Comparative efficacy of some entomopathogenic fungi as biological control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in north-east arid zone of Nigeria.

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ABSTRACT

Four strains of entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces sp* and *Nomuraea rileyi* were evaluated for pathogenicity against *Bemisia tabaci* in the laboratory *invitro* using four concentrations of conidia/ml.Thirty whiteflies were used for each set of experiment for each strain of entomopathogenic fungi. They were divided into six groups out of which one group is a control. Each treatment was replicated five times on monthly bases for each concentration along with control. *Bemisia tabaci* mortality was recorded at two days interval for ten days. Mortality data were analyzed using the Two-Way Analysis of Variance and significant means were differentiated by Duncan Multiple Range Test (DMRT). Compared with the control group, all strains had a significantly increased mortality rate of Whitefly ($P < 0.05$). The most effective strain was *B. bassiana* in all the concentrations (mortality range between 81.6±0.01 – 95.2±0.44 % at 10 days post treatment (DPT) followed by *N. rileyi* (63.6±0.41 - 78.2±0.18) %, least performance was noticed in *P. species* (42.6±0.01 - 68.2±0.44 %). This study has shown that the potential of entomopathogenic fungi especially *B. bassiana* and *N. rileyi* has raised a hope to be harnessed as alternative control measure of *Bemisia tabaci* at larval stage with further research.

Key Words: *Bemisia tabaci*, entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae, Nomuraea rileyi*, *Paecilomyces sp*, bio control.

INTRODUCTION

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) popularly known as whitefly is widely distributed in almost all ecological zone and is one of the most intractable and damaging insect pest in many agricultural and horticultural crops including vegetables, ornamentals and field crops (Oliveira *et al.*, 2001, Stansly and Naranjo, 2010). *Bemisia tabaci* is highly polyphagous; damaging a broad range of crops by directs feeding on phloem sap and excretes honeydew on leaves and fruits of crops under attack. The sticky, sugary surface forms a substrate for the growth of black sooty molds that stain crops and covers the leaves; thus impairing photosynthesis (Hogeenhout *et al.*, 2008). The resulting stickiness and discoloration greatly reduce the values of agricultural crops such as ornamentals, vegetables and cottons (Hequet *et al.*, 2007). *B. tabaci* is a vector of several important families of plant viruses and transmit over 100 virus species of plants (Jones, 2003, Hogeenhout *et al.*, 2008). Damaging effect of *B. tabaci* has been a major challenge to agriculture not only in the Arid Zone but worldwide due to significant yield loss in crops pests (Berlinger, 1986).

Control measures throughout the world cost billions of dollars each year (Pawar *et al.*, 1986) and for Centuries in the past, farmers including foresters and environmentalist have searched for crops that can survive and produce in spite of not only *B. tabaci* but other insect pests and diseases they transmit, sometimes by action as simple as collecting seeds from the highest yielding plants in their field. Most of the times selections were unscientific and the result obtained are in terms of resistance is unpredictable. Though the discovery of scientific improvement in plant breeding techniques base on knowledge of genetics and the use of chemical pesticides for the control of *B. tabaci* and other insect pests have made significant impact by improving the resistance of many crops to important insect pests and diseases (Ubi, 2013), however, the processes involved in making crosses, back crosses, and progeny selection has been time consuming and made it difficult to react quickly to the evolution and resurgence of pests and virulent pathogen races.

With the advent of genetic engineering and tissue culture biotechnology (Hoy, 1992; Ubi, 2013), it became very possible to transfer a single or multiple alien gene(s) of desired characteristics into a plant of interest more quickly and deliberately to produce transformed (transgenic) plants that could be resistance to insect pests through expression of the introduced gene without limiting it within plant families as in the case of conventional

breeding (Hoy, 1992; Ubi, 2013). However, the processes involved are still very costly in terms of financial burden, time and technical know-how.

Throughout the world, growers routinely use insecticides against *B. tabaci* and these have included broad-spectrum chemicals such as organophosphate, carbornates and pyrethroids. The use of these chemicals for the control of this insect species has been shown to have a significant impact on crop production. However, the use of the chemicals pesticides is becoming more problematic in many areas of crop production and vector control due to development of resistance of these insect pests to chemical pesticides and the accumulation effects of these pesticides to human health and the environment is becoming an issue of concern. *Bemisia tabaci* have shown resistance to over 40 active ingredients of the major synthetic insecticide groups in many countries of world (Gunning *et al.,* 1992, Armes *et al.,* 1992, Ahmad *et al.,* 1997 and Bues *et al.,* 2005). Synthetic insecticide therefore is not expected to retain its efficacy indefinitely even in areas where resistance have not been reported or investigated. Overdependence on a particular group of chemicals is one of the most important reasons for the rapid development of resistance among insects (Chu *et al.,* 2010) and also, the over use and increase dosages of insecticides against resistant insect pest populations could lead to disruption of natural enemies of both the target and secondary pest species, thereby generating the notorious "pesticide treadmill" effect. In lieu of this therefore, there is an urgent need to research on alternative means of control insect pests other than depending on conventional use of synthetic insecticides hence the focuses of this study.

In many parts of the world, research is currently being conducted on the development of bio insecticides based on entamopathogenic fungi and transgenic host plant for the control of insect pests (Horowitz *et al.,* 2014). The use of entomopathogenic as bio-pesticides has been shown to have more advantageous over conventional insecticides being used for insect pest control because in addition to environmental benefits, the chances of resistance being developed against insect pathogenic entities are said to be far less compared to synthetic insecticide (Horowitz *et al.,* 2014) hence the need to evaluate its efficacy in the control of *Bemesia tabaci* in Arid Zone Ecology. These studies therefore assess the efficacy of four strains of entomopathogenic fungi to control *Bemesia tabaci* on tomato and cotton crops.

Materials and methods

Study area:

The study was conducted in three States of the Sudano-Sahelian ecological zone of Nigeria *viz:* Bauchi, Borno and Gombe States (Fig. 1). These three States share some similar agro-ecology, which is characterized by unimodal rainfall ranging between 12 mm to 1200 mm annually, temperature ranges between 18.4 and 34°C, altitude of 600–1800 m (http://www.infonet-biovision.org/default/ct/690/agrozone). Vegetation is mainly Sudano-savannah grass land making the region suitable for the cultivation of various crop varieties including vegetables, bulbs, grain and tree crops and also suitable for high rate of multiplication and infestation of crops by insect pests. In each State, two major areas of farming activities were selected *viz:* Wuro wasse and Toro (Bauchi); Tudun wada and COAG farms (Borno); and Kwadon and Dadin kowa (Gombe).

Bauchi is the Capital city of Bauchi State and is located in the Sudan-Savannah region of north-east Nigeria at Latitude 10º18'N and Longitude 9º50'E. Gombe on the other hand is the capital city of Gombe State and is located at Latitude 10º 17' N and Longitude 11º10' E while Maiduguri is the Capital of Borno State and is located in the Sahel Savannah region of North-East Nigeria at Latitude 11º 05' N and Longitude 13º 05' E and at about 350 m above sea level.

The temperature for the three States varies with time and location of an area. In Bauchi for instance the mean daily maximum temperatures range from 29.2°C in July and August to 37.6°C in March and April. The mean daily minimum ranges between 11.7°C in December and January to about 24.7°C in April and May. While in Gombe temperature ranges between 22 and 34°C and has similar pattern of monthly distribution like Bauchi. In Maiduguri, the rainy season months are May to September/October with its peak in August. Humidity ranges

from about 28 per cent to 46 per cent. Monthly rainfall ranges from 0.0 mm in December - April, to about 343 mm in August. Onset of the rains varies but more often in April while they end virtually by October. The sunshine hours range from about 5.1 hours in July - September to about 10.9 - 12 hours in the remaining months (http://www.infonet-biovision.org/default/ct/690/agrozones).

Test of efficacy of Entomopathogenic fungi as biological control agents.

Four strains of entomopathogenic fungi were evaluated in this study and pure culture strains were obtained from the culture collection of the Agricultural Research Council's Plant Protection Research Instituted (PPRI) South Africa. The strains include: *B. bassiana*, *M. anisopliae*, *P. species* and *N. rileyi*. Isolates selection was based on the report that they are among the most virulent entomopathogen against many insect species in other ecological zones (Myint, 1997; Jenkins, 1995). Isolates were cultured in the laboratory in the Department of Biological Sciences, University of Maiduguri on Sabouraud Dextrose Agar (SDA), supplemented with 1% yeast extract and kept for 10 days at 25±1 ºC, 70 % relative humidity (RH), 12:12 (Light: Darkness) photoperiod and pH 6.7 as described by (Hatting, 2012). Then conidia were harvested at days 5, 10 and 15 as described by Horowitz *et al*., (2014).

Laboratory bioassay of Entomopathogenic fungi on Whitefly:

Four concentrations of conidia suspension for each strain were used for the bioassay *viz:* 1 x 10^6, 1 x 10^7, 1 x 10^8 and 1 x 10^9 conidia/ml.

30 whiteflies nymph were used for each set of experiment for each strain of entomopathogenic fungi. They were divided into five groups of six members. Each group was made up of 6 nymphs. Each group was introduced into a petri dish containing moistened filter paper and fresh top leaves obtained from tomato plant. The moistened filter paper for each group was impregnated with a 0.5 ml spore suspension for each concentration except the control group. To prevent the flies from escaping, the petri dishes were covered with insect proof nets on top for ventilation. Control flies were placed into Petri dishes containing filter paper impregnated only with a 0.5 ml aqueous Tween. The flies in group 1 to 4 were treated

with 0.5 ml spore suspension of each concentration at two days interval for 10 days while the filter paper were kept moist to prevent leaves from desiccation by adding 15 ml of distilled water uniformly over the surface of net cover alternated with fungi application days. All treated and control groups were observed on daily bases for 10 days to detect dead whiteflies.

Data analysis

Data were analyzed using the Two-Way Analysis of Variance (Sokal and Rohlf 1969) and significant means were differentiated by Duncan Multiple Range Test (DMRT).

RESULTS

The results of mortality response of *Bemisia tabaci* to the evaluated entomopathogenic fungi is presented in tables 1 – 4. The results showed that all the four strains of Entomopathogenic fungi had a pathogenic effect on the Whitefly nymph. Compared with the control group, all strains had a significantly increased mortality rate in the Whitefly ($P < 0.05$). The mortality rate increases for all strains as concentration and time (days) of exposure of the Whitefly to the fungi increased. Generally, the effectiveness of all the strains begun from 4 days post treatment (DPT), however, significant effect was recorded after six days post treatment. The most effective strain was *B. bassiana* in all the concentrations (mortality range between 81.6±0.01 – 95.2±0.44 % for nymph (Table 1), followed by *N. rileyi* (63.6±0.41 -78.2±0.18).

able 1. % Mean ± S.D mortality of *B. tabaci* exposed to *Beauveria bassiana*

		Concentration (conidia/ml)			
ETD	Control	10^6	10^7	10^8	10^9
2	2.6±1.21[a]	9.8±2.03[a]	7.6±0.01[a]	11.8±1.03[a]	17.6±0.50[a]
4	3.6±1.21[a]	18.4±1.02[b]	26.8±1.31[b]	32.3±1.30[b]	38.3±0.44[b]
6	6.8±2.02a[b]	39.5±2.04[c]	47.2±0.31[c]	60.6±0.21[c]	70.1±0.30[c]

8	9.0±2.03[b]	40.8±1.03[c]	63.5±1.00[d]	75.0±0.13[d]	77.8±0.03[c]	
10	15.7±2.33[c]	81.6±0.01[d]	86.4±2.05[e]	92.8±0.02[e]	95.2±0.44[d]	

Means in the same column with the same letters in each table are not significantly different at P = 0.05 by DMRT, DS = developmental stage, ETD = exposure time in days.

Table 2. % Mean ± S.D mortality of *B. tabaci* exposed to *Nomuraea rileyi*

		Concentration (conidia/ml)				
DS	ETD	Control	10^6	10^7	10^8	10^9
Larvae/Nymph	2	1.8±2.11[a]	3.5±2.13[a]	12.4±0.01[a]	9.6±1.04[a]	14.6±0.54[a]
	4	3.6±1.30[b]	14.8±1.03[b]	32.8±1.11[b]	38.8±1.30[b]	44.3±0.41[b]
	6	8.8±2.02[c]	33.5±2.03[c]	53.2±0.31[c]	58.7±0.61[c]	60.1±0.30[c]
	8	12.3±2.03[c]	48.8±1.03[d]	62.5±1.33[c]	60.0±0.33[cd]	70.8±0.03[d]
	10	14.6±2.33[c]	63.6±0.41[e]	88.4±2.07[d]	68.8±0.23[d]	78.2±0.18e

Means in the same column with the same letters in each table are not significantly different at P = 0.05 by DMRT, DS = developmental stage, ETD = exposure time in days.

Table 3. % Mean ± S.D mortality of *Bemeisia tabaci* exposed to *Metarhizum anisopolae*

		Concentration (conidia/ml)				
DS	ETD	Control	10^6	10^7	10^8	10^9
Larvae/Nymph	2	6.3±2.01[a]	8.5±2.23[a]	12.4±0.03[a]	9.6±1.01[a]	14.6±0.88[a]
	4	9.2±1.33[a]	18.8±1.00[b]	28.8±1.21[b]	32.8±1.3[b]	42.3±0.41[b]
	6	9.8±2.22[a]	40.5±2.03[c]	53.2±0.22[c]	58.7±0.71[c]	68.1±0.33[c]

8	12.3±1.03[a]	51.8±0.03[d]	60.0±1.35[d]	62.5±0.33[c]	70.8±0.08[c]
10	14.6±2.33[ab]	60.6±0.31[e]	63.4±2.02[d]	68.8±0.68[d]	68.8±0.68[c]

Means in the same column with the same letters in each table are not significantly different at P = 0.05 by DMRT, DS = developmental stage, ETD = exposure time in days.

Table 4. % Mean ± S.D mortality of *B. tabaci* exposed to *Paecilomyces species*

		Concentration (conidia/ml)				
DS	ETD	Control	10^6	10^7	10^8	10^9
Larvae/Nymph	2	3.6±1.21[a]	9.8±2.03[a]	10.5±0.01[a]	12.6±1.03[a]	14.6±0.50[a]
	4	3.6±1.21[a]	18.8±1.02[b]	22.6±1.31[b]	28.8±1.30[b]	28.8±0.44[b]
	6	6.8±2.02[a]	30.5±2.04[c]	32.2±0.31[c]	34.7±0.21[cd]	60.8±0.30[c]
	8	9.3±2.03[b]	40.8±1.03[d]	42.5±1.00[d]	60.0±0.13[d]	62.8±0.32[c]
	10	14.0±2.33[b]	42.6±0.01[d]	44.4±2.05[d]	48.8±0.02[c]	68.2±0.44[cd]

Means in the same column with the same letters in each table are not significantly different at P = 0.05 by DMRT, DS = developmental stage, ETD = exposure time in days.

DISCUSSION

Recently, implementation of entomopathogenic fungi for biological control of insects has increased, and a few of them are available commercially (Ferron *et al.*, 1991; Shah & Pell, 2001). The results obtained from this study using bioassay revealed that all the four strains of entomopathogenic fungi evaluated had a pathogenic effect on the *B. tabaci* nymph *invitro*. Compared with the control group, all strains had a significantly increased mortality rate in the *B. tabaci* ($P < 0.05$). However, *B. bassiana* and *N. rileyi* outperformed the strains of *M. anisopolae* and *P. species* causing a mean mortality >90 % compared to <80 % respectively in the laboratory. The mortality rate increased significantly for all the fungi strains as the time (days) of post exposure of the Whitefly to the fungi increased. This finding seems to agree

with the report of Samson *et al.*, (1988), Lacey *et al.*, (2001) in a study on biological control by entomopathogenic fungus that in most cases mortality rate of insect pest to the fungi was dose dependent with regard to cornidia/ml concentration used. Although no insecticidal compounds has been isolated from entomopathogenic fungi to be responsible for the death of insect, however, fungal spores or conidia have been reported to cause infection in insect by accessing to the host through the cuticle and it involves complex biochemical interactions between the host and the fungus (Samson *et al.*, 1988; Lacey *et al.*, 2001). Despite the fact that the mechanism of infection has not been fully understood however, it has been suggested that extracellular chitinases act as the virulence factor in fungal entomopathogenicity. According to Lacey *et al.* (2001) the successful use of entomopathogenic fungi as insect control agents ultimately depends on the use of the right propagule, formulated in an optimal manner and applied at an appropriate dosage and time, and also the presence of a susceptible host stage.

The finding of *B. bassiana* in this study as the most pathogenic of the evaluated fungi strains (mortality rate 81.6 % to 95.2 %), followed by *N. rileyi* (63.6 % to 88.4 %) at the lowest and highest dose concentration suggest that these strains might be highly lethal to whitefly larvae and thereby could have a great potential as a microbial control agents of whitefly at nymphal developmental stages. This finding agree with Pirali- Kheirabadi *et al*., (2007) in a study on biological control of *Boophilus annulatus* by *B. bassiana* and other entomopathogenic fungi, that mortality rate of host was dose dependent with regard to the conidial concentration used, where mortality rate (90 % -100 %) was recorded at 6 to 11 DPT lethal time for $\geq$ 50 % mortality. The pathogenicity of *B. bassiana* to different species of ticks has been found that this fungus has considerable potential as a microbial control agent for the management of animal ticks (Kaaya *et al.*, 1996; Zhioua *et al.*, 1997). Hornbostel *et al.* (2004), in a study on the sub lethal effects of *B. bassiana* on engorged larval, nymphal, and adult Ixodes scapularis, revealed that *B. bassiana* reduces fitness (fecundity and body mass) in all the active stages of development and showed that its impact as a bio control agent might be higher than that suggested by direct mortality alone. Although this study have shown that entomopathogenic fungi have great potential in the management of *B. tabaci* by attacking and killing the whitefly, however, the mechanism of inducing pathogenic effect has not been studied and isolation of the insecticidal compounds from these fungi is also yet to be done. Therefore, future research on the functional and biochemical characterization of the fungi is of primary goal.

CONCLUSION

The results obtained from the bioassay showed that all the four strains of Entomopathogenic fungi had a pathogenic effect on the Whitefly the nymph. Compared with the control group and all the strains had a significantly increased mortality rate in the Whitefly. The mortality rate increases for all strains as concentration and time (days) of exposure of the Whitefly to the fungi increased. The high pathogenicity exhibited by entomopathogenic fungi especially *B. bassiana* and *N. rileyi* to white fly population in this study suggest that they could be potentially harnessed as alternative control measure with further research.

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