INTERACTIONS OF SOME REGISTERED AGROCHEMICALS IN NIGERIAN FARMING SYSTEMS WITH ENTOMOPATHOGENIC FUNGI, *METARHIZIUM ANISOPLIAE* AND *ISARIA FARINOSA*

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ABSTRACT

Entomopathogenic fungi, *Metarhizium anisopliae* and *Isaria farinosa* are biocontrol agents (BCA) widely reported for the management of insect pests, and they are potential components of Integrated Pest Management (IPM) systems. Compatibility of their infective conidia with low rates of four agrochemicals; Champ-DP (Fungicide), Uproot (Glycophosphate herbicide,), DP-Force (Organophosphate insecticide) and Sniper (Methylphosphate insecticide) were evaluated *in vitro*. The conidia were cultured on Sabouraud Dextrose Agar (SDA) containing 0, 10, 15, 25, 50 and 100 ppm of each agrochemical and incubated at ambient temperature (mean temperature=27 °C) for 12-14 days. Growth lag times, growth rates and conidia densities of the isolates were evaluated. All the agrochemicals significantly ($P<0.05$) extended the lag time of *M. anisopliae*, but eventual rates of growth and conidiation increased significantly ($P<0.05$). Variabilities in the levels of interactions of the agrochemicals with the fungi were concentration-dependent. DD-Force significantly ($P<0.05$) increased conidiation rates at the five concentrations and appeared to be the most compatible with *M. anisopliae* among the four agrochemicals. *I. farinosa*, on the other hand, was relatively incompatible with the agrochemicals and caused 'growth' or 'no growth' responses at different concentrations. The *I. farinosa* isolate could not grow at the lowest concentration (10 ppm) of the herbicide (Uproot) and 15 ppm of the insecticide (Sniper) but tolerated up to 25 ppm of Champ-DP (Fungicide) and DD-Force (Insecticide). Conidiation of the *I. farinosa* was null at all concentrations of the agrochemicals except Champ-DP treatments which allowed some conidia production at 10-25 ppm. However, 8-32% inhibition of conidiation occurred at these concentrations. The agrochemicals were compatible with *M. anisopliae* but relatively toxic to *I .farinosa* by inhibition of sporulation and growth at most concentrations.

Keywords: Agrochemicals, Entomopathogenic fungi, Compatibility, Growth, Lag time, Conidiation

INTRODUCTION

Entomological pests pose significant threats to agriculture in tropical environments particularly Nigeria, with a population of over 100 million people who are largely dependent on indigenously-produced crops. Insect infestations cause severe field and post-harvest losses of food crops (Nwajiuba, 2012; Tefera, 2012). Repeated applications of chemical insecticides are employed in the management of rapidly increasing pest populations where cultural control measures fail to minimize pest incidents. However, the use of chemical insecticides are of considerable environmental concerns (Edwards, 2013; Perry *et al*., 2013) and are capable of causing development of resistant pest strains where they are repeatedly used (Perkins, 2012).

Agriculture in Nigeria is predominantly rain-fed, making it highly vulnerable to climate change (Enete and Amusa, 2010). Changes in temperature and precipitation patterns could result in shifts in land use patterns, change of farming systems and a consequent breakdown of bio-geographical barriers. These may, in the long run, lead to emergence of new pests and failure of novel control methods. The environments in which crops would be grown in the next decade are thus expected to change significantly, with unpredictable pest problems.

Development of sustainable Integrated Pest Management (IPM) system using biocontrol agents as a component, alongside existing conventional methods may be promising. *Metarhizium anisopliae* and *Isaria farinosa* are cosmopolitan entomopathogenic fungi. They are anamorphs of Hypocreales which are virulent against important tropical agricultural pests (Shahid, 2012; Borisade, 2015; Borisade and Magan, 2015) and they are natural pest population regulators wherever they exist freely (Augustyniuk-Kram, 2012). They occur in nature as insect pathogens and are capable of adapting to

life as saprophytes and endophytes (Borisade, 2016). Many strains have been isolated from the soil, which is the major source of entomopathogenic fungi biodiversity, plant parts and insects (Samson *et al.,* 2013).

Based on the diverse ecological niche of this fungal group, it is important that studies are conducted to evaluate the impact which herbicides, fungicides and insecticides may exert on their growth characteristics and conidiation, both of which are the key indices of pathogenicity and secondary spread. In addition, generating information on interactions between entomopathogenic fungi and conventional chemical insecticides that are often used in farming systems in Nigeria is clearly necessary for the biodiversity conservation of entomopathogenic fungal species and their eventual adoption into the IPM System.

The use of fungal-based biocontrol agents (BCAs) as a stand-alone insecticide is safe but may be slow in action. Thus, it may not adequately replace conventional chemical insecticides (CCI) under conditions of rapidly-growing pest populations. Fungal-based BCAs may be better suited for use in the form of CCI-BCA composite applications. However, there is strong evidence that agrochemicals influence growth, infectivity and overall efficacy of fungal BCAs. Oliveira *et. al.* (2003) reported that Chlorpyrifos and Endosulfan formulations inhibited 100% germination of *B. bassiana*. Similarly, Silva *et. al.* (2013) reported significant variabilities in growth and conidiation of *M. anisopliae* which were treated with different insecticides, herbicides and fungicides.

These studies and some other previous ones evaluated compatibility of entomopathogenic fungi with chemicals at manufacturers' recommended application rates. As far as we know, interactions of fungal propagules with sub-lethal dose of insecticides have not been studied, particularly under the tropical agro-climate. Effects of agrochemicals on biocontrol agents are variable and may be dependent on species and strains of fungal agents, pesticide concentration (Schumacher and Poehling, 2012) and other interacting biotic and abiotic factors (Borisade and Magan, 2014).

Many agro-ecological regions of Africa have been predicted as potential climate change hot-spots (Dina *et al*., 2014) with potential adverse impacts on crop productivity (Knox *et al*., 2012) and changes in distribution and dynamics of insect pests (Karuppaiah and Sujayanad, 2012; Bebber *et al.,* 2013). Thus, we propose an IPM system where fungal-based biocontrol agents will compliment low rates (barely lethal dose) of conventional agrochemicals in order to minimize adverse environmental impacts. Combining BCAs with low rates of CCI is expected to significantly reduce environmental concerns that are related to the use of chemical pesticides, widen the range of target pests, alleviate problems of development of resistant strains of pests and improve overall efficacy.

Several groups of agrochemicals are applied in the Nigerian farming systems for the management of insect pests, plant disease pathogens and suppression of weeds. The aim of this study was to test the toxicity of low concentrations of some of these chemicals to *I. farinosa* and *M. anisopliae* isolates and evaluate compatibility indices.

MATERIALS AND METHODS

Source of Fungi and Agrochemicals

Entomopathogenic *M. anisopliae* (275.86DC) was provided by Professor Tariq Butt, Swansea University, UK while *I. farinosa* strain (ARSEF 6069) was supplied by the United States Department for Agriculture (USDA). Agrochemicals which are registered for use in Nigerian horticultural crops farming systems were purchased from retail agro-stores in South Western Nigeria. Table 1 shows the list of the agrochemicals and their recommended application rates.

Table 1. Description of agrochemicals used for *in vitro* compatibility bioassays with *M. anisopliae* and *I. farinosa*

Trade name	Formulation	Chemical group	Technical name	Type of agrochemical	Concentration of active ingredient	Recommended dose
Uproot	EC	Glycophosphate	Isopropylamine	Herbicide	480 g/liter Isopropylamine salt	3-6 liters/ha
Sniper	EC	Methyl Phosphate	2,3-Dichlorovinyl dimethyl phosphate (DDVP)	Insecticide	1000 g/liter DDVP)	3-15 ml in 200 ml water
DD-Force	EC	Organophosphate	Dichlorovinyl dimethyl phosphate	Insecticide	DDVP1000EC	0.63-2 liters/ha
Champ-DP	WG	Copper Hydroxide	Copper Hydroxide	Fungicide	57.6% Copper Hydroxide	Not indicated

EC=Emulsifiable concentrate, WG=Wettable granules

Bioassays were carried out in the Plant Protection Laboratory, Ekiti State University, Nigeria (7.7212° N, 5.2575° E).

Fungal Culture and Preparation of Conidia Suspension

The fungal isolates were cultured on sterile Sabouraud Dextrose Agar (SDA) in 9 cm Petri-dishes. Incubation was done at ambient temperature (mean temperature = 27 °C) inside sealable polythene bags for 14 days. Thereafter, 10 ml sterile de-ionized water containing 0.02% Tween 80 was poured on the culture and conidia were gently dislodged using Drigalsky spatula. Conidia suspension was poured into 25 ml Universal Bottle and standardized to 1.0 x 10^7 conidia ml^{-1} by serial dilution and evaluation of conidia concentration using Neubauer haemocytometer and viewed under the compound microscope (Borisade, 2015).

Initial Growth and Conidiation Rates

Prepared sterile SDA in 9 cm Petri-dishes were inoculated at the centre with 10 µl of conidia suspension containing 1.0 x 10^7 conidia ml^{-1} using micropipette (Eppendorf 1-20 µl). Triplicate plates were inoculated, sealed with parafilm to prevent moisture loss from the agar surface and incubated at ambient temperature in the dark. Radial extension was measured along pre-marked orthogonal axes after initial 48 hours incubation period and this continued daily for 14 days or until the surface of the plate was fully covered. Radial extension (mm) against the period of growth (days) was fitted into a linear model to estimate growth rates (Borisade and Magan, 2014). Conidia from 21 days culture were harvested into universal bottles with 10 ml sterile de-ionized water containing 0.02% Tween 80. Serial dilutions were made and spore count was done using Improved Neubauer Haemocytometer under X40 magnification of Microscope (OLYMPUS BHTUBH-2).

Preparation of Agrochemical Modified-SDA

Calculated amounts of the agrochemicals (Champ-DP, DD-Force, Sniper and Uproot) were added to sterile and molten SDA at 50 °C and poured into 9 cm Petridishes. SDA media containing 10, 15, 25, 50 and 100 ppm of each agrochemical were prepared in triplicates and kept at 4 °C for 2-3 days until required for bioassay. These concentrations were chosen because they (a) are far less than the recommended rates (b) were within the concentration range detectable on insect cuticle and insect haemolymph and (c) showed significant toxicity to some important tropical pests in our preliminary bioassays. The control consisted of Petridishes containing standard SDA prepared with Reverse Osmosis (RO) water (without agrochemical). The water activity (a_w) of the media at the various concentrations of the agrochemicals in the PDA were confirmed using a_w meter (Aqua Lab Dew Point Water Activity meter 4TE, Accuracy = ± 0.0003) and they were within target (0.995 – 0.993 a_w). This is necessary to ensure that eventual variabilities in growth and conidiation were due to actual toxicity of chemicals to the fungi rather than effects of water stress.

Effect of Agrochemicals on Lag time, Growth Rate and Conidiation

Prepared agrochemical modified-SDA media in 9 cm Petridishes and the control were inoculated at the centre with 10 µl of conidia suspension

(containing 1.0 x 10^7 conidia ml^{-1}) from pure colony using Micropipette and this was replicated three times. The plates were sealed with parafilm and incubation was done under ambient temperature in the dark. Measurement of radial extension along pre-marked orthogonal axes was done starting from the first 48 hours, and this continued daily for 14 days or until about ¾ of the agar surface was covered by the colony. Growth rates were estimated using a linear model as described previously. Lag time was estimated from the equation of the regression curve by regressing y-axis to zero. Conidia from 21 days culture were harvested using sterile deionized water containing 0.02% Tween 80 and estimation of conidia concentration was done using a Haemocytometer and x40 objective of microscope. Thereafter, conidia density was calculated as:

$$\text{Conidia density} = \frac{\text{Estimated number of conidia}}{\text{Total colony area (Sqcm)}}$$

Toxicity and Compatibility Indices

Differences in growth and conidiation rates in the treatments were calculated relative to control and expressed as percentages. The agrochemicals were considered toxic and incompatible with the fungal propagules at concentrations where concomitant inhibition of growth and conidiation occurred.

Statistical Analysis

The experiment was a Completely Randomized Block Design with three replicates for evaluation growth, conidiation rates and interactions with agrochemicals. Growth and conidiation data were analysed for compliance with the requirements of parametric tests; normality of the data was tested using the Shapiro-Wilks and Kosmogorov-Smirnoff's Tests. Equality of variance of the means was examined using Leven's Test. Where the data failed one of the requirements of a parametric statistical model, a Kruskal-Wallis Test (the non-parametric equivalent of 2-way Analysis of Variance, ANOVA) with rank transformed data was used. Conidiation data was log-transformed (Log_{10} conidia-density) and analysed using a 2-way ANOVA. Where ANOVA showed a significant difference, a *Post-hoc* test was performed to separate the means using Fisher's Least Significant Difference (LSD) (α=0.05). Data were analysed using the SPSS-21, Statistical Package.

RESULTS

Growth Rate and Conidia Density at Ambient Temperature

The rates of growth of *M. anisopliae* and *I. farinosa* were 1.6 mm day^{-1} and 0.5 mm day^{-1} respectively (Figure 1). The log phase of growth are shown in the figure for each replicate plate on which radial extension was measured. The R^2 values were within 0.9871-0.9987. Fluctuations in ambient temperature during the growth period are shown in Figure 2. The minimum temperature recorded was 27.9 °C while the maximum was 34.3 °C and the mean daily temperature was approximately 27 °C. Conidia density of *M. anisopliae* and *I. farinosa* were 5.25 x 10^3 and 5.37 x 10^4 conidia cm^{-2} colony area respectively.

Effect of Agrochemicals on Growth Rate

Figure 3 shows the effect of the agrochemicals based on Glycophosphate (herbicide), Methyl-phosphate (insecticide), and Copper hydroxide (Fungicide) at five concentrations (10, 15, 20, 50 and 100 ppm) on growth rates of *M. anisopliae* and *I. farinosa*. The effect of these agrochemicals on *M. anisopliae* was similar. A Kruskal-Wallis test showed there was no significant variabilities in its rate of growth in relation to the choice of agrochemical (Champ-DP, n = 18; Uproot, n = 18, Sniper, n = 18; DD-Force, n = 18) χ^2 (3, 72) = 4.709, P = 0.194. DD-Force had the highest median score (Md=42.14), followed by Sniper (Md = 39.89), Uproot (Md = 35.89) and Champ-DP (Md = 28.08), but without statistical significance. The five tested concentrations of the agrochemicals (10, 15, 20, 50 and 100 ppm) similarly had no significant effect on growth rate of *M. anisopliae* (10 ppm, n=12; 15 ppm, n=12; 25 ppm, n=12; 50 ppm, n=12; 100 ppm, n=12) χ^2 (4, 72) =4.45, P=0.348. However, the highest median score (Md = 1.8778) was recorded at 100 ppm concentration, followed by 50 ppm (Md = 1.7396) while the lowest median score was the control, 0 ppm (Md = 1.4018). Comparably, all the agrochemicals had significant and dramatic effects on the growth of *I. farinosa*. There was total inhibition of *I. farinosa* growth by the herbicide (Uproot) at the five tested concentrations. There was no growth at 50 and 100 ppm of Champ-DP (Fungicide) and DD-Force (insecticide). Similarly, 15-100 ppm of Sniper (insecticide) caused 100% inhibition of growth.

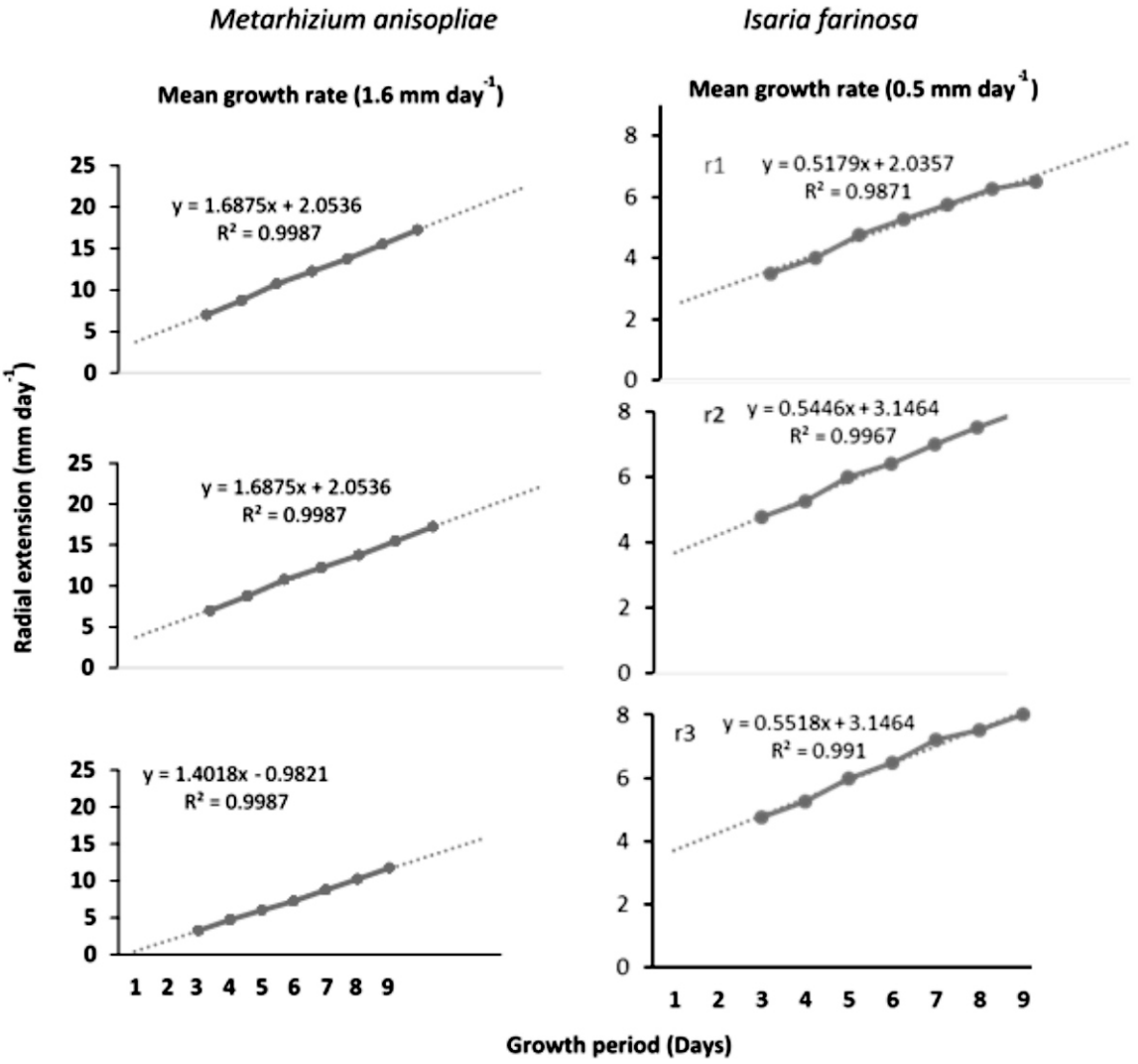

Figure 1. Typical linear model used for estimation of growth rate of *M. anisopliae and I. farinosa*. r_1, r_2 and r_3 are replicate plotted graphs of radial extension against growth period. Coefficient of x in the linear equation is the slope of the curve, (=growth rate). R^2 represent goodness of fit of the curves as its value tends towards unity.

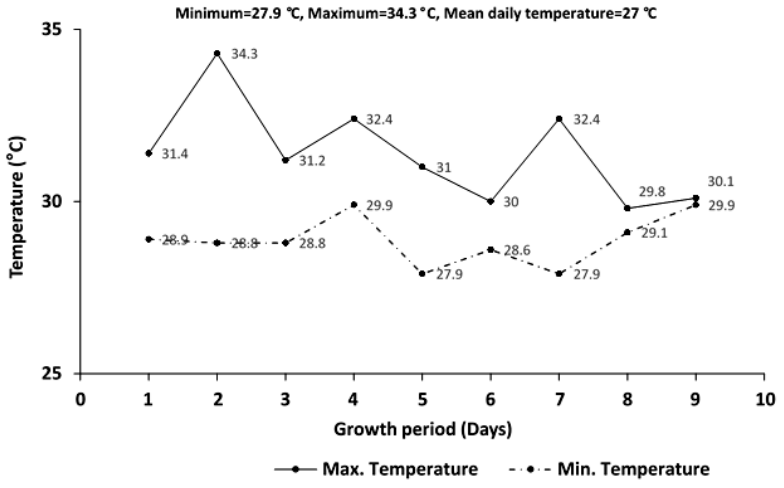

Figure 2. Fluctuations in daily ambient temperature during growth period of *M. anisopliae* and *I. farinosa*

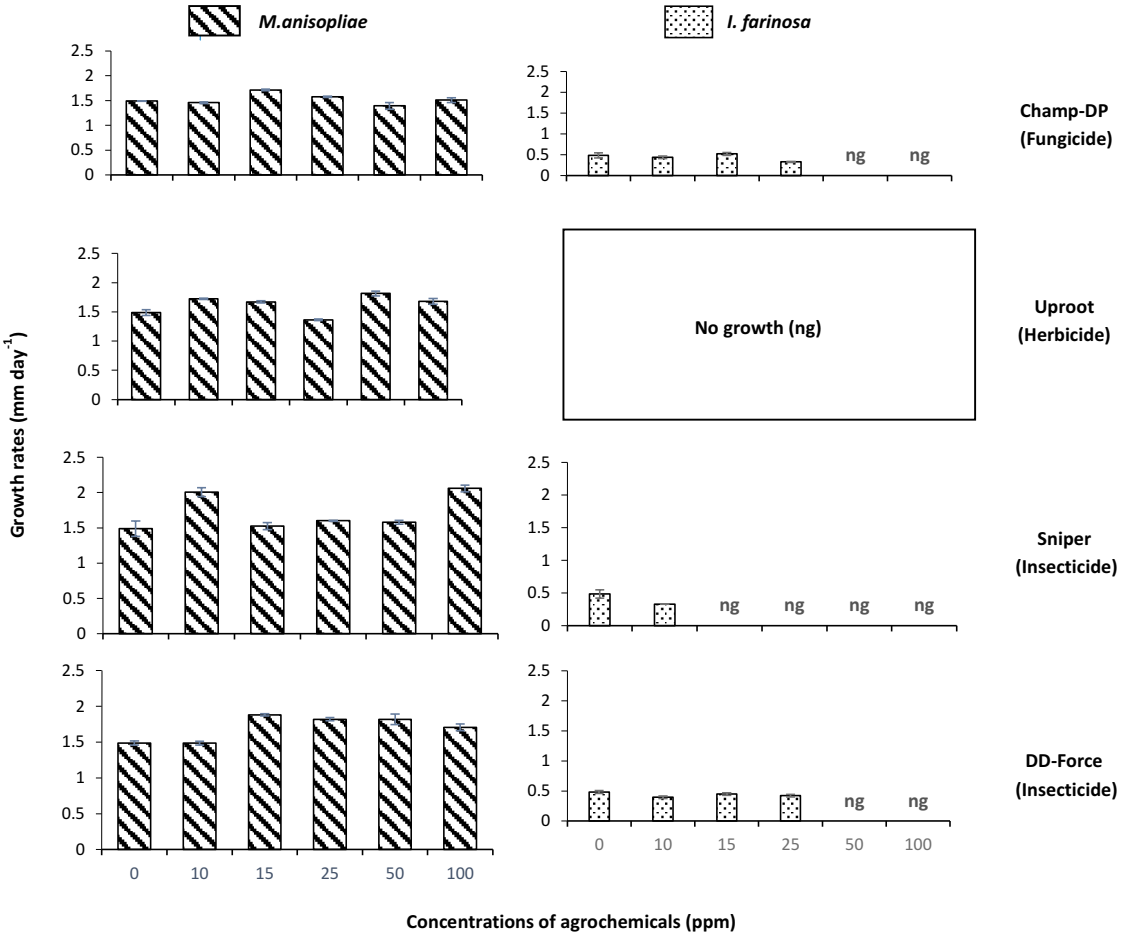

Figure 3. Effect of different concentrations of agrochemicals on *in vitro* growth of *M. anisopliae* and *I. farinosa.*

ng: no growth.

Effect of Agrochemicals on Conidia Density

The four agrochemicals significantly (P=0.011) interacted with conidiation, the effect being more dramatic on *I. farinosa* and concentration-dependent (Table 2). The conidia density of the two isolates at different concentrations of the agrochemicals are shown in Table 3. *Isaria farinosa* produced significantly higher number of conidia cm^{-2} colony area (4.73) compared with *M. anisopliae* (3.72) in the control. However, at certain agrochemical concentrations, conidia density increased significantly compared with the control. For example, conidia densities at 10, 15, 25 and 50 ppm of DD-Force (insecticide) were 4.75, 5.01, 5.45 and 5.42 conidia cm^{-2} colony area and they were significantly higher than the control (0 ppm, 3.72 conidia cm^{-2} colony area). However, 100 ppm of DD-Force significantly reduced conidia density compared with 10-50 ppm concentrations. In contrast, *I. farinosa* could not sporulate at 10-25 ppm DD-Force (insecticide) and 10 ppm of Sniper, although growth occurred at these concentrations. Champ-DP (Fungicide) caused no significant inhibition of conidiation of *I. farinosa* at 10-25 ppm and this was the concentration range where growth was recorded with the fungicide treatment.

Table 2. ANOVA Table of the effect of different concentrations of agrochemicals on sporulation rates of *M.anisopliae* (Ma275.86DC)

Source of Variation	*SS*	*DF*	*MS*	*F*	*P-value*	*Significance*
Agrochemicals	1.553023435	5	0.310604687	1.353433372	0.295969776	ns
Concentrations	3.618622571	3	1.206207524	5.255946172	0.01117379	*
Error	3.442408324	15	0.229493888			
Total	8.614054331	23				

ns: not significant; *Significant (P=0.05)

Table 3. Effect of five concentrations of different agrochemicals on conidia density (Log_{10} conidia cm^{-2} colony area) of *M. anisopliae* and *I. farinosa*

		Agrochemicals			
	Concentrations (ppm)	Champ-DP (Fungicide)	Uproot (Herbicide)	Sniper (Insecticide)	DD-Force (Insecticide)
M. anisopliae	0	3.72b	3.72b	3.72c	3.72b
	10	4.90a	4.19b	3.87c	4.75b
	15	4.65a	3.52b	4.37c	5.01b
	25	3.82b	3.78b	3.90c	5.45a
	50	4.10b	4.20b	4.82b	5.42b
	100	4.82a	4.82a	5.56a	4.70b
	Concentrations (ppm)	**Champ-DP**	**Uproot**	**Sniper**	**DD-Force**
I. farinosa	0	4.73a	4.73a	4.73a	4.73a
	10	4.46a	ng	ns	ns
	15	4.65a	ng	ng	ns
	25	4.52a	ng	ng	ns
	50	ng	ng	ng	ng
	100	ng	ng	ng	ng

Mean values followed by same alphabet in same column are not significantly different (P<0.05).
ng: no growth
ns: there was growth but no sporulation

Effect of Agrochemicals on Lag Time

Figure 4 shows the effect of agrochemical concentrations on lag period of growth of *M. anisopliae* and *I farinosa*. All the agrochemicals significantly increased the lag time of *M. anisopliae* at 25-100 ppm. For example, the lag time in the control (0 ppm) was 1.7 days and this increased to 3.6 and 4.4 days at 50 and 100 ppm respectively. In contrast, the lag time of *I. farinosa* decreased from 7 days in the control to 2.4 days at 25 ppm of Champ-DP. At all the agrochemical concentrations where growth of *I farinosa* occurred, the lag time decreased significantly (P<0.05) compared with the control.

Compatibility Based on Rates of Inhibition of Growth and Conidiation

Toxicity or compatibility based on concomitant inhibition of growth and conidiation is shown in Table 4. The agrochemicals were non-toxic to *M.anisopliae* at 10-100 ppm. At certain concentrations, significant increase in the growth rate and conidia density occurred. For example, at 15 ppm of Champ-DP there was 15% increase in growth rate and 20% increase in conidia density. Inhibition of growth (8%) and increased conidia density (2%) was recorded at 25 ppm of Uproot (herbicide). The two insecticides, Sniper and DD-Force also increased growth and conidia densities

of *M. anisopliae* at the five concentrations. However, the agrochemicals increased the lag time of *M. anisopliae* differentially and variabilities in the levels of interaction were further modulated by concentration.

In contrast, all the agrochemicals were incompatible with *I. farinosa* (ARSEF 6609) except at 10-15 ppm of Champ-DP. The herbicide, Uproot caused 100% inhibition of growth of *I. farinosa* at the five concentrations thus, showing the highest toxicity among the agrochemicals.

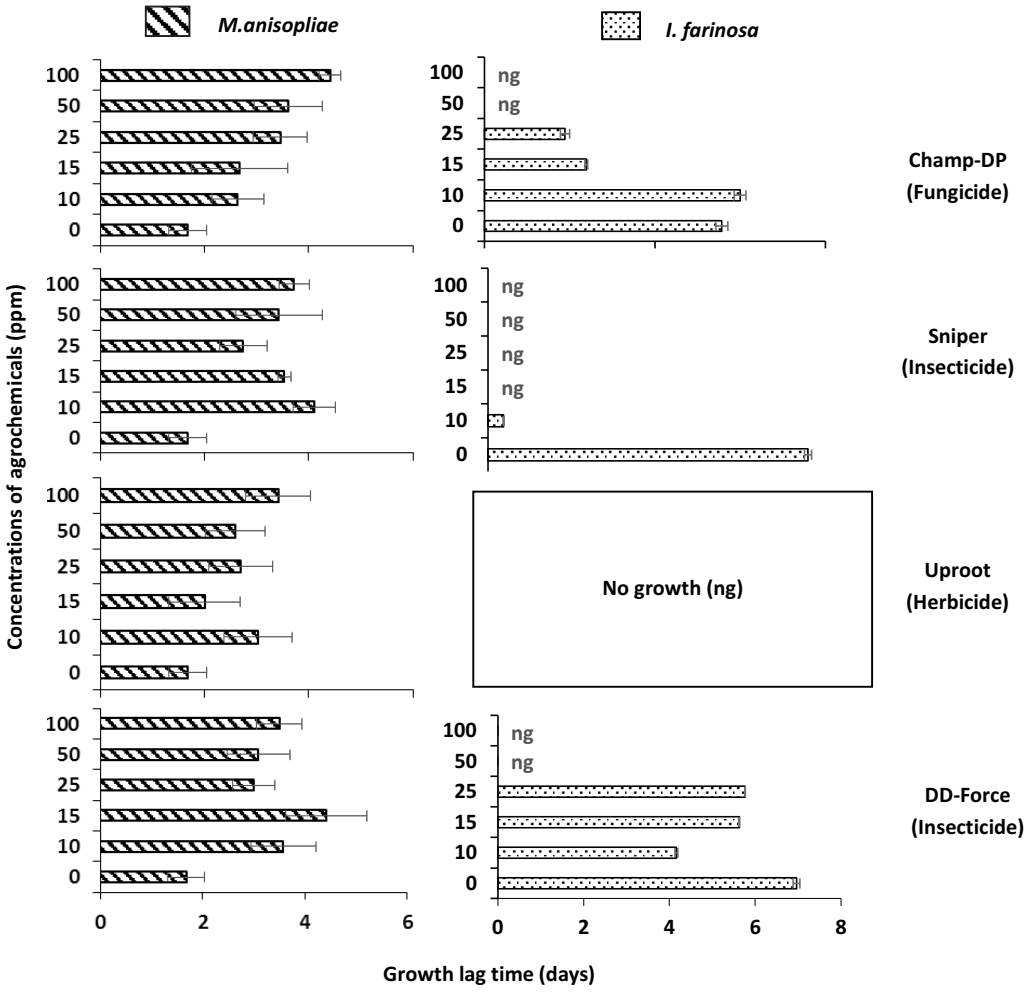

Figure 4. Effect of different concentrations of four agrochemicals on growth lag time of *M. anisopliae* and *I. farinosa*.
ng: no growth

Table 4. Toxicological classification of agrochemicals to *M. anisopliae* and *I. farinosa* relative to control (%).

	Agrochemicals															
	Champ-DP				Uproot				Sniper				DD-Force			
Concentration (ppm)	GR	SPR	LGT	C	GR	SPR	LTG	C	GR	SPR	LTG	C	GR	SPR	LTG	C
M.anisopliae (Ma 275.86 DC)																
10	*2.2	24	57	ct	16	11	144	ct	35	4	80	ct	1	22	113	ct
15	15	20	59	ct	12	*6	110	ct	2	15	19	ct	26	26	163	ct
25	6	3	106	ct	*8	2	63	ct	8	5	60	ct	22	32	78	ct
50	*7	9	115	ct	22	11	104	ct	6	23	54	ct	22	31	84	ct
100	1	23	162	ct	13	23	121	ct	38	33	103	ct	15	21	108	ct
I. farinosa (ARSEF 6609)																
10	*9	*6	8	nc	*100	ng	ng	nc	*32	*100	*95	nc	*18	*100	*40	nc
15	8	*2	*57	ct	*100	ng	ng	nc	*100	ng	ng	nc	*7	*100	*19	nc
25	*32	*4	*66	nc	*100	ng	ng	nc	*100	ng	ng	nc	*12	*100	*17	nc
50	*100	ng	ng	nc	*100	ng	ng	nc	*100	ng	ng	nc	*100	ng	ng	nc
100	*100	ng	ng	nc	*100	ng	ng	nc	*100	ng	ng	nc	*100	ng	ng	nc

KEY TO THE TABLE

GR: growth
SPR: sporulation
LGT: lag time
C: compatibility rating
ct: compatible
nc: not compatible
ng: no growth.
Asterik (*) on values in GR and SPR columns indicate inhibition
Asterik (*) on values in LTG column indicate reduction in lag time
Values without asterik show % increase relative to control

Similarly, the insecticide (Sniper) caused 32% growth inhibition at 10 ppm and growth was null at 15-100 ppm. Champ-DP (fungicide) allowed some growth of *I. farinosa* at 10-25 ppm but conidiogenesis was suppressed. Similarly, this strain could grow at 10 ppm of Sniper and 10-25 ppm of DD-Force but there was no conidiation at these concentrations.

DISCUSSION

The results of this study could serve as a guide in selecting entomopathogenic fungal candidates for further studies and field trials. None of the agrochemicals had adverse effect on growth rate and conidiation of *M. anisopliae* at the low concentrations but lag time was significantly extended. On the other hand, *I. farinosa* was inhibited differentially; conidiation of *I. farinosa* at concentrations of agrochemicals where growth occurred were either null (100% inhibition of conidiogenesis) or significantly inhibited compared with the control. We concluded that the *I. farinosa* isolate (ARSEF 6609) was incompatible with most of these agrochemicals even at concentrations below the levels recommended by the manufacturers.

It appeared there was no direct relationship between growth and conidiogenesis and the mechanism of interaction of chemicals with fungal species which permitted vegetative growth but deterred conidiation has not been fully understood. Compatibility or toxicity of the agrochemicals to the fungi in relation to growth rates and conidiation are consistent with earlier findings (Cuthbertson *et. al.*, 2005; Silva *et. al.*, 2013), but there are no data to compare variabilities in lag time caused by agrochemicals

The insecticides which were compatible with *M. anisopliae* can be combined at low concentrations with bio-propagules and tested against target pests such that, adverse environmental impacts which are often associated with use of chemicals are

minimized or eliminated completely. The levels of compatibility which were observed suggests that the *M. anisopliae* strain might be adoptable into Integrated Pest Management (IPM) system for the management of important agricultural pests under the tropical climate. However, there is need to search for more of compatible indigenous species and to conduct studies on development of appropriate formulations to preserve viability of conidia to enhance infectivity. Low concentrations of CCI + BCA combinations need to be tested on specific agricultural pests to evaluate efficacy and range of susceptible pests, particularly under field conditions.

Fungal growth rate may affect virulence while conidiation is essential for secondary spread and persistence. Fast growing fungi may have considerable capability to overcome host defenses, utilize host nutrients very quickly, intoxify the haemolymph and cause mortality within a relatively shorter time. Secondary spread on the other hand may eliminate the need for repeated application of BCAs after the initial inundation. Slowly-growing fungal strains or strains with extended lag time therefore, may give room for an insect host to exploit its defensive mechanism; such as shedding of cuticles before a successful appresorial formation and breaching of cuticle barriers, to evade pathogen.

Based on the results of this study, agrochemicals are capable of interfering with lag time (the time taken by fungi to adjust to its environment before rapid growth). For example, the lag time of *M. anisopliae* significantly increased relative to control by most of the agrochemicals, without adverse effect on eventual rates of growth. In contrast, DD-Force (insecticide) reduced the lag time of the *Isaria* strain from 6.9 days in the control to 4.6 days at 10 ppm, without any significant reduction in the rate of growth but conidiogenesis was 100% inhibited. At this concentration (10 ppm of DD-Force), it may be suggested that the insecticide would deter secondary spread but not initial infection and virulence. It is important that effect of agrochemicals on lag time be considered as an important factor in classification of chemical compatibility with fungal BCAs.

Classification of toxicity in the current report was based on the ability of agrochemicals to inhibit both growth and conidiation and we are of the opinion that assessment of toxicity in a particular study should be peculiar. In the current study, we made use of low concentrations of agrochemicals (10-100 ppm) to simulate concentrations which may be present on insect cuticle or encountered in the haemolymph by entomopathogenic fungi after a spaced and alternated spray of fungal propagules, followed by chemicals or vice versa. Under such scenarios; germination, lag time, growth rate and conidiation (%), may all be important in the classification of compatibility.

Silva *et. al.* (2013) simulated in-tank mix of recommended rates of agrochemicals with conidia of *M. anisopliae* to evaluate effects on germination, growth and conidiation using the biological index (BI) formula described by Rosi-Zalaaf (2008). Alves *et al.* (1998) proposed BI formula for classification of toxicity of agrochemicals to entomopathogenic fungi by relating vegetative growth and conidiogenesis to a control (%). In another study, Neves *et al.* (2001) acknowledged the merits of this formula and suggested that conidial viability need to be considered in calculating compatibility values. Oliveira *et al.* (2003) also reported that Alves *et al.* (1998) BI formula failed to consider viability of conidia (% conidia germination) as an important compatibility factor and therefore suggested the need for a review. In view of the importance of viability of conidia as a factor in classification of toxicity of chemicals to fungi, Rosi-Zalaaf (2008) proposed a BI formula relating vegetative growth of fungal colony, germination and conidiation to a control (%).

The result of this study represents one of the occasions where none of the proposed BI formulae was adequate to accommodate increase in growth and conidiation or lag time responses in relation to concentrations of agrochemicals. Lag time is an important virulence factor in classifying toxicity or compatibility. The formula of Alves *et al.* (1998) for example was based on percentage inhibition of radial extension (colony size) and number of conidia relative to control (%) without any consideration for lag time and the actual rate of growth. However, it may be possible to

eliminate the effect of lag time by fitting into the BI model the following parameters: (a) the actual rate of growth (mm day^{-1}) relative to control (%), rather than colony diameter (mm) relative to control (%) and (b) conidia density (Conidia cm^{-2} of colony area) relative to control (%), rather than mere number of conidia (conidia yield) relative to control (%). Some of the tested agrochemicals caused >100% increase in lag time of *M. anisopliae,* for example at 100 ppm of Uproot and DD-Force, but the eventual rates of growth were comparable with the control. In some treatments (which also involved *M. anisopliae*), extended lag time occurred but faster rates of growth and higher conidia production were stimulated; such as recorded at 50 ppm of Uproot. Fungal species are known to respond to chemical and water stress by producing large numbers of conidia to compensate for poor mycelial proliferation (Borisade and Magan, 2014).

Compatibility of agrochemicals with entomopathogenic fungi is known to vary with fungal isolates and strains and concentrations of active ingredients. Strains which are intolerant to low concentrations of these chemicals, such as the *I. farinosa* being reported in this study, may find usefulness in organic agricultural IPM systems. However, use of agrochemicals in areas where such strains occur naturally may affect their biodiversity, based on the current observations. In nature, we expect that fungitoxic or fungistatic actions of agrochemicals would diminish over time in the presence of other biotic and abiotic interactions capable of degrading these chemicals. Addition of agrochemicals into growth substrate followed by a cross-check of the a_w of the substrate before inoculation, can be considered the best method of evaluation of toxicity of chemicals to fungal BCAs. Carrier substances in agrochemicals are capable of modifying osmotic environments or substrate a_w. Therefore, in addition to the bio-toxicity of active ingredients to biocontrol fungi, water stress (a_w) could further modulate germination rates, lag time, growth rates and conidiation. For example, Silva *et al* (2013) simulated in-tank mixing of fungal conidia with agrochemicals by vigorous vortexing for 2 minutes followed by constant agitation in a rotary shaker at 214 rpm and 25 °C temperature for 3 hours, before spraying on growth media. It is noteworthy that the effect of a_w of the solution in which the conidia was suspended for more than 3 hours, was not considered in the bioassay as an important factor capable of affecting the outcome. Failure of propagules to germinate after such treatments may be due to hyper-osmotic shock rather than bio-toxicity interactions. Hallworth and Magan (1999) reported that a_w of substrates significantly affected growth of *M.anisopliae* in addition to the effect of a_w modifying solutes. Similarly, Borisade and Magan (2014) demonstrated variabilities in germination rates, growth and conidiation of six isolates of *Beauveria bassiana*, five isolates each of *Isaria farinosa* and *M. anisopliae* and a strain of *Isaria fumosorosea* in relation to a_w. The results showed that most strains of *M. anisopliae* failed to germinate at $a_w < 0.96$.

After all, Silva *et al.* (2013) reported significant differences in compatibility ratings of two fungicides (Flint and Priori) with the same active ingredient (Strobilurin), but in different forms as (a) wettable granule (WG) and (b) emulsifiable concentrate (EC). The two fungicides showed varied degrees of toxicity (compatibility indices were 46.5 and 72.6 respectively) to *M. anisopliae* when their concentrations were calculated based on the same volume rate (200 L ha^{-1}). This suggests that formulations and handling method may also affect compatibility of agrochemicals with BCAs in addition to bio-toxic effects. Different formulations of agrochemicals with the same active ingredient may present dissimilar osmotic environments when prepared at the same labeled rates. This may be responsible for variabilities in the growth characteristics of *M. anisopliae* that was treated with the WG and EC formulations of Strobilurin. Practically, to avert germination problems, the spraying period of agrochemicals may be alternated with application of BCAs after time interval that is sufficient for germination of conidia or on the other hand, avoid soaking of fungal propagules in chemical mixture. It is technically possible to check the a_w profile of a fungal biocontrol agent and that of the prepared chemical mixture before taking a decision on mode of application (in-tank mix vs alternated spraying regimes).

CONCLUSION

In developing an IPM system for the management of a particular pest, it is important that agrochemicals which are compatible with BCAs are selected in addition to choosing the best methods of handling, in order to preserve or enhance conidia viability, infectivity and virulence against the target pest.

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