



## EFFECT OF POWDERY FORMULATION OF ENTOMOPATHOGENIC FUNGI ON RICE PLANTS INFESTED WITH AFRICAN RICE GALL MIDGE (*AfRGM-Orseolia oryzivora*)

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### ABSTRACT

**Background:** *Orseolia oryzivora* (AfRGM), is a serious insect pest of rain-fed and irrigated lowland rice in Africa which has resulted in huge yield losses on rice farms. The widespread usage of chemical pesticides for pest management has raised ecological and human health concerns, prompting a quest for alternatives.

**Objectives:** This work was conducted to assess entomopathogenic fungi effect for controlling of *Orseolia oryzivora* using powdery formulation for easy application by the farmer on the field.

**Methods:** Powdery formulation of Entomopathogenic fungi – EPF (*Beauveria bassiana* and *Metarhizium anisopliae*) prepared in laboratory, were applied to the test plants to control adult AfRGM. The experimentation was established using a Completely Randomized Design (CRD) method, which was replicated thrice inside cages in the screen house. Level of infestation was monitored while the agronomic data and physiological parameters were taken and analyzed.

**Results:** Plants treated with *B. bassiana* Bba 5653 had the lowest tiller infestation with highest non-infested tiller followed by *B. bassiana* Bba 5654 and *B. bassiana* Bba 326 with percentage non-infested tiller of 89.14%, 85.94% and 76.49% respectively.

**Conclusion:** Powdery formulation of *B. bassiana* effectively controlled AfRGM without negative effect on chlorophyll content, stem girth, grain weight, leaf length and breadth.

**Keywords:** *Beauveria bassiana*, *Metarhizium anisopliae*, *Orseolia oryzivora*, Entomopathogenic fungi, Rice, Powdery

### INTRODUCTION

It is impossible to overstate the importance of rice (*Oryza* spp.) for human and faunal consumption because it accounts for a significant portion of global calorie consumption (FAO, 2013). It covers 10% of all cereal-growing territory in Sub-Saharan Africa (SSA) and accounts for 15% of overall cereal production (Nwanzeet *al.*, 2006). “Rice is grown by around 20 million farmers in SSA, and about 100 million people rely on it for their livelihood” (Nwanzeet *al.*, 2006). “Rice output in the region climbed at a 4 percent annual rate from 1985 to 2003, compared to only 2.4 and 2.5 percent for maize and sorghum, respectively” (Okochap and Nuga, 2007). In SSA, it is farmed on 8.5 million hectares, accounting for 5.5 percent of global rice production. Rice is grown in nearly all African countries, although two of the countries, Nigeria and Madagascar, take

more than half of the rice land (Okochap and Nuga, 2007). However, a variety of pests and diseases limit its production. Nwilene *et al.* (2017) and Brenière (1983) identified a number of arthropods (Dipteran and Lepidopteran) that impair rice productivity both in the field and after harvest. *Orseolia oryzivora* Harris & Gagné (Diptera: Cecidomyiidae), the African Rice Gall Midge (AfRGM), one of the most common insect pests on the rice farm, usually attacks the rainfed and irrigated lowland rice plantation in Africa” (Nwilene *et al.*, 2006). Farmers' fields have had severe yield losses of about 25–100% within three months (Oyetunji *et al.*, 2014a), plummeting rice yield. Insect-induced losses must be combated in order to improve food security in Africa and improve the continent's reliance on rice. A variety of strategies have been developed to combat

the threat of pest infestation on the field. Many of these methods are constrained in one way or another (Oyetunji et al, 2019). Synthetic pesticides have been used for pest management on a regular basis, causing ecological concern and health challenges, prompting a quest for different control methods (Oyetunji et al, 2019). The farmers waste large parcel of land could be utilized for rice cultivation on insect prevention by planting *Paspallums crobiculatum* of 1-2m across the farm's border to enhance early building-up of AfRGM parasitoids (*Apprositus prosirae* and *Platygaster diplosisae*) (Nwilene et al., 2006). With all of these flaws, it is critical to find ecologically friendly solutions that do not pose a health concern and do not waste farm lands to combat this insect. As a result of this, the use of entomopathogenic fungi as biocontrol agent is necessary as alternative to other methods. Entomopathogenic fungi (EPF) are a rich source of bioactive chemicals that can be used to manage pests (Ginsberg et al., 2002; Kaaya et al., 1996 and Kaaya, and Munyinyi, 1995). Although the use of entomopathogenic fungi on this pest has been reported to have achieved excellent results (Oyetunji et al., 2019), a farmer-friendly strategy to the technology's deployment is required. This study used two entomopathogenic fungi namely *Beauveria bassiana* and *Metarhizium anisopliae* with each containing three strains to evaluate the efficacy of powdery formulation in managing adult AfRGM in rice-based cropping systems with the aim of controlling the insect pest in a farmer friendly manner to enhance rice production and food security in the region.

## Materials and methods

The materials used for the study include; Glass slides, cover slip, lactophenol in cotton blue solution, microscope, petri dish, distilled water, cork borer, chloramphenicol, spirit lamp, film wrap, potato dextrose agar, 70% ethanol, needle and syringe, Aluminium foil, Hand gloves, beaker, conical flask, measuring cylinder, sterile soil, 5-litre size plastic buckets, wooden cage, screen house, three strains of two fungi -*Beauveria bassiana* and *Metarhizium anisopliae*, SPAD metre, metre rule, Carbofuran, seeding tray, harvesting bags and ITA 306 rice variety.

Seed of susceptible rice variety (ITA 306) was planted in a box containing sterile paddy soil

collected from AfricaRice. After 21 days of planting, four seedlings were transplanted into pots and placed in the screening cages. With three replications, the research was set up using Completely Randomized Design (CRD). As a treatment, each fungus' lyophilized mycelium (0.05g) containing the conidia was prepared and applied on the plants while five male and female AfRGM were introduced in each cage. The treatments comprise three *Beauveria bassiana* stains (BA=Bba 326, BB=Bba 5653, BC=Bba 5654), three *Metarhizium anisopliae* strains (MA=Meta31, MB=IC30, MC=IC20), CW=Water treatment, CF= Female treatment alone, CM=Male treatment only, CCF=Synthetic insecticide, and UIF= Uninfested control.

To evaluate incidence and severity at various levels, a sample of four hills was chosen and the number of galls was counted weekly from 21 to 70 days after transplantation (DAT). The Standard Evaluation System (SES) was used to rank rice resistance based on the mean percent tiller infestation (IRRI, 1996). Chlorophyll content and stem girth were measured weekly, together with tiller infestation, to determine the treatment's likely effect on the plants. Other agronomic and yield characteristics recorded are the tillers number per plant, number of panicle, and yield. The data were evaluated to establish efficiency of the EPF on AfRGM by calculating percentage mean tillers (IRRI, 1996).

The incidence was calculated as follows:

$$\%HI = \sum_{n=1}^{\infty} \frac{HG}{TH} \times 100$$

(HI-hill infestation, HG-Hills with Galls, TH- Total no of hill estimated)

The severity was calculated as follows:

$$\%TI = \sum_{n=1}^{\infty} \frac{TG}{TT} \times 100$$

(TI-Tiller infestation, TG-Tillers with Galls, TT- Total no of Tillers estimated)

The percentage of uninfested tillers was also calculated for each treatment. The non-infestation percentage over the control was also determined as follows:

% Non-infestation advantage over control =

$$\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Yield parameters, such as panicle length, panicle number, grain number, and hundred grain weights, were also measured. Data collected was analyzed using SAS 9.2 package. General linear model has been used for the analysis of variance (ANOVA), and specific difference between variables was found using Fisher's Least Significant Difference (LSD) to separate the means at a significance level of  $P=0.05$ .

## Results

It was observed in this study that as the rice plant grew, the level of infestation increased first before it reduced. Plants treated with *B. bassiana* generally had the least tiller infestation among all the plants with EPF application with *B. bassiana* (5654) having the lowest amount of tiller infestation ( $P \geq 0.05$ ); the infestation in *B. bassiana* 5653 was slightly higher. Throughout the study, the plants with female gall midge and water control without EPF exhibited the highest tiller infection (Table 1).

Figure 1 shows the non-infested tiller's percentage advantage over the control. Apart from non-infested plants and male treated plants, which both had a 64.74 percent non-infested tiller advantage over the control, *B. bassiana* Bba 5653 and *B. bassiana* Bba 5654 had 46.85 percent and 41.58 percent non-infested tiller advantage over the control, respectively.

The chlorophyll level was found to be high during the pre-reproductive phase. The plants treated with *B. bassiana* Bba 5654 had the maximum chlorophyll content of 34.04 SPAD metres at the beginning of data collection during vegetative stage, followed by non-infested plants with a value of 32.09 SPAD metres and plants treated with male AFRGM having 31.5 SPAD metres. *B. bassiana* Bba 5654 had the maximum mean chlorophyll content of 35.81 SPAD metre among the EPF treated plants, followed by *B. bassiana* Bba 5653 and *B. bassiana* Bba 326 with 33.71 SPAD metre and 30.2 SPAD metre, respectively (Figure 2).

The results of the stem girth measurements showed the effect of the variables on the stem girth at the vegetative stage. The stem girth of the plants treated with powdery formulation of conidial EPF was shown in Figure 3. Plants with male gall midge and those treated with

*B. bassiana* Bba 326, and *B. bassiana* Bba 5653 all had the largest stem girth of 1.1cm, followed by uninfested plants with 0.94cm, 0.94cm, and 0.75cm, respectively. The average stem girth of uninfested plants was 1.57cm, followed by male infected plants (0.88cm), *B. bassiana* Bba 5654 (0.87cm), *B. bassiana* Bba 5653 (0.85cm), and *B. bassiana* Bba 326 (0.81cm) (Figure 3).

The result of the impact of dry conidial entomopathogenic fungi on plant height, leaf length, and leaf breadth revealed that plants treated with *B. bassiana* Bba 5653 grew to a height of 84.56cm, followed by plants with male gall midge (82.02cm), uninfested plants (76.27cm), and *B. bassiana* Bba 5654 (72.57cm). The least plant height was observed in the water treated control plants which grew only to a height of 10.42cm (Table 2). Plants with *B. bassiana* Bba 326 had the longest flag leaf of 41.37cm, followed by uninfested plants (38.56cm), plants treated with *B. bassiana* Bba 5654 (38.21cm), and plants treated with synthetic pesticide (37.46cm), with the water treated control having the shortest flag leaf of 9.25cm (Table 2). The leaf width also followed a similar pattern. The plants treated with *B. bassiana* Bba 5654 had the highest leaf breadth of 0.02cm while the water-treated control had the least (Table 2).

The results on the effects of plant treatments on several yield parameters, such as panicle length, panicle number, grain number, and hundred grain weight are shown on Table 3. Plants with male gall midge had the longest panicle length of 29.00cm, followed by the uninfested plants with 25.66cm panicle length and plants treated with *B. bassiana* 326 with 24.00cm panicle length. Plants with female gall midge alone had no panicle at all (that is, they were without panicle formation). The panicle number followed a similar pattern. Plants treated with male gall midge had the highest number of panicles (mean value - 7.66), followed by *B. bassiana* Bba 5654-treated plants (mean value - 4.33), and uninfested plants (mean value - 4.33). Plants with female gall midge only and with water as control had no panicles (that is, they did not produce panicles) (Table 3). The grain number and weight per hundred grain followed the same pattern. Plants treated with male gall midge had a grain number of 623 grains and a hundred grain weight of 2.56g, respectively, while plants treated with female gall midge solely and those treated with water control had 0.00 grain number and 0.00 grain

weight since they did not produce panicle (Table 3).

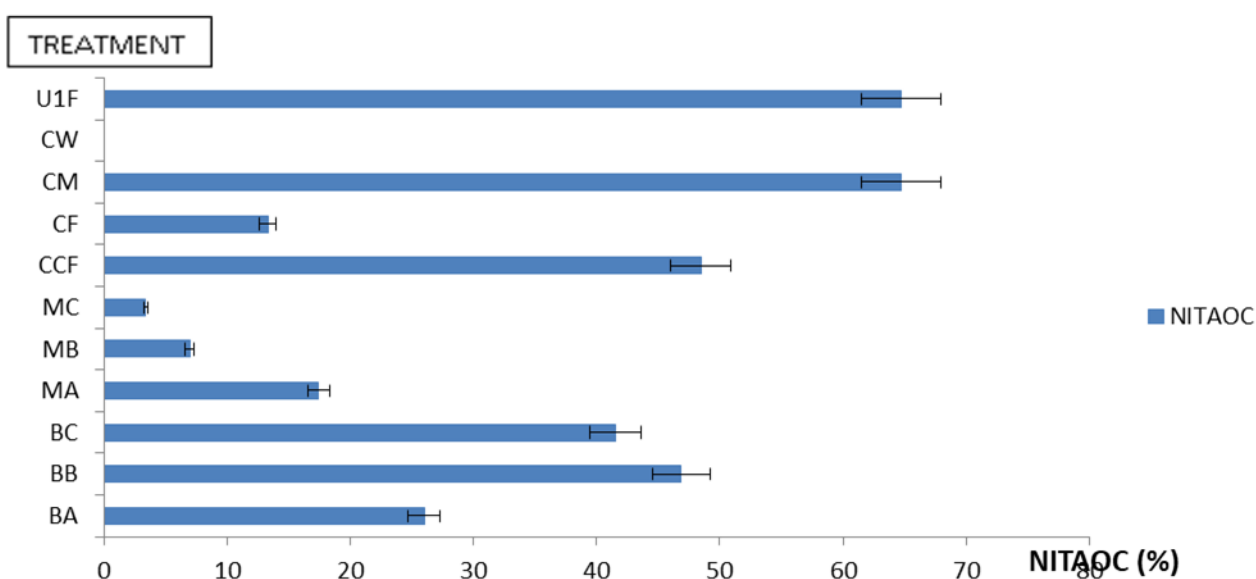
The Pearson correlation coefficients of the powdery formulation of entomopathogenic fungus in this investigation were displayed in Table 4. The mean chlorophyll content, mean stem girth, plant height, flag leaf breadth, panicle length, panicle number, and grain number were all inversely associated. The chlorophyll content at 49 days after

transplanting (49 DAT) and the mean chlorophyll content demonstrated favourable connection with grain weight. Plant height, flag leaf length, leaf breadth, panicle length, panicle number, and grain quantity were all positively linked with grain weight (Table 4).

**Table 1:** Percentage tiller infestation of AfRGM-infested rice plants treated with powdery formulation of entomopathogenic fungi

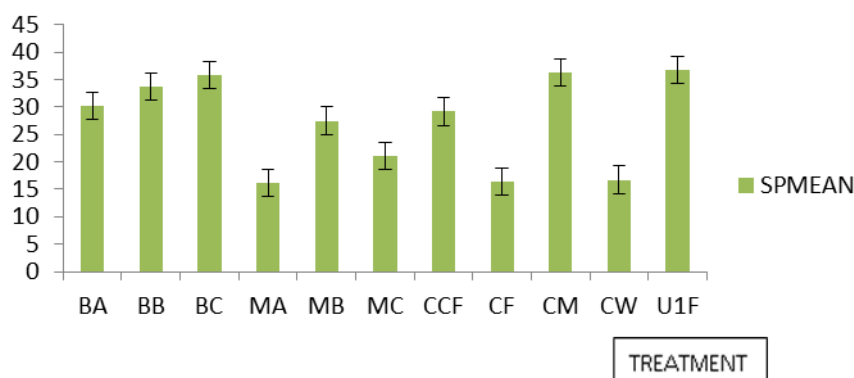
TRT	TI21DAT=Tiller Infestation 21 Days After Transplanting							
	TI21DAT (%)	TI28DAT (%)	TI35DAT (%)	TI42DAT (%)	TI49DAT (%)	TI56DAT (%)	TI63DAT (%)	TI70DAT (%)
BA	18.25	26.19	27.46	32.76	31.48	20.19	17.86	13.89
BB	2.08	13.89	9.8	11.11	16.67	11.11	11.11	11.11
BC	10.83	21.67	20.2	11.94	17.17	14.93	10.62	5.13
MA	7.22	20.91	30.81	38.15	29.32	38.27	37.03	27.88
MB	7.41	34.65	39.56	34.02	40.33	45.18	45.18	34.1
MC	9.57	29.71	44.25	44.56	47.32	49.17	57.14	48.3
CCF	0	0	0	0	4.17	22.1	23.78	29.1
CF	2.08	15.01	19.84	33.45	33.6	45.6	50.66	49.46
CM	0	0	0	0	0	0	0	0
CW	1.67	27.08	31.16	37.65	62.11	66.13	51.08	47.52
UIF	0	0	0	0	0	0	0	0
MEAN	5.65	17.19	20.28	22.14	25.65	29.91	29.40	23.32
LSD	12.85	23.31	26.08	24.6	28.97	28.11	25.02	31.18
CV	76.35	79.63	75.51	65.23	66.32	55.17	49.97	78.53

Keys:BA=Bba 326, BB=Bba 5653, BC=Bba 5654, MA= Meta31, MB=IC30, MC=IC20, CW= Water treatment, CF= Female treatment only, CM=Male treatment only, CCF=Synthetic insecticide, UIF= Uninfested control, Tiller Infestation and DAT= Days after Transplanting; TRT= Treatment



**Figure 1:** Effect of powdery formulation of entomopathogenic fungi on non-infested tiller advantage over the control of rice plants  
 Keys:BA=Bba 326, BB=Bba 5653, BC=Bba 5654, MA= Meta31, MB=IC30, MC=IC20, CW= Water treatment, CF= Female treatment only, CM=Male treatment only, CCF=Synthetic insecticide and UIF= Uninfested control; NITAOC= Non-infested tiller advantage over control

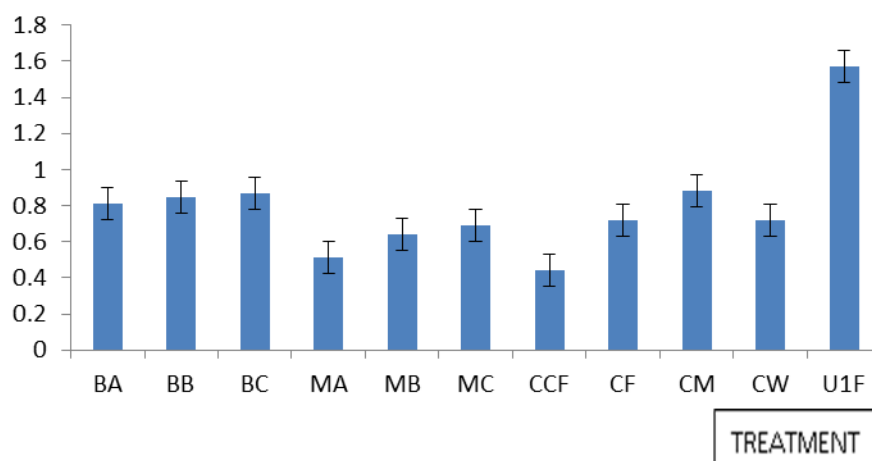
### Chlorophyll content



**Figure 2:** Chlorophyll content of AfRGM-infested rice plants treated with powdery formulation of entomopathogenic fungi

Keys:BA=Bba 326, BB=Bba 5653, BC=Bba 5654, MA= Meta31, MB=IC30, MC=IC20, CW= Water treatment, CF= Female treatment only, CM=Male treatment only, CCF=Synthetic insecticide and UIF= Uninfested control

### Stem girth (cm)



**Figure 3:** Effect of powdery formulation of entomopathogenic fungi on rice stem girth

Keys:BA=Bba 326, BB=Bba 5653, BC=Bba 5654, MA= Meta31, MB=IC30, MC=IC20, CW= Water treatment, CF= Female treatment only, CM=Male treatment only, CCF=Synthetic insecticide and UIF= Uninfested control

**Table 2:** Growth parameter of AfRGM-infested rice plants treated with powdery formulation of entomopathogenic fungi

TREATMENT	PLANTHEIGHT (cm)	LEAFLENGTH (cm)	LEAFBREATH (cm)
BA	77.53	41.37	2.16
BB	84.56	34.42	2.20
BC	72.573	38.21	2.22
MA	23.54	19.65	0.50
MB	55.09	29.7	1.55
MC	40.22	28.06	1.63
CCF	59.63	37.46	1.63
CF	20.42	17.35	0.02
CM	82.02	36.78	1.98
CW	10.42	9.25	0.02
UIF	76.27	38.56	1.95
MEAN	54.76	30.07	1.44
LSD	17.28	9.4	0.55
CV	18.54	18.37	22.71

Keys:BA=Bba 326, BB=Bba 5653, BC=Bba 5654, MA= Meta31, MB=IC30, MC=IC20, CW= Water treatment, CF= Female treatment only, CM=Male treatment only, CCF=Synthetic insecticide and UIF= Uninfested control

**Table 3:** Yield parameter of AfRGM-infested rice plants treated with powdery formulation of entomopathogenic fungi

TREATMENT	PANICLE LENGTH (cm)	PANICLE NUMBER	GRAIN NUMBER	GRAIN WEIGHT (g)
BA	24.00	4.00	319.0	2.56
BB	23.33	4.00	293.0	2.73
BC	23.33	4.33	354.0	2.5
MA	24.33	3.00	271.3	2.33
MB	24.33	3.00	272.7	2.3
MC	23.33	2.33	139.3	2.56
CCF	23.33	2.33	165.7	2.2
CF	0.00	0.00	0.0	0.0
CM	29.00	7.67	623.0	2.6
CW	0.00	0.00	0.0	0.0
UIF	25.66	4.33	378.7	2.4
MEAN	20.06	3.18	256.16	2.01
LSD	2.73	2.55	253.9	0.35
CV	8.09	47.22	58.2	10.45

Keys:BA=Bba 326, BB=Bba 5653, BC=Bba 5654, MA= Meta31, MB=IC30, MC=IC20, CW= Water treatment, CF= Female treatment only, CM=Male treatment only, CCF=Synthetic insecticide and UIF= Uninfested control

**Table 4:** Pearson Correlation Coefficients of AfRGM-infested rice plants treated with powdery formulation of entomopathogenic fungi

	TI49 DAT	TIM EAN	SP49 DAT	SPM EAN	GRT 49DAT	GRT MEAN	PLTH GHT	LEF LENGT	LEF BRET	PAN LENT	PAN NUM	GRAIN NUM	GRAI NWT
TI49DAT	1												
TIMEAN	0.9***	1											
SP49DAT	-0.59**	-0.57**	1										
SPMEAN	-0.69***	-0.72***	0.93***	1									
GRT49DAT	-0.50*	-0.60**	0.68***	0.76***	1								
GRTMEAN	-0.3	-0.4	0.2	0.34	0.39	1							
PLTHGHT	-0.59*	-0.64***	0.87***	0.87***	0.78***	0.32	1						
LEFLENGT	-0.62*	-0.601	0.80***	0.80***	0.64***	0.28	0.92***	1					
LEFBRET	-0.47*	-0.52*	0.84***	0.83***	0.72***	0.24	0.89***	0.90***	1				
PANLENT	-0.46*	-0.42*	0.65***	0.62***	0.51**	0.14	0.70***	0.70***	0.76***	1			
PANNUM	-0.46*	-0.51*	0.59*	0.63***	0.64***	0.3	0.68***	0.61	0.66***	0.76***	1		
GRAINNUM	-0.42*	-0.48*	0.53*	0.58*	0.58*	0.3	0.63*	0.55*	0.59*	0.72***	0.98***	1	
GRAINWT	-0.45	-0.42	0.65***	0.61*	0.50*	0.11	0.70***	0.72***	0.81***	0.94***	0.67***	0.61*	1

Keys:TI49DAT = Tiller Infestation 49 Days After Infestation, TI70DAT = Tiller Infestation 70 Days After Infestation, and TI80DAT = Tiller Infestation 80 Days After Infestation TIMEAN is an abbreviation for Mean Tiller Infestation. MEANCHL= Mean Chlorophyll, CHL70DAT=Chlorophyll at 70 Days After Transplanting, CHL49DAT=Chlorophyll at 49 Days After Transplanting, CHL70DAT=Chlorophyll at 70 Days After Transplanting, CHL70DAT=Chlorophyll at 70 Days After Transplanting LEFLENT= Leaf Length, LEFBRET= Leaf Breadth, GRAINWT=100-Grain Weight. PANLENT= Panicle length, LEFLENT= Leaf Length, LEFBRET= Leaf Breadth, GRAINWT=100-Grain Weight. At P = 0.05, 0.01, 0.001, values with asterisks are significant; values without asterisks are not significant.

## Discussion

As the rice plants grew, the level of infestation increased, according to this study. This may be due to the growth of the insect population, which resulted in the emergence of different generations of gall midge. The extent of the decline in the infestation varied depending on the control capacity or ability of each of the treatment. In this study, *Beauveria bassiana* had a better control impact on

adult gall midges than *Metarhizium anisopliae*. This is in line with the discoveries of Oyetunji *et al.*(2019), who employed *M. anisopliae* to regulate the effects of AfRGM on rice fields. In addition, Kaaya and Munyinyi, (1995) reported that “the entomopathogenic fungi were effective as bio-control against Tsetse flies”. The powdered *B. bassiana* formulation was observed to be efficient against adult gall midges in the study. This shows

how easily it penetrated and formed germ tubes in the mature AfRGM. "Entomopathogenic fungi are considered the most adaptable biological control agents on extensive host range of insects due to their ability to penetrate during infestation" (Hu and Leger, 2002). Much research has been done on the use of entomopathogenic fungus in controlling different arthropods (Harris *et al.*, 2000); but the impact of entomopathogenic fungi on Dipteran, including Gall midge, has received little consideration. However, from this study, it can be established that using dry conidials of entomopathogenic fungus, specifically *B. bassiana* Bba 5653, had a positive effect in controlling the infestation of AfRGM in rain-fed and irrigated lowland rice plantations. Zahn *et al.* (2013) also observed that *B. bassiana* strains were highly effective in the management of Citrus Thrips (Thysanoptera: Thripidae) on California Blueberries. However, *B. bassiana* has demonstrated inconsistent success in suppressing thrips and a variety of other insect species in laboratory and greenhouse studies which shows different levels of accomplishment (Jacobson *et al.*, 2001; Ugine *et al.* 2005; Frantz and Mellinger, 1998, Murphy *et al.* 1998, Azaizeh *et al.* 2002, Stanghellini and El-Hamalawi 2005).

Worthy of note is the fact that the EPF had no negative effects on the plants' physiology, agronomy, or yield parameters in AfRGM adult infested plants, according to the study. Plants treated with AfRGM had significantly lower chlorophyll content than those in the control group without AfRGM. As a result, each entomopathogenic fungus had no discernible effect on plant chlorophyll content reduction. At the vegetative stage, the chlorophyll content was generally high. This is consistent with the discoveries of Chrispaul *et al.* (2010) and Oyetunji *et al.* (2012, 2014b), who found that during the early phase of leaf growth, chlorophyll, protein, and structural compound synthesis was high, resulting in high energy producing rates to support energy needs by the plants. Furthermore, there have been no instances of gall midge having a deleterious impact on rice plant chlorophyll levels (Oyetunji *et al.*, 2019). Similarly, the application of EPF on rice plants had no effect on the chlorophyll concentration as observed in this study. In a study piloted by Oyetunji *et al.*, (2012), both *Botryodiplodia theobromae* and

*Trichoderma* sp applications on some rice varieties resulted in a drop in rice plant chlorophyll level. The study underscored that the fungi caused rot in the rice plants and even collaborated with termites on rice plants, resulting in even more damage (Oyetunji *et al.*, 2012). As a result, it is possible that those fungi were rot-causing fungi in some way. However, EPF utilized in this study was not found to be pathogenic. Entomopathogenic fungi have not been found to have pathogenic effect (Include a reference here). This could explain why there was no drop in chlorophyll content in the treatments. The EPF reduced AfRGM infestation, which resulted in an increase in chlorophyll content of the treatments, allowing the level of chlorophyll to be controlled.

According to this study, rice plants with male gall midge had the most panicles, followed by those treated with *B. bassiana* Bba 5654. Plants treated with *B. bassiana* had greater panicles and grain weight than those treated with *M. anisopliae* in EPF. This indicated EPF's long-term effect in preventing mature AfRGM infestations on rice plants. According to Zahn *et al.*, (2013), *B. bassiana* was successfully used as a biocontrol agent for the management of Citrus Thrips in California Blueberries. In addition, Stanghellini and El-Hamalawi (2005) and Ugine *et al.* (2005) found *B. bassiana* to be an excellent biocontrol agent.

While panicle number was related to leaf breadth, plant height and panicle length, grain number was related to plant height, leaf breadth, and grain weight in a substantial and favourable manner.

In conclusion, the strains of *Beauveria bassiana* performed better than the strains of *Metarhizium anisopliae* powdery formulation. Therefore, strains of *Beauveria bassiana* especially Bba 5653 and Bba 5654 are recommended for use in managing the infestation of AfRGM in insect based cropping system without negative effect on plant physiology, yield or agronomic parameters of the plants. More research is needed on other EPF and the use of adhesive agent to make the effect last longer during raining season in rainfed lowland ecology.

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