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Evaluation of bioefficacy potential of entomopathogenic fungi against the whitefly (*Bemisia tabaci* Genn.) on cotton under polyhouse and field conditions

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ABSTRACT

The whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), is becoming a serious problem on Bt cotton. It causes enormous crop loss through its direct feeding and as a vector of cotton leaf curl virus. Chemical-dependent management is harming the environment and increased insecticide resistance is often observed in the fields. Identification of most virulent strains of entomopathogenic fungi (EPF) is essential to serve as an important component of an IPM program for management of *B. tabaci*. Compared to *B. tabaci* adults, the nymphal stage is reported to be more susceptible to entomopathogens, and targeting nymphs also helps vector management. We evaluated the bioefficacy of EPF and chemical pesticides against nymphs of *B. tabaci* on Bt cotton under polyhouse and field conditions. The bioefficacy index (BI) was considered as a mechanism to select the most effective EPF strains for field evaluation. The highest nymphal mortality under polyhouse conditions was recorded for *Metarhizium anisopliae* NA-01299 (86.7%), *Beauveria bassiana* MT-4511 (85.1%), *Cordyceps javanica* IT-10498 (81.1%), IT-10499 (81%), and *B. bassiana* NA-0409 (78.2%) relative to other EPF strains, spiromesifen (69.6%), buprofezin (62.2%) and pyriproxyfen (52.7%) at 7-days-post-spray treatment (DAS). However, among all the EPF, the highest BI was recorded in *C. javanica* IT-10499 (77%), IT-10495 (75.4%), *Fusarium verticillioides* IT-10493 (74.6%), and *B. bassiana* MT-4511 (73.1%). The pooled data of two-year field trials (2017–18 & 2018–19) revealed that the highest nymphal mortality was recorded for MT-4511 (85%), IT-10499 (83.2%), and pyriproxyfen 10% EC (78.6%) at 7-DAS. The BI-based selection of EPF proved to be a useful predictor of field efficacy. A sequential spray of the selected EPF would be a vital approach for resilient and sustainable integrated management of the *B. tabaci* nymphal population under field conditions.

1. Introduction

The whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), is one of the most destructive insect pests among the world's top 100 invasive organisms (De Barro et al., 2011). It causes severe economic damage on about 900 plant species directly by feeding on plant sap and indirectly by transmitting about 111 plant viruses especially begomoviruses (family Geminiviridae) (Navas-Castillo et al., 2011; Polston et al., 2014). Worldwide, 42 distinct cryptic species of *B. tabaci* are grouped based on a 3.5% pairwise divergence in mtCOI sequences (Naveen et al., 2017; Kanakala and Ghanim, 2019). One invasive group MEAM1 (biotype B), and eight other genetic groups including Asia-I, Asia-I-India, Asia II-1, Asia II-5, Asia II-7, Asia II-8, and Asia II-11 are reported in India (Crowder et al., 2010; Ellango et al., 2015). Among these, Asia-I, Asia II-1, Asia II-8, and Asia-II-11 feed on cotton in India with the dominance of Asia-I and Asia-II-1 in the northern cotton growing zone of India (Ellango et al., 2015; Biswas et al., 2020).

B. tabaci assumed its pest status on cotton in 1905 in India. Until now, six whitefly outbreaks have been reported in different cotton-growing states in India. A severe outbreak occurred in the northern cotton-growing zone during 2015–16 which caused 50–60% yield loss in cotton (Kranthi, 2015; Singh et al., 2016). *B. tabaci* also causes indirect

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yield loss in the cotton crop (81.4 to 88.4%) by transmitting cotton leaf curl disease (CLCuD) in the northern cotton-growing zone of India (Monga, 2014; Sain et al., 2020; Monga and Sain, 2021). The polyphagous nature and virus-transmission capability of whitefly contribute to its management difficulties (De Barro et al., 2011). More than 50% reduction of insecticide usage on cotton in India was achieved after the adoption of Bt cotton technology from 2003 to 2006 (Krishna and Qaim, 2012). However, insecticides' demand for cotton has increased from 2374 tonnes in 2006 to 6372 tonnes in 2011. This has happened due to increased attacks of sucking pests on Bt cotton hybrids coupled with their increased levels of insecticide resistance (Gutierrez et al., 2015; Naveen et al., 2017). About 35 chemical insecticides are registered in India for the management of *B. tabaci*, but it has acquired resistance to many of them (Kranthi et al., 2002; Whalon et al., 2016, Naveen et al., 2017). The increased use of chemical insecticides is creating a negative effect on human health and the environment (Chitra et al., 2006; Akthar et al., 2009; Tomer et al., 2014). Ultimately, this is resulting in the growing demand for safer, resilient, and sustainable approaches, which can be a substitute for chemical pesticides.

Mycoinsecticides including entomopathogenic fungi (EPF) play a vital role as an alternative to chemical pesticides in integrated pest management (IPM) and insecticide resistance management (IRM) programmes in a more resilient and sustainable manner. Researchers in the past have demonstrated EPF as potential biopesticides. They are target-specific, highly reproductive, and their resting structures-producing capabilities (chlamydospore/blastospore) ensure their long-term survival under stressful conditions (Sandhu et al., 2012). About 750 species of EPF in 90 genera can infect insect-pests and mites, and about 20 species can infect whiteflies (Scorsetti et al., 2008; Ramanujam et al., 2014). Among these, only a few EPF like *Beauveria bassiana* (Bals-Criv.) Vuill., *Cordyceps farinosa* (Bally) Kepler, B. Shrestha & Spatafora [=*Isaria farinosa* (Holmsk.) Fr.], *Metarhizium anisopliae* (Metschn.) Sorok. and *Akanthomyces lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha [=*Lecanicillium lecanii* (Zimm.) Zare & W. Gams] are being studied for whitefly management (Lacey et al., 2015; Borisade and Mahan, 2015; Sain et al., 2019b). However, in India, only one bioinsecticide (*A. lecanii*) is commercially available for the management of whitefly on cotton (Sain et al., 2019a).

Studies conducted in the recent past have revealed that the second instar nymphal and adult of *B. tabaci* are the first and second most susceptible life stages for EPF, respectively (Cuthbertson et al., 2005; Quesada-Moraga et al., 2006). Consequently, we hypothesized that the identification of highly virulent EPF for targeting the nymphs becomes the most vital component for the successful management of whiteflies. This would lead to a reduction of both the nymphal and adult population in cotton fields. Therefore, this study was conducted for evaluation of the relative bioefficacy of EPF and chemical insecticides against whitefly nymphs under polyhouse, and field conditions. Further, it was intended to select the most virulent EPF based on the bioefficacy index (BI) for field use. The EPF exhibiting the best field efficacy would be utilized as a potential bioinsecticide component in an IPM/IRM program for whitefly vector and the cotton leaf curl disease (CLCuD) in the cotton fields.

2. Materials and methods

The current study was conducted during the rainy seasons of 2016–17 and 2018–19 at ICAR- Central Institute for Cotton Research, Regional Station (ICAR-CICR RS), Sirsa (Haryana) India (29°32′36.1″N 75°02′18.8″E). The incidence of natural infestation of *B. tabaci* occurs seasonally (from May to November) in the northern Indian cotton-growing region, and its population remains at its peak from July to September.

2.1. Fungal strains, insecticides, and whitefly population

The EPF strains used in this study were procured from the National Agriculturally Important Microbial Culture Collection (NAIMCC; Mau, Uttar Pradesh, India) [*B. bassiana* (Bb) - NAIMCC-F-0409; *M. anisopliae* (Ma) NAIMCC-F-01299 and from the Microbial Type Culture Collection (MTCC; Chandigarh, India) [*B. bassiana* (Bb) MTCC-4511, MTCC-4565, MTCC-6098, MTCC-6097, MTCC-4102]. Four indigenous cultures of *Fusarium verticillioides* (Sacc.) Nirenberg (=*Fusarium moniliforme* J. Sheld) (Fv) (ITCC-10493), *Cordyceps javanica* (Cj) (formerly *Isaria javanica*) (ITCC-10495, ITCC-10498, ITCC-10499) were collected, isolated from *B. tabaci* cadavers during a survey in the northern cotton-growing zone of India, identified based on morphological characteristics and molecular methods using ITS1 and ITS4 primers (White et al., 1990; Humber, 2012), and submitted to the Indian Type Culture Collection (ITCC), New Delhi (Table 1). All purified EPF strains were maintained in Petri plates containing Sabouraud Dextrose Agar (Hi-Media) amended with 0.2% yeast (SDYA) in a BOD incubator at 25 ± 2°C and 6:18 hr light: dark regime. Commercially available insecticides and bio-formulations such as spiromesifen 22.9%SC (Bayer Crop Science India Ltd.), flonicamid 50%WG (UPL Ltd.), diafenthiuron 50%WG (Syngenta India Ltd.), buprofezin 25%SC (Rallis India Ltd. A Tata Enterprise), pyriproxyfen 10%EC (Syngenta India Ltd.), talc-based formulation of *A. lecanii* 0.2% WP [Indore Biotech Inputs & Research (P) Ltd.], and *Azadirachta indica* oil (Azadirachtin 300 ppm SC) (*T*-Stanes and Company Ltd.) were obtained from the local market and used in this study for the comparison with the EPF strains.

The whitefly adult population was collected from the cotton experimental fields of ICAR-CICR-RS and farmers' fields in Sirsa, Haryana (India) and maintained on *Gossypium hirsutum* L. cultivar HS-6 in a polyhouse at the research station. The total DNA was extracted from whitefly samples for molecular identification. The partial mitochondrial cytochrome oxidase I (mtCOI) gene of whitefly was amplified using CI-J and TL2 pair of allele-specific primers (Simon et al., 1994; Dinsdale et al., 2010). As a result, three sequences of the partial mtCOI gene of three whitefly samples collected from polyhouse and field were MN329161, MN329162, and MN329162. These sequences were submitted to NCBI, analyzed and compared with reference sequences from NCBI-GenBank to ascertain the genetic group status of whitefly populations.

2.2. Screening of bioefficacy of EPF under laboratory and polyhouse conditions

Mycelial growth and sporulation were examined in the laboratory for determining the cumulative bioefficacy of each EPF strain. For this, a small 5 mm bit of each EPF culture was inoculated in Petri plates (90 mm) containing SDYA and streptomycin sulfate (20 μg L^{-1}) and incubated for 10 days at 25 ± 2 °C in the darkness. For each treatment, three replications (Petri plates) were maintained. The mycelial growth of each EPF culture was measured 10 days after inoculation (DAI). The average of two perpendicularly measured diameters of each colony was

Table 1
List of fungal cultures procured and used for the virulence and field study.

Fungal isolates	Accession numbers*/NCBI GenBank Accession Number
Beauveria bassiana	MTCC-4565/JQ266124.1
Beauveria bassiana	MTCC-4511/JQ266218.1
Beauveria bassiana	MTCC-6097/JQ266161.1
Beauveria bassiana	MTCC-6098/JQ266162.1
Beauveria bassiana	NAIMCC-F-00409
Cordyceps javanica	ITCC-10495/MG976232.1
Cordyceps javanica	ITCC-10498
Cordyceps javanica	ITCC-10499/MG976234.1
Fusarium verticillioides	ITCC-10493/MG976231.1
Metarhizium anisopliae	NAIMCC-F-01299
Metarhizium anisopliae	MTCC-4102

*MTCC = Microbial Type Culture Collection, Indian Institute of Microbial Technology; NAIMCC = National Agriculturally Important Microbial Culture Collection; ITCC = Indian Type Culture Collections.

recorded. To estimate spore production, a 5 mm disc of actively growing (10 days-old) culture was cut using a sterilized cork borer was put into 0.1 ml of 0.02% aqueous Tween 80 (PEG-80 sorbitan monolaurate, Hi-Media) and then into a test tube containing distilled water, and the spores were carefully scraped from the disc utilizing a fine paintbrush. The spore suspension was collected and vortexed to achieve a homogeneous suspension. The suspensions were diluted with sterile distilled water to make the basic concentration (10 ml) of approximately 10^{-7} conidia/ml. The other concentrations were prepared from 10^{-3} to 10^{-6} using a serial dilution procedure. For each suspension, spores were counted using an improved Neubauer hemocytometer. For each treatment, three replications (5 mm culture disc) were used separately.

For evaluation of polyhouse and field bioefficacy, the conidia of each EPF strain were harvested from SDYA by flooding the sterile 0.01% (v/v) Tween 80 solution and stirring with a glass rod. Three Petri plates containing 10 days old active culture of each EPF strain were used for making the conidial stock solution, which was vigorously vortexed and filtered through quadruple layers of nylon cheesecloth. The filtered suspension (10 ml) was agitated, and conidial concentration was observed individually under a compound microscope at 400X magnification using an improved Neubauer hemocytometer. The conidial suspension (1×10^7 conidia ml^{-1}) with 0.01% (v/v) surfactant (Tween 80, Hi-Media) was utilized for bioefficacy assays. The performance of the conidial viability of each EPF suspension was confirmed based on their germination at 24 hr after incubation on the SDYA medium (Hi-Media) (>95% germination) before the inception of every bioassay (Wraight et al., 2007).

Comparative bioefficacy of each EPF and the chemical insecticides recommended for whitefly management in India were evaluated under polyhouse following the nymphal mortality bioassay test (Sain et al., 2019b). Pest-free 30 days-old potted cotton plants (cultivar HS-6) with 5–6 primary leaves were used to obtain a whitefly nymphal population comprising individuals of a similar age. The potted plants were kept inside a whitefly rearing polyhouse containing whitefly-infested plants for egg-laying. Adult whiteflies were separated from these plants 24 hr after incubation using the portable low-pressure air pump and transferred to another screen-house for the next 10 days until nymphs reached the 2nd instar (0.30–0.44 mm in length and 0.18–0.36 mm in width) (Mascarin et al., 2013). Afterward (10 days after egg-laying), the randomly selected third instar nymphs (40–50 nymphs/leaf) were marked on the abaxial surface of leaves with a waterproof marker (a circle around each nymph).

EPF strains, chemical insecticides, and a commercial formulation of *A. lecanii* were evaluated for their bioefficacy under polyhouse conditions (Table 2). The EPF (pure culture 10^7 conidia ml^{-1}) and chemicals were sprayed onto the leaves of cotton plants having marked nymphs The control was sprayed with 0.01% Tween 80 solution only. In the polyhouse, 30 ± 2 °C temperature, 75 ± 2% relative humidity (RH), and a diurnal day/night cycle of 16/8h were maintained. For each treatment, three replications were implemented and each replication contained three potted plants with three leaves on each (27 leaves/treatment). The nymphal mortality was recorded before spray treatments and at 3, 5, and 7 days post-spray treatment (DAS) with the help of a 20X hand magnifying lens. Nymphal bodies exhibiting an opaque or greenish-white colour with shiny and brownish colored eyes, or visible honeydew droplets appearing on the excretions, were considered to be alive. In contrast, nymphal bodies with a yellowish-brown appearance and a shriveled body shape were considered to be dead. Every time, 50 randomly sampled nymphs per leaf (circle mark using a waterproof marker) for screening each EPF strain and treatment were analyzed. The experiment was repeated twice with all the treatments. Because this bioassay used a uniform population of nymphs, Schneider-Orelli's formula- Corrected mortality% = [(% mortality in treatment – % mortality in control)/(100 – % mortality in control)] × 100, was used to correct the mortality data before the analysis of variance (Puntener, 1981).

To choose the best EPF for further field experiments, the data on virulence/nymphal morality under polyhouse conditions, mycelial growth, and sporulation were considered for calculating the overall bioefficacy index (BI) (Sain et al., 2019b): BI = 0.35 × MG + 0.15 × SP + 0.50 × MO. For this formula, MG = mycelial growth in mm at 10 DAI, SP = sporulation 1×10^8 conidia ml^{-1} at 10 DAI, and MO = nymphal mortality at 7 DAS. The weighting for each factor (mycelial growth 15%, sporulation 35%, and nymphal mortality 50%) was given in percentage for BI (Sain et al., 2019b).

Table 2
Comparative bioassay of entomopathogenic fungi and chemical pesticides against whitefly nymphs under polyhouse conditions.

Treatments	Mycelial growth*	Spore concentration (10^7/ml)@	Corrected nymphal mortality (mean ± SE) over control$		
			3 DAS**	5 DAS	7 DAS
B. bassiana MT-4565	62.4 ± 1.7[a]	14.7 ± 1.1	21.0 ± 0.6[a]	50.5 ± 0.9[a]	75.5 ± 2.2 [a]
B. bassiana MT-4511	59.3 ± 1.2 [a]	65.3 ± 1.7[a]	55.2 ± 0.5[b]	68.6 ± 2.6	85.1 ± 1.2[b]
B. bassiana MT-6097	51.7 ± 1.7	43.9 ± 0.5	35.8 ± 1.2[c]	49.1 ± 1.2 [a b]	58.8 ± 1.1
B. bassiana MT-6098	18.9 ± 1.2	20.1 ± 0.6[b]	21.0 ± 0.9 [a]	50.5 ± 1.1 [a]	75.5 ± 1.4 [a]
B. bassiana NA-409	64.0 ± 2.3 [a]	69.9 ± 3.4	20.3 ± 0.6 [a]	62.1 ± 0.6 [d]	78.2 ± 1.3 [a]
C. javanica IT-10495	81.0 ± 1.7[b]	54.7 ± 1.2[c]	42.4 ± 1.1 [d]	73.9 ± 1.2[c]	77.6 ± 1.4 [a]
C. javanica IT-10498	69.7 ± 1.2	34.7 ± 1.1[d]	49.3 ± 1.1 [e]	78.3 ± 1.1	81.1 ± 1.2[c]
C. javanica IT-10499	80.1 ± 1.7[b]	56.6 ± 1.2[c]	38.6 ± 1.2[cdf]	61.7 ± 0.8 [d]	81.0 ± 1.2[c]
F. verticillioides IT-10493	76.5 ± 1.1[b]	63.3 ± 1.5[a]	42.0 ± 1.1	76.3 ± 1.0[c]	76.7 ± 0.4 [a]
M. anisopliae NA-01299	64.0 ± 2.4 [a]	34.9 ± 1.7[d]	77.4 ± 2.0	82.7 ± 0.7	86.7 ± 1.2[b]
M. anisopliae MT-4102	39.7 ± 0.6	21.6 ± 0.6[b]	56.2 ± 1.3[b]	59.1 ± 1.7 [e]	70.6 ± 1.5 [d]
Buprofezin 25%SC (1.6 ml/l)	–	–	44.8 ± 1.1 [ef]	55.7 ± 1.4 [e]	62.2 ± 1.3
Spiromesifen 22.9% w/w SC (1 ml/l)	–	–	35.1 ± 0.6 [cf]	56.5 ± 1.3 [e]	69.6 ± 0.9[c]
Pyriproxyfen 10% EC (2.5 ml/l)	–	–	40.4 ± 0.5 [cd]	49.4 ± 1.2 [af]	52.7 ± 07 [d]
Flonicamid 50% WG (0.4 g/l)	–	–	41.2 ± 1.1[df]	42.1 ± 1.1 [g]	52.0 ± 1.7 [d]
Commercial *A. lecanii* (2 g/l)	–	–	37.4 ± 1.1 [cd]	46.3 ± 1.2 [bf]	46.8 ± 0.6 [e]
A. indica oil (Azadirachtin 300 ppm) @5 ml/l	–	–	20.9 ± 0.6[a]	38.5 ± 0.8 [g]	48.2 ± 0.9 [de]
F- value	122.38	165.83	212.53	111.75	107.75
Total df	32	32	50	50	50
LSD	4.92	4.50	2.96	3.59	3.67
P-value ((P < 0.05)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE(d)	2.36	2.16	1.45	1.76	1.79

**DAS = Days after spray inoculation; The experiment was repeated twice on different occasions, each time with 50 nymphs for each treatment and EPF strain.
*Mycelial growth diameter was measured (in mm) at 10 days post-inoculation from the Petri plates.
@The spore concentration per ml solution was measured at 10 days post-inoculation using a 5 mm mycelial disc (x 10^7 conidia/ml/5 mm disc) from the Petri plates.
$Different letters along with mortality data within the column indicate significant differences between treatments ($P \leq 0.05$).

2.3. Screening of bioefficacy under field conditions

Nine treatments including the best-performing strains of *B. bassiana, C. javanica, F. verticillioides, M. anisopliae*, three chemical insecticides, one each of *A. indica* oil, *A. lecanii* commercial formulation, and positive control (water spray) were used to evaluate the comparative bioefficacy under field conditions (Table 3). The experiment was laid out in a randomized complete block design with three replications and repeated twice during the 2016–17 and 2018–19 crop seasons with similar treatments. Each field plot (size 6.37 × 3 m) comprised of 144 cotton plants of the Bt cotton hybrid NCS 855 BGII with a spacing of 67.5 cm between rows and with an interplant spacing of 60 cm within rows. A two-meter gap was maintained around each plot and a barrier of the four-meter-height plastic sheet was used to prevent the cross drifts of spray treatments. The spray treatments were applied using a manually operated knapsack sprayer with deflector nozzles. All the treatment plots were properly randomized in three replications before the treatment applications. The recommended package of practices was followed for raising the cotton crop except for insecticidal sprays. The nymphal population was recorded/marked (live/dead) prior to spray treatment, and thereafter at 3-, 5- and 7- DAS.

A non-uniform population of whitefly nymphs was observed, and hence to correct for control mortality Sun-Shepard's formula was used (Puntener, 1981). For this formula, corrected mortality % = [(Mortality % in treated plot ± Change % in control plot population)/100 ± Change % in control plot population)] × 100. For this formula, Change % in control plot population = (Population in control plot after treatment - Population in control plot before treatment/Population in control plot before treatment) × 100.

2.4. Statistical analysis

The percentage values data were arcsine transformed before statistical analysis. The experimental data from laboratory, polyhouse and field experiments were statistically analyzed using analysis of variance (ANOVA) of completely randomized design and randomized complete block design, respectively. Treatment effects were compared with Least Significant Difference Test (LSD) at $P \leq 0.05$ with the help of online computer software OP Stats (Sheoran et al., 1998).

3. Results

3.1. In vitro mycelial growth and sporulation of EPF

The results of the laboratory study revealed that the greatest mycelial growth (diameter) at 10 DAI was observed in *C. javanica* IT-10495 (81.0 mm), IT-10499 (80.1 mm), and *F. verticillioides* IT-10493 (76.5 mm) strains. However, in these three EPF strains, the difference in mycelial growth was non-significant ($P < 0.05$). The highest conidial population ml^{-1}/5 mm disc (10^7 conidia ml^{-1}) (3.35 at $P < 0.05$) was recorded in *B. bassiana* NA-0409 (69.9 × 10^7), MT-4511 (65.3 × 10^7), and *F. verticillioides* IT-10493 (63.3 × 10^7) (Table 2).

3.2. Comparative bioassay of bio-insecticides and chemical insecticides under polyhouse

All three sequences of the partial mtCOI gene of whitefly samples had 94–95% nt identity with the sequences of whitefly cryptic species Asia II-1 and were clustered in the phylogenetic group Asia II-1. The partial mtCOI gene sequence accession numbers MN329161, MN329162, and MN329162 serve as an identifier for the submitted sequence, and allow the readers to retrieve the sequence upon reading the journal article in the future.

Under polyhouse conditions, the whitefly nymphal mortality was ranged between 70.6 and 86.7% with the highest by *M. anisopliae* NA-01299 (86.7%) followed by *B. bassiana* MT-4511 (85.1%), *C. javanica* IT-10498 (81.1%), and IT-10498 (81%) at 7 DAS. The nymphal mortality by chemical pesticides ranged between 52 and 69.6%, with the highest by spiromesifen (69.6%) and buprofezin (62.2%). Overall, the highest BI was recorded with *C. javanica* IT-10499 (77%), IT-10495 (75.4%), *F. verticillioides* IT-10493 (74.6%), and *B. bassiana* MT-4511 (73.1%). The EPF strains with the highest BI among all EPF were selected for field trials (Table 2; Fig. 1).

3.3. Comparative field bioassay of bio-insecticides and chemical insecticides

The EPF with a higher BI (under laboratory and polyhouse bioassays) were compared with the commercial chemical pesticides and formulation of *A. lecanii* for their field efficacy. The selected EPF strains and chemical treatments were significantly superior in terms of nymphal mortality over control under field conditions during 2017–18 and 2018–19 (Table 3). Pooled results revealed that every treatment performed in the same trend during both years. The highest nymphal mortality recorded by *B. bassiana* MT-4511, *C. javanica* IT-10499, and pyriproxyfen (2.5 ml L^{-1}) at seven-DAS (Table 3; Fig. 2). Among all treatments, the pooled nymphal mortality was ranged between 57.3 and 83.2%, with the highest by *B. bassiana* MT-4511 (85%), *C. javanica* IT-10499 (83.2%), and pyriproxyfen (2.5 ml L^{-1}) (78.6%). These treatments were statistically on par with each other but significantly superior to diafenthiuron, commercial formulations of *A. indica* oil, and *A. lecanii*. Also, the treatments NA-01299 (77.8%), IT-10493 (74.7%), and

Table 3
Effect of selected entomopathogenic fungal strains and chemical pesticides on whitefly nymphal mortality under field conditions (2017–18 and 2018–19).

Treatments	Corrected nymphal mortality (mean ± SE) over control (1 × 10^6)*					
	2017–18			2018–19		
	3 DAS	5 DAS	7 DAS	3DAS	5 DAS	7DAS
Diafenthiuron 50% WP @ 1 g/l	31.3 ± 1.5	45.8 ± 3.3^{a}	62.6 ± 2.1^{a}	26.2 ± 1.3	42.3 ± 2.1^{a}	53.5 ± 1.3^{a}
Pyriproxyfen 10% EC @ 2.5 ml/l	23.5 ± 0.8^{a}	54.8 ± 2.9	76.9 ± 2.8 ^{ab}	40.2 ± 2.2	72.6 ± 2.8^{b}	80.3 ± 1.4^{b}
A. lecanii commercial formulation 0.1% WP @ 5 g/l	18.8 ± 1.2^{bc}	27.7 ± 1.3	67.9 ± 3.7^{a}	43.0 ± 1.2	67.0 ± 2.3^{b}	73.4 ± 1.0^{bc}
A. indica oil (Azadirachtin 300 ppm) @5 ml/l	16.1 ± 1.2^{c}	42.9 ± 2.3^{a}	50.7 ± 3.3^{a}	18.9 ± 0.7	58.5 ± 2.0^{ab}	64.0 ± 2.3^{ac}
C. javanica IT-10499 @5 ml/l/10^6	26.3 ± 2.5a	65.3 ± 2.5^{b}	81.8 ± 3.9^{ab}	54.1 ± 2.4	79.3 ± 3.0^{b}	84.7 ± 2.9^{b}
M. anisopliae NA-01299 @5 ml/l/10^6	44.3 ± 2.4	44.6 ± 2.1^{a}	76.1 ± 1.8^{a}	43.2 ± 1.9	72.3 ± 1.1^{ab}	79.4 ± 3.0^{b}
F. verticillioides IT-10493 @5 ml/l/10^6	22.5 ± 2.0^{ab}	45.3 ± 1.7^{a}	71.4 ± 2.0^{ab}	43.3 ± 1.3	70.5 ± 2.7^{b}	78.0 ± 1.8^{b}
B. bassiana MT-4511 @5 ml/l/10^6	43.6 ± 3.4	62.4 ± 1.3^{b}	83.7 ± 2.8^{b}	37.1 ± 1.2	75.2 ± 0.6^{b}	86.3 ± 2.3^{b}
Water spray	11.7 ± 0.4	9.8 ± 0.2	14.7 ± 1.6	8.5 ± 0.9	10.5 ± 0.5	11.4 ± 1.2
F-values	50.56	72.87	22.02	85.85	102.31	196.10
Total df	26	26	26	26	26	26
LSD	4.8	6.07	8.75	4.66	6.54	5.16
P-value ((P < 0.05)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE(d)	2.261	2.841	4.093	2.181	3.061	2.412

*DAS = Days after spray; the experiment was repeated twice during 2016–17 and 2018–19 crop seasons for each treatment and EPF strain.
$Different letters along with treatment data indicate significant differences between treatments ($P \leq 0.05$).

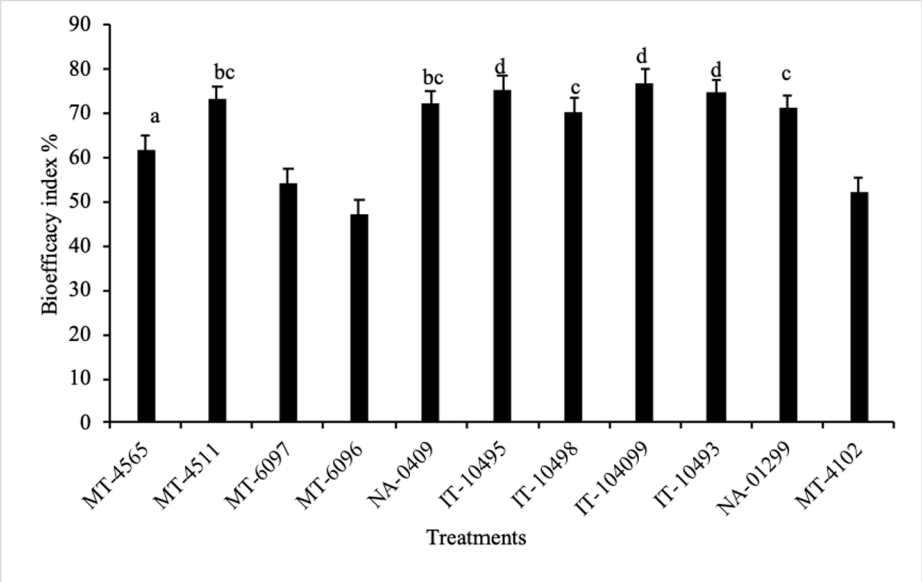

Fig. 1. Bioefficacy index of entomopathogenic fungal strains against whitefly nymphs under polyhouse conditions. The values are means and standard deviation (n = 11) and were analyzed using a one-way analysis of variance (ANOVA). Different letters indicate statistically significant differences between treatments ($P < 0.05$). Error bars represent the standard error of the mean total mortality (SEm 0.76).

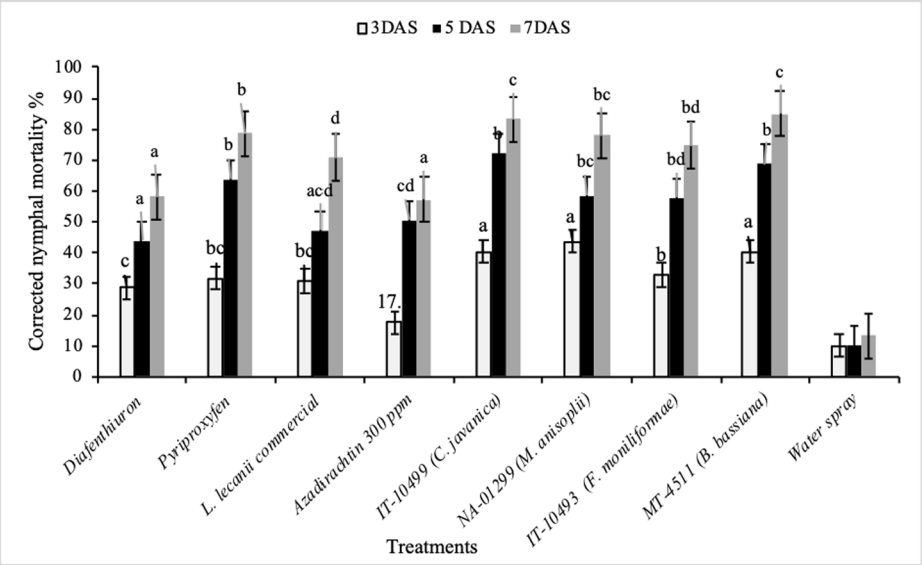

Fig. 2. The pooled effect of selected entomopathogenic fungal strains and chemical pesticides on whitefly nymphal mortality under field conditions. The values are means of two years of repeated experiments and standard deviation (n = 9) and were analyzed using a one-way analysis of variance (ANOVA). Different letters indicate statistically significant differences between the treatment pooled mortality at 3rd, 5th, and 7th DAS ($P < 0.05$), individually. DAS = days after spray. Error bars represent the standard error of the mean of nymphal (SEm for 3, 5 and 7 DAS are 5.8, 5.4, and 2.6, respectively).

pyriproxyfen (78.6%) were superior to commercial bio-formulations and control and were statistically on par with each other (Fig. 2).

4. Discussion

The indiscriminate uses of chemical insecticides have been reported to cause several health problems including, pesticide poisoning, skin allergies, cancer in human beings, deleterious effect on natural enemies, environment and pesticide resistance in insect-pests (Kranthi et al., 2002; Chitra et al., 2006; Akthar et al., 2009; Tomer et al., 2014). Therefore, biopesticide investigation is warranted and it is proved that EPF referred to as bioinsecticides are effective and environmentally safe alternatives to control many important insect-pests sustainably. Before this study, many EPF species are utilized for management of the *B. tabaci* considering their virulence as the major factor for their selection (Cuthbertson et al., 2005; Quesada-Moraga et al., 2006; Borisade and Mahan, 2015; Sain et al., 2019b). However, both mycelial growth and conidial production are important factors in perpetuation, causing infection and proliferation. Both of these factors and metabolic virulence factors (insect mortality) are integral parts of pest management (Sain et al., 2019a). Hence, in this study, we hypothesized that the BI (mycelial growth, conidial production, virulence) for selecting the best EPF strain for predicting the probable highest field efficacy.

In this study, all the EPF strains showed variability in mycelial growth, sporulation, and nymphal mortality. The native strain of *C. javanica* exhibited higher mycelial growth, while strains of *B. bassiana* recorded consistently higher conidial production. The highest nymphal mortality was observed with *B. bassiana* MT-4511, *C. javanica* IT-10498, and IT-10499. This may be because the native strains are more adapted to their native ecosystem. Conidia come into contact with the host insect, germinate, penetrate the cuticle, and mycelia growth takes place during penetration and host infection

(Joseph et al., 2010). Once the EPF enters inside the host, it has to develop its mycelium which produces metabolites that affect the insect's behavior or acts as a killing agent of the host (Vey et al., 2002; Joseph et al., 2010; Leao et al., 2015). Spores–the first and last stage of the life cycle of the EPF–and mycelial growth play a vital role in the virulence potential of an EPF, and such studies on proliferation and bioassay should be carried out before choosing any EPF for effective management of whitefly under field conditions.

Additionally, the nymph stage is the most susceptible life stage of *B. tabaci* followed by the second instar and adult whiteflies, respectively (Cuthbertson et al., 2005). The nymphal stage sucks the sap of leaves and secretes honeydew which encourages the development of sooty mold on leaves. The leaf blackening due to sooty mold reduces yield and quality through decreasing photosynthesis of crop plants (Polston et al., 2014). Hence, in this study, we evaluated the EPF against the whitefly nymphal stages (Asia-II-1; 3-4th instar) which are the most susceptible stage to EPF as well as non-carrier stages for cotton leaf curl virus. The targeted whitefly population used in this study was a cryptic species Asia II-1 which has developed resistance against several chemical insecticides (Ellango et al., 2015; Naveen et al., 2017; Kanakala and Ghanim, 2019; Biswas et al., 2020).

The polyhouse experiment showed that all the EPF strains (*B. bassiana, C. javanica, M. anisopliae, F. verticillioides*) caused nymphal mortality ranging from 58.8 to 86.7%. The higher nymphal mortality was observed with *M. anisopliae* NA-01299, *B. bassiana* MT-4511, *C. javanica* IT-10498, IT-10499, and *B. bassiana* NA-0409 (1x10^7 conidia ml^{-1}) at 7 DAS than spiromesifen and buprofezin. Our results are in line with the results of other researchers who worked on EPF against *B. tabaci*. Vicentini et al. (2001) reported that *B. bassiana* caused up to 25.7% nymphal mortality and 92.3% at 7 and 14 DAS, respectively in a bioassay conducted with melon leaves. Different isolates of *B. bassiana* caused 3–85% mortality of 4th instar nymphs (10^7 conidia ml^{-1}) (Quesada-Moraga et al., 2006). *Metarhizium* formulations reduced the nymphal and adult population ranging from 85.8 to 92.7% in controlled conditions (Batta, 2003). Similarly, the isolates of *B. bassiana* and *C. fumosorosea* (*I. fumosorosea*) are reported to cause 71–86% mortality of *B. tabaci* biotype B nymphs within 8 d (Mascarin et al., 2013). Eslamizadeh et al. (2015) found that the mortality of *B. tabaci* eggs and 2nd, 3rd, and 4th instars nymphs by the *C. fumosorosea* (*Paecilomyces fumosoroseus*) isolates ranged from 42 to 91, 38 to 90, 37 to 89, and 41 to 86%, respectively.

In this study, *F. verticillioides* IT-10493 from *B. tabaci* caused 76.7 and 78% nymphal mortality under polyhouse and field conditions, respectively. *F. verticillioides* has been reported to infect and causes quite good mortality in whitefly and other insect hosts (Humber, 1992). However, this can infest and produce mycotoxins on maize and other food sources (Glenn et al., 2001; Matny, 2014). We have not observed pathogenic symptoms of *F. verticillioides* IT-10493 on cotton during the bioassay test. However, we took intensive care while evaluating this strain for field efficacy. Henceforth, the *F. verticillioides* IT-10493 strain needs to be further tested for its infestation and production of mycotoxins on other crops and food sources before applying it under field conditions.

The pathogenicity or virulence of a microbial bioagent may not be the single factor for its success in managing insect pests in field conditions. The bioefficacy potential of bioinsecticides relies on their virulence and proliferation (conidia production, mycelia growth, and metabolite production). For the development of an effective formulation, good physical properties (sufficient conidia not less than 1 million ml^{-1}, long-term storage stability, high residual activity) are required. The formulation must be with biological agent functional throughout storage and provide consistently effective control of target pests after application (Jackson et al., 2010; Mascarin et al., 2010; Batta, 2016; Sain et al., 2018). Sufficient conidial production by an EPF and their germination are some of the most important components which affect its shelf-life, proliferation, and virulence. Hence, sporulation, mycelial growth, and virulence are the most essential components for the successful management of target pests. The selection of a potential EPF for applying in fields involves all these three parameters. Our study showed that a single strain generally may not perform all three activities uniformly. Hence, to choose the best EPF for field study, these three parameters i) the virulence/nymphal morality under polyhouse conditions, ii) mycelial growth, and iii) sporulation under laboratory were considered to calculate the overall BI of EPF strains (Sain et al., 2019b; Sain et al., 2019a). In this study, we selected the best EPF strains *C. javanica* IT-10499, IT-10495, *F. verticillioides* IT-10493, and *B. bassiana* MT-4511 which showed the highest BI among all the EPF, for evaluating their field efficacy and compared with commercial insecticides and biopesticides. Interestingly, these strains also turned out to be the best under field conditions. The highest nymphal mortality was recorded by *B. bassiana* MT-4511, *C. javanica* IT-10499, and pyriproxyfen during 2017–18 and 2018–19 (pooled results). These treatments were statistically on par with each other but significantly superior to diafenthiuron and other treatments. Also, the nymphal mortality values by *M. anisopliae* NA-01299 and *F. verticillioides* IT-10493 were on par with pyriproxyfen.

Similarly, Quesada-Moraga et al. (2006), observed up to 85% mortality of the fourth instar nymphs of *B. tabaci* (10^7 conidia ml^{-1}) by *B. bassiana* strains. Several researchers have confirmed the potential of *C. fumosorosea* and *B. bassiana* for the microbial control of whiteflies (Vicentini et al., 2001; Quesada-Moraga et al., 2006; Zimmermann, 2008; Cabanillas and Jones, 2009). *Metarhizium* formulations reduced whitefly nymph and adult population ranging from 30 to 92.2% under field conditions (Batta, 2003). The efficacy of *A. lecanii* is reported to be similar to *B. bassiana* in reducing whitefly population in tomato crops, which ranged from 56 to 87% at 0.25 and 3.2 × 10^6 conidia ml^{-1} (Karthikeyan and Selvanarayanan, 2011). Ibrahim et al. (2011) found 50 and 96.5% mortality by *B. bassiana* within 3.4 and 7 days, respectively (BotaniGard®), while native isolates of *M. anisopliae* caused 50 and 88% mortality in 4.5 and 7 days, respectively (Ibrahim et al., 2011). However, lower mortality values ranging from 0.92 to 69% by Quesada-Moraga et al. (2006). Mascarin et al. (2013) obtained 71–86% mortality with *B. bassiana* and *C. fumosorosea* (10^7 conidia ml^{-1}) within 8 days. The research findings of Quesada-Moraga et al. (2006), Ibrahim et al. (2011), and Mascarin et al. (2013) are similar to our study, where we observed nymphal mortality between 78.6 and 85% at 7 DAS by *C. javanica* IT-10499 and *B. bassiana* MT-4511 (10^7 conidia ml^{-1}). In this study, IT-10493 (*F. verticillioides*) also showed 74.7% nymphal mortality, which is a new finding and needs to be further evaluated for its environmental safety issues. The variability in nymphal mortality in similar EPF could be due to the difference in environmental factors (temperature and relative humidity) which affects the virulence of EPF under field conditions (Sosa-Gómez and Alves, 2000).

Khan et al. (2003) reported that 0.5% and 1% concentration of botanical oil were effective in reducing whitefly infestation and CLCuV disease severity. Also, a lower incidence of CLCuV (16.6%) was reported in the imidacloprid treatment compared to 42.6% in the untreated control (Humza et al., 2016). Hence, the management of whitefly at its nymphal stage using EPF would certainly reduce adult populations, which are vectors for CLCuD. Further, multiple applications of EPF can provide enhanced mortality (more than 90%) (Wraight et al. (2000).

Interestingly, two of the potential biocontrol strains in our study were *B. bassiana* MT-4511, *C. javanica* IT-10499, and they showed similar levels of biocontrol capacity both in polyhouse and field experiments (2017–18 and 2018–19). Ultimately, this study provided a better understanding of the selection of EPF by performing laboratory and polyhouse experiments and using BI before conducting a field evaluation trial. The selected EPF strains turned out to be the best strains under field conditions. Thus, our hypothesis of considering BI analysis of laboratory and polyhouse data would suggest that the BI method would be a useful predictor of field efficacy. Furthermore, studies are underway to identify effective formulation techniques to optimize the efficacy of EPF strains under field conditions, and for adoption in the IPM of whitefly in cotton.

5. Conclusion

Targeting the nymphal stage as it is the most susceptible to EPF, prior to its adult stage is a more desirable option for its management. We found that the BI facilitated the identification of the most effective EPF and proved to be a useful predictor of field efficacy. Using only the mortality bioassay data for the selection of EPF, the promising EPF of this study would have been rejected. With this, we were able to find the most effective EPF *B. bassiana* MT-4511, *C. javanica* IT-10499, which showed better field efficacy than pesticides. The IT-10499 and MT-4511 have been shown compatibility with the recommended dose of *A. indica* oil, Pongamia oil, spiromesifen, flonicamid, buprofezin, and pyriproxyfen. MT-4511 is also found compatible with diafenthiuron, profenophos, and imidacloprid (Sain et al., 2019b). A sequential spray of chemicals with these selected EPF can be employed to manage the B. tabaci nymphal population (Asia-II-1) and CLCuD severity under field conditions. Future investigations must be conducted on the development of an effective commercial formulation, yield assessments, and economic analyses for obtaining better results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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