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EFFICACY OF Moringa oleifera EXTRACT, Trichoderma asperellum, A SYNTHETIC FUNGICIDE AND CATTLE DUNG AMENDMENT IN THE INTEGRATED MANAGEMENT OF RICE BLAST DISEASE

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Abstract

This study evaluated the effect of integrated disease management on the incidence and severity of blast disease, and growth performance of rice. *Moringa oleifera* extract, *Trichoderma asperellum*, cattle dung and a synthetic fungicide were evaluated in sixteen treatment combinations using a susceptible IRAT 109 rice cultivar. The pot experiment was laid out in a randomized complete block design with three replications at the rooftop screenhouse of the Department of Crop Protection and Environmental Biology, University of Ibadan between May and October, 2019. Treatment with combination of *M. oleifera* extract, cattle dung and blastforce had the lowest disease incidence and mean severity of 16.8 and 7.5%, respectively which was significantly (p<0.05) lower than the standard positive check. Inoculated plants that were treated with a combination of *T. asperellum, M. oleifera* and cattle dung produced the highest yield of 7.6 tons/ha relative to control. This study showed that the combined application of naturally occurring biopesticides and cattle dung was more effective than single treatment in the management of rice blast disease.

Keywords: Blast disease, Biopesticide, Fungicide, Moringa oleifera, IRAT 109, Rice, Yield.

Introduction

Rice (*Oryza sativa* L.), is one of the three most important cereal crops grown as a primary source of food for over 3.5 billion people of the world's population and Nigeria accounts for about 8 million tonnes, which is the largest rice producer in Africa (FAO, 2019). The crop is known to be attacked by many diseases which cause huge losses annually, probably because it is usually produced in humid regions where climatic conditions are favourable for fungal infection. Among fungal diseases of rice, blast caused by *Magnaporthe oryzae* Couch. has been reported in more than 85 countries with over 30% of the annual rice harvest lost to infection (Dagdas *et al.*, 2012). When young rice seedlings are colonized by rice blast pathogen, the whole plants often die and if it spreads to other parts of the plants, it results in nearly total loss of the rice yield (Kapoor and Abhishek, 2014).

The disease can be managed by use of fungicides, planting of resistant cultivars, biotechnological methods and through integrated approach (Ribot *et al.*, 2008; Hasan *et al.*, 2016). However, indiscriminate and repeated application of fungicides has led to pathogen resistance and bioaccumulation in the food chain

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which discourage the adoption of this method. Resistant cultivars have been reported to be very effective in preventing fungal diseases, but new races of fungi that break down the resistance of rice cultivars make them vulnerable to diseases and thus unreliable (Hubert *et al.*, 2015). Other management practices such as use of biological control agents, organic manure adopted singly and crop rotation have been found to be of limited use because of wide host range of the pathogen in most rice growing areas.

Moringa oleifera is a fast-growing evergreen plant in the tropics, which has been reported to possess antimicrobial properties (Anwar *et al.*,2007; Giri*et al.*, 2010; Dania and Thomas, 2019). *Trichoderma* species abound in the rhizosphere and have been widely used in the control of plant diseases (Ritika and Utpal, 2014; Khalid, 2017; Dania and Gbadamosi, 2019). The application of organic manure to the soil enhances plant vigour and increases resistance to diseases (Amos *et al.*, 2015). Integrated disease management is a cost effective approach to disease management of plants by combining biological, cultural and chemical methods with a minimum adverse effect on the environment. This study, therefore, evaluated the effect of integrated disease management on the incidence and severity of blast disease, and also growth and yield of rice.

Materials and Methods

Experimental site and sources of experimental materials

The experiment was conducted in the Phytopathology laboratory and roof top screenhouse of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Ibadan, Nigeria between May and October, 2019. An isolate of *Trichoderma asperellum* and a susceptible IRAT 109 rice cultivar were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, a synthetic fungicide (Blastforce) was purchased from an Agrotech store, *M. oleifera* leaves were obtained from the university premises while wheat seeds were purchased from the open market. Cattle dung used in this experiment was obtained from the Teaching and Research Farm of the University of Ibadan.

Preparation of growth medium and isolation of Magnaporthe oryzae

Dehydrated Potato Dextrose Agar (PDA) powder was prepared by dissolving 39 g/Lof sterile distilled water in a conical flask sealed with cotton wool and wrapped with aluminum foil. The mixture was autoclaved at a temperature of 121°C and a pressure of 1.05 kg/cm² for 15 minutes. The medium was allowed to cool to about 45°C and afterwards 1 ml of lactic acid was added to inhibit the growth of bacteria contaminants. Twelve milliliter of the medium was dispensed per Petri dish aseptically in a laminar flow hood and allowed to set for 25 minutes.

Leaf samples of rice plant showing blast symptoms were collected from the rice field at International Institute of Tropical Agriculture (IITA), Ibadan and brought to the laboratory for isolation of the pathogen. The leaves were cut into smaller sizes measuring $3\text{mm} \times 2\text{mm}$ using a sterile scalpel. The leaves were surface-sterilized with 10% sodium hypochlorite for one minute, rinsed in three changes of sterile distilled water and blot-dried on sterile filter paper before plating on PDA. The inoculated plates were incubated at $28 \pm 2^{\circ}$ C for 4 days (Dania *et al.*, 2015).

Identification of *M. oryzae* involved microscopic examination of the fungus and examining the cultural and morphological characteristics of the fungus using standard mycological charts (Barnett and Hunter, 2000; Kariaga*et al.*, 2016) and following confirmation at the mycological herbarium of IITA.

Pathogenicity test

Four seeds of a susceptible rice cultivar IRAT 109 were sown in experimental pots; these were separately filled with 10 kg of sterilized sandy loam soil and later thinned to two stands per pot at one week after germination. Conidia were harvested from a two-week old culture isolates after full sporulation on PDA. An aliquot of 0.5 ml of a polyethylene sorbitol ester (Tween 80) and 10 ml of sterile distilled water were added to each Petri dish, to facilitate the detachment of the pathogen conidia and mycelia from the culture medium. The inoculum suspension was filtered through Whatman No 1 filter paper. The seedlings were inoculated with an inoculum concentration of 1×10^6 at three weeks after sowing using a hand sprayer. Control treatment were sprayed with only sterile distilled water. Inoculated plants were kept at 27-28°C in a screenhouse under alternating 12 h light and darkness, till 14 days after inoculation. Re-isolation and re-inoculation were done to establish Kock's postulates.

Preparation of treatments

Wheat seeds were soaked in sterile distilled water for 24 hours followed by decanting and washing with three changes of sterile distilled water. The seeds were then bottled and autoclaved at 1.05 kg/cm² pressure and 121°C for 30min to kill any associated seed-borne pathogen. The sterilised seeds were allowed to cool for 5 h and then inoculated with a 4-day old culture of *T. asperellum* and incubated at 27-28°C for 10 days to allow for mycelial ramification on the wheat seeds. *Moringa* leaves were air-dried for two weeks and ground to powder and 200g of it was dispersed (20% w/v) in 1 litre of sterile distilled water (SDW). The extract was left for 12 hours before use (Dania and Thomas, 2019). The synthetic fungicide (Blastforce) was prepared by dissolving 0.3g in 11itre of sterile distilled water according to manufacturer's instruction, which served as positive check. Cattle dung was applied at 20 g per pot and mixed thoroughly with the soil, which was equivalent to 7.5 tonnes/ ha⁻¹ (Sudarsono*et al.*, 2014).

Quantification of fungal spores and inoculation of rice plants

Spore suspension of *M. oryzae* was prepared by dispensing 10 ml of sterile distilled water to the Petri dish containing a 10-day-old culture of the fungus to dislodge the conidia and was filtered through a layer of cheese-cloth. Spores were counted by dispensing 0.1 ml of inoculum on a hemacytometer. Spore concentration was adjusted to 10⁶ spore/ml according to Dania et al. (2015) and healthy rice plants with fully developed first four leaves were inoculated at 21 days. Plants inoculated with *M. oryzae* without any amendment served as control, while inoculated plants treated with Blastforce fungicide only served as positive check. Inoculated plants were covered with transparent polythene sheets overnight for 12 hours to create relative humidity needed to initiate infection. Five grammes of T. asperellum that has fully ramified on the carrier substrate were weighed and mixed with the top soil at one week after inoculation. Cattle dung was applied on the soil as previously described. M. oleifera leaf extract and Blastforce were applied as foliar spray at one week after inoculation. Data on growth parameters such as number of leaves, number of tillers, plant height, number of panicles, disease incidence and severity, were collected weekly, while yield parameters such as grain yield, grain weight, shoot dry weight, root dry weight and 100 seed weight were collected at the end of the experiment.

Determination of disease incidence and severity

Disease incidence was determined by counting the number of infected plants and expressed as percentage of the total number of plants assessed in each treatment:

Disease incidence = $\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$

Disease severity was assessed using the modified method of IRRI (2002):

1= No lesion observed;

2= Small brown specks of pinpoint size without sporulating spots;

3= Roundish to slightly elongated necrotic sporulating spots;

4= Narrow or slightly elliptical lesion;

5= Rapidly coalescing small, whitish lesion without distinct margins;

6= Severe blighting of leaves of infected plants;

Mean severity was determined according to the modified method of Asad*et al.* (2010):

Mean severity = $\frac{\text{Sum of numerical ratings on a tree} \times 100}{\text{Total No. of disease symptoms on tree} \times y}$

Where y = Number of severity rating

Evaluation of treatments for the management of blast disease of rice

The experiment was laid out in a randomized complete block design with sixteen treatment combinations and three replications. Each pot was filled with 10 kg of sterilized soil. Five seeds were sown directly in each pot and later thinned to 2 seedlings per pot after one week of germination. *Trichoderma asperellum*, *M. oleifera*, cattle dung and blastforce each alone or their combinations were considered as treatments as follows:

- T₀= Rice plant inoculated without spray (control)
- $T_1 =$ Inoculated + *T*. asperellum
- $T_2 =$ Inoculated + Moringa oleifera
- $T_3 = Inoculated + cattle dung$
- $T_4 =$ Inoculated + Blastforce
- T_5 = Inoculated + *T*. asperellum + *M*. oleifera; T_1 + T_2
- T_6 = Inoculated + *T. asperellum* + cattle dung; T_1 + T_3
- T_7 = Inoculated + *T. asperellum*+ Blastforce; T_1 + T_4
- T_8 = Inoculated + *M. oleifera* + cattle dung; T_2 + T_3
- T_9 = Inoculated + *M. oleifera* + Blastforce; T_2 + T_4
- T_{10} = Inoculated + Cattle dung + Blastforce; T_3 + T_4
- T_{11} = Inoculated + *T. asperellum* + *M. oleifera* + cattle dung; $T_1+T_2+T_3$
- T_{12} = Inoculated + *M. oleifera* + cattle dung + Blastforce; $T_2+T_3+T_4$
- T_{13} = Inoculated + *T. asperellum*+ Blastforce + *M. oleifera*; T_1 + T_4 + T_2
- T_{14} = Inoculated + *T*. *asperellum* + cattle dung + Blastforce; $T_1+T_3+T_4$
- T_{15} = Inoculated + *T. asperellum* + *M. oleifera* + cattle dung +Blastforce; $T_1+T_2+T_3+T_4$

Data analysis

Percentage data were transformed into arcsine angles to produce the approximate constant variance. All numerical data were analyzed using the Statistical Analysis System (SAS) Version 9.4 and means were separated according to Duncan Multiple Range Test (DMRT) at 5% level of significance.

Results and Discussion

Isolation and Pathogenicity test

Three fungal genera, *Aspergillus*, *Rhizopus* and *Magnaporthe* were isolated from the infected leaf samples in the laboratory. *Aspergillus* spp. and *Rhizopus* spp. were saprophytes or secondary invaders with very low frequencies of isolation. *Magnaporthe oryzae* was predominant and present in all infected leaf samples. The pathogen was characterised by the brown mycelia with grayish brown concentric rings when grown on Potato Dextrose Agar (PDA) culture (Figure 1a). The microscopic view showed a blue hyaline, spindle-shaped with tapering ends conidia with four septa singly and jointly fitted together (Figure 1b).

At two weeks after inoculation with M. oryzae, the leaves showed small brown spots at initial stage of infection which rapidly coalesced and developed into extensive spindle-shaped lesions with white central part and brownish borders typical of the disease (Figure 2). These results confirm previous reports of various authors that implicated M. oryzae as the causal organism of rice blast disease (Hajano et al., 2011; Rajput et al., 2017).



Fig. 1. Cultural (a) and morphological (b) characteristics of Magnaporthe oryza.



1= No symptom





3= Slightly elogated necrotic leasion





whitish lesion



6= Severe blighting of leaves in infected plants



Table 1. Effect of Moringa oleifera extract, Trichode incidence and severity of rice blast disease	erma asperellum, b	olastforce fungicide	e and cattle dung	amendment on the
tarona tar		Disease i	ncidence (%)	
Ireaunent	Vegetative	Reproductive	Maturity	MS (%)
Inoculated and untreated rice plants (Control) (T_0)	30.0(33.20)a	32.5(34.72)a	45.8(49.08)a	51.2(53.31)a
$T. asperellum (T_1)$	16.7(24.12)b	22.2(28.71)b	26.50(30.97)ab	11.2(18.49)b
Moringa oleifera (T2)	22.2(28.71)ab	27.0(31.13)ab	27.2(31.24)ab	14.2(21.70)b
Cattle dung (T ₃)	27.2(31.24)a	27.2(31.24)ab	30.0(33.20)ab	16.7(24.05)ab
Blastforce (T ₄)	22.2(28.71)ab	22.4(28.93)b	27.5(31.55)ab	14.2(21.70)b
T. asperellum + Moringa oleifera (T5)	16.7(24.12)b	27.5(31.55)ab	27.5(31.55)ab	14.2(21.70)b
T. asperellum + cattle dung (T_6)	12.5(17.89)bc	22.2(28.71)b	22.2(28.71)b	10.2(18.49)b
T. asperellum + Blast force (T_7)	20.1(26.71)b	23.1(28.76)b	28.8(33.02)ab	14.5(21.68)b
<i>M. oleifera</i> + cattle dung (T_8)	16.7(24.12)b	22.2(28.71)b	22.2(28.71)b	10.2(18.49)b
$M.oleifera + Blastforce (T_9)$	24.7(29.87)ab	27.5(31.55)ab	27.5(31.55)ab	14.2(21.70)b
Cattle dung + Blastforce (T_{10})	12.5(17.89)bc	22.2(28.71)b	32.5(34.72)ab	20.1(26.71)ab
<i>T. asperellum</i> + <i>M. oleifera</i> + cattle dung (T_{11})	27.2(31.24)a	27.2(31.24)ab	32.5(34.72)ab	20.1(26.71)ab
<i>M. oleifera</i> + cattle dung+ Blastforce (T_{12})	12.5(17.89)bc	13.2(21.39)bc	16.8(24.14)c	7.5((13.83)bc
T. asperellum + Blastforce + M . oleifera (T ₁₃)	22.2(28.71)ab	22.8(28.93)b	23.1(28.71)bc	10.8(19.149)b
<i>T. asperellum</i> + cattle dung + Blastforce (T_{14})	22.3(28.80)ab	25.3(30.78)ab	27.5(31.55)ab	14.2(21.70)b
<i>T. asperellum</i> + <i>M. oleifera</i> +cattle dung + Blastforce (T_{1i}	15) 16.7(24.12)b	16.7(24.12)bc	22.2(28.71)b	10.2(18.49) b
$S.E(\pm)$	0.53	0.42	0.27	0.61
CV(%)	3.84	4.77	2.38	5.42
MS = Mean Severity. Means with the same letter along a data were transformed into arcsine angles (in parenthesis)	column are not sign) before analysis of v	ufficantly different ac variance (ANOVA).	cording to DMRT a	at P≤0.05. Percentage

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Effect of treatments on incidence and severity of rice blast disease

Treatment T_{12} a combination of *M. oleifera* extract, cattle dung and Blastforce had the lowest disease incidence and mean severity of 16.8 and 7.5%, respectively which was significantly lower than T_4 with single application of the standard Blastforce fungicide that served as positive check (Table 1). Control plants (without treatment application) recorded the highest disease incidence and mean severity of 45.8 and 51.2%, respectively at maturity stage. Mean severity among the treatments ranged from 7.5 to 20.1%, which was significantly lower than the control (51.2%). Significant disease suppression was obtained in the plots treated with T_{12} (combination of cattle dung, *M. oleifera* extract and Blastforce).

The efficacy of *Moringa oleifera* agrees with previous report of Mazidet al. (2011) that the botanical produces phenolic compounds that are actively involved in disease resistance following the entry of pathogens into host plants. The toxicity of tannins and proanthocyanidins had direct effect on pathogens and these metabolites might also enhance resistance by contributing to healing of wounds through lignification of cell. (Lattanzioet al.,2006). The application of botanicals and bioagents has been identified as a sustainable tool in the management of plant diseases (Adandonon set al., 2006). Rice blast is a systemic disease and *Trichoderma* species possessed the inherent ability to migrate through the stem and in the process reduced the incidence and severity of maize stalk rot disease (Sobowaleet al., 2007). The significant reduction in disease incidence and severity in this study is consistent with the findings of Chang et al. (2007) that organic manure enhanced vigorous growth in plants which increased the resistance threshold with a corresponding decrease in the rate of susceptibility to diseases

Effect of treatments on growth and yield of rice plants inoculated with *Magnaporthe oryzae*

The various growth parameters responded differently to all the treatments. Rice plants treated with T14 (*T. asperellum*, cattle dung and Blastforce fungicide} had the best overall growth performance with 27.0 tillers and reaching a height of 57.3 cm at the vegetative stage compared to treatment T_4 which served as the positive check (Table 2). This was followed by treatment T_{12} (*M. oleifera* extract, cattle dung and Blastforce) with 23.3 and 56.6 cm tillers and height respectively.

However, inoculated plants that were treated with a combination of *T*. *asperellum*, *M*. *oleifera* and cattle dung produced the highest yield of 7.6 tonnes/ha⁻¹, which was significantly higher than the control, followed by treatment with combined application of cattle dung and Blastforce (Table 3).

Treatment	No. of	No. of	Plant	Root dry	Shoot dry
TAURINA	leaves	tillers	height (cm)	weight (g)	weight (g)
Plants inoculated with pathogen only (Control) (T ₀)	43.3b	16.3b	55.8ab	7.3a	15.0ab
$T.$ asperellum (T_1)	46.0b	19.3ab	42.8bc	6.5ab	11.9b
$M.$ oleifera (T_2)	42.3b	16.7b	46.6b	5.6ab	11.5b
Cattle dung (T ₃)	<i>57.7</i> a	23.0ab	47.9b	5.5b	15.0ab
bastforce (T ₄)	43.3b	20.7ab	44.0b	4.8b	11.7b
T. asperellum + M. oleifera (T_5)	50.0ab	20.7ab	53.7ab	4.9b	13.4ab
<i>T. asperellum</i> +cattle dung (T_6)	54.7a	22.7ab	50.1ab	6.2ab	19.0a
<i>T. asperellum</i> +Blastforce (T_{7})	49.7ab	21.0ab	54.1ab	5.3b	14.5ab
<i>M. oleifera</i> + cattle dung (T_8)	51.0ab	22.0ab	51.8ab	5.8ab	11.8b
M. oleifera +Blastforce (T9)	37.3bc	15.0b	47.8b	4.8b	13.7ab
Cattle dung + Blastforce (T_{10})	51.3ab	20.7ab	50.9ab	5.1b	15.4ab
T. asperellum + M. $oleifera$ + $cattle dung (T_{11})$	44.3b	18.7ab	45.6b	7.6a	17.3ab
<i>M.</i> oleifera + cattle dung + Blastforce (T_{12})	58.0a	25.3a	56.5a	7.1a	16.0ab
T. asperellum+ blastforce + M. oleifera (T_{13})	51.7ab	23.0ab	45.8b	5.8ab	17.1ab
<i>T.</i> asperellum + cattle dung + Blastforce (T_{14})	61.7a	27.0a	57.3a	6.8ab	18.4ab
<i>M. oleifera</i> + cattle dung + Blastforce (T_{15})	40.0b	17.3b	43.3bc	5.4b	15.8ab
S.E	1.5	0.8	1.3	0.3	1.5
CV (%)	3.88	9.6	5.7	2.9	6.3

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Table 3. Effect of <i>Moringa oleifera</i> extract, <i>Trichoderma asperellum</i> , Bla rice yield	astforce fungici	ide and cattle	cattle dung a	amendment on
Treatments	No. of	Grain	100 grain	Grain yield
	panicles	wt (g)	wt (g)	(t/ha ⁻¹)
Inoculated and untreated rice plants (Control (T ₀)	6.0ab	22.8d	4.1	4.3b
$T. asperellum (T_1)$	7.3ab	23.0d	4.0	6.5a
$M.$ oleifera (T_2)	8.0ab	18.6d	4.0	5.6ab
Cattle dung (T ₃)	11.7a	38.4ab	4.1	5.5ab
Blastforce (T ₄)	8.0ab	28.5c	4.4	4.8b
T. asperellum + M . oleifera ($_{T5}$)	7.7ab	29.3c	4.2	4.9b
<i>T. asperellum</i> + cattle dung (T_6)	9.3a	36.5b	4.1	6.8a
<i>T. asperellum</i> + Blast force (T_7)	7.7ab	29.6c	4.1	5.3ab
<i>M. oleifera</i> + cattle dung (T_8)	9.0a	32.0bc	4.1	5.8ab
M. oleifera + Blastforce (T ₉)	6.7ab	18.1de	4.0	4.8b
Cattle dung + Blastforce (T_{10})	9.0a	35.4b	4.1	5.3ab
T. asperellum+ M. oleifera + cattle dung (T_{11})	10.7a	34.9b	4.3	7.6a
M. oleifera + cattle dung+ Blastforce (T ₁₂)	8.7ab	25.2cd	4.0	7.1a
T. asperellum + Blast force + M. oleifera (T_{13})	8.7ab	32.7bc	4.0	5.8ab
T. asperellum + cattle dung + Blast force (T_{14})	11.7a	41.5a	4.2	6.2ab
T. asperellum + M. oleifera + CD + Blastforce (T_{15})	7.0ab	38.2ab	4.1	5.4ab
S.E (±)	0.5	1.2	0.1	0.7
CV (%)	4.4	7.8	1.2	3.6
$CD = Cow$ dung. Means with the same letter along a column are not signific DMRT at $P \le 0.05$	cantly different	according to		

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Treatments T_{11} and T_{12} with cattle dung combinations produced the highest grain yield per hectare. Organic manure has been reported to improve soil structure, enhance better aeration, and provides essential nutrients for plant growth (Chang *et al.*, 2007). Cattle dung is an important source of bio-fertilizer, which enhances soil fertility and stimulates the activities of beneficial soil microbes with an overall increase in yield and the essential nutrients released from cattle dung such as phosphorus and potassium uptake had also been reported to increase yield and yield components (Andi and Nur, 2013). Previously, the application of cattle dung, *T. harzianum* and neem extract in various combinations has been found to be more effective in the management of bacterial blight disease of cowpea than treatment with single application of synthetic bacteriomycin (Dania and Oni, 2019).

Treatment combinations with *T. asperrellum* also significantly influenced rice growth and yield in this study. This is consistent with previous findings of Ritika and Utpal (2014) that *Trichoderma* species rapidly proliferated in the rhizosphere, thereby enhancing growth, development, crop productivity, resistance to abiotic stress, and the assimilation and utilization of nutrients by plants. Similarly, Harman (2006) and Faheem *et al.* (2010) reported that crop yield in fields could increase significantly after the addition of *Trichoderma* species, especially when seeds were previously treated with *Trichoderma* spores before planting in the field

Trichoderma asperellum had been reported to enhance optimization of available nutrients in the soil and thus was a good bio-stimulator of plant growth improving crop yield (Lopez-Bucio*et al.*, 2015).

Plants inoculated with the pathogen and treated with a combination of M. *oleifera* extract, cattle dung and Blastforce had significantly lower disease incidence and mean severity. Integrated management of rice blast disease using the combination of T. *asperellum*, cattle dung and blastforce significantly increased the number of leaves, tillers, panicles and grain weight which were the major determinants of good crop yield. Therefore, the combined application of naturally occurring biopesticides and cattle dung along with a synthetic fungicide is strongly recommended in the management of rice blast disease.

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