Archives of Phytopathology and Plant Protection

ISSN: 0323-5408 (Print) 1477-2906 (Online) Journal homepage: https://www.tandfonline.com/loi/gapp20

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To cite this article: Olumoye E. Oyetunji, Christopher Odebode, Francis Nwilene, Abou Togola & Manuele Tamo (2019): Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the adult African rice gall midge (AfRGM – *Orseolia oryzivora*), Archives of Phytopathology and Plant Protection, DOI: 10.1080/03235408.2019.1681633

To link to this article: https://doi.org/10.1080/03235408.2019.1681633

Published online: 06 Nov 2019.

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ARCHIVES OF PHYTOPATHOLOGY AND PLANT PROTECTION
https://doi.org/10.1080/03235408.2019.1681633

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Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the adult African rice gall midge (AfRGM – *Orseolia oryzivora*)

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ABSTRACT

Rice is an important staple crop whose production is limited by array of insect pests and diseases. African rice gall midge (AfRGM) *Orseolia oryzivora* Harris & Gagné (Diptera: Cecidomyiidae) is a major insect pest of lowland rice ecology in Africa. Heavy yield losses have been recorded in many farmers' rice fields. Use of synthetic insecticides has fostered environmental and human health concern that initiates a search for alternative control measures such as Entomopathogenic fungi (EPF) – *Beauveria bassiana* and *Metarhizium anisopliae*. The experiment was laid out on completely randomised design (CRD) with three replications. The study showed *M. anisopliae* IC30 had the greatest control effect on adult AfRGM with 90.58% of non-infested tillers. The percentage of non-infested tiller advantage over the control followed the same trend with *M. anisopliae* IC30 having the greatest value of 50.72%. Tiller infestation had significant negative correlation with chlorophyll content, leaf breadth and grain number.

ARTICLE HISTORY
Received 10 May 2018
Accepted 21 September 2019

KEYWORDS
Entomopathogenic; fungi; adult AfRGM; infestation; B. bassiana; M. anisopliae

Introduction

Rice (*Oryza spp.*) is one of the most important cereal crops for human and livestock consumption. It accounts for over 20 per cent of global calorie intake. It is the world's most important food crop being the staple food of over 50 per cent of the world population particularly of India, China and a number of other countries in Africa and Asia (Food and Agriculture Organization [FAO] 2013). Rice ranked the second most important cereal crop in terms of production after maize (FAO 2013). Rice occupies 10 per cent of the total land under cereals and contributes

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15 per cent of total cereal production in Sub-Saharan Africa (SSA). About 20 million farmers in SSA grow rice, and about 100 million people depend on it for their livelihoods (Nwanze et al. 2006). From 1985 to 2003, the region's rice production increased at an annual rate of 4 per cent, compared to only 2.4 and 2.5 per cent for maize and sorghum, respectively. Rice is grown on 8.5 million hectares in SSA, equal to 5.5 per cent of the global rice area. Almost all of the region's 38 countries grow rice, but two regions, Nigeria and Madagascar, account for 60 per cent of the rice land (Okochap and Nuga 2007). Rice (*Oryza spp.*) is one of the most important cereal crops for human and livestock consumption accounting for over 20 per cent of global calorie intake. And it is the world's most important food crop being the staple food of over 50 per cent of the world population (Nguyen 2004). Despites its importance, its production is limited by array of pests and diseases. Series of pests affect rice production both on the field and at post-harvest stages (Brenière 1983; Nwilene et al. 2017). One of the major insect pests on the field is African rice gall midge (AfRGM). AfRGM *Orseolia oryzivora* Harris & Gagné (Diptera: Cecidomyiidae) is a major insect pest primarily of rainfed and irrigated lowland rice in Africa. Heavy yield losses of 25%–100% have been recorded in farmers' fields (Nwilene et al. 2006) thereby reducing the production capacity to very low ebb in an endemic situation. Thus, there is need to tackle insect-induced losses to enhance food security in Africa to aid their dependence on rice.

A lot of approaches have been devised to combat the menace of insect infestation on the field. Many of these approaches are limited in one facet or the other. Repeated use of synthetic insecticides for pest control has fostered environmental and human health concern that initiates a search for alternative control measures. Planting of *Paspallum scrobiculatum* of 1–2 m around the border of the field to boost the rapid production of the AfRGM parasitoids namely *Platygaster diplosisae* and *Apprositus prosirae* (Nwilene et al. 2006; Oyetunji et al. 2014a) did not leave the farmers without wasting a good portion of the field that could have been used for rice production for pest exclusion. With all these shortcomings, there is a need to look for environmental-friendly approaches without associated health risk to control this pest. This is the reason why the search for entomopathogenic fungi (EPF) is needful. EPF are considered as a rich source of bioactive compounds for controlling pests (Ansari et al. 2011; Ginsberg and Ewing 1989; Kaaya and Munyinyi 1995; Kaaya et al. 1996; Ginsberg et al. 2002). This study aimed at managing adult AfRGM since they are the causative agent in the spread of the pest across the field areas, using three strains each of the two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in rice-based cropping systems.

Materials and methods

Paddy soil was collected from AfricaRice lowland rice field and sterilised at the Nematology Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan. The sterile soil was filled into pots when sufficiently cool. Susceptible rice variety (ITA 306) was sown at the rate of one seedling per hill with 20-cm spacing of four hills per pot. The seeds were sown in the seed boxes, and seedlings were transplanted to the pots containing sterile paddy soil 21 days after seeding. Four seedlings were transplanted in a pot on a spacing of 20 cm between hills with one seedling per hill. The pot containing seedling was placed in the screening cages. A basal dose of NPK at the rate 40:40:40 kg ha^{-1} was applied at transplanting while topdressing of urea 40 kg ha^{-1} was applied at 20 days after transplanting (DAT). The experiment was laid out using completely randomised design (CRD) with three replications. Each fungal strain (0.05 g) was prepared with 9 mL of sterile distilled water. The treatments were applied separately four DAT, using hand-held sprayer after which five adults AfRGM were introduced. The treatments include the three stains of *B. bassiana* (BA = Bba 326, BB = Bba 5653, BC = Bba 5654), three strains of *M. anisopliae* (MA = Meta31, MB = IC30, MC = IC20), CW = Water treatment, CF = Female treatment only, CM = Male treatment only, CCF = Synthetic insecticide and UIF = Uninfested control.

During the period, data were collected on tiller infestation, panicle number plant height, leaf length, leaf breadth, Panicle number, panicle length, grain number, grain weight and yield. Non-infested tillers and the percentage of the non-infestation tiller advantage over the control (NITAOC) were also calculated per treatment. The data were subjected to Analysis of Variance (ANOVA) and general linear model (GLM) using the SAS 9.2.

Fisher's least-significant difference (LSD) was to separate means at a significance level of $p < 0.05$.

Results

The results showed increase in the level of infestation as the plant grows and inoculum multiplied on it. It showed that the three strains of *M. anisoplae* have greater control effect on the adult gall midge than the strains of *B. bassiana* in this study (Table 1). *M. anisopliae* -IC30 strain has the greatest control effect on the adult gall midge in this study followed by strain Meta31 and IC20, respectively (Figure 1). Therefore, the percentage of uninfested tiller was high on the plants treated with IC30 (90.58%) followed by IC20 (78.87%) and *B. bassiana* -Bba 326. IC30 had the highest non-infested tiller advantage over the control (NITAOC) percentage with 50.72% followed by IC20 with

Table 1. Percentage tiller infestation of adult AfRGM on rice treated with entomopathogenic fungi.

Treatment	TI21DAT (%)	TI28DAT (%)	TI35DAT (%)	TI42DAT (%)	TI49DAT (%)	TI56DAT (%)	TI63DAT (%)	TI70DAT (%)
BA	12.37	10.10	43.06	36.68	23.44	32.50	32.50	31.02
BB	4.00	10.39	29.90	29.82	55.56	65.38	67.47	66.72
BC	6.22	16.21	32.59	71.43	37.96	43.06	59.72	48.20
MA	25.33	23.40	31.67	34.72	27.50	27.04	27.04	27.04
MB	13.43	18.93	11.88	7.66	7.55	5.41	6.08	6.08
MC	2.50	2.63	26.74	21.39	30.23	29.90	29.90	28.70
CCF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CF	1.00	20.89	47.22	42.01	41.37	49.17	47.08	48.04
CM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CW	14.56	6.92	33.67	36.11	51.52	61.97	61.97	63.92
UIF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MEAN	7.22	9.95	23.34	25.44	25.01	28.58	30.16	29.07
Lsd	19.22	18.75	36.26	34.42	35.20	33.70	38.84	37.50
CV	10.86	84.65	71.24	83.89	78.94	82.45	82.97	83.18

BA = Bba326, BB = Bba5653, BC = Bba5654, MA = Meta31, MB = IC30, MC = IC20, CW = Water treatment, CF = Female treatment only, CM = Male treatment only, CCF = Synthetic insecticide, UIF = Uninfested control, TI = Tiller Infestation and DAT = Days after transplanting.

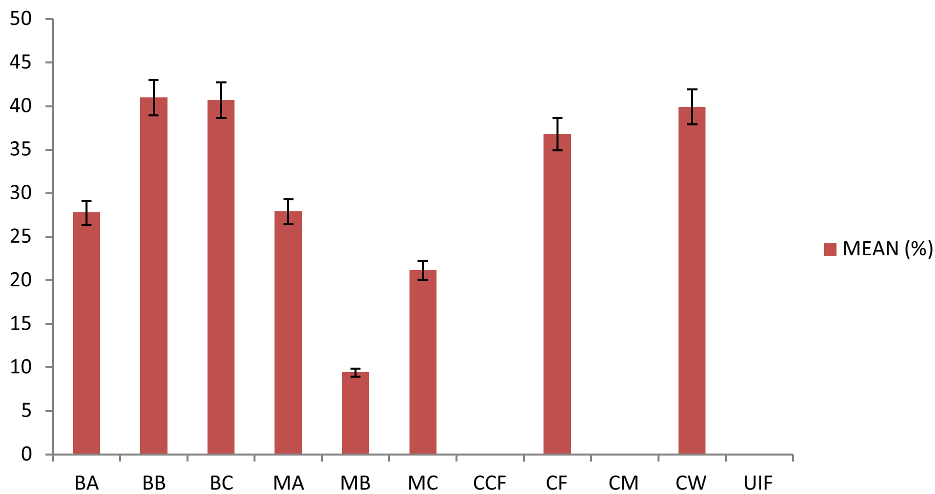

Figure 1. Percentage of mean tiller infestation by adult AfRGM on the treatment. Where BA = Bba 326, BB = Bba 5653, BC = Bba 5654, MA = Meta31, MB = IC30, MC = IC20, CW = Water treatment, FC = Female treatment only, CM = Male treatment only, CCF = Synthetic insecticide and UIF = Uninfested control.

31.23% (Figure 2). *M. anisoplae* has been found useful for the control of termite in rice base cropping system (Nwilene et al. 2008). Minshad et al. (2011) established that *M. anisopliae* was very effective for controlling Biting Midge.

While there was a significant ($p < 0.05$) reduction on the plant height of the plant treated with AfRGM, there was no resultant negative effect of EPF on the plant height. Some of the EPF-treated plants had equal plant height and leaf length with the uninfected control. The plants treated with *M.*

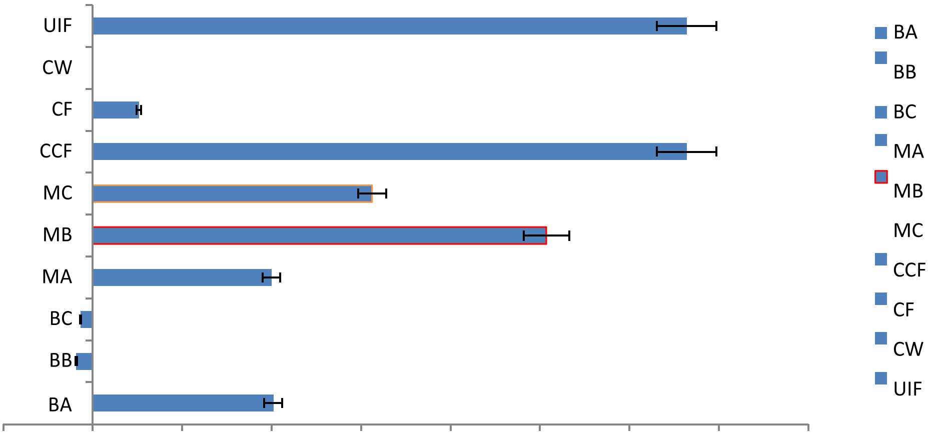

Figure 2. Percentage of non-infested tillers advantage over the control (NITAOC). Where BA = Bba 326, BB = Bba 5653, BC = Bba 5654, MA = Meta31, MB = IC30, MC = IC20, CW = Water treatment, FC = Female treatment only, CM = Male treatment only, CCF = Synthetic insecticide and UIF = Uninfested control.

Table 2. Effect of the entomopathogenic fungi on yield and agronomic parameter.

Treatment	Plant height (cm)	Leaf length (cm)	Leaf breadth (cm)	Pan num	Pan length	Grain num	Grain wt
BA	100.00	41.72	1.16	1.00	25.67	90.00	3.10
BB	94.33	20.80	1.10	1.00	27.17	68.30	2.37
BC	101.50	33.06	1.59	1.33	30.67	148.00	2.80
MA	100.17	33.68	1.32	5.33	27.22	540.30	2.93
MB	88.34	30.98	1.45	4.67	28.00	596.00	3.03
MC	103.88	33.44	1.29	5.33	25.57	368.70	3.63
CCF	107.67	32.43	1.65	7.33	23.84	753.70	2.67
CF	77.33	28.08	0.94	2.00	16.92	217.00	2.13
CM	104.72	30.93	1.30	8.33	21.76	557.70	2.50
CW	16.67	25.93	0.36	0.00	0.00	0.00	0.00
UIF	103.42	31.34	1.44	9.00	20.56	709.70	2.70
MEAN	90.73	31.13	1.24	4.12	22.49	368.13	2.53
Lsd	18.48	9.56	0.29	4.49	4.37	493.00	0.96
CV	27.39	15.99	27.50	74.55	11.41	71.26	21.22

Where BA = Bba 326, BB = Bba 5653, BC = Bba 5654, MA = Meta31, MB = IC30, MC = IC20, CW = Water treatment, FC = Female treatment only, CM = Male treatment only, CCF = Synthetic insecticide and UIF = Uninfested control; Lsd = Least.
Significant difference and CV = coefficient of variation.

anisopliae strains Meta 31 and IC20 had significantly higher panicle number (5.33) and grain number than the control (Table 2).

The experiment revealed the effect of AfRGM infestation and EPF on the agronomic parameters. The agronomic parameters evaluated in this study were plant height, flag leaf length, and flag leaf breadth (Table 2). While there was a significant (0.005) reduction on the plant height of the plant treated with AfRGM, there was no resultant negative effect of EPF on the plant height. Indeed, some of the EPF-treated plants had equal plant height with the uninfested control. While the flag leaf length

Table 3. Pearson's correlation coefficients of entomopathogenic fungi on adult gall midge.

	TI49DAT	TI70DAT	MEANTI	PANNUM	PANLENT	PLTHGT	LEFLENT	LEFRET	GRAINNUM	GRAINWT
TI49DAT	1									
TI70DAT	0.75***	1								
MEANTI	0.86***	0.93***	1							
PANNUM	−0.58***	−0.67***	−0.69***	1						
PANLENT	−0.23	−0.21	−0.17	0.17	1					
PLTHGT	−0.39	−0.38	−0.38	0.51**	0.86***	1				
LEFLENT	−0.21	−0.35	−0.25	0.24	0.30	0.37	1			
LEFBRET	−0.59***	−0.53**	−0.58***	0.51**	0.75***	0.76***	0.33	1		
GRAINNUM	−0.49*	−0.61***	−0.59***	0.87***	0.18	0.43	0.27	0.49**	1	
GRAINWT	−0.16	−0.23	−0.15	0.25	0.79***	0.79***	0.37*	0.56**	0.24	1

Where TI49DAT = Tiller infestation at 49 days after infestation, TI70DAT = Tiller infestation at 70 days after infestation, MEANTI = Mean tiller infestation, CHL49DAT = Chlorophyll at 49 days after transplanting, MEANCHL = Mean chlorophyll, CHL70DAT = Chlorophyll at 70 days after transplanting, PANLENT = Panicle length, LEFLENT = Leaf length, LEFBRET = Leaf breadth, 100-GRAINNUM = Grain number, GRAINWT = Grain weight. *, **, *** significant at $p = 0.05$, 0.01, 0.001; values without asterisks are not significant.

of *B. bassiana* strain Bba 326 was significantly higher (41.72 cm) than the strain Bba 5653 (20.80 cm), there was no significant difference in the flag leaf length among the strains of *M. anisopliae*. There was significant difference in the leaf length of plant treated with the *B. bassiana* strain Bba 326 (41.72 cm) and the treated control (25.93 cm). However, there was no significant difference in the flag leaf length of the plants treated with other EPF and the water-treated control. Generally, the leaf breadth of each EPF-treated plant was significantly higher than the leaf breadth of the water-treated control. The plants treated with synthetic insecticide had the highest leaf breadth of 1.65 cm, followed by the plants treated with *B. bassiana* strain Bba 5654 with 1.59 cm and plants treated with *M. anisopliae* strain IC30 with 1.45 cm leaf breadth (Table 2).

Table 3 shows the association of all morphological traits estimated by phenotypic correlation coefficient. The Tiller infestation at 49 DAT had positive correlation with the mean tiller infestation and tiller infestation at 70 DAT. Mean tiller infestation had negative correlation with chlorophyll content at 49 DAT, chlorophyll content at 70 DAT, leaf breath and grain number. However, mean tiller infestation had negative correlation with panicle length, plant height, leaf length but not with significant effect. While panicle number had significant and positive correlation with plant height, leaf breadth and grain number, panicle length had positive correlation with plant height, leaf breadth and grain weight.

The study showed that the chlorophyll content during the vegetative stage of each of the treatments was high and gradually decreased as the plants approached senescence. It was observed in this study that all the plants not treated with AfRGM had high chlorophyll content (>120 nmol/cm^2), while those treated with adult AfRGM had significantly low chlorophyll content. The chlorophyll content of the plant

Table 4. Chlorophyll content of rice infested with adult AfRGM and treated with entomopathogenic fungi days after transplanting.

Treatment	42DAT (nmol/cm^2)	49DAT (nmol/cm^2)	56DAT (nmol/cm^2)	63DAT (nmol/cm^2)	70DAT (nmol/cm^2)
BA	103.47	70.90	72.60	70.07	65.07
BB	77.97	65.57	55.40	47.70	46.43
BC	84.93	56.70	50.90	49.70	47.60
MA	79.40	103.87	89.50	81.60	69.23
MB	81.43	62.03	66.67	63.90	58.97
MC	91.37	86.03	74.33	70.87	63.33
CCF	121.10	132.60	130.23	110.20	105.10
CF	110.90	90.90	82.30	90.10	80.40
CM	120.83	134.07	107.57	131.43	120.47
CW	104.03	106.53	83.40	91.93	82.50
UIF	119.73	143.53	122.50	129.07	115.10
MEAN	99.56	95.70	85.04	85.14	77.65
Lsd	43.85	50.34	50.90	52.45	46.36
CV	16.50	30.87	28.88	32.31	31.74

BA = Bba326, BB = Bba5653, BC = Bba5654, MA = Meta31, MB = IC30, MC = IC20, CW = Water treatment, CF = Female treatment only, CM = Male treatment only, CCF = Synthetic insecticide, UIF = Uninfected control, SP = Spad meter and DAT = Days after transplanting.

treated with Bba 5653 was low generally throughout the experiment followed by that of Bba 5954. On the average, the plants treated with *Beauveria* had slightly higher chlorophyll content than the plants treated with *M. anisopliae*. At 70 DAT, three treatments had chlorophyll content above 100 nmol/cm^2. The rice plants treated with male gall midge only had the highest chlorophyll content of (120.47 nmol/cm^2) followed by uninfested plants with 115.1 nmol/cm^2 and then the plants treated with synthetic insecticide of 105.1 nmol/cm^2.

The chlorophyll content of the rice plants treated with only female gall midge and water treatments was low (Table 4). At the end of the experiment, uninfested plants had the highest mean chlorophyll content (125.99 nmol/cm^2) followed by male-infested plants (122.87 nmol/cm^2) and plants treated with synthetic insecticide (119.85 nmol/cm^2) (Table 4). It therefore means that the gall midge has serious effect on the chlorophyll content of rice leaf.

Discussion

In this study, there was increase in the level of infestation as the plant continued to grow. This is not far-fetched from the build-up of the insect population that gave rise to different generations. Although there were reductions in the level of infestation of the EPF when compared with the control, there were variations in the level of the reduction based on the nature of each treatment. *M. anisopliae* had greater control effect on the adult gall midge than that of *B. bassiana* in this study. Although there was no prior study on the use of entomopathogenic fungi for control of adult AfRGM, but according to Kaaya and Munyinyi (1995), the entomopathogenic fungi were effective as biocontrol against some pests such as including Tsetse flies. *M. anisopliae* IC30 was found to have recorded low pest infestation in the amount of gall formed in the tillers. This depicts that it was easy for it to penetrate and form germ tube in the adult AfRGM. Entomopathogenic fungi are potentially the most versatile biological control agent, due to their wide host range that often results in natural epizootics. An attractive feature of these fungi is that infectivity is by contact, and the action is through penetration; *M. anisopliae* is the second most widely exploited entomopathogenic fungus in biocontrol attempts, and it is known to attack over

Two hundred species of insects belong to orders Coleoptera, Dermoptera, Homoptera, Lepidoptera and Orthoptera (Hu and St. Leger 2002). Much work had been conducted on the use of entomopathogenic fungi to control various classes of pests (Harris et al. 2000), but little attention was put to the effect of entomopathogenic fungi on dipteran of which gall midge is one. However, this study showed the positive effect of the use of entomopathogenic fungus especially *M. anisopliae* IC30 to controlling adult stage of African Rice Gall Midge. This is in accordance with a study conducted by Minshad et al. (2011), where he used entomopathogenic fungus as a biological control for an important vector of livestock disease – the *Culicoides* biting midge, and found it very useful. Minshad et al. (2011) established that *M. anisopliae* was very effective for controlling biting Midge. Revankar et al. (1999) also established the effect of *M. anisopliae* which was used as biocontrol mechanism in immunocompetent hosts as it caused sinusitis in the host.

The study showed that in the AfRGM adult-infested plants, the EPF had no negative effect on the chlorophyll content of the plant. The plants in the control without AfRGM had high chlorophyll content, while those treated with adult AfRGM had significantly low chlorophyll content. It therefore means that each entomopathogenic fungi had no significant effect on the reduction of the chlorophyll content of the plants. The chlorophyll content at the beginning was high at vegetative stage. This is in accordance with the findings of Oyetunji et al. (2012, 2014b) and Chrispaul et al. (2010), who reported that during early stages of leaf growth, synthesis of chlorophyll, proteins and structural compounds were high resulting in high catabolic rates to support energy needs by the plants. Although no work has been reported on the effect of EPF on chlorophyll content of any plant, but according to the study conducted by Oyetunji et al. (2012), it was reported that both *Botryodiplodia theobromae* and *Trichoderma sp.* inoculation in some rice plant caused reduction in chlorophyll content. He emphasised that the fungi caused rot in the plants and also worked in synergy with the termites on rice plant, thereby causing more deterioration. As a result, this could be that those organisms were causative organisms of rot in one form or the other, while the EPF used in this case were not traced to be pathogenic. This may be the reason for none reduction in the chlorophyll content in the treatments. Thus, photo-synthase and photosynthesis were in full course and so there was no reduction. Neither the enzymes nor the biochemical reactions were affected or denature in any way by the EPF. Thus, the physiological processes were in order. Reduction in the chlorophyll content in some treatments was as a result of pest infestation.

The EPF reduced the infestation of AfRGM which caused reduction in the chlorophyll content thereby having control on the level of chlorophyll. In the same vein, the EPF did not have any negative effect on the stem girth but were able to indirectly reduce pest infestation which could have drastically reduced the stem girth. The study showed that the plants treated with *Metarhizium* had more panicle numbers and grain weight than the plants treated with *B. bassiana*. This is as a result of the effect of the EPF on the adult gall midge infestation. This revealed the standing effect of EPF to controlling the infestation of adult AfRGM on rice plant. This is in accordance with the findings of Zhioua et al. (1997); Zhioua et al. (1999) and Zimmerman (1993) showed great potential of *M. anisopliae* as biocontrol. However, mean tiller infestation had negative correlation with panicle length, plant height, leaf length but not with significant effect. While panicle number had significant and positive correlation with plant height, leaf breadth and grain number, panicle length had significant and positive correlation with plant height, leaf breadth and grain weight. *M. anisoplae* IC30 can be used to controlling AfRGM adult on rice without any negative effect on the plant physiology or agronomy.

Acknowledgement

The authors gratefully appreciate Nematology laboratory, IITA for assistance rendered during the preparation of the culture in the laboratory.

Disclosure statement

No potential conflict of interest was reported by the authors.

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