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Induced host plant resistance in cauliflower by *Beauveria bassiana*

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Abstract
Endophytic fungi, which live within host plant tissues asymptomatically, are important mediators of plant-herbivore interactions. We tested whether *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), an entomopathogenic fungus could colonize the cauliflower (*Brassica oleracea* L. var. *botrytis*) after fungal spores suspension applied as foliar spray. Further, we assessed whether the oviposition behavior and development of *Plutella xylostella* L. (Lepidoptera: Plutellidae) was affected by endophytic *B. bassiana*. After inoculation, re-isolation of fungus on selective media revealed that colonization of cauliflower by *B. bassiana* increased consistently with the time but after reaching a threshold level of 70%, rate of colonization became constant. Sequence characterized amplified region (SCAR) marker assay was used to identify the presence of *B. bassiana* in treated plant samples and a nucleotide BLAST search of the endophytic fungus showed 100 % similarity with the *B. bassiana* sequence. Scanning electron microscope (SEM) images of the treated leaf surface revealed that although numerous conidia were present only few actually germinated. SEM and light microscopy of inner surface of treated leaf epidermis showed the random and crisscross growth of fungal hyphae. The secondary metabolite profiles of treated, control and pure fungus culture from ethyl acetate extracts were investigated using gas chromatography-mass spectrometry. Chromatographic separation yielded 9, 9 and 12 compounds in treated, control and pure culture of *B. bassiana* extracts respectively. In the laboratory experiments, *P. xylostella* did not show any preference in laying eggs on the treated and untreated cauliflower plants. However, no larvae were able to develop on treated leaf.

Keywords: Beauveria bassiana; Cauliflower; Endophytes; Biological control; Plutella xylostella

1. Introduction
The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a worldwide pest of Brassica crops [1]. According to a study by Zalucki *et al*. [2], The annual cost of controlling *P. xylostella* in Brassica vegetables is US $1.4 billion worldwide, rising to US $2.7 billion if yield losses due to *P. xylostella* are taken into account and to US $4-5 billion if losses and control costs of *P. xylostella* in the canola industry are included. In India, a loss of US $ 16 million every year was recorded due to diamondback moth damage [3]. *Plutella xylostella* has a long history of becoming resistant to insecticides, beginning with DDT in 1950 [4]. Since then, no new product has remained effective for more than a few years when applied intensively [5]. However, indiscriminate and intensive use of insecticides has led to the destruction of natural enemies from Brassica crop agro-ecosystem. Since single control tactic is bound to failure, development of integrated pest management program underpinned by natural enemies is desirable.
Cauliflower (*Brassica oleracea* var. *botrytis*) is the major vegetable produced and consumed in India. In 2012, India planted cauliflower and broccoli in 38 thousand ha and produced around 7 million Metric tons of cauliflower and broccoli and stood second only to China in total production [6]. Majority of cauliflower crop farms are small and situated near to the metropolitan cities owing to high demands for fresh vegetables in these cities. Due to the consumer preference for immaculate cauliflower curd, farmers usually spray pesticides extensively to meet the market expectations [1]. Pesticides residues in the vegetables pose serious health risk.
Endophytes are the microorganisms that can colonize the plant asymptomatically. Several studies have reported that the entomopathogenic fungus *Beauveria bassiana* can survive as an endophyte in several plant species, viz., maize [7-15], tomato [16], *Theobroma gileri* [17], banana [18], coffee [19, 20], cocoa [21], date palm [22], in the bark of *Carpinus caroliniana* [23], in seeds and needles of *Pinus monticola* [24], and in opium poppies [25].

Studies have demonstrated that endophytic *B. bassiana* can act as a guard for the plant by producing an array of different bioactive metabolites that provide protection to the plant from different pests. Bing and Lewis [8]. in 1991 reported that artificial injection of granular formulation of *B. bassiana* into the corn stem conferred the season-long suppression of corn borer. Akello *et al.* [18]. in 2007 reported that endophytic *B. bassiana* in banana significantly reduced larval survivorship of banana weevil, *Cosmopolites sordidus*.

Several studies have been done to evaluate the efficacy of introduced endophytic fungi against foliar pest *P. xylostella*. The unspecialized endophytic fungi *Acremonium alternatum*, causes significant increase in mortality and reduced relative growth rates of *P. xylostella* larvae feeding on the cabbage leaf inoculated with *A. alternatum* [26]. Similarly, Batta [27], Reported the ability of *Metarhizium anisopliae* (Metch.) Sorokin (Ascomycota: Hypocreales) to internally colonize the *Brassica napus* plants and efficacy of endophytic *M. anisopliae* against the *P. xylostella*. Lohse *et al.* [28]. in 2015 evaluated different fermentation and formation strategies for better establishment of *Beauveria bassiana* as an endophyte in oilseed rape plants, one of the preferred hosts of *P. xylostella*. Current available knowledge on beneficial brassica endophytes and their prospects in the development of additional protective traits in brassica crops plants have been reviewed [29]. Considerable work has been done to use *B. bassiana* against *P. xylostella* through foliar applications [30-32]. However, to the best of our knowledge, no study has been carried out to evaluate the efficacy of endophytic *B. bassiana* against *P. xylostella*. Therefore, in the present study we first used microbiological, molecular, light and electron microscopic, and GC-MS techniques to verify the internal colonization of cauliflower by *B. bassiana*. We then examined the effectiveness of endophytic *B. bassiana* against *P. xylostella*.

2. Material and Methods

2.1 Insects

Pupae of *P. xylostella* were collected from the cauliflower fields planted in the horticulture farm, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. At the time of these studies, the colonies of *P. xylostella* had been maintained on cauliflower leaves for 37 generations at ambient temperature (mention temp- 28±2 °C) at the insectary, Department of Agricultural Entomology, TNAU.

2.2 Plants

Cauliflower seeds (*B. oleracea* L. var. *botrytis*, Shobha F1, East-West Seed International, India) were surface sterilized by immersing the seeds in 2% sodium hypochlorite for 2 min, followed by 2 min in 70% ethanol and then rinsing in sterile distilled water three times. In order to evaluate the efficiency of sterilization 100 µl of last rinsing water was transferred on potato dextrose agar (PDA) and plate was incubated for 10 days at 25°C. Three sterilized seeds were sown in 1.5 L plastic pots containing autoclaved mixture of soil, vermicompost and coir peat in the ratio 1:1:2 and placed in green house. One week after germination, two comparatively weak seedlings were removed and single seedling was maintained under greenhouse at 20–25°C, 60–80% RH, and with a 12-h photoperiod conditions until used. There were total 120 seedling pots, arranged in completely randomized design.

2.3 Fungus

B2 isolate of *B. bassiana* used in this study was obtained from the Department of Plant Pathology, TNAU. The virulence of obtained fungal culture was verified by passing it through *P. xylostella*. A monosporic mother culture of the B2 isolates was maintained on selective potato dextrose agar (PDA) slant at 25^0C in the dark. Selective media was prepared by adding cetyl trimethyl ammonium bromide (CTAB) as reported by Posada *et al*. [33]. and 1ml antibiotic solution per liter. The antibiotic stock solution consisted of 0.02 g of each of three antibiotics (tetracycline, streptomycin, and penicillin) dissolved in 10 ml of distilled water [34]. To obtain a spore suspension, the isolate from the mother culture was grown on selective PDA Petri plates for 15 days at 25^0C in the dark. Conidia from 15-day-old cultures were harvested by scraping conidia from Petri plates into an aqueous sterile solution of 0.002% Tween 80. The obtained conidial suspensions were filtered through several layers of cheesecloth to remove mycelium mats and with the help of hemocytometer, the final concentration was adjusted to $2X10^8$ conidia/ ml. To assess the viability of obtained conidia, 10 µl of 10 times diluted conidia suspension was inoculated on the surface of selective PDA plate kept at 25 ^{0}C. After 48h, the percent of germinating conidia were counted under the dissecting microscope on a randomly selected area of the plate. Any conidia solution having germination below 90% was not used in the bioassays.

2.4 Inoculation of cauliflower plants

Colonization of cauliflower by *B. bassiana* was determined after 2, 4, 7, 10, 12 and 14 days of inoculation. Randomly selected seedlings were treated with conidial suspension or with water containing 0.002% Tween 80. In all the bioassays, cauliflower plants at two true leaves stage were inoculated by spraying the adaxial surface of the fully expanded leaves to run-off with a conidial suspension ($2X10^8$ conidia/mL) containing 0.002% Tween 80 using a hand atomizer. The control plants were sprayed with water containing 0.002% Tween 80. After spraying all individual plants were covered with clear plastic cover for 24h to maintain relative humidity. After 2, 4, 7, 10, 12 and 14 days of inoculation treated plants were uprooted, cleaned and surface sterilized in 70% ethyl alcohol (2 min), followed by 2% sodium hypochlorite solution ($NaClO_3$) (2 min), and rinsed thrice with sterile distilled water. Both control and treated leaves were excised, chopped into small pieces ($1mm^2$) and approximately 40 equal leaf pieces obtained from each seedling were plated onto the individual selective PDA media plate. After 2 weeks, presence of *B. bassiana* was confirmed by the presence of mycelia and conidia emerging from the plant tissues and percentage of colonization was calculated based on the number of leaf samples showing fungal out growth to the total number of leaf pieces. There were 5 treatment and 5 control replicates at every observation interval.

2.5 Determination of endophytic colonization by molecular, light and electron microscopic techniques

To observe the germinating conidia on the leaf surface, two days post inoculation, sprayed leaf was observed under the conventional SEM (JEOL.JSM-35) at 20 kV. In order to observe the endophytic hyphae of *B. bassiana* in sprayed leaves, leaf samples were observed 7 days after inoculation. The leaf tissue was soaked in 10% potassium hydroxide (KOH) solution overnight to soften and clear the tissue. Using forceps and razor blade upper epidermis of the leaf tissue was peeled. The inner surface of the obtained peel was coated with gold particles in a Polaron E5100 sputter coating unit. Photographs were taken with a JEOL. JSM-35 SEM at 20 kV.

In case of light microscopy, epidermal peel was obtained as specified above. The peeled epidermis was placed on a microscope slide and stained with aniline blue solution (0.325 g aniline blue, 100 ml water, 50 ml water, 50 ml 85% lactic acid). The slide was heated for 30 sec. on low flame spirit lamp, covered and observed under a light microscope at 100X magnification.

For molecular studies, 7 days post inoculation, DNA was extracted from *B. bassiana* treated, untreated cauliflower leaves (negative control) and pure *B. bassiana* culture used in treating cauliflower (positive control) by CTAB method [35]. In order to verify the accuracy of surface sterilization of treated leaves, water obtained from last rinsing of treated leaf was also subjected to PCR analysis. Extracted DNA was subjected to PCR amplification using *B. bassiana* specific SCAR primers [36]. The primers used were SCA 15441 F5' TTCCGAACCCGGTTAAGAGAC and SCA 15441 R 3' TTCCGAACCCATCATCCTGC. The PCR profile used was 5 min. initial denaturation at 94 ^{0}C; 40 cycles of denaturation at 94 ^{0}C for 1 min., annealing at 60 ^{0}C for 1 min, elongation at 72 ^{0}C for 2 min and a final elongation at 72 ^{0}C for 10 min. Following PCR amplification, an aliquot (5 µl) of PCR reaction mixture from each sample was electrophoresed on a 1.5% agarose gel, visualized by ethidium bromide staining and scored by comparison to a 100 kb DNA ladder. The gel was viewed on a transilluminator to identify samples with amplification products. PCR product of DNA obtained from the surface sterilized treated leaf and conidia of mother culture was sequenced. The obtained nucleotide sequences were compared with other *B. bassiana* species using Blast search from the NCBI Genbank database and the sequence was deposited in GenBank (Accession Number KR 363631).

2.6 Comparison of secondary metabolite profile of treated leaf, untreated leaf and mother culture of *B. bassiana* through GC-MS

Seven day post inoculation, one control or treated seedling was randomly selected and surface sterilized as discussed above. Leaf samples were freeze dried at -20 ^{0}C. Total of 2 g freeze dried samples were grounded in mortar and pestle in 20 ml of ethyl acetate. The mixture was shaken (150 rpm) at room temperature on an orbital shaker for 24 h. Ethyl acetate extracts were transferred into Eppendorf tubes and centrifuged at 5000 rpm for 10 min. 1 ml of supernatant was collected and filtered through 0.45 µm filter into GC vial for gas chromatography-mass spectrometry (GC-MS) analysis (Shimadzu GCMS 2010 QP PLUS). For analysis of *B. bassiana* metabolites, 2g conidia of *B. bassiana* were harvested by scraping conidia from one week old Petri plates into 20 ml ethyl acetate and analyzed as described above.

2.7 Insect Bioassay

In order to assess the implications of endophytic *B. bassiana* on *P. xylostella* 30 control or treated plants were treated in the same way as described above. Seven days post treatment, one treated and control plant was randomly selected and was excised from the base of the plant near the soil surface and surface sterilized, as described above. After drying the sterilized plants in the laminar flow hood for 3 hours, one leaf from the plants were placed upright in 25ml conical flask, filled with distilled water. Two flask containing inoculated or control leaves were placed inside the screened cage (0.3 X 0.3 X 0.3 m). Two pairs of newly emerged *P. xylostella* adults (2 males and 2 females) were released into screened cages. After 24 h, the number of eggs laid on each leaf were recorded. Egg bearing leaf replicates were used to determine larval survival at 96h and 120 h. Experiment was replicated 15 times.

2.8 Statistical analyses

Data from the bioassays to compare the frequency of colonization of cauliflower by *B. bassiana,* was arcsine transformed before conducting ANOVA analysis and Tukey's test was used to make multiple comparisons of the mean. Data from laboratory assessment of endophytic *B. bassiana* against *P. xylostella* were analyzed using the Student's *t*-test. Data for all statistical calculations were performed with the R version 2.15.1 package [37]. Mean in all analyses were separated at, $\alpha = 0.05$.

3. Results

3.1 Inoculation of cauliflower plants

Beauveria bassiana was re-isolated from all cauliflower leafs that were inoculated with the fungal suspension of $2X10^8$ spores/ml (Fig. 1A). The percentage (mean ±standard error) of leaf pieces showing fungal growth when placed on *B. bassiana* selective medium was 30±2.24 at 2nd day, 44.4± 1.40 at 4thday, 70.75± 2.58 at 7th day, 71.4± 3.50 at 10th day, 70.8±2.24 at 12th day and 70.2± 3.68 at 14th day after treatment (Table 1). In the intervals after 7th day, percentage of re-isolation of the fungus was significantly (Tukey test, $P<0.05$) higher than the previous sampling intervals. Leaf pieces from controls did not exhibit any sign of *B. bassiana* growth when placed on *B. bassiana* selective medium.

Table 1: Colonization of Cauliflower leaves by *B. bassiana*

Number of days	% colonization by *B. bassiana*
2	30.00±2.24 a
4	44.40±1.40 b
7	70.75± 2.58 c
10	71.40±3.50 c
12	70.80±2.24 c
14	70.20± 3.68 c

Means± (SE) followed by the same alphabet are not significantly different ($P<0.05$, n=5), (n= sample size)

3.2 Determination of endophytic colonization by molecular, light and electron microscopic techniques

Fungal conidia present on the leaf surface germinated after rehydration. Fig.1C illustrates early germination and the formation of a germ tube from a single conidium. SEM images revealed that although numerous conidia were observed on the leaf surface, only few actually germinated (Fig.1 B). Conidia germinating on stomata were also observed (Fig. 1D). Examination of hyphae on the inner surface of epidermis through LM and SEM of the leaf showed that typically hyphae were growing parallel to the plant cell wall but in few cases, they penetrated the cell. (Fig. 1E, F, G).

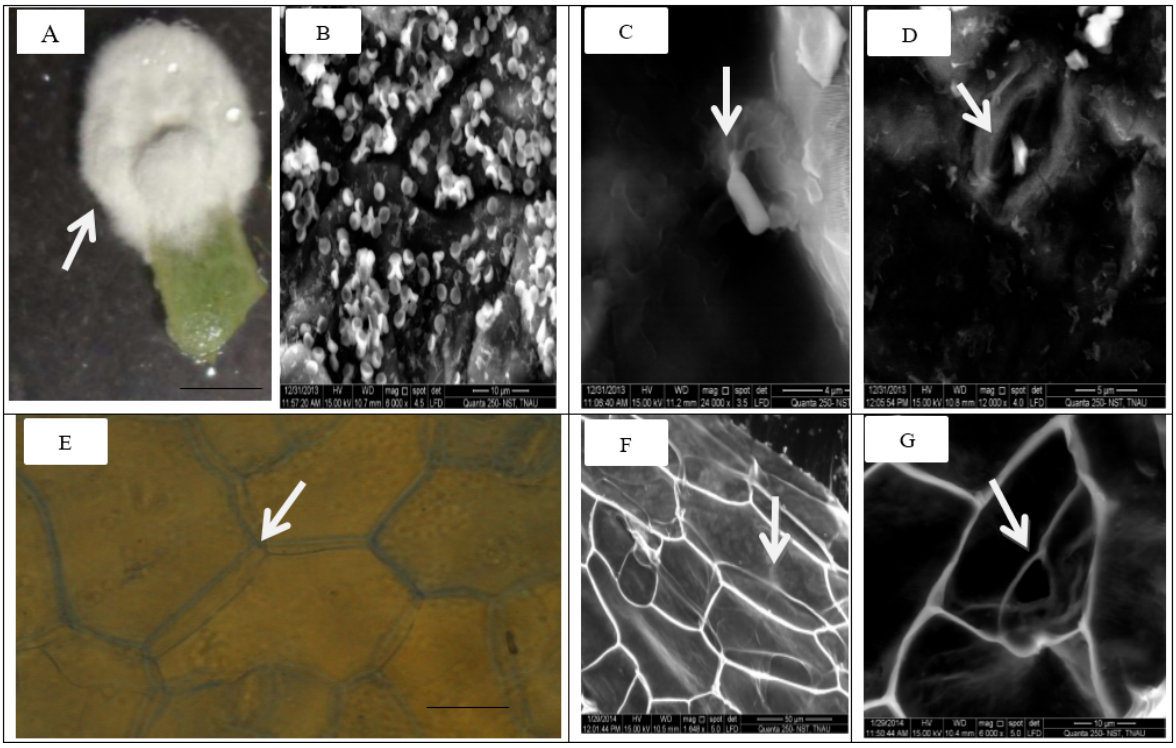

Fig 1: A. Re-isolation of *B. bassiana* from leaf fragments of treated cauliflower on selective PDA plate (arrow) bar, 20µm; B. leaf surface and *B. bassiana* conidia, C. Germinating conidia with very short germ tube at penetration site; D. Germinating conidia on stomata; E. Light micrograph of a cauliflower leaf epidermal cells showing hyphae of *B. bassiana* (arrow) bar, 20µm; F & G Electron micrograph of the inner surface of leaf epidermis showing *B. bassiana* hyphae (arrow head)

PCR amplification of total genomic DNA obtained from the treated and mother culture *B. bassiana* yielded clear, consistent, and discrete banding patterns corresponding to *B. bassiana*. However, no bands corresponding to *B. bassiana* were detected in control plants and water (Fig 2).

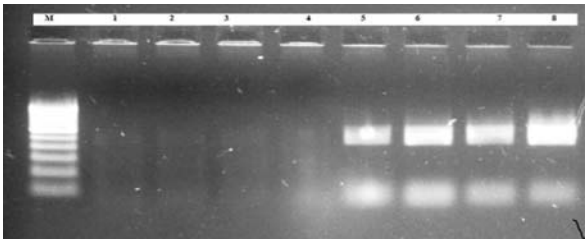

Fig 2: PCR amplification using primers specific for *B. bassiana* gene. Lane M-100 bp DNA ladder, lane 1and 2, Control untreated plant leaf, 3 and 4 water obtained from last rinsing while surface sterilization of treated leaf, lanes 5 and 6 DNA obtained from the surface sterilized treated leaf and lane 7 and 8 DNA obtained from conidia of mother culture, *B. bassiana*

3.3 Comparison of secondary metabolite profile of treated leaf, untreated leaf and mother culture of *B. bassiana* through GC-MS

The number of compounds detected from mass spectra analysis showed nine, nine and twelve Compounds in treated, control and pure culture of *B. bassiana* respectively (Table 2).

3.4 Laboratory assessment of endophytic *B. bassiana* against *P. xylostella*

In the oviposition choice test, the number of *P. xylostella* eggs laid on treated or untreated control plants were not significantly different (t = -0.14, df = 26.56, p-value = 0.89) (Table 3). However, only 7 larvae reared on treated cauliflower leaves survived after 96h and none survived after 120 h, whereas on control cauliflower leaves on an average 16 larvae survived after 96h and 15 after 120h (Table 3).

Table 2: List of the metabolites identified in 100% ethyl acetate extracts of *B. bassiana*, inoculated and control plants and pure culture *B. bassiana*.

sample	Peak	Retention time (min)	Area (mV*min)	Height (mV)	Name of compound
Inoculated leaf extracts	1	36.674	691234	105022	2,6,10- Trimethyl, 14-ethylene-14-pentadecene
	2	37.236	84063	14767	Pentadecanal
	3	37.684	248734	31872	3,7,11,15- Tetramethyl 1,2-hexadecen-1-ol
	4	45.792	945962	46229	Heptane,1,1'-oxybis-
	5	47.317	1155713	94182	v 2-tert-Butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzenel) phenol
	6	47.925	508074	42187	Hexadecanoic acid, dodecyl ester
	7	48.627	1469628	394391	1,2-Benzenedicarboxylic acid
	8	49.682	306566	46333	Teracontane-1,40-diol
	9	50.474	2038146	105614	Behenic acid, cyanomethyl ester
Control	1	36.674	727487	125252	2,6,10-trimethyl,14-ethylene-14- pentadecene

leaf extracts	2	37.236	130558	22309	Pentadecanal
	3	37.684	223067	36861	3,7,11,15- Tetramethyl 1,2-hexadecen-1-ol
	4	38.217	1224985	112961	N,N-dimethyl-1- nonadecanamine
	5	43.568	3457661	117439	1H-Purin-6-amine,((2-fluorophenyl), methyl)
	6	46.727	5296901	406692	1,22-Docosanediol
	7	47.93	3289590	269932	Tetrakis (2,3-ditert-butylphenyl-)4,4-biphenylene diphosphonate
	8	48.625	1699237	438097	1,2 Benzenedicarboxylic acid
	9	50.52	9167535	709874	Tetracontane
Beauveria bassiana extracts	1	39.389	15098594	450100	p ,p-dioctyldiphenylamine
	2	40.92	17120963	515032	Tetratriacontane
	3	46.929	2445634	252598	Octacosane
	4	47.214	1628911	178055	4a, 7,7,10a Tetramethyldodecahydrobenzo(f)chromen-3-ol
	5	47.558	4709773	555646	Tetratetracontane
	6	47.85	8341929	1754615	Tetracontane
	7	47.966	2840861	782715	1H-Purin-6-amine,((2-fluorophenyl), methyl)
	8	48.638	1118999	307719	1,2-Benzenedicarboxylic acid, diisooctyl ester
	9	49.547	571021	81287	Tetracosane,3-ethyl
	10	49.662	1067556	168115	Dotriacontane
	11	50.108	2017298	184710	15-Isobutyl-(13.alpha.H)-isocopalane
	12	50.746	12472698	1984927	Tetracontane

Table 3: Ovipositional preference and larval survival of *P. xylostella* on *B. bassiana* inoculated and unsprayed Control cauliflower leaves in the laboratory

Plant type	n	Mean (± SE) number of eggs laid per leaf during 24 h	Mean (±SE) total larvae surviving per leaf after (a*)	
			96 h	120h
Treatment	15	20.6± 2.00 a	7.0±0.45 a	0.00 a
Control	15	21.5± 2.70 a	16±.87 b	15±1.20 b

Numbers followed by different letters in the same column are significantly different using paired t test (P > 0:05). a* Eggs laid on leaves control and treatments were kept to determine larval survival after 96h and 120 h. Numbers averaged 21 and 22 eggs per replicate for treatment and control, respectively.

4. Discussion

This study demonstrated that *B. bassiana* can establish as an endophyte in cauliflower leaves, by foliar application of *B. bassiana* conidia. Initially colonization of cauliflower by *B. bassiana* increased consistently with time but after reaching a threshold level of 70%, rate of colonization became constant. Endophytic relationships are highly dependent on genotypic interactions (host and endophytes), environmental conditions and ecology of the diverse population of multiple endophytes in the plant [38-41]. Reasons for the constant rate of colonization of cauliflower by *B. bassiana,* after a threshold level (70%) of colonization achieved is not clear but one possible mechanism might be direct competition for available plant-derived resources.

The cauliflower plants examined for endophytic presence of *B. bassiana* through PCR amplification with fungal specific SCAR primers gave the positive results. A nucleotide BLAST search of the obtained sequences were 100 % identical with the other reported *B. bassiana* sequences present in the NCBI database [42]. No band was observed in the control and water obtained from last rinsing of treated leaf while performing surface sterilization. PCR results confirmed the endophytic presence of *B. bassiana* in the cauliflower leaf tissues and verified the accuracy of surface sterilization method used to kill the conidia present on the surface of treated cauliflower leaves.

Electron microscope images of the surface of the inoculated cauliflower leaves revealed that *B. bassiana* do not require any precise location on the leaf epidermis for germination. After imbibing moisture from the surrounding, conidia germinated on the leaf surface, exerting mechanical pressure on the outer cell, leading to the rupturing of the cell and penetration of the hyphae into the leaf tissues (Fig1.C). After penetration, the primary hyphae develop rapidly into a branched, multicellular mycelial network (Fig1. E, F). Hyphae were found to be growing directly into neighboring epidermal cells and/or grow into the inter- cellular spaces (Fig1. G). No extensive mycelium growth was observed on the cauliflower leaf surface as observed in corn [14]. No conidial formation or emergence of *B. bassiana* in or from cauliflower plant tissues was observed.

Compounds from multiple metabolite classes can be altered in response to the inoculation with fungal endophytes; however, these responses can be variable. Many of the compounds considered have physiological significance or bioactive properties, which could impact habitat interactions and can provide protection to the plant from pests [43]. The profile of secondary metabolites detected in the GC-MS of ethyl acetate extracts of pure culture of *B. bassiana*, inoculated and control plants were found to be significantly different. A total of nine, nine and twelve compounds were detected in treated, control and pure culture of *B. bassiana* respectively (Table 2), except for few which were common; all compounds detected were different in each treatment. This change in phytochemistry may be related to the use and availability of resources in the host tissues and *B. bassiana* may be depleting or fortifying the levels of important minerals leading to downstream changes in metabolism. An induced systemic response seen in the laboratory analysis about the effects of endophytic *B. bassiana* on *P. xylostella* also seems likely due to the observed changes in phytochemistry. We were not able to identify any known *B. bassiana* metabolite in GS-MS analysis, it might be because of the difference in method employed, different strain of *B. bassiana* or different solvent used.

In the choice bioassay, *P. xylostella* did not show any significant preference for oviposition, when presented with the choice of treated or untreated control plants. However, no larvae could survive on inoculated cauliflower plants. No mycelium growth was observed from the dead larvae. The larval mortality over time developed on treated cauliflower plants could be attributed to the internal growth of the fungus

in the plant tissues following germination and penetration and production of secondary metabolites [34]. Another report by Batta [27]. On endophytic action of *Metarhizium anisopliae* infesting *Brassica napus* plants against *P. xylostella* reported 63.3 percent larval mortality after 4 weeks of the fungus inoculation. Lower mortality they obtained might be because they used second instar *P. xylostella* and different fungus and plant species model. No reports are yet available on the exact mode of action of fungal endophytes.

Whether *B. bassiana* completes its life cycle in the cauliflower plant remains ambiguous as no efforts were made to study the conidia production by fungus in the plant. The present study provides a comprehensive step-by-step method to verify the intentional colonization of cauliflower plant by *B. bassiana* via microbiological, molecular and light and electron microscopic methods and subsequently assessing the effect of endophytic fungus on insect herbivore. Our results support the view that the extensively studied *B. bassiana* have a broader ecology and the potential to affect the performance of insect herbivores, when living within plants. We recommend that further studies to assess the effect of the endophytic *B. bassiana* on plant photochemistry should be carried out with broad range of solvents and using different analysis methods. Future field level studies on specific plant- fungus-insect interactions should be carried out to assess the direct and indirect effects of endophytic entomopathogens on target regulations of insect pests.

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