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Efficacy of different isolates of entomopathogenic fungi against *Brevicoryne brassicae* (Linnaeus) at different temperatures and humidities

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ABSTRACT: **The virulence of twenty-five isolates of entomopathogenic fungi consisting of ten belonging to *Beauveria bassiana* (Bals.) Vuill., seven to *Verticillium lecanii* (Zimmerman), five to *Metarhizium anisopliae* (Metschinikoff) Sorokin, two to *Nomuraea rileyi* (Farlow) Samson and one to *Paecilomyces fumosoroseus* (Wize) Brown and Smith originating from a wide range of insect species was investigated in laboratory bioassays on cabbage aphid, *Brevicoryne brassicae* (Linnaeus) at different regimes of temperature (20, 25 and 30°C) and relative humidity (75, 85, 90 and 95 %). All fungal isolates except *N. rileyi* isolates were pathogenic to the aphid, but in varying degrees. Among three levels of temperature tested, aphid mortality was significantly higher at 25°C than 20 and 30 °C. Aphid mortality decreased with decreasing relative humidity. Among all isolates in all combinations of temperature and relative humidity, four isolates of *V. lecanii*, V.I-1, V.I-2, V.I-6, and V.I-7 showed higher virulence to *B. brassicae*. In multiple dose bioassays, lowest LT_{50} was obtained from V.I-7 isolate. The highest virulence of V.I-7 isolate of *V. lecanii* to *B. brassicae* suggests that the isolate would be a potential candidate as a microbial control agent for the cabbage aphid.**

KEY WORDS: *Brevicoryne brassicae*, entomopathogenic fungi, humidity, temperature, *Verticillium lecanii*

INTRODUCTION

Entomopathogenic fungi are of considerable importance in crop pest control because of their ability to infect a wide range of insects like aphids, whiteflies, leaf miners, weevils, grasshoppers and cockroaches (Rabindra, 1999). Fungi are the only insect pathogens currently used for control of aphids (Latge and Papierok, 1988).

The cabbage aphid, *Brevicoryne brassicae* (Linnaeus) is most common in cabbage and is widely distributed in temperate regions of the world. Many genera of cruciferae are colonized by this aphid (Blackman and Eastop, 2000). It damages the crop by direct feeding and also acts as a vector for about 20 plant viruses, including cabbage black ring spot, cabbage ring necrosis, cauliflower mosaic and radish mosaic virus (Blackman and Eastop, 2000). This aphid remains one of the most important pests of horticultural and oil-seed brassicae crops

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Table 1. Fungal isolates evaluated in the study

Sl. no.	Fungi/Isolate code	Host/ Stage/ Crop	Place of collection
1	*B. bassiana* (Bb-1)	ITCC Culture	IARI, New Delhi
2	(Bb-2)	Unknown identity	Unknown identity
3	(Bb-3)	*Neochetina bruchi* /Adult/ Water hyacinth	PDBC water tanks, Bangalore, Karnataka
4	(Bb-4)	*Spodoptera litura* / Larva/Lab. reared	Bangalore, Karnataka
5	(Bb-5)	*Hypothenemus hampei* / Larva & Adult / Infested berries/ Coffee	Chettalli, Madikeri, Karnataka
6	(Bb-6)	Tree Hopper /Adult / *Cassia fistula*	Bangalore, Karnataka
7	(Bb-7)	*Plocaedrus ferrugineus* / Grub/ Cashew	NRCC, Puttur, Karnataka
8	(Bb-8)	*Dicladispa armigera* / Rice	Guwahati, Assam
9	(Bb-9)	Dipteran insect / Rice	Guwahati, Assam
10	(Bb-10)	*Cnaphalocrosis medinalis* / Rice	Ludhiana-Punjab
11	*M. anisopliae* (Ma-1)	Unknown identity	Unknown identity
12	(Ma-2)	*Amsacta albistriga* / Larva / Groundnut	Davanagere, Karnataka
13	(Ma-3)	Oryctes rhinoceros / *Grub / Coconut*	CPCRI, Kasaragod, Kerala
14	(Ma-4)	*Plocaederus ferrugineus* / Grub / Cashew	NRCC, Puttur, Karnataka
15	(Ma-5)	*Holotrichia serrata* / Sugarcane/ Grub	SBI, Coimbatore (TN)
16	*N. rileyi* (Nr-3)	Achaea janata / Larva / Cotton	Chinthamani, Karnataka
17	(Nr-38)	*Spodoptera litura* / Cabbage	Guntur, Andhra Pradesh
18	*P. fumosoroseus*	Tetranichus urticae	Bangalore, Karnataka
19	*V. lecanii* (Vl-1)	IIHR isolate	IIHR, Hessaraghatta
20	(Vl-2)	*Coccus viridis*/ Adult / Citrus	IIHR Res.Station, Chettalli, Karnataka
21	(Vl-3)	*Coccus viridis*/ Adult / Coffee	CCRS, Cherttalli, Karnataka
22	(Vl-4)	*Hemileia vastatrix*/ Rust spots/ coffee	CCRS, Chettalli, Karnataka
23	(Vl-5)	Mealy bug/ Grape	Pune, Maharashtra
24	(Vl-6)	*Rhophalosiphum maidis*/ Maize aphid	Bangalore, Karnataka
25	(Vl-7)	*Bemisia tabaci*/ Whitefly	Bangalore, Karnataka

despite all attempts to control its infestation (Sing *et al.*, 1994).

Several studies have been carried out using entomopathogens, predators and parasitoids to control cabbage pests other than *B. brassicae*. Cabbage aphid has developed resistance to insecticides (CABI, 2002). This indicates the need for studies on natural enemies of this aphid. Compared to other natural enemies, only limited studies have been carried out on the efficacy of entomopathogenic fungi for biological control of *B. brassicae*. Hence there is an urgent need to identify suitable entomofungal pathogens for this pest that are compatible with other natural enemies of this pest and other cabbage pests.

MATERIALS AND METHODS

Fungal isolates

Twenty-five fungal isolates used in this study were obtained from the Project Directorate of

Biological Control (PDBC). These include *Beauveria bassiana* (Bals) Vuill (10 isolates), *Verticillium lecanii* (Zimmerman) Viegas (7 isolates), *Metarhizium anisopliae* (Metschinikoff) Sorokin (5 isolates), *Nomuraea rileyi* (Farlow) (2 isolates) and one isolate of Paecilomyces *fumosoroseus* (Wise) Brown et Smith. Detailed information about these fungal isolates are given in Table 1. (Footnotes)

Preparation of inoculum

Initially, a preliminary pathogenicity test was conducted on *B. brassicae* with 25 fungal isolates and pathogenic isolates were identified. The pathogenic isolates were re-isolated from infected aphids and maintained as stock culture on Sabouraoud dextrose agar with yeast extract (SDAY) medium. Stock cultures were stored in a refrigerator at 5°C (Chandler, 1993). Then each isolate was grown on Petri-plates on Potato Dextrose Agar (PDA) medium for 14 days at 25 ± 1° C in A B.O.D. incubator. Conidial suspension of each isolate was prepared by washing colonies on PDA plates with 10 ml of aqueous solution of Tween-80 (0.05%). The resulting conidial suspension was filtered through a double-layered muslin to remove mycelial bits. Conidia were counted using a haemocytometer under microscope and conidial concentration was adjusted to 1 x 10^7/ml conidia. Prior to bioassays, the viability of conidia of each isolate was assessed according to the method of Schading *et al.* (1995). Conidial batches with more than 85 per cent viability were used in the bioassays.

Maintenance of insects

B. brassicae were *collected* from the cabbage field and mass reared in the net house on 2-3 month old cabbage plants. 10-days-old adults were used for bioassay studies.

Bioassay procedure

The bioassay method adopted had two major components. An initial single dose screening assays followed by multiple dose assays. Healthy cabbage leaves were given washing of with distilled water for 10 minutes, followed by sterilization in sodium hypochlorite solution (0.25%) for 3 minutes. The leaves were then given three washes with sterile distilled water and air-dried in a laminar flow chamber (LFC). The detached leaves were then placed aseptically and individually over sterilized 1% agar medium in Petri-plates. Inoculation was carried out by immersing the aphids in the conidial suspension in a Buchner funnel for 5-10 second, followed by transfer to a sterile filter paper. Twenty aphids / replication were transferred to the leaf discs with the help of a brush. Then the Petra-dishes were incubated at different temperatures (20, 25 and 30 °C) and humidities (75, 85, 90 and 95 %) in a growth chamber at 12:12 h photoperiod.

Observations on the mortality of the aphids were recorded at 24 h interval for a period of 7 days. All newborn nymphs and dead adults were removed daily. Dead adults were transferred to Petri-dishes over a sterile moistened filter paper for development of external mycelial growth on the aphids.

Multiple-dose-bioassays

Four promising isolates which showed higher virulence to *B. brassicae* in single dose bioassay were selected and assessed further in a multiple-dose-mortality response. The bioassay was carried out using six different spore concentrations containing 10^4, 10^5, 10^6, 10^7, 10^8, 10^9 spores/ml. Bioassay was carried out in a similar manner as described earlier.

Statistical analysis

Analysis of variance (ANOVA) was used to analyze percentage mortality data after arcsine transformation to normalize the data. Percentage mortality (at 7-day-post-treatment) was also adjusted for natural mortality in controls using Abbott (1925) formula before analysis and was then analyzed using three-way analysis of variance for a completely randomized design. Means were compared using Duncan's multiple range test (P=0.05). LC_{50} values were computed with corresponding 95 percent confidence limits (Finney, 1964).

RESULTS AND DISCUSSSION

In pathogenicity test, all the isolates of the fungi except *N. rileyi* isolates were found pathogenic to *B. brassicae* and selected for further studies.

Effect of temperature

Aphid mortality was significantly affected by temperature (F=25.78, P<0.01) and isolate (F=73.85, P<0.01) with a significant temperature *isolate interaction (F=4.37, P <0.01). It was observed that among three regimes of temperature tested, mortality at 25°C was significantly higher (56.44%) than at 20°C and 30°C with (53.67 and 54.33%, respectively). Temperature not only regulates the physiology of the fungus and insect, but also the ability of the fungus to infect the host. Dimbi *et al.* (2004) found that six *M. anisopliae* isolates were most effective at 30°C to fruit flies while lower

Table 2. Mortality of *B. brassicae* caused by different fungal isolates at 20°C and four relative humidity regimes

Fungal isolates		Relative humidity (%)			
		75	85	90	95
B.bassiana	(Bb-1)	25.00(±0.00) fgh	40.00(±3.46) egh	45.10(±1.96) fgh	50.98(±1.96) gh
	(Bb-2)	38.46(±1.92)cd	74.08(±2.00)bc	80.39(±3.40)c	82.35(±3.40)c
	(Bb-3)	19.23(±3.33)h	36.20(±4.00)gh	43.14(±1.96)gh	47.06(±5.88)gh
	(Bb-4)	26.93(±1.92)efgh	38.05(±2.00)gh	45.10(±1.96)efgh	47.06(±3.40)gh
	(Bb-5)	36.54(±3.33)cd	48.10(±2.00)ef	52.94(±3.40)ef	54.90(±1.96)fgh
	(Bb-6)	30.77(±3.33)defg	42.30(±2.00)efg	49.02(±3.92)efg	50.98(±3.92)gh
	(Bb-7)	19.23(±3.33)h	36.10(±3.46)gh	43.14(±3.40)gh	45.10(±3.92)h
	(Bb-8)	34.62(±1.92)cde	32.08(±5.92)h	43.14(±1.96)gh	50.98(±3.92)gh
	(Bb-9)	23.08(±1.92)gh	32.05(±2.00)h	39.22(±1.96)h	43.14(±1.96)h
	(Bb-10)	26.93(±3.84)efgh	40.11(±3.46)fgh	47.06(±5.88)fgh	52.94(±5.88)fgh
M. anisopliae	(Ma-1)	38.46(±1.92)cd	74.13(±3.46)bc	82.35(±1.96)c	86.27(±1.96)bc
	(Ma-2)	19.23(±3.33)h	36.20(±2.00)gh	43.14(±2.00)gh	43.14(±3.92)h
	(Ma-3)	23.08(±3.84)gh	36.21(±2.00)gh	41.18(±1.96)gh	47.06(±3.39)gh
	(Ma-4)	32.69(±3.84)cdef	50.03(±2.00)e	54.90(±3.40)e	56.86(±3.92)fg
	(Ma-5)	26.93(±1.92)efgh	40.53(±346)fgh	49.02(±1.96)efg	52.94(±3.40)fgh
V. lecanii	(Vl-1)	67.31(±1.92)b	76.25(±3.46)ab	82.35(±1.96)c	86.27(±3.92)bc
	(Vl-2)	67.31(±1.92)b	78.01(±3.92)ab	84.31(±2.00)bc	86.27(±1.96)bc
	(Vl-3)	36.54(±3.33)cd	60.55(±3.46)d	80.39(±1.96)c	62.75(±1.96)ef
	(Vl-4)	34.62(±1.92)cde	48.05(±2.00)ef	66.67(±3.92)d	70.59(±5.88)de
	(Vl-5)	30.77(±0.00)defg	66.06(±2.00)cd	72.55(±3.40)d	78.43(±1.96)cd
	(Vl-6)	65.39(±0.00)b	82.00(±3.46)ab	90.20(±1.96)ab	96.08(±1.96)ab
	(Vl-7)	75.00(±1.92)a	84.55(±2.00)a	96.08(±1.96)a	100.00(±0.00)a
P. fumosoroseus		36.39(±1.92)cd	72.07(±5.29)bcd	91.39(±1.96)ab	88.24(±3.40)bc

Means followed by the similar letters in the columns are not significantly different at 5%.

Table 3. Mortality of *B. brassicae* caused by different fungal isolates at 25°C and four relative Humidity regimes

Fungal isolates		Relative humidity (%)			
		75	85	90	95
B.bassiana	(Bb-1)	30.19(±1.89)d-h	40.39(±1.92)ij	44.00(±5.29)fgh	49.02(±1.96)efgh
	(Bb-2)	43.39(±0.00)c	76.92(±3.33)abc	86.05(±2.00)b	90.20(±1.96)b
	(Bb-3)	20.75(±3.26)h	36.54(±3.33)j	44.10(±1.98)fgh	45.10(±3.33)gh
	(Bb-4)	39.62(±1.88)cd	48.08(±5.77)fghi	52.23(±3.46)efg	52.94(±6.79)efg
	(Bb-5)	37.73(±5.66)cde	48.08(±3.33)fghi	54.02(±2.00)ef	56.86(±1.96)ed
	(Bb-6)	30.19(±1.89)d-h	44.23(±1.92)hij	50.20(±1.98)efgh	50.98(±2.00)efgh
	(Bb-7)	22.64(±4.99")h	34.62(±1.92)j	42.52(±2.00)gh	47.06(±3.39)fgh
	(Bb-8)	35.85(±1.89)cde	42.31(±3.33)hij	50.00(±7.21)efgh	56.86(±1.96)ed
	(Bb-9)	24.53(±1.89)gh	36.54(±3.33)j	40.30(±3.46)h	43.14(±1.96)h
	(Bb-10)	28.30(±2.00)efgh	42.31(±0.00)hij	48.15(±5.29)efgh	56.86(±3.92)ed
M. anisopliae	(Ma-1)	41.51(±4.99)c	78.85(±5.09)ab	86.25(±2.00)b	86.27(±1.96)b
	(Ma-2)	22.64(±1.89)h	34.62(±3.85)j	44.10(±2.00)fgh	45.10(±2.00)gh
	(Ma-3)	26.41(±0.00)fgh	36.54(±5.77)j	40.00(±3.46)h	45.10(±1.96)gh
	(Ma-4)	33.96(±3.77)c-g	51.92(±1.92)efgh	58.11(±3.46)ed	62.75(±3.39)d
	(Ma-5)	26.41(±3.26)fgh	42.31(±0.00)hij	46.15(±3.46)fgh	54.90(±2.00)def
V. lecanii	(Vl-1)	66.04(±3.26)b	73.08(±1.92)c	82.08(±3.46)b	90.20(±3.33)b
	(Vl-2)	66.04(±2.00)b	78.85(±1.92)ab	86.05(±4.00)b	90.20(±2.00)b
	(Vl-3)	69.81(±1.89)ab	59.62(±3.33)de	66.20(±1.98)cd	72.55(±1.92)c
	(Vl-4)	39.62(±1.89)cd	51.92(±1.92)efgh	68.21(±2.00)c	72.55(±2.00)c
	(Vl-5)	33.96(±3.30)c-g	67.31(±5.09)cd	72.11(±2.00)c	78.43(±1.92)c
	(Vl-6)	67.92(±1.89)ab	86.54(±1.92)a	98.04(±1.98)a	100.00(±0.00)a
	(Vl-7)	75.47(±3.77)a	84.62(±1.92)a	100.00(±0.00)a	100.00(±0.00)a
P. fumosoroseus		37.51(±1.99)cde	76.85(±3.84)bc	100.00(±0.00)a	94.12(±3.39)b

Means followed by the similar letters in the columns are not significantly different at 5%.

temperature delayed onset of disease but did not affect total mortality. Ekesi *et al.* (1999) noted that there was significant decrease in fungal infection of *Megalurothrips sjostedti* (Trybom) by *M. anisopliae* isolates at 20°C in comparison to 25 and 30°C. Our results, therefore, support previous reports (Dimbi *et al.*, 2004; Ekesi *et al.*, 1999) that the rate of disease development increases with increase in temperature until an optimum level is reached (Tables 2, 3 and 4).

Effect of relative humidity

Aphid mortality caused by fungal isolates was significantly affected by relative humidity ($F=53.62$, $P<0.01$). Aphid mortality increased with increasing relative humidity (RH) but it was not at the same rate for all isolates. At 75 percent RH maximum aphid mortality was caused by V.l-7 isolate (at 20 and 25°C) and V.l-1, V.l-2, V.l-6 and V.l-7 (at 30°C) and minimum mortality by B.b-3, B.b-7 and M.a-2 (at 20°C) and

Table 4. Mortality of *B. brassicae* caused by different fungal isolates at 30°C and four relative

Fungal isolates	Relative humidity (%)			
	75	85	90	95
B.bassiana (Bb-1)	26.93(±1.92)defgh	40.03(±3.46)efg	44.25(±2.00)efgh	48.46(±2.00)defg
(Bb-2)	40.39(±1.92)b	74.08(±2.00)b	86.08(±4.00)c	88.77(±0.00)ab
(Bb-3)	17.31(±1.10)h	36.20(±2.00)fgh	44.11(±2.00)efgh	46.25(±3.46)efg
(Bb-4)	26.93(±1.89)defg	38.05(±1.92)efgh	46.15(±3.46)efgh	48.50(±2.00)defg
(Bb-5)	36.54(±3.33)bcd	44.10(±2.00)ed	52.50(±3.46)ef	54.33(±2.00)de
(Bb-6)	32.69(±1.89)bcde	48.88(±3.33)d	50.65(±3.33)efg	52.25(±3.46)def
(Bb-7)	21.16(±1.92)fgh	36.24(±1.92)fgh	42.40(±2.00)fgh	44.05(±2.00)fg
(Bb-8)	30.77(±3.33)b-f	42.25(±2.00)def	50.21(±5.29)efg	52.70(±6.00)def
(Bb-9)	25.00(±6.66)efgh	32.66(±4.00)h	40.46(±3.46)gh	42.25(±2.00)g
(Bb-10)	26.93(±2.00)defgh	38.10(±2.00)efgh	46.00(±5.29)efgh	50.80(±1.92)defg
M. anisopliae (Ma-1)	38.46(±1.92)bc	74.10(±2.00)b	82.50(±6.00)c	86.55(±1.92)b
(Ma-2)	21.16(±1.89)fgh	34.35(±3.46)gh	38.59(±2.00)h	42.90(±2.00)g
(Ma-3)	25.00(±3.33)efgh	36.23(±1.92)fgh	42.85(±3.46)fgh	44.00(±3.46)fg
(Ma-4)	34.62(±1.92)bcde	48.47(±2.00)d	54.05(±1.89)e	56.25(±2.00)d
(Ma-5)	25.00(±3.33)efgh	38.21(±1.92)efgh	48.44(±3.46)efgh	54.11(±3.46)de
V. lecanii (Vl-1)	65.39(±3.33)a	76.46(±0.00)ab	84.12(±1.92)c	88.95(±1.92)ab
(Vl-2)	67.31(±5.08)a	80.39(±1.92)ab	84.15(±1.92)c	88.08(±0.00)ab
(Vl-3)	34.62(±1.89)bcde	60.08(±2.00)c	66.14(±2.00)d	70.25(±1.92)c
(Vl-4)	34.62(±1.92)bcde	48.00(±3.46)d	64.65(±3.46)d	70.78(±0.00)c
(Vl-5)	30.77(±3.33)b-f	62.50(±1.92)c	72.05(±2.00)d	76.50(±1.92)c
(Vl-6)	71.15(±3.33)a	82.25(±1.92)a	98.54(±1.92)ab	96.00(±2.00)a
(Vl-7)	69.23(±1.92)a	82.05(±1.92)a	100.00(±0.00)a	96.25(±3.46)a
P. fumosoroseus	36.39(±1.99)bcd	74.31(±3.84)b	95.00(±0.00)b	96.80(±4.00)a

Means followed by the similar letters in the columns are not significantly different at 5%.

B.b-3 (at 25 and 30°C). Whereas at 85 percent RH, maximum aphid mortality was observed in V.l-7 (at 20 and 30°C) and V.l-6 (at 25°C) and minimum mortality was seen in B.b-9 and B.b-7 (at 20 and 30°C) and B.b-7 (at 25°C). Aphid mortality at 90% RH was highest in V.l-7 (at 30°C), V.l-7 and *P. fumosoroseus* (at 25°C) and was lowest in B.b-9 (at 20 and 30°C) and M.a-3 (at 25°C). At 95% RH, aphid mortality was highest in V.l-7 (at 20°C), V.l-6 and V.l-7 (at 25°C) and *P. fumosoroseus* (at 30°C). High humidity is required by many fungi for spore germination and sporulation but some studies have shown laboratory infections by *B. bassiana* at low humidity (Hastuti *et al.*, 1999). Our results are similar to Benz (1987) and Hastuti *et al.* (1999) who demonstrated that entomopathogenic hyphomycetes are limited by ambient humidity.

Table 5. Probit analysis of dose-mortality response of *B. brassicae* to selected isolates

Fungal isolate	*χ^2	Regression equation	LC_{50} (Spores/ml)	Fiducial limits (Spore/ml)	Relative activity
V.I-1	4.05	Y=-2.61+0.49X	$2.1x10^5$	$9.2x10^4$ - $4.5x10^5$	1.19
V.I-2	5.70	Y=-3.22+0.59X	$2.5x10^5$	$1.2x10^5$ - $4.6x10^5$	1.00
V.I-6	3.00	Y=-2.39+0.56X	$1.9x10^4$	$5.9x10^3$ - $4.4x10^4$	13.16
V.I-7	2.08	Y=-2.80+0.68X	$1.2x10^4$	$4.3x10^3$ - $2.7x10^4$	20.80

* Not significant

Multiple dose assays

The multiple-dose-mortality response showed that V.I-7 isolate of *V.lecanii* had a significantly lower LC_{50} (1.2×10^4 conidia/ml) than other isolates and that *B. brassicae* exhibited differential susceptibility to the fungal isolates. Thus comparison of LC_{50} values indicated that V.I7 isolate is the most virulent isolate to *B. brassicae* with the lowest LC_{50} followed by V.I-6, V.I-1 and V.I-2 isolates (Table 5).

Diseases form an important component of the natural enemy complex of aphids. *V. lecanii* is the only hyphomycetes fungus commonly found as a natural pathogen of aphids. (Milner, 1997). Zhang *et al.* (2001) have tested *V. lecanii* on *B. brassicae*, *P. xylostella*, *Pieris brassicae* and *Tetranychus urticae* and found that pathogenicity to *T. urticae* and *B. brassicae* was significantly higher than to *P. xylostella* and *P. brassicae*. This fungus has been found pathogenic to aphids, when applied as aqueous extract or formulated products such as wettable powder and oil based formulations under glasshouse as well as field conditions (Alavo *et al.*, 2002). Vehrs and Parrella (1991) evaluated *V. lecanii* for the control of *Aphis gossypii* on chrysanthemum in laboratory as well as glasshouse conditions and noticed high level of mortality of apterae adults after 6 days of treatment with 2.4 x 10^6 conidia/ml.

Selection of the most virulent entomopathogenic fungal isolate across a wide range of temperature and humidity for management of cabbage aphid was the main objective of this study. Accordingly, the results of this study have provided useful baseline data to develop biological control of this aphid with most promising fungal isolates identified under the test.

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