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In vitro compatibility of entomopathogenic fungi with insect growth regulators

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Abstract
The present investigation, was carried out to test the compatibility of two entomopathogenic fungi *viz.*, *M. anisopliae* and *B. bassiana* with three insect growth regulators *viz.*, novaluron 10EC, chlorfluazuron 5.4 EC and buprofezin 25 SC. The results obtained on the radial growth, percent inhibition of mycelial growth and spore production confirmed that buprofezin 25 SC at its 50 percent of field recommended dose was found compatible with both the fungi. Amongst the treatments, comprised of the maximum mycelial growth at 6 days after inoculation was observed in *M. anisopliae* grown on the media containing buprofezin 25 SC at its 50 percent field recommended dose (69.00 mm) and was followed by the treatment of *M. anisopliae* with buprofezin 25 SC at its 100 percent of field recommended dose (68.00 mm). In case of percent inhibition of mycelia growth of fungi, the lowest percent inhibition (0.96 percent) was observed when *M. anisopliae* was grown on media incorporated with buprofezin 25 SC at its 50 percent field recommended dose followed by the treatment of *B. bassiana* grown on media incorporated with buprofezin 25 SC at its 50 percent field recommended dose (3.87 percent). Mean conidia production of fungi was found maximum in *M. anisopliae* (7.23×10^7) and *B. bassiana* (6.76×10^7) grown on media without incorporation of insect growth regulators. However, when these fungi grown on media incorporated with insect growth regulators, highest mean conidia production was observed in *B. bassiana* grown on media incorporated with buprofezin 25 SC at its 50 percent field recommended dose.

Keywords: Compatibility, entomopathogenic fungi, insect growth regulators, radial diameter

Introduction
Entomopathogenic fungi (EPF) are the microorganisms that are pathogenic to insects. They typically cause the infection when particular insect comes in the contact with spores of the fungal species. Russian zoologist Elie Metchnikoff found *Metarhizium anisopliae* (previously *Entomophthora anisopliae*) in the late nineteenth century Similarly, Agostino Bassi in 1835 was established *Beauveria bassiana* (formerly *Botrytis bassiana*). Chitin synthesis inhibitors is one of the groups of insect growth regulators found to be effective in inhibiting chitin synthesis *in vivo* by blocking the chitin synthetase enzyme activity. Novaluron acts by both ingestion and contact (Ishaaya *et al.,* 2003) [4]. Chlorfluazuron acts by inhibiting biosynthesis of chitin in the cuticle of insects which leads to cause the loss of cuticle elasticity and firmness, and it is also acts as anti-moulting agent results in abortive moulting. Buprofezin is one of the first IGRs which mainly against sucking pests. It expresses its activity at the time of moulting due to which insects are not able to shed their cuticle and die during this process (De Cock and Degheele, 1998) [2]. Combination of EPF with chemical pesticides not only reduce time to kill the target pests but also produce additive or synergistic effects provided they should be compatible with each other. Generally, factors responsible for determining compatibility of EPF with insecticides or pesticides are spore production and toxicity index of EPF. On the basis of compatibility, we can use compatible EPF and chemical pesticide combinations for control of target pests (Sain *et al.,* 2022) [7]. This study will help to understand compatible IGR and EPF combinations so that they can be used in management of different pests.

2. Material and Methods

2.1 Effect of insect growth regulators on mycelial diameter of *M. anisopliae* and *B. bassiana*

The *in vitro* compatibility of entomopathogenic fungi with insect growth regulators was tested using poisoned food technique and recorded vegetative and reproductive growth of fungus. Accordingly, quantity of each insect growth regulator as per their field recommended and half of the field recommended dose of each insect growth regulator were calculated (for 60 ml potato dextrose agar medium). It was added separately in 250 ml conical flask containing molten potato dextrose agar which was dispense in three petriplates (7 cm diameter) in order to maintain three replications. After solidification of PDA 5 mm disc of either *Metarhizium anisopliae* or *Beauveria bassiana* was placed in the centre of each petri plate. They were incubated at ambient room temperature in laminar air flow and observations of mycelial growth of fungal colony in millimetre (mm) on 1st, 2nd, 3rd, 4th, 5th and 6th day/days after inoculation on PDA were measured. Similarly, reproductive growth of fungus was calculated by counting spores produce by fungi in each treatment after 15 days of inoculation.

2.2 Percent inhibition of entomopathogenic fungi treated with insect growth regulators

Percent inhibition of both entomopathogenic fungi *viz., M. anisopliae* and *B. bassiana* was calculated separately by considering the treatments of *M. anisopliae and B. bassiana* alone as control. It was calculated by using the formula given by Vincent (1947) [9] and recorded data of mycelial growth of fungi.

$$I = \frac{(C - T)}{C} \times 100$$

Where,
I= Percent inhibition (%)
C= Growth of fungus in control plate (mm)
T= Growth of fungus in treatment plate (mm)

2.3 Effect of insect growth regulators on spore production of *M. anisopliae* and *B. bassiana*

To study the effect of IGR on spore production of entomopathogenic fungi, 5 mm disc of 10 days old culture of *M. anisopliae* and *B. bassiana* grown on PDA plates, containing desired concentrations of an IGRs were put in test tubes containing 10 ml sterilized distilled water and 0.01% Tween 80. Mixed thoroughly using vortex mixer, strained from double layer sterilized muslin cloth and after that conidia were counted by using haemocytometer. Number of conidia per ml was calculated using following formula;

$$\text{No. of spores per m l} = \frac{\text{Dilution factor} \times \text{total no.of cells counted}}{\text{No.of squares counted} \times 2.5 \times 10^{-7}}$$

3. Results and Discussion

3.1 Effect of insect growth regulators on mycelial diameter of *M. anisopliae* and *B. bassiana* at 6 DAI

The data obtained regarding mycelial diameter of fungi recorded in different treatments at six days after inoculation was significant. The treatment T_1- *Metarhizium anisopliae* recorded highest mycelial diameter (69.67mm) however, it was at par with T_8- *Metarhizium anisopliae* + Buprofezin 25 SC (50% RD) (69.00 mm) and T_7- *Metarhizium anisopliae* + Buprofezin 25 SC (100% RD) (68.00 mm). The latter treatment was also at par with treatment T_4- *Metarhizium anisopliae* + Novaluron 10 EC (50% RD) (62.33 mm) and T_3- *Metarhizium anisopliae* + Novaluron 10 EC (100% RD) (59.67 mm). The next treatment in sequence which recorded more fungal diameter was T_2- *Beauveria bassiana* (51.67 mm) which was at par with T_{14}- *Beauveria bassiana* + Buprofezin 25 SC (50% RD) (49.67 mm). The latter treatment however, was also found at par with T_{13}- *Beauveria bassiana*+ Buprofezin 25 SC (100% RD) (46.33 mm), T_{10}- *Beauveria bassiana* + Novaluron 10 EC (50% RD), T_6- *Metarhizium anisopliae* + Chlorfluazuron 5.4 EC (50% RD) (both recorded the equal mycelial growth of 43.67 mm) and T_9- *Beauveria bassiana*+ Novaluron 10 (42.67 mm). However, latter treatment was again found at par with T_5- *Metarhizium anisopliae* + Chlorfluazuron 5.4 EC (100% RD) (40.67 mm). Significantly lowest fungal diameter of 32.00 mm was recorded in T_{11}- *Beauveria bassiana* + Chlorfluazuron 5.4 EC (100% RD) which was statistically not different from treatment T_{12}- *Beauveria bassiana* + Chlorfluazuron 5.4 EC (50% RD) (34.67 mm).

Compatibility of half the field recommended dose of buprofezin 25 SC to *Metarhizium anisopliae* and *Beauveria bassiana* was reported by Sain *et al.* (2019) [8]. Similarly, Reddy *et al.* (2020) [6] reported more radial growth of *Metarhizium anisopliae* and *Beauveria bassiana* in the treatments comprised of half the recommended dose of Buprofezin than the treatments comprised of full recommended dose of Buprofezin. Joshi *et al.* (2018) [5] tested the compatibility of *Beauveria bassiana* and *Metarhizium anisopliae* with novaluron 10 EC and found Novaluron at recommended dose was compatible with both the fungi.

Table 1: Effect of insect growth regulators on mycelial diameter of *M. anisopliae* and *B. bassiana*

Sr. No.	Treatments	Concentration of IGRs (%)	Mean mycelial diameter (mm) 6 DAI
T_1	*M. anisopliae*	-	69.67 (8.38)
T_2	*B. bassiana*	-	51.67 (7.22)
T_3	*M. anisopliae* + Novaluron 10 EC (100% RD)	0.015%	59.67 (7.76)
T_4	*M. anisopliae* + Novaluron 10 EC (50% RD)	0.0075%	62.33 (7.93)
T_5	*M. anisopliae* + Chlorfluazuron 5.4 EC (100% RD)	0.01%	40.67 (6.42)
T_6	*M. anisopliae* + Chlorfluazuron 5.4 EC (50% RD)	0.005%	43.67 (6.64)
T_7	*M. anisopliae* + Buprofezin 25 SC (100% RD)	0.037%	68.00 (8.28)
T_8	*M. anisopliae* + Buprofezin 25 SC (50% RD)	0.0185%	69.00 (8.34)
T_9	*B. bassiana*+ Novaluron 10 EC (100% RD)	0.015%	42.67 (6.57)
T_{10}	*B. bassiana* + Novaluron 10 EC (50% RD)	0.0075%	43.67 (6.65)
T_{11}	*B. bassiana*+ Chlorfluazuron 5.4 EC (100% RD)	0.01%	32.00 (5.70)
T_{12}	*B. bassiana* + Chlorfluazuron 5.4 EC (50% RD)	0.005%	34.67 (5.93)
T_{13}	*B. bassiana*+ Buprofezin 25 SC (100% RD)	0.037%	46.33 (6.84)
T_{14}	*B. bassiana* + Buprofezin 25 SC (50% RD)	0.0185%	49.67 (7.08)
C.D. (p=0.01)			0.37
SEm (±)			0.09

* Figures in parenthesis are √n+0.5 values
RD: Recommended Dose
** DAI: Days after Inoculation

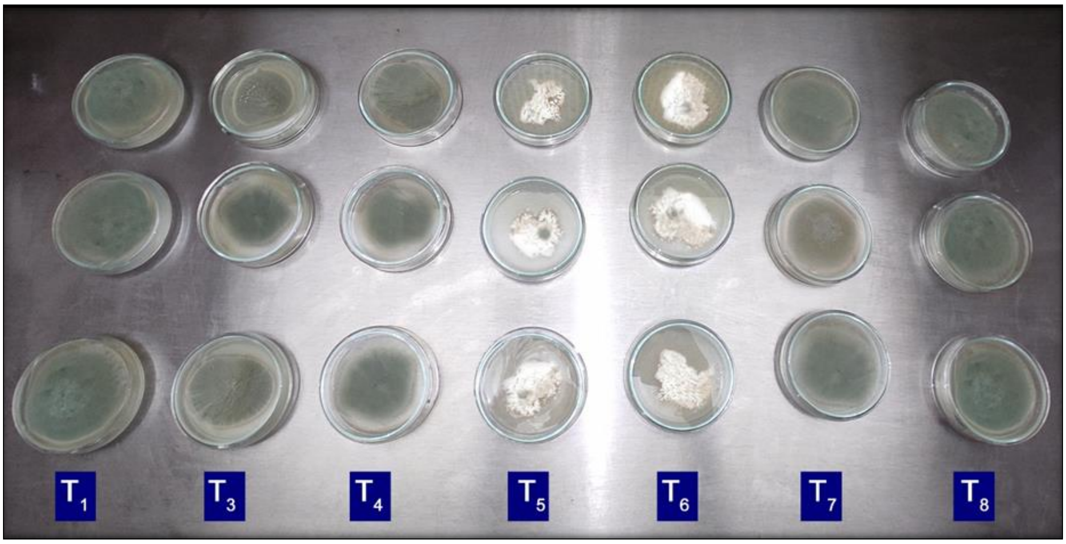

Fig 1: Growth of *M. anisopliae* on PDA incorporated with insect growth regulators at 6 DAI

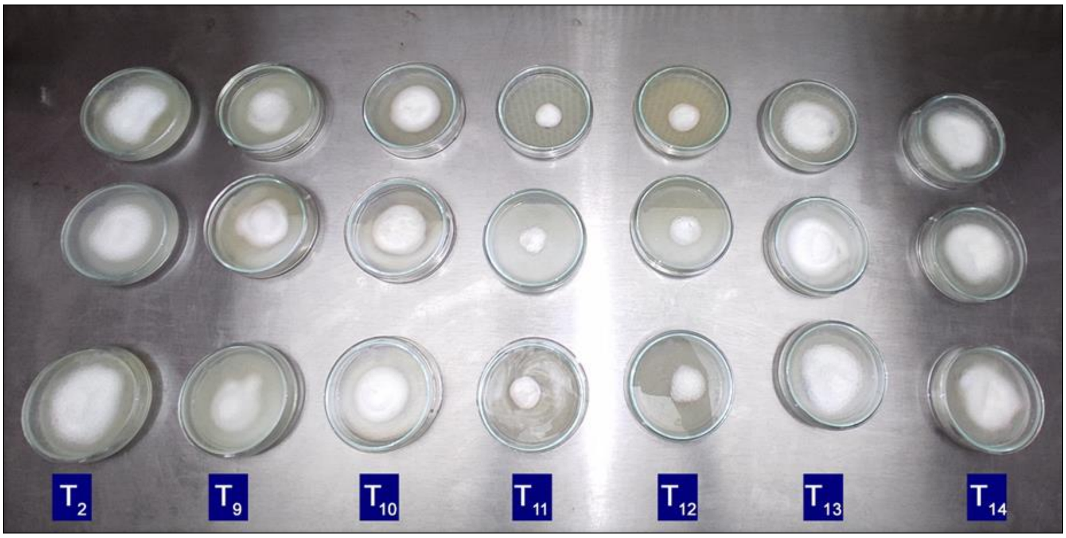

Fig 2: Growth of *B. bassiana* grown on PDA containing insect growth regulators at 6 DAI

3.2 Percent inhibition of entomopathogenic fungi treated with insect growth regulators

As data shown in the table 2, percent inhibition of mycelial growth of *M. anisopliae* was least when it was grown on media incorporated with buprofezin 50% RD *i.e.,* 0.96 percent followed by buprofezin 100% RD (2.39percent), novaluron 50% RD (10.53 percent) and novaluron100% RD (14.35 percent). Percent inhibition of mycelia growth of *M. anisopliae* was higher when it was grown on media incorporated with Chlorfluazuron 50% RD and 100% RD *i.e.,* 37.31 percent and 41.62 percent, respectively.

Table 2: Percent inhibition of mycelial growth of *M. anisopliae* treated with insect growth regulators at 6 DAI

Sr. No.	Treatments	Conc. (%)	Mean mycelial growth (mm)	Percent inhibition
1.	*M. anisopliae* + Novaluron 10 EC (100% RD)	0.015	59.67	14.35
2.	*M. anisopliae* + Novaluron 10 EC (50% RD)	0.0075	62.33	10.53
3.	*M. anisopliae* + Chlorfluazuron 5.4 EC (100% RD)	0.01	40.67	41.62
4.	*M. anisopliae* + Chlorfluazuron 5.4 EC (50% RD)	0.005	43.67	37.31
5.	*M. anisopliae* + Buprofezin 25 SC (100% RD)	0.037	68.00	2.39
6.	*M. anisopliae* + Buprofezin 25 SC (50% RD)	0.0185	69.00	0.96
7.	Control (*M. anisopliae*)	-	69.67	-

RD: Recommended dose

Data from table 3 revealed that all the insect growth regulators evaluated were caused some inhibitory actions on the mycelial growth of fungus *B. bassiana*. Least percent inhibition of mycelial growth of *B. bassiana* (3.87 percent) was recorded by the action of buprofezin at its 50% RD. Buprofezin at its 100% RD stood next in terms of less inhibition of mycelial growth (10.33 percent). Next treatment in order of exhibiting less inhibition of mycelial growth of *B. bassiana* was novaluron at its 50% RD (15.48percent) followed by novaluron at its 100% RD (17.41percent). However, maximum percent inhibition of mycelial growth of *B. bassiana* were recorded in the treatments comprised of chlorfluazuron 50% RD (32.90 percent) and 100% RD (38.07 percent).

Table 3: Percent inhibition of mycelial growth of *B. bassiana* treated with insect growth regulators at 6 DAI

Sr. No.	Treatments	Conc. (%)	Mean mycelial growth (mm)	Percent inhibition
1.	*B. bassiana*+ Novaluron 10 EC (100% RD)	0.015	42.67	17.41
2.	*B. bassiana* + Novaluron 10 EC (50% RD)	0.0075	43.67	15.48
3.	*B. bassiana*+ Chlorfluazuron 5.4 EC (100% RD)	0.01	32.00	38.07
4.	*B. bassiana* + Chlorfluazuron 5.4 EC (50% RD)	0.005	34.67	32.90
5.	*B. bassiana*+ Buprofezin 25 SC (100% RD)	0.037	46.33	10.33
6.	*B. bassiana* + Buprofezin 25 SC (50% RD)	0.0185	49.67	3.87
7.	Control (*B. bassiana*)	-	51.67	-

RD: Recommended dose

Reddy *et al.*, (2020) [6] reported 5.53% and 6.52% inhibition in mycelial growth of *Metarhizium anisopliae* and *Beauveria bassiana* due to 50% RD and 15.71% and 19.96% at 100% RD of buprofezin, respectively. Joshi *et al.* (2018) [5] reported 24.32% and 23.45% inhibition in mycelial growth of *Metarhizium anisopliae* and *Beauveria bassiana* in the treatment of recommended dose of novaluron 10 EC which is in accordance with the present findings.

3.3 Effect of insect growth regulators on spore production of *M. anisopliae* and *B. bassiana*

Table 4 indicates that all treatments showed reductions in conidia production than that of T_1- *M. anisopliae* (7.23×10^7 spores per ml) and T_2- *B. bassiana* (6.76×10^7spores per ml). Amongst different treatments wherein PDA is incorporated with insect growth regulator, treatment T_8- *M. anisopliae* + Buprofezin 25 SC (50% RD) and T_{14}- *B. bassiana*+ Buprofezin 25 SC (50% RD) recorded statistically equal conidial production *i.e.,* 6.71×10^7 spores per ml and 6.57 $\times10^7$ spores per ml, respectively. It was followed by T_{13}-*B. bassiana*+ Buprofezin 25 SC (100% RD) with 6.43 $\times10^7$ spores per ml and was at par with T_7-*M. anisopliae* + Buprofezin 25 SC (100% RD) with 6.40 $\times10^7$ spores per ml. The next effective treatment in order of spore production was T_9-*B. bassiana*+ Novaluron 10 EC (100% RD) with 5.11 $\times10^7$ spores per ml and was at par with T_{10}-*B. bassiana*+ Novaluron 10 EC (50% RD) with 4.75 $\times10^7$ spores per ml andT_3-*M. anisopliae* + Novaluron 10 EC (100% RD) with 4.56 $\times10^7$ spores per ml. The latter two treatments were also at par with T_4-*M. anisopliae* + Novaluron 10 EC (50% RD) with 4.24 $\times10^7$ spores per ml. The treatment T_6- *M. anisopliae* + Chlorfluazuron 5.4 EC (50% RD) recorded comparatively less spores *i.e.,* 1.89×10^7 per ml and was at par with T_{12}- *B. bassiana*+ Chlorfluazuron 5.4 EC (50% RD) with 1.78×10^7 spores per ml and T_{11}-*B. bassiana*+ Chlorfluazuron 5.4 EC (100% RD) with 1.61×10^7 spores per ml. The latter two treatments were also at par with T_5-*M. anisopliae* + Chlorfluazuron 5.4 EC (100% RD) which recorded lowest spores *i.e.*, 1.24×10^7 per ml.

Present findings got strong support from the findings of Cuthbertson *et al.* (2005) [1] who revealed that exposure of spores of entomopathogenic fungi *Lecanicellium lecanii* to buprofezin provided an acceptable level of spore germination. Hassan *et al.* (1994) [3] also found that buprofezin was relatively harmless to *Lecanicellium muscarium*.

Table 4: Effect of insect growth regulators on spore production of *M. anisopliae* and *B. bassiana*

Treat. No.	Treatments	Conidia production ($\times10^7$)			Mean conidia prod. (10^7)
		R I	R II	R III	
T_1	*M. anisopliae*	7.40	6.30	8.00	7.23
T_2	*B. bassiana*	7.10	6.50	6.68	6.76
T_3	*M. anisopliae* + Novaluron 10 EC (100% RD)	4.82	4.54	4.32	4.56
T_4	*M. anisopliae* + Novaluron 10 EC (50% RD)	4.51	4.30	3.90	4.24
T_5	*M. anisopliae* + Chlorfluazuron 5.4 EC (100% RD)	1.40	1.20	1.12	1.24
T_6	*M. anisopliae* + Chlorfluazuron 5.4 EC (50% RD)	2.10	1.80	1.78	1.89
T_7	*M. anisopliae* + Buprofezin 25 SC (100% RD)	6.50	6.31	6.40	6.40
T_8	*M. anisopliae* + Buprofezin 25 SC (50% RD)	6.10	6.81	6.80	6.57
T_9	*B. bassiana*+ Novaluron 10 EC (100% RD)	5.00	5.20	5.12	5.11
T_{10}	*B. bassiana* + Novaluron 10 EC (50% RD)	4.82	4.72	4.70	4.75
T_{11}	*B. bassiana*+ Chlorfluazuron 5.4 EC (100% RD)	1.70	1.62	1.50	1.61
T_{12}	*B. bassiana* + Chlorfluazuron 5.4 EC (50% RD)	1.80	1.78	1.76	1.78
T_{13}	*B. bassiana*+ Buprofezin 25 SC (100% RD)	6.41	6.50	6.39	6.43
T_{14}	*B. bassiana* + Buprofezin 25 SC (50% RD)	7.00	6.71	6.42	6.71
C.D. (p=0.01)					0.70
SEm (±)					0.18

RD: Recommended dose

4. Conclusion

Results revealed that the entomopathogenic fungus *M. anisopliae* grows more rapidly than *B. bassiana* on PDA media. The growth of both these fungi is adversely affected when PDA media is incorporated with either half or full dose of any of the three insect growth regulators tested. However, amongst the three insect growth regulators buprofezin 25 SC was found most compatible with both the fungi followed by Novaluron 10 EC while chlorfluazuron 5.4 EC was less compatible as it recorded least fungal growth of both entomopathogenic fungi. Similarly, half recommended dose of an insect growth regulator was found more compatible than its full dose. The highest number of spores were recorded in *M. anisopliae* grown on media without incorporation of IGR and was followed by *B. bassiana* grown on media without incorporation of IGR. When both the fungi were grown on the PDA media incorporated either with half or full dose of IGRs, maximum spore production by the fungi were recorded when they were grown on media incorporated with buprofezin 25 SC at its half of field recommended dose.

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6. References
1. Cuthbertson AG, Walters KF, Deppe C. Compatibility of the entomopathogenic fungus *Lecanicillium muscarium* and insecticides for eradication of sweet potato whitefly, *Bemisia tabaci*. Mycopathologia. 2005;160:35-41.
2. De Cock A, Degheele D. Buprofezin: a novel chitin synthesis inhibitor affecting specifically planthoppers, whiteflies and scale insects. Insecticides with Novel Modes of Action: Mechanisms and Application. 1998;74-91.
3. Hassan SA, Bigler F, Bogenschu tz H, Boller E, Brun J, Calis JNM, *et al*. Results of the sixth joint pesticide testing programme of the IOBC/WPRS- working group ''Pesticides and Beneficial Organisms''. Entomophaga. 1994;39:107-119.
4. Ishaaya I, Kontsedalov S, Horowitz AR. Novaluron (Rimon), a novel IGR: potency and cross-resistance. Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America. 2003;54(4):157-164.
5. Joshi M, Gaur N, Pandey R. Compatibility of entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae with selective pesticides. Journal of Entomology and Zoology Studies. 2018;6(4):867-872.
6. Reddy BN, Lakshmi VJ, Laha GS, Maheswari TU. Compatibility of entomopathogenic fungi with buprofezin for management of brown planthopper, *Nilaparvata lugens* Stal (Delphacidae: Hemiptera) in rice. Journal of Plant Development Sciences. 2020;12(1):35-38.
7. Sain SK, Monga D, Kranthi S, Hiremani NS, Nagrale DT, Kumar R, *et al*. Evaluation of the bioefficacy and insecticide compatibility of entomopathogens for management of whitefly (Hemiptera: Aleyrodidae) on upland cotton under laboratory and polyhouse conditions. Neotropical Entomology. 2022;51(4):600-612.
8. Sain SK, Monga D, Kumar R, Nagrale DT, Hiremani NS, Kranthi S. Compatibility of entomopathogenic fungi with insecticides and their efficacy for IPM of *Bemisia tabaci* in cotton. Journal of Pesticide Science. 2019;44(2):97-105.
9. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947 Jun 21;159(4051):850.