Original Research Article

Characterization of growth and virulence of five Nigerian isolates of entomopathogenic fungi using Galleria mellonella larvae for pathogenicity testing

Abstract

Entomopathogenic fungi (EF) are naturally occurring insect population regulators,with several species that are exploited as biocontrol agents against insect pests. Five EF consisting of two strains of *Isaria farinosa*, (IF-1 and IF-II) and one strain each of *Metarhizium anisopliae*, *Beauveria bassiana* and *Entomophaga grylli*. (Sensu lato) were isolated from soil using *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Herbst) larvae as bait. They were cultured on standard Potato Dextrose Agar (PDA) (Sigma-Aldrich, UK) and identified based on phenotypic appearance and micro-morphology. Growth rates (mm day^{-1}), number of conidia per cm^2 colony area after incubation for 14 days at ambient temperature (25±2°C), viability of conidia (% germination), based on 24-hours incubation period and virulence of the infective conidia against *Galleria mellonella* were evaluated. The data on growth was subjected to analysis of Variance (ANOVA) procedure and means were separated using Tukeys Honestly Significant Difference (P=0.05). The number of conidia produced by *Entomophaga* grylli was (7.0×10^5 conidia cm^2 per colony area), while the isolate of *M. anisopliae* produced (5.2×10^4 conidia cm^2 per colony area). The number of conidia produced by the two isolates of *I. farinosa*, IF-1 and IF-2 and *B. bassiana* were 9.4×10^4, 7.2×10^4 and 2.1×10^5 conidia cm^2 per colony area respectively. Eighty percent of *E. grylli* conidia germinated after incubating for 24 hours at 25°C while 100% germination occurred in the other fungal isolates. There were statistically significant variabilities in the rates of growth of the EF isolates $F(4,2.064) = 12.97$, $P=0.001$. The *B. bassiana* isolate had the fastest growth rate, with statistically significant value of 3.3 mm day^{-1}. The rates of growth of the two *I. farinosa* isolates: IF-I, IF-II and *M. anisopliae* were comparable, being 1.53, 1.4 and 1.28 mm day^{-1} respectively, without statistically significant difference. The growth rate of *E. grylli* was 2.0 mm day^{-1}, which was significantly higher than the growth rates of *I. farinosa* and *M. anisopliae*. The mean percentage mortality values of, *G. mellonella* larvae treated with

1×10^{8}conidia ml^{-1} of the infective conidia of *I. Farinosa*, IF-1, IF-2, *M. anisopliae*, after five days were 70, 60, 60% respectively while *E. grylli* and *B. bassiana* caused 50% mortality. The results suggest that the five isolates examined can potentially be developed into experimental formulations and tested against important horticultural pests in future studies.

Keywords: Conidiation, Growth rate, Virulence, Isolation, Mortality

Introduction

Entomopathogenic fungi are natural pest population regulators, freely occurring in soil and some important members of the species, especially the anamorphs of Hypocreales: *Beauveria bassiana*, *Isaria farinosa* and *Metarhizium anisopliae*, have been isolated from dead insects (Servim *et al.*, 2010), soil (Zimmermann, 2008), tree barks (Shahid *etal*., 2012) and many have been developed into bio-pesticides for the management of pest populations.

Increaseddevelopment of insect'sresistance to chemical pesticides and environmental toxicity problems havepromoted interests in search for alternative, environment-friendly pesticides that can be exploited as stand-alone pest control agents or as a componentof integrated pest management (IPM) system (Kimberly and Seow, 2017).

Development of sustainable Integrated Pest Management (IPM) system using fungal biocontrol agents as a component, alongside existing conventional methods under tropical climate is promising(Borisade, *et al.*, 2016).However, success of entomopathogenic fungal biopesticides under field conditions in the tropics depends largely on their ability to exploit the relatively short,favourable windows of temperature and relative humidity available for growth and infectivity (Vega *et* al., 2009; Borisade and Magan, 2015).It is expected that entomopathogenic fungal biopesticides developed from non-tropical bioactive propagule may not be perfectly adaptablefor use under tropical agro ecological conditions, where temperatures and relative humidity often fall outside the critical limits for growth and infection.Borisade and Magan (2014)reported the critical upper and lower temperature and water activity (a_w) boundaries for nineteen strains of entomopathogenic fungi, including *B. bassiana, I. farinosa, M. anisopliae* and *I. fumosorosea*isolated from tropical and temperate environments and demonstrated that species from tropical environments showed better adaptability to marginal temperatures and $a_w$and variabilities in the rates of growth,

sporulation and virulence of some of the species was temperature and relative humidity (RH)-dependent.

As far as we know, eco-physiology of Nigerian isolates of entomopathogenic fungal species and characterization of growth, sporulation and pathogenicity have not been reported. However, studies on thebiocontrol efficacyof experimental formulation of *M. anisopliae*(*Ma* 275.86DC),non-indigenous but tropical isolate showed promising results under ambient temperature and relative humidity when tested against the banana weevil, *Cosmopolites sordidus*in the laboratory (Borisade *etal*., 2018) and the tomato whitefly, *Bemisia tabaci* under field conditions.

The aim of this study was to isolate entomopathogenic fungal species from Nigerian forest soil using the rustred flour beetle, *Triboliumcastaneum* (Coleoptera: Tenebrionidae) larvae for baiting, characterize growth, sporulation and evaluate virulence of the isolates using *Galleria mellonella* larvae for pathogenicity testing.

Materials and Methods

Source of *Galleria mellonella* and *Tribolium castaneum* larvae

*Galleria mellonella*and *T. castaneum* were obtained from the Department of Crop Science, Plant Protection Unit, Faculty of Agricultural Sciences, Ekiti State University Ado-Ekiti, Nigeria. The *G. mellonella*larvae were reared on diet containing rice bran, wheat grit, ricegrit, bee wax and honey while *T. castaneum* larvae were reared on wheat flour.

Isolation and identification of entomopathogenic fungi

Soil samples were collected from 1-5 cm top soil of the experimental garden, Department of Plant Biology, Federal University of Technology Minna. The soil was spread evenly on a flat surface and air-dried for two weeks.The soil was thereafter homogenized and weighed into

fivedisposable plastic Petridishes (60 g per Petridish). The soil in each Petridish was sprayed with 5 ml sterile distilled water to moisten the surface. Ten larvae (second instar larvae)of *T. castaneum* were introducedinto each Petri dish and the lid was replaced. The set-up was incubated at ambient temperature (25 ± 2°C) in the dark and dead larvae was checked daily. Dead larvae were removed, rinsed in sterile distilled water containing 0.02% Polyoxyethylenesorbitan monooleate(Tween-80®)as surfactant. The rinsed larvae were placed on sterile filter paper to absorb water droplets and thereafter surface sterilizedby dippingin 0.02 % sodium hypochlorite solution for 2-3 seconds, rinsed in three changes of sterile distilled water and air dried on filter paper for ten minutes.The larvae samples were arranged on filter paper at 2 cm apart and further surface-disinfectedby spraying with80% ethanol.

The surface-sterilized larvae were placed singly on freshly prepared PotatoDextrose agar (PDA) media (Sigma-Aldrich, UK)containing 0.01% chloramphenicol, inside 9 cm disposable Petridish.The Petridishwas sealed with Parafilm and incubated in the dark at ambient temperature for 2-3 days. Fungal out-growths from the larvae were sub-cultured and pure culture of each isolate was prepared using single-spore isolation technique.The isolates were identified using their micro-morphological characteristics and phenotypic appearance with reference to identification guides (Samson *etal*., 1988).

Measurement of growth rates

One µl of conidia suspension containing 1.0×10^{3}conidia ml^{-1}, prepared from 14 days old culture was inoculated at the centre of sterile PDA plates using Micropipette and replicated three times. The plates were sealed with Parafilm to prevent moisture loss fromthe agar surface and incubated at ambient temperature in the dark. After 24 hours, radial extension was measured daily along two orthogonal axes marked at the back of the plates for a period of 7-10 days or until three quarter (¾) of the media surface was covered by the mycellia.The

radial extension values (mm) were plotted against the period of growth (days)to estimate growth rate, using a linear model (Borisade and Magan, 2015).

Evaluation of sporulation rates

Five agar plugs were randomly taken from 14 days old culture using 1 cm cork borer. The agar plugs were placed in 20 ml disposable sterile Falcon bottles and 10 ml sterile distilled water containing 0.02% Tween 80® water was added. The Falcon bottles were vortexed for 2-3 minutes to dislodge the conidia intothe water. Estimation of the number of conidia in the suspension was done using a Neubauer Haemocytometer and microscope. The number of conidia cm^{-2} colony area was calculated as:

$$\frac{Estimated\ conidia\ number}{Colony\ area}$$

Determination of conidia viability

Viability of conidia was determined by spread plating10µl of conidial suspension containing 1×10^{6}conidial ml^{-1} on fresh PDAplates.Three portions on the SDA plates were covered with sterile coverslip,the plates were sealed with Parafilm and incubated at ambient temperature for 24 hours.The coverslip area were viewed under microscope under x40Objectiveand the percentage of conidia showing development of germ tube was estimated for 50 randomly counted conidia per coverslip field.The conidia were scored as viable when the germ tubes are half the size of the conidia.

Evaluation of pathogenicity of conidia

Conidia suspensionwas prepared from 14 days old culture of each isolate and standardized to 1×10^{6}conidial ml^{-1}. *Galleria mellonella*larvae were dipped into the conidia suspension and placed on filter paper inside a modified Petridish (Borisade *et al.,* 2018). Each Petridish contained 10 larvae and replicated three times. The Petridishes were arranged inside a

cupboard and equilibrated to 95-98 % humidityby placing two beakers containing of 500 ml distilled water each (Borisade and Magan, 2015).Mortality of larvae was recordeddaily and the mean percentage values of cumulative mortality were plotted against the incubation period to definethe trends of larvae morality.

DataAnalysis

The data on growth rates of the fungal isolates was checked for compliance with the requirement of Parametric Tests and subjected to Analysis of variance (ANOVA). Where there were significant differences, a Post-hoc test was conducted and means were separated using Tukey's Honestly Significant Difference (HSD), P=0.05% (IBM SPSS) and graphs were plotted using Microsoft Excel2013.

Results

Entomopathogenic isolates

Fourmorphologically distinctentomopathogenic isolates were obtained and consisted of *Entomophaga*sp, *Beauveriabassiana*, *Metarhiziumanisopliae* and *Isariafarinosa*(Sensu lato).Considering the phenotypic appearance and colony morphology, the *I.farinosa* were two separate strains and they were assigned names as *I. farinosa*-I (IF-I) and *I. farinosa*-II (IF-II) (Figure 1)

Viability of conidia, growth and sporulation rates

Germinations rates of conidia of the entomopathogenic isolates is shown in Figure 2. Germination of *B. bassiana,* I.*farinosa*-II and *M. anisopliae* was 100% while the *Entomophaga* sp. had 80% germination after 24 hours of incubation. However, no germination occurred in *I.farinosa*-I after 24 hours of incubation. There were statistically

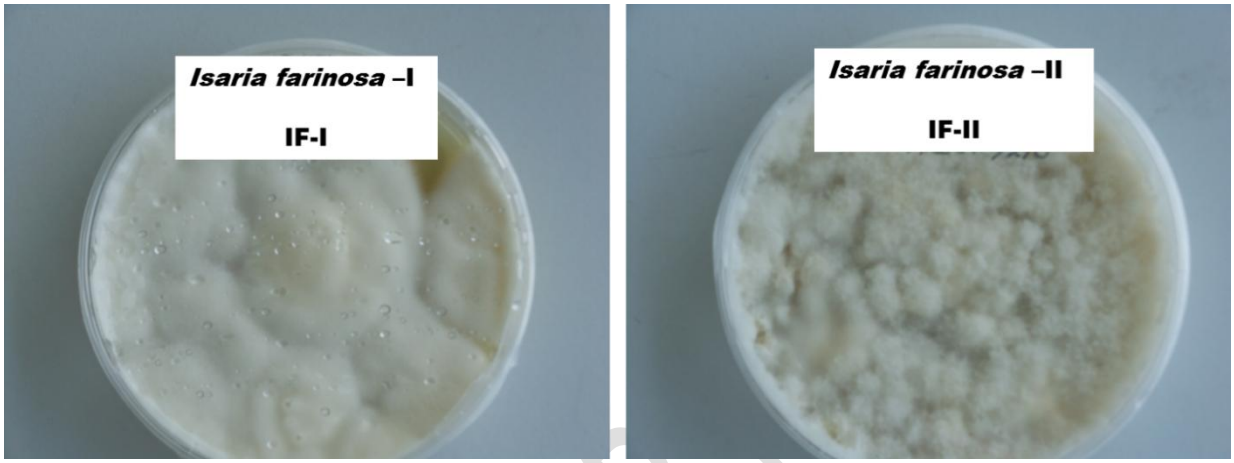

Figure 1. *Isaria farinosa* isolates showing variabilities in growth morphology on SDA media.

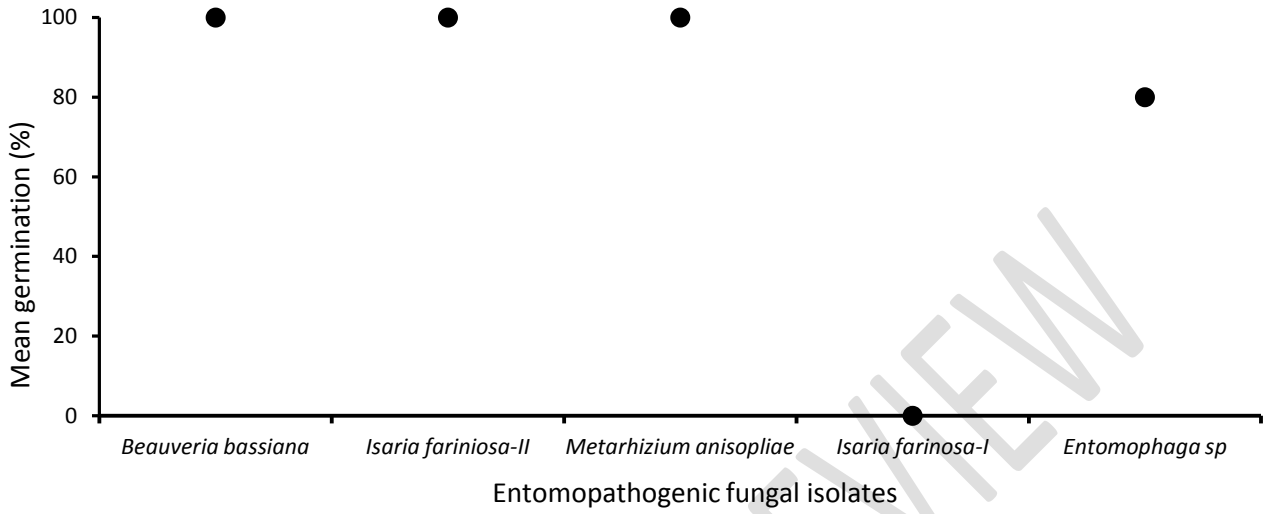

Figure 2.Germination rates of five entomopathogenic fungal conidia on SDA media after incubation for 24 hours.

significant variabilities in the rates of growth of the *I. farinosa*isolates F(4, 2.064) =12.97, P=0.001. The *B. bassiana* isolate had the fastest growth rate, with statistically significant value of 3.3 mm day^{-1}. The rates of growth of the two *I. farinosa* isolates: IF-1, IF-II and *M. anisopliae* were comparable, being 1.53, 1.4 and 1.28 mm day^{-1} respectively, without statistically significant difference. The growth rate of *E. grylli* was 2.0 mm day^{-1}, which was significantly higher than the growth rates of *I. farinosa* and *M. anisopliae* (Figure 3). The number of conidia produced by *Entomophaga* grylli was 7.0×10^5 conidia cm^{-2} colony area), while the isolate of *M. anisopliae* produced 5.2×10^4 conidia cm^{-2} colony area. The number of conidia produced by the two isolates of *I. farinosa*, IF-1 and IF-2 and *B. bassiana* were 9.4×10^4, 7.2×10^4 and 2.1×10^5 conidia cm^{-2} colony area respectively (Figure 4).

Pathogenicity of isolates against *G. mellonella* larvae

All the isolates of EF infected and caused mortality of *G. mellonella* larvae during a five-day incubation period and the highest percentage mortality was caused by *M. anisopliae*. The mean percentage mortality of, *G. mellonella* larvae treated with 1×10^8conidia ml^{-1} of the infective conidia of *I. Farinosa*, IF-1, IF-2, *M. anisopliae*, were 70, 60, 60% respectively while *E. grylli* and *B. bassiana* caused 50% mortality after five days (Figure 5)

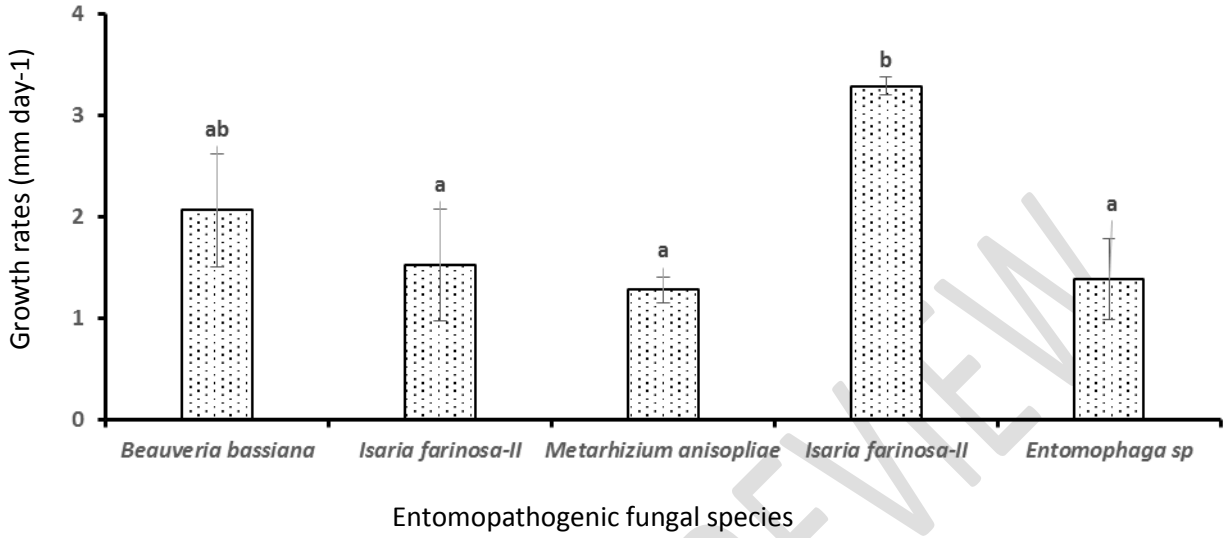

Figure 3. Growth rates of five entomopathogenic fungi cultured on SDA media and incubated at ambient temperature

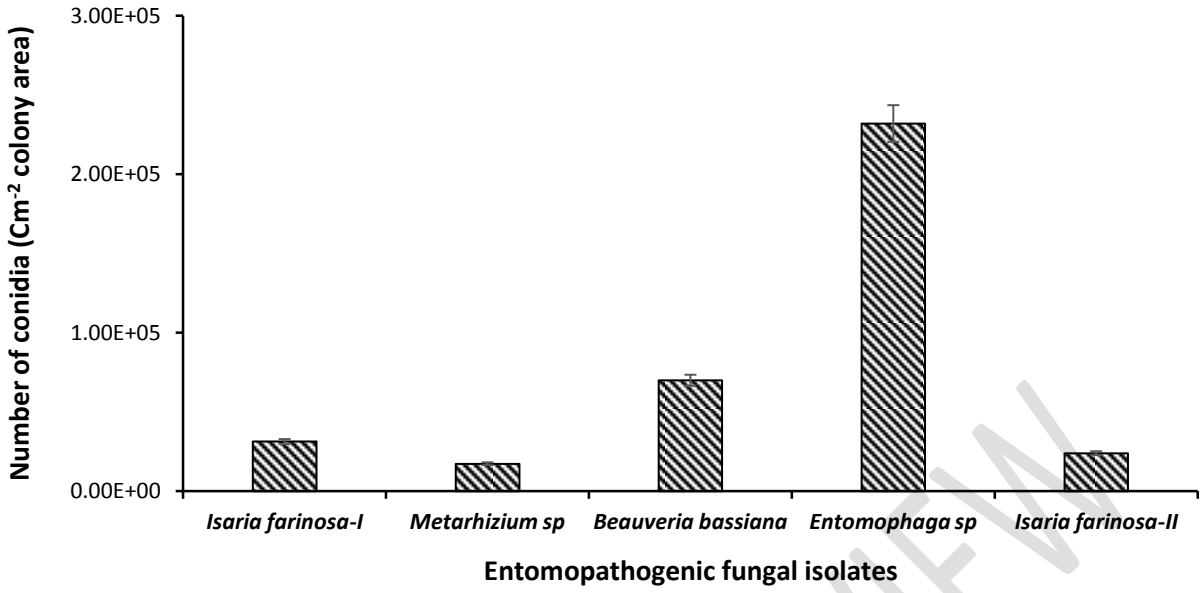

Figure 4: Conidia production rates of five entomopathogenic isolates on Sabouraud Dextrose Agar after incubation for 14 days

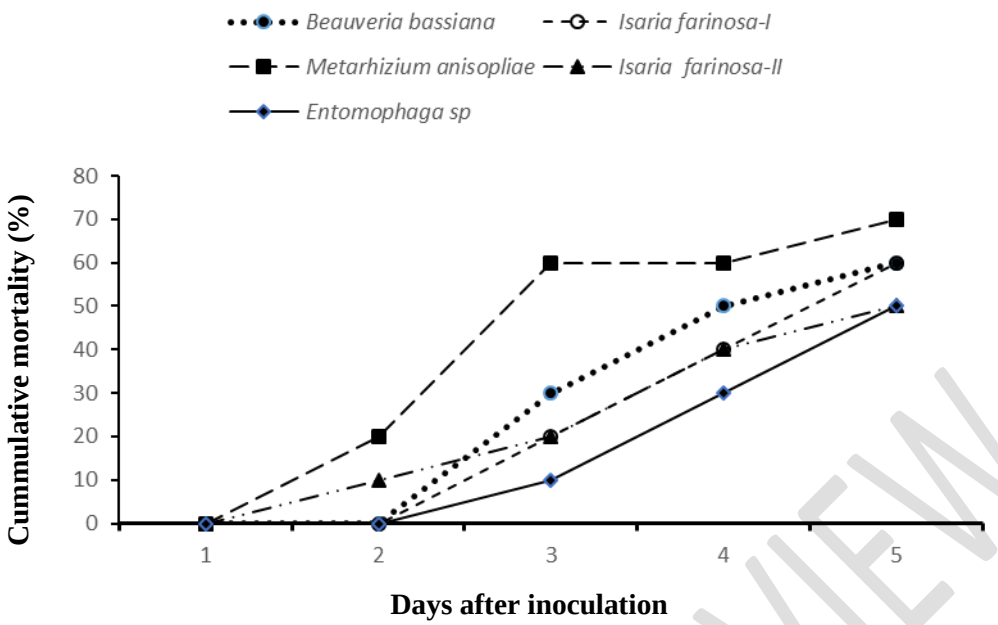

Figure 5: Cumulative mortality of *Galleria mellonella* inoculated with infective spores of five different entomopathogenic fungi

Discussion

Five distinct isolates of entomopathogenic fungi(EF) which comprised of *Entomophaga* sp,*B.bassiana, M.anisopliae* and two strains of *I.farinosa* were isolated from the soil samples, an indicationthatEF species which can be potentially developed into biopesticides in the futureare present and welldistributed in the soil.The soil sample from which the fungi were isolated was collected from a single spot, it is expected thatgreater diversity of isolates would occur if a wider area is profiled. Nussenbaum and Lecuona (2012) reportedhigh distribution and occurrence of *B. bassiana* and *I. farinosa*in soilsamples collected from different locations.Diversity of EF in soil is attributableto tolerance to a wide range of climatic conditions(Nouri Aiin *et al*., 2014) and other anthropogenic factors, such as land use practices (Borisade, *et al.* 2016).

The conidia of all the isolates except IF-I germinated after incubation for 24 hours at rates that ranged between 70% - 100%. Germination is an important characteristic of EF which necessary for infection and the rate at which it occurs often correlate with virulence (Borisade *et al*., 2016; Hussain *etal*., 2017).Evaluation of germination in this study was based on a 24-hour incubation period, during which IF-Ifailed to germinate. Yeo *etal*. (2003) and Borisade *etal*. (2015) reported that failure of entomopathogenic fungi to germinate within a 24-hourincubation period does not imply the spores are not viable,as optimal conditions required for germination and growthare well differentiated among species and strains. Lag time is known to vary among EF and those that are same species often show a wide differentiation in abiotic requirements, especially temperature and water activity that permits germination and growth (Borisade and Magan, 2014). It is interesting that the isolate (IF-I) which failed to germinate after incubating for 24 hours caused 60% mortality among the host population

within five days. However, there is no literature to explain the relationship between the time taken by EF to adjust to its environment prior to germination (Lag time) and eventual virulence, except the contrasting reportin Borisade, *et al.* (2016), where the relationship between lag time, infectivity and virulence of EF was studied and it was suggested that lag time need to be considered as an important factor in characterization of virulence of EF that are exploited in biocontrol programmes.

The variabilities in virulence in this study is similar to earlier reports by Nussenbaum and Lecuona (2012),where mortality rates of 32to 100% was recorded in boll weevil inoculated with the infective conidia of *B. bassiana* strains. Similarly, Domingues da Silva (2001) recorded 15% to 83% mortality in boll weevil populations infected by *B. bassiana*. Susceptibility of insects to entomopathogenic fungi may be dependent on the type of host, host resistant factors, innate virulence of the EF and interacting abiotic factors (Santiago-Álvarez*etal*., 2006).*Galleria* larvae has been adopted in several studies in modelling virulence of EF and results obtained in the laboratoryusing Galleria-model pathogenicity assay often correlate with results from field trials (Kavanagh and Reeves, 2004).

The EF isolates sporulated under the prevailing ambient conditions on artificial media. This suggests that these speciespossessthe complementary attributethat is required for theirdevelopment into biocontrol agents (BCAs). The basic characteristics of EF that determine their suitability for being developed into biopesticides is the ability to produce large numbers of conidia that are environmentally stable and infective (Shah and Pell. 2012; Jackson *etal*., 2012). Production of large conidia of EF is often achieved using solid fermentation systems (SF) on artificial media that are readily available and cheap (Mascarin *etal*., 2013; Borisade et al., 2017). However, the physical and nutritional requirements for conidia production are more stringent than those required for mycelial growth(Moore, 1996).

It is therefore important that eco-physiology of the species be studied to determine the optimal conditions in favour of conidiation.

Conclusion

The results suggest that the five isolates examined can potentially be developed into experimental formulations and tested against important horticultural pests in future studies.

However, there is the need for more studies on eco-physiology of the species, especially their temperature boundaries within which they can grow and infect, water activity profiles and relative humidity (RH) relations. Studies are required on development of experimental formulations, which can be further tested to generate data on the host range of the species and evaluation of safety to humans and aquatic species before embarking on field trials.

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References

Abdel-Hadi A, & Magan, N. (2009). Influence of physiological factors on growth, sporulation and ochratoxin A/B production of the new *Aspergillusochraceus* grouping. *World Mycotoxin Journal* 2: 492 – 434.

Borisade, O.A. & Magan, N. (2014). Growth and Sporulation of entomopathogenic fungi *Beauvaria bassiana, Metarhizium anisopliae, Isaria farinosa* and *Isaria fumosorosea* strainsinrelation to water activity and temperature interactions. *Biocontrol Science and Technology*, 24(9), 999 – 1011

Borisade, O.A & Megan, N. (2015). Resilience and Relative Virulence of Strains of Entomopathogenic Fungi under interaction of Abiotic Stress. *African Journal of Microbiology Research*, 9(14), 988- 1000.

Borisade O.A, Oso, A.A. & Falade, M.J. (2016). Interactions of some registered agrochemicals in Nigerian farming systems with entomopathogenic fungi, *Metarhiziumanisopliae* and *Isariafarinosa*. *Ife Journal of Science*, 18(4), 949 – 961.

Borisade O.A, Oso, A.A. & Falade, M.J. (2017). Yield of *Metarhizium anisopliae* conidia on four tropical cereals and in-vitro evaluation of virulence against cowpea weevil, *Callosobruchus maculatus. Journal of Advances in Microbiology*, 4(4):1-9.

Borisade, O.A., Oso, A.A., & Falade, M.J. (2018). Growth characterisation and virulence of non- tropical isolates of *Metarhizium anisopliae* against Banamna weevil. *Cosmopolitessordidus* (Coleoptera: Curculionidae) *in vitro*. *MAYFEB Jorunal of Agricultural Science,* 1, 1- 9.

Domingues da Silva, C. A. (2001). Seleçȧo de isolados de *Beauvaria bassiana* patogènicos ao bicudo-do-algodoeiro. Preq. Agropec. *Bras*. 36(2)243 – 247.

Hussain, A., Rizwan-ul-Haq, M., & Aljabr, A.M. (2017). Susceptibility, Antioxidant Defense and growth Inhibitory of *Rhynchopohorus ferrugineus Olivier* (Coleoptera: Curulionidae) against the virulence of *Metarhizium anisopliae* Isolates. *Universal Journal of Plant Science*, 5(2), 17 – 23.

Jackson, M.A., Dunlap, C.A., & Jaronski, S.T. (2010). Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. *BioControl*, 55(1), 129 – 145.

Kavanagh, K., & Reeves, E.P. (2004). Exploiting the potential of insects for *in vivo* pathogenicity testing of microbial pathogens. *FEMS Microbiology Reviews* 28(1), 101 - 112.

Kimberly Moon San Aw & Seow Mun Hue. (2017). Mode of Infection of Metarhizium spp. Fungus and Their Potential as Biological Control Agents. *Journal of Fungi*, 3(30), 1-20.

Kryukov, V.Y., Yaroslavtseva, O.N., Whitten, M.M.A., Tyurin, M.V., Ficken, K.J., Greig, C., Melo, N.R., Glupov, V.V., Dubovskiy, I.M & Tariq, M.B. (2016). Fungal infection dynamics in response to temperature in the lepidopteran insect *Galleria mellonella*. *InsectScience* 25(3), 454 – 466.

Mascarin, G.M., Kobori, N.N., Quintela, E.D., & Delalibera Jr, I. (2013). The virulence of entomopathogenic fungi against *Bemisiatabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. *BiologicalControl* 66(3), 209 – 218.

Moore, E. (1996). Fundamentals of the fungi. Prentice Hall, New Jersey, USA. pp. 574.

Mweke, A., Ulrichs, C., Nana, P., Akutse, K, S., Fiaboe, K.K.M., Maniania, N.k. & Ekesi, S. (2018). Evaluation of entomopathogenic Fungi *Metarhiziumanisopliae*, *Beauvariabassiana*, and *Isaria* sp. For the Management of *Aphiscraccivora* (Hemiptera: Aphididdae).*Journal of Economy Entomology,* 20(10), 1- 8.

NouriAiin, M., Askary, H., Imani, S. &Zare, R. (2014). solation and characterization ofentomopathogenic fungi from hibernating sites of Sunn Pest (*Eurygasterintegriceps*) on Ilam Mountains, Iran. *International Journal of Current Microbiology and Applied Sciences*, 3(12), 314 – 325.

Nussenbaum, A.L & Lecuona, R.E. (2004). Selection of *Beauvaria bassiana sensu lato and Metarhizium anisopliae sensu lato* isolates as microbial control agents against the boll weevil *(Anthonomus grandis)* inArgentina. *Journal of invertebrate Pathology*, 110,1-7.

Samson, R.A., Evans, H.C. & Latge, J.P. (1988). Atlas of entomopathogenic fungi. Springer - Verlag, Berlin, Heidelberg, New York, 187 pp.

Santiago-Álvarez, C., Maranhão, E.A., Maranhão, E. &Quesada-Morag, E. (2006). Host plant influences pathogenicity of *Beauveria bassiana* to *Bemisia tabaci* and its sporulation on cadavers. *JournalofBio-Control*, 51, 519.

Servim, A., Dermir, I., Höfte, M., Humber, R.A., & Dermirbag, Z. (2010). Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. *Biocontrol*, 55(2), 279 – 297.

Shahid, A.A., Rao, A.Q.,Bakhsh, A.&Husnain, T. (2012). Entomopathogenic fungi as biological controllers:New insights into their virulence and pathogenicity. *Archives of Biological Science, Belgrade*, 64(1), 21 - 42.

Shah, P.A., &Pell, J. K. (2012). Entomopathogenic fungi as biological control agents. *Journal of Applied Microbiology and Biotechnology*, 61(5-6), 413 - 420.

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S., ... & Pell, J. K. (2009). Fungal entomopathogens: new insights on their ecology. *Fungal Ecology*, 2(4), 149 - 159.

Yeo, H., Pell, J.K., Alderson, P.G., Clark, S.J., & Pye, B.J. (2003). Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi on their pathogenicity to two aphid species. *Pest Management science*: formerly *Pesticicdes Science* 59(2), 159 – 165.

Zimmermann, G. (2008). The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosa*) and the *Isariafumosorosea* species complex (formerly *Paecilomycesfumosoroseus*): biology, ecology and use in biological control. *Biocontrol science and technology*, 18(9), 865 – 901.

Zimmermann, G. (1986). The Galleria bait method of detection of entomopathogenic fungi in soil. *Journal of Applied Entomology*. 102, 213-215.

NouriAiin, M., Askary, H., Imani, S. &Zare, R. (2014). solation and characterization ofentomopathogenic fungi from hibernating sites of Sunn Pest (*Eurygasterintegriceps*) on Ilam Mountains, Iran. *International Journal of Current Microbiology and Applied Sciences*, 3(12), 314 – 325.

Nussenbaum, A.L & Lecuona, R.E. (2004). Selection of *Beauvaria bassiana sensu lato and Metarhizium anisopliae sensu lato* isolates as microbial control agents against the boll weevil *(Anthonomus grandis)* inArgentina. *Journal of invertebrate Pathology*, 110*:* 1-7.

Samson, R.A., Evans, H.C. & Latge, J.P. (1988). Atlas of entomopathogenic fungi. Springer - Verlag, Berlin, Heidelberg, New York, 187 pp.

Santiago-Álvarez, C., Maranhão, E.A., Maranhão, E. &Quesada-Morag, E. (2006). Host plant influences pathogenicity of *Beauveria bassiana* to *Bemisia tabaci* and its sporulation on cadavers. *JournalofBio-Control*, 51, 519.

Servim, A., Dermir, I., Höfte, M., Humber, R.A., & Dermirbag, Z. (2010). Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. *Biocontrol*, 55(2), 279 – 297.

Shahid, A.A., Rao, A.Q.,Bakhsh, A.&Husnain, T. (2012). Entomopathogenic fungi as biological controllers:New insights into their virulence and pathogenicity. *Archives of Biological Science, Belgrade*, 64(1), 21 - 42.

Shah, P.A*.,* &Pell, J. K. (2012). Entomopathogenic fungi as biological control agents. *Journal of Applied Microbiology and Biotechnology*, 61(5-6), 413 - 2.

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S., ... & Pell, J. K. (2009). Fungal entomopathogens: new insights on their ecology. *Fungal Ecology*, 2(4), 149 - 159.

Yeo, H., Pell, J.K., Alderson, P.G., Clark, S.J., & Pye, B.J. (2003). Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi on their pathogenicity to two aphid species. *Pest Management science*: formerly *Pesticicdes Science* 59(2), 159 – 165.

Zimmermann, G. (2008). The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosa*) and the *Isariafumosorosea* species complex (formerly *Paecilomycesfumosoroseus*): biology, ecology and use in biological control. *Biocontrol science and technology*, 18(9), 865 – 901.

Zimmermann, G. (1986). The Galleria bait method of detection of entomopathogenic fungi in soil. *Journal of Applied Entomology*. 102: 213-215.