Investigation of bio fungicidal properties of crude extracts of neem leaf against blast disease of Fingermillet(*Eleusine coracana*)

ABSTRACT

The efficacy of neem leaf extract against fingermillet blast pathogen *Magnaporthe grisea* was assessed *invitro*. The neem leaf extract significantly inhibits the pathogen mycelial growth and melanin biosynthesis. Neem leaf extract shows 84.33 % inhibition of the pathogen over control. Volatile test infer that no volatile compounds were found to be involved in growth inhibition and melanin production inhibition.In field experiments foliar spray with neem leaf extract 5%,10% and 15% along with chemical Tricyclazole 0.1% and Picoxystrobin 6.78% + Tricyclazole 0.1 % was done. Data recorded on leaf blast PDI at 15, 25 and 40 days after transplanting revealed that lower leaf blast PDI of 17.72, 16.45 and 15.45 respectively was recorded in neem leaf extract(T1,T2,T3) which is comparatively similar with chemical treatment 1 and 2 PDI 13.00 and 10.90. In neem leaf extract treated field a significant yield increase was also observed.

Key words: Fingermillet, blast, neem leaf extract,botanicals.

1. INTRODUCTION

Finger millet (*Eleusine coracana*) is one of the important millet crops of India. Finger millet plays an important role in the dietary needs as well as incomes of many rural households. It accounts for about 12 per cent of the global millet area(reference). It is the most important small millet in the tropics and is cultivated in many states in general. It is considered as poor man's crop since it is often grown under low-input systems with near-optimum yield levels. Besides having capacity to withstand biotic and abiotic stresses. Under vulnerable conditions it is plagued by some of the diseases such as blast, blight, foot rot, smut, rust and mosaic. Of the several diseases that affect finger millet, blast is not only wide distributed in almost all the finger millet growing regions of the world, but also it is the most destructive disease. The fungus attacks different plant parts like leaf, neck and fingers, and the disease is accordingly termed as leaf blast, neck blast, and finger blast. Use of resistant varieties, alone may not form a durable, long-lasting solution in mitigating disease [1].

Most of the farmers in our country are highly depending on the chemical pesticides and fungicides to control the pest and disease of millet crops. Harmful effect of these chemicals brings the emergency call for ecofriendly and hazard free sustainable agriculture. The integration of scientific and traditional knowledge would help to develop technologies which are need based, better problem solving, locally available, easily acceptable, cost effective, convincing and credible to the agricultural farmers. Recent scenario has aroused interest in indigenous technical knowledge (ITK). These ITKs are based on experiences which gathered momentum through generations and are being developed and improved through informal experimentation. Indigenous technology is used by the native inhabitants of a country or region and it constitutes an important part of its cultural heritage.In Trichy district of Tamilnaduapplication of neem leaf extract at the level of 5%, 10% and 15% is practiced by farmers and had excellent control of fingermillet blast in field condition. Several studies have reported the importance of organic approaches for improving the yield and management of diseases. The present study was to scientifically analyze the mode of action of neem leaf extract against fingermillet blast pathogen [2].

2.MATERIALS AND METHODS

2.1. Isolation of Pathogen

Finger millet leaves showing typical symptoms of blast were collected from the field. Leaves with typical blast symptoms were first washed in tap water and then cut into small bits of 2 mm size, containing the blast lesion along with a portion of healthy tissue surrounding the lesion. These bits were surface sterilized with 0.1 per cent sodium hypochlorite solution for two min followed by three changes of sterilized distilled water. These bits were placed on RYLA medium (Ragi yeast lactose agar medium) under aseptic conditions and incubated at 27 °C and watched for the growth of colony. After fourteen days of incubation, a small loop of fungal culture from the colonies was picked and put on a clean slide containing a drop of lacto phenol. The slide was observed under low and high power objectives for the presence of conidia.

2.2. Ragi Yeast Lactose Agar medium

For culturing the isolate of *Magnaporthe grisea* in all the laboratory experimental studies, standard Ragi Yeast Lactose Agar (RYLA) medium was used. The composition of RYLA used is given below.

Ground ragipowder	20 g
Yeast	1 g
Lactose	5 g
Agar-Agar	20 g
Distilled water	1000 ml (volume to make up)

Twenty grams of ground ragi powder was boiled in water. The extract obtained was filtered using muslin cloth. Twenty grams of agar, one gram of yeast and five grams of lactose were dissolved in extract and the final volume was made up to 1000 ml by adding distilled water. The required quantity of the prepared medium was dispensed into number of conical flasks, plugged with non-absorbent cotton and wrapped with paper. The flasks containing the medium were sterilized using autoclave at 1.1 kg cm $^{-2}$ pressure for 15 min.

2.3. Poisoned Food Technique

The efficacy of the neem leaf extract on *Magnaporthe grisea* was studied by poison food technique. Known quantity of neem leaf extract added to RYLA medium to get a known concentration of 5%. Then it was poured in a sterile petri plate under aseptic condition and solidified. After solidification mycelial disc of 9mm actively growing culture of *Magnaporthe grisea* was aseptically placed onto the medium at the center of the plate which serves as treatment and the RYLA medium with the test fungus alone acts as a control. Chemical control 1 – 0.1% Tricyclazole and Chemical control 2 is 6.78% Picoxystrobin + 0.1%Tricyclazole.The plates were incubated at room temperature for 48 hrs. The percent inhibition was measured using the formula,

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Where, Dc = Average diameter of fungal growth (cm) in control
Dt = Average diameter of fungal growth (cm) in treatment

2.4. Volatile test

The efficacy of volatiles of neem leaf, fruit, seed and fruit pericarp on *Magnaporthe grisea* was studied by partitioned pertiplate technique. Known quantity of neem leaf, fruit, seed and fruit pericarp was placed on one half of the plate. On another side RYLA medium was added under aseptic condition and solidified. After solidification mycelial disc of 9mm actively growing culture of *Magnaporthe grisea* was aseptically placed onto the medium at the center of the plate which serves as treatment and the RYLA medium with the test fungus alone acts as a control. Then the plate is incubated under 27°C for three days and the observations were taken.

2.5. Evaluation of efficacy of neem leaf extract against blast of fingermillet under field conditions

Field experiments were conducted in *Kharif* 2019 and 2020 at Agricultural college and research institute, Eachangkottai. The experiment was conducted in randomized block design with 6 treatments,three replications were maintained for each treatment. Foliar spray was done as per the treatment schedule with three concentration 5%,10% and 15% i.e. Three sprays of neem leaf extract at 15 days interval starting from 15 days after transplanting against leaf blast and two sprays (once at 50 % flowering followed by second at 15 days later) for neck and finger blast management. Foliar spray with the chemical Tricyclazole 0.1% (T4 chemical check 1) and Picoxystrobin 6.78% + Tricyclazole 0.1 % (T5 chemical check 2) was also used as check for comparison. All normal agronomical practices were followed at regular intervals. Observations were made regularly for the appearance and development of symptoms. The disease incidence was assessed at 15 days interval. In addition, yield parameter like grain yield and BC ratio from each treatment were also recorded.

$$\text{Percent disease index} = \frac{\text{sum of individual rating} \times 100}{\text{Number of leaves observed} \times \text{max grade (9)}}$$

3. RESULT AND DISCUSSION

The efficacy of neem leaf extract against fingermillet blast pathogen *Magnaporthe grisea* was assessed by poisoned food technique. The neem leaf extract significantly inhibits the pathogen as shown in table 1. Neem leaf extract 5% shows 84.33 % inhibition against the pathogen over control. With respect to Chemical controls Tricyclazole 0.1 % (chemical control 1) has exhibited 88.88 % inhibition over control. Similarly Picoxystrobin 6.78% + Tricyclazole 0.1 % (chemical control 2) has exhibited 100 % inhibition (Fig 1 and Fig. 2). In case of neem leaf extract treated pathogen apart from mycelial growth reduction, melanin synthesis inhibition was also observed.

Table 1. Efficacy of neem leaf extract against ragi blast pathogen *Magnoporthe grisea invitro*

S.No	Treatment	Particulars	Finger millet blast incidence		
			Mycelial growth in (mm)	% zone inhibition	Sporulation x10^2/ml
1	T_1	Neem15%	13.30	85.22	6.98
2	T_2	Neem10%	13.50	85.00	7.25
3	T_3	Neem 5%	14.10	84.33	8.55
4	T_4	Tricyclazole 0.1%	10.00	88.88	0.00
5	T_5	Picoxystrobin 6.78% + Tricyclazole 0.1 %	0.00	100.00	0.00
6	T_6	Control	90.00		124
		CD (P=0.05)	0.28		0.24

Average of three replication

Fig 1. Efficacy of neem leaf extract against fingermillet blast pathogen *Magnaporthe grisea*

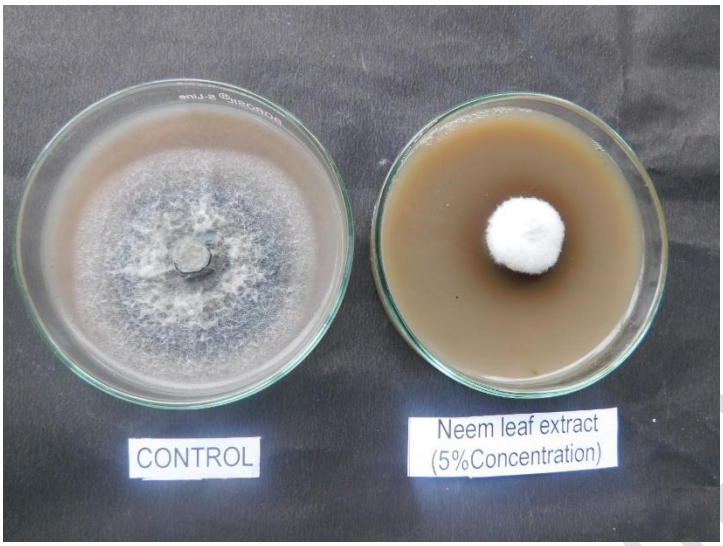
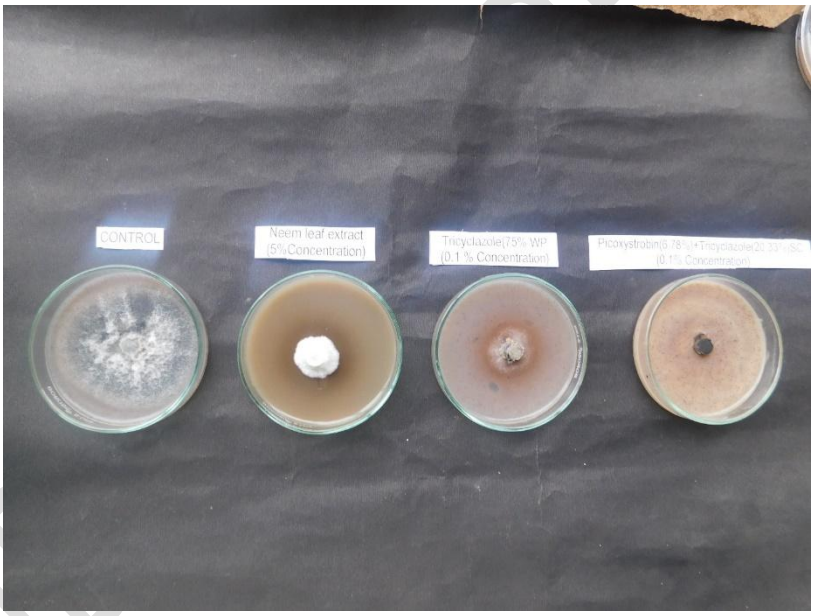
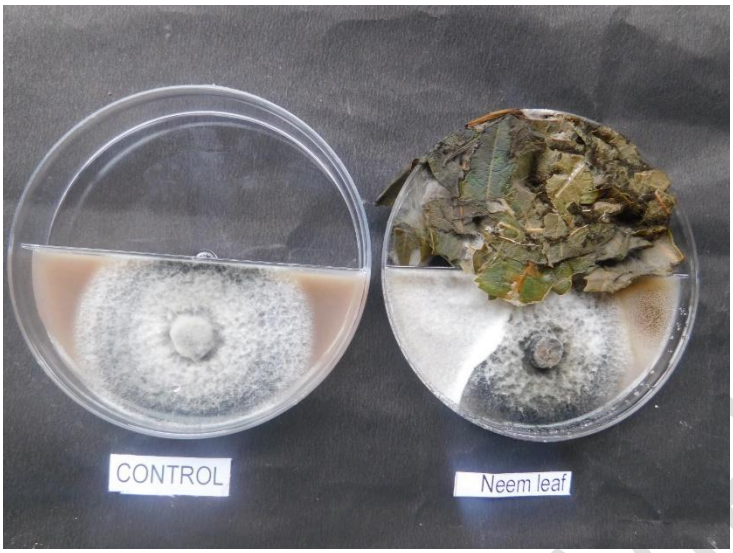


Fig 2. Efficacy of neem leaf extract and fungicides against fingermillet blast pathogen *Magnaporthe grisea*



In volatile test the partitioned petri plates containing neem leaves, fruits, seeds and pericarp resemble same as that of control there was no change in color or growth of the pathogen. Hence, from this we infer that no volatile compounds were found to be involved in growth reduction and melanin production inhibition. (Fig 3.)

Fig 3. Efficacy of neem leaves volatiles against fingermillet blast pathogen *Magnaporthe grisea*



3.1. Evaluation of efficacy of neem leaf extract against blast of fingermillet under field conditions

Field evaluation of neem leaf extractwith three concentration 5%,10% and 15%along with Tricyclazole 0.1% (T4 chemical check 1) and Picoxystrobin 6.78% + Tricyclazole 0.1 % (T5 chemical check 2) were carried out. Significant reductionin finger millet blast was recorded and presented in Table 2 and Fig 1.

Table 2. Efficacy of neem leaf extract against ragi blast pathogen *Magnoporthe grisea* in field.

S. No	Treatment	Particulars	Finger millet blast PDI %			Percent disease control
			2019	2020	Mean	
1	T_1	Neem15%	15.70 (23.34)	15.20 (22.94)	15.45	72.65
2	T_2	Neem10%	16.20 (23.73)	16.70 (24.12)	16.45	70.88
3	T_3	Neem 5%	16.72 (24.13)	18.72 (25.63)	17.72	68.63
4	T_4	Tricyclazole 0.1%	12.50 (20.70)	13.50 (21.55)	13.00	76.99
5	T_5	Picoxystrobin 6.78% + Tricyclazole 0.1 %	10.50 (18.90)	11.30 (19.64)	10.90	80.70
6	T_6	Control	54.00 (47.29)	59.00 (50.18)	56.50	
		CD (P=0.05)	0.29	0.28		

Data in parenthesis shows Arc sine percentage transformation. Average of three replication

Table 3. Efficacy of neem leaf extract on grain yield and cost benefit ratio of finger milletin field during 2019-2020.

S.No	Treatment	Particulars	Grain yield q/ha	B:C ratio	Mean

			2019	2020	Mean	2019	2020	
1	T_1	Neem15%	19.72	19.50	19.61	1.23	1.21	1.22
2	T_2	Neem10%	19.43	19.23	19.33	1.21	1.20	1.21
3	T_3	Neem 5%	19.31	19.15	19.23	1.20	1.19	1.20
4	T_4	Tricyclazole 0.1%	20.80	20.50	20.65	1.30	1.28	1.29
5	T_5	Picoxystrobin 6.78% + Tricyclazole 0.1 %	23.20	22.80	23.00	1.45	1.42	1.44
6	T_6	Control	13.65	13.20	13.42	0.66	0.65	0.66
	CD (P=0.05)		0.35	0.32				

Data recorded on leaf blast PDI at 15, 25 and 40 days after transplanting (DAT) revealed that lower leaf blast PDI of 17.72, 16.45 and 15.45 respectively. was recorded inT1,T2,T3, which is comparatively similar with chemical treatment 1 and 2 PDI 13.00 and 10.90 respectively. whereas in untreated check leaf blast PDI was 56.50%. The maximum grain yield per hectare was recorded in the treatment T523.00q/ha followed by T4 20.65 q/ha. In neem leaf extract treated field on an average of 19 kg/ha was recorded (Fig .2).

Melanin is produced by many organisms, including pathogenic fungi, where it can play a role as an important virulence factor. The appressorium is a highly pigmented and thick-walled structure formed by *M. oryzae* during infection. It is a site of intense melanin production and an important intermediate organ in the host infected by *M*. *oryzae*[1]. Fungal melanin with versatile functions is frequently associated with pathogenicity and stress tolerance. In rice blast fungus, *Magnaporthe oryzae*, DHN melanin is essential to maintain the integrity of the infectious structure, appressoria. These results suggest that the neem leaf extract treatment inhibits melanin synthesis, thereby reducing the ability of *M. grisea* to infect fingermillet plant.Our results are in accordance with previous findings by [2,3,4]. In fungi, normal synthesis of melanin favors increased fungal tolerance to abiotic and biological environmental stresses, including radiation, extreme temperatures, dryness, metal toxicity, and host immune responses.

In vitro screening of plant extracts/ products and a synthetic product *i.e.* garlic oil, neem oil, neem leaf, parthenium leaf, turmeric rhizome and garlic bulb extracts were effective in inhibiting spore germination and growth of *P. grisea*[5]. Thecrude extracts obtained from leaves of all the test plants possessed antifungal principles for the growth of *P. grisea* . The crude extracts of *Impatiens balsamina* and *Tagetes erecta* recorded 85.64 and 83.82% reduction in the dry weight of mycelium respectively of *P. grisea* over untreated control [6]. The extent of antifungal effect noted in the crude aqueous preparation of leaf of other plants in terms of percent reduction over control were *Solanum nigrum* (79.04%), *Allium satiivum* (76.54%), *Ricinus communis* (75.40%), *Ocimum sanctum* (73.80%), *Nerium indicum* (74.44%) and *Azadirachta indica* (69.02%). All the extracts showed the antisporulant action and mycelial growth reduction over control against the test fungus [7,8,9,10,11].

4. CONCLUSION

Neem leaf extract shows 84.33 % inhibition of the pathogen over control. Volatile test infer that no volatile compounds were found to be involved in growth inhibition and melanin production inhibition. In field experiments foliar spray with neem leaf extract 5%,10% and 15% along with chemical Tricyclazole 0.1% and Picoxystrobin 6.78% + Tricyclazole 0.1 % was done. Data recorded on leaf blast PDI at 15, 25 and 40 days after transplanting revealed that lower leaf blast PDI of 17.72, 16.45 and 15.45 respectively was recorded in neem leaf extract(T1,T2,T3) which is comparatively similar with chemical treatment 1 and 2 PDI 13.00 and 10.90. In neem leaf extract treated field a significant yield increase was also observed. Through this study it was indicated thatnatural neem products which are environmentally safe and it can be used as an alternative to chemicals formanagement of blast disease in Fingermillet.

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