Antifungal Effects of Selected Bio-Controls Against *Rice Blast Fungus (Magnaporthe Oryzae)*

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Abstract

Rice blast caused by *Magnaporthe oryzae* is a devastating fungal disease of rice globally affecting all plant parts and seeds. Use of fungicides is the major management method of the disease; however, fungicides have negative impacts on environment and human health. The objective of this study was to determine the potential efficacy of *Trichoderma* isolates, BG-1, SYA-E, BRO-2, SYA-C, EM-B and vermiwash against rice blast fungus *(M. oryzae).* The assay was carried out in the laboratory in a Completely Randomized Design (CRD) with three replications. *M. oryzae* fungus was isolated from naturally infected rice tissues of variety ITA-310 in west Kano irrigation scheme by sporulation of the pathogen on a moist chamber and then streaked on 2% water agar media and cultured for five days. Both *Trichoderma* and *M. oryzae* isolates were cultured in PDA (Potato Dextrose Agar) for 7 days and inhibition tested using dual culture method. A disc plug of 8 mm radius of *M. oryzae* and 5 *Trichoderma* isolates were picked from the periphery of the mycelium and placed 7cm apart in 9cm PDA plates under aseptic conditions. Rice straw vermiwash harvested after 14 days from the vermicomposting structure and diluted to 10% was streaked at the edge of the agar plate with a test pathogen aseptically. The inoculated petri dishes were incubated at 25±2 ºC and data on radial growth in mm of the 5 *Trichoderma spp* and vermiwash against M. oryzae colonies was collected at 48hrs, then after every 24hrs for 7 days. The 6 bio controls were significantly different for antifungal activity at $p \leq 0.0001$. However, the control was significantly different to all the biocontrol's at $p \leq 0.05$. The biocontrol that exhibited the highest antifungal activity against M. oryzae were Vermiwash, EM-B, SYA-C, and BRO-2 with inhibition of 72.81%, 71.49% and 71.05%, respectively exhibiting potential to be used as controls for rice blast disease. Additional research of the selected best performing *Trichoderma* isolates and vermiwash need to be evaluated under green house and field experiments to suppress rice blast disease for enhanced rice productivity.

Key words; Vermiwash, Biocontrol, Invitro, Isolates, Rice Blast

INTRODUCTION

Suppression of diseases is a biological process involving interaction of the host plant, pathogen and the environment. Different strategies can be used individually or in combination with others in a view to manage the plant diseases (Manivannan *et al.,* 2012.).Chemical pesticides and fungicides have been extensively used for control of the diseases and pests successfully however; a shift in the attitude towards use of this chemical products in agriculture due to strict regulation on some fungicides, miss use of the chemicals, residue left overs on the crops and spread of diseases to natural ecosystem have been noted.

This has prompted researchers to focus on finding alternatives to synthetic chemicals in controlling pest and diseases referred to as biological controls. Biocontrol's are based on plant parts /products, microorganism such as bacteria viruses and fungal and bacterial enzymes inhibitors (Manivannan *et*

al., 2012.). They are cost effective; give protection throughout the cropping period, effective in controlling specific plant diseases, less toxic to plants and safe to the environment. Fungus *Magnaporthe oryzae* is a devastating air borne pathogen that attacks rice at all stage of growth infecting the aerial parts of the plant (Mutiga *et al.,* 2017). The disease is managed by use of fungicides such as carbendazims, benzoyl, and tricocyclazole among others however; these chemicals have diverse effect on the environment, human health as well as development of resistance by the pathogen. Biological control of rice blast fungus majorly relies on use of antagonistic mycoparasites and induction of host plant resistance.

Trichoderma *sp* are key biocontrol agents of plant pathogenic fungi also known as mycoparasites with a potential to directly affect fungal pathogens and induce resistance in plants. Over 300 different species of Trichoderma have been morphologically and genetically identified, and known to spread widely over different ecosystems. This mycroparasite have gained popularity as a biocontrol because of: secretions of lytic cell wall degrading enzymes CWDE`s, root nutrient competition and induction of systemic resistance in plants. Crude extracts of Trichoderma isolates indicated antimicrobial activities towards spore germination of all plant pathogenic fungi.(Tamandegani *et al.,* 2020)
Vermiwash a by-product of vermicomposting has exhibited potential in its application as a biocontrol agent against pest and disease (Che Sulaiman & Mohamad, 2019). Other studies have proved that organic amendments when applied to plants induces resistance of some pest attacks and disease resistance compared to synthetic fertilizer (Arancon *et al.,* 2005).

Past studies have shown that major factors determining efficacy of compost vermiwash in inhibiting progression of plant disease is their composition of microbial communities linked to organic wastes (Fernández-Gómez *et al., 2011).* Vermiwash has microorganisms that can suppress plant diseases through complex mechanism like antibiosis, competition through production of massive siderophores, induce systemic resistance (ISR) as well as acquired systemic resistance (ASR). (Mehta *et al.,* 2014)
Consistent performance of vermiwash was noted on a number of trials over a wide condition of the rhizosphere, however, minimum research have been conducted against foliar plant pathogens. Therefore, studies of foliar spray of vermiwash against foliar plant pathogens needs further validation (Khan et al., 2015).

Material and methods

Collection of fungal spores from rice blast diseased leaves in the field.

Sampling of the paddy fields was done in West Kano and Busia counties where naturally infected leaves were identified and collected. The infected rice tissues carefully packed in dry brown, small sized coin envelopes and taken to the laboratory for preservation and isolation. Water agar (WA) solid medium (2%) was prepared by dissolving 20g of agar powder in 1000 ml of water, autoclaved at 121^0C for 15 minutes and cooled down to 50°C. 12mls of the media was dispensed into 6-cm-diameter plastic dishes waiting for isolation process. Rice bran agar and 2% water agar media was prepared by mixing 1000 ml of water with 20g rice bran and 20g of agar powder, autoclaved and cooled down to 50°C. Approximately 12 ml of the media was dispensed into 6-cm-diameter plastic petri dishes and allowed to solidify in readiness for sporulation of the fungus.(FEI *et al.,* 2019). Rice blast fungus was isolated from infected rice tissues by sporulation of the pathogen on a moist chamber and then streaked on 2% water agar media. Spores were identified by observing lesions of the diseased rice tissues using a dissecting microscope in a laminar airflow hood. A single spore was picked with a sterile wire loop, put in plates containing water agar media and incubated for 7 days at 25^{o}C until germination. The germinating single spores of 4mm^2 was cut and transferred into petri dish containing Rice Bran Agar

(RBA) media. Under a sterile condition in a laminar airflow hood, steel forceps was used to pick 5 pieces of sterilized Whatman filter paper cut into $2mm^{2,}$ and put around the germinating spores. The petri dish was containing the pathogen was incubated at an average temperature of 25 ºC with a 12-hour photoperiod for 21 days. The colonized Whatman filter papers were peeled off from the media, placed on lidded plastic container containing silica gel desiccant for 10 days at room temperature to dry. The dried filter papers containing the fungus were put in sterile coin envelops inside a lidded plastics and stored at -20^{o}C for future use.

Determination of antifungal effects of the six-biocontrol agents on *M. oryzae* was conducted at KALRO-Sugar Research Institute in Kisumu County. The biocontrol comprised 5 *Trichoderma* spp and rice straw vermi juice at 10% dilution. The assays were carried out in the laboratory and layed out in a Complete Randomized Design (CRD) with 3 replications. *M. oryzae* isolate and the biocontrols were cultured in Potato Dextrose Agar (PDA) for 7 days and inhibition tested using a dual culture method. Using a sterile cork-borer, 8 mm disc plug of *M. oryzae* and the *Trichoderma* isolates were picked from the periphery of the colony and placed 7cm apart in a PDA plates (9cm in diameter) under aseptic conditions. The inoculated petri dishes were further incubated at 25±2 ºC temperature and radial growth of *M. oryzae* colonies measured after 7days. Vermiwash was collected from the vermicomposting structure after 14days and diluted to 10%, streaking was done at the edge of the agar plate with a test pathogen in a laminar airflow chamber. The petri-dishes with the test pathogen were put in the growth chamber at 25 ± 2°C, with 12 h day/night cycle. The diameter of the *M. oryzae* colony was measured at 48 and 72 hrs after inoculation according to Akinnuoye-Adelabu *et al* (2019).

After 21 days of incubation at 25^{o}C with 12-hour photoperiod plate culture of *M.oryzae* were colonized by the fungus and a dusty grey appearance mycelium seen to have grown occupying the culture plates and the Whatman filter papers (Zewdu, 2021). About 3ml of distilled and sterile water was added on the 21 days dish with *M.oryzae* and plastic cell scrapper was used to gently harvest mycelium and fungal conidia for observation. Conidial cell count/concentration was performed using a hemocytometer and biological microscope at a 100-magnification. Observed was a conidiophore that appeared swollen with a fusiform shape with 2 septet and translucent colors. (Zewdu, 2021)

Figure 2: *Dusty grey mycelial growth on petri dish and germinated single M oryzae spore observed under microscope*

A total of the 6-biocontrol agents comprising of five *Trichoderma* isolates identified as BG-1, SYA-E, BRO-2, SYA-C, EM-B and vermiwash were tested for antifungal activity against rice blast fungus

M. oryzae. These micro-organisms are documented as having potential to inhibit the growth of several fungal plant pathogens including *Pyricularia spp.* The radial growth of the colony was compared against the controls and the antagonistic effect of the selected biocontrol agents against *M.oryzae* calculated according to (Yadav, 2018)

$$\text{percentage inhibition, } PI\ (\%) = \frac{C - T}{C} \times 100\%$$

Where, PI= C = Pathogen radial growth in cm in control; T = Radial growth in cm in treated plates.

RESULTS AND DISCUSSIONS

Out of the 6 bio controls agents 2 of them Vermiwash at (72.9%) and one *Trichoderma* isolate EM-B (71.3%) showed maximum inhibition *against M..oryzae* and further carried forward to green house and field experiments. The rest of the four isolates exhibited different degrees up to 71.01% of growth inhibition as shown in table 1 below.

Table Percentage growth inhibition or *Trichoderma* isolates and Vermiwash against rice blast fungus *(Oryzae*). Results indicate mean of the three replications with standard deviation

SNo	Biocontrol	Radial Growth(mm)	% Growth Inhibition	
1	BG-1	7.11 ±0.057	70.8±	B
2	SYA-E	7.17±0.193	68.4±	B
3	BRO-2	7.11±0.840	70.08±	B
4	SYA-C	6.64±0.0918	71.01±	B
5	EM-B	6.51±0.0154	71.3±	B
6	V. JUICE	6.16±0.0750	72.9±	B

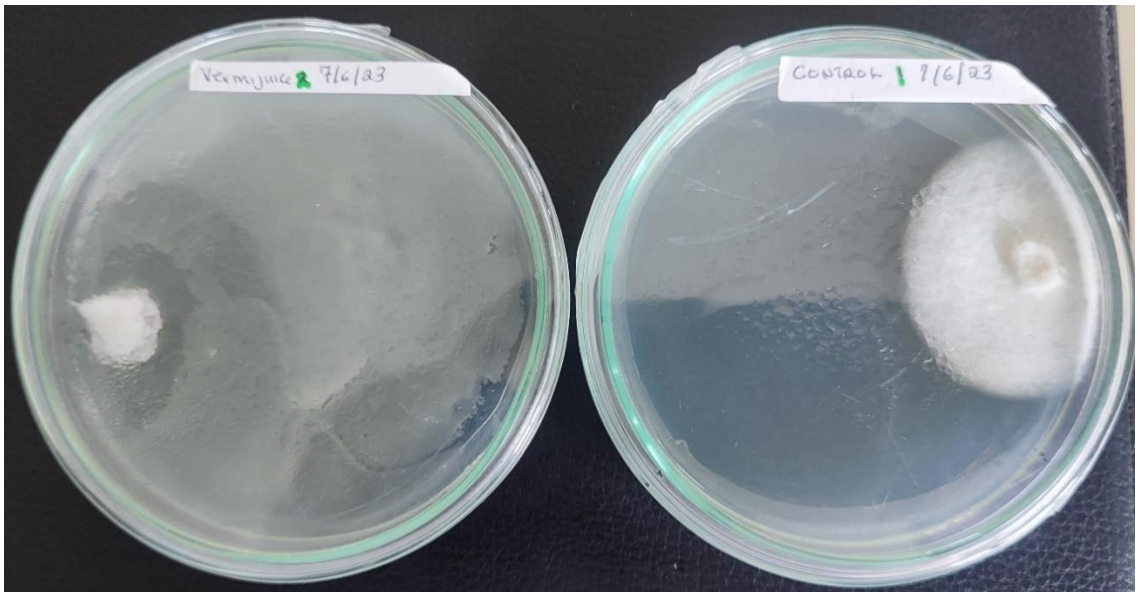

Figure 3: *Dual culture assay of M.oryzae streaked with vermiwash*

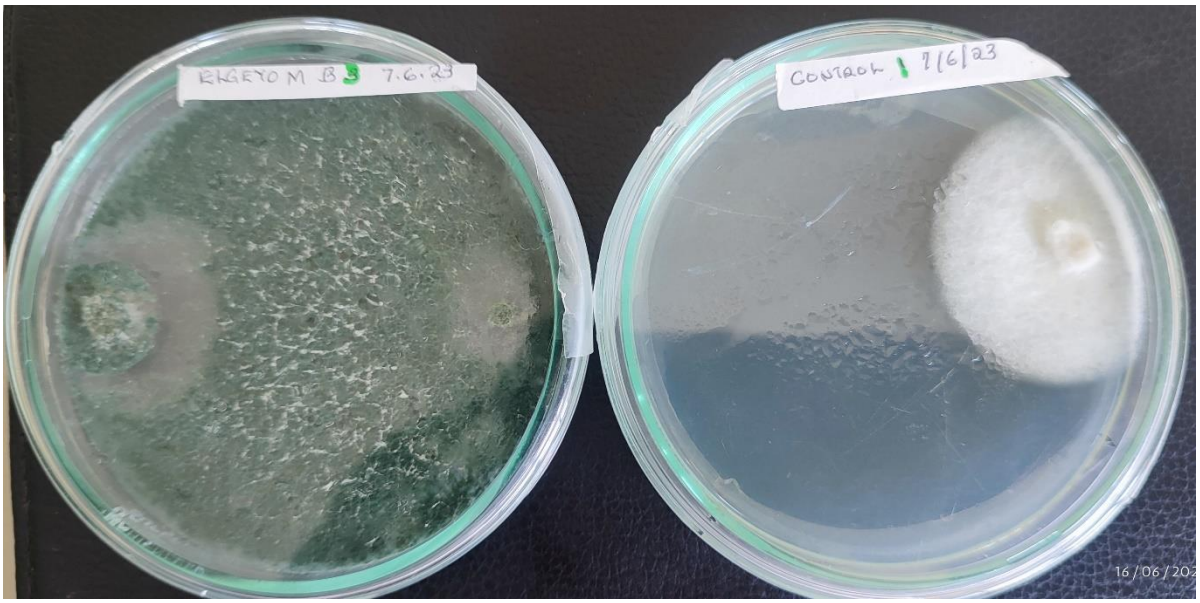

Figure 4: *Dual culture assay of M.oryzae with Trichoderma isolate EM*

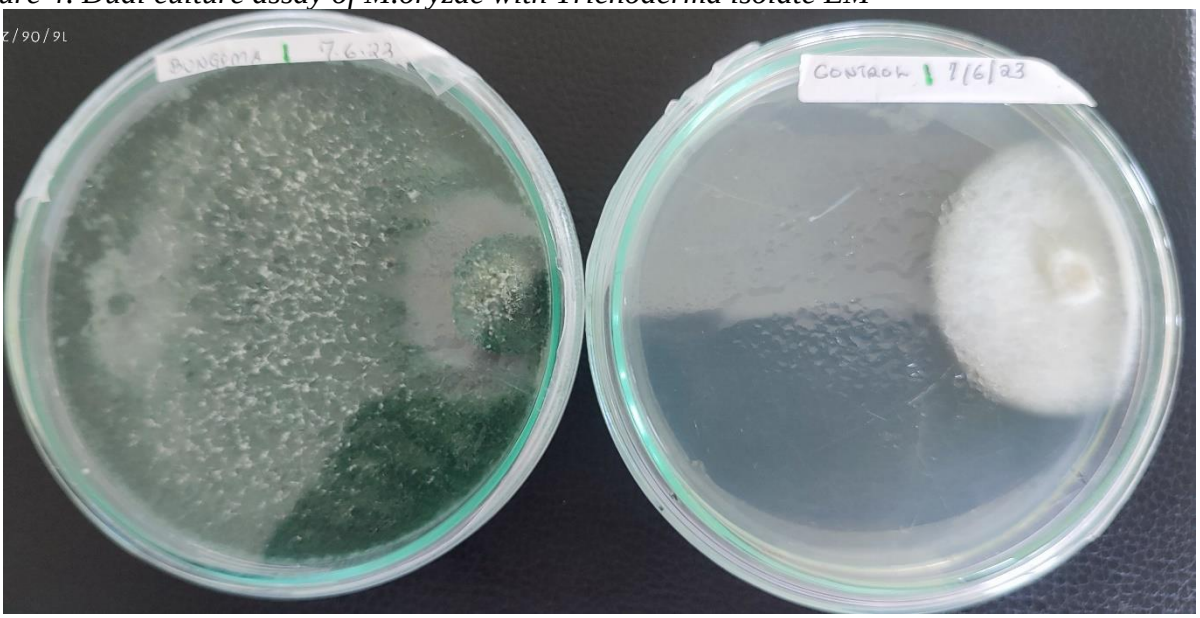

Figure 5: *Dual culture of M oryzae with Trichderma isolate BRO*

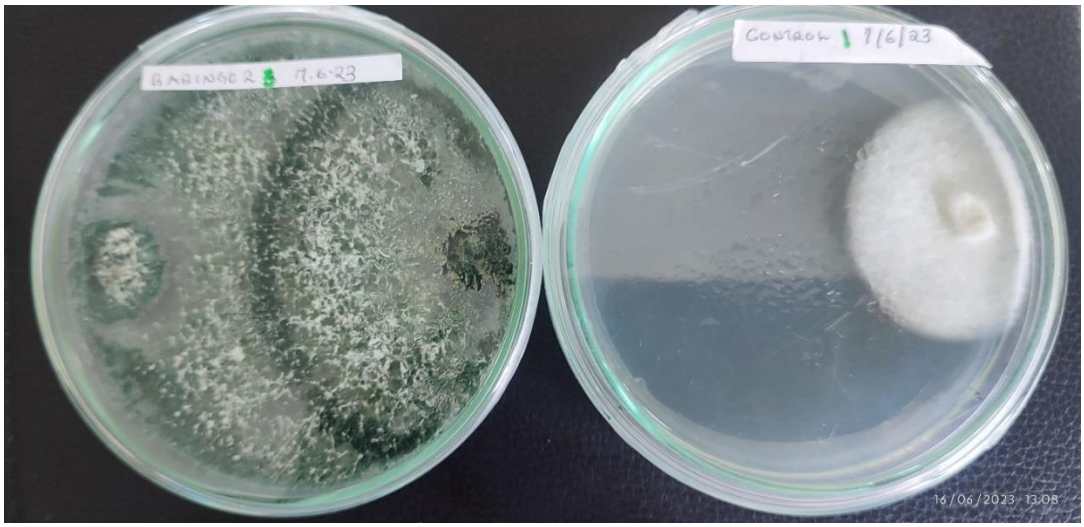

Figure 6: ***Dual culture of M oryzae with Trichderma isolate BRO2***

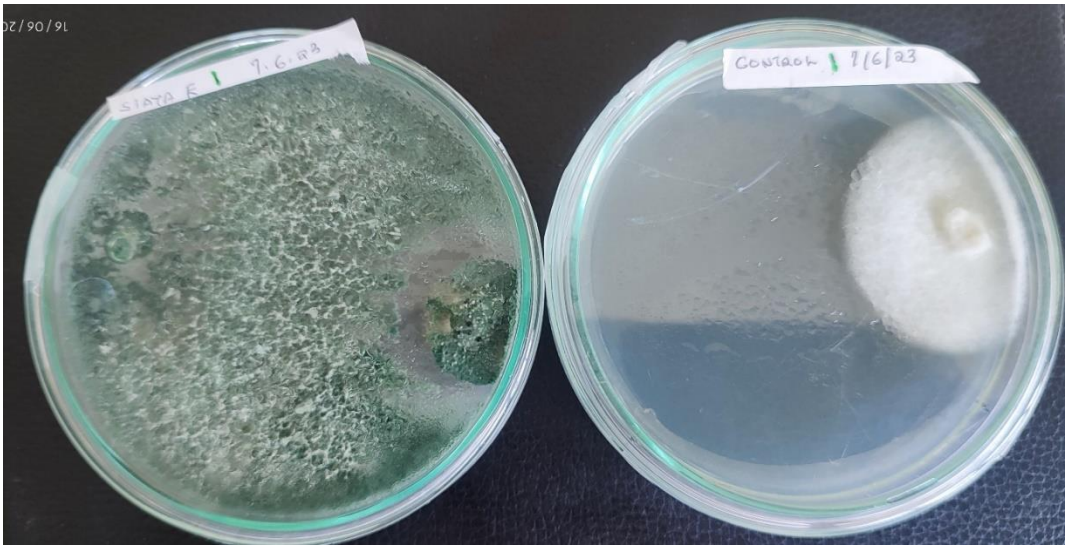

Figure 7: ***Dual culture of M oryzae with Trichoderma isolate SYA-E***

DISCUSSION

The study was conducted to evaluate the effectiveness of *Trichoderma Spp* and rice straw vermiwash on invitro growth of rice blast fungus *Magnapothe oryzae.* The findings revealed that Trichoderma EM-B isolates and rice straw vermiwash exhibited a high potential of inhibiting the growth rice blast fungus *M.oryzae* under laboratory conditions. These findings are in agreement with studies conducted on vermiwash indicating that the presence of beneficial micro biota boosts plant growth through production of plant growth enzymes and hormones that indirectly suppresses plant pest and diseases (Gudeta *et al*., 2021), Vermiwash exhibited an ability to control bacterial and fungal phytopathogens both invitro and in-vivo according to (Yadav, 2018). Application of vermiwash on above ground parts of plants as foliar spray and below ground as soil drench have been studied to understand the mechanism behind suppression of plant pest and diseases. It is recorded that vermiwash contains enzymes, nutrients and other secretions of the worms that suppress the pest when used as a foliar

application (Joshi *et al*., 2015). Other invitro studies have shown the effect vermiwash on common fungal and bacterial phytopathogens, two bacterial isolates from vermiwash identified as *Bacillus sp* and *Burkholderia sp* exhibited a significant zone of inhibition against fugal phytopathogens like *Fusarium solani* in Brinjal,*alteraria, solani* in tomato. that were isolated from diseased leaves and seeds of various plants(Pattnaik *et al*., 2015). Vermiwash has the potential to suppress both foliar and root pathogen in cucumber and tomato where various concentration of vermiwash were drenched into the growth medium and found to significantly suppress damage of root by the pathogen *Fusarium oxysporum.*

CONCLUSION

Rice straw vermiwash and EMB *Trichoderma* isolates were found to be the most effective biocontrol agents against *M oryzae* phytopathogen. The two biocontrol agents exhibited the potential against rice blast diseases invitro and were selected for further trials in the green house and field experiments

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