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# Efficacy of Essential Oil of *Aegle marmelos* based Amaext-eo, A Formulated Product against *Pyricularia grisea* Causing Blast Disease of Rice

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**In order to enhance the efficacy of essential oil of *Aegle marmelos a present study* was under taken. The essential oil extract was combined with a formulating agent (coded B+) and named Amaext-eo, bioassayed under *in-vitro* condition against *Pyricularia grisea* Sacc. causing blast disease of rice. The Product was found to inhibit conidial germination completely at 0.01% concentration and mycelial growth at 1% concentration whereas, the extract alone could inhibit the germination only partially at the same concentration. The formulated product Amaext-eo retained its fungitoxicity till 24 months storage period in all treatments. In a separate test the efficacy of the product was also assed in the green house and under field condition and compared with the standard fungicide carbendazim. This formulated product has therefore improved the efficacy of fungitoxicity compared to the unformulated botanical extract under *in-vitro* and *in-vivo* condition and so found comparable with standard fungicide carbendazim (Bavistin 50%wp).**

**Key words:** Blast Disease, Rice, *Aegle marmelos*, Oil.

The expanded use of synthetic chemicals in industry negatively impacts environmental and public health due to their slow decomposition and accumulation in the environment. Consequently, there is a growing interest in natural products for use as alternative pesticides and as antimicrobial agents because they have a lower impact on the environment and they meet consumer demand for greener products. The use of biological compounds extracted from plants may be an alternative to conventionally used fungicides to control phytopathogenic fungi. Since plant extracts such as essential oils are can serve as alternative to synthetic chemicals. Essential oils are mixtures of volatile compounds obtained by distilling an essence prepared from natural materials[7]. Some essential oils have useful activities, including antimicrobial, spasmolytic, and insect-repelling properties [4,5,12]. Consequently, there have been attempts to use essential oils for food preservation, pharmacological purposes and aromatherapy [6,14]. Essential oils have been used by many workers for controlling fungi, bacteria, viruses and insect pests [19]. The essential oils are known for possessing antifungal and antibacterial properties[20]. The antimicrobial properties of essential oils invariably depend on the chemical nature of the constituents

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present in them. Essential oils, being lipophilic in nature can easily penetrate deeper through living tissue unbarred by the selective permeability of the cell membrane, hence they are of interest in the management of fungal diseases.

However, the products prepared from green plants if not formulated, tends to lose their efficacy due to decomposition [16,17,26]. Therefore the present work being reported as hereunder is aimed at developing an appropriate formulation for enhancing the efficacy and shelf life of such botanical products in the instant case so as to prevent the spread of the disease and pest without losing time in spraying such botanical fungicide like Amaext-eo in the instant case which has not lost its effectivity even after storage for certain period. The present research report therefore describes about the efficacy of essential oil of *Aegle marmelos*, with a selected formulating agent a surfactant, coded B+, and examines its suitability against the test pathogen *P. grisea*, the incitantly agriculturally important food crops such as rice blast.

## MATERIALS AND METHODS

### Preparation of essential oil extract

Fresh leaves of *A. marmelos* Corr. Were collected, washed thoroughly and loaded in Clevenger's apparatus mixing sterilized double distilled water(1:1w/v). The essential oil was then collected in a clean glass vial and dehydrated using $Na_2SO_4$ anhydrous. Extract thus recovered was treated as 100% essential oil extract (EO). These were kept at $2^0$c and this extract was then diluted from 100% to, 10%, 1%, 0.1%, 0.01%, and 0.001% and utilized for further studies.

### Preparation of formulated product

The formulating agent, a surfactant (coded $B^+$) was also diluted from 100% to, 10%, 1%, 0.1%, 0.01%, 0.001%, 0.0001%, and 0.00001% and each of these dilutions were combined separately with each dilutions of EO(1:1v/v) to be treated as Amaext-eo 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[15]) 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 $24^0$ C, and stored at $4^0$ C for further studies.

### Bioassay test

### Conidial germination test

Aliquots, 0.1 ml from each concentrations viz., 10%, 1%, 0.1%, 0.01%, 0.001% and 0.0001% of Amaext-eo, 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 glass slide with equal quantity and incubated in moist chamber at $24^0$C for 24hours. Observations on conidial germination (%) and the pattern of fungitoxicity were recorded after 24 hours 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

Amaext-eo, the formulated product was combined with sterilized melted OMA medium separately so as to get the final concentration of 10%, 1%, 0.1%, 0.01% and 0.001%. The extract mixed medium was poured into the petriplates aseptically and left for 5 days to allow ethanol to evaporate. Removed the contaminated plates, if any. Actively growing mycelia of *P. grisea* was cut with a sterile cork-borer (0.5mm) and inoculated separately in the center of each such petriplates aseptically. All such plates were incubated at $28\pm2^o$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 [21]. No mycelial growth was accorded numerical value 0.1cm, for

the purpose of statistical analysis.

**Shelf-life effect**

Shelf-life effect of Amaext-eo, FA and EO each at 10% concentration were stored at room temperature for fresh, 6, 12, 18 and 24 months in a cleaned, sterilized glass vial with air tight stopper and compared with fresh. The product was then bioassayed separately against test pathogen *P.grisea* through conidial germination at 10%, 1%, 0.1%, 0.01%, 0.001% and 0.0001% concentrations in the same way as stated earlier in text. Appropriate controls were 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 24 hours 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 variety 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 sprays inoculated on twenty-five-day old seedlings. EO, formulating agent (B+ coded) and Amaext-eo, 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, in percentage. Data obtained were statistically analyzed.

**Field experiment**

Feeds of blast susceptible rice cultivar HR 12 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 7x2.5 m plots with a spacing of 15 x 15 cm 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. EO, FA (B+ coded) and Amaext-eo (@ 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 2009-2011. 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**

Amaext-eo, treatment exhibited complete conidial germination inhibition (2%) in *P.grisea* conidia at 0.01% FA concentrations with all tested combination of EO (Table 1). The treatments 0.001% - 0.0001% of FA registered complete inhibition with 10%-0.1% of EO combination. Germination varied in a wide range from 40%- 98% in other treatments, but produced destructive toxic pattern such as reduction, granulation, thin and coiled germ tube compared to normal 98% in either control . Control FA and EO registered no conidial germination upto 0.1% concentration respectively. Another control

(Ethanol) registered normal [98% (81.87) ± 0.46] conidial germination.

**Poisoned food technique**

All the other treatments with Amaext-eo did significantly reduce the mycelial growth compared to control [4.5cm (15.91) ±0.32]. Amaext-eo (EO+FA) produced complete mycelial growth inhibition (0.1cm) at 1% concentration against test pathogen *P.grisea* and significantly reduced mycelial growth at each tested concentrations. EO or FA alone registered complete inhibition at 1% concentration respectively (Table 2).

**Shelf-life test**

Amaext-eo, at 0.1% concentration and EO and FA at 1% concentration respectively showed complete inhibition (2%) against test pathogen *P.grisea* till 24 months storage period (Table 3). Amaext-eo retained its fungitoxicity up to 24 months storage period in all treatments. Deformities patterns viz., granulated cytoplasm of conidia, thin, reduced, coiled and granulated germ tube were recorded up to 0.001% concentration of formulated product and EO alone till 6 months storage period. All the other treatments registered normal conidial germination and found at par with control [98% (81.87) ±0.55].

**Greenhouse test**

EO, FA and Amaext-eo were sprayed at 0.01, 0.1 and 1% concentrations and compared with standard fungicide carbendazim at recommended

**Table 1.** Fungitoxic effect of a formulated product Amaext-eo against *Pyricularia grisea*

| Treatment (FA/EO) | Concentration(%) 10% | 1% | 0.1% | 0.01% | 0.001% | 0.0001% | Control (FA) |
|---|---|---|---|---|---|---|---|
| | Conidial germination(%) | | | | | | |
| 10% | 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] |
| 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] |
| 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] | 40(39.23) [bg] |
| 0.001% | 2(8.13) [a] | 2(8.13) [a] | 2(8.13) [a] | 50(45) [bg] | 70(56.79) [b] | 98(81.87) | 80(63.44) [b] |
| 0.0001% | 2(8.13) [a] | 2(8.13) [a] | 2(8.13) [a] | 60(50.77) [bg] | 80(63.44) [bef] | 98(81.87) | 98(81.87) |
| Control(EO) | 2(8.13) [a] | 2(8.13) [a] | 2(8.13) [a] | 10(18.44) [bg] | 60(50.77) [bef] | 98(81.87) | |
| Control(Ethanol) | 98(81.87) | 98(81.87) | 98(81.87) | 98(81.87) | 98(81.87) | 98(81.87) | |

C.D. at P= 0.05 = 0.46 for interaction between individual treatments of EO, 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%; [a]= completely inhibited conidia, [b]= reduced germ tube, [g]= granulated germ tube, [e]= thin germ tube, [f]= coiled germ tube

**Table 2.** Fungitoxicity of Amaext-eo against *P.grisea* mycelial growth

| Concentration (%) | Mycelial growth(cm/cm$^2$) Amaext-eo(EO+FA) | EO | FA |
|---|---|---|---|
| 10 | 0.1(0.01) | 0.1(0.01) | 0.1(0.01) |
| 1 | 0.1(0.01) | 0.1(0.01) | 0.1(0.01) |
| 0.1 | 1.4(1.54) | 3(7.07) | 2.2(3.80) |
| 0.01 | 2.5(4.91) | 3.5(9.62) | 3.7(10.75) |
| 0.001 | 3.2(8.04) | 4(12.57) | 4.2(13.86) |
| Control | 4.5(15.91) | 4.5(15.91) | 4.5(15.91) |

C.D. at P= 0.05 = 0.32 for interaction between individual treatments of EO, FA and Amaext-eo; Data in parentheses represents area of the mycelial growth in cm$^2$ computed through 3.14×r$^2$ method; complete inhibition is represented by 0.1 cm/0.01cm$^2$

**Table 3.** Shelf-life effect of a formulated product Amaext-eo against *Pyricularia grisea*

| Treatments | Storage period(months) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration | Fresh | | | 6 | | | 12 | | | 18 | | | 24 | | |
| (%) | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III |
| | Conidial germination(%) | | | | | | | | | | | | | | |
| 10 | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) |
| 1 | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) |
| 0.1 | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | $2^{a}$(8.13) | 80 $^{bg}$(63.4) | $2^{a}$(8.13) | 96 $^{bg}$(78.4) | 98(81.9) | $2^{a}$(8.13) | 98(81.9) | 98(81.9) |
| 0.01 | $2^{a}$(8.13) | $10^{bg}$(18.44) | $40^{be}$(39.2) | $2^{a}$(8.13) | $30^{bg}$(33.2) | $80^{be}$(63.4) | $35^{bge}$(36.2) | $60^{bge}$(50.7) | 98(81.9) | $40^{bef}$(39.2) | 98(81.9) | 98(81.9) | $50^{bef}$(45) | 98(81.9) | 98(81.9) |
| 0.001 | $50^{bg}$(45) | $60^{bg}$(50.77) | $80^{be}$(63.4) | $55^{bg}$(47.8) | $80^{bg}$(63.4) | 98(81.9) | $70^{bge}$(56.7) | 98(81.9) | 98(81.9) | $75^{bef}$(60) | 98(81.9) | 98(81.9) | $75^{bef}$(60) | 98(81.9) | 98(81.9) |
| 0.0001 | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) |
| Control (Ethanol) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) | 98(81.9) |

C.D. at P= 0.05 = 0.55 for interaction between individual treatments of EO, FA and Amaext-eo; I= Amaext-eo; II= EO; III= FA; 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; e= thin germ tube; f= coiled germ tube

**Table 4.** Fungitoxic performance of EO, FA and Amaext-eo extracts on reduction of rice blast disease in green house from the year 2009-2011

| Year | Foliar blast score(%) 2009 | | | | 2010 | | | | 2011 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Extract/ Concentrations (%) | EO | FA | Amaext-eo | Carbendazim | EO | FA | Amaext-eo | Carbendazim | EO | FA | Amaext-eo | Carbendazim |
| 0.01 | 15 | 18 | 13 | - | 17 | 21 | 14 | - | 20 | 24 | 15 | - |
| 0.1 | 10 | 15 | 7 | 5 | 11 | 14 | 7 | 6 | 13 | 16 | 9 | 7 |
| 1 | 1 | 1 | 1 | - | 1 | 1 | 1 | - | 1 | 1 | 1 | - |
| Control | 67 | 67 | 67 | 67 | 70 | 70 | 70 | 70 | 80 | 80 | 80 | 80 |

C.D. at P =0.05 = 1.37 for interaction between individual treatments of EO, FA and Amaext-eo. Noinfection is accorded the value 1.0 for the purpose of statistical analysis

**Table 5.** Fungitoxic performance of Amaext-eo on rice blast disease reduction(%) and grain yield(kg/ha) under field conditions

| Treatment | Concentration(%) | Year | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2009 | | 2010 | | 2011 | |
| | | DS (%) | GY (kg/ha) | DS (%) | GY (kg/ha) | DS (%) | GY (kg/ha) |
| EO | 0.1% | 23 | 780 | 24 | 775 | 26 | 760 |
| FA(B+) | 0.1% | 29 | 540 | 33 | 525 | 34 | 510 |
| Amaext-eo | 0.1% | 6 | 840 | 7 | 835 | 8 | 800 |
| Carbendazim | 0.1% | 5 | 824 | 6 | 800 | 6 | 795 |
| Untreated | 0.1% | 75 | 236 | 78 | 226 | 80 | 220 |

C.D. at P =0.05 = 2.20 for interaction between individual treatments of EO, FA and Amaext-eo; Data in parantheses represents angular value; DS=disease score; GY=Grain yield

dose to check the spread of foliar blast disease of rice in green house on a blast susceptible variety HR12 (Yr. 2009-2011). All the treatments did significantly reduce the disease compared to control (67-80%±1.37). Amongst the extracts, Amaext-eo displayed no foliar blast infection at 1% concentration in three year. Amaext-eo was found to reduce the disease significantly at par with carbendazim (5-7%± 1.37) at 0.1% concentration and maximum disease score was recorded in FA (18-24%±1.37) followed by EO treatment(15-20%±1.37) at 0.01% concentration in three year . Disease score in control ranged from 67-80%±1.37 (Table 4).

**Field experiment**

Effect of the formulated product, Amaext-eo, EO and FA (coded B+) sprayed at 0.1% concentration were evaluated for the control of rice blast disease in field on a blast susceptible variety HR12 (Yr. 2009-2011 during wet season). All the treatments significantly reduced foliar blast compared to control (75-80% ± 2.20). Independently in all the three years, Amaext-eo reduced the disease (6-8% ± 2.20) which was comparable with a standard fungicide carbendazim (5-6% ± 2.20) at 0.1% concentration but the disease percentage was significantly higher in FA(B+) (29-34% ± 2.20) followed by EO(23-26%±2.20) in three year, highest yield was reported in Amaext-eo (800-840Kg/ha±2.20 ) followed by Carbendazim (795-824Kg/ha±2.20 ). Untreated check produced lowest yield in the range (220-236 Kg/ha ±2.20), in three years (Table 5).

## DISCUSSION

The use of many synthetic fungicides has been cautioned due to their pollutive effects, non-biodegradability and residual toxicities. Most of these fungicides have become a popular target of conservationists and are treated to be one of the most vital man-made pollutants [1]. During recent years, many essential oils have been found as potent antimicrobial agents [23]. Since such antifungal essential oils have penetration action, these may especially be used to control seed-borne fungal pathogens. The volatility, ephemeral nature and biodegradability of these compounds of angiosperms will be especially advantageous if they are developed as pesticides. The physical nature of essential oils, low molecular weight substances with pronounced lipophilic tendencies, is believed to allow them to penetrate cell membranes quickly. Also, essential oils themselves have been known to contain various compounds such as terpenoids and phenolic compounds, such as thymol, carvacrol, and eugenol, which exert high antimicrobial activity in their pure forms [8,11]. In addition, the antimicrobial activities of oregano, savory, and thyme are likely due to their high contents of thymol and carvacrol, which are one of the most efficient volatile antibacterial agents known so far [13]. Hence, the antimicrobial components contained within essential oils, together with their high efficiency in penetrating microbial cell membrane, are believed to exert their antimicrobial activity against the plant pathogenic

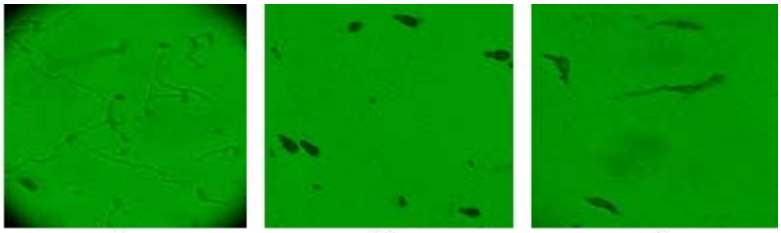


**Fig. 1.** Formulated product Amaext-e showing fungitoxic pattern in *P.grisea,* A= Normal germination (Control), B= Completely inhibited conidia, C= Reduced and granulated germ tube

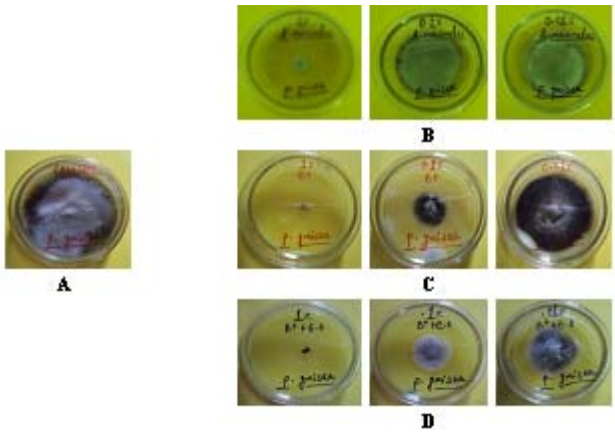


**Fig.2.** Photograph showing Performance of Amaext-eo; Plate-D formulated product, compared with EO Plate-B, and FA Plate-C, respectively. Plate- A showing *P.grisea* with no mycelial growth inhibition (control)

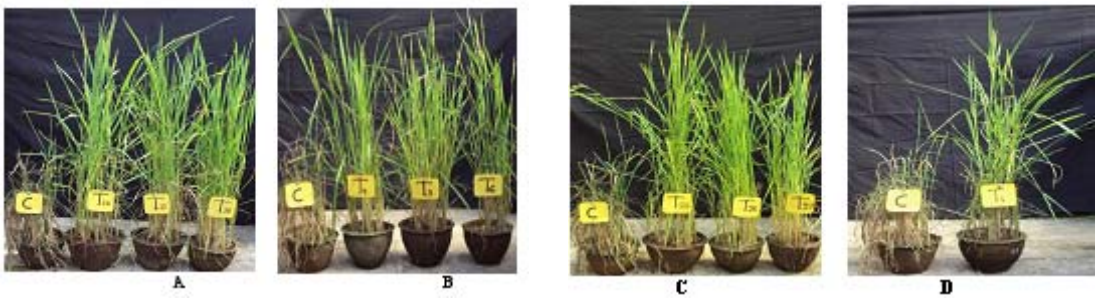


**Fig. 3.** Showing prevention of rice blast in green house in A = Essential oil extract (T16, T17 & T18); B= Formulating agent B+ (T4 , T5 & T6); C= Amaext-eo (T25, T26& T27 ) at 1, 0.1 and 0.01 percentage concentrations respectively in that order, in each set under green house test compared with a standard fungicide Carbendazim; D= at 0.1%(T1) and C= represents control in each case

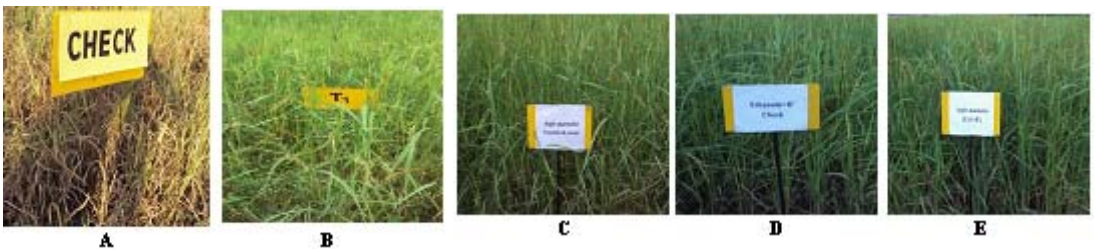


**Fig. 4.** Showing prevention of rice blast in field; A= control sprayed only with water; B= a standard fungicide carbendazim; C= essential oil extract; D= formulating agent B+; E = formulated product Amaext-eo at 0.1% concentration

and health related microorganisms tested in this study [3,9,25].

Botanically derived products, when not utilized fresh even if proving effective as the crop protectant [2,10,18,22], as they tend to decompose rapidly hence, makes the choice difficult to use them as substitute to the synthetic agrochemicals. Both enzymic and non-enzymic components accelerates the process of decomposition and the disintegration of the macerated or otherwise extracted product, derived from plant origin. Present work is therefore being reported as hereunder aimed to develop an appropriate formulation of this botanical fungicide so as to use effectively at the time of need.

The product Amaext-eo, 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&2 and Fig 1&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 24 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. Amaext-eo 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&5 and Fig 3&4). Thus the formulated product developed and reported herewith possess the potential to be deployed in blast disease management strategy.

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