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Research Article

Fusarium pallidoroseum: A potential entomopathogenic agent for the biological management of *Aphis gossypii*

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Abstract
With rising need of switching over to sustainable agricultural practices, utilization of entomopathogenic fungi (EPF) as biocontrol agents, provides better substitute against chemical pesticides- having several side-effects. Therefore, an attempt have been made to explore the potential EPF fungi that could be incorporated into IPM practices for control of *Helicoverpa armigera* Hubner and *Aphis gossypii* Glover. Regarding this, an entomopathogenic fungus, *Fusarium pallidoroseum* (Cooke) Sacc, was isolated from natural population of *H. armigera* infesting chickpea (*Cicer arietinum* L.) and explored efficacy under *in-vitro* & field conditions. The findings of present investigation shows efficacy of *F. pallidoroseum* as potential biocontrol agent against okra aphid (*A. gossypii*), as it inflicted initial mortality of 43.33% nymphs on 2nd day and lead to complete annihilation (93.33%) of nymph population on 8th day of spore suspension application at 1x10^{10} spores/mL concentration. The observations against adult okra aphid clearly demonstrated that spraying of 1 x 10^{10} spores/mL of *F. pallidoroseum* resulted 66.67% mortality after 8th days of spraying. Increased mortality was recorded with increase in spore suspension concentrations. The LC_{50} & LC_{90} value for *F. pallidoroseum* against nymphs of *A. gossypii* was recorded 3.79 x 10^5 and 2.74 x 10^8, respectively. The findings were used to develop formulations (1 × 10^4 to 1 × 10^{10} spore suspension/mL conc), and tested at field-level. The results showed that formulation at 1 x 10^{10} spores/mL conc was most effective against *A. gossypii,* recorded 93.33% mortality of nymphs & 66.67% mortality of adults; could be used under IPM practices.

Keywords: *Aphis gossypii*, Bio-efficacy, Entomopathogen, *Fusarium pallidoroseum*, *Helicoverpa armigera*, Bioagent

INTRODUCTION

During the last few years, *H. armigera* and *A. gossypii* are considered serious pests causing considerable losses under changing agro-climatic conditions in India, despite heavy uses of chemical pesticides (EPPO, 2006; Patel and Purohit, 2013; Ghosal *et al*., 2012; Khating *et al*., 2016; Nagamandla *et al*., 2017; Singh and Dhiman, 2018; Rathee and Dalal, 2018 and Yaqoob *et al*., 2019). *A. gossypii* (Homoptera: Aphididae) is a major sucking pest of okra, causing considerable losses in the form of curling and distortion of the young leaves. Also, the presence of nymphs and adults, their shed skins and honeydews decrease the aesthetic quality of the crop. Advancement in the field of biological management of major pests of pulses, especially against the pod borer *H. armigera*, has been significantly in the recent past (Mehrvar *et al.,* 2008; Ahmad and Ansari 2013; Jarrahi and Safavi 2016; Mora *et al*., 2017; Kalvnadi *et al*., 2018 and Goncalves *et al*., 2020). Productivity of chickpea crop is greatly affected by chickpea pod borer *H. armigera*, which damages up to 90-95% crop because of its high fecundity, nomadic behavior, polyphagous feeding nature and induced resistance against major groups of insecticides (Mishra *et al*., 2013). This pod has reported the yield loss of up to 400Kg/ha- borer, with 30-40% average pods damaged during favorable environment conditions; which causes reluctant to cultivate chickpea among the farmers (Hussain, 2007).

However, the continued development of natural populations resistant to chemical insecticides indicates that

further chemicals and or biological agents must be investigated for their efficacy against these insects. Considering the ill effects of chemicals and increased application costs, the biocontrol method such as use of fungal pathogens is desirable. Although, there were lots of evidence on the occurrence and efficacy of entomopathogens such as *Metarrhizium* spp and *Beauveria* spp*,* still there are several gaps in the identification and morphological characterization of natural enemies and their exploitation as bio-management tools with reference to agro-climatic niches. These fungal bio-control agents would offer a new approach to combat natural population of insect pest*,* while protecting the efforts and investments of the marginal farmers (Pawar and Borikar, 2005; Lingappa *et al*., 2005; Ahmad and Ansari 2013; Jarrahi and Safavi 2016; Kalvnadi *et al*., 2018; Mohammed *et al*. 2018; Javed *et al*. 2019; Nazir *et al*. 2019 and Litwin *et al*. 2020). The indispensable step in the development of an effective fungal microbial biocontrol agent is careful assessment and selection of the most suitable isolate, based on virulence against host insect. Factors such as temperature, pH, humidity and other environmental factors have a great significance in the incidence, severity and epidemiology of the disease (Patel and Purohit, 2013; Khating *et al*., 2016; Nagamandla *et al*., 2017; Singh and Dhiman, 2018; Rathee and Dalal, 2018 and Yaqoob *et al*., 2019). The absence of significant correlation between field and laboratory outcomes have made it complicated to visualize the genuine efficacy of entomopathogen against target and non-target insects due to different environmental conditions. Thus in the present investigation, an attempt have been made to explore an alternate entomopathogenic biocontrol agent from natural environment; which could be used as an eco-friendly, efficient , cost-effective biocontrol agent as well as can reduce the agricultural losses.

MATERIALS AND METHODS

Study area

Nineteen villages of the Tehsil Bakshi Ka Talab (BKT), district Lucknow; were randomly selected for collection of the diseased *Helicoverpa* larvae. Bakshi Ka Talab is geographically located at the North Latitude 26°59′0 and East Latitude 80°53′0″ E. It is situated at the distance of 25 km away from the Lucknow, at the National Highway 24. The temperature of this area was 42°C to 45°C while in winter season temperature fell down between 5°C -8°C and the elevation of BKT was 124m (407 ft). Total number of villages in this Tehsil was 185 (Fig. 1).

Collection of infected Helicoverpa armigera larvae

Frequent field visits were made to the selected villages of BKT, and collected the diseased specimens of *Helicoverpa* larvae. The samples thus collected were kept properly and brought to the Biocontrol Laboratory, University of Lucknow; for further identification of natural enemies.

Isolation of entomopathogenic fungi

The infected larvae were surface sterilized with 0.1% (w/v) mercuric chloride solution and rinsed thrice in sterile distilled water. The sterilized infected larva was placed separately into sterilized petriplates, containing potato dextrose agar with streptomycin sulphate (2.5µg/mL), for isolation of entomopathogenic fungi at 28±2^{o}C for three days. The culture was further purified by growing single spore on PDA plates and maintained at 4 ^{0}C in refrigerator.

Effect of different media on isolated entomopathogen

The isolated entomopathogenic fungus was further cultured on a different medium such as Czapek-Dox agar, potato dextrose agar, MYEA, agar, coconut milk agar (5 and 10 %), Martin agar medium, Molish Agar medium, Sabarouds agar medium, Richards medium and Asthana and Hawkers medium; for detailed study of morphological characteristics. A mycelial disc of 3 mm diameter was transferred and inoculated centrally onto different culture plates containing different medium in 3 replicates and incubated at 28 ± 2°C. The colony appearances and pigmentations were assessed after 2 weeks of incubation, while growth rate was measured daily until fully grown. Average dry weight of the three replicate was taken as standard value for comparing the growth in different media.

Effect of temperature and pH on isolated pathogen

The effect of temperature and pH was assessed by analyzing the *in vitro* growth rate on potato dextrose broth at different temperature and pH, respectively. For observing temperature effect, the PDB containing flasks were inoculated with an equal amount of fungal inoculum (20µl of test entomopathogen at the concentration 10^6 spores /mL). The flasks were incubated at different temperatures 20, 24, 28, 32, 35 and 40 ^{0}C after inoculation for fifteen days. The dry weight of mycelial mats was measured 15 days after inoculation.

For observing pH effect, before sterilization the pH of the medium was adjusted in the range of pH-5 to pH-9 by using 1M solution of HCl and NaOH. The flasks were inoculated with mycelial disc (3mm) of entomopathogen from the 7 days old culture. Flasks containing PDB of different pH were incubated at 28±2^{0} C. Average dry weight of the three replicates was taken as standard value for comparing the growth of fungus at different pH.

Scanning electron microscopy

For SEM observations, mycelia and conidia of *F. palli-*

doroseum were fixed with 5% cold buffered glutaraldehyde for 24 h at room temperature. The samples were washed with sodium cacodylate buffer for 30 min and subsequently fixed with 2% osmium tetraoxide for 24h at 20 °C, dehydrated in a graded ethanol series for five minutes each and sputter coated with gold palladium. The images of *F. pallidoroseum* were obtained in the scanning electron microscope (JOEL, Japan, Model-JSM 6490 LV) at the Department of Environmental Science, Babasaheb Bheemrao Ambedkar University, Lucknow. The details regarding applied voltage, magnification used and the size of the content of the images were implanted on the photographs itself.

In vitro bio-efficacy against *Helicoverpa armigera* and *Aphis gossypii*

Two hundred mL of culture medium was taken in a 500 mL conical flask, autoclaved at 120 ^{0}C (15 lbs) for 20 minutes. The flasks were inoculated with a six mm disc of two week's old *F. pallidoroseum* grown on PDA media. The flasks were incubated at 28±2 ^{0}C in an incubator orbital shaker at 120± 10 rpm. The spore suspension was prepared by mixing the fungal mat in a mixer-grinder and mixed with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent (Rombach *et al.,* 1986). The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005). From the stock spore suspension, serial dilutions were made to obtain the require concentrations by using haemocytometer. Spore concentrations of 1x 10^{10}, 2x 10^9, 2x 10^8, 2x 10^7, 2x 10^6, 2x 10^5, 2x 10^4 and water spray as control were evaluated against *H. armigera* and *A. gossypii,* respectively (Shophiya *et al.*, 2014 and Jayasimha *et al.*, 2012). The lethality of the concentration was recorded by observing the percentage mortality of the okra aphid; at regular interval of two, four, six and eight days, after spraying.

Statistical analysis

Data on effect of temperature and pH on the growth of fungus were subjected to ANOVA, using Statistical Analysis System Version 9.0 (SAS 2002); however, for mortality data, Abbot's formula was used to calculate corrected mortality. From the corrected mortality data, the probability integral of the chi square distribution, regression equation, slope and lethal concentrations (LC_{50} and LC_{90}) were calculated; so that the efficacy and accuracy of the *F. pallidoroseum* can be standardized as an effective entomopathogen.

RESULTS AND DISCUSSION

During Rabi season; diseased *Helicoverpa* larvae were collected from chickpea crop of the vegetable growing areas of the study site - Bakshi Ka Talab block, Lucknow district. The samples thus collected were subjected for detailed *in vitro* investigation at the Bioconrol Laboratory, Department of Botany, University of Lucknow.

Out of total 1250 larvae collected from fields, 32% mortality was recorded due to tachnid flies, *Campoletis chlorideae,* nuclear polyhedrosis virus and fungal infection. *F. pallidoroseum* was isolated from third instar infected larvae of *H. armigera* for the first time in agro-climatic condition of Lucknow district of Uttar Pradesh, India.

Microscopic characteristics

The isolated fungus showed growth of dense, compact aerial mycelia, initially yellowish-orange colour and later turned white on potato dextrose medium (Fig. 2).

Fig. 1. *Study area- Bakshi Ka Talab , Lucknow.*

(A) **(B)** **(C)**

Fig. 2. *Colony morphology of Fusarium pallidoroseum at 100X magnification (A) on PDA (B) sporodochia (C) Hyphae showing typical mesoconidia.*

Orange to peach pigmentation around the colony on the reverse side of the Petridishes was also observed. After incubation at 28 ± 2°C temperature for 14 days, this isolate also produced definite sporodochia with macroconidia. Hyaline septate hyphae with buldged compartments, conidiophores and phialides were observed microscopically. Cylindrical phialides with small collaret was observed as a constituent of a complex branching system. Monophialides as well as polyphailides conditions, were observed. Macroconidia produced in sporodochia on phailides were long, 3-9 septate, sickle shaped, smooth and possess a significant foot cell. Those born in the aerial mycelium were slightly curved, 3-7 septate and without notched. Fusiform mesoconidia were also observed in the culture which looked like 'rabbit ears' were abundant in the aerial mycelia.

Scanning electron microscopy

A big mass of hyphae was observed together with macroconidia, mesoconidia and microconidia at 1000X and 3500X magnification examined by SEM. Infection hyphae with appresorium (measuring about 5.21 µm) were observed among the mycelial mat (Fig. 3 A), along with the polyphialides (Fig. 3 B) and chlamydospore (Fig. 3 C) at 6000X and 2500X magnification, separately. The length of macroconidia was found approximately 11.26 µm (Fig. 3 D). Short spindle-shaped microconidia were also present. The mycelial organization revealed by SEM also showed an extracellular material around the hyphae which was seen as a flocculent material over the cells or as a fine fibrils attaching hyphae to each other, resembling a biofilm (Fig. 3 E). The round structure seen in Fig. 3 F, under 2500X is a chlamydospore with a diameter of about 2.56 µm.

Effect of different media

The results on suitability of different synthetic and semisynthetic media in solid state on the growth and sporulation of the fungus are represented in Table 1.

The fungus grew rapidly on solid agar medium like Czapek-Dox medium, potato dextrose medium and MYEA medium as cottony flattened colonies with yellowish-orange sporulation and produces high number of macroconidia and microconidia. Spindle-shaped mesoconidia and microconidia were observed in the aerial mycelium. The potato dextrose agar medium recorded maximum mycelial growth (80.00 mm) followed by Czapek Doxagar medium (77.66 mm) and Coconut water agar medium (10% coconut water) (75.33mm). The characteristic growth pattern of fungus was not observed on coconut agar medium with 5-10% coconut water and agar medium but production of macroconidia was found maximum.

Effect of temperature and pH

The optimum temperature for growth of *F. pallidoroseum* was found 28 ^{0}C (dry mycelial weight 264.51 mg) followed by 32^0 C (dry mycelial weight 203.12 mg) and 24^{0}C (184.43 mg). The minimum dry mycelial weight was obtained at 40^0 C (92.47mg) after fifteen days of inoculation which shows that higher temperature inhibited the growth of fungus (Graph 1 & Table2).

The results revealed that the fungus could grow and sporulate in wide range of pH, i.e., from pH-5.0 to pH -9.0, in liquid potato dextrose medium (Graph 1). The dry mycelial weight was significantly higher at pH 8.0 (444.33mg) followed by pH 7 (334.42 mg) after fifteen days of inoculation. All the experimental data are significantly different from each other (Graph 2 &Table: 3).

Bio-efficacy of *F. pallidoroseum* against *Helicoverpa armigera*

The research results showed that *F. pallidoroseum* could initiate infection on susceptible *Helicoverpa* larvae only when the host surface was injured. This indi-

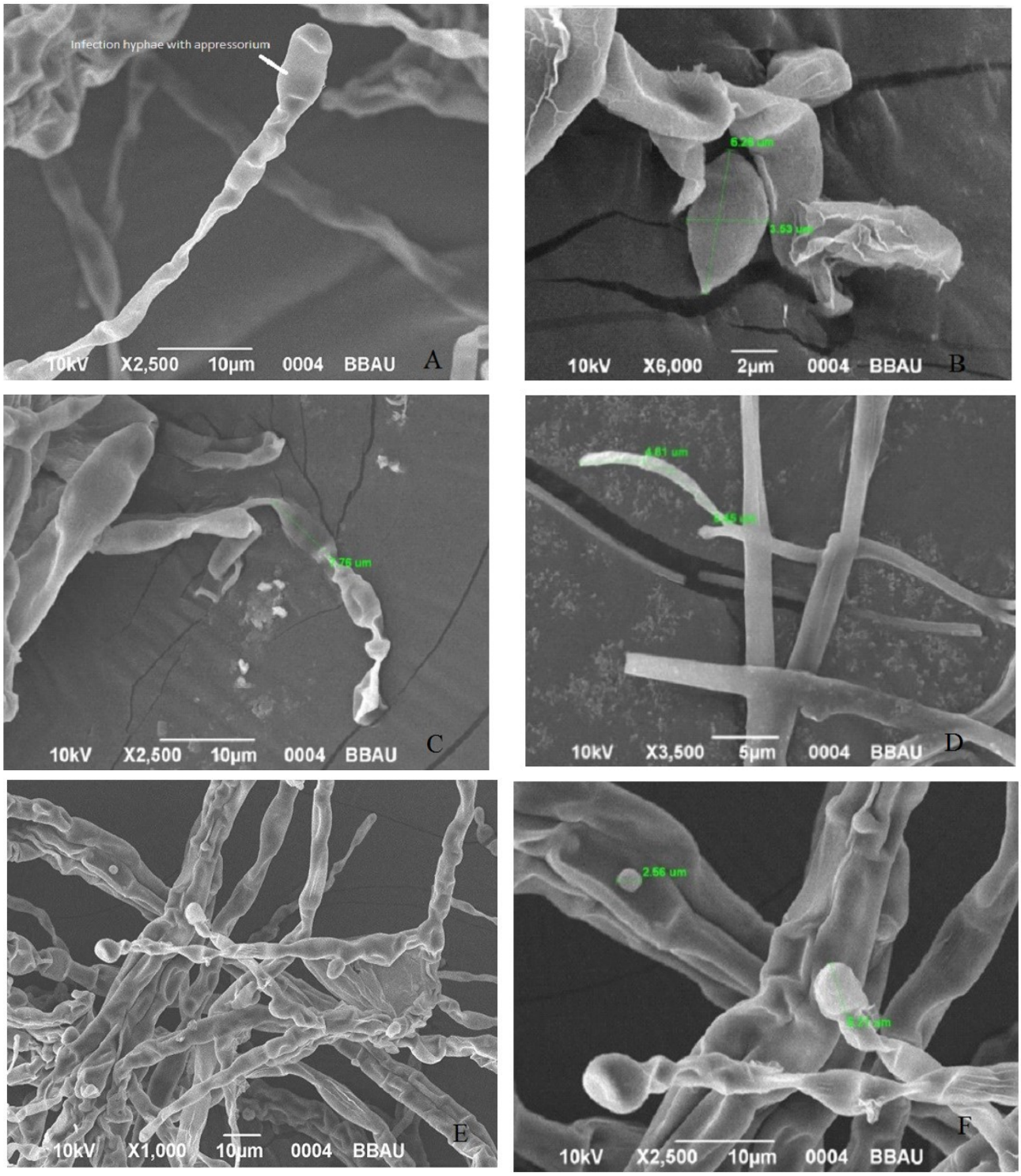


Fig. 3. *Scan electron micrographs of F. pallidoroseum grown on PDA (A). Infection hyphyae with appresorium (B). Polyphialides (6000X) (C). Chlamydospore (2500X) and (D). Macroconidia (3500X) (E). An extracellular material around the hyphae (F). Microconidia and chlamydospores (2500X) .*

cates that this is a weak pathogen of this pest.

Bio-efficacy of *F. pallidoroseum* against *Aphis gossypii*

Bio-efficacy studies on okra aphid, *A. gossypii* clearly indicate that this fungus might be used successfully for the control of *A. gossypii* in okra by incorporating it in integrated pest management strategies. Among the different fungal concentrations, the least per cent mortality of nymphs was noticed in 1 × 10^6 spore suspension (46.67%) to 1 × 10^4 spore suspension (36.6%) as against 93.33% in 1 x 10 ^{10} spores per mL after 8 days after spraying (Graph 3). The result also revealed that two days after spray, all the treatments differed signifi-

Table 1. Growth of *Fusarium pallidoroseum* on different solid media

Medium	Conidia production		Colour of colony	Radial growth after 15 days mm)
	Macroconidia	Microconidia		
Potato dextrose agar	++	++++	White wooly growth and orange tan in reverse	80.0 ± 0.171
Agar 2%	-	++++	Only white mycelium	80.0 ± 0.185
Czapek Dox Medium (Difco and BBL)	++	+++	Orange cousin growth with yellowish orange in reverse	77.66± 0.168
MYEA (malt yeast extract)	+	+++	pink cousin growth with yellowish orange in reverse	72.33±0.159
Coconut water 5% with agar	++	+	Only white mycelium	67.00±0.188
Coconut water 10% with agar	++	+	Only white mycelium	75.33±0.196
Martin Agar medium	+	++	pink growth with yellowish orange in reverse	69±0.169
Molish Agar medium	+	-	Only white mycelium	22±0.086
Sabouraud Agar medium	+	+	Only white mycelium	31±0.121
Richards medium	++	-	Orange cousin growth with yellowish orange in reverse	5.8±0.001
Asthana & Hawkers medium	++	++	White wooly growth and orange tan in reverse	72 ±0.173

Radial growth* each value is the mean of six replication and represented as mean ±SE, Sporulation represented by 0 - 20%, + 20-40%, ++ 40-60%, +++ 60-80%, ++++ 80-100%

Table 2. ANOVA for effect of different temperature on the growth of *Fusarium pallidoroseum*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	127648	7	18235.5	9.72688	0.00053	2.24902
Within Groups	74990.1	40	1874.75			
Total	202639	47				

Table 3. ANOVA for effect of media pH on the growth of *Fusarium pallidoroseum.*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	196392	7	28056	4.8266	0.00085	2.31274
Within Groups	186009	32	5812.79			
Total	382401	39				

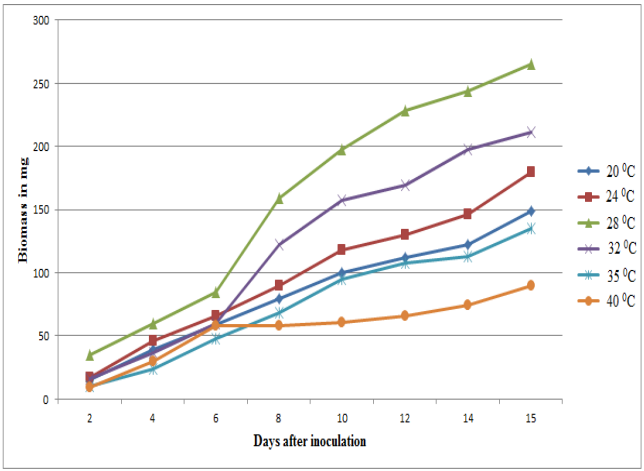
cantly with each other. Nymphs were more susceptible than adults. At six days after spraying, nymphs and adults showed sluggish movement, internal and external infection and profuse sporulation. A maximum of 93.33 % mortality of nymphs and 66.67% adult mortality was recorded at 1 x 10^{10} spores per mL concentration which were at par with other treatments. In general, an inclination in mortality was observed with advancement of time with most spore concentrations, indicating a linear positive association between mortality and days of observation. The other treatment also resulted in significantly higher mortality of *A. gossypii nymphs* and adults than the control.

Bioassays of *F. pallidoroseum* against the nymphs and adults of *A. gossypii* under laboratory conditions revealed a range of variation in their biological activity. Probit analysis of mortality data enabled calculation of the dose–response relationships for nymphs, obtaining the following equations and LC_{50} with 95% fiducial limits: y = 0.448±0.036- 2.50, x^2 = 13.067, and 3.79 x 10^5 spores/ mL (Fiducial limit 1.84 x 10^5–7.12 x 10^5).The fit of the transformed data was acceptable using the chi-squared test (Table 4).

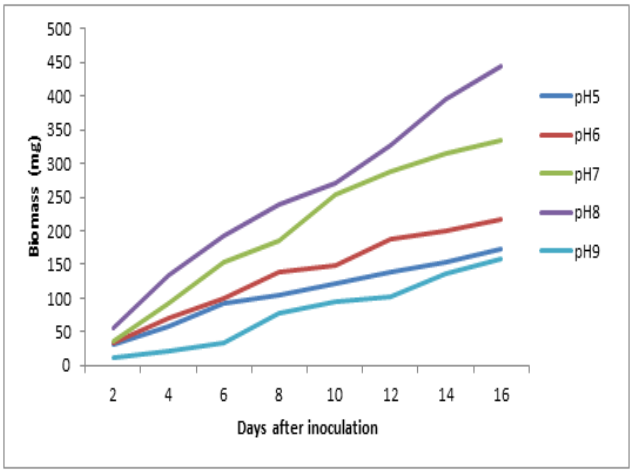
We can conclude that the isolated entomopathogen has a high potential as a biological control agent in the strategic management of *A. gossypii*. Thus, it can be

Table 4. Probit analysis of concentration-mortality response of the nymphs to *F. pallidoroseum*

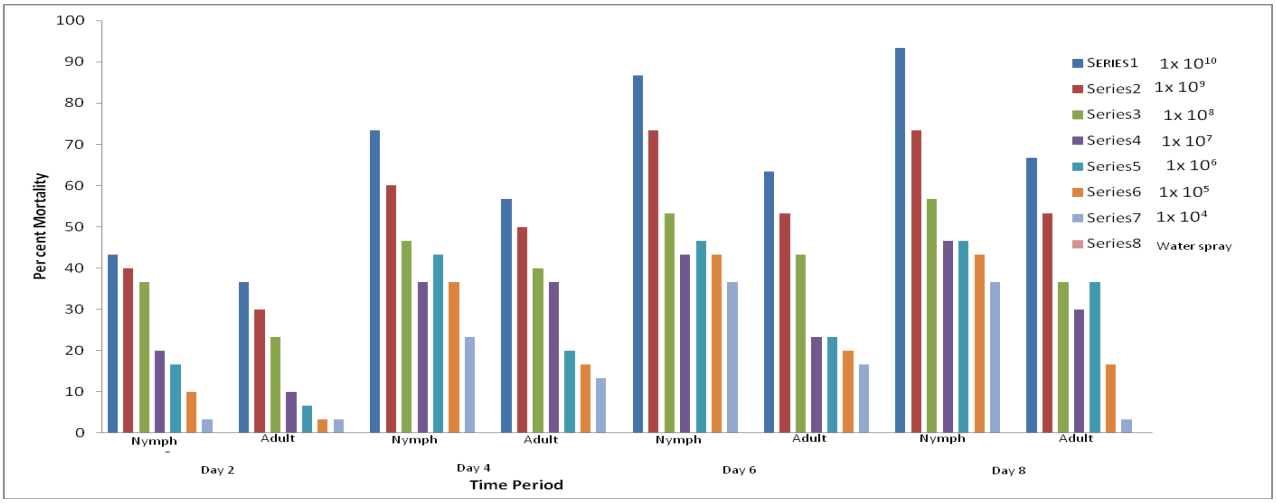
LC_{50}	Fiducial limit		LC_{90}	Fiducial limit
	Lower	Upper		Lower
$3.79x10^5$	$1.84x10^5$	$7.12x10^5$	$2.74x10^8$	$1.13x10^8$



Graph 1. *Effect of different temperature on the growth of F. pallidoroseum.*



Graph 2. *Effect of pH on the growth of F. pallidoroseum.*



Graph 3. *Efficacy of F. pallidoroseum on the nymphs and adults of A. gossypii.*

inferred that further research is needed on the possibility of developing indigenous formulation by the application of synergists or diet enhancers in increasing its virulence under field conditions.

Literature revels that the researches on *Fusarium* against insects has led to the discovery of new species (Freeman *et al*., 2013a; Aoki *et al*., 2018; Aoki *et al*., 2019; da Silva *et al*., 2020) and of numerous remarkable interactions between *Fusarium* and insects (Freeman *et al*., 2013b; Kasson *et al*., 2013; O'Donnell *et al*., 2016; Toki *et al*.,2016) demonstrating that fungi often solely studied as plant pathogens could also play supplementary roles in nature for which we don't know the biological significance.

Further, many *Fusarium* species have been recorded to be competent in controlling agricultural insect pests; causing high mortality rates and having fast action and profuse sporulation (Ganassi *et al*., 2001; Torres-Barragan *et al*., 2004; Munshi *et al*., 2008; Abdul-Wahid and Elbanna, 2012; Fan *et al*., 2015; Tosi *et al*., 2015; da Silva *et al*., 2016; Anwar *et al*., 2017; Velez *et al*., 2019; da Silva *et al*.,2020; Diniz *et al*., 2020; de Lima *et al*., 2021). Although, interactions of *Fusarium* spp as an entomopathogenic fungi have received greater attention in the recent years, but much remains to be explored as did in the current investigation.

Scanning electron microscopy

During current observation of SEM studies, appressoria formation by *F. pallidoroseum* is consistent with the

findings of Nair and Corbin (1981). Direct penetration of cuticle via infection pegs (appressoria) might also be the mode of attack, as was evident from SEM study. The observation by SEM have already been recorded against different *Fusarium spp.* Similar result in SEM study was recorded in case of *F. solani* and *F. oxysporum* with the difference lieing in size and septation of macroconidia and microconidia facilitating taxonomic classification at the species level (Ciampi *et al*., 2009; Shahnazi *et al*., 2012; Husien, 2019).

Effect of different media

Similarly, it was revealed that potato dextrose agar medium supported the best growth of *Trichoderma terrean, Colletotrichum gloeospiroides, Beaveria bassiana* and *F. pallidoroseum* (Shehu and Ibrahim, 2014). Growth characters of *F. oxysporum* f. sp. *gerberae* studied on different solid media indicated that the growth was maximum on Oat meal agar followed by Richards's agar, Czapek's Dox agar, and Potato Dextrose agar supported maximum growth of fungal colony (Rajirani et al., 2000; Rajirani 2001; Chittem and Kulkarni, 2008; Mezzomo *et al*., 2018; Westphal *et al*., 2021). These studies revealed that among the solid substrates, leafy substrates, bran and oil seed cakes, and the liquid substrates tested mature coconut water supported maximum biomass and macrconidia. Increased virulence of spore suspensions prepared from coconut media was found due to the abundance of macroconida in them.

Bio-efficacy of *F. pallidoroseum* against *Aphis gossypii*

Field experiments conducted to estimate efficacy of *F. pallidoroseum* to manage *A. craccivora* using different spore formulations discovered that 82% mortality of mite, *Calepitrimerus azadirachtae* by *F. samitectum* at 2.3 x 10^9 spores per mL (Navik *et al*., 2015). A significantly enhanced proline level in plants infected by *F. pallidoroseum* also showed their role as plant growth promoter (Srivastava *et al.,* 2011). Monga *et al*., 2010 also reported *F. pallidoroseum* to cause 80-95% mortality of *cotton mealybug, Phenacoccus solenopsis* Tinsley.

F. semitectum Berk and Ravenel was reported to be effective against many sucking pests viz., sugarcane wooly aphid, *Ceratovacuna lanigera* Zehntner (Aswini, 2007 and Nagaratha, 2004), on cowpea aphid, *A. craccivora* Koch (Roopa Rani, 2008) and tobacco aphid, *Myzus persicae* (Sulzer) (Asharani, 2009). Further, bio-efficacy of *Fusarium pallidoroseum* against cowpea sucking pests such as *Aphis craccivora* and *Riptortus pedestris* was very effective entomopathogen against *A. craccivora* and *R. pedestris* respectively and showed consistently higher mortality with increase in the exposure time; however, against *Beauveria bassiana* and *Metarhizium anisopliae* it was recorded moderately effective (Kavitha and Faizal, 2020; Singh and Kaur 2020; Tarekegn *et al.,* 2020).

Furthermore, majority of the isolates of *F. oxysporum* species complex have also been tested against Lepidoptera insects the mortality rates recorded from low to high (Ali-Shtayeh, and Jamous, 2003; Sun and Liu, 2008; Baidoo and Ackuaku, 2011), and from moderate to high against insects of the orders Coleoptera and Hemiptera (Torres-Barrag_an *et al*., 2004; Qi *et al*., 2011; Ameen, 2012; Qi *et al*., 2016; Anwar *et al.,* 2017; Sharma and Marques 2018).

These fungal bio-control agents would offer a new approach to combat natural population of insect pest, while protecting the efforts and investments of the marginal farmers (Lingappa *et al*., 2005; Freeman *et al*., 2013; Aoki *et al*., 2018; Sharma and Marques , 2018; da Silva *et al*., 2020).

Conclusion

The findings of the present investigation shows that spraying of spore formulation of *Fusarium pallidoroseum*, at 1 x 10^{10} spores/ mL conc was the most effective against *A. gossypii,* recorded 93.33% mortality of nymphs and 66.67% mortality of adults; could be used under IPM practices. Further, after multilocational field trials as well as synergistic effects of the selected strains of *F. pallidoroseum,* in combination with selected chemicals and their toxicity to the target pests (i.e. still in progress); an effective, ecofriendly, cost effective biocontrol agent could be explored for transferring the technology to the farmers.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abdul-Wahid, O.A. & Elbanna, S.M. (2012). Evaluation of the insecticidal activity of *Fusarium solani* and *Trichoderma harzianum* against cockroaches; Periplaneta America-

na. *African Journal of Microbiology Research,* 6, 1024–1032. DOI:10.5897/ajmr-11-1300.

2. Ahmad, S. & Ansari, M. S. (2013) Acute toxicity and sublethal effects of a pyrethroid (cypermethrin) on survival, development and fitness of *Helicoverpa armigera*. *Archives of Phytopathology and Plant Protection* 46, 1726–1739. https://doi.org/10.1017/S1742758413000246
3. Ali-Shtayeh, M. S., Mara'i, A. B. B. & Jamous, R. M. (2003). Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. *Mycopathologia*, 156(3), 235-244.
4. Ameen, M. K. M. (2012). Screening of *Fusarium* isolates pathogenicity *in vitro* by using the larvae of *Galleria Mellonella* L. *Journal of Basrah Researches* (Sciences), 38(3).
5. Anwar W, Haider MS, Shahid AA, Mushtaq H, Hameed U, Rehman MZU, Iqbal MJ. 2017. Genetic diversity of *Fusarium* isolated from members of *Sternor rhyncha* (Hemiptera): entomopathogens against *Bemisia tabaci*. Pakistan Journal of Zoology 49: 639–645. [Crossref], [Web of Science ®], [Google Scholar]
6. Aoki, T., Kasson, M.T., Berger, M.C., Freeman, S., Geiser, D.M. & O'Donnell, K. (2018). *Fusarium oligoseptatum* sp. nov., a mycosymbiont of the ambrosia beetle *Euwallacea validus* in the Eastern US and typification of *F. ambrosium*. *FUSE* 1, 23e39. doi: 10.3114/fuse.2018.01.03
7. Aoki, T., Smith, J.A., Kasson, M.T., Freeman, S., Geiser, D.M.,Geering, A.D. & O'Donnell, K. (2019). Three novel Ambrosia *Fusarium* Clade species producing clavate macroconidia known (*F. floridanum* and *F. obliquiseptatum*) or predicted (*F. tuaranense*) to be farmed by *Euwallacea* spp. (Coleoptera: Scolytinae) on woody hosts. *Mycologia* 1e17. https://doi.org/10.1080/00275514.2019.1647074
8. Asharani, Manjunatha, A., M., Mohan, I. Naik, Shivanna, B. K., Gayathridevi, S. & Pradeep, S. (2009). Evaluation of fungal pathogen, *Fusarium semitectum* Berk and Ravenel against tobacco aphid under laboratory and greenhouse conditions. *Karnataka Journal of Agricultural Sciences* 22 (3-Spl. Issue), 495-498.
9. Aswini, G.V., Manjunatha, M. & Mohan, I. Naik (2007). Evaluation of fungal pathogen, *Fusarium semitectum* Berk and Ravenel against sugarcane woolly aphid under laboratory and greenhouse conditions. *Karnataka Journal of Agricultural Sciences* 20(4), 767-770.
10. Baidoo, P.K. & Ackuaku, S.K. (2011). The effects of spore concentrations of entomogenous fungi on larval mortality and development of the maize stem borer Eldana saccharina Walker (Lepidoptera: Pyralidae). *J. Appl. Biosci*. 47, 3221e3229. http://hdl.handle.net/123456789/11106
11. Chittem, K. & Kulkarni, S. (2008). Effect of Media on the Growth of *Fusarium oxysporum* f. sp. *gerberae* and *Fusarium oxysporum* f. sp *dianthi. Karnataka J. Agric. Sci*. 21(2), 303-304.
12. Ciampi, L., Jaun Nissen, M., Venegas, E., Fuentes, R., Costa, M., Schobitz, R., Alvarez, D. & Alvarado Pilar (2009). Identification of two species of *Fusarium* link that cause wilting of colored callas (*Zantedeschiaa ethiopica* L. Spreng.) cultivated under greenhouse conditions in Chile. Chilean *Journal of Agricultural Research* 69(4), 516-525.
13. da Silva Santos, A. C., Diniz, A. G., Tiago, P. V. & de Oliveira, N. T. (2020). Entomopathogenic Fusarium species: a review of their potential for the biological control of insects, implications and prospects. *Fungal Biology Reviews*, 34(1), 41-57. https://dx.doi.org/10.1016/j.fbr.2019.12.002
14. da Silva Santos, A. C., Oliveira, R. L. S., da Costa, A. F., Tiago, P. V. & de Oliveira, N. T. (2016). Controlling *Dactylopius opuntiae* with *Fusarium incarnatum–equiseti* species complex and extracts of *Ricinus communis* and *Poincianella pyramidalis*. *Journal of Pest Science*, *89*(2), 539-547. https://doi.org/10.1007/s10340-015-0689-4
15. de Lima, I. J., Carneiro Leão, M. P., da Silva Santos, A. C., da Costa, A. F. & Tiago, P. V. (2021). Production of conidia by entomopathogenic isolates of Fusarium caatingaense on different vegetable substrates. *Biocontrol Science and Technology*, 31(2), 206-218.
16. European and Mediterranean Plant Protection Organization (2006). Distribution maps of Quarantine pests, *Helicoverpa armigera*. On-line Retrieved from ww.eppo.org/Quarantine/insects/*Helicoverpaarmigera*/HELIAR_ma p.ht m
17. Fan Y., Ortiz-Urquiza, A., Garrett, T., Pei1, Y. & Keyhan, N.O. (2015). Involvement of a caleosin in lipid storage, spore dispersal, and virulence in the entomopathogenic filamentous fungus, *Beauveria bassiana*. *Environmental Microbiology,* 17(11), 4600–4614. https://doi.org/10.1111/1462-2920.12990
18. Freeman, S., Protasov, A., Sharon, M., Mohotti, K.M., Eliyahu, M., Okon-Levy, N., Maymon, M. & Mendel, Z., (2013a). Obligate feed requirement of *Fusarium* sp. nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea aff. fornicata*. *Symbiosis* 58, 245e251.
19. Freeman, S., Sharon, M., Maymon, M., Mendel, Z., Protasov, A., Aoki, T., Eskalen, A., O'Donnell, K., (2013b). *Fusarium euwallaceae* sp. nov.—a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia* 105, 1595e1606. https://doi.org/10.3852/13-066
20. Ganassi, S., Moretti, A., Stornelli, C., Fratello, B., Bonvicini Pagliai, A.M., Logrieco, A. & Sabatini, M.A. (2001). Effect of *Fusarium*, Paecilomyces and *Trichoderma* formulations against aphid *Schizaphis graminum*. *Mycopathologia* 151, 131e138.
21. Ghosal, A., Chatterjee, M. L. & Manna, D. (2012). Studies on some insecticides with novel mode of action for the management of tomato fruit borer (*Helicoverpa armigera* Hub.). *Journal of Crop and Weed*, 8(2), 126-129.
22. Gonçalves Diniz, A., Barbosa, L. F. S., Santos, A. C. D. S., Oliveira, N. T. D., Costa, A. F. D., Carneiro-Leão, M. P., & Tiago, P. V. (2020). Bio-insecticide effect of isolates of *Fusarium caatingaense* (Sordariomycetes: Hypocreales) combined to botanical extracts against *Dactylopius opuntiae* (Hemiptera: Dactylopiidae). *Biocontrol Science and Technology*, 30(4), 384-395. https://doi.org/10.1080/09583157.2020.1720601
23. Husien, H. (2019). Isolation, Identification, Pathogenicity bioassay and Mass production of Indigenous Isolate of Entomopathogenic Fungi against Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) (Doctoral dissertation, Palestine Technical University-Kadoorie).
24. Hussain, A. (2007). Efficacy of some synthetic and biopesticides against pod borer. *Agricultural Research and Extension* pp 10.
25. Jarrahi, A. & Safavi, S. A. (2016). Fitness costs to *Heli-*

coverpa armigera after exposure to sub-lethal concentrations of *Metarhizium anisopliae sensu lato*: Study on F1 generation. *Journal of invertebrate pathology*, 138, 50-56. http://www.sciencedirect.com/science/article/pii/S0022201116300556

26. Javed K, Javed H, Mukhtar T & Qiu D. (2019). Pathogenicity of some entomopathogenic fungal strains to green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae). *Egypt J Biol Pest Control*. 29(92), 1–7. https://doi.org/10.1186/s41938-019-0183-z
27. Jayasimha, G.T., Rachana, R.R., Rajkumar, V.B. & Manjunatha M (2012). Evaluation of fungal pathogen, *Fusarium semitectum* Berk and Ravenel against okra aphid, *Aphis gossypii* Glover under laboratory and green house conditions. *Pest Management in Horticultural Ecosystems* 18(2), 139-142.
28. Kalvnadi, E., Mirmoayedi, A., Alizadeh, M., & Pourian, H. R. (2018). Sub-lethal concentrations of the entomopathogenic fungus, *Beauveria bassiana* increase fitness costs of Helicoverpa armigera (Lepidoptera: Noctuidae) offspring. *Journal of invertebrate pathology*, 158, 32-42. http://dx.doi.org/10.1016/j.jip.2018.08.012
29. Kasson, M.T., O'Donnell, K., Rooney, A.P., Sink, S., Ploetz, R.C.,Ploetz, J.N., Konkol, J.L., Carrillo, D., Freeman, S., Mendel, Z.,Smith, J.A., Black, A.W., Hulcr, J., Bateman, C., Stefkova, K.,Campbell, P.R., Geering, A.D.W., Dann, E.K., Eskalen, A., Mohotti, K., Short, D.P.G., Aoki, T., Fenstermacher, K.A., Davis, D.D. & Geiser, D.M., (2013). An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus Euwallacea on avocado and other plant hosts. *Fungal Genet. Biol*. 56, 147e157. https://doi.org/10.1016/j.fgb.2013.04.004
30. Kavitha, S.J. & Faizal MH (2020). Bio-efficacy of entomopathogens on major sucking pests in cowpea (Vigna unguiculata L.). *Journal of Entomology and Zoology Studies*, 8(4), 694-698.
31. Khating SS, Kabre GB & Dhainje A.A. (2016). Seasonal incidence of sucking pests of okra along with natural enemies in Khandesh region of Maharashtra. *Asian Journal of Biosciences,* 11(2), 269-272. DOI : 10.15740/HAS/AJBS/11.2/269-272.
32. Lingappa, S., Saxena, H. & Devi Vimala, P.S. (2005). Role of biocontrol agents in management of *Helicoverpa armigera* (Hubner). In: Hem Saxena, A. B. Rai, R. Ahmad and Sanjeev Gupta eds. Recent advances in *Helicoverpaarmigera* Management. Indian Society of Pulses Research and development, IIPR, Kanpur, pp 159-184.
33. Litwin, A., Nowak, M. & Rozalska, S. (2020) Entomopathogenic fungi: unconventional applications. *Rev Environ Sci Biotechnol* 19, 23–42. https://doi.org/10.1007/s11157-020-09525-1
34. Mehrvar, A., Rabindra, R.J., Veenakumari, K. & Narabenchi, G.B. (2008). Molecular and biological characteristics of some geographic isolates of nucleopolyhedrovirus of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Entomological Society of Iran* 28, 39-60.
35. Mezzomo, R., Rolim, J.M., Poletto, T., de Oliveira, M.B., Lazarotto, M., Fátima, M. & Brião Muniz (2018). Mycelial growth and sporulation of fusarium spp. pathogenic to ilex paraguariensis in different culture media and under exposure to different light levels. *Scientia Agraria* 19(1),14. DOI: 10.5380/rsa.v19i1.55844.
36. Mishra, K., Singh, K. & Tripathi CPM (2013). Management of pod borer (*Helicoverpa armigera*) infestation and productivity enhancement of gram crop (*Cicer aritenium*) through vermiwash with biopesticides. *World Journal of Agricultural Sciences,* 9(5), 401-408. DOI: 10.5829/idosi.wjas.2013.9.5.1749
37. Mohammed AA, Kadhim JH & Kamaluddin ZNA (2018). Selection of highly virulent entomopathogenic fungal isolates to control the greenhouse aphid species in Iraq. *Egypt J Biol Pest Control* 28(71), 1–7. https://doi.org/10.1186/s41938-018-0079-3
38. Monga, D., Kumhar, K.C. & Kumar, R. (2010). Record of *Fusarium pallidoroseum* (Cooke) Sacc. on Cotton Mealybug, *Phenacoccus solenopsis* Tinsley. *Journal of Biological Control,* 24(4), 366–368. https://doi.org/10.18311/jbc/2010/3588
39. Mora MAE, Castilho AMC & Fraga ME (2017). Classification and infection mechanism of entomopathogenic fungi. *Arq Inst Biol* 84, 1–10. https://doi.org/10.1590/1808-1657000552015
40. Munshi, N.A., Barkat, Hussain, Malik, G.N., Musavir, Yousuf. & Fatima, N. (2008). Efficacy of entomopathogenic fungus *Fusarium pallidoroseum* (Cooke) Sacc. against Gypsy moth (*Lymantria obfuscate* Walker). *Journal of Entomology* 5, 59-61. DOI: 10.3923/je.2008.59.61
41. Nagamandla, R.S., Jha, S. & Latha N.S. (2017) Insect pests of tomato and their weather relations under open and cover cultivation. *Int J Curr Microbiol Sci* 6(9), 368–375. https://doi.org/10.20546/ijcmas.2017.609.046
42. Nair, J. & Corbin, J.B. (1981). Histopathology of *Pinusradiata* seedling infected by *Colletotrichumacutatum* f. sp. *pinea*. *Phytopathology* 71(8), 777–783.
43. Navik, O.S., Manjunatha, M., Kumaraswamy, M.C. & Latha, M. (2015). Efficacy of entomoathogenic fungi and acaricidal molecules on mite, *Calepitrimerus azadirachtae* Channa Basavanna (Acari: Eriophyidae) on neem. *Journal of Eco-friendly Agriculture* 10(1), 53-57.
44. Nazir, T., Basit, A., Hanan, A., Majeed, M.Z. & Qiu, D. (2019). *In vitro* pathogenicity of some entomopathogenic fungal strains against green peach aphid *Myzus persicae* (Homoptera: Aphididae). *Agron,* 9(7), 1–12. https://doi.org/10.3390/agronomy9010007.
45. O'Donnell, K., Libeskind-Hadas, R., Hulcr, J., Bateman, C.,Kasson, M.T., Ploetz, R.C., Konkol, J.L., Ploetz, J.N., Carrillo, D.,Campbell, A., Duncan, R.E., Liyanage, P.N.H., Eskalen, A.,Lynch, S.C., Geiser, D.M., Freeman, S., Mendel, Z., Sharon, M., Aoki, T., Coss_e, A.A. & Rooney, A.P. (2016). Invasive Asian *Fusariume Euwallacea ambrosia* beetle mutualists pose a serious threat to forests, urban landscapes and the avocado industry. *Phytoparasitica* 44, 435e442. https://doi.org/10.1007/s12600-016-0543-0
46. Patel DR & Purohit MS.(2013). Influence of different weather parameters on aphid, Melanaphis sacchari infesting kharif Sorghum. *International Journal of Plant Protection* 6(2), 484-486. http://www.researchjournal.co.in/online/IJPP.htm
47. Pawar, V.M. & Borikar, P.S. (2005). Microbial options for the management of *Helicoverpaarmigera* (Hubner) In: "Recent Advances in *Helicoverpa* Management (Hem Saxena, A. B. Rai, R. Ahmad and Sanjeev Gupta eds.) Indian Society of Pulses Research and Development,

IIPR, Kanpur, pp 193-231.
48. Qi, H., Wang, J., Endoh, R., Takeuchi, Y., Tarno, H. & Futai, K. (2011). Pathogenicity of microorganisms isolated from the oak platypodid, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). *Appl. Entomol. Zool*. 46, 201e210.
49. Qi, J., Aiuchi, D., Tani, M., Asano, S. I. & Koike, M. (2016). Potential of entomopathogenic *Bacillus thuringiensis* as plant growth promoting rhizobacteria and biological control agents for tomato Fusarium wilt. *Int J Environ Agric Res*, *2*(6), 55-63.
50. Rajirani, O.P. (2001). Production and evaluation of the fungus, *Fusarium pallidoroseum* (Cooke) Sacc. As biopesticide against pea aphid, *Aphis craccivora* Koch. *PhD thesis*. Kerala Agricultural University, Thrissur, pp. 136.
51. Rathee, M. & Dalal, P. (2018). Emerging Insect Pests in Indian Agriculture. Indian Journal of Entomology, 80(2), 267-281. DOI No.: 10.5958/0974-8172.2018.00043.3
52. Rejirani, O.P., Mathai, S. & Peethambaran, C.K. (2000). Evaluation of different naturally available substrates for mass production of the entomopathogenic fungus, *Fusarium pallidoroseum* (Cooke) Sacc. In: Proceedings of the 12th Kerala Science Congress, Thiruvananthpuram, pp. 27-29.
53. Rombach, M.C., Aguda, R.M., Shepard, B.M. & Roberts, D.W. (1986). Infection of rice brown plant hopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by field application of entomopathogenic Hyphomycetes (Deuteromycot ina). *Environmental Entomology* 15, 1070-1073. DOI:10.1093/EE/15.5.1070
54. Roopa Rani, V. (2008). Bio ecology of aphid, *Aphis craccivora* Koch and evaluation of fungal pathogen, *Fusarium semitectum* Berk and Ravenel against cowpea aphid. *M. Sc. (Agri.) Thesis*. University of Agricultural Sciences, Bangalore (India)
55. Statistical Analysis System (2002). SAS Software: Version 9.1.SAS Institute, Cary, NC
56. Sasidharan, K.R. & Varma, R.V. (2005). Laboratory evaluation of *Beauveria bassiana* (Balsamo) Vuillemin against *Indrabela quadrinata* Walker (Lepidopteron: Metarbelidae) a key pest of *Casuarina equisetifolia* L. in Tamil Nadu. *Journal of Biological Control,* 19, 197-200
57. Shahnazi, S., Meon, S., Vadamalai, G., Ahmad, K. & Nejat, N. (2012). Morphological and molecular characterization of Fusarium spp. associated with yellowing disease of black pepper (Piper nigrum L.) in Malaysia. *J Gen Plant Pathol* 78, 160–169. DOI 10.1007/s10327-012-0379-5.
58. Sharma, L., & Marques, G. (2018). *Fusarium*, an entomopathogen—A myth or reality?. *Pathogens*, *7*(4), 93. doi:10.3390/pathogens7040093
59. Shehu, K. & Ibrahim, M. (2014). Influence of culture media and light regimes on the growth of *Helminthosporium fulvum*. *The Experiment* 27(2), 1863-1866.
60. Shophiya, J., Nancy, Sahayaraj, K., Kalaiarasi, J.M.V. & Shirlin Jebitta M. (2014). Biocontrol potential of entomopathogenic fungus *Beauveria bassiana* (balsamo) against *Pericalliaricini* (Fab.) (Lepidoptera: Arctiidae) larvae. *Biolife* 2(3), 813-824
61. Singh, H. & Kaur, T. (2020). Pathogenicity of entomopathogenic fungi against the aphid and the whitefly species on crops grown under greenhouse conditions in India. *Egypt J Biol Pest Control* 30 (84). https://doi.org/10.1186/s41938-020-00287-0
62. Singh, N. & Dhiman S. (2018). Quality and quantity loss by aphid infestation in vegetable crops grown under protected cultivation in Ladakh region. *Defense Life Science Journal*, 3(1), 71-74. DOI : 10.14429/dlsj.3.11516
63. Srivastava, S., Singh, V.P., Kumar, R., Srivastava, M., Sinha, A. & S Simon (2011). In vitro evaluation of carbendazim 50% WP, antagonists and botanicals against Fusarium oxysporum f. sp. psidii associated with rhizosphere soil of guava. Asian Journal of Plant Pathology, 5 (1), 46-53. http://dx.doi.org/10.3923/ajppaj.2011.46.53
64. Sun, B. D., & Liu, X. Z. (2008). Occurrence and diversity of insect-associated fungi in natural soils in China. *Applied soil ecology*, 39(1), 100-108.
65. Tarekegn Fite, Tadele Tefera, Mulugeta Negeri, Tebekew Damte & Waktole Sori (2020) Evaluation of *Beauveriabassiana, Metarhiziumanisopliae* and *Bacillus thuringiensis* for the management of *Helicoverpaarmigera* (Hubner) (Lepidoptera: Noctuidae) under laboratory and field conditions, *Biocontrol Science and Technology*, 30(3), 278-295, DOI: 10.1080/09583157.2019.1707481
66. Toki, W., Kawakita, A. & Togashi, K. (2016). Presence of weed fungus in a non-social beetleefungus cultivation mutualism. *Ecol. Entomol*. 41, 253e262. DOI: 10.1111/een.12293
67. Torres-Barrag_an, Anaya, A.L., Alatorre, R., Toriello, C. (2004). Entomopathogenic fungi from 'El Eden' Ecological Reserve, Quintana Roo, Mexico. *Mycopathologia* 158, 61e71. https://doi.org/10.1023/B:MYCO.0000038424.013 6 9 .c 8
68. Tosi, L., Beccari, G., Rondoni, G., Covarelli, L. & Ricci, C. (2015). Natural occurrence of *Fusarium proliferatum* on chestnut in Italy and its potential entomopathogenicity against the Asian chestnut gall wasp *Dryocosmus kuriphilus*. *J. Pest. Sci.* 88, 369e381. https://doi.org/10.1007/s10340-014-0624-0
69. Velez, de A.,, Augusto, B., Diniz, A. G., Barbosa, L. F. S., da Silva Santos, A. C., da Costa, A. F., & Tiago, P. V. (2019). Potential of *Fusarium incarnatum-equiseti* species complex isolates with *Chenopodium ambrosioides* and *Enterolobium contortisiliquum* extracts to control *Dactylopius opuntiae*. *International Journal of Tropical Insect Science*, 39(2), 131-138. https://doi.org/10.1007/s42690-019-00014-9
70. Westphal, K.R., Heidelbacha, S., Zeuner, E.J., Jensena, M.R., Nielsen, M.E., Vestergaarda, S.Z., Bekker, N.S., Skovmark, J., Olesen, C.K., Thomsen, K.H., Niebling, S.K., Sørensen, J.L. & Sondergaard, T.E. (2021). The effects of different potato dextrose agar media on secondary metabolite production in *Fusarium*. *International Journal of Food Microbiology* 347(2).00.000. https://doi.org/10.1016/j.ijfoodmicro.2021.109171
71. Yaqoob Munazah, Qurat ul Ain & Ayoub Liyaqat (2019). Seasonal incidence of aphid, *Aphis gossypii* infesting okra and its relation with weather parameters. *Journal of Entomology and Zoology Studies*, 7(4), 672-674.