 1 percent.

However, Cow urine showed significantly higher inhibition

(86.67%) at 20 percent concentration and the efficacy was decreased with decrease in concentration *i.e*; at 15, 10, and 5 percent, it showed 80.74, 60.74 and 38.15 percent inhibition respectively.

While, Cow dung exhibited higher inhibition (82.56%) at 20 % concentration and the efficacy was decreased with decrease in concentration *i.e*; at 15, 10 and 5 percent, it showed 71.48, 61.11 and 46.67 percent inhibition respectively.

Whereas, Beejamrutha showed significantly higher inhibition (67.41%) at 7 percent concentration and the efficacy was decreased with decrease in concentration *i.e*; at 5, 3 and 1 percent, it exhibited 50.37, 29.63 and 20.74 percent respectively.

Among all the natural preparations tested, Jeevamrutha proved to be completely effective in inhibiting the growth of the pathogen at all the four concentrations imposed followed by Modified panchagavya completely effective at 4, 3 and 2 percent concentrations. Based on the above results, are there two natural preparations were found to be effective and used for further field studies.

All the concentrations of six natural preparations were significantly differed with control.

Among all the natural preparations tested, Jeevamrutha proved to be completely inhibiting the growth of the pathogen at all the four concentrations imposed. Modified panchagavya at its three higher concentrations showed the complete inhibition. Based on the above results, these two natural preparations were found to be effective and used for further field studies.

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

S. No	Treatments	Concentrations	Radial Growth cm	% Inhibition
1.	Cow Urine	5%	5.57	38.15 (38.13)
		10%	3.53	60.74 (51.18)
		15%	1.73	80.74 (63.96)
		20%	1.20	86.67 (68.56)
2.	Cow Dung	5%	4.80	46.67 (43.07)
		10%	3.50	61.11 (51.40)
		15%	2.57	71.48 (57.70)
		20%	1.57	82.59 (65.32)
3.	Panchagavya	1%	2.37	73.70 (59.13)
		2%	1.43	84.07 (66.49)
		3%	0.00	100.00 (90.00)
		4%	0.00	100.00 (90.00)
4.	Modified Panchagavya	1%	1.47	83.70 (66.17)
		2%	0.00	100.00 (90.00)
		3%	0.00	100.00 (90.00)
		4%	0.00	100.00 (90.00)
5.	Jeevamrutha	1%	0.00	100.00 (90.00)
		2%	0.00	100.00 (90.00)
		3%	0.00	100.00 (90.00)
		4%	0.00	100.00 (90.00)
6.	Beejamrutha	1%	7.13	20.74 (27.06)
		3%	6.33	29.63 (32.96)
		5%	4.47	50.37 (45.19)
		7%	2.93	67.41 (55.17)
	Control		9.00	0.00 (0.00)
	C.D P=O.OS		0.17	1.90
	S.Em ±		0.06	0.67
	SE d		0.09	0.94
	C.V %		4.36	1.57

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

Table 2: Different natural preparations and their concentrations used.

S. No.	Natural compounds	Concentrations used Percent (%)
1	Cow Urine	5, 10, 15, 20
2	Cow Dung	5, 10, 15, 20
3	Panchagavya	1, 2, 3, 4
4	Modified Panchagavya	1, 2, 3, 4
5	Jeevamrutha	1, 2, 3, 4
6	Beejamrutha	1, 3, 5, 7

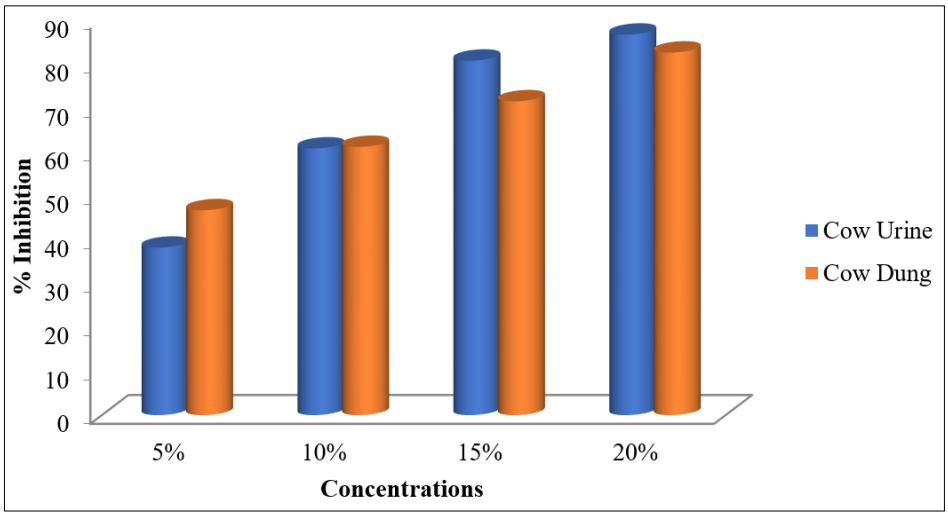

Fig 1: Evaluation of bio efficacy of natural preparations (Cow urine and Cow dung) against *C. lunata* using poisoned food technique

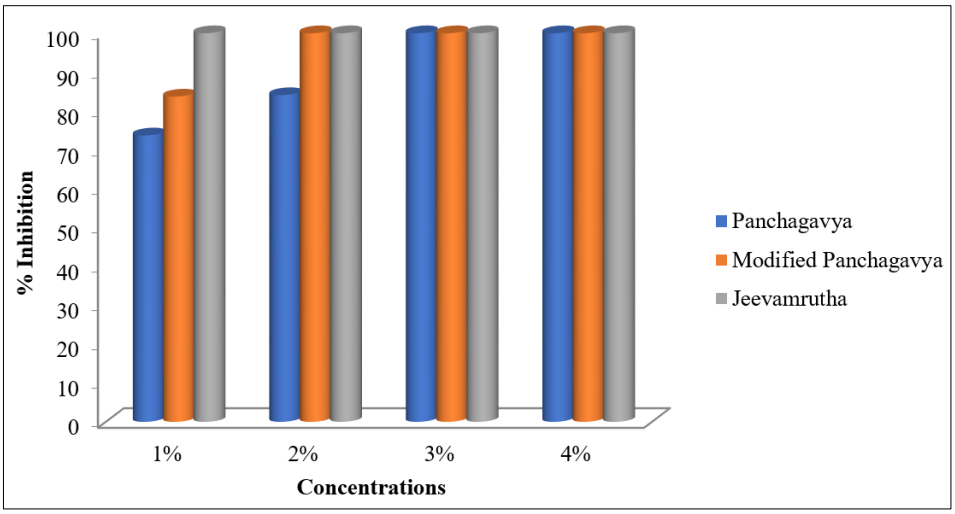

Fig 2: Evaluation of bio efficacy of natural preparations (Panchagavya, Modified Panchagavya and Jeevamrutha) against *C. lunata* using poisoned food technique

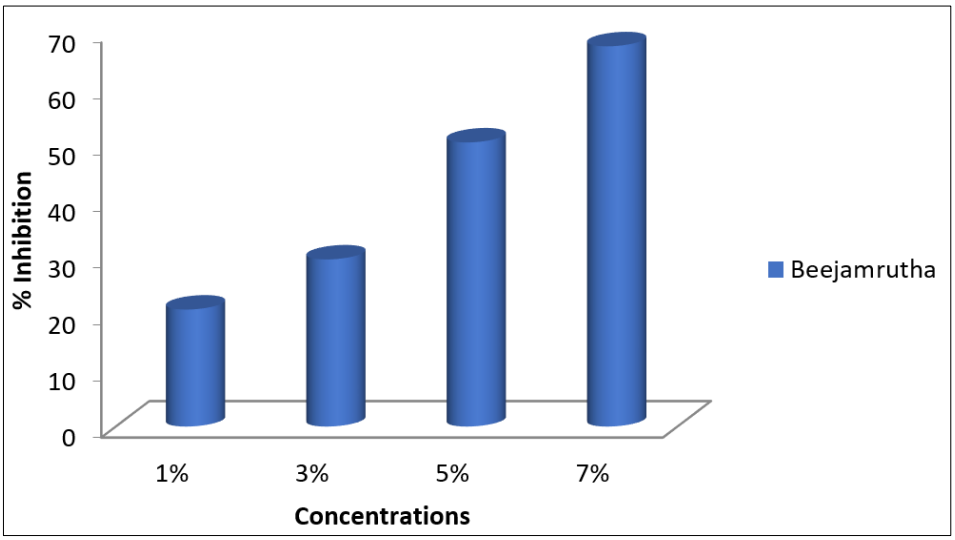

Fig 3: Evaluation of bio efficacy of natural preparations (Beejamrutha) against *C. lunata* using poisoned food technique

This study summarized and concluded as follow

Totally six natural compounds *viz*; Cow urine, Cow dung, Modified panchagavya, Panchagavya, Beejamrutha and Jeevamrutha evaluated against the mycelial growth of predominantly occured pathogen *C. lunata*. Among all the natural preparations tested, Jeevamrutha proved to be completely effective in inhibiting the mycelial growth at low concentrations *i.e.*, 3 and 4 percent followed by Modified

panchagavya completely effective in inhibiting the mycelial growth at low concentration (4%). Based on the above results, these two natural preparations were found to be effective and used for further field studies.

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