

AMALAB-E, A FORMULATED BOTANICAL PRODUCT POTENTIAL AGAINST RICE BLAST INCITANT *PYRICULARIA GRISEA*

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ABSTRACT

Formulated organic product was developed by combining a formulating agent (coded A+) with ethanolic extract of *Aegle marmelos* and screened against *Pyricularia grisea*, causing blast disease of rice. The bioassay test conducted through standard conidial germination exhibited MIC of *A. marmelos* extract at 0.1% and mycelial growth at 1%, whereas, the combined formulated Product registered MIC at 0.01% and at 1% respectively in that order. Thus, formulated product improved the efficacy of this botanical product which possesses the potential to be utilized for the management of one of the most destructive diseases of rice blast.

INTRODUCTION

Rice is one of the staple food crops and grown in about 11% of the total cultivated land area globally. It serves as the major food for nearly half of world's population. However the crop is attacked by one of the most destructive diseases called blast incited by *Pyricularia grisea* Sacc that reduces the yield significantly when it occurs in countries where rice is grown. Synthetic fungicides in the past served as an effective protectant, but hazards, associated with it innately, increasing cost and non availability of appropriate quality and the quantity at the time of need to the end users, has created many fold problems in its manufacturing, use and after use to our environment and non-targetted organism including the human beings. The products prepared from green plants constitute a suitable alternative to synthetic agrochemicals (Lapis and Dumancas, 1979; Tewari, 1986; Tewari, 1995; Singh, 1997; Amadioha, 2000; Kamalkannan *et al.*, 2001; Tewari and Mishra, 2003; Okigbo and Emoghene, 2004; Paraskeva *et al.*, 2008; Satish *et al.*, 2010). These products are environmentally non-pollutive and non hazardous and being organic in origin decompose easily thus have no residual effect. Nevertheless, there exist a wide scarcity of formulated botanical products as fungitoxicant especially against this serious fungal diseases. It was therefore decided to develop a suitable formulated botanical product so as to enable its feasibility for practical purpose. The present research report therefore describes about the efficacy of a botanical (*A. marmelos*) extract with a selected formulating agent a surfactant, coded A+, and examines its suitability against the test pathogen *P. grisea*, the incitant of rice blast.

MATERIALS AND METHODS

Preparation of ethanolic extract

Fresh leaves of *Aegle marmelos* Corr. were collected, washed in sterilized distilled water and oven dried at $45 \pm 2^\circ\text{C}$ to get 1kg dried powder. These powder was added to 95% ethanol (1:5w/v) in separate flasks closed with rubber stopper and incubated on a shaker over night at room temp. The extract was then collected in a round bottom rotary-vacuum flash-evaporator flask and excess of ethanol was evaporated at reduced pressure by flash-evaporator. Extract thus recovered in syrup form weighing 130g and was treated as 100% ethanolic mother extract (EME). This extract was then diluted from 100% to, 10%, 1%, 0.1%, 0.01%, 0.001%, 0.0001%, and 0.00001% and utilized for further studies.

Preparation of formulated product

The formulating agent, (a surfactant coded A+) was similarly diluted from 100% to, 10%, 1%, 0.1%, 0.01%, 0.001%, 0.0001%, and 0.00001% and each of these dilutions were combined with serially diluted EME(1:1v/v) to be treated as Amalab-e and utilized for further studies.

Isolation of rice blast incitant, *P. grisea*

Actively growing fresh spindle shaped leaf lesions of rice blast having brown margins and ashy grey centres, were collected from a susceptible variety Lalat, cut into small pieces, surface sterilized in 0.1% sodium hypo chloride solution for 30 seconds, washed thoroughly with sterile distilled water thrice and dried on sterilized blotting paper before transferring it to previously prepared Oat meal agar medium (Oat meal-30g; Agar-Agar-20g; Biotin and Thiamine in traces; Distilled water-

1L; Padmanabhan *et al.*, 1967) aseptically in petriplate. The *P. grisea* isolate thus obtained was confirmed through Kotch's postulate, purified by single spore isolation and maintained on OMA slants. These slants were incubated for seven days at 24°C, and stored at 4°C for further studies.

Bioassay test

Conidial germination test

Aliquots, 0.1mL from each concentrations viz., 100%, 10%, 1%, 0.1%, 0.01%, 0.001%, 0.0001% and 0.00001% of Amalab-e, the formulated product was pipetted out on to cavity slides separately and evaporated to dryness. Conidial suspension of 7day old pure culture of the test pathogen *P. grisea* with 30-35 conidia per microscopic field (under low power magnification) were placed separately on each glass slide with equal quantity and incubated in moist chamber at 24°C for 24h. Observations on conidial germination (%) and the pattern of fungitoxicity were recorded after 24h of incubation. Appropriate controls were maintained keeping three replications in each case and the experiment was repeated thrice. Data on germination was transformed to angular value and statistically analyzed.

Poisoned food technique

Amalab-e, the formulated product was combined with melted OMA media separately so as to get the final concentration of 10%, 5%, 1%, 0.1%, 0.01%, 0.001%. The extract mixed media were poured into the petriplates aseptically and left for 24h to check contaminations if any. Actively growing mycelia of *P. grisea* was cut with a sterile cork-borer and inoculated separately in the center of each such petriplates aseptically. All such plates were incubated at $28 \pm 2^\circ\text{C}$ for seven days. Appropriate controls were maintained keeping three replications in each case and the experiment was repeated thrice. The mycelial growth (cm) was observed and recorded when it grew to periphery in control petriplates and was computed through $3.14 \times r^2$ methods (Tewari and Shukla, 1990). No mycelial growth was accorded numerical value 0.1cm, for the purpose of statistical analysis.

Shelf-life effect

Shelf-life effect of Amalab-e, and EME stored at room temperature for 1, 3, 6, 9 and 12 months in a cleaned, sterilized glass vial with air tight stopper. The product was then separately diluted to 1%, 0.1%, 0.01%, 0.001% and 0.0001% concentrations and bioassayed against conidial germination of *P. grisea* in the same way as stated earlier in text. Appropriate control was maintained keeping three replications in each case and the experiment was repeated thrice. Observations on conidial germination (%) and the pattern of fungitoxicity were recorded after 24h of incubation. Data on germination was transformed to angular value and statistically analyzed.

Dose response relationships studies under *in-vivo* condition Green house experiment

Healthy seeds of a blast susceptible rice HR12 were sown in 19cm diameter earthen pots filled with 3 kg sterilized soil mixed with compost. Pots were watered twice daily with tap water and ammonium sulphate was applied after 20days of sowing @1g/pot to accelerate the disease development. Conidial suspension from 7-day old culture of *P. grisea*

(containing 30-35 conidia per microscopic field under low power magnification) prepared as described earlier and spray inoculated on twenty-five-day old seedlings. Freshly prepared EME, formulating agent (A+ coded) and Amalab-e, diluted to 1, 0.1 and 0.01% concentrations in aqueous suspension. These were sprayed thrice each at weekly interval separately on twenty-seven-day old seedlings showing initial blast symptoms. Standard fungicide carbendazim @ 0.1% and sterilized distilled water were sprayed in the same way to serve as standard check and control respectively. The experiment was repeated thrice keeping three replications in each treatment. Observations on disease score was recorded on the fifth day of the last spraying, based on SES 0-9 scale. Data obtained were statistically analyzed.

Field experiment

Seeds of blast susceptible rice cultivar HR12 were sown in lines on raised seed beds. Twenty five days old seedlings were transplanted in a randomized block design @ two seedlings per hill in a 7x2.5m plots with a spacing of 15 x 15cm between hills and rows. Gap filling was done 7 days after transplanting. A gap of 1 m was left all around between plot to plot. The plots were fertilized with N120, P60 and K60 /ha as a basal dose. EME, FA (A+ coded) and Amalab-e (@ 0.1% for spraying was prepared as stated earlier. The extract was sprayed at weekly intervals three times beginning from initial symptom development of blast *i.e.* after 15th day of transplanting. Standard fungicide carbendazim @ 0.1% and sterilized distilled water were sprayed in the same way to serve as standard check and control respectively. All sprayings were carried out during morning hours to avoid scorching heat of the sun. Three replications were maintained for each treatments and the experiment was repeated thrice during the wet seasons of 2008-2010. The leaf area damaged on the top three leaves barring flag leaves in three tillers per plant was recorded 7 days after last spraying in percentage on five plants in each plot randomly leaving the border line all around. Data were statistically analyzed.

Statistical analysis

The data on conidial germination, mycelial growth, disease score and grain yield of FA and botanical have been taken as individual treatment and was statistically analysed after transforming the data to angular values. Hence, there is only one CD provided to compare between the treatment means for all FA and botanical. The treatment mean values have been provided in a tabular form for a better and quick comparison and also to economize space in publication of the paper.

RESULTS

Conidial germination

Amalab-e, showed bursting and complete inhibition (2%) in *P. grisea* conidia at 100% and 0.01% FA concentrations respectively with all tested combination of EME. Control registered normal [98% (81.87) \pm 0.30] conidial germination. There was an inverse relationship recorded on germination increase with decrease in concentrations from 0.001% to 0.00001% of FA when combined with 10-0.01% concentrations of EME (Table 1). Varying degree of deformities

Table 1: Fungitoxic effect of a formulated product Amalab-e against *Pyricularia grisea*

EME/FA Concentration(%)	100	10	1	0.1	0.01	0.001	0.0001	0.00001	Control	Formulating agent (%)
	Conidial germination(%)									
100	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *	2(8.13) *
10	2(8.13) *	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a
1	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a
0.1	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a
0.01	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a
0.001	2(8.13) ^a	2(8.13) ^a	30(33.21) ^b	74(59.34) ^b	91(72.54) ^b	98(81.87)	98(81.87)	98(81.87)	98(81.87)	20(26.56) ^{bg}
0.0001	2(8.13) ^a	35(36.27) ^{bg}	67(54.94) ^{bg}	93(74.66) ^{bg}	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)
0.00001	2(8.13) ^a	50(45) ^b	94(75.82) ^b	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)
Control (EME)	2(8.13) *	2(8.13) ^a	2(8.13) ^a	2(8.13) ^a	30(33.21) ^b	60(50.77) ^b	98(81.87)	98(81.87)	98(81.87)	98(81.87)
Control (Ethanol)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)	98(81.87)

C.D. at p= 0.05 = 0.30 for interaction between individual treatments of EME, FA and formulated botanical product; Data in parentheses represents the transformed angular values; complete inhibition is represented as 2% and normal conidial germination is represented as 98%; *complete bursting; ^acompletely inhibited conidia; ^breduced germ tube; ^ggranulated germ tube

Table 2: Fungitoxic potential of EME, formulating agent (A+ coded) and their combined effect (EME+A+) against mycelial growth (cm²) of *Pyricularia grisea* through poisoned food technique

Extracts	Concentration (%)													
	10		5		1		0.1		0.01		0.001		Control	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A
EME	0.1	0.01	0.1	0.01	0.1	0.01	3.2	8.045	3.9	11.95	4	12.571	4.5	15.910
Formulating agent A+ (in coded)	0.1	0.01	0.1	0.01	0.1	0.01	2.5	4.910	3.7	10.75	4	12.571	4.5	15.910
Combined effect of (EME+A+)	0.1	0.01	0.1	0.01	0.1	0.01	1	0.785	2.2	3.802	3.2	8.045	4.5	15.910

C.D. at p= 0.05 = 0.21 for interaction between individual treatments of EME, FA and Amalab-e; 'D' =diameter(cm), 'A' =Area of mycelia growth (cm²) computed through 3.14 x r² method; complete inhibition is represented by 0.1 cm/0.01cm²

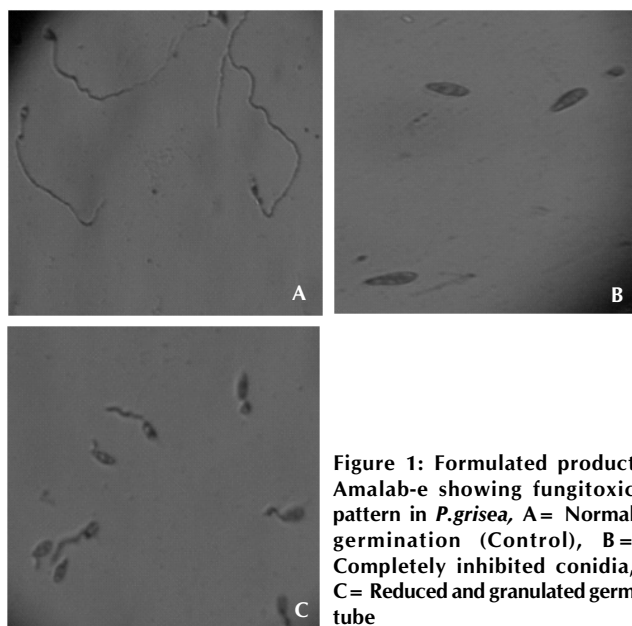


Figure 1: Formulated product Amalab-e showing fungitoxic pattern in *P.grisea*, A= Normal germination (Control), B= Completely inhibited conidia, C= Reduced and granulated germ tube

were observed in all other treatment.

Poisoned food Technique

On combining EME with the corresponding concentrations of FA (A+), there was complete inhibition of mycelia growth (0.1cm) up to 1% concentration against the test pathogen *P.grisea* (Table 2). All the treatments did significantly reduce the mycelial growth compared to control (4.5(15.91) ± 0.21). The combined botanical products displayed either at par or greater fungitoxic effect than EME or FA tested alone.

Shelf-life effect

Amalab-e, upto 0.01% and EME, upto 0.1% concentrations respectively showed complete inhibition (2%) in *P. grisea* till 12 months storage period (Table 3). At 0.01% in EME and 0.001% concentration of formulated product significantly reduced germ tube growth [30%(33.2)-60%(50.7) ± 1.2] in all the storage periods. All the other treatments including EME at 0.001% (for 10 months) registered normal conidial germination and found at par with control [98% (81.87) ± 1.2].

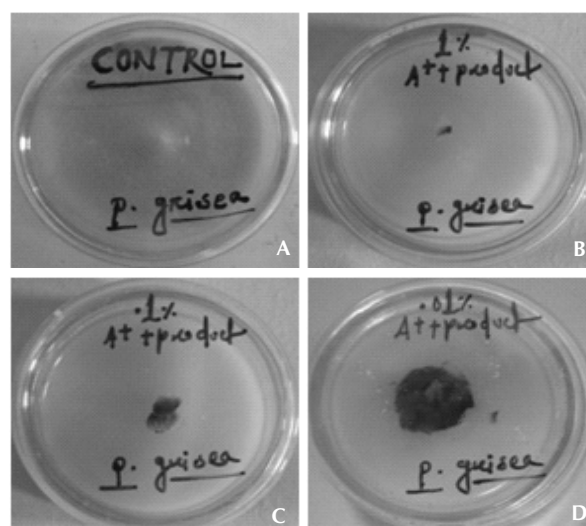


Figure 2: Photograph showing performance of Amalab-e; Plate- A showing *P.grisea* with no mycelial growth inhibition (control), Plate- B, C, D showing formulated product (EME+A+) with three different concentrations

Table 3: Shelf-life effect of a formulated product Amalab-e against *Pyricularia grisea*

Treatments concentration (%)	Storage period(months)		Conidial germination(%)		Fresh concentration	
	Amalab-e	EME	Amalab-e	EME	Amalab-e	EME
1	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a
0.1	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a	2(8.1) ^a
0.01	2(8.1) ^a	30(33.2) ^{bg}	2(8.1) ^a	36(36.8) ^{bg}	2(8.1) ^a	40(39.2) ^{bg}
0.001	30(33.2) ^{bg}	60(50.7) ^{bg}	40(39.2) ^{bg}	60(50.7) ^b	40(39.2) ^{bg}	60(50.7) ^b
0.0001	98(81.8)	98(81.8)	98(81.8)	98(81.8)	98(81.8)	98(81.8)
Control (Ethanol)	98(81.8)	98(81.8)	98(81.8)	98(81.8)	98(81.8)	98(81.8)

C.D. at p = 0.05 = 1.2 for interaction between individual treatments of EME, Amalab-e; Data in parentheses represents the transformed angular values; complete inhibition is represented as 2% and normal conidial germination is represented as 98%; ^a = completely inhibited conidia, ^b = reduced germ tube, ^g = granulated germ tube

Dose response relationships studies under *in-vivo* condition

Green house experiment
EME, FA and Amalab-e were sprayed at 0.01, 0.1 and 1% concentrations and compared with standard fungicide carbendazim at recommended dose to check the spread of foliar blast disease of rice in green house on a blast susceptible variety HR12 (Yr. 2008-2010). All the treatments did significantly reduce the disease compared to control (6-6.8 ± 1.2). Amongst the extracts, Amalab-e displayed least foliar blast (0.5-0.6) at 1.0% concentration in the year 2010. Amalab-e was found to reduce the disease significantly at par with carbendazim (0.9-1.0 ± 1.2) at 0.1% concentration and maximum disease score was recorded in EME and found significantly at par with FA treatment (2.7-2.6 ± 1.2) at 0.01% concentrations in three year (Table 4). Disease score in control ranged 6-6.8 on 0-9scale.

Field experiment

Effect of the formulated product, Amalab-e, EME and FA (coded A+) sprayed at 0.1% concentration were evaluated for the control of rice blast disease in field on a blast susceptible variety HR12 (Yr. 2008-2010). All the treatments significantly reduced foliar blast compared to control (77-80% ± 2.46). Independently in all the three years, Amalab-e reduced the disease (11-13% ± 2.46) which was comparable with a standard fungicide

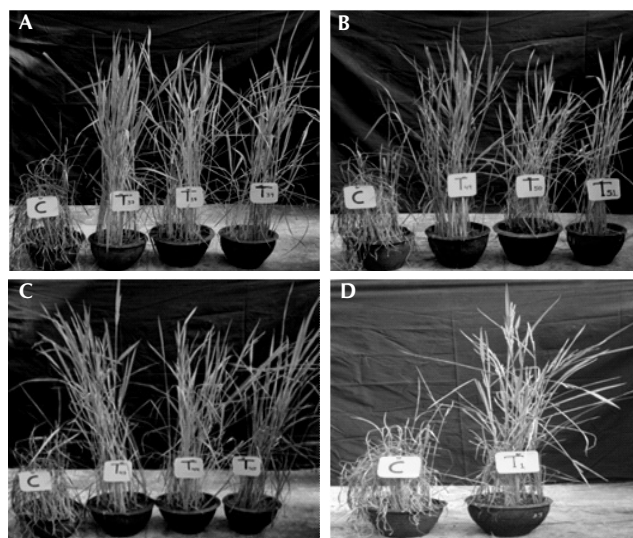


Figure 3: Showing prevention of rice blast in green house in A = Ethanolic mother extract (T37, T38 and T39); B= Formulating agent A+ (T49, T50 and T51); C= Amalab-e (T43, T44 and T45) at 1, 0.1 and 0.01 percentage concentrations respectively in that order, in each set under green house test compared with a standard fungicide Carbendazim; D= at 0.1%(T1) and C= represents control in each case

carbendazim (11-12% ± 2.46) at 0.1% concentration but the disease percentage was significantly higher in FA(A+) (30-33% ± 2.46) followed by EME (22-26% ± 2.46) in three year, highest yield was reported in Amalab-e (1400-14500Kg/ha ± 2.46) followed by Carbendazim (1300-1330Kg/ha ± 2.46). Untreated check produced lowest yield in the range (575-620 Kg/ha ± 2.46), in three years (Table 5).

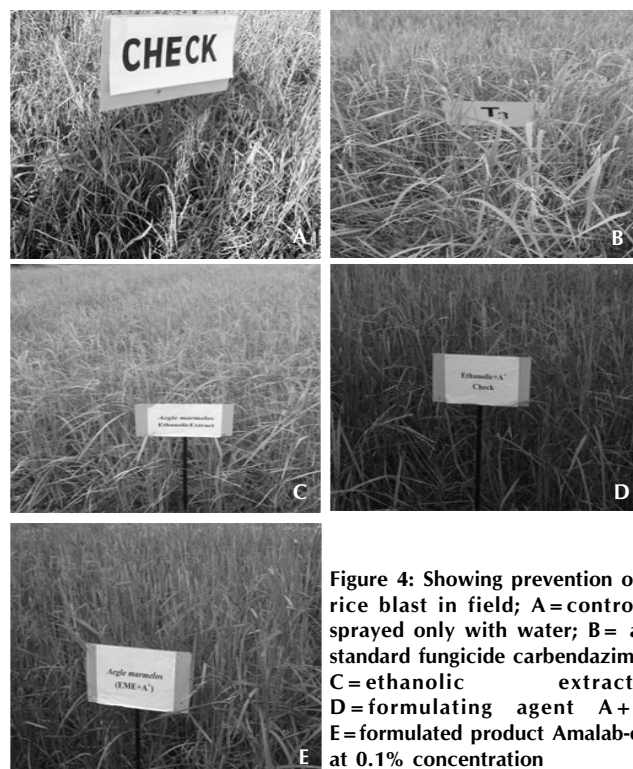


Figure 4: Showing prevention of rice blast in field; A=control sprayed only with water; B= a standard fungicide carbendazim; C= ethanolic extract; D= formulating agent A+; E= formulated product Amalab-e at 0.1% concentration

Table 4: Dose response relationships of EME, FA and Amalab-e extracts on reduction of rice blast (HR12) in green house from 2008 to 2010

Foliar blast score(0-9Scale)	2008				2009				2010				
	Extract/ Concn	EME	FA	Amalab-e	Carbendazim	EME	FA	Amalab-e	Carbendazim	EME	FA	Amalab-e	Carbendazim
0.01		2.7	2.5	1.3	-	2.7	2.6	1.3	-	2.6	2.5	1.2	-
0.1		1.5	1.6	0.9	0.9	1.4	1.6	0.7	1.0	1.4	1.6	0.9	1.0
1		1.3	1.2	0.6	-	1.1	1.3	0.6	-	1.0	1.2	0.5	-
Control		6	6	6	6	6.5	6.5	6.5	6.5	6.8	6.8	6.8	6.8

C.D. at $p = 0.05 = 1.34$; *For EME control, an equal amount of ethanol was added and diluted as in any other treatment and all the other treatment in control were sprayed only with water

Table 5: Fungitoxic performance of Amalab-e on rice blast disease reduction(%) and grain yield(kg/ha) under field conditions

Treatment	Concentration(%)	Year		Year		Year	
		2008	2009	2009	2010	2010	2010
		DS(%)	GY(kg/ha)	DS(%)	GY(kg/ha)	DS(%)	GY(kg/ha)
EME	0.1%	25	1120	26	1110	22	1140
FA(B+)	0.1%	30	940	32	920	33	900
Amaext-e	0.1%	13	1400	12	1420	11	1450
Carbendazim	0.1%	12	1300	11	1325	11	1330
Untreated	0.1%	77	620	78	600	80	575

C.D. at $p = 0.05 = 2.46$ for interaction between individual treatments of EME, FA and Amalab-e; Data in parantheses represents angular value; DS = disease score; GY = Grain yield

Table 6: Weather parameters from September to December (2008-2010)

Year/Month	Monthly average climatic parameters								
	Max(temp °c)	Min(temp °c)	RF (mm)	RH -I (%)	RH-II (%)	Wind Speed (kmph)	E (mm)	SS Hrs	
2008	September	31.15	27.98	11.30	91.8	73.6	5.48	4.34	4.2
	October	31.63	25.89	0.761	90.54	62.06	2.90	4.39	6.50
	November	29.61	19.31	0	90.5	52.86	2.23	3.89	6.54
	December	28.64	17.23	0	95.22	49.19	1.43	3.70	5.23
	Average	30.25	22.60	3.01	92.01	59.42	3.01	4.08	5.61
2009	September	32.14	26.38	10.39	93.26	76.2	3.17	4.2	3.99
	October	31.25	24.91	2.62	91.06	56.03	2.38	4.03	6.76
	November	29.73	21.79	1.78	91.13	54.93	2.38	3.84	6.37
	December	27.95	14.94	0	95.35	44.64	1.45	3.79	6.65
	Average	30.26	22.00	3.69	92.7	57.95	2.34	3.96	5.94
2010	September	31.18	26.16	4.53	93.6	74.66	3.18	4.31	4.65
	October	30.19	22.81	6.94	93.93	76.06	3.85	3.55	5.29
	November	30.09	20.15	1.29	94.43	63.23	2.41	3.52	5.81
	December	26.01	15.9	0.929	92.87	52.41	2.76	3.57	6.11
	Average	29.36	21.25	3.42	93.70	66.59	3.05	3.73	5.46

Max temp°C-Maximum temperature, Min temp°C-Minimum temperature, RF-Rain fall, RH-Relative humidity, E-Evaporation, SS Hrs-Sunshine hours

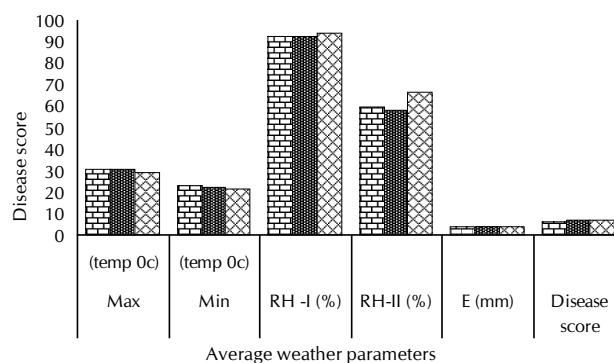
Weather parameters

Weather parameters viz., Max (temp °C), Min (temp °C), RF (mm), RH-I (%), RH-II (%), Wind Speed (kmph), E (mm) and SS (Hrs) were retrieved from the autoweather station of the Agrometrological unit of the Department of crop production at Central rice research Institute, Cuttack, Odisha, India for the period of September to December (Yr. 2008-2010) and recorded that average of months in a year recorded decline in minimum temperature and evaporation in a range from 22.60 to 21.25 and 4.08 to 3.73 respectively. Relative humidity-I registered increase from 92.01 to 93.70 percent (Table 6).

DISCUSSION

Botanically derived products have been reported to exhibit their antimicrobial potential (Tewari and Nayak, 1991; Hiremath *et al.*, 1993; Sharma *et al.*, 1999; Okigbo and Nmeke, 2005; Parekh *et al.*, 2006; Hulley *et al.*, 2010) but these being organic in origin, tend to decompose faster when applied on the surface of the plant for the management of diseases under

■ 2008 Average Sep-Dec ■ 2009 Average Sep-Dec ▣ 2010 Average Sep-Dec


Figure 5: Average weather parameters vis-a-vis disease score

in-vivo condition. In order to improve the efficacy of such product and avoid rapid loss in antimicrobial activity, a study was conducted to develop appropriate formulation.

The product Amalab-e, derived from the leaf extract of a

A. marmelos not only completely inhibited the conidial germination and mycelia growth of pathogens but also produced a variety of conidial distortions (Table 1 and 2 and Figs. 1 and 2). Prominent patterns of conidial distortions were reduction and granulation in germ tube length. The shelf-life of the value added formulated product also retained its fungitoxicity for a period of 12 months, in all the treatments (Table 3) and thereby appreciably enhanced its keeping quality which could therefore be stored to be utilized safely by the end users at the time of need. Though, the antimicrobial activity of *A. marmelos* (bael) has been found to have proven activity but against other pathogens (Balakumar et al., 2011; Gheisari et al., 2011). This report is specific to the rice blast pathogen *P. grisea* with the studies carried out in detail as indicated various experimentation presented under result which is first report particularly that includes shelf-life of the formulated product and the toxic effect under *in-vivo* condition. Amalabe was also screened for the control of rice blast under *in-vivo* condition at Central Rice Research Institute, Cuttack. This product effectively reduced foliar blast and was found comparable to a standard synthetic fungicide carbendazim both in green house and under field conditions (Table 4 and 5; Figs. 3 and 4).

Nevertheless, there is little information on the effect of changed climate on pest bio-diversity at large and against *P. grisea* causing rice blast in particular. Hence, the threat perception on increasing the disease /pest pressure continues. However, as observed in the present case, especially when minimum temperature and evaporation declined, though, in a narrow range and relative humidity–I (%) increased (Table 6; Fig. 5), rice blast disease pressure clearly registered an increase. Thus the formulated product developed and reported herewith possess the potential to be deployed in blast disease management strategy.

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REFERENCES

- Amadioha, A. C. 2000. Fungitoxic effects of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato Arch. *Phytopathol. Pflanz.* **10**: 1-9.
- Balakumar, S., Rajan, S., Thirunalasundari, T. and Jeeva, S. 2011. Antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes. *Asian Pac. J. Trop. Biomed.* 309-312.
- Gheisari, H. R., Amiri, F. and Zolghadri, Y. 2011. Antioxidant and antimicrobial activity of Iranian Bael (*Aegle Marmelos*) fruit against some food pathogens. *Int. J. Current Pharmaceutical Res.* **3(3)**: 85-88.
- Hiremath, S. P., Badami, S., Swamy, H. K. S. and Biradar, J. S. 1993. Antimicrobial activity of various extracts of *Acalypha indica* (Euphorbiaceae). *Indian J. Microbiology.* **33(1)**: 75-77.
- Hulley, I. M., Viljoen, A. M., Tilney, P. M., Van Vuuren, S. F., Kamatou, G. P. P. and Van Wyk, B. E. 2010. Ethnobotany, leaf anatomy, essential oil composition and antibacterial activity of *Pteronia onobromoides* (Asteraceae). *South African J. Botany.* **76**: 43-48.
- Kamalkannan, A., Shan mughan, V., Surendran, M. and Srinivasan, R. 2001. Antifungal properties of plant extract against *Pyricularia grisea* the rice blast pathogen. *Indian Phytopath.* **54(4)**: 490-492.
- Lapis, D. B. and Dumancas, E. E. 1979. Survey of higher plants for fungicidal properties against *Pyricularia oryzae*. *Cav. Philippine phytopath.* **15**: 23.
- Okigbo, R. N. and Emoghene, A. O. 2004. Antifungal activity of leaf extracts of some plant species on *Mycosphaerella fijiensis* Morelet, the causal organism of black sigatoka disease of Banana (*Musa acuminata*). *KMITL Sci. J.* **4**: 20-31.
- Okigbo, R. N. and Nmeka, I. A. 2005. Control of yam tuber with leaf extracts of *Xylopiya aethiopicum* and *Zingiber officinale*. *Afr. J. Biotechnol.* **4(8)**: 804 – 807.
- Padmanabhan, S. Y., Chakrabarti, N. K., Mathur, S. C. and Veeraghavan, J. 1967. Physiologic specialization in *Pyricularia oryzae* Cav. *Proc. Ind. Acad. Sci.* **LXV**: 63-73.
- Paraskeva, M. P., Van Vuuren, S. F., Van Zyl, R.L., Davids, H. and Viljoen, A.M. 2008. The *in vitro* biological activity of selected South African *Commiphora* species. *J. Ethnopharmacology.* **119**: 673-679.
- Parekh, J., Karathia, N. and Chanda, S. 2006. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. *Afr. J. Biomed. Res.* **9**: 53-56.
- Satish, S., Raghavendra, M. P., Mohana, D. C. and Raveesha, K. A. 2010. *In vitro* evaluation of the antifungal potentiality of *Polyalthia longifolia* against some sorghum grain moulds. *J. Agri. Tech.* **6(1)**: 135-150.
- Sharma, R., Phookan, A. K. and Bhagabati, K. N. 1999. Effect of some plant extracts in the management of sheath blight disease of rice. *J. Mycology and Plant Pathology.* **29(3)**: 336-339.
- Singh, R. A. 1997. Current status of rice blast in India and Challenges ahead. *Indian Phytopathology.* **50(2)**: 186-191.
- Tewari, S. N. 1986. A new technique for bioassay of natural plant product. *Current Sci.* **55 (22)**: 1137-1140.
- Tewari, S. N. and Shukla, H. S. 1990. Improved method for bioassay of pesticides. Integrated Pest Management Diseases. *Int. Rice Res. Newsl.* **15**: 26-27.
- Tewari, S. N. and Nayak, M. 1991. Antimicrobial activity of four different plant extracts against three fungal pathogens of rice. *Trop. Agric.* 373-375.
- Tewari, S. N. 1995. *Ocimum sanctum* L., a botanical fungicide for rice blast control. *Trop. Sci.* **35**: 263-273.
- Tewari, S. N. and Mishra, M. 2003. Fungitoxic potential of aromatic rice against *Pyricularia grisea*. *Indian Phytopath.* **56(3)**: 289-290.