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Research Article

Identification of entomopathogenic fungi from insect pests of mango and their efficacy against mango hoppers

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ABSTRACT: Surveys were made in the Sindhudurg and Ratnagiri districts of the Konkan region of Maharashtra, India during 2017-2018 for the collection of mummified insect samples from different mango orchards in order to isolate entomopathogenic fungi. The collected mummified insect samples were brought to the laboratory and incubated on PDA plates. Those samples showing growth on PDA plates were sub-cultured until pure fungus growth was obtained. They were tested for their pathogenicity in the laboratory and 14 samples were found pathogenic to mango hoppers. They were sent to NFCCI, Agarkar Research Institute, Pune for identification. Out of the 14 entomopathogenic samples identified, 7 were from Genus *Fusarium*, 4 were mixed cultures of *Fusarium* and *Aspergillus*, and one sample each from Genus *Trichoderma*, *Mucor* and *Nigrospora*. Studies showed that Fusarium species were the most common entomopathogenic fungi associated with mango pests, especially mango hoppers. Laboratory evaluation of these samples showed that amongst different isolated entomogenus fungi, *Fusarium semitectum* sample 16(1) was more pathogenic to mango hoppers than other fungal samples. Field evaluation of this sample also proved its efficacy.

KEYWORDS: Entomopathogenic fungi, *Fusarium semitectum*, mango hoppers, *Nigrospora oryzae, Trichoderma asperellum*

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Abbreviations

PDA: Potato Dextrose Agar
CFU: Colony Forming Unit
gm.: Gram
DAI: Days After Inoculation
DAS: Days After Spraying
SE: Standard error
CD: Critical difference

INTRODUCTION

Fungi are common pathogens of insects and are major regulators of insect populations in nature. Various kinds of fungi are associated with insects, either directly such as entomopathogenic fungi, or indirectly which grow on honeydew excreted by sucking insects or cultivated by ants in their nest for nutrition. In contrast with saprophytic fungi, parasitic fungi attack living organisms, penetrate their outer defences, invade them and obtain nourishment from living cells, thereby causing diseases and sometimes death of the host (Zimmermann, 2007; Laznaik *et al.*, 2012; Corallo *et al.*, 2021). Certain fungi form highly specialized parasitic relationships with insects (Mannino *et al.*, 2019). Entomopathogenic fungi employ different strategies to invade insect hosts and evade their immune response. In recent times, about 90 genera or almost 700 fungal species are considered insect-infecting fungi that represent all the major classes of fungi (Hajek & Leger, 1994). These fungi are still insufficiently exploited and it is important to identify them and investigate their potential in biological control of insect pests.

Mango *(Mangifera indica)* is a major commercially grown fruit crop of the South Konkan region of Maharashtra (India). Various insect pests such as mango hoppers, thrips, mealybugs, hairy caterpillars, stem borers, fruit flies etc. can cause heavy damage to this crop. Among these mango hopper (*Idioscopus* spp.) is the most serious and destructive pest which was first reported in India by Distant in 1908. Twenty species of hoppers are reported on mango in the Konkan region of Maharashtra (Dalvi *et al.*, 1992) and up to 48% yield loss is reported due to this pest in the region (Godase

et al., 2004). Different chemical insecticides are extensively used for the control of this pest. However, due to the disadvantages of chemical pesticides and table-purpose use of mango fruits, farmers are now demanding effective eco-friendly biopesticides. The hot and humid conditions of this region are advantageous to make use of entomopathogenic fungi for pest control. Several species of fungi were found to be pathogenic to mango hoppers. Kumar *et al.* (1983) isolated two fungi *Verticillium lecanii* (Zimm.) Viegas and *Isaria tax* from mango hopper, *Idioscopus clypealis* (Lethierry) from India. Similarly, Srivastava and Tandon (1986) also reported two fungi *Beauveria bassiana* (Balsamo) Vuillemin and *V. lecanii* from mango hopper. Rombach and Roberts (1989) reported *Hirsutella versicolor* (Petch) on mango hopper in the Philippines while Tripathi *et al.* (1990) found *B. bassiana* infesting mango hopper in India. Verghese *et al.* (2002) reported *B. bassiana* from mango stone weevil. Visalakshy *et al.* (2010) reported epizootics of *Entomophthora* spp. on mango inflorescence hopper *Idioscopus nitidulus* (Walker) at Bengaluru. Sayed and Dunlap (2019) tested six isolates of *B. bassiana* and one isolate of *Metarhizium anisopliae* (Metchnikoff) Sorokin against *Aulacaspis tubercularis* (Newstead) and *Icerya seychellarum* (Westwood) in mango. Probit analysis of data at 95 % confidence limits of LC50 and LT50 showed significant differences in the susceptibility of nymphs of both insects to the different fungal isolates. Considering all, the present study was planned to investigate a diversity of fungal entomopathogens in mango agroecosystems and the potential of their isolates as bioagents for the control of mango hoppers.

MATERIALS AND METHODS

Collection of samples

Intensive surveys of mango orchards for the collection of samples of insects infested with fungi were undertaken in the Sindhudurg and Ratnagiri districts of Maharashtra. Different villages from these two districts were surveyed during the flowering season of mango and samples were collected at weekly intervals. While collecting samples, emphasis was given to collecting the samples from those mango orchards which were less intensively managed with chemical pesticides. Mycoses insects which were found stuck to the back side of leaves were collected. At the most 3 to 4 samples were collected from each site and each mycosed insect sample was kept in a separate glass vial and details about samples like place of collection, date of collection, host etc. were recorded. Each sample was given a separate identification number and was brought to the Biocontrol Laboratory, Regional Fruit Research Station, Vengurle for further investigation. Collected samples were further scrutinized and a total of 113 samples were kept for isolation in the laboratory at room temperature and relative humidity which ranged between 25 ± 3°C and 75 ± 3 %, respectively.

Isolation of fungi

In the laboratory, the mummified samples were first surface sterilized by immersing them in a 5% sodium hydrochloride solution for a few seconds and rinsing them thrice with sterilized water. Then the specimens were cut open in a sterilized plate and a small portion of infected haemolymph/tissue was streaked on Potato Dextrose Agar plates with the help of an inoculating needle. Out of a total of 113 samples collected; 23 samples showed growth on PDA plates. These samples were subcultured until pure colony growth of the fungus was obtained. The pure colonies of entomo-pathogenic fungi were again subcultured on PDA slants and stored in the refrigerator.

Pathogenicity test

For the evaluation of samples for the pathogenicity test, 23 samples isolated were allowed to grow on PDA plates for 10 days. Two discs of fungus (0.5 cm diameter) along with PDA were bored out with the help of a cork borer. It was then macerated in 1 l water and then it was sprayed with a hand sprayer on mango hoppers in pot culture in the laboratory. For spraying one-year-old mango saplings were selected and placed in cages. 10 nymphs of mango hopper (*Idioscopus niveosparsus*) collected from previously identified unsprayed mango trees near the laboratory were released on mango saplings. The next day they were sprayed with fungal suspension using a hand sprayer and from seven days after spraying up to the next seven days dead mango hoppers from each treatment were observed and collected daily. From the dead hopper, fungi were isolated on PDA plates. Samples showing growth identical to their mother culture on PDA were marked out for identification.

Identification of fungal isolates

The fungal isolates which passed the above-described pathogenicity test were again cultured on PDA slants and these fresh 14 samples were sent to the National Fungal Culture Collection of India, Agharkar Research Institute, Pune (Maharashtra, India) for identification. The samples were identified based on their morphological characteristics following Booth (1971).

Laboratory evaluation of different fungal samples

The growth performance of 14 identified entomo-pathogenic fungal isolates was studied under laboratory conditions. For this, all the fungal samples were grown on PDA plates. After 10 days, a 0.5 cm circular disc of fungus along with PDA was bored out with the help of a cork borer and transferred to the centre of another fresh PDA plate under

aseptic conditions. The Centre of a 0.5 cm circular fungal disc was marked on each Petri plate with a marker pen. It was then inoculated at room temperature. The radial growth of fungal samples was measured with the help of a measuring scale from the marked centre to the longest and shortest periphery of fungal growth. By calculating the average value we recorded the radial growth of each sample daily up to eight days.

To evaluate the efficacy of the isolates against mango hopper, a pot experiment with three replications and 15 treatments under laboratory conditions was conducted. 10 mango hopper nymphs were released on mango saplings placed in cages. Fungal suspensions of 14 isolates along with water in the control treatment were sprayed on mango hoppers with a hand sprayer. Suspensions were prepared again by macerating two discs of fungus (one cm diameter) in 1 l of water, from 10 days old isolates. This 1 l suspension was used to spray on 3 saplings as 3 replications. Mortalities in each treatment and each replication were recorded at 10 days after spraying and accordingly per cent mortality was calculated.

Field evaluation of isolates against mango hoppers

Because of the limitation of mango trees for the conduction of experiments, only those samples which were found promising in laboratory evaluation were selected for evaluation under field conditions. For this, a separate field experiment was conducted at Mango Research Farm, Regional Fruit Research Station, Vengurle district Sindhudurg in randomised block design with seven treatments and three replications. Selected fungal isolates were multiplied in potato dextrose broth, incubated in a shaker for 10 days and then mixed with an equal volume of talc powder. CFU (Colony Forming Unit) count of formulations was adjusted to standard commercial formulations, *i.e.*, 2 x 10^9 per gram and then used for field trial. Each formulation was sprayed with 5 gm/l water on the mango plant at the flowering stage with the help of a foot sprayer. Hopper population before spraying and at 3, 7 and 10 days after spraying was recorded. For counting the hopper population two plants per treatment were selected and from each plant, five inflorescences were selected along the periphery of each plant at equal distances. The inflorescence of the mango plant was held between the thumb and index finger at the base. The tip of the inflorescence was gently taped with the other hand. Due to tapping the nymphs of the hopper moved backward and congregated at the base of inflorescence where they were counted with the help of a magnifying lens.

RESULTS

Pathogenicity test

All of the 23 samples isolated from different insect pests of mango were sprayed on mango hoppers to test their pathogenicity. 14 of them passed the pathogenicity test and were sent for identification to the Agharkar Research Institute, Pune. The details of these samples are described in Table 1.

Out of 14 entomopathogenic isolates identified, 4 isolates were *Fusarium semitectum* Berk. and Ravenel, three isolates were *F. monilifor*me J. Sheld, four isolates were mixed cultures *F. moniliforme* and *Aspergillus* spp. and one isolate each *Trichoderma asperellum* Samuels, Lieckf. and Nirenberg, *M. hiemalis* Wehmer and *N. oryzae* (Berk. and Broome) Petch. From the table, it is obvious that *Fusarium* species are the most common fungal entomopathogen in this research.

Laboratory evaluation of different fungal samples

The growth performance of 14 entomopathogenic isolates on PDA plates under laboratory conditions is presented in Table 2. From the table, it is revealed that isolate 6(4) (*T. asperellum*) is very vigorous in growth and completely spread on the PDA plate in just 5 Days After Inoculation (DAI) followed by isolate 18(4) (*F. semitectum*). Other isolates took 8 to 9 days to spread completely in the plate.

Evaluation of different fungal samples against mango hoppers (Pot Experiment)

To search out the most effective entomopathogen for control of the mango hopper, the efficacy of 14 isolates that passed the pathogenicity test was evaluated against the mango hopper in the laboratory by conducting a pot experiment. Per cent, mortality of mango hoppers in different treatments in pot experiment was evaluated and presented below (Figure 1). From the graph, it is seen that all the isolates tested recorded significantly higher mortality over control except isolate S2 [*T. asperellum* 6(4)], S3 [*M. hiemalis* 12(2)] and S9 [*N. Oryzae* 21(3)] which were at par with control. The highest per cent mortality of mango hoppers (86.77%) was observed in isolate S5 [*F. semitectum* 16(1)] and was followed by isolate S6 [*F. semitectum* 18(3)] and S10 [*F. semitectum* 22(2)]. Latter isolates were also found at par with isolate S7 [*F. semitectum* 18(4)]. The remaining isolates recorded less than 50% mortality. Thus, from the result it is obvious that all the *F. semitectum* isolates were effective against mango hoppers and isolate 16(1) of this fungus was more virulent than others.

Evaluation of different fungal cultures against mango hoppers (Field Experiment)

Entomopathogens that showed more virulence in laboratory evaluation were selected for further testing under field conditions. Three different isolates of *F. semitectum* and one isolate of *T. asperellum* were tested along with standard commercial formulations of *M. anisopliae* and *Lecanicellium*

Table 1. Details of 14 entomopathogenic fungal samples

S. No.	Sample no.	Date of collection	Location/Provider	Crop	Host	Fungal species identified	Growth performance on PDA	
							Vigour	Colour
1	1(2)	5/12/17	Plot no. 4 Mango div. RFRS. Vengurle	Mango	Hopper	*F. moniliforme* J. Sheld	+	White
2	6 (4)	19/12/17	Mr. Jagdish Vaidya (Aadeli)	Mango	Hopper	*Trichoderma* sp. aff. *T. asperellum* Samuels, Lieckf. and Nirenberg	+++	Yellowish white with green spores
3	12 (2)	18/01/18	Mr. Sumit Bhosle Talawde	Mango	Hopper	*Mucor* sp. aff. *M. hiemalis* Wehmer	+	White
4	13 (1)	18/01/18	Mr. Dilip Aaklekar	Mango	Dipteran fly	*F. moniliforme* J. Sheld	+	White
5	16 (1)		Dapoli Horticulture	Mango	Hopper	*Fusarium* sp. aff. *F. semitectum* Berk. and Ravenel	+	White
6	18(1)	05/02/18	Mr. D. R. Bhagat	Mango	Pupa of mango shoot borer	*F. semitectum* Berk. and Ravenel	+	Brownish
7	18(4)	05/02/18	Mr. D. R. Bhagat	Mango	Hopper	*F. semitectum* Berk. and Ravenel	++	Brownish
8	20	05/02/18	Mr. Prabhudesai (Padave)	Mango	Hopper	*F. moniliforme* + *Aspergillus* sp.	+	white
9	21 (3)	08/02/18	Mr. Janardhan Teli (Vaghotan)	Mango	Dipteran fly	*N. oryzae* (Berk. and Broome) Petch	+	Black
10	22(2)	08/02/18	Mr. Ajit Shirodkar (Padel)	Mango	Hopper	*Fusarium* sp. aff. *F. semitectum* Berk. and Ravenel	+	White
11	22(3)	08/02/18	Mr. Ajit Shirodkar (Padel)	Mango	Hopper	*F. moniliforme* + *Aspergillus* sp.	+	White
12	23 (1)	08/02/18	Sub Regional Mango Research Station, Girye	Mango	Hopper	*F. moniliforme* + *Aspergillus* sp.	+	White
13	23 (2)	08/02/18	Sub Regional Mango Research Station, Girye Rameshwar	Mango	Dipteran fly	*F. moniliforme* + *Aspergillus* sp	+	White
14	24 (2)	22/02/18	Mr. Ramcharndra Jambhekar (Pombhurle, Devgad)	Mango	Hopper	*F. moniliforme* J. Sheld	+	Brownish

lecanii while the control was untreated. The results of the field evaluation of isolates are presented in Table 3.

Data from Table 3 shows that the hopper population before treatment varies from 23.33 to 29 per 10 inflorescences in different treatments and statistically does not differ from each other. Hopper population recorded at 3 days after application of treatments (3DAS) also does not differ statistically from each other. However, at 7 days and 10 days after application of treatments, all three *F. semitectum* isolates recorded significantly lower hopper populations than control and were equally effective to commercial formulations of *M. anisopliae* and *L. lecanii*. Amongst all, fungal isolate *F. semitectum* 16(1) recorded the lowest hopper population, *i.e.*, 19.33 and 25.33 per ten inflorescences at 7 DAS and 10 DAS, respectively and emerged as the most effective treatment in controlling the hopper population. Thus based on pot as well as field experiments, it can be concluded that *F. semitectum* is a potential entomopathogen which can be exploited for the control of mango hoppers in the mango agro ecosystem in the Konkan region of Maharashtra.

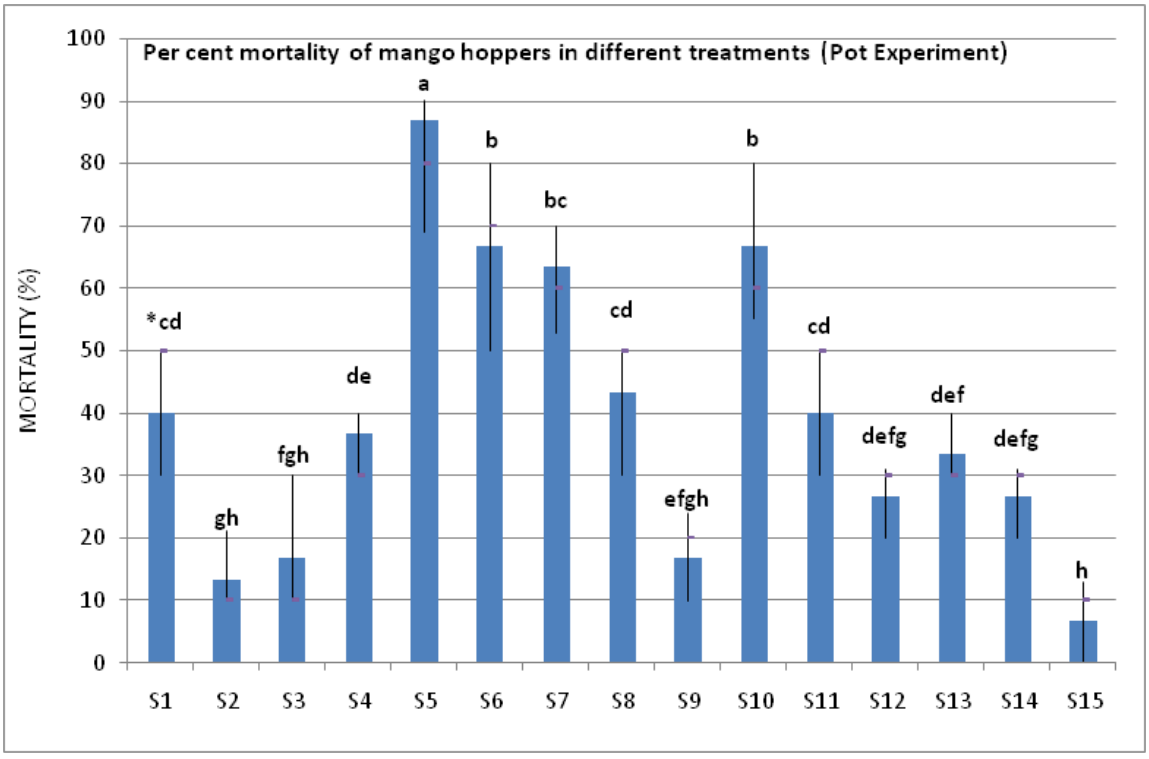
DISCUSSION

Pathogenicity test

Teetor-Barsch & Robert (1983) reviewed the entomogenous Fusarium species and reported that a large number of *Fusarium* species show either strong or weak pathogenic reactions in various insect groups. Field observations of natural mortalities as well as pathogenicity

Table 2. Radial growth of different fungal isolates on PDA plate under laboratory conditions

S. No.	Fungal isolates	Radial growth (in cm) of fungus						
		2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI
1	*F. moniliforme* 1(2)	2.00	2.98	3.85	4.65	5.6	6.45	7.35
2	*T. asperellum* 6(4)	2.73	4.78	6.23	8.00	-	-	-
3	*M. hiemalis* 12(2)	1.89	2.38	2.98	3.65	4.55	5.4	6.35
4	*F. moniliforme* + mixed 13(1)	2.10	3.15	3.95	4.90	5.35	6.68	7.50
5	*F. semitectum* 16(1)	1.96	3.08	4.13	4.83	5.70	6.50	7.45
6	*F. semitectum* 18(1)	1.76	2.40	3.33	4.45	5.50	6.70	7.15
7	*F. semitectum* 18(4)	2.78	3.90	5.75	7.35	7.85	8.00	-
8	*F. moniliforme* + *Aspergillus* sp. 20	2.38	3.30	4.45	5.25	6.25	7.35	8.0
9	*N. Oryzae* 21(3)	1.00	1.78	3.53	4.35	5.25	6.25	7.15
10	*F. semitectum* 22(2)	1.43	2.60	3.80	4.68	5.95	7.05	8.0
11	*F. moniliforme* + *Aspergillus sp.* 22(3)	2.45	3.73	4.63	5.63	7.00	7.73	8.0
12	*F. moniliforme* + *Aspergillus* sp. 23(1)	2.05	2.75	3.73	4.90	5.85	6.30	6.83
13	*F. moniliforme* + *Aspergillus* sp. 23(2)	1.58	2.40	3.40	4.15	5.15	6.35	7.35
14	*F. moniliforme* 24(2)	2.18	3.20	4.15	5.20	6.08	6.53	6.98



* Note: treatments followed by different letters differ significantly ($p < 0.05$)

S1- *F. moniliforme* 1(2)	**S2-** *T. asperellum* 6(4)	**S3-** *M. hiemalis* 12(2)
S4- *F. moniliforme* + Mixed 13(1)	**S5-** *F. semitectum* 16(1)	**S6-** *F. semitectum* 18(1)
S7- *F. semitectum* 18(4)	**S8-** *F. moniliforme* +*Aspergillus sp.* 20	**S9-** *N. Oryzae* 21(3)
S10- *F. semitectum* 22(2)	**S11-** *F. moniliforme* + *Aspergillus* sp. 22(3)	**S12-** *F. moniliforme* + *Aspergillus* sp. 23(1)
S13- *F.moniliforme* +*Aspergillus* sp. 23(2)	**S14-** *F. moniliforme* 24(2)	**S15-** Control (Water spray)

Figure 1. Per cent mortality of mango hopper 10 days after treatment with different entomopathogenic fungi in a pot experiment.

Table 3. The population of mango hoppers in different treatments in field trials (2018-19)

S. No.	Fungi /isolates	Number of mango hoppers/10 inflorescence			
		Before Spray	**3 DAS**	**7 DAS**	**10 DAS**
T1	*T. asperellum* 6(4)	27.33 *(5.22)	26.33 (5.13)	26.67 (5.16)	32.33 (5.68)
T2	*F. semitectum* isolate 16(1)	29.00 (5.38)	24.00 (4.89)	19.33 (4.39)	25.33 (5.01)
T3	*F. semitectum* isolate 18(1)	25.00 (4.96)	25.67 (5.04)	25.00 (4.99)	30.0 (5.47)
T4	*F. semitectum* isolate 22(2)	23.33 (4.79)	22.67 (4.71)	21.00 (4.52)	28.67 (5.35)
T5	*M. anisopliae*	23.67 (4.84)	23.67 (4.86)	22.33 (4.72)	30.00 (5.44)
T6	*L. lecanii*	24.00 (4.87)	22.67 (4.73)	21.33 (4.57)	30.33 (5.51)
T7	Control	23.67 (4.84)	28.00 (5.24)	38.67 (6.19)	46.00 (6.75)
	F test	**Non Sig.**	**Non Sig.**	**Sig.**	**Sig.**
	SE	**-**	**-**	**0.35**	**0.31**
	CD at 1%	**-**	**-**	**1.49**	**1.33**

*figures in the parenthesis are square root transformed values.

tests indicated strong pathogenic reactions in Homopterans and Dipteran insects while weak reactions in Lepidopteran and Coleopteran insects. Gupta *et al.* (1991) isolated beauvericin as an insect toxin from *F. semitectum* and *F. moniliforme* var. subglutinans. Torres *et al.* (2002) isolated *F. moniliforme* and *Aspergillus* spp. from different insect pests from the tropical and agricultural area of El Eden Ecological Reserve, Quintana Roo, Mexico. Guesmi-Jouini *et al.* (2010) reported *F. semitectum* to be pathogenic to the artichoke aphid *Capitophorus elaeagni* (Del Guercio) (Hemiptera: Aphididae). Santos *et al.* (2020) also reviewed the potential of *Fusarium* species for the biological control of insects and reported that the genus includes many strong entomopathogenic agents like *F. incarnatum-equiseti, F. fujikuroi, F. solani* and *F. oxysporum*.

Podder and Ghosh (2019) proved the larvicidal activity of spores of *T. asperellum* and its crude and methanolic extracts against mosquito larvae. Balogun and Fagade (2004) investigated dead cadavers of grasshoppers *Zonocerus variegates* (L.) and reported their association with eight fungal species. Amongst the eight species of fungi, *Mucor* reported a 13 % incidence. In the case of *N. oryzae* no direct evidence of entomopathogenicity has been reported in the literature reviewed. However, Thakur *et al.* (2014) reported the adverse effect of endophytic *N. oryzae* on the survival and fitness of *Spodoptera litura* (F.) larvae in cabbage.

Evaluation of fungal cultures against mango hoppers (pot and field experiment)

The pathogenicity of *F. semitectum* to mango hopper observed during the present investigation found strong support in the work of Parker *et al.* (1996) who tested the suspension of fungus *F. semitectum* and found it pathogenic to thrips *Scritothrips dorsalis* Hood. High mortality observed in different isolates of *F. semitectum* in pot experiment is supported by the work of Jayasimha *et al.* (2014) and Galil *et al.* (2019) who reported more than 70 per cent mortality due to *F. semitectum* against okra leaf hopper and bean and wheat aphids under greenhouse conditions. Mikunthan and Manjunatha (2006) sprayed *F. semitectum* at 1 x 10^9 spores/ml on chilly and observed mycosis and mortality in sucking pests such as chilli thrips *S. dorsalis*, broad mite *Polyphagotarsonemus latus* (Banks), sugarcane wooly aphids *Ceratavacuma laningera* Zehntner, spiraling whitefly *Aleyrodicus disperses* Russell, whitefly *Bemisia tabaci* Gennadius, and *Aleyrodes gossypii* Fitch and coconut mite *Aceria guerreronis* Keifer. They further reported that it did not cause mortality in lepidopteran larvae and ladybird beetle *Menochilus sexmaculatus* Fabricus, predatory mite *Amblyseius ovalis* (Evans) and larval parasitoid *Goniozus nephantidis* (Muesebeck). It failed to infect larvae of *Bombyx mori* L., and *Apis indica* F. and did not influence the composting ability and growth of earthworm *Eisenia fetida* (Savigny). Similarly, the results of the field trial can be compared with those of Aiswariya *et al.* (2007) which reported 75.21% and 64.40% mortality due to this fungus at 4.2 x 10^9 spores/ml in nymphs and adults of spiralling whitefly under laboratory conditions. However, in field conditions, it caused 18.06 %, 21.84 % and 19.67 % mortality in eggs, nymphs and adults of spiralling whitefly at 6.2 x 10^{15} spores/ml, respectively.

CONCLUSION

In mango agroecosystems of the Konkan region of Maharashtra, various species of *Fusarium* were found to be pathogenic to the mango hopper**.** *Fusarium semitectum* isolate 16(1) was found to be more pathogenic to mango hopper and proved its efficacy in laboratory as well as field studies.

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