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RESEARCH ARTICLE

Characterisation of *in vitro* cultured strains of *Beauveria bassiana* and *Metarhizium anisopliae* through poison food technique to evaluate their pesticide tolerance and genetic variability

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Abstract

Entomopathogenic fungi have high potential for efficient suppression of a variety of arthropod pests. However, their field application is easily affected by the potential inhibitory effects of various pesticides. The compatibility of two fungicides and 6 insecticides with *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin was determined under laboratory conditions, adopting the poison food technique. The growth, sporulation and bioefficacy of the fungi grown on the poison food media were assessed. In order to develop pesticide-tolerant strains, both fungi were grown separately in poisoned media continuously for 10 passages with an increase in concentrations of pesticides. The DNA isolated from the fungal cultures was subjected to DNA fingerprinting and PCR amplification reactions using 10 primers. A genetic similarity matrix was constructed using Jaccard's similarity coefficient values and this matrix was subjected to an unweighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendrogram using the average linkage procedure to assess variability induced by pesticide exposure. The results showed that although growth was inhibited, both fungi survived in all pesticide-amended media. *Beauveria bassiana* recorded the maximum radial growth (9 cm) in 13 days, followed by the cultures grown in imidacloprid, carbosulfan, carbofuran and chlorpyriphos and showed statistical significance from the other treatments. Polymorphism was higher for *B. bassiana* cultured in carbendazim. The polymorphism exhibited in *B. bassiana* was higher (83.19 %) compared to *M. anisopliae* (38.46 %). Among the 10 universal fungal primers evaluated, RFu-10 was found to give maximum polymorphism.

Keywords: *Beauveria bassiana*; *Metarhizium anisopliae*; pesticide tolerance; poison food technique; polymorphism

Introduction

Entomopathogenic fungi (EPF) are cosmopolitan natural enemies of arthropod pests and they are effective for the regulation of insect pests in agroecosystems in an eco-friendly manner. These microbial insecticides effectively control insect pests because of their high virulence, broad host range and can be isolated from wide ranges of soil habitats, particularly cultivated and forest soils existing in diverse habitats (1). *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are promising entomopathogenic fungi extensively used for the biocontrol of a wide range of economically important insect pests (2, 3).

In integrated pest management programmes (IPM), a pivotal role is given to biocontrol agents and sometimes it may require applications of biocontrol agents along with chemical pesticides. Integration of a selected strain of entomopathogenic fungi with selective insecticides can improve the control efficiency of the pests. Simultaneously, the pesticide dosages can be reduced, which minimises the risks of environmental contamination and delays the expression of insecticide resistance in insect pests (4). Application of insecticides and fungicides in combination with these entomogenous fungi or separately may affect the effectiveness of the latter through their effect on growth, sporulation and germination (5). In this context, we must have sufficient knowledge of the compatibility of these fungi with pesticides. Moreover, if fungi with pesticide tolerance could be developed, it would enhance their scope in IPM programmes.

Today, the most sought-after strategy for pest management is IPM, wherein integrating biological, chemical, cultural and mechanical measures for the management of the noxious pests. We cannot totally supplant chemical pesticides with bioagents for pest management in the immediate future. Developing aggressive and pesticide-tolerant strains of EPFs by conventional and/or genetic approaches can improve the field persistence and efficacy of EPFs in pesticide-treated agro-ecosystems by combining them with biocontrol agents, which impair the efficacy of both (6). Diverse ways can be used to improve EPFs pesticidal capabilities and reduce the negative effects of these chemicals. Henceforth, the compatibility of the entomopathogenic fungi with pesticides needs elucidation to harness their full potential in pest management programmes. The present investigation was conducted to test the toxicological impact of chemical pesticides and fungicides on entomopathogenic fungi, to study the compatibility of fungi with pesticides and to develop and characterise pesticide-tolerant strains of *B. bassiana* and *M. anisopliae*.

Materials and Methods

The compatibility of 2 fungicides and 6 insecticides with *B. bassiana* and *M. anisopliae* was determined under laboratory conditions, adopting the poison food technique (7). The growth, sporulation and bio-efficacy of the fungi grown on the poison food media were assessed. The experiment was conducted as a completely randomised design (CRD) with 3 replications. The pesticides used are carbendazim 0.1 %, carbofuran 3G, carbosulfan 6G, chlorpyriphos 0.03 %, imidacloprid 0.006 %, lambda cyhalothrin 0.025 %, malathion 0.15 % and mancozeb 0.2 %. The required quantity of the chemicals was measured and added separately to 100 mL of sterilised PDA in conical flasks in its molten stage when the temperature was below 45 °C and mixed thoroughly. The media thus prepared was poured into sterile 9 cm Petri plates inside the laminar air flow chamber and allowed to solidify. Circular fungal discs of 5 mm size, cut from the growing outer edges of a 14-day-old culture of the respective fungi, using a sterile cork borer, were placed in the centre of these Petri plates with solidified poisoned PDA. Fungus inoculated into media without pesticide served as the control. The inoculated plates were incubated at room temperature.

Observations on the radial growth of the fungi were measured daily until the fungi fully covered the control plates. Both fungi were grown separately in poisoned food media continuously for ten passages with an increase in concentrations of pesticides. The mycelium of *B. bassiana* and *M. anisopliae* harvested from a 15-day-old potato dextrose agar culture poisoned with the chemicals was used for DNA isolation. DNA was isolated using the GenElute plant genomic DNA miniprep kit (Sigma). 20 mg of the mycelium was transferred to a microcentrifuge tube and ground in 350 µL of lysis solution A and 50 µL of lysis solution B using a micro pestle. The stock DNA was properly labelled and stored at 4 °C.

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1 µL of 6 X gel-loading buffer (0.25 % bromophenol blue, 30 % sucrose in TE buffer pH 8.0) was added to 5 µL of DNA. The samples were loaded to 0.8 % agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg mL^{-1} ethidium bromide. Electrophoresis was performed with 0.5 X TBE as electrophoresis buffer at 75 V until the bromophenol dye front had migrated to the bottom of the gel. The gels were visualised in a UV transilluminator (Genei) and the image was captured under UV light using the gel documentation system (Bio-Rad).

PCR amplification reactions were carried out in a 20 µL reaction volume, which contained 1X PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTP (dATP, dGTP, dCTP and dTTP), ~10 ng DNA, 0.4 µL of taq DNA polymerase enzyme (Genei), 0.1 mg mL^{-1} BSA and 5 pM of primer. The reaction was carried out in triplicate to obtain concurrent results. 10 primers were used for the reactions. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR system 9700, applied biosystems). The PCR products were checked in 1.5 % agarose gels prepared in 0.5 X TBE buffer containing 0.5 µg mL^{-1} ethidium bromide. The PCR product was scored for the presence (+) or absence (-) of bands. The number of monomorphic bands and the number of polymorphic bands were recorded. Thus, the banding pattern of all 10 primers for the eighteen samples, as given in Table 1, was scored as 1 and 0 in the Excel sheet and then subjected to further statistical analysis. A genetic similarity matrix was constructed using Jaccard's similarity coefficient values and this matrix was subjected to an unweighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendrogram using the average linkage procedure. All these computations were carried out using NTSYS-pc version 2.02 software and the dendrogram constructed was used to assess the association and distance between the fungi cultured in different chemicals under study (8).

Results and Discussion

Fungal growth

When *B. bassiana* was cultured in poisoned food media, 2 DAI, the growth of the fungus in the control Petri plates without chemicals reached 1.54 cm and was on par with the mean radial growth of the fungus in imidacloprid poisoned media (1.36 cm) (Table 2). The

Table 1. Mean sporulation of *Beauveria bassiana* in poisoned food media for continuous 10 passages

Sl. No.	Treatments	Spore count (spores $mL^{-1} \times 10^8$)									
		I at 13 DAI	II at 13 DAI	III at 15 DAI	IV at 15 DAI	V at 18 DAI	VI at 16 DAI	VII at 18 DAI	VIII at 19 DAI	IX at 21 DAI	X at 21 DAI
T_1	Carbendazim	0.04 (0.22)	0.03 (0.17)	0.01 (1.20)	0.03 (0.18)	0.06 (0.24)	0.06 (0.25)	0.02 (0.17)	0.02 (0.16)	0.01 (0.10)	0.009 (0.009)
T_2	Mancozeb	0.03 (0.19)	0.02 (0.15)	0.01 (1.04)	0.008 (0.093)	0.02 (0.12)	0.01 (0.12)	0.01 (0.12)	0.01 (0.12)	0.008 (0.089)	0.008 (0.009)
T_3	Carbofuran	19.82 (4.45)	12.29 (3.51)	9.56 (3.09)	11.27 (3.36)	13.11 (3.62)	26.39 (5.14)	19.87 (4.46)	14.20 (3.77)	11.05 (3.32)	11.39 (3.38)
T_4	Carbosulfan	7.65 (2.77)	10.03 (3.17)	13.38 (3.66)	28.40 (5.33)	52.99 (7.28)	59.26 (7.69)	42.48 (6.52)	48.17 (6.94)	37.94 (6.16)	34.76 (5.89)
T_5	Imidacloprid	35.45 (5.95)	33.57 (5.79)	41.43 (6.44)	68.88 (8.29)	69.67 (8.35)	84.33 (9.18)	70.99 (8.43)	62.69 (7.92)	57.87 (7.61)	56.31 (7.50)
T_6	Chlorpyriphos	1.41 (1.19)	0.75 (0.87)	0.16 (0.40)	0.19 (0.43)	0.22 (0.47)	0.22 (0.47)	0.21 (0.45)	0.28 (0.53)	0.32 (0.57)	0.31 (0.56)
T_7	Lambda cyhalothrin	0.26 (0.51)	0.15 (0.39)	0.16 (0.40)	0.37 (0.61)	0.54 (0.74)	0.39 (0.62)	0.43 (0.66)	0.48 (0.69)	0.44 (0.66)	0.39 (0.63)
T_8	Malathion	0.27 (0.52)	0.24 (0.49)	0.13 (0.36)	0.06 (0.24)	0.10 (0.32)	0.13 (0.36)	0.10 (0.32)	0.09 (0.31)	0.08 (0.28)	0.08 (0.28)
T_9	Untreated control	1108.31 (33.29)	441.59 (21.01)	244.76 (15.64)	229.49 (15.15)	223.89 (14.96)	222.67 (14.92)	222.45 (14.92)	213.57 (14.61)	213.53 (14.61)	208.81 (14.45)
	C.D (p = 0.05)	(10.614)	(3.054)	(1.373)	(2.422)	(1.607)	(1.753)	(1.906)	(2.044)	(2.265)	(6.348)

Figures in parentheses are square root transformed values.

Table 2. Mean growth of *Beauveria bassiana* in poisoned food media for continuous 10 passages

Sl. No.	Treatments	Growth of the fungus (cm)									
		I at 13 DAI	II at 13 DAI	III at 15 DAI	IV at 15 DAI	V at 18 DAI	VI at 16 DAI	VII at 18 DAI	VIII at 19 DAI	IX at 21 DAI	X at 21 DAI
T_1	Carbendazim	4.15 (2.04)	3.89 (1.97)	4.12 (2.03)	3.10 (1.76)	3.27 (1.81)	3.62 (1.90)	3.21 (1.79)	3.36 (1.83)	3.53 (1.88)	3.20 (1.79)
T_2	Mancozeb	2.84 (1.68)	2.67 (1.63)	2.05 (1.43)	2.41 (1.55)	2.49 (1.58)	2.89 (1.70)	2.96 (1.72)	2.18 (1.48)	2.34 (1.53)	2.44 (1.56)
T_3	Carbofuran	5.82 (2.41)	5.81 (2.41)	5.59 (2.37)	5.65 (2.38)	5.74 (2.39)	5.17 (2.27)	5.31 (2.30)	5.53 (2.35)	5.24 (2.29)	5.51 (2.35)
T_4	Carbosulfan	6.89 (2.63)	6.19 (2.49)	6.27 (2.51)	5.78 (2.40)	5.94 (2.44)	5.45 (2.34)	5.66 (2.38)	5.82 (2.41)	5.46 (2.34)	5.85 (2.42)
T_5	Imidacloprid	8.27 (2.88)	8.49 (2.91)	8.37 (2.89)	7.46 (2.73)	8.28 (2.88)	7.87 (2.81)	8.10 (2.85)	7.79 (2.79)	8.18 (2.86)	8.29 (2.88)
T_6	Chlorpyriphos	5.51 (2.35)	4.17 (2.04)	4.12 (2.03)	4.28 (2.07)	3.71 (1.93)	4.19 (2.05)	4.34 (2.08)	4.27 (2.07)	4.52 (2.13)	4.72 (2.17)
T_7	Lambda cyhalothrin	4.22 (2.05)	3.17 (1.78)	3.06 (1.75)	3.35 (1.83)	2.71 (1.65)	2.76 (1.66)	2.96 (1.72)	2.34 (1.53)	2.56 (1.60)	2.97 (1.72)
T_8	Malathion	2.72 (1.65)	2.63 (1.62)	1.95 (1.39)	2.11 (1.45)	2.39 (1.55)	2.72 (1.65)	2.45 (1.57)	2.73 (1.65)	2.23 (1.49)	2.64 (1.63)
T_9	Untreated control	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)
	C.D (p = 0.05)	(0.052)	(0.061)	(0.048)	(0.066)	(0.098)	(0.047)	(0.044)	(0.047)	(0.045)	(0.038)

Figures in parentheses are square root transformed values.

carbofuran and carbosulfan-treated media recorded a mean growth of 1.26 and 1.21 cm, respectively and were statistically on par. The growth of the fungus in the media poisoned with chlorpyriphos, lambda cyhalothrin and mancozeb was 0.93, 0.83 and 0.79 cm and these were on par. The least growth was observed in the case of carbendazim and malathion, which recorded a mean growth of 0.67 and 0.50 cm, respectively. Good compatibility of the insecticide, imidacloprid 0.006 %, with *B. bassiana* and *M. anisopliae* was evident from the radial growth of these fungi in the media poisoned with imidacloprid 0.006 %, which did not differ significantly from that in the control in the observations taken 2 and 5 days after inoculation, though a significant difference in growth was seen in the observations taken later.

Metarhizium anisopliae recorded the highest mycelial growth in the control plate two days after inoculation and had a mean growth of 1.26 cm, followed by the growth in imidacloprid and carbofuran mixed media, which produced 1.12 and 1.09 cm, respectively and showed statistical similarity (Table 3). *Metarhizium anisopliae* in carbosulfan recorded a mean growth of 1.05 cm, while that in chlorpyriphos and mancozeb recorded a mean radial growth of 0.69 and 0.63 cm respectively and were on par. Relatively lower growth was observed in the case of lambda cyhalothrin, carbendazim and malathion, in which the fungus had a mean growth of 0.57, 0.53 and 0.53 cm respectively and were on par. Among all the pesticides tested, malathion 0.15 % was the most inhibitory to the growth of *B. bassiana* and *M. anisopliae*. The mean radial growth of *B. bassiana* was 2.72 cm at 13 DAI and that of *M. anisopliae* was 3.53 cm at 15 DAI. Some insecticides are known to antagonise, while some others are known to synergise the activities of these fungi. Research indicates that fungicides in general were antagonistic to *B. bassiana* at varying intensities (9).

Beauveria bassiana and *M. anisopliae* grown continuously in varying doses of two fungicides, viz. carbendazim and mancozeb and 6 insecticides, viz. carbofuran, carbosulfan, imidacloprid, chlorpyriphos, lambda cyhalothrin and malathion, showed that there was inhibition in the growth of both the fungi, but they grew on all the media mixed with pesticides. *Beauveria bassiana* recorded the maximum radial growth (9 cm) in 13 days, followed by the fungi grown in imidacloprid, carbosulfan, carbofuran and chlorpyriphos, which recorded a mean radial growth of 8.27, 6.89, 5.82 and 5.51 cm respectively and showed statistical significance from the other treatments (Table 2). The growth of the fungus on lambda cyhalothrin mixed media was 4.22 cm and was on par with the fungus cultured in carbendazim, which produced a mean radial growth of 4.15 cm. When the fungus was grown on media mixed with the fungicide, mancozeb and the insecticide malathion, the growth was very low and recorded 2.84 and 2.72 cm, respectively and were statistically on par. Even in the tenth passage of the fungi, a similar trend was observed with a maximum of 9 cm growth recorded for the control within 21 days. Imidacloprid, carbosulfan, carbofuran, chlorpyriphos, carbendazim, lambda cyhalothrin, malathion and mancozeb poisoned media recorded a mean radial growth of 8.29, 5.85, 5.51, 4.72, 3.20, 2.97, 2.64 and 2.44 cm, respectively and exhibited significant statistical difference. Inglis et al. stated that the inhibitory properties of insecticides and fungicides vary both between and within chemical classes (10). However, a similarity in performance of the fungi cultured in carbofuran and carbosulfan, both belonging to the carbamate group, was observed in the present study.

When *M. anisopliae* was cultured in poison mixed media, the maximum growth of 9 cm was recorded for the control treatment without pesticide exposure on the fifteenth day and it was followed by the growth of the fungus in imidacloprid mixed media, which recorded a mean radial growth of 6.37 cm and exhibited a significant statistical difference from the other treatments (Table 3). The fungi cultured with carbosulfan and carbofuran recorded a mean growth of 5.63 and 5.46 cm respectively and were on par. Lambda cyhalothrin-poisoned media had a mean growth of 4.89 cm of *M. anisopliae*. The media mixed with chlorpyriphos and mancozeb had recorded a mean growth of 4.33 and 4.14 cm, respectively, of the fungus and were on par. Relatively lower growth was observed in the media poisoned with malathion, which recorded a mean radial growth of 3.53 cm and was on par with that in carbendazim (3.31 cm), which had the lowest growth among the different treatments. During the tenth passage, the maximum growth of 9.00 cm was recorded for the control treatment on 26 days, followed by that grown in imidacloprid, carbosulfan and

Table 3. Mean growth of *Metarhizium anisopliae* in poisoned food media for continuous ten passages

Sl. No.	Treatments	Growth of the fungus (cm)									
		I at 15 DAI	II at 15 DAI	III at 17 DAI	IV at 17 DAI	V at 17 DAI	VI at 23 DAI	VII at 21 DAI	VIII at 24 DAI	IX at 23 DAI	X at 26 DAI
T_1	Carbendazim	3.31 (1.82)	3.12 (1.77)	2.99 (1.73)	3.13 (1.77)	3.28 (1.81)	3.43 (1.85)	3.26 (1.81)	3.48 (1.86)	3.83 (1.96)	3.99 (2.00)
T_2	Mancozeb	4.14 (2.03)	4.26 (2.06)	4.18 (2.04)	4.24 (2.06)	4.22 (2.05)	4.27 (2.07)	4.31 (2.08)	4.29 (2.07)	4.37 (2.09)	4.26 (2.06)
T_3	Carbofuran	5.46 (2.34)	5.15 (2.27)	5.23 (2.29)	5.24 (2.29)	5.26 (2.29)	5.25 (2.29)	5.20 (2.28)	5.37 (2.32)	5.39 (2.32)	5.31 (2.30)
T_4	Carbosulfan	5.63 (2.37)	5.48 (2.34)	5.39 (2.32)	5.25 (2.29)	5.29 (2.30)	5.53 (2.35)	5.56 (2.36)	5.86 (2.42)	5.74 (2.39)	5.71 (2.39)
T_5	Imidacloprid	6.37 (2.52)	6.35 (2.52)	6.44 (2.54)	6.48 (2.55)	6.38 (2.53)	6.63 (2.58)	6.73 (2.59)	6.67 (2.58)	6.63 (2.58)	6.97 (2.64)
T_6	Chlorpyriphos	4.33 (2.08)	4.13 (2.03)	4.05 (2.01)	4.17 (2.04)	4.27 (2.07)	4.38 (2.09)	4.53 (2.13)	4.37 (2.09)	4.29 (2.07)	4.23 (2.06)
T_7	Lambda cyhalothrin	4.89 (2.21)	4.51 (2.12)	4.43 (2.10)	4.37 (2.09)	4.23 (2.06)	4.08 (2.02)	4.06 (2.02)	4.12 (2.03)	3.92 (1.98)	3.84 (1.96)
T_8	Malathion	3.53 (1.88)	3.33 (1.83)	3.26 (1.81)	3.12 (1.77)	3.09 (1.76)	3.09 (1.76)	3.29 (1.81)	3.25 (1.80)	3.12 (1.77)	3.09 (1.76)
T_9	Untreated control	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)
	C.D (p = 0.05)	(0.073)	(0.059)	(0.057)	(0.069)	(0.063)	(0.046)	(0.058)	(0.059)	(0.067)	(0.040)

Figures in parentheses are square root transformed values.

carbofuran poisoned media, which recorded a mean radial growth of 6.97, 5.71 and 5.31 cm respectively and had a significant difference when compared to the other treatments. The fungi grown with mancozeb and chlorpyriphos mixed media recorded a mean growth of 4.26 and 4.23 cm respectively and were on par. A mean growth of 3.99, 3.84 and 3.09 cm was observed for the fungi cultured with carbendazim, lambda cyhalothrin and malathion respectively.

Sporulation

The spore count of *B. bassiana* in the control with no chemical treatment was significantly high and recorded a mean spore count of 1108.31 × 10^8 spores mL^{-1} and differed significantly from the other treatments (Table 1). A mean spore count ranging between 35.45 × 10^8 to 0.03 × 10^8 spores mL^{-1} was observed in the media treated with imidacloprid, carbosulfan, carbofuran, chlorpyriphos, malathion, lambda cyhalothrin, carbendazim and mancozeb respectively and were on par. In the tenth continuous passage, the highest spore concentration was recorded in the control treatment with a spore load of 208.81 × 10^8 spores mL^{-1}, followed by that grown with imidacloprid, carbosulfan and carbofuran mixed media, which had a concentration of 56.31 × 10^8, 34.76 × 10^8 and 11.39 × 10^8 spores mL^{-1} and differed significantly from the other treatments. The spore count obtained from the fungi cultured with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb mixed media ranged from 0.39 × 10^8 to 0.008 × 10^8 spores mL^{-1} and were statistically on par.

The spore count of *M. anisopliae* in the pure culture without any chemical treatments in the first subculturing was significantly higher and recorded a concentration of 84.67 × 10^8 spores mL^{-1}, followed by that grown with imidacloprid mixed media, which had a spore load of 26.73 × 10^8 spores mL^{-1} and differed significantly from the other treatments (Table 4). The concentration of spores present in the fungi cultured with carbosulfan, carbofuran, carbendazim, chlorpyriphos, mancozeb, lambda cyhalothrin and malathion ranged from 1.47 × 10^8 to 0.02 × 10^8 spores mL^{-1} respectively and were on par. During the tenth subculturing of the fungi, the fungi grown as control recorded maximum concentration of spores and it was 67.74 × 10^8 spores mL^{-1} and was on par with that grown with imidacloprid-poisoned media, which had a mean spore count of 51.60 × 10^8 spores mL^{-1}. The spore count of fungi grown with carbosulfan and carbofuran mixed media was 5.62 × 10^8 and 2.70 × 10^8 spores mL^{-1} respectively and were statistically on par. A mean spore count ranging from 0.25 × 10^8 to 0.01 × 10^8 spores mL^{-1} was recorded in the cultures grown with chlorpyriphos, mancozeb, carbendazim, lambda cyhalothrin and malathion mixed media respectively and were on par.

Polymorphism

Among the 10 primers tested, the primer RFu-10 was found to give maximum polymorphism of 100 % (Fig 1). The primer RFu-4 produced the maximum number of scorable bands (15), whereas the primer RFu-7 obtained only one scorable band. *Beauveria bassiana* exhibited a polymorphism of 82.30 %. In the case of *M. anisopliae*, the primer RFu-7 induced maximum polymorphism of 40.00 %. Out of the 10 primers evaluated, the primer RFu-2 produced the maximum scorable bands (11 Nos.) and the primer RFu-6 developed the minimum number of scorable bands (5 Nos.). The polymorphism exhibited by the fungus is 26.58 % (Fig. 2).

Jaccard's similarity coefficient values for each pairwise comparison between the fungi cultured in different chemicals were calculated and a similarity coefficient matrix was constructed. The matrix was subjected to the unweighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendrogram using the average linkage procedure. All these computations were carried out using NTSYS-pc version 2.02 software. Polymorphism was higher for *B. bassiana* cultured in carbendazim. The polymorphism exhibited in *B. bassiana* was higher (83.19 %) compared to *M. anisopliae* (38.46 %) (Fig. 3). Among the ten universal fungal primers evaluated, RFu-10 was found to give maximum polymorphism. Variations in the genetic profile of both fungi were seen after ten passages through the poisoned media with high and varying concentrations of insecticides belonging to different groups. It was observed that variations in the genetic makeup of the fungi were induced more by the insecticides of the synthetic pyrethroid group.

Table 4. Mean sporulation of *Metarhizium anisopliae* in poisoned food media for continuous 10 passages

Sl. No.	Treatments	Spore count (spores mL^{-1} × 10^{8})									
		I at 15 DAI	II at 15 DAI	III at 17 DAI	IV at 17 DAI	V at 17 DAI	VI at 23 DAI	VII at 21 DAI	VIII at 24 DAI	IX at 23 DAI	X at 26 DAI
T_1	Carbendazim	0.14 (0.37)	0.17 (0.41)	0.17 (0.41)	0.26 (0.51)	0.32 (0.57)	0.26 (0.51)	0.17 (0.41)	0.17 (0.41)	0.19 (0.43)	0.19 (0.44)
T_2	Mancozeb	0.06 (0.25)	0.10 (0.32)	0.16 (0.40)	0.17 (0.41)	0.20 (0.45)	0.14 (0.38)	0.15 (0.39)	0.22 (0.47)	0.21 (0.45)	0.22 (0.47)
T_3	Carbofuran	1.39 (1.18)	1.68 (1.29)	1.83 (1.35)	1.42 (1.19)	1.55 (1.24)	1.27 (1.13)	1.24 (1.11)	1.82 (1.35)	1.77 (1.33)	2.70 (1.64)
T_4	Carbosulfan	1.47 (1.21)	2.61 (1.62)	6.45 (2.54)	6.73 (2.59)	6.72 (2.59)	4.66 (2.16)	4.42 (2.10)	5.08 (2.25)	6.17 (2.48)	5.62 (2.37)
T_5	Imidacloprid	26.73 (5.17)	39.69 (6.30)	59.83 (7.74)	59.19 (7.69)	56.36 (7.51)	38.19 (6.18)	27.61 (5.25)	39.24 (6.26)	50.06 (7.08)	51.60 (7.18)
T_6	Chlorpyriphos	0.09 (0.31)	0.16 (0.39)	0.17 (0.41)	0.23 (0.48)	0.45 (0.67)	0.57 (0.76)	0.34 (0.58)	0.22 (0.47)	0.27 (0.52)	0.25 (0.49)
T_7	Lambda cyhalothrin	0.03 (0.16)	0.05 (0.22)	0.04 (0.21)	0.06 (0.24)	0.05 (0.22)	0.04 (0.20)	0.03 (0.16)	0.03 (0.16)	0.03 (0.17)	0.02 (0.15)
T_8	Malathion	0.02 (0.16)	0.02 (0.16)	0.02 (0.14)	0.01 (0.11)	0.009 (0.094)	0.01 (0.11)	0.007 (0.009)	0.02 (0.15)	0.009 (0.099)	0.01 (0.11)
T_9	Untreated control	84.67 (9.20)	75.31 (8.68)	64.31 (8.02)	75.64 (8.69)	88.55 (9.41)	72.13 (8.49)	51.74 (7.19)	47.12 (6.86)	66.63 (8.16)	67.74 (8.23)
	C.D (p = 0.05)	(3.922)	(2.422)	(1.123)	(0.754)	(0.591)	(0.903)	(1.483)	(0.997)	(1.020)	(1.200)

Figures in parentheses are square root transformed values.

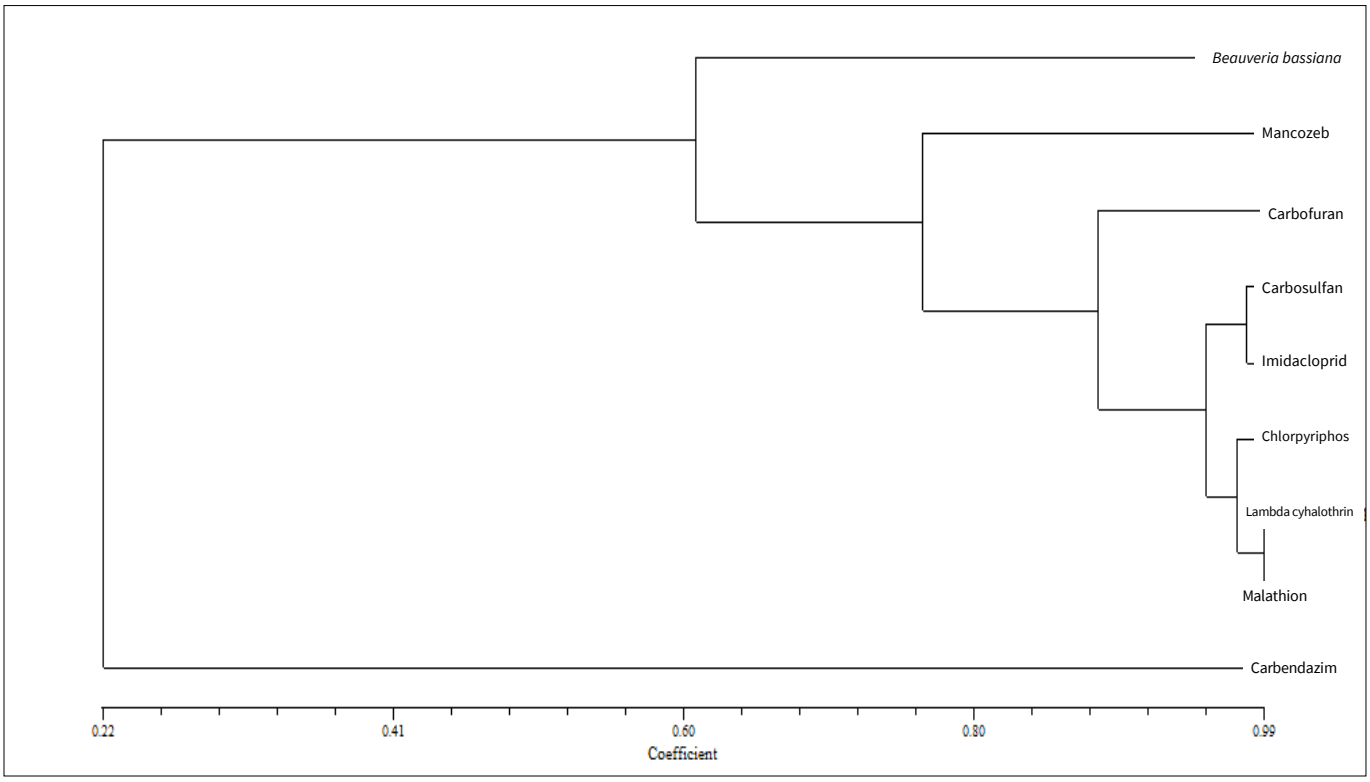


Fig. 1. Dendrogram generated from molecular analysis of *Beauveria bassiana* cultured in poison media for 10 passages.

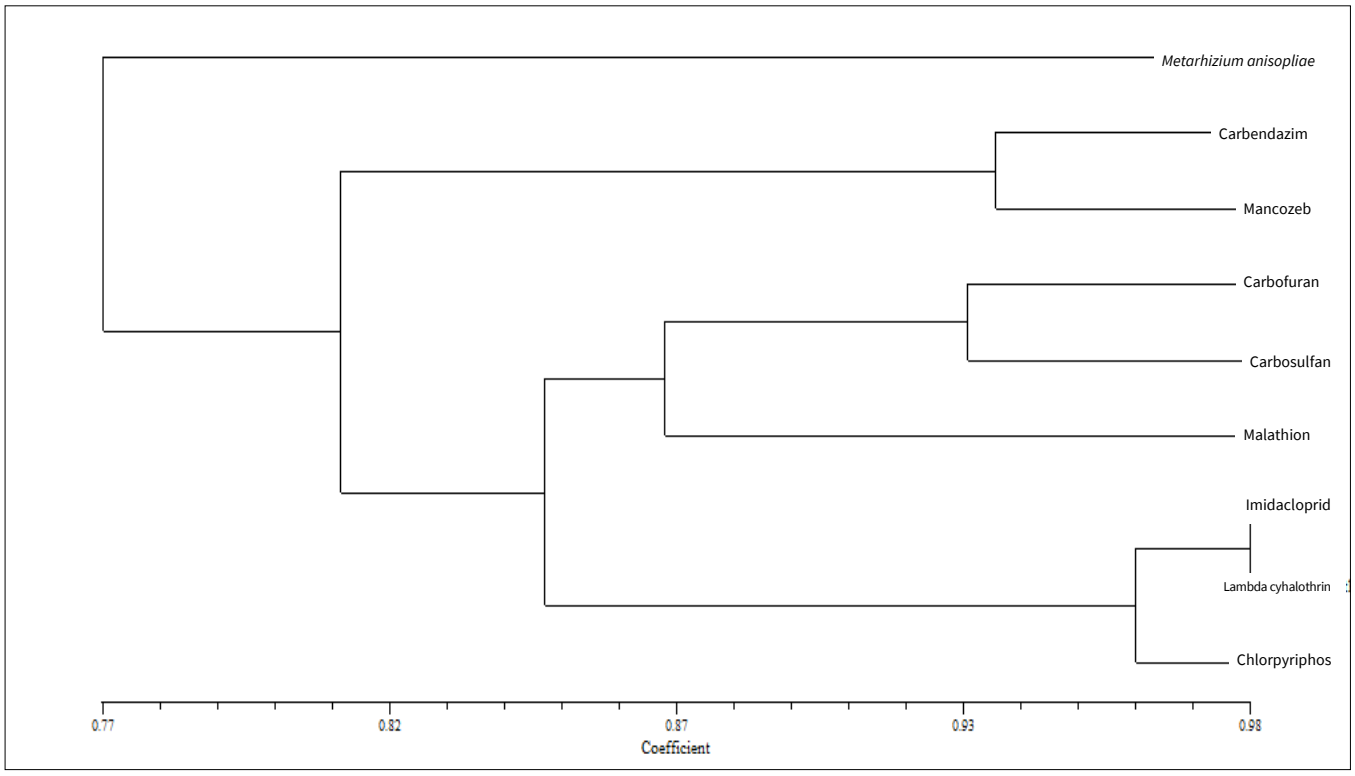


Fig. 2. Dendrogram generated from molecular analysis of *Metarhizium anisopliae* cultured in poison media for 10 passages.

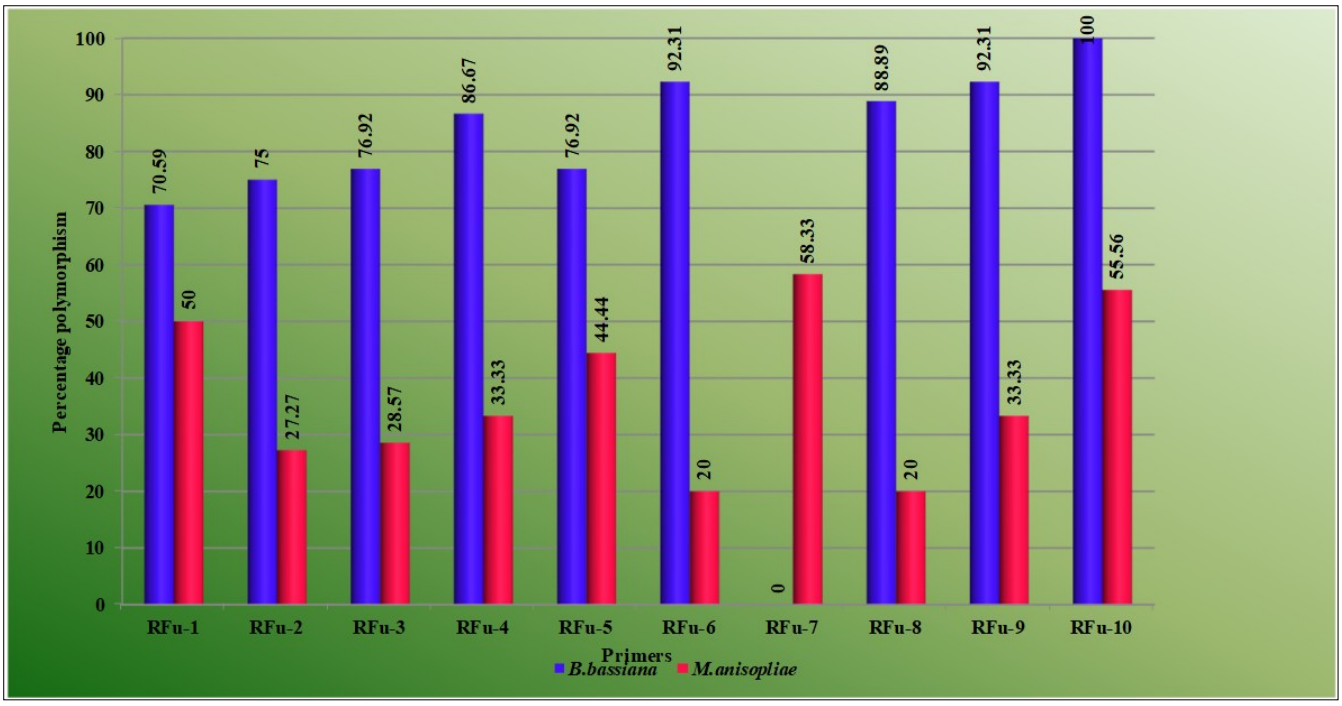


Fig. 3. Polymorphism exhibited by *Beauveria bassiana* and *Metarhizium anisopliae* in the 10 primers.

Conclusion

The study showed that both fungi tolerated high doses of pesticides, though their growth, sporulation and bioefficacy were reduced. *Beauveria bassiana* and *Metarhizium anisopliae* tolerated up to 32 times the recommended field dose of imidacloprid, while tolerance to other pesticides ranged from 8–16 times the field dose, indicating the feasibility of integrating *Beauveria bassiana* with these chemicals in pest management programmes. Subculturing reduced spore production in both fungi, with a greater decline in *B. bassiana* and *M. anisopliae* isolates, which showed pleomorphic deterioration under repeated subculturing. Co-application of entomopathogenic fungi with low doses of insecticides can be integrated as a component of IPM programme.

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Compliance with ethical standards

Conflict of interest: Author do not have any conflict of interests to declare.

Ethical issues: None

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