ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY

Research Article

Biomass Production and Field Trial of Entomopathogenic Fungi *Metarhizium anisopliae* (Metschn.) against Some Insect Pest of Agricultural Importance

Aliyu HU[1]*, Isma'il S[2], Yakubu MN[2], Deba FA[2], Ladan MA[2], Haruna US[3], Tahir F[2] and Abdulhameed A[2]

[1]*Department of Biological Sciences, Federal University of Kashere, Gombe, Nigeria*

[2]*Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria*

[3]*Department of Food Science and Technology, Federal Polytechnic, Bauchi, Nigeria*

***Corresponding Author:** Aliyu HU, Department of Biological Sciences, Federal University of Kashere, Gombe, Nigeria.

Received: January 12, 2022; **Published:** March 28, 2022

Abstract

Insect pests of agricultural crops have remained a major cause of reduced yields worldwide. The strain used in the current study was obtained from isolates collected from across Nigerian geographical zones and cultured in the Ecology laboratory of the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria. The fungus was initially grown on Potato Dextrose Agar medium and the conidia from the Petri dishes harvested and suspended in Tween 80 which was held in sterile distilled water. The conidia was subsequently mass produced using pre-cooked rice and rice husks. Field application was achieved by suspending the conidia in distilled water and using conventional back pack spray equipment. Rice grain had significantly higher spore counts than the rice husk. Even though significantly higher mortalities > 70% were recorded for both the substrates at both 7 and 10 days post treatment. The rice grain recorded higher percentage mortality against all the insect pests tested. This research showed the potentials of indigenous *Metarhizium anisopliae* as an effective insecticides of whiteflies, aphids, weevils and thrips that infest and destroy vegetables crops.

Keywords: *Metarhizium anisopliae; Biomass Production; Insecticides*

Introduction

Insect pests have remained a major limiting factor in the production of crops, vegetables and other agricultural produce worldwide [26]. Farmers in Nigeria rely heavily on synthetic chemical pesticides for the control of these pests on their farm. Chemical pesticides have been reported to have negative impact on man, animals and the environment leading to different forms of neurological ailments, cancer, environmental pollution and residual effect on crop produce among others [3,24]. These and other factors such as pest increased resistance to pesticides or their dose have necessitated the search for and use of safe, reliable and environmentally friendly alternatives such as biopesticides.

Entomopathogenic fungi have gained widespread recognition for use as biological control agents against a number of insect species globally. They are ecofriendly [1,13,27] and have high virulence and broad host range [14,21]. A number of mycoinsecticides based on *Beauveria bassiana* (Balsamo), *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Verticillium lecanii* (Zimm.) Viegas. have been exploited to control a number of insect pests of crops [5,6,20,31]. As well, *Metarhizium anisopliae* has also been exploited widely in the control of several insect pests worldwide [7]. However, Production of adequate quantities of good quality inoculums has been a challenge in the mass production of these fungi. *Metarhizium anisopliae* has this advantage of being produced en mass because of the nature of its conidia. Mass production of conidia of entomopathogenic fungi in large quantities may be achieved through several methods depending on the quantity that is desired [4]. It could be for laboratory experimentation and field-testing during which relatively small quantities of the inoculums are required and for commercial purpose, where a labour-intensive approach is usually adopted. Fungal entomopathogens have been produced in large quantities sufficient enough for niche markets in China, America and elsewhere [4,12,24]. For better efficiency, it is advised to isolate and use indigenous isolates of fungal entomopathogens due to the fact that they are better adapted to local environmental conditions [13,15].

Development of simple and reliable production system follows the basic multiplication procedure which has been proven reliable. It is the most viable technique for mass production and involves the use of a diphasic strategy where the fungal inoculum is produced in liquid culture, and subsequently used for inoculating the solid substrate for the production of conidia. The liquid fermentation is used to produce blastospore [18,25] and mycelium forms [17] and the solid phase is carried out in a solid substrate, which has a large surface area for aeration and physically supports the fungus to produce conidia, and it is also used as a source of nutrients [17]. The commonly used solid substrate is parboiled rice but this is expensive [17] and influences the price of the end product. Conidial production using rice as a substrate is approximately about 1×10^9 conidia g^{-1}. An added advantage would be the mass production of entomopathogenic fungal spores to be produced at low cost with high yield of the spores, that must be viable, virulent and with persistent propagules [24].

Aim of the Study

The aim of the current study therefore, was to evaluate comparative mass production of conidia of *Metarhizium anisopliae* using rice grain (pre-cooked) and rice husks as well as to test the pathogenicity of spores produced against some insect pest of vegetables on the field.

Materials and Methods

Selection of strain

Metarhizium anisopliae was obtained from fungi stock culture collection of Ecology laboratory, ATBU Bauchi. The spore suspension was prepared based on the method adopted by Erper., *et al*. 2016. The isolate was cultured on PDA potato dextrose agar plates and incubated at 25°C for 14 days until sporulation of the fungus occurred. The spores were harvested and placed in 10 ml of distilled water containing Tween 80 (0.1%). The suspension was filtered through a Whatman number 1filter paper to remove the mycelia and other associated debris, it was then mixed by vortex to obtain the inoculums culture. The spores were counted using a Neubauer Hemocytometer at 400 x magnification.

Liquid state fermentation

Liquid medium was prepared by suspending 130g dehydrated PDB Potato dextrose broth in 2000 ml of distilled water and heated to dissolve it completely. The medium was autoclaved at 15 lbs pressure (121°C) for 15 minutes. It was allowed to cool down and the lukewarm medium was poured into four conical flasks. The media were inoculated with 1 ml of *Metarhizium anisopliae* spore suspension each. The flasks were incubated at 25°C for 3 days. The spore suspension was subjected to spore counting as above.

Solid state fermentation

Rice husks were collected from rice mill in Bauchi while rice grains were bought from the market in Bauchi. Rice husk was soaked with distilled water and drained, 500g each was weighed and subsequently placed in each autoclavable bag and autoclaved. The rice grains were washed with clean water and pre-cooked. Subsequently 500g of the pre-cooked rice was weighed and placed into each autoclavable bag for autoclaving. The bags were allowed to cool and taken to the laminar flow chamber where they were each inoculated with 1 ml of spore suspension produced from the liquid phase. Bags were then plugged with ventilated caps to reduce contamination and incubated at

25°C for 12 days. At the end of the conidia production process, the bags were removed from the incubators and separately emptied onto trays and were placed in the drying room. And in order to determine the level of production, 1g each of the rice husk and pre-cooked rice (rice grain) with conidia was taken and a mixture of 100 ml sterile distilled water and Tween 80 (0.05% v/v) was prepared. The conidia obtained from the suspension were counted. And to obtain the spores as powder, the media were dried with the fungus so that the spores may separate from the substrate. The spores harvested from this procedure was preserved [18].

Enumeration of spores

To enumerate the spores, 1g block each of the rice husks and pre-cooked rice was cut aseptically and placed in to a conical flask containing 0.1% Tween 80 solution. A drop of conidial suspension of the fungus was placed on the Neubauer hemocytometer for spore count. A cover glass was put over the grid carefully so as to eliminate air bubbles from entering between cover glass and slide. The conidia were counted using a high-resolution research microscope in the middle square of the hemocytometer which contains 25 groups of 16 small squares and each about 0.2 mm square.

Field trial

Four important pests of vegetables namely Whiteflies, Aphids, weevils and thrips was sprayed with the fungal spores. Field trial was laid out in a completely randomized block design with four replications and mortality of the pests was observed after 7 days and 10 days post exposure to fungal spores. The whole experiment was repeated on another day. All the agronomic practices with recommended doses of fertilizers were maintained to make sure the plants were in good condition. The isolate was applied at the dose of 100 g/l for rice grain (pre-cooked) and that obtained from rice husk respectively. Application was done after planting at 3 days interval during pest incidence. Plant infestation with whiteflies, aphids, weevils and thrips were recorded after 7 days and 10 days post application of the *Metarhizium anisopliae* and the reduction in plant infestation was observed and recorded.

Statistical analysis

Data obtained from the study were transformed where necessary using x=log (x) for ease of analysis. The conidial production of entomopathogenic fungi from different media as well as pest reduction produced by the action of the spores were subjected to statistical analysis using SPSS and GraphPad Prism Version 8. The means were separated using Brown-Forsythe test at $P < 0.05$ level of significance.

Results

Potato dextrose broth was successfully used to produce high quality inoculums in the liquid phase fermentation with an average spore production of 15.26×10^6 spores/ml and this was subsequently used as inoculum for mass production of the *Metarhizium anisopliae* on the solid media namely rice grain (pre-cooked) and rice husk that were evaluated in this study.

Results in figure 1 show that rice grain (pre-cooked) produced the highest number of mean spores count of 1×10^{12} compared to rice husk with mean spores count of 5.02×10^{11}.

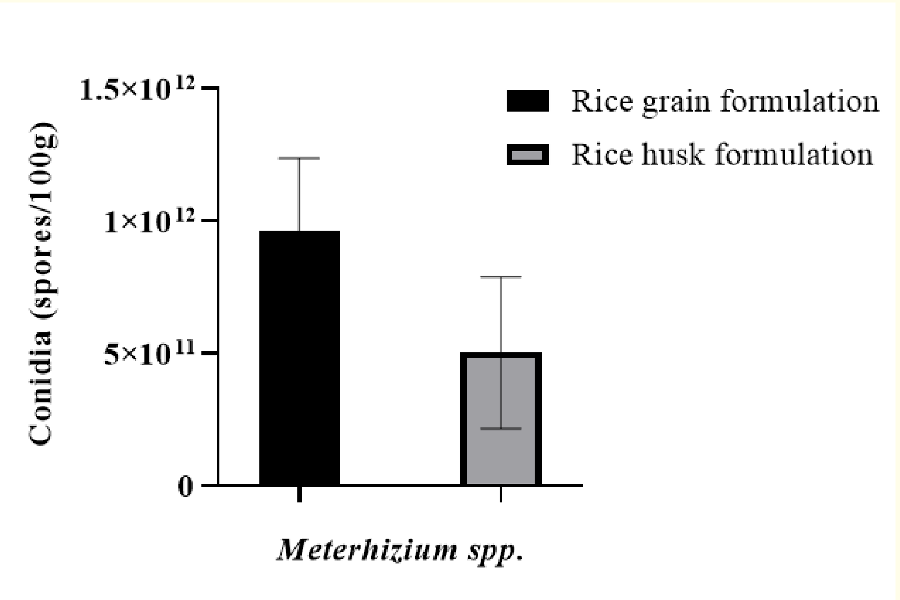

Figure 1: *Comparative spore counts of conidia of Metarhizium anisopliae cultured on rice grain (pre-cooked) and rice husk.*

Virulence of *Metarhizium anisopliae* on insect pests

There was generally very high mortality on all the insect pests treated with the conidia obtained from the two substrates on which the Metarhizium anisopliae was grown and harvested from. Virulence of the isolate was assessed based on reduction of insect pests infesting plants on the fields 7 days and 10 days post treatment with fungal conidia.

Conidia cultured on rice grain (pre-cooked) produced its highest activity against whiteflies with a mortality of 93.50% followed closely by thrips with a mortality of 92.29%, it produced a mortality of 90.80 in weevils and 89.41% in aphids at 7 days post exposure to the fungal conidia.

Subsequently, an increase in mortality of the pests was also observed 10 days post exposure of the pests to the fungal conidia as the mortality of whiteflies increased to 97.98%, mortality of thrips increased to 95.98% while those of aphids increased to 95.67% and weevils 90.80%.

At 7 days post exposure of pests to fungal conidia produced on rice husk, the highest virulence was recorded for thrips with a mortality of 98.13%, followed closely by aphids with a mortality of 88.90%, this was followed by weevils with a mortality of 78.00% and finally whiteflies with a mortality of 73%.

At 10 days post exposure of the pests to the fungal conidia, mortality of thrips and weevils remained at 98.13% and 78% respectively while those of whiteflies and aphids increased to 80.4% and 97.73% respectively.

	Post treatment with Conidia on rice grain				Post treatment with Conidia on rice husk			
Pretreatment Incidence of pests	Mortality after 7 days	% mortality	Mortality after 10 days	% mortality	Mortality after 7 days	% mortality	Mortality after 10 days	% Mortality
Whitefly (50)	46.75	93.50	48.5	97.00	36.5	73.00	40.20	80.40
Aphids (150)	134.12	89.41	143.5	95.67	132.6	88.90	146.6	97.73
Weevils (15)	13.62	90.80	13.62	90.80	11.70	78.00	11.70	78.00
Thrips (112)	103.37	92.29	107.5	95.98	109.90	98.13	109.90	98.13

Table 1: *Mortality of pests of Capsicum spp. treated with conidia of Metarhizium anisopliae produced on rice grain (pre-cooked) and rice husk.*

Discussion

Environmentally friendly insect pest management practices demand that indigenous organisms that have potential biological control activity be isolated from the local environment and exploited accordingly as reported by Zaki and Jacques (2020). Production of biomass of *Metarhizium anisopliae* on solid media showed significant variation at $P < 0.05$ level of significance with conidia produced on rice grain (pre-cooked) having the highest number of spores compared to those produced on rice husk. Higher spore production on precooked rice was also reported by Sahayaraj., *et al*. (2008) who also recorded higher fungal spores on rice grain after evaluating several agricultural products and byproducts. It is also important to note that the spores produced on both substrates in the current study differ significantly in their quantity and virulence. The higher virulence for example of spores cultured on rice grains recorded substantial reduction in the population of whiteflies and aphids. This could be attributed to higher carbohydrate contents of the rice grain than that of the rice husk.

Similar report on aphids and whiteflies was observed by Sigh and Kaur (2020); Vu., *et al*. (2007). On the contrary, Ansari., *et al*. (2008) reported that media of growth had no effect on the role of virulence of *Metarhizium anisopliae* against thrips.

Quality of conidia which depends on nutrients from its food source could affect efficacy as is observed in the case of conidia produced on rice husk [9,11,24].

Over all, both products produced promising results on the field in terms of percentage mortality of insects treated. The use of indigenously isolated *Metarhizium anisopliae* in this study, has shown the need to further determine the potentials of other indigenous culture that are adapted to the natural environment with possibly higher virulence than their exotic counterpart. Different fungi have different pathogenic potential in their ability to infect and kill insects as a result of metabolic and genetic differences inherent to each isolate due to their adaptation to their original environment [11]. This observation further buttresses the fact that indigenous isolates are better suited for the control of local pest populations.

Since using rice grain (pre-cooked) is expensive compared to using rice husks, rice husk can be used as an alternative to rice grain (pre-cooked) for large scale spore production in *Metarhizium anisopliae* for field application against important insect pests of crops since efficiency of spores produced is within the acceptable limits. Conidia produced on rice grain produced efficiency of between 90.8% to 97% after 10 days post exposure while that produced on rice husk produced mortality of 78% to 98.13%. this result is in agreement with that observed by [2] who observed that *Metarhizium anisopliae* produced a mortality of 85-96% in thrips 11 days post exposure to the fungal spores. [27] also made similar observations. [8] successfully controlled and managed thrips using *Metarhizium anisopliae* in both laboratory bioassay as well as on the field. There were significant differences in the reduction of insect pests infesting chili after exposure to fungal conidia. It is also important to note that the isolate is effective against weevils and as such it could be used in the management and control of these important pests as also observed by [16].

Conclusion

The use of *Metarhizium anisopliae* in the biological control of insect pests in a part of northern Nigeria provided promising results and is good news to Nigerian agriculture. The biomass production potential of the isolate was established successfully on rice grain (pre-cooked) and rice husk with conidia cultured on rice grain showing higher efficacy compared to that cultured on rice husk. This study therefore provided a positive outcome that the isolate was effective against whiteflies, aphids, weevils and thrips and hence it has the potential to be developed into a mycopesticide for its use in integrated pest management and control programs.

Acknowledgement

The authors are grateful to the Department of Biological Sciences of Abubakar Tafawa Balewa University, Bauchi for providing us with the work bench and their expert support and to TETFUND/NRF (ATBU/TETFUND/NRF2019/BIO/03) for full funding of the research.

Bibliography

1. Abdel-Raheem M. “Insect control by entomopathogenic fungi, and chemical compounds”. Book, Lambert Academic Publishing (2015): 76.

2. Ansari MA., *et al*. “Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis*, in plant-growing media”. *Entomologia Experimentalis et Aplicata* 127.2 (2008): 80-87.

3. Aliyu HU and Fidelis LK. "Occurrence of insect pathogenic fungi in some locations within Federal University of kashere, Gombe State, Nigeria". *Jewel Journal of Scientific Research* 6.1-2 (2021): 34-37.
4. Alves SB and Pereira RM. "Production of *Metarhizium anisopliae* and *Beauveria bassiana*". *Ecosustania* 14 (1989): 188-192.
5. Avery PB., *et al*. "Effect of different photoperiods on the infectivity and colonization of Paecilomyces fumosoroseus". *Journal of Insect Science* 4 (2004): 38.
6. Babu V., *et al*. "Laboratory studies on the efficiency of neem and the entomopathogenic fungus *Beauveria bassiana* on *Spodoptera litura*". *Entomology* 56 (2001): 56-63.
7. Chen Z-H., *et al*. "Efficacy of *Metarhizium anisopliae* isolate MAX-2 from Shangri-la, China under desiccation stress". *BMC Microbiology* 14 (2014): 4.
8. Culzar S., *et al*. "Combined effect of entomopathogens against Thrips tabaci Lindeman (Thysamoptera: Thripidae): Laboratory, greenhouse and field trials". *Insects* 12.5 (2021): 456-473.
9. El-Sufty R., *et al*. "Pathogenicity of the fungus *Beauveria bassiana* (Bals.) Vuill to the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (Col.Curculionidae) under laboratory and field conditions". *Egyptian Journal of Biological Pest Control* 19.1 (2009): 81-85.
10. Erper I., *et al*. "Evaluation of some entomopathogenic Fungi for controlling the green shield bug, *Palomenaprasina* L. (Heteroptera:Pentatomidae)". *Egyptian Journal of Biological Pest Control* 26 (2016): 573.
11. Hou FJ., *et al*. "Virulence evaluation of entomopathogenic fungi against the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Dryopthoridae)". *Malaysian Applied Biology* 47.5 (2018): 25-30.
12. Feng MG., *et al*. "Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status". *Biocontrol Science and Technology* 4.1 (1994): 3-34.
13. Gebremariam A., *et al*. "Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, *Beauveria bassiana* (Balsam) Vuillemin, and *Metarhizium anisopliae* (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticides". *Heliyon* 7.5 (2021): e07091.
14. Gurlek S., *et al*. "Isolation and characterization of *Beauveria* and *Metarhizium* spp. from walnut fields and their pathogenicity against the codling moth, *Cydiapomonella* (L.) (Lepidoptera:Tortricidae)". *Egyptian Journal of Biological Pest control* 28.1 (2018): 50.
15. Hernando-Trejo A., *et al*. "Effects of native entomopathogenic fungal strains and neem extract on *Spodoptera frugiperda* on maize". *Southwestern Entomology* 44.1 (2019): 117-124.
16. Khun KK., *et al*. "Integration of entomopathogenic fungi into IPM programs: studies involving weevils (Coleoptera: Curculionoida) affecting horticultural crops". *Insects* 11.10 (2020): 659.
17. Kruger RD., *et al*. "Solid Substrate Production and Formulation of an Isolate of *Metarhizium anisopliae* for biological Control of Stem Bug *Tibraca lombativentris*". *World Applied Sciences Journal* 32.7 (2014): 1242-1251.
18. Latifian M., *et al*. "Mass production of entomopathogenic fungi *Metarhizium anisopliae* by using agricultural products based on liquid-solid diphasic method for date palm control". *International Journal of Farming and Allied Sciences* 3 and 4 (2014): 368-372.

19. Maniania NK., *et al*. "A field trial of the entomogenous fungus *Metarhizium anisopliae* for control of onion thrips, *Thrips tabaci*". *Crop Protection* 22.3 (2003): 553-559.

20. Melekan N., *et al*. "Evaluation of entomopathogenic fungi *Beauveria bassiana* and *Lecnic iummuscariumon* different nymphal stages of greenhouse whitefly *Trialeurodes vaporariorum* in greenhouse conditions". *Biharean Biology* 9.2 (2015): 108-112.

21. Mishra S., *et al*. "Morpho-molecular characterization and virulence determination of entomopathogenic fungal isolates native to Indian subcontinent". *Medical Mycology* 1 (2015): 3.

22. Mohammed AA and Hatcher PE. "Effect of temperature, relative humidity and aphid developmental stage on the efficacy of the mycoinsecticide Mycota R against Myzuspersicae". *Biocontrol Science and Technology* 26.10 (2016): 1379-1400.

23. Mohammed AA., *et al*. "Selection of highly virulent entomopathogenic fungal isolates to control the greenhouse aphid species in Iraq". *Egyptian Journal of Biological Pest Control* 28 (2018): 71.

24. Prasad CS and Pal R. "Mass production and economics of entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecaniion* agricultural and industrial waste". *Scholars Journal of Agricultural and Veterinary Sciences* 1.1 (2014): 28-32.

25. Riaz ABID., *et al*. "Intra-specific variability among *Metarhizium anisopliae* strains in their ability to produce blastospores in liquid culture media". *Pakistan Journal of Botany* 45.3 (2013): 1099-1103.

26. Robles-Acosta., *et al*. "Entomopathogenic fungi as biological control agents of *Phyllocoptruta oleivora* (Protigmata: Eriophyidae) under greenhouse conditions". *Florida Entomologist* 102.2 (2019): 303-308.

27. Santos TS., *et al*. "Entomopathogenic Fungi Biomass Production and Extracellular Biosynthesis of Silver Nanoparticles for Bioinsecticide Action". *Applied Sciences* 11.6 (2021): 2465.

28. Sahayaraj K and Namasivayam KRS. "Mass production of entomopathogenic fungi using agricultural products and by products". *African Journal of Biotechnology* 7.12 (2008): 1907-1910.

29. Singh H and Kaur T. "Pathogenicity of entomopathogenic fungi against the aphid and the whitefly species on crops grown under green house conditions in India". *Egyptian Journal of Biological Pest Control* 30 (2020): 84.

30. Soundrapandian P and Chandra R. "Mass production of entomopathogenic fungi *Metarhizium anisopliae* (Deuteromycota; Hyphomycetes) in the laboratory". *Research Journal of Microbiology* 2.9 (2007): 690-705.

31. Tanada Y and Kaya HK. "Insect pathology". Academic, San Diego, CA (1993).

32. Zaki O and Jacques P. "Limiting factors of mycopesticides development". *Biological Control* 144 (2020): 104220.

Volume 10 Issue 4 April 2022