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Efficacy of several species of fungi bioagents and fungicides against *Magnaporthe oryzae in vitro*

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ABSTRACT

On rice (*Oryza sativa* L.), more than 70 diseases caused by fungi, bacteria, viruses, and nematodes have been listed. Among them, the blast disease caused by *Magnaporthe oryzae* B. Couch (syn. *Pyricularia oryzae* Cavara) seriously threatens global rice production worldwide. Effective management strategies such as the use of resistant cultivars and the application of fungicides individually cannot sustainably contain rice blast disease, because of the emergence of resistance genes and environmental threats. Therefore, applying bioagents to reduce *M. oryzae* infections, complete other strategies, and reduce pesticide dependence. The present work was carried out to evaluate the potential of not only plant rhizosphere-associated fungi in controlling *Magnaporthe oryzae* but also the effectiveness of five fungicides in coculture *in vitro*. Twenty-three biocontrol fungi mainly *Trichoderma* and *Penicillium* evaluated on six *M. oryzae* isolates were found to reduce pathogen growth in dual culture assay. The results obtained highlighted the good ability of bioagents to inhibit the growth of *M. oryzae* by 82.7 to 97% at 7 days after incubation. In addition, the fungicides tested namely Amistar, Horizon, Opu, Spartaktak, and Signum successfully inhibited the mycelial growth of *M. oryzae*. The major outcome of our study was evidence that all *M. oryzae* isolates were susceptible to the fungicides tested through their concentration (ppm) of 50% (EC50). No pathogen isolate was hypersensitive or resistant to fungicides with the EC50 average values of 0.24, 0.25, 0.28, 0.33, and 0.38 mg/l respectively for Spartak, Signum, Horizon, Amistar, and Opus. These good bioagents must be tested on plants in the greenhouse and the best of them could be used in IPM strategy with the combination of resistant cultivars and a reasoned use of fungicides for blast disease control.

Keywords: Rice, *M. oryzae*, bioagents, fungicide, EC50, *in vitro.*

INTRODUCTION

The main challenge of agriculture is the production of high quantity and quality food, safe, and affordable for a growing world population. Each plant species is frequently affected by hundreds of different pathogens (fungi, bacteria, mollicutes, viruses, insects, and nematodes) (Liu and Wang, 2016; Soura et

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al. 2020). Rice blast caused by the fungus *Magnaporthe oryzae* (syn : *Pyricularia oryzae* cavara) is the most devastating disease that can be measured using several different parameters, particularly its cosmopolitan geographical distribution (Ballini et al., 2008).

Despite the advent of improved varieties and increased trade, an increase in crop diseases has been observed (Lepoivre, 2003). On the other hand, monoculture, ignorance of the modes of contamination, the conditions conducive to the appearance of the diseases, the biology of pathogens, and their rapid development would cause significant economic losses (Hien, 2022). According to Thakur (2016), about 36.5% of the total losses are due to all kinds of pests (pathogens, insects, and weeds), of which 14.1% are caused by diseases. Farmers try to limit the risks by using current management strategies based on crop inadequate rotation, the continuous application of fungicides, and resistant varieties (TeBeest et al., 2015). According to Nguyen et al. (2016), cultivars with durable resistant genes are scarce in the field because of the pathogen's ability to evolve to overcome resistant genes. Also, the continuous use of chemical fungicides leads to the appearance of resistant strains of the pathogen under the pressure of natural selection, as well as the risks of toxicity, and ecotoxicity (Compant et al., 2005 ; Ngyen et al., 2016). According to Ballini et al. (2008), the costs of fungicides to control blast disease were estimated at 160 million Euros in Japan. However, its uses are still needed in the context of severe attacks and in IPM strategy.

Biological Control Agents (BCAs) are considered to be one of the best strategies, a better alternative to conventional agriculture, and a viable solution to current food challenges (Koné, 2022). A synthesis of available data suggested that viruses, bacteria, fungi, and oomycetes antagonistic are used to control a wide range of plant pathogens, on various crops, and are exploited as biopesticides (Moya et al., 2016; Köhl et al., 2019). BCAs are applied to control plant pathogens, where they act via a range of modes of action (Köhl et al., 2019). Most BCAs exhibit several mechanisms that may affect the disease triangle directly, indirectly, or synergistically (Wang et al., 2018). The direct mechanisms may prevent the growth or activity of the pathogens through competition for space and nutrients, antibiosis, production of hydrolytic enzymes, inhibition of pathogen-produced enzymes or toxins, and through ISR defense mechanisms (Conrado and Santos, 2017). Whereas, the indirect mechanisms may involve nitrogen fixation, the production of regulators such as auxins, cytokinins, and gibberellins, etc (Chagas et al., 2018). Their enormous diversity, associated with their ability to synthesize different secondary metabolites for pathogens control, promote plant growth, and enhance plant fitness has prompted research on these BCAs (Schiffers, 2011).

In this study, we highlight the contribution of BCAs, and fungicides as a strategy for controlling *M. oryzae in vitro*. Managing plant diseases using BCAs is a promising and environmentally friendly approach that can be used alone or in combination with other approaches in the context of Integrated Pest Management (IPM) in sustainable agriculture (Dubey et al., 2015). With this background information, the current study intended to evaluate the efficiency of the selected bioagents and fungicides in *M. oryzae* inhibition *in vitro* condition. The implementation of this strategy will permit to minimization of dependence on chemical fungicides in blast disease management and meet food demand in an environment-friendly approach.

MATERIALS AND METHODS

Antagonist bioagents dual culture with *Magnaporthe oryzae*

This work was conducted in the laboratory of phytopathology (FYMY) of the Catholic University of Louvain (UCL), Belgium, from February to August 2016. Twenty-three (23) isolates of biological control agents, from the PROBIOM project of the Catholic University of Louvain (UCL) isolated from the rhizosphere of maize seedlings, were

used in this study. These BCAs were screened for their performance in controlling fungal pathogen populations. The antagonism experiment was conducted using the dual culture technique (Romeiro, 2007) confrontation with six isolates of *M. oryzae* on PDA media *in vitro*, and ten 10 isolates for fungicides test. These bioagents were composed of 16 isolates of *Trichoderma*, 3 *Penicillium*, 2 *Fusarium*, 1 *Epicoccum*, and 1 *Cladosporium* (Table 1). Pathogen and bioagents were placed on opposite sides of a Petri dish, maintaining a distance of 3 cm. The evaluation of the inhibition exerted by the BCA on the pathogen was estimated after 6, 13, and 21 days of incubation according to the following formula:

Icd (%) = 100 x (1-(rcd/rt))

Icd: Percentage of inhibition of mycelial growth of the pathogen by direct contact,

rcd: Average radius of the pathogens in the presence of the candidate;

rt: Mean radius of control pathogens.

Isolates of *Magnaporthe oryzae* collection

In vitro control strategies of pathogenic fungi require their prior isolation from the host tissues and their culture on an appropriate medium. A judicious choice of the plant sample and its superficial disinfection, the deposit of the lesion on a nutrient medium often selective to obtain the pure culture (germination of a single conidium) are the steps of isolation of pathogens in general (Lepoive, 2003). Thus, leaf samples of *O. sativa* and *Oryza longistaminata* with blast lesions from Mali were collected (Figure 1) and used on RAL (Rice-Agar-Yeast) and PDA (Potato-Dextrose-Agar), before being incubated at 22°C temperature. The monospore cultures were derived from monospore clones, and germination (Table 2).

Fungi molecular identification

Molecular identification was performed based on fungal Genomic DNA extracted from the mycelial and conidium harvested from the Petri dishes containing PDA according to the inuPREP Plant DNAKit kit. The extracted DNA was recovered and purified in 1.5 ml tubes for PCR test. Molecular markers ITS4 and ITS5 rDNA regions were amplified, and the sequencing was done by the Macrogen company.

In phylogenetic analyses, the appropriate taxa for the analyses were initially selected following BLAST searches of GenBank (http://www.ncbi.nlm.nih.gov/). The sequence datasets were compared with similar sequences from other species in the *Magnaporthe* species already available in GenBank and mentioned by (Klaubauf et al., 2014). An overview of strains used in this study is presented in Table 3 above that were obtained from public databases. The phylogenetic analyses were done using the Molecular Evolutionary Genetics Analysis (MEGA) software approach we built automatically the phylogenetic tree and the evolutionary history was inferred using the Maximum Parsimony method (Kumar et al., 2018).

Fungicides

Five of the most used and currently recommended fungicides for cereals representing different groups were selected. Between them, Amistar (Azoxystrobin 250 g/l) from Strobilurins chemical family, which is a polyvalent systemic and translaminar fungicide is distributed by Syngenta Crop. Its approval number is L1382-56; 8898/B (Phytoweb. be and Sage pesticide). Horizon (Tebuconazole 250 g/l), chemical family Triazole, is a systemic fungicide. Its approval numbers are 8354P/B, No L 01258-017 and formulated as an aqueous emulsion (EW), based on 25% of Tebuconazole (Sage pesticide). Opus (Epoxiconazole 125 g/l) also belongs to the chemical family Triazole, systemic fungicide, foliar preventive, and curative. The approval number is 8472/ L01270042. Epoxiconazol (125 g/l) is use in Emulsifiable Concentrate (EC), manufactured by BASF AG (Phytoweb. be). About, Spartak (Prochloraz 450 g/l), is a broad-spectrum penetrating fungicide for controlling cereal diseases with the approval number L 00916-04; 7322P/B. It is formulated

in Emulsifiable Concentrate (EC) containing 45% Prochloraz and 6,7% pyraclostrobin distributed by BASF /l and). It is a unique Belgium (Phytoweb.be). Signum is composed of pyraclostrobin (Qol) and boscalid (SDHI), two complementary molecules (Boscalid 267 g fungicide with a broad spectrum of action, specifically for fruits and vegetables. The approval number is 9429P/B. It is formulated in the form of granules to be dispersed in water (WG) and distributed by BASF Belgium (Phytoweb.be) (Table 3).

Effects of fungicides on spore germination and mycelial growth

To determine the effectiveness of fungicides and their median effective concentration (EC50) against *M. oryzae* (i.e. the level of susceptibility of the isolates), five fungicides were tested on ten isolates of the pathogen. These tests were performed on potato dextrose agar (PDA) amended with four-fold concentrations of each fungicide from 0.01, 0.1, 1, and 10 mg/L. Three Petri dishes were used for each treatment and the tests were repeated twice. In each Petri dish, 3 discs of mycelium were placed on the agar equidistant from the edge of the Petri dishes, and on the other discs, mycelium facing down (Figure 2). Three control treatments without fungicide were also inoculated with each pathogen isolate. The Petri dishes were then incubated in the darkness at 22°C and the mycelial growth of the different isolates measurements were done at, 3, 7, and 10 days after incubation (DAI). EC50 values were calculated from the regression equation of each treatment by applying the value of 50% of the mean diameters of mycelial growth of the controls to the formula.

Statistical analysis

The bioassays were run in a completely randomized design with three replications, and three plates of each pathogen isolate without an antagonist or fungicide used as control. All data were collected and prepared in Microsoft Excel. All data were tested for normality and homogeneity before analysis of variance (ANOVA). Statistical analysis of variance (ANOVA) was performed using R software, and the averages were compared with the Scott Knott test served to determine statistically significant differences which were accepted on the 95% significance level ($P < 0.05$). The percentage of fungi growth inhibition was obtained from the germination of the control treatment.

Also, the lethal dose for fungi germination expressed about the concentration of fungicides (ppm) that caused 50% inhibition (EC50) was determined. The method of probit-log dosage ($y = alogx + b$) analysis was utilized for determining the EC50 assuming that the tolerance of fungi to concentrations of fungicides is a normal distribution (Sena et al. 2013). The method consists in estimating the linear regression of the probit value, corresponding to the percentage inhibition of fungi growth on log concentration of fungi.

Table 1: Strains of biological control agents tested.

Codes	Fungi	morphological Id	Total plant sample
BC627	*Trichoderma*	Trico 3 green	477
BC487	*Trichoderma*	Trico 3 green	469
BC562	*Trichoderma*	Trico big balls	470
BC474	*Trichoderma*	Trico green dark	466
BC580	*Trichoderma*	Trico 3 green	280
BC592	*Trichoderma*	Trico 3 green	465

BC584	*Trichoderma*	Trico green dark	473
BC528	*Trichoderma*	Trico green dark	466
BC656	*Trichoderma*	Trico fine green	473
BC681	*Trichoderma*	Trico fine green	469
BC518	*Trichoderma*	Trico 3 green	465
BC170	*Trichoderma*	Trico green dark	469
BC664	*Trichoderma*	Trico green dark	465
BC768	*Trichoderma*	Trico 3 green dark	473
BC628	*Trichoderma*	Trico fine green	473
B008	*Trichoderma*		332
BC195	*Penicillium*	Pen green	425
BC573	*Penicillium*	Pen green /orange	483
BC754	*Penicillium*	Pen orange/ green/black	382
BC187	*Fusarium*	*F. mauve* pts black	258
BC621	*Fusarium*	*F. poae* gray	332
BC706	*Epicoccum*	Epicoccum	280
BC51	*Clodosporium*	Clodo/Pen green	300
BC852	*Bionectria*	*B. ochroleuca* (green)	287

Id: identification.

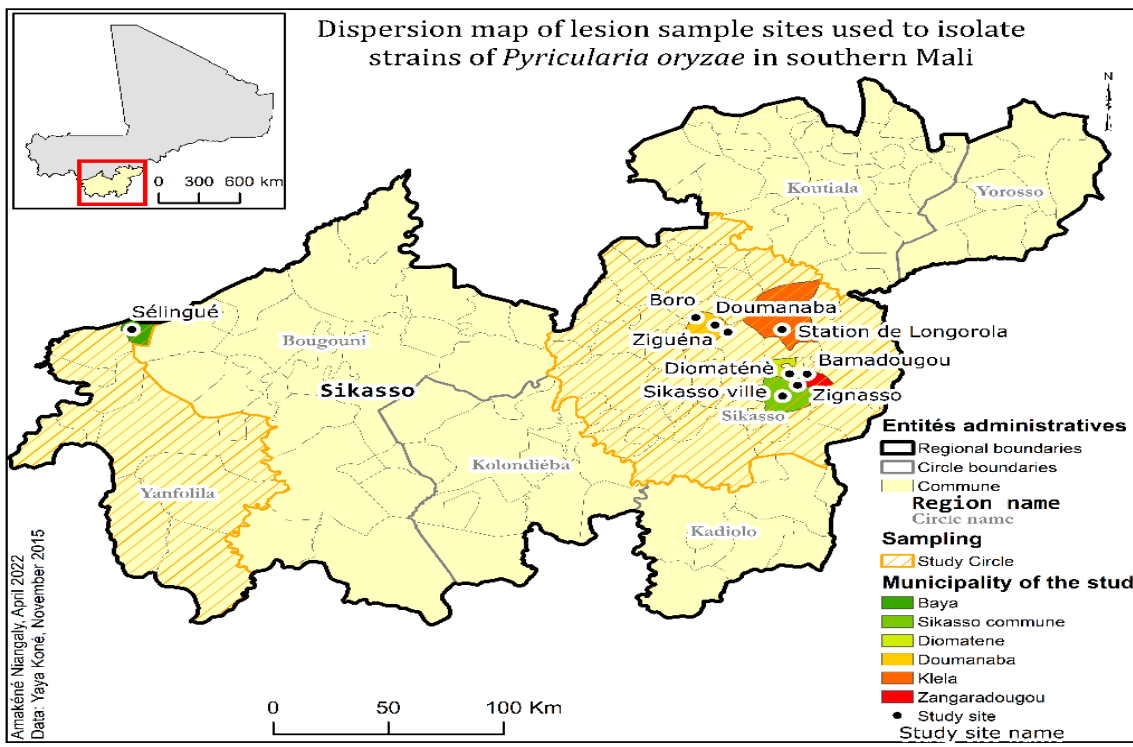

Figure 1: Distribution map of plant tissues sampling area in South of Mali.

Table 2: Isolates of *Magnaporthe oryzae* with the localities.

Isolates codes	Localities	Field	Isolates codes	Localities	Field
M01	Station of Longorola	Greenhouse II	**M13**	Sikasso city	Field 4
M02	Station of Longorola	EMD	**M14**	Station of Longorola	Greenhouse 1
M03	Sélingué	Sector B21	**M15**	Station of Longorola	Canal I
M04	Sélingué	Sector B17	**M16**	Sikasso city	4 ponts
M05	Sélingué	Sector A15	**M17**	Sikasso city	BCEAO
M06	Sélingué	Sector A18	**M18**	Bamadougou	Field 3
M07	Sélingué	Sector A22	**M19**	Sélingué	Sector A21
M08	Sélingué	Sector A24	**M20**	Sélingué	Sector A17
M09	Zignasso	Field 2	**M21**	Sélingué	Sector A19
M10	Bamadougou	Field 6	**M22**	Sélingué	Sector B12
M11	Bamadougou	Field 7	**M23**	Sélingué	Sector B16
M12	Sikasso city	Field 3	**M24**	Sélingué	Sector B11

Table 3: The fungicides use.

Commercial name	Active mater	Concentration	Chemical family	Formulation
Amistar	Azoxystrobin	250g/l	Strobilurin	SC
Horizon	Tebuconazole	250g/l	Triazoles	EW
Opus	Epoxiconazole	125g/l	Triazoles	EC
Spartak	Prochloraz	450g/l	Imidazole	EC
Signum	Boscalid + Pyraclostrobin	267g/l + 67g/l	Strobilurin	WG

SC : suspension concentrate, EW : Emulsion-in-Water, EC : emulsifiable concentrates WG : Water-dispersible granules.

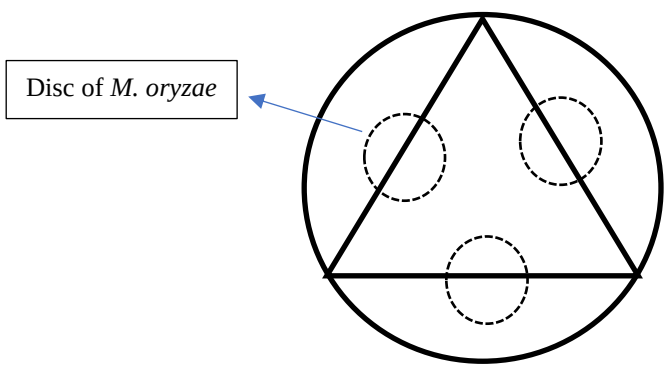

Figure 2: Method of *Magnaporthe oryzae* growing on culture media amended with fungicides.

RESULTS

Phylogenetic analysis of DNA sequence for *Magnaporthe* species identification.

ITS, the dataset was analyzed using the Maximum Parsimony method. The result of the phylogeny analysis showed that the single locus phylogeny of the internal transcribed spacer (ITS) region placed all *Magnaporthe* isolates from *O. sativa* and *Oryza longistaminata* into *Magnaporthe* oryzae (Figure 3). According to Chaverri et al. (2015), misidentification of a cryptic taxon by the use of a collective name may have negative consequences for strategic matters in the industry, plant quarantine, human, and animal health. Therefore, for a good identification, it is necessary to combine ITS with other markers such as the largest subunit of RNA polymerase II (RPB1), large subunit (LSU) of rDNA, and translation elongation factor 1-alpha (TEF1).

The alignment showed that all the sequences were identical except for 2 places. On one side, the M15 and M16 strains have the Guanine substituted by thymine (Single Nucleotide Polymorphism (SNP)). On the other hand, the M18 and M20 differ from the others by having a Guanine in the place of Alanine at another place in the sequence of all the 10 strains sequenced, there are 3 different types of sequence which testify to a certain diversity.

Effectiveness of bioagents on mycelial growth of *Magnaporthe oryzae*

BCA strains of the temperate region were cocultured with six isolates of *M. oryzae*. Mycelial growth inhibition of *M. oryzae* was measured at 13 DAI and the type of interaction occurring between the antagonistic fungi was observed using the scale rating proposed by Scauflaire et al. (2011), and Mayaka et al. (2019). The results showed the inhibition rate of each isolate of *Magnaporthe* per BCA (Table 4). It suggested that the *Magnaporthe* isolates tested have the same behavior in dual culture with all the bioagents used. ANOVA showed at 13 DAI, 21 of the BCAs inhibit the growth of the pathogen between 82.74 to 96.98%. The other four bioagents including *Fusarium* BC187, *Cladosporium* BC51, *Penicillium* BC754, and *Bionectria* BC851 which inhibit *M. oryzae* mycelial growth below 70% seem to be not interesting enough in this case (Figure 4).

Therefore, as *M. oryzae* is a polycyclic agent, it is important to focus on a precise and fairly short time of inhibition at which the pathogen can be controlled by the bioagent without causing major damage to the production and winning the time. A period of 21 DAI would be long for the control of blast because when the environmental conditions are favorable can quickly infest the rice crop. It is, for this reason, this study limits the data at 13 DAI.

Evaluation of the sensitivity of *M. oryzae* to different concentrations of fungicides

This result represents the evolution of the means of eight isolates of the pathogen with different fungicides (Figure 5). This test showed that all the fungicides tested have a good inhibitory action on the mycelial growth of *M. oryzae* at 7 days after incubation. Doses 0.1; 1 and 10 mg/L seem to be a good action on the control of the pathogen because the percentage of inhibition was high for all the fungicides in comparison to the 0.01 mg/L. No *Magnaporthe* strain showed hypersensitivity or resistance to the fungicides tested. Of the 5 fungicides tested, the least effective at low concentrations were those usually used in the control of blast disease, namely Opus and Amistar (Figure 5).

Effets of fungicides on spore germination and mycelial growth

The effectiveness of five fungicides such as Amistar (Azoxystrobin), Horizon (Tebuconazole), Opus (Epoxiconazole), Sportak (Prochloraz), and Signum (Boscalid+Pyraclostrobin) was evaluated on nine isolates of *M. oryzae*. Table 6 illustrates the Effective Concentration 50 (EC50) of fungicide against *M. oryzae* on a solid culture medium (PDA) containing different concentrations of fungicides in the function of time. On the dishes amended with fungicides,

tiny poor-growing fungal colonies were observed at the highest rate of 10 mg/L. The concentration of fungicides (ppm) that caused 50% inhibition (EC50) of pathogen was determined based on the linearization of the curves which show the percentage inhibition of the pathogen growth in the function of the concentration at 7 DAI. Thus, the concentrations were transformed into logarithms using the equation y = alogx + b (Table 6) after linearization (Figure 6). Therefore, (**y)** represents the percentage of growth inhibition of the pathogen and (**x)** is the value of EC50 to look for. Spartak (Prochloraz) showed the lowest EC50 value and was followed by Signum, Horizon, and Amistar with respectively 0.24, 0.25, 0.28, and 0.33. The most inefficient was Opus (Epoxiconazole) with 38 (Table 5). These values showed that all tested products have good inhibitory action on the growth of the different isolates of *M. oryzae*. None of the isolates showed resistance to the fungicides tested. However, the EC50s of the M23 and M16 strains are higher than those of the others for almost all the fungicides used. Amistar (Azoxystrobin) belongs to the group of strobilurin fungicides, with a systemic and translaminar action on the plant.

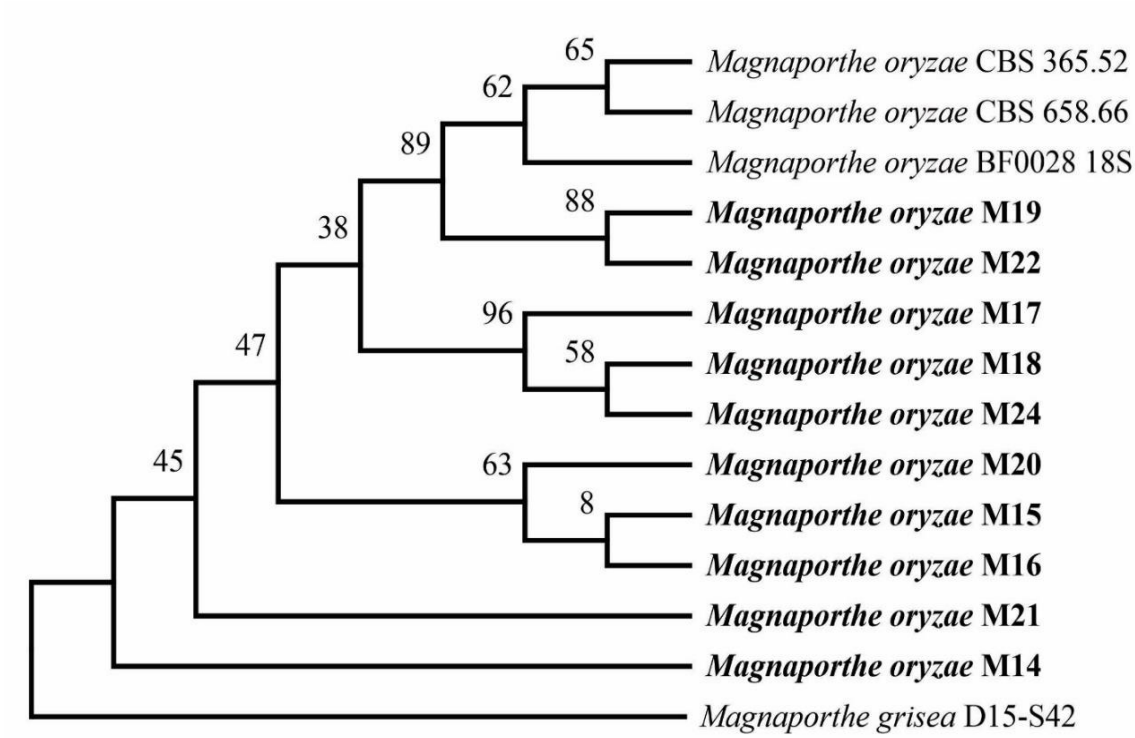

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Figure 3: Phylogenetic tree inferred from a Maximum Parsimony analysis of taxa based on analyses of a concatenated alignment of ITS sequence of rADN of 14 strains representing the genus *of Magnaporthe* from suthern of Mali.

Table 4: Inhibition rate of each isolate of *Magnaporthe oryzae* in function of bioagents.

Codes	BCA genus	M01	M05	M08	M09	M10	M13	Mean	Grouping	StDev
B008	*Trichoderma*	96.77	97.14	96.88	97.5	96.85	97.12	97	A	0
BC474	*Trichoderma*	96.77	97.14	96.88	95.83	96.85	97.12	96.87	AB	0.28
BC664	*Trichoderma*	96.77	97.14	96.88	96.67	95.79	97.12	96.83	AB	0.28
BC627	*Trichoderma*	96.77	97.14	96.88	97.5	96.85	95.2	96.87	AB	0.56
BC487	*Trichoderma*	96.77	97.14	96.88	96.67	96.85	95.2	96.81	AB	0.85
BC681	*Trichoderma*	96.77	97.14	96.88	97.5	96.85	94.24	96.87	AB	0.85
BC592	*Trichoderma*	96.77	97.14	96.88	94.17	96.85	97.12	96.87	AB	0.28
BC656	*Trichoderma*	96.77	97.14	96.88	94.17	96.85	97.12	96.87	AB	0.28
BC628	*Trichoderma*	96.77	97.14	96.88	97.5	96.85	93.28	96.87	AB	0.28
BC584	*Trichoderma*	95.7	97.14	96.88	95	95.79	95.2	95.75	ABC	0.28
BC768	*Trichoderma*	96.77	97.14	96.88	94.17	96.85	92.12	96.81	ABC	0.82
BC706	*Epicoccum*	96.77	97.14	96.88	89.17	96.85	88.47	96.81	ABC	0.28
BC621	*Fusarium*	96.77	94.29	96.88	94.17	96.85	89.43	95.53	ABC	0.49
BC170	*Trichoderma*	93.55	94.29	96.88	96.67	93.69	90.39	93.99	ABC	0.28
BC528	*Trichoderma*	93.55	94.29	96.88	93.33	95.79	90.39	93.92	ABC	0.56
BC580	*Trichoderma*	94.62	97.14	96.88	93.33	93.69	88.47	94.16	ABC	0.75
BC518	*Trichoderma*	95.7	89.52	96.88	93.33	91.59	82.71	92.46	CDE	1.49
BC562	*Trichoderma*	86.02	85.71	87.5	90.83	92.64	90.39	88.95	DE	1.02
BC573	*Penicillium*	93.55	70.48	96.88	88.33	93.69	84.63	90.94	EF	3.43
BC195	*Penicillium*	91.4	80	62.5	89.17	90.54	88.47	88.82	F	2.44
BC187	*Fusarium*	60.22	65.71	57.29	65.83	62.15	69.74	63.93	G	1.76
BC51	*Cladosporium*	54.84	56.19	52.08	68.33	61.09	49.09	55.52	H	1.41
BC754	*Penicillium*	45.16	54.29	46.88	61.25	44.27	56.77	50.59	I	2.54
BC851	*Bionectria*	35.48	45.71	47.92	57.5	60.04	47.17	47.55	I	2.95

For the grouping column, the values followed by the same letter are not significantly different at a threshold of 0.05, according to Tukey's test. DAI : indicate day after incubation ; StDev: represent standard deviation of each percentage average. The different colors represent the percentage of efficiency of each bioagent isolate: dark green (more than 90%); light green and dark blue (89 to 70%); and light blue and red (less than 70%).

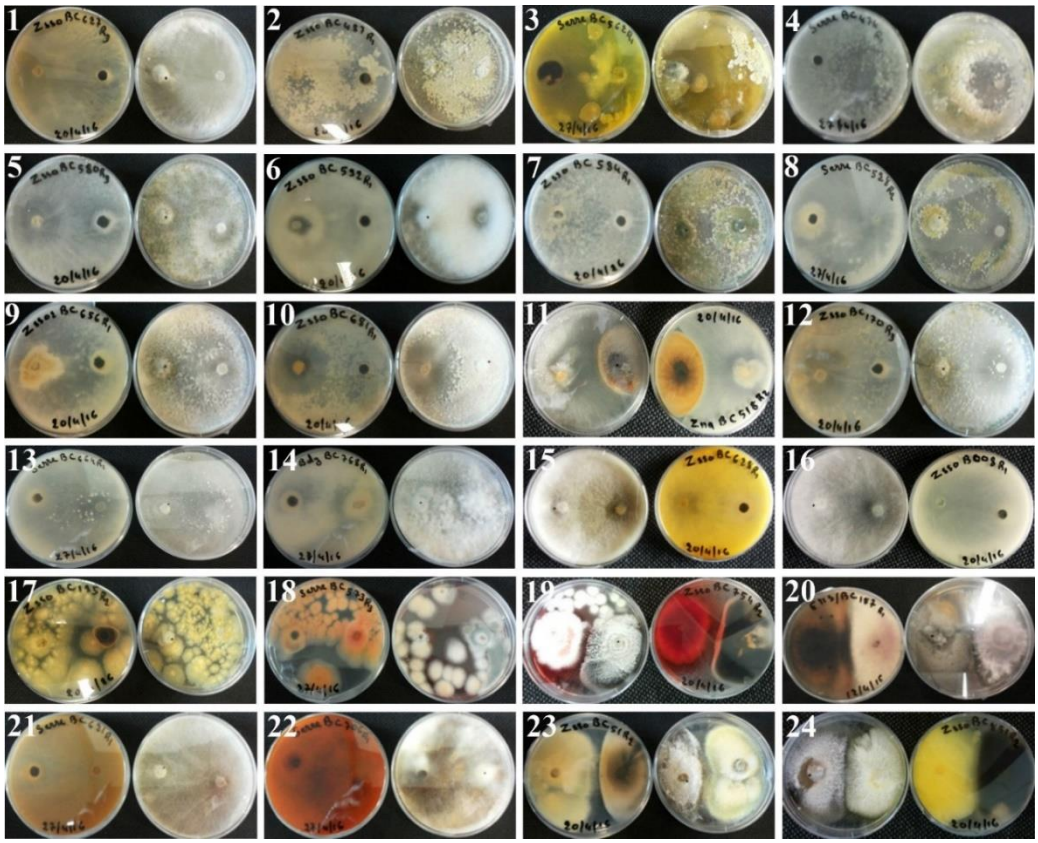

Figure 4: Dual culture of *M. oryzae* to evaluate its antagonism potential and systems.
In these Petri dishes, the fungi *M. oryzae* is in black color and the other colors represent the bioagents. Using scale of the type of antagononism proposed by Scauflaire et al. (2015), three different types of interaction between antagonists were observed viz /Antagonists touch each other; // : Presence of inhibition zone. For three BCAs, a zone of inhibition appears between the candidate and the pathogen, including *Penicillium* (BC754), *Fusarium* (BC187), and *Cladosporium* (BC51); A: colonization of the pathogen. Other candidate BCAs colonized the mycelium of the pathogen mainly by *Trichoderma* and constitutes the best candidate. Another type of antagononism was observed between BC851 and *M. oryzae* touched each other without a zone of inhibition.

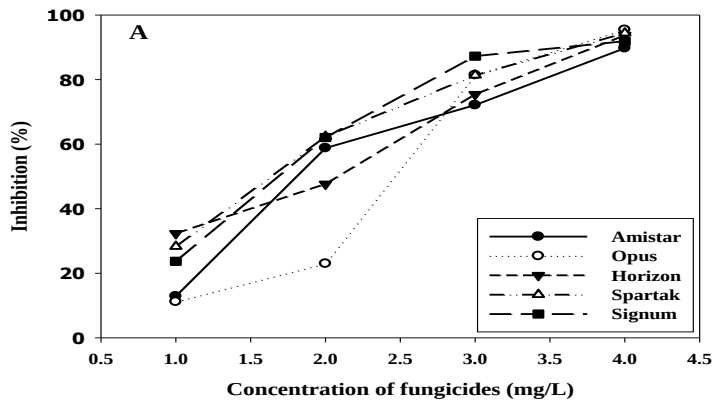

Figure 5: Percentage inhibition of fungicides on *M. oryzae* growth at different fungicide concentrations.

Table 5: Effective Concentration 50 (EC50) value of fungicides against *Magnaporthe oryzae*.

Fungicides	Equations	EC50 (y = alogx + b)
Amistar	y = 24.39x - 2.6089	0.33
Horizon	y = 21.183x + 9.3071	0.28
Opus	y = 31.118x - 25.048	0.38
Spartak	y = 21.73x + 12.253	0.24
Signum	y = 22.975x + 8.7714	0.25

y : represent the percentage of growth inhibition of the *M. oryzae*
x : the value of EC50 to look for.

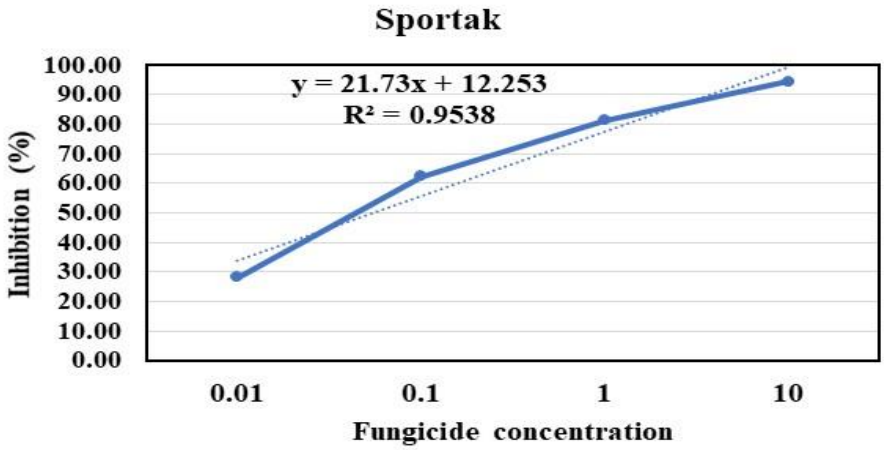

Figure 6: Example of linearization curve for *Magnaporthe oryzae* on Spartak product.

DISCUSSION

The result of dual culture showed that 21 of the BCAs inhibit the growth of the pathogen between 82.74 to 96.98% at 13 DAI. The other four bioagents including *Fusarium* BC187, *Cladosporium* BC51, *Penicillium* BC754, and *Bionectria* BC851 which inhibit *M. oryzae* mycelial growth below 70% seem not to be interesting enough in the study. An emerging technique of interactions between microorganisms, which is often termed 'co-culturing', imparts stress on microorganisms by forcing them to compete for resources, and this may serve to stimulate the biosynthesis of unique secondary metabolites, which may provide a competitive advantage to these organisms (Conrado and Santos, 2017). It has been shown that co-culturing can activate novel biosynthetic pathways with signaling molecules, indicating that the physical interactions between fungi are important for the activation of new metabolites (Chagas et al., 2018). Koné et al. (2020) reported that five strains of *T. harzianum* inhibited the growth of the pathogen with antagonism coefficients between 0.50 and 0.78. Some trains of *T. harzianum* were effective against *Fusarium solani*, *Fusarium oxysporum*, and *Aspergillus niger* in dual culture, which inhibited the pathogen growth by 71-84% in 5 days (Dabiré et al., 2016). In addition, Ghorri et al. (2013), showed the efficacy of two *Trichoderma* isolates (sp1 and sp2) against strains of *Fusarium* wilt with mycelial growth inhibition rates greater than 65% at 6 days (Ghorri, 2016). Furthermore, *T. harzianum* T-aloe inhibited the growth of *Sclerotinia sclerotiorum* at the rate of 56.3% in protecting soybeans (Zhang et al. 2016). The results of all these studies are consistent with this study. Fungi could

overcome competition in various ways, such as rapid growth, sporulation, stress recovery, and negation of inhibitors (Koné, 2022). In this present study, three different types of interaction between antagonists were observed such as competition for nutrients in which antagonists touch each other, antibiosis (presence of inhibition zone between antagonists) using the scale of the type of antagonism proposed by Scauflaire et al. (2015). Before starting another step of this research, the better identification of these BCA is paramount as advocated by C inhibited at 300 ppm (Lamrani et al., 2012). This result is confirmed by Bahous et al. (2006), who demonstrated that azoxystrobin applied in a single treatment protected rice leaves against five fungal pathogens for at least two weeks. However, recent studies provide solid evidence, that many resistance problems with fungicides are demonstrated. According to Dorigan et al. (2019), *Pyricularia graminis-tritici* on wheat was assessed to be resistant to the triazole fungicides tebuconazole and epoxiconazole phenotypically based on EC50 values and molecular analysis of the presence of mutations in the CYP51A gene. A study by Wang et al. (2008) showed that Tebuconazole used against *Rhizoctonia solani* on rice inhibits the growth of mycelium with an EC50 value of 0.51 mg/l. Also, 58.03 and 62.53 mg/l in EC50 of Tebuconazole has a long persistence effect on rice plants inoculated with *R. solani*. However, it should be noted that the two tests converge concerning the low EC50 values to control these both pathogens. The test of six fungicides against two maize diseases found that azoxystrobin inhibited fewer pathogens with an EC50 value greater than 0.1 mg/l for maize. two pathogens (*F. subglutinans* and *F. tempera*) (Shin et al., 2014). In addition, Kim et al. (2003) revealed through *in vitro* tests, 25 isolates *Pyricularia grisea* of resistant to the levels of Azoxystrobin and got the EC50 ranging from 2.93 to 24.89 mg/l. Compared to our study, the EC50 of this fungicide is 0.16mg/l. This implies that strains from Mali are sensitive to Azoxystrobin. Also, in the test of Chen et al. (2013) on 90 different Epoxiconazole concentrations ranging from 0.11 to 0.86 mg/l on *M. oryzae* isolates, they found that the pathogen germinated at 0.11 to 35 mg/l and the good inhibitory effects started at 0.46 mg/l.. Whereas in the present study, the EC50 of Epoxiconazole was 0.38mg/l. We hypothesized that this fact is due to the scarcity application of fungicides to control rice blast disease in Mali. This order was retained in the present study where Sportak and Horizon have EC50 values lower than Amistar on *M. oryzae* isolates of Mali. According to Serge et al. (2021), mycelial growth of *Phytophthora katsurae* was dependent on the products and its concentrations. It had been suggested that the routinely use of chemical fungicides causes several negative effects such as the development of pathogen resistance to the applied agents, and their nontarget environmental impacts (Compant et al., 2005), in addition to the pathogens mutant selection strains (Nguyen et al., 2016). Management of rice blast has relied on treatment with fungicides because resistant cultivars become vulnerable within a few years after release (Ballini et al., 2008). Nguyen et al. (2016) reported that cultivars with durability are scarce in the field because of the pathogen's ability to evolve to overcome resistance genes. Therefore, these fungicides should be alternated with others with different modes of action to avoid pathogen resistance.

Conclusion

Most of the bioagents used in this study have shown promising results in the laboratory against *M. oryzae in vitro*, which could be an alternative, a complement, and/or in combination with another strategy. Rice seeds soaking in the suspensions of bioagents and/or their introduction before the arrival of the agent could be useful. The fungicides should be alternated with others with different modes of action to avoid pathogen resistance. Understanding, optimization, and combination of control strategies including varietal improvement, agronomic management, biocontrol, and the rational use of synthetic fungicides would be an integrated, and environmentally friendly approach accessible to farmers. In order to have a decision rule on the application of the efficient BCAs of this study, trials in the greenhouse, station, and then in the field could be envisaged with

Trichoderma, *Penicillium* and *Epicoccum* isolates.

COMPETING INTERESTS

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this manuscript.

AUTHORS' CONTRIBUTIONS

YK was responsible for organizing the data, writing the paper, and making the figures. MBS was responsible for writing the paper and organizing the data. AN was responsible for mapping and organizing the figures. KD was responsible for overseeing the project and writing/editing the manuscript. MK was responsible for collecting the sample of diseased leaves in the field and the georeferncement.

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