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Lethal and sub lethal effects of indigenous isolates of *Metarhizium anisopliae* (Metchnikoff) Sorokin against *Spodoptera litura* (Fabricius)

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Abstract
Lethal and Sub Lethal Effects of indigenous isolates of *Metarhizium anisopliae* (Metchnikoff) Sorokin against *Spodoptera litura* (Fabricius) was evaluated by leaf dip method during the period of August 2015 to February 2016. A total of three isolates (SSB, SBvB and SBvD) of *M. anisopliae* from 30 samples were obtained based on their pathogenicity test against *S. litura*. Among the three isolates, SSB (76.67 %) and SBvB (63.33 %) showed highest mortality whereas the least mortality was found in the SBvD (50.00 %) isolate at six days after treatment at the concentration of 3x 10 ^{8} cfu/1000 ml. Per cent pupation, adult emergence and fecundity (23.33, 6.67, 0.00 respectively) were very poor in the SSB depicting the high virulence whereas the highest was found in the SBvB (50.00, 23.33, 23.00, respectively) isolate. The order of mortality and sub lethal effect of these isolates were SSB>SSvB>SSvD. This paper adds the some more isolates of entomopathogenic fungi for the management of soil inhabiting insect pests. The isolate SSB performed better under laboratory conditions and need to be evaluated under field conditions for their promotion against soil dwelling and defoliating insect pests.

Keywords: *Metarhizium anisopliae, Spodoptera litura*, local isolates

1. Introduction
Historically the use of synthetic chemicals is still one of the major plant protection tools for insect pest management in horticultural crops [5]. The discovery of insecticidal property of DDT during 1939 and series of other group of insecticides has led to era of pesticides which made the farmers to depend on them since the speed of kill of insect pest was rapid. More than 60 per cent of pesticides are used in agriculture sector for crop protection among which, insecticides share larger percentage compared to fungicides, bactericides and herbicides [19]. Due to indiscriminate use of chemical pesticides affected both biotic and abiotic ecosystem and increased cost of chemical pesticides forced the researchers to search an alternative. Therefore, in 1975 the concept of Integrated Pest Management (IPM) came into existence. Among the various IPM practices, uses of microbial pesticides are considered to be a promising alternative to chemical pesticides [18]. Recently the use of entomopathogens *viz.*, bacteria, nematodes, virus and fungi for insect pest management in cultivated crops is popularizing. Among these, Entomopathogenic Fungi (EPF) is gaining more importance and has been extensively studied as key regulatory factors in insect populations and as agents for bio control [9, 13]. These are found to be more advantageous because of its host specificity and non- interference with non-target ecosystems. They exhibit a high genetic variability, thus being able to inhibit pest resistance to the pathogens and also they can kill the host just by coming in contact with host (cuticle) whereas bacteria, nematodes and virus require natural openings like mouth, anus and spiracles or by ingestion of food [1].
About 90 genera and more than 700 species of fungi, dispersed in various taxonomic groups, have been identified as insect pathogens [9]. Successful programmes of microbial control using EPF to combat arthropod pests in soils and aquatic environments have been developed, principally utilizing the genera *Metarhizium*, *Beauveria*, *Sporothrix*, *Lecanicillium*, *Nomuraea*, *Hirsutella*, *Aschersonia*, *Isaria*, *Paecilomyces*, and *Enthomophthora* [1]. Among the genera, *Metarhizium anisopliae* (Metchnikoff) Sorokin commonly known as green muscardine fungus is gaining more importance because of its wider host range infecting almost all soil inhabiting insects like root grubs, termites, scarabids etc [4, 10]. Since the entomopathogenic fungi exhibit

high level of variation among the isolates in relation to their pathogenicity, optimal temperature and viability at different climatic conditions, the regional specific or local isolates are need to be identified [21]. This paper reports the use of indigenous isolates of *M. anisopliae* for their lethal and sub lethal effects against *Spodoptera litura* under laboratory conditions.

2. Materials and Methods

2.1 Collection and isolation of *M. Anisopliae*

A roving survey was conducted at different villages of Belagavi districts of Karnataka state during the period of August 2015 to February 2016 covering major area of vegetable crops. During the survey, soil samples from vegetable fields and naturally infested insect specimens were collected and brought to the laboratory of Entomology KRC College of Horticulture Arabhavi and isolated the *M. anisopliae*. A total of 30 places included six taluks of Belagavi district and in each taluk five villages and one field for each village were selected for soil sampling and collection of insect samples.

2.2 Isolation of *M. anisopliae* through serial dilution

About 0.2 g of soil sample was placed in a 1.5 ml micro centrifuge tube with 1.3 ml of 0.02% Tween-80 solution and vortexes for 15 minutes. The resulting suspension was serially diluted (10^{-9}) and plated on selective medium. After incubation for 6 days at 25ºC, the putative entomopathogenic fungi were selected by morphological characteristics (aspects of the colonies, such as color, diameter and mycelia texture) [16].

2.3 Isolation of *M. anisopliae* from surface sterilized insect samples

The symptomatic insect specimens for mycoses were brought to the laboratory from various vegetable growing fields. Upon death or mycosis the specimens were surface sterilized using one per cent sodium hypochlorite, followed by 70 per cent alcohol and three repeated changes of sterile distilled water. They were then inoculated on selective media and kept under incubation for one week under room temperature. On development of fungal mycelia they were transferred to fresh media for further growth. The pure cultures thus developed were stored under refrigeration for further study [14].

2.4 Rearing of *Spodoptera litura*

Wild population of the test insect, *S. litura* was collected from vegetable fields of KRCCH, Arabhavi and light sources of nearby hostels. The culture of the test insect was maintained on castor, *Ricinus communis* leaves under laboratory conditions in plastic tubs. The larvae of second instars were taken from the culture, as and when required. All the experiments were conducted in the Bio control laboratory, Department of Entomology, KRC College of Horticulture Arabhavi.

2.5 Bioassay study

To determine the bio-efficacy and sub lethal effects for isolated *M. anisopliae*, a bioassay test was conducted and a preliminary experiment was run in order to decide the final concentrations for the bioassay.

Two ml of spore suspension with different formulations (3 x 10^3, 3 x 10^4, 3 x 10^5, 3 x 10^6, 3 x 10^7 and 3 x 10^8 cfu/1000ml) were prepared. The efficacy of *M. anisopliae* against *S. litura* was determined by using the leaf dip method. Leaf discs of 9cm diameter were dipped in spore suspension for two minutes and air dried. Ten 2rd instar larvae of *S. litura* were released in a petri dish (10cm diameter) and allowed to feed on the treated leaf disc. Larvae were maintained at room temperature 27.0 ± 1.0 ºC and the relative humidity of 70.0 ± 5.0% RH. Another group, 10 larvae were allowed to feed the leaf disc treated with distilled water and reared under the above mentioned conditions, served as control [3]. The isolates which showed pathogenicity (mortality) were further investigated for their effect on growth and developmental parameters of *S. litura*. A chronic feeding experiment was conducted wherein freshly treated castor leaves were fed for two days to the second instar larvae of *S. litura* followed by untreated leaves until the initiation of pupation. The observations were recorded as pupation (%), adult emergence (%) and fecundity (%).

2.6 Statistical analysis

The data of the experiment on mortality and sub lethal effects were analyzed by ANOVA, Completely Randomized Design (CRD) and means were separated by DMRT (p=0.01).

3. Results and Discussion

Thirty soil samples and 30 insect specimens were collected from six taluks (five villages in each taluk) of Belagavi district. A total of 18 isolates (60%) from 30 soil samples and six isolates (20%) from 30 insect samples were identified as entomopathogenic fungi based on their morphological and colony characteristics. Further, to confirm the entomopathogenic fungi, *M. anisopliae* a pathogenicity test against *S. litura* was undertaken in the laboratory conditions. Out of 24 isolates screened for their pathogenicity test based on morphology and colony structure, only three isolates from soil samples coded as SSB, SBvB and SBvD yielded positive results causing death of the test insect. However, none of the isolates obtained from the insect samples yielded positive results of pathogenicity. The study on distribution of *M. anisopliae* was conducted in Tamil Nadu by Sahayaraja and Borgio [15] with a collection of 40 soil samples from agricultural and horticultural fields. Among the samples collected, the presence of *M. anisopliae* was noticed in 50 per cent of the samples**.** The Diversity of EPF in Spain was observed by Moraga *et al.* [11]. Out of 244 soil samples, 175 soil samples possessed the EPF. Of the 175 soil samples, 104 yielded *B. bassiana* (42.6 %), 18 yielded *M. anisopliae* (7.3 %), and 53 samples (21.7 %) harbored both fungi. Similarly, investigation for the distribution of soil borne EPF in crop fields was conducted in Iran. Among 150 soil samples collected, EPF occurred at 78 per cent of soil samples from which 40 per cent of *B. bassiana*, 21 per cent *M. anisopliae* and 17 per cent had both species. They found that occurrence and distribution of EPF was not significantly affected by pH and texture of soil [6].

In the present study all the isolates were found to cause mortality on third day of treatment. However, the significantly highest mortality of *S. litura* was recorded by the isolate SSB followed by SBvB and SBvD respectively. Since the development of mycelial growth on dead cadaver was very poor in all the three isolates, they were placed on agar medium to observe for the growth of *M. anisopliae* (Table 1). All the three isolates (SSB, SBvB and SBvD) obtained from the pathogenicity test were screened for their virulence against *S. litura* under laboratory conditions. These isolates which showed the varied per cent mortality were further investigated for their effect on growth and development of *S.*

litura under laboratory conditions.

3.1 Mortality response of *S. litura*

The mortality of *S. litura* significantly increased at three and six days after treatment in all the three isolates of *M. anisopliae* (SSB, SBvB and SBvD). The per cent mortality was found to be highest at 3 X 10^8 cfu/1000 ml compared to other concentrations. While the lowest per cent mortality of *S. litura* was found at 3 X 10^3 cfu/1000 ml at three and six days after treatment. No significant difference was observed with respect to mortality between the concentrations of 3 X 10^5 and 3 X 10^6 cfu/1000 ml at different intervals of time in all the local isolates of *M. anisopliae*. Similarly, no appreciable mortality of *S. litura* was noticed in the lower concentration (3 X 10^3 cfu/1000 ml) of *M. anisopliae* and it was *on par* with the control treatment at three and six days after treatment in all the isolates of *M. anisopliae*. Mortality of *S. litura* in control treatment was noticed after six days after treatment (DAT). However, it was significantly lowest compared to all other concentrations of *M. anisopliae* (Table 2; Fig. 1).

Ansari *et al.* [2] reported the *M. anisopliae* isolate; CLO 53 and CLO 54 caused the highest mortality of 90 per cent after 10 weeks post inoculation when compared to other isolates collected by them. The ICIPE 78 and MA/GPK isolates of *M. anisopliae* were most virulent and showed highest (77.9% - 82.6%, respectively) mortality compared to others [20]. Virulence of *M. anisopliae* isolate (FT83) against the second instar larva of beet army worm was temperature dependent and increased from 20-30° C but decreased at 35° C [8]. The mortality of second instar nymph of brinjal white fly was more compared to fourth instar nymphs by the isolate (GJ4) of *M. anisopliae* [12].

3.2 Effect of *M. anisopliae* on growth and development of *S. litura*

The isolate, SSB was the most virulent strain of *M. anisopliae* recording lowest per cent pupation and adult emergence (23.33 and 6.67 respectively) with no fecundity at the concentration of 3×10^8 cfu per 1000 ml. The isolate SBvD recorded highest per cent pupation, adult emergence and fecundity (50.00, 23.33 and 23.00 respectively) at the concentration of 3×10^8 cfu per 1000 ml indicating its least virulency. The order of sub lethal effect of these isolates were SSB>SBvB>SBvD (Table 3).

A graph with per cent pupation, adult emergence and fecundity was plotted against concentration and mortality. Negative correlation was observed in both concentration and mortality. Hence both were inversely proportional to per cent pupation, adult emergence and fecundity (Fig 2 and 3). Gutierrez *et al.* [7] reported that Ma2, Ma8 and Ma16 isolates of *M. anisopliae* were most virulent at the concentration of 10^8 CFU/ml and showed the reduced adult emergence of Mexican fruit fly in the field-cage condition. Similarly, Thangavel *et al.* [17] reported that *M. anisopliae* recorded more ovicidal effect (37.3%) at $2x10^9$conidia/ml against exotic spiraling whitefly.

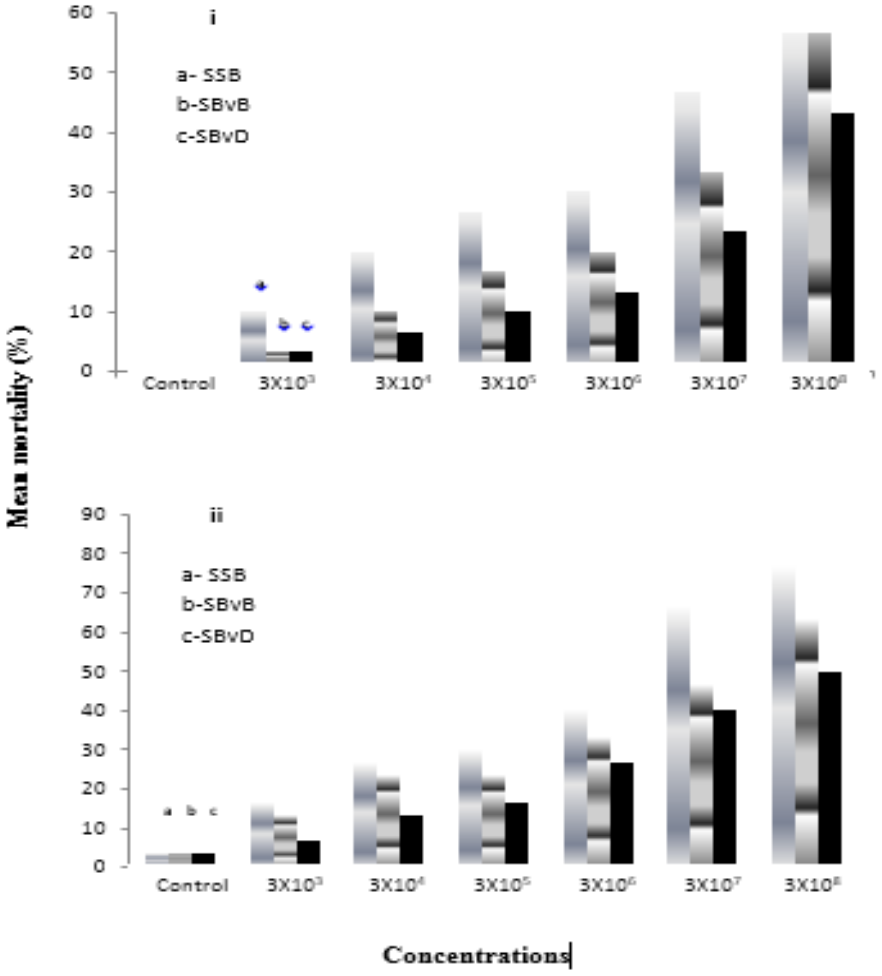

Fig. 1: Concentration - mortality response of *Spodoptera litura* to SSB, SBvB and SBvD at (i) three and (ii) six days after treatment.

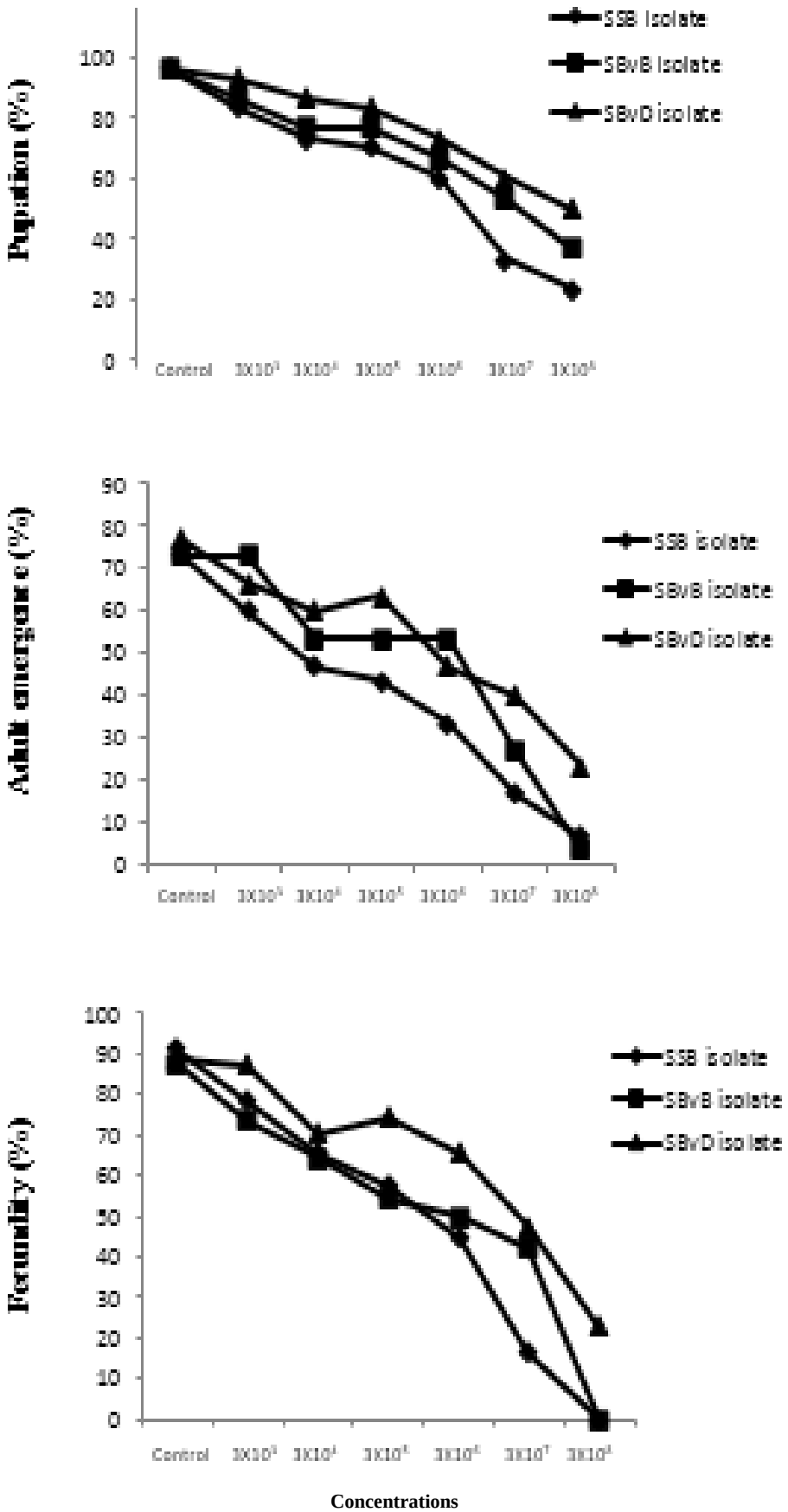

Fig. 2: Per cent pupation, adult emergence and fecundity- concentration response of *Spodoptera litura* to SSB, SBvB and SBvD isolates.

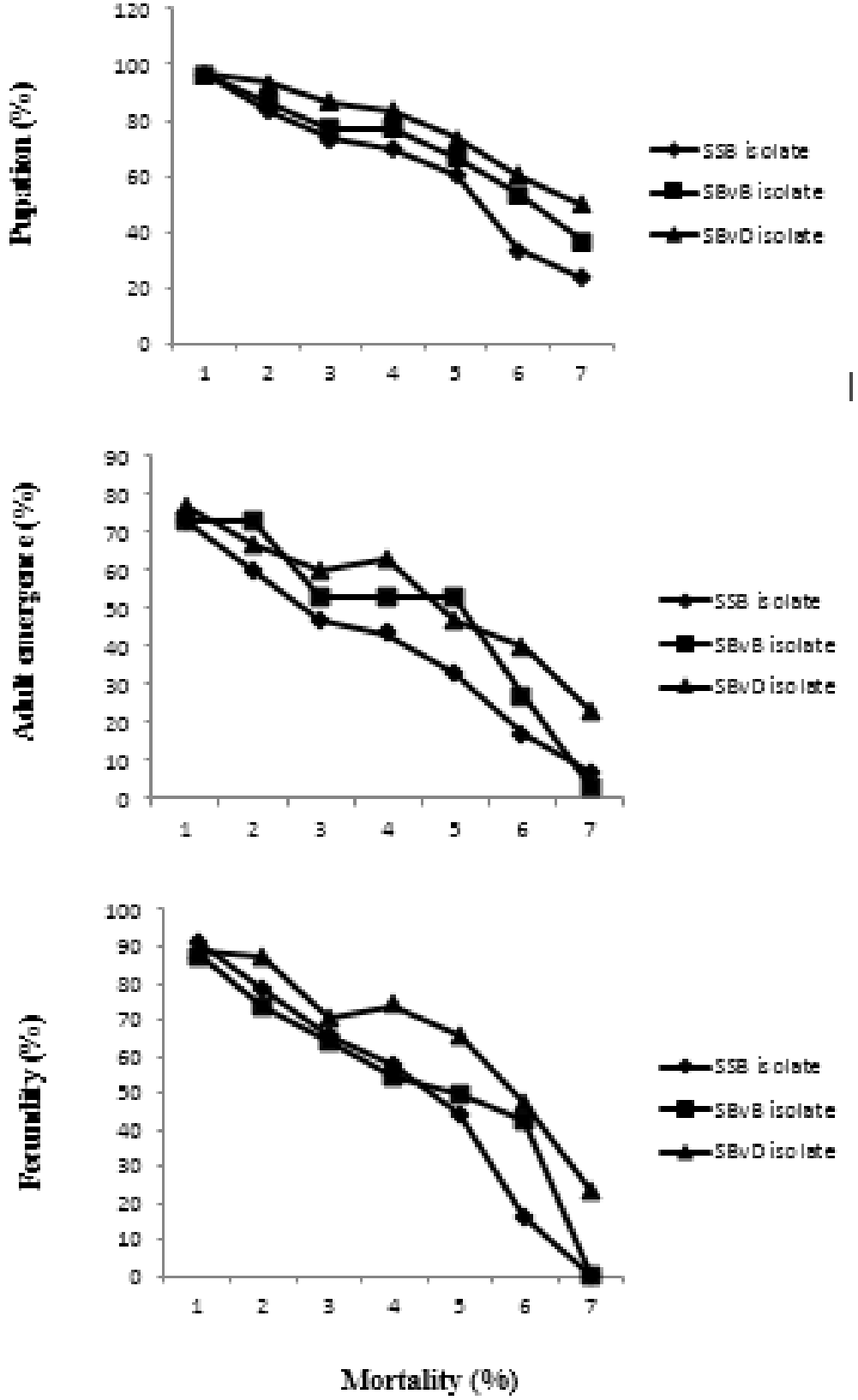

Fig 3: Per cent pupation, adult emergence and fecundity- Mortality response of *Spodoptera Litura* to SSB, SBvB and SBvD isolates.

Table 1: Pathogenicity test of local isolates of *Metarhizium anisopliae* against *Spodoptera litura* under laboratory condition.

SL. NO.	Isolates	Infectivity	Time taken to kill test insect
1.	SGD	-Ve	-
2.	SGP	-Ve	-
3.	SGA	-Ve	-
4.	SGG	-Ve	-
5.	SCN	-Ve	-
6.	SCH	-Ve	-
7.	SCB	-Ve	-
8.	SHAm	-Ve	-
9.	SHB	-Ve	-
10	SHAb	-Ve	-
11.	SBvD	+Ve	2 days
12.	SBvH	-Ve	-
13.	SBvB	+Ve	3 days
14.	SSM	-Ve	-
15.	SSB	+Ve	3 days
16.	SBhSp	-Ve	-
17.	SBhSM	-Ve	-
18.	SBhB	-Ve	-
19.	GKBSFB	-Ve	-
20.	GGCL	-Ve	-
21.	CEBFB	-Ve	-
22.	HHDBM	-Ve	-
23.	BKBAW	-Ve	-
24.	SYTLM	-Ve	-

Table 2: Bio-efficacy of local isolates of *Metarhizium anisopliae* against *Spodoptera litura*.

Treatments	Concentrations (Spores/1000 ml)	Mortality (%)					
		3 DAT			6 DAT		
		SSB	SBvB	SBvD	SSB	SBvB	SBvD
T_1	3×10^8	56.67 (48.93)a	56.67 (48.85)a	43.33 (41.07)a	76.67 (61.22)a	63.33 (52.78)a	50.00 (45.00)a
T_2	3×10^7	46.67 (43.08)ab	33.33 (35.22)b	23.33 (28.78)ab	66.67 (54.78)a	46.67 (43.08)ab	40.00 (39.23)ab
T_3	3×10^6	30.00 (33.00)bc	20.00 (26.67)bc	13.33 (21.14)bc	40.00 (39.15)b	33.33 (35.01)bc	26.67 (31.00)bc
T_4	3×10^5	26.67 (31.00)bc	16.67 (23.86)c	10.00 (15.00)bcd	30.00 (33.21)b	23.33 (28.78)cd	16.67 (23.86)c
T_5	3×10^4	20.00 (26.07)cd	10.00 (18.43)c	6.67 (12.29)cd	26.67 (31.00)bc	23.33 (28.78)cd	13.33 (21.14)cd
T_6	3×10^3	10.00 (15.00)d	3.33 (6.14)d	3.33 (6.14)cd	16.67 (23.86)c	13.33 (21.14)d	6.67 (12.29)de
T_7	Control (Distilled water)	0.00 (0.00)e	0.00 (0.00)d	0.00 (0.00)d	3.33 (6.14)d	3.33 (6.14)e	3.33 (6.14)e
$F_{6,12}$		*	*	*	*	*	*
S. E m±		4.22	3.06	4.78	2.76	3.80	3.53
CD		13.00	9.43	14.71	8.52	11.70	10.90
CV		14.84	13.37	26.60	7.70	12.23	13.37

Figures in parenthesis are angular transformed values/ Arc sin transformed value
* F-test is significant at 5 per cent probability, ** F-test significant at 1 per cent probability
The values in the column following same alphabet letters are not significantly different from each other
DAT - Days after treatment

Table 3: Effect of local isolates of *Metarhizium anisopliae* on growth and development of *Spodoptera litura*

Treatments	Concentrations (Spores/1000 ml)	Pupation (%)			Adult emergence (%)			Fecundity (%)		
		SSB	SBvB	SBvD	SSB	SBvB	SBvD	SSB	SBvB	SBvD
T1	3×10^8	23.33 (28.78)d	36.67 (37.22)d	50.00 (45.00)e	6.67 (12.29)e	3.33 (6.14)c	23.33 (28.78)e	0.00 (0.00)e	0.00 (0.00)e	23.00 (23.98)c
T2	3×10^7	33.33 (35.22)d	53.33 (46.92)cd	60.00 (50.77)de	16.67 (23.86)d	26.67 (31.00)b	40.00 (39.15)d	16.33 (4.94)e	42.33 (40.56)d	47.33 (43.47)bc
T3	3×10^6	60.00 (50.85)c	66.67 (54.99)bc	73.33 (59.00)cd	33.33 (35.22)c	53.33 (47.01)a	46.67 (43.08)cd	44.33 (43.90)d	49.67 (44.85)cd	65.67 (54.24)ab
T4	3×10^5	70.00 (56.79)bc	76.67 (61.72)abc	83.33 (66.14)bc	43.33 (41.15)bc	53.33 (46.92)a	63.33 (52.78)b	56.67 (49.93)cd	54.33 (47.49)cd	74.00 (59.59)ab
T5	3×10^4	73.33 (59.00)bc	76.67 (61.22)abc	86.67 (68.86)bc	46.67 (43.08)bc	53.33 (46.92)a	60.00 (50.77)bc	65.67 (54.68)c	64.33 (53.37)bc	70.33 (57.37)ab
T6	3×10^3	83.33 (66.14)b	86.67 (66.14)ab	93.33 (77.71)ab	60.00 (50.77)ab	73.33 (59.21)a	66.67 (54.78)ab	78.33 (64.13)b	73.33 (59.04)b	87.33 (71.27)a
T7	Control (Distilled water)	96.67 (83.86)a	96.67 (75.00)a	96.67 (83.86)a	73.33 (59.00)a	73.33 (59.00)a	76.67 (61.22)a	91.00 (73.27)a	87.67 (69.83)a	88.67 (70.70)a
	$F_{6,12}$	*	*	*	*	*	*	*	*	*
	S. Em±	2.76	4.45	3.53	3.13	3.99	2.32	2.32	2.57	6.06
	CD	8.52	13.74	10.89	9.66	12.30	7.14	7.18	7.93	18.68
	CV	5.03	7.67	5.42	8.23	9.33	4.88	5.54	5.65	11.04

Figures in parenthesis are angular transformed values/ Arc sin transformed values
* F- test is significant at 5% probability, ** F- test is significant at 1% probability
The values in the column following same alphabet letters are not significantly different from each other

4. Conclusion

The results of the present findings showed that all the three isolates were able to cause disease showing the average mortality and effect on growth and development. However, the isolate SSB of *M. anisopliae* performed better under laboratory conditions and need to be evaluated under field conditions for their promotion against soil dwelling and defoliating insect pests.

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6. Reference

1. Alves SB, Lopes RB. Controle microbiano de pragas na América Latina: avanços e desafi os. Piracicaba: FEALQ. 2008; 414.
2. Ansari MA, Vestergaard S, Tirry L, Moens M. Selection of highly virulent fungal isolate, *Metarhizium anisopliae* CLO53, for controlling *Hoplia philanthus.* Journal of Invertebrate Pathology. 2004; 85:89-96.
3. Boopathi T, Karuppuchamy P, Kalyanasundaram MP, Mohankumar S, Ravi M. Pathogenicity, ovicidal action and median lethal concentrations (LC_{50}) of entomopathogenic fungi against exotic spiraling whitefly, *Aleurodi*cus disperses. Russell. Journal of Pathology. 2013; http://dx.doi.org/10.1155/2013 /393787.
4. Culliney TW, Grace JK. Prospects for the biological control of subterranean termite (Isoptera: Rhinotermitidae) with special reference to *Coptotermes formosamus*. Bulletin of Entomological Research. 2000; 90: 9-21.
5. Damos P, Colomar LAE, Loriatti C. Integrated Fruit Production and Pest Management in Europe: The Apple Case Study and How Far We Are From the Original Concept? Insects. 2015; 6:626-657.
6. Derakhshan A. Natural Occurrence and Distribution of Soil borne Entomopathogenic Fungi in Shahrood Region, Northeast of Iran. International Meeting on Soil Fertility Land Management. Agroclimatology. 2008; 873-877.
7. Gutierrez RL, Luz ATDL, Ochoa JM, Dominguez OR, Pescador AR, Edwards ML *et al*. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastreha ludens* (Diptera: Tephritidae): laboratory and field trials. Journal of Economic Entomology. 2000; 93:1080-1084.
8. Han JH, Jin BR, Kim JJ, Lee SY. Virulence of entomopathogenic fungi *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* for the microbial control of *Spodoptera exigua*. Mycobiology. 2014; 42:385-390.
9. Inglis GD, Goettel MS, Butt TM, Strasser H. Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N. (eds.). Fungui as Biocontrol Aggents. Wallingford. CAB International. 2001; 23–69.
10. Mahapatra GK, Sreedevi K. Indigenous approaches for the management of termite and white grub in upland rice. Current Biotica. 2014; 8(1):97-108.
11. Moraga EQ, Cortesb JAN, Maranhaoa EAA, Urquizaa AO, Alvareza CS. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological research. 2007; 111 (8):947–966.
12. Norhelina L, Sajap AS, Mansour SA, Idris AB. Infectivity of five *Metarhizium anisopliae* (Deuteromycota: Hyphomycetales) strains on whitefly, *Bemesia tabaci* (Homoptera: Aleyrodidae) infesting brinjal, *Solanum melongena* (Solanaceae). Acedamic Journal of Entomology. 2013; 6:127-132.
13. Quesada-Moraga E, Ruiz-Garcia A, Santiago-Alvarez C. Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Matarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). Journal of Economic Entomology. 2006; 99:1955– 1966.
14. Reji Rani OP, Shifa BS, Soni KB, Sudharma K. Isolation and screeing of indigenous entomopathogenic fungi aganist sucking pests of vegetables. International Journal of Applied and *Pure Science* Agriculture. 2015; 1:9-17.
15. Sahayaraja K, Borgio JF. Distribution of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina:Hyphomycetes) in Tamil Nadu, India, its biocontrol potential on *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). Arch. Phytopathology Plant Protection. 2006; 42(5):424-435.
16. Shin TY, Choi JB, Bae SM, Koo HN, Woo SD. Study on selective media for isolation of entomopathogenic fungi. International Journal of Industrial Entomology. 2010; 20: 7-12.
17. Thangavel B, Palaniappan K, Pillai KM, Subbarayalu M, Madhaiyan R. Pathogenicity, ovicidal action and median lethal concentrations (LC_{50}) of entomopathogenic fungi against exotic spiraling whitefly, *Aleurodicus disperses*. Russell. Journal of Pathogens. 2013; 1-7.
18. Thomas MB, Read AF. Can fungal biopesticides control malaria? Nature Review Microbiology. 2007; 5:377-383.
19. Wahab S. Biotechnological approaches in the management of plant pests, diseases and weeds for Sustainable Agriculture. Journal of Biopesticides. 2009; 2:115-134.
20. Wekesa VW, Maniania NK, Knapp M, Boga HI. Pathogenecity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi.* Experimental and Applied Acarology. 2005; 36:41-50.
21. Yeo H, Pell JK, Alderson PG, Clark SJ, Pye BJ. Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species. Pest Management Sciences. 2003; 59:156–165.