UNIVERSIDAD AUTÓNOMA DE NUEVO LEÓN
FACULTAD DE CIENCIAS BIOLÓGICAS

ENTOMOPATHOGENIC ENDOPHYTE Beauveria bassiana PROMOTES EARLY FLOWERING AND DROUGHT SURVIVAL IN Arabidopsis thaliana AND Zea mays AND CONTROL Spodoptera frugiperda POPULATION IN Zea mays

Tesis presentada por
LAIJU KUZHUPPILLYMYAL PRABHAKARANKUTTY

Como requisito parcial para obtener el grado de

DOCTOR EN CIENCIAS CON ORIENTACIÓN EN MICROBIOLOGÍA

AGOSTO 2020

UNIVERSIDAD AUTÓNOMA DE NUEVO LEÓN

FACULTAD DE CIENCIAS BIOLÓGICAS

DEPARTAMENTO DE MICROBIOLOGÍA E INMUNOLOGÍA

ENTOMOPATHOGENIC ENDOPHYTE *Beauveria bassiana* PROMOTES EARLY FLOWERING AND DROUGHT SURVIVAL IN *Arabidopsis thaliana* AND *Zea mays* AND CONTROL *Spodoptera frugiperda* POPULATION IN *Zea mays*

BY

M.C. LAIJU KUZHUPPILLYMYAL PRABHAKARANKUTTY

THESIS

As a partial requirement to obtain the Ph.D degree in Science with Microbiology accentuation

CIUDAD. UNIVERSITARIA **August 2020**

ENTOMOPATHOGENIC ENDOPHYTE *Beauveria bassiana* PROMOTES EARLY FLOWERING AND DROUGHT SURVIVAL IN *Arabidopsis thaliana* AND *Zea mays* AND CONTROL *Spodoptera frugiperda* POPULATION IN *Zea mays*

Thesis Committee

Director: Dr. María Julissa Ek Ramos

Secretary: Dr. Patricia Tamez Guerra

First Vocal: Dr. Ricardo Gómez Flores

Second Vocal: Dr. Carlos Eduardo Hernández Luna

Third Vocal: Dr. José Ángel Merino Mascorro

ENTOMOPATHOGENIC ENDOPHYTE *Beauveria bassiana* PROMOTES EARLY FLOWERING AND DROUGHT SURVIVAL IN *Arabidopsis thaliana* AND *Zea mays* AND CONTROL *Spodoptera frugiperda* POPULATION IN *Zea mays*

Thesis Direction

Dr. María Julissa Ek Ramos

Director

Dr. Julio S. Bernal

External Director

This work was developed in the Laboratory of Biological Formulations of the Department of Microbiology and Immunology of the Faculty of Biological Sciences under the direction of Dr. María Julissa Ek Ramos and Dr. Julio S. Bernal (external director) and in coordination with Dr. Patricia Tamez Guerra and Dr. Ricardo Gómez Flores and the support of Consejo Nacional de Ciencia y Tecnología (CONACYT No: 449643)

ACKNOWLEDGEMENTS

First, I would like to express my gratitude towards Consejo Nacional de Ciencia y Tecnología (CONACYT) for economically supporting me during my Ph.D program (scholarship -No 449643).

I am sincerely thanking Dr. Maria Julissa Ek Ramos for opening a new adventure in my scientific life by accepting me as her very first postgraduate student. I feel so honored about that. I do not have enough words to express my intimate and sincere thanks to her for being an excellent mentor and guide throughout my four years of the stay.

It is my pleasure to say big and sincere thanks to two great doctors Dr. Patricia Tamez Guerra and Dr. Ricardo Gómez Flores who helped me throughout this period, not only academically, but also in my personal life. I feel so honored to work with them and there are not enough words to express my gratitude towards them.

I take this opportunity to thank Dr. Cristina Padilla Rodriguez for opening the doors to this department. Taking this opportunity to extend my gratitude towards Dr. Julio Bernal who accepted the invitation to be my external director.

Extend my sincere gratitude, love, and respect towards Sr. Fernando Ferrera for giving me the opportunity to do my field trials in his land without giving single money. He himself prepared the land, provided the watering facility, and gave his personal staff to help me around. Sr. Antonio Padilla, his wife Adriana and kids Ivan and Lilly, there are no words to express my gratitude towards them.

In addition, I take this opportunity to express my thankfulness to all my doctors and my friends over here that have helped me one or in another way to complete this course, academically, emotionally, etc. Dr. Alonso Alberto Orozco Flores, Dr. Rahim Foroughbakhch Pounavab, Dr. Jorge Villareal, Dr. Cesar Iván Romo Sáenz, Dr. Jorge Dávila, Dr. Luisa Solis Soto, Dr. Ángel Merino, Dra. Liceth Villarreal Treviño, Dr. Laura María Trejo Ávila, Dr. Carlos Hernández Luna, Dr. Norma Zamora, Dr. Lupita González. Special thanks to my Friends and lab mates Nora Mares, Sra Rossy Navarro, Adán Galindo, Verónica Padilla, Juan Ballesteros, Patricia Calleja, Fabiola Venegas, Any Gutiérrez, Nereida Rivera, Alberto Aguayo, Oziel Zúñiga,

Angello Martínez, Andrea Abrego, Rosa Flores, Ana Barajas, Dennisse Aguilar, Diana Ruiz, Sofia Berni, Jessica Seis, Jonathan Chapa, Verónica López, Brenda Vargas, Evelyn Bueno, María Luisa Sánchez, Dianelys Sotolongo, Servando Cantú, Ricardo Romero, Gerardo Palacios, Brizeth Medellin, Oscar Flores, Esteban López, Ileana Meléndez. My family friends in Monterrey, Queretaro, Houston, and Mc. Allen, who always encouraged me by saying, you can do it, go on. My little girls' friend's family members, always cheering me up, whenever they feel that I am down. My family members, from long distance, always supporting me with their kind and powerful words.

My sincere and intimate gratefulness to Dr. Norma Laura Heredia and Dr. José Santos García Alvarado who believed in my strength to go out of the comfort zone and open a new area of investigation, without forgetting Dr. Bindu Krishnan and Dr. Elva Teresa Aréchiga Carvajal, who opened my path to them.

Finally, my family here with me, who always sees my anger, frustration, sadness, and my happiness. My husband Bharathan who inspired me to study again giving me all the support, allowing me to work on weekends, who took me to General Teran, all weekends, from February to June, to check our field experiments. He never got tired of that. Thank you so much. The list will not finish if I start to say. Now the most important persons who have allowed me to do all these are my little girls Kalyani and Bhavani. If they would not have given me the permission, I could not have finished this course. And my Mommy, my brother even though they are so far away from me they always inspired me telling me that you can do it. And yes, I did it.

Thank God.

DEDICATION

This work is primarily dedicated to God

Then to
Bharathan Vellayikodath Soumyan
Kalyani Vellayikodath Kuzhuppillymyal
Bhavani Vellayikodath Kuzhuppillymyal
Late. Prabhakarankutty Kuzhuppillymyal Ayyappan
Prasanna Karuthala Subramayan
Libin Kuzhuppillymyal Prabhakarankutty
Soumyan Vellayikodath Gopalan
Late Nalini Muthukattu Kochappu

INDEX

Section **Page**

LIST OF THE TABLES

LIST OF THE FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

°C	-	Degree Celsius
Hyd1 and Hyd2	-	Hydrophobin proteins 1 and 2
PR	-	Pathogenesis-related
Ct	-	*Colletotrichum tifiediae*
PSR	-	Phosphate Starvation Response
PGP	-	Plant Growth Promotion
F2	-	Segregating Population
FAW	-	Fall Army Worm
Pr1 and Pr2	-	Pathogen-related Proteins 1 and 2
MAD1 and MAD2	-	Mitotic Arrest Deficient Proteins 1 and 2
DAMPs	-	Danger-Associated Molecular Patterns
PAMPs	-	Pathogen-Associated Molecular Patterns
ETI	-	Effector-Triggered Immunity
PTI	-	PAMPs-Triggered Immunity
SDS	-	Sodium Dodecyl Sulphate
2D-DIGE	-	Two-Dimensional Difference Gel Electrophoresis
MS	-	Mass Spectrometry
MALDI	-	Matrix-Associated Laser Desorption/Ionization
ESI	-	Electron Spray Ionization
mL	-	Milliliter
µL	-	Microliter
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
MC	-	Methyl Cellulose
CS	-	Corn Starch
CC	-	Negative control (Untreated)

CMC	-	Negative control with only methyl cellulose
CCS	-	Negative control with only cornstarch
MCGHA	-	*B. bassiana* strain GHA with methyl cellulose
MCPTG4	-	*B. bassiana* strain PTG4 with methyl cellulose
MCPTG6	-	*B. bassiana* strain PTG6 with methyl cellulose
CSGHA	-	*B. bassiana* strain GHA with cornstarch
CSPTG4	-	*B. bassiana* strain PTG4 with cornstarch
CSPTG6	-	*B. bassiana* strain PTG6 with cornstarch
UANL	-	Universidad Autónoma de Nuevo León
CDMX	-	Ciudad de México
UNAM	-	Universidad Nacional Autónoma de México
CIMMYT	-	Centro Internacional de Mejoramiento de Maíz y Trigo
USA	-	United States of America
h	-	hours
m	-	meters
cm	-	centimeters
fig.	-	Figure
%	-	Percentage
kDa	-	Kilo Dalton
±	-	more or less
=	-	equals to
≥	-	greater than or equal to
≤	-	less than or equal to

ABSTRACT

As an alternative to chemical pesticides the use of artificially inoculated endophytes in seeds have the advantage of controlling insect pests at a low application cost since little inoculum is required and protect the fungus from adverse environmental conditions. Using *Beauveria bassiana* as seed treatment in *Zea mays* plants establish them as endophytes in plants and enhance plant growth, induce drought tolerance, and can control insect pest *Spodoptera frugiperda*. The aim of the present study was to establish *B. bassiana* strains GHA, PTG4 and PTG6 as an endophyte in *Z. mays* and *Arabidopsis thaliana* to evaluate its effect on plant growth and yield, resistance to drought, tolerance to *S. frugiperda* and analyze the proteins present in *B. bassiana-Z. mays* interaction. Results showed that plants treated with *B. bassiana* strains GHA and PTG4 flowered earlier than untreated and PTG6 treated plants. Fresh and dry weight of *B. bassiana* plants showed significant difference compared with untreated plants. Fifty percent of the *B. bassiana*-treated plants recovered their vigor with watering after a gradual drought stress. *S. frugiperda* larvae fed on *B. bassiana*-treated plants were late to reach their pupal stage and female moths were born with wing deformities and observed parthenogenesis, also controlled *S. frugiperda* population in the field studies. *B. bassiana*-treated plants guttation fluid contained proteins with 100 kDa to 150 kDa.

Key words: *B. bassiana*, biological control, multitrophic interactions, protein effectors, proteomics.

RESUMEN

Como una alternativa a los plaguicidas químicos el uso de endófitos artificialmente inoculados en las semillas tiene la ventaja de controlar las plagas a un costo de aplicación reducido, ya que se requiere poco inoculo y también protege el hongo contra los factores abióticos. El uso de *Beauveria bassiana* en los tratamientos de las semillas de las plantas potencia su establecimiento como endófito, para que funcione como promotor de crecimiento, inductor de tolerancia a la sequía y para controlar la población de *Spodoptera frugiperda* en *Z. mays*. El objetivo de este presente estudio fue establecer *B. bassiana* como endófito en las plantas de *Z. mays* y *Arabidopsis thaliana* y evaluar su efecto en el crecimiento, la resistencia, a la sequía, y a la tolerancia a *S. frugiperda* y analizar las proteínas que están presentes en la interacción de *B. bassiana-Z. mays*. Resultados mostraron que las plantas tratadas con las cepas GHA y PTG4 florecieron antes que las plantas no tratadas y tratadas con la cepa PTG6, y también se observó una diferencia significativa en el peso fresco y peso seco de las plantas tratadas con *B. bassiana*. Mas de 50% de las plantas tratadas con *B. bassiana* se recuperó su vigor después del estrés por sequía. Las larvas de *S. frugiperda* que se alimentaron de las plantas tratadas con *B. bassiana* tardaron en llegar al estadio de pupa. Las hembras presentaron deformidades en las alas, y se observó partenogénesis. Los estudios en el campo también se pudo controlar la población de *S. frugiperda*. Las gotas de savia de la xilema de las plantas tratadas con *B. bassiana* contienen proteínas con tamaño de 100 kDa - 150 kDa.

Palabras clave: *B. bassiana*, control biológico, efectores proteicos, interacciones multitróficas, proteómica.

INTRODUCTION

Microbial control is the most viable alternative to synthetic chemical pesticides (Quesada et al., 2014). In recent years, there is a growing appreciation of the prevalence, ecological significance, and management of potential fungal endophytes. Fungal endophytes are fungi that internally colonize plant tissues without causing evident damage or disease. Several studies suggest that fungal endophytes are involved in many beneficial interactions with their hosts, providing protection against a variety of stressors, including herbivores, pathogens, heat, and drought (Ek-Ramos et al. 2013). Asymptomatic fungal endophytes are abundant and widespread in monocot and dicot plants. Amongst the potentially beneficial fungal endophytes are several specially known to be natural enemies of insects (entomopathogens). Entomopathogenic fungal endophytes have been isolated from a variety of different plant species and tissues. They are classified as no-Clavipitaceae, referring to fungal endophytes that are usually transmitted horizontally. Clavipitaceae endophytes, on the other hand, are found in grasses and are vertically transmitted (Castillo Lopez et al. 2014). As endophytes, several Clavipitaceae entomopathogens including *Beauveria bassiana*, *Lecanicillium lecanii*, *Metarhizium anisoplae, Purpureocillium lilacinum,* and *Isaria* sp. have negative effects on insect pests when *in planta*, antagonize to plant pathogens and promote plant growth. The activity of *B. bassiana* has received attention due to its negative effects on a variety of insect herbivores including cotton aphid (Castillo Lopez et al. 2014). Furthermore, effects of these fungi on herbivores when present within the plants as endophytes may represent a new venue for their use in pest management.

The main focus of this study was to understand the mechanisms involved during the beneficial interaction of fungal endophytes and their plant hosts, under biotic stress conditions where tolerance mechanisms have been developed by the economically important crop maize (*Zea mays*) and plant model *Arabidopsis* (*Arabidopsis thaliana*) and to control *Spodoptera frugiperda* using different strains of *B. bassiana*. To assess this, we performed one-dimensional electrophoresis to determine the different proteins profiles that are involved in these interactions. The results from this study are novel and have a contribution to the current knowledge regarding plant tolerance against drought and insect pests and provided new information on the beneficial interaction between fungal endophytes and their host plants. This study also provided information about the use of guttation liquids for the analysis of proteins.

ANTECEDENTS

Fungi have been known to be a rich repository of medicinally important compounds since the discovery of penicillin. Today, the range of drugs derived from fungi stretch from antibiotics to immune suppressants to anti-cholesterol drugs (statins). Despite plants remain the major source of drugs or their lead molecules, with every new bioactive molecule reported from a plant source, there are reports of endangered status or even extinction of medically important plants due to over-harvesting. Hence, the focus has turned toward fungi namely "endophytes" which reside within these medicinally important plants and from which may have acquired their potential medicinal values (Venugopalan and Srivastava 2015).

Endophytes

The term endophyte was coined by Heinrich Anton de Bary in 1884 (Venugopalan and Srivastava 2015). Although the term "endophyte" has several definitions, it is widely accepted that endophytes are microorganisms present in the plant tissues without causing any apparent symptoms (Ownley, Gwinn, and Vega 2010). Endophytic fungi often referred to as "symptomless fungi" occur ubiquitously in plants. They reside in intercellular spaces of stems, petioles, roots and leaves of plants without causing any obvious negative effects (Mohana Kumara et al., 2014).

The earliest records of the presence of endophytic fungi have come from the 400-million-year-old fossils of the early Devonian Rhynie chert deposits, which suggest that endophyte-plant associations may have evolved along with the evolution of higher plants. Modern day studies on endophytic fungi can be traced back to mid-19th century. Based on differences in evolutionary relatedness, taxonomy, plant hosts and ecological functions, the endophytic fungi are classified as the Clavipitaceae or grass endophytes (C-endophytes) and the non-Clavipitaceae endophytes (NC-endophytes) of vascular plants, ferns, conifers, and angiosperms, with the latter being further separated into three functional classes based on host colonization patterns, mechanism of transmission between host generations, *in planta* biodiversity levels and ecological functions (Venugopalan and Srivastava 2015). Evidence suggests that these fungal endophytes significantly improve host plant tolerance to drought, insect, diseases, and nematodes, and in exchange, plants provide protection, nutrition, and dissemination of the fungi. A few benefits to plants are also conferred by non Clavipitaceae endophytes (Castillo Lopez et al. 2014).

The endophyte community within a plant is determined by fungi (genotype, competitive potential, tissue specificity, infection location) and host (genotype, variations in plant defenses, geographical location). Numerous studies have shown that endophyte richness and diversity are influenced by a vast array of abiotic and biotic factors including microclimate, microhabitat, and geographic location. A plant species may seem to be homogenous, but spatial and genetic differences render some plant more, and others less, susceptible to endophyte infection and subsequent colonization. For example, changing environmental conditions can influence host specificity. Ahlholm et al. in 2002 reported host-endophyte interactions in birch tree (*Betula* spp.), where the tree genotype directly influenced the presence of the fungal endophyte *Ventura ditricha*. Host genotype-enhanced resistance or increased susceptibility has been studied extensively within *Arabidopsis thaliana*, especially in relation to pathogens (Currie et al. 2014).

Endophytes benefit their hosts through improved tolerance to biotic stress such as drought, enhanced photosynthesis and transpiration, protection against pathogens through induced plant systemic resistance and the deterrence of phytophagous invertebrates. These benefits arise directly from endophyte metabolism or indirectly through production of compounds that alter host's physiology. Conversely, plant does not always benefit from the presence of endophytes, and in some cases, plant fitness increased with the endophyte was absent. To unravel the complexity of endophyte-plant system, we must "think of individual plants as an ecosystem of interacting microbes" (Currie et al. 2014).

Plants responds to drought stress through a range of physiological and biochemical changes and research has shown that fungal endophytes are able to increase a host plant's tolerance to drought stress, possibly through the enhancement of root development and leaf growth, regulating the opening and closing of stomata, osmotic regulation and improvement of the anti-oxidation protection system (Currie et al. 2014).

For crop plants, soil salinity is one of the most significant abiotic stresses, as it reduces crop yield by more than 50% (Currie et al. 2014). Several non-Clavipitaceae entomopathogens, including *Beauveria bassiana, Lecanicillium lecanii, Metarhizium anisopliae* and *Isaria* (*Paecilomyces*) spp. have negative effects on insect pests, when *in planta* may antagonize plant pathogens, and promote plant growth. The endophytic activity of *B. bassiana* has received attention due to its negative effects on a variety of insect herbivores including cotton bollworm. The fungus *Purpureocillium lilicinum* has been mainly considered a nematophagous, egg-parasitizing fungus, especially against

the root-knot nematode, *Meloidogyne incognita*, and several other plant-parasitic nematode species including *Radopholus simillis*, *Heterodera* spp., and *Globodera* spp. (Lopez and Sword 2015).

B. bassiana has been reported as an endophyte in maize, potato, cotton, cocklebur and jimsonweed tomato, *Theobroma gileri*, in the bark of *Carpinus caroliniana*, in the seeds and needles of *Pinnus monticola*, in opium poppy, on date palm, coffee, in bananas, cocoa beans, and in coffee seedlings (Vega et al. 2008).

Other entomopathogenic fungi have also been reported as endophytes, including *Verticillium lecanii* in an *Araceae*, *V. lecanii* and *Paecilomyces farinosus* in the bark of *C. caroliniana*; *Paecilomyces* sp. in *Musa acuminata* and in rice, and *Paecilomyces varioti* in mangroves. *Cladosporium*, another genus containing insect pathogenic species has been reported as an endophyte in *Festuca*, various grasses, mangroves, *Cuscuta reflexa* Roxb, *Abutilon indicum*, and *Calotropis gigantean*, *M. acuminata*, wheat, oak*, Ilex*, cacti and in apples (Vega et al. 2009).

Beauveria bassiana

The genus *Beauveria* contains at least 49 species of which approximately 22 are considered pathogenic. *B. bassiana*, a white muscadine fungus is the most historically important of commonly used fungi in this genus. Originally known as *Tritirachium shiotae*, this fungus was renamed after the Italian lawyer and scientist Agostino Bassi who first implicated it as the causative agent of a white muscadine disease in domestic silkworms (Rai et al. 2014).

B. bassiana is a fungus that grows naturally in soils throughout the world and acts as a pathogen on various insect species, causing white muscadine disease, therefore belongs to the entomopathogenic fungi. An interesting feature of *Beauveria* spp. is the broad host specificity of many isolates. Hosts of agricultural and forest significance include Colorado potato beetle, codling moth, and several genera of termites, American boll worm and *Helicoverpa armigera* (Rai et al. 2014).

Scientific classification of *B. bassiana* (http://eol.org) revised on June 12th, 2020.

Kingdom: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Cordycipitaceae

Genus: Beauveria

Species: B. bassiana

B. bassiana is the most appreciated endophytic fungal entomopathogen to date, with a widespread commercial availability as a potent mycopesticide. Fungus establishes itself as an endophyte either naturally, e.g., by stomatal penetration, or with the aid of inoculation methods such as soil drenches, seed coatings and immersions, radicle dressings, root and rhizome immersions, stem injection, and foliar and flower sprays. Hence it is widely acknowledged as a success in a variety of plants such as grasses, agricultural crops, tomato, cotton, corn, and potato; the medicinal group including opium poppy, cocoa, and coffee; and trees such as *Carpinus caroliniana* and western white pine (Singh et al. 2015).

B. bassiana **as an entomopathogen**

Some endophytes belong to genera that include fungal entomopathogens such as *Beauveria* (Vega et al. 2008). The entomopathogenic mitosporic ascomycete, *B. bassiana* is an important natural pathogen of insects and it has been developed as a microbial insecticide for use against many major arthropod pests in agricultural, urban, forest livestock and aquatic environment. It has been developed as a microbial insecticide for use against many major pests including lepidopterans and orthopterans. About 33.9% of the mycoinsecticides are based on *B. bassiana,* followed by *Metarhizium anisopliae, Isaria fumosorosea,* and *Beauveria brongniartii* (Khan et al. 2012).

The endophytic habit of *B. bassiana* may provide benefits to both plant and fungus. It is well known that plant species has a significant impact on shaping plant-associated microbial communities. As suggested by the bodyguard hypothesis, plant gains thorough reduction of damage against herbivorous insects or plant diseases; fungus benefits through protection from environmental stress, acquisition of limiting nutrients from endophytic colonization as well as

exudates on plant surfaces, and use of plant surface as a staging platform for insect parasitism (Ownley et al. 2010).

B. bassiana has also been found naturally as an endophyte in several plant species and has been artificially introduced into many others. Artificial introduction of *B. bassiana* as an endophyte has been successful in maize, cacao, date palm, coffee, banana, radiata pine, fava beans, opium poppy, cotton, the common bean, and tomato (Greenfield et al. 2016).

Mechanism of action of entomopathogenic *B. bassiana*

B. bassiana infects insects via attachment of cells, namely spores or aerial conidia, to host surface. All life stages of the fungus appear to be infectious including hyphae, serial conidia, single-celled blastospores (produced during saprophytic growth under certain nutrient conditions) and submerged conidia. The extent to which these latter fungal forms infect insects in nature is unknown; asexually produced (aerial) conidia are the main dispersal and infectious structures, capable of resisting to a greater extent, more than hyphae and blastospores, various abiotic stress. *B. bassiana* conidia are hydrophobic, binding to similarly hydrophobic insect epicuticle or waxy layer, structure rich in hydrocarbons, fatty acids, and wax esters. As a nonmotile organism, targeting of insects by fungus is considered a passive event, with airborne, water dispersed, and/or presence in substrate over which insects would forage, i.e., leaf surface and soil, mediating initial contact with potential hosts. Thus, infection can be viewed as an opportunistic program initiated by conidia that happen to find themselves on a host cuticle. In this respect, although preferential site of infection, typically those where sclerotization of cuticle is lower, that is mouthparts and anus, have been noted for many insects, the fungus can initiate infection essentially anywhere on host cuticle. This contrasts with other microbial pathogens, which must be ingested and/or utilize more specific route of entry into the host (Ortiz and Keyhani 2016).

Spore germination and successful infection by *B. bassiana* relies on various factors e.g., susceptible host, host stage and certain environmental factors such as temperature and humidity. Generally, germination of *B. bassiana* conidia starts after about 10 hrs. and is completed in 20 hrs. at 25°C (Keswani, Singh, and Singh 2013).

To enable infection, conidia must adhere to the host cuticle and subsequently germinate to form a germ tube and an appressorium. The cuticle can then be breached using a combination of mechanical pressure and enzymatic degradation, enabling hyphae to grow through the cuticle and

invade the insect hemocoel, where they switch to growth as single-celled blastospore. These cells circulate freely through the hemocoel, where they exploit the hemolymph for nutrition and secrete toxins that eventually kill the host. Successful invasion requires that the fungal cells evade or overcome immune responses, both during growth through the cuticle and especially in hemocoel. When host is killed, the cuticle must be breached again from inside to allow escape from insect body and sporulate on the cadaver, enabling conidia to be dispersed and start a new infection (Valero-Jiménez et al. 2016).

Hydrophobic interactions predominate with *B. bassiana* conidia containing an outermost or rodlet layer comprised of proteins known as hydrophobin. At least two hydrophobin proteins Hyd1 and Hyd2 have been characterized in *B. bassiana* (Ortiz and Keyhani 2016). Studies show that Hyd1 is localized to aerial surface and submerged conidia, and at the base of germinating conidia, but was not detected on blastospores. Likewise, Hyd2 is found on conidial aerial surface and at the base of the germinating conidia. However, Hyd2 was not found on submerged conidia and blastospores. In addition, neither Hyd1 nor Hyd2 were observed on hyphae or hyphal bodies (Ortiz and Keyhani 2016).

A specific adhesion gene (Mad1), mediating attachment to insect cuticles has been characterized in the related entomopathogenic fungus, *Metarhizium robertsii*, and although a homolog has been identified in *B. bassiana* genome, its contributions to adhesion to insect surfaces and/or virulence has yet to be characterized. The extent to which this initial binding is consolidated with additional factors and the cue(s) used by the fungus to initiate its infection program remains unknown (Ortiz and Keyhani 2016).

It is likely that more than one mode of action is operative in suppression of plant disease by *B. bassiana.* Isolates of the fungus are known to produce numerous secondary metabolites like beauvericin, beauverolides, bassianolides, oosporein, cyclosporin A, and oxalic acid, with antibacterial, antifungal, cytotoxic, and insecticidal activities. Effects of these compounds on microorganisms and insects have been reported. Recently, another antimicrobial compound, bassianolides, from *B. bassiana* fermentation culture under low nitrogen conditions, was characterized. Bassianolides has activity against fungi and Gram-positive cocci. Antibiosis assays with *B. bassiana* against various plant pathogens *in vitro* have been reported. For example, *B. bassiana* strain 11-98 suppresses plant disease cause by the soil borne plant pathogen *Rhizoctonia solani* and *Pythium myriotylum* and its application to tomato seeds gave protection against

damping off (Ownley et al. 2010). Similarly, cotton seed treatments reduced the severity of *R. solani* damping off in seedlings (Ownley et al. 2010).

Proposed mechanism of action of other known fungal endophytes (*Piriformospora indica*, *Colletotrichum,* and others)

Piriformospora indica is a model organism used in mycorrhizal research (Shrivastava and Varma 2014). *P. indica*, a root colonizing and growth promoting basidiomycete fungus, was recognized in the Indian Thar desert. *P. indica* has been found to be a potent new candidate symbiont for providing enormous growth-promoting activity to a broad spectrum of plants, including agricultural and medicinal crops. In this perspective, *P. indica* has become a paramount tool in improving the productivity of several crops such as *Brassica campestris* sp. chinensis *Lycopersicon esculentum*, *Hordeum vulgare*, *Piper nigrum*, *Glycine max*, *Cicer arietinum*, *Arabidopsis* sp., *Oryza sativa,* and *Nicotiana tabacum* under natural and/or stress conditions (Ansari, Gill, and Tuteja 2014).

P. indica has shown to be very versatile and has the potential to colonize a variety of different plants, but research to understand molecular mechanisms underlying symbiosis, has basically been done on the agronomical important monocotyledonous crop plant barley (*Hordeum vulgare*) and the dicotyledonous genetic model plant *Arabidopsis thaliana*. During colonization of roots, plant host induces the expression of defense-related genes, which is in turn reprogrammed and actively suppressed by the *P. indica* own survival genes and to establish the beneficial interaction. Furthermore, it has been demonstrated that in *P. indica*-colonized roots, expression of various defense-related genes, like pathogenesis-related (PR) genes, ethylene signaling molecules, and ethylene-targeted transcription factors are mildly up regulated during the initial stages of colonization, but down regulated in the later stages, supporting the idea that either the fungus does not stimulate extensive defense gene expression for longer periods of time, or they down regulate them after an initial activation period (Trivedi et al. 2016).

Mechanisms behind the unique action of *P. indica* suggest that fungal interactions are characterized by increase in efficiency of nutrient uptake from soil due to better hyphal penetration as compared to thicker root hairs. Plants deliver phosphor assimilates to fungus and during mycorrhizal association; plants acquire phosphates from extensive network of extra radical hyphae. Interaction of *P. indica* with plant alters pathway for nitrogen metabolism, thereby helping

plants to absorb more nitrogen. This phenomenon gives higher resistance to water deficiency and makes plants drought tolerant. Enhanced growth of plants under mycorrhizal conditions amplifies its starch requirement. This starch is obtained from deposition in root amyloplasts and then, it is interpreted that one of the major starch degrading enzymes, the glucan- water di kinase, is activated by *P. indica* (Shrivastava and Varma 2014).

Uptake and transportation of important macronutrients like iron, zinc, manganese, copper, etc., are also regulated by the fungus. Along with this, beneficial phytohormones are synthesized by plant associated with *P. indica*. The cumulative effect of macro/micronutrients and phytohormones regulates plant metabolism leading to value addition, early flowering, plant growth promotion, etc. (Shrivastava and Varma 2014).

Colletotrichum species are extensively studied as model organisms for research into genetics. The biotrophic life strategies adopted by *Colletotrichum* species may also contribute to their prominence as symptomless endophytes of living plant tissues (Cannon et al. 2012).

The genus *Colletotrichum* (Sordariomycetes, Ascomycota) comprises around 600 species attaching over 3,200 species of monocot and dicot plants. These pathogens use the following multistage hemi biotrophic infection strategy: dome shaped appressoria first puncture host surface using a combination of mechanical force enzymatic degradation, bulbous biotrophic hyphae enveloped by an intact host plasma membrane then develop inside living epidermal cells, and finally, the fungus switches to necrotrophy and differentiates thin, fast growing hyphae, and kill and destroy host tissues (O'Connell et al. 2012).

Although most characterized species of fungal genus *Colletotrichum* are destructive pathogens, a species known as *C. tofieldiae* (Ct) is an endemic endophyte in natural *Arabidopsis thaliana* populations in central Spain. Colonization by Ct initiates in roots and systemically spreads into shoots. Ct transfers the macronutrient phosphorous to shoots, promotes plant growth, and increase fertility only under phosphorous-deficient conditions, a nutrient status that might have facilitated the transition from pathogenic to beneficial lifestyles. The host's phosphate starvation response (PSR) system controls Ct root colonization and is needed for plant growth promotion. PGP also requires PEN2 – dependent indole glycosylates metabolism, a component of innate immune responses, indicating a functional link between innate immunity and the PSR system during beneficial interactions with Ct (Hiruma et al. 2016).

Arabidopsis thaliana and *Zea mays* plant models

Arabidopsis model has been extensively used to study plant-pathogen interactions, particularly to elucidate defense responses (Verma et al. 2014). *Arabidopsis* has been established as an important model system for studying plant biology and plant-microbe interactions (Martinuz et al. 2015). In the year 2000, the first plant genome was completely sequenced. The plant species that was selected for sequencing is known as *Arabidopsis* or mouse-earned cress, shortened from its full Latin name *Arabidopsis thaliana*. *Arabidopsis* is not a crop species, but it belongs to the Brassicaceae family, which include oilseed rape, cabbage, mustard, turnip, and cauliflower. It was selected because *Arabidopsis* is quite easy to grow in the lab, as it has a relatively small genome (Nobuta and Meyers 2005).

A. thaliana has a broad natural distribution throughout Europe, Asia, and North America. Many different ecotypes have been collected from natural populations. The entire life cycle, including seed germination, formation of a rosette plant, bolting of the main stem, flowering, and maturation of the first seeds is completed in 6 weeks. Flowers are 2 mm long, self-pollinate as the bud opens, and can be crossed by applying pollen to the stigma surface. Seeds are 0.5 mm in length at maturity and are produced in slender fruits known as siliques. Seedlings develop into rosette plants that range from 2 to 10 cm in diameter, depending on growth conditions. Leaves are covered with small unicellular hairs known as trichomes that are convenient models for studying morphogenesis and cellular differentiation (Meinke et al. 1998).

By resolving the genome sequence, the high number of natural and artificially generated mutants, and the relatively ease of use under laboratory conditions, *A. thaliana* has over the past decades developed into a key model plant for plant biology research at every level, which not only covers genomics, transcriptomics and proteomics but also all of the others, like phenomics, metabolomics, interactomics and ionomics. *A. thaliana* is also used to characterize the microbiome of its rhizosphere in studies involving plant-microbe interrelationships and to unravel the mechanisms leading to disease, mutualism, or symbiosis (Martinuz et al. 2015).

When an organism is not known to colonize *Arabidopsis*, it is important that the organism being examined shows the same interactions phenotypes in the model when compared with original host. A good example of this is the beneficial basidiomycete *Piriformospora indica* which has a large host range. However, like for arbuscular mycorrhiza, *Arabidopsis* is only used as a model for non-

host resistance. It illustrates that there may be limitations for using a model plant in studying plant-microbe interactions, particularly when tripartite interactions are involved (Martinuz et al. 2015).

Analysis of the *Arabidopsis* genome revealed 1,528 tandem arrays containing 4,140 individual genes, with arrays ranging up to 223 adjacent members. Thus 17% of all genes of *Arabidopsis* are arranged in tandem arrays. (The Arabidopsis Genome Initiative 2000)

Significant advances in understanding plant growth and development have been made by focusing on the molecular genetics of this simple angiosperm. The 120 mega base genomes of *Arabidopsis* are organized into 5 chromosomes and contains an estimated 20,000 genes. More than 30 mega bases of annotated genomic sequence have already been deposited in GenBank by the consortium of laboratories in Europe, Japan, and the United States. The entire genome was sequenced in the year 2000, which enhanced the value of *Arabidopsis* as a model for plant biology (Meinke et al. 1998).

One of the original ideas behind using *Arabidopsis* as a model system was to facilitate the identification of related genes of importance in crop plants (Meinke et al. 1998).

Scientific classification of *A. thaliana*
https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=23041#null
revised on June 25th, 2020.

Kingdom: *Plantae*
Subkingdom: *Viridiplantae*
Class: *Magnoliopsida*
Order: *Brassicales*
Family: *Brassicacease*
Genus: *Arabidopsis*
Species: *A. thaliana*

Maize has a long history of genetic and genomic tool development and is considered one of the most accessible higher plant systems. With fully sequences genome, a suite of cytogenetic tools, methods for both forward and reverse genetics, and characterized phenotype markers, maize is amenable to studying questions beyond plant biology. Major discoveries in the areas of

transposons, imprinting and chromosome biology came from work in maize. Moving forward in the post-genomic era, this classic model system will continue to be at the forefront of basic biological study (Nannas and Dawe 2015).

The term *maize* (*Zea mays* ssp. *mays*) is often used synonymously with *corn,* particularly in the United States and about its agricultural use. Whereas both terms are correct, maize is a name that refers uniquely to this plant (Nannas and Dawe 2015). Maize is a giant grass that was domesticated from wild grasses (teosintes) about 9,000 years ago, in southwestern Mexico. This process involved seed enlargement, elimination of the protective hard fruit case surrounding the seed, enhancement of husk leaves to protect an enlarged cob, development of non-shattering structures to keep seed on the cob, switching of seed placement on the plant and reduced shoot branching to permit greater nutrient allocation to seeds. These changes so profoundly affected seed dispersal and germination that domesticated maize does not survive in the wild, intimately tying maize genetics to human selection and migration (Johnston-Monje and Raizada 2011).

Many varieties or "races" differ in physical properties, but generally maize is a single-stalk plant that grows to approximately 8 feet tall with about 20 long, narrow leaves growing individually from nodes along the stalk. Several characteristics make it an attractive genetic system. It is easy to culture on any scale, from a few plants in pots to many acres. It can be grown successfully year-round in greenhouses and growth chambers with proper lighting; it is also quite handy and can be grown outdoors under a range of conditions, from tropical to temperate climates. Maize is a naturally out crossing species, which makes its genetic architecture more like other out crossing organisms such as humans rather than self-pollinating plants. Whereas its genetics are like humans, maize retains the major strength of plant genetics, including the ability to self-cross and quickly produce homozygotes or F2 populations (Nannas and Dawe 2015).

It is likely that every plant species harbors endophytes, and indeed seeds of many plant species have been reported harboring endophytes. Plant seeds usually fall to the soil, a microbially rich habitat, and lie dormant waiting for environmental cues to germinate, possibly recruiting surface microbes to help protect against degradation or predation. As seeds begin to germinate, seed endophytes may be important founders of the seedling microbial community as shown in rice, eucalyptus, and maize (Johnston-Monje and Raizada 2011).

During maize evolution, domestication, breeding and migration, some endophytes were lost, and it is also possible that endophytic genomes may have been modified, phenomena that might

contribute to the increase disease susceptibility of modern maize. Modern maize is susceptible to various pathogens including *Fusarium, Spodoptera*, etc. (Mousa et al. 2015). A promising alternative strategy to manage *Fusarium* outbreaks and reduce mycotoxin contamination may be using biological antagonists. Johnston-Monje and Raizada 2011 reported that wild, traditional, and modern maize possess endophytes that combat pathogens including *F. graminearum in vitro*. Other studies have identified other biological control agents that combat *F. graminearum,* including *Bacillus* and *Pseudomonas* spp. However, most of this research is preliminary and effective commercial biological control is not currently available (Mousa et al. 2015).

Inoculation of *B. bassiana* has been developed in *Z. mays* to control *Ostrinia nubilalis*, the European corn borer using aqueous and granular formulations. Furthermore *B. bassiana* has been found as endophyte in several plant species, and probably plays an important role to avoid attack of insect-pests implants (Verma et al. 2014).

Scientific classification of *Z. mays*
https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=42269#null
revised on June 25th, 2020.

Kingdom:	*Plantae*
Subkingdom:	*Viridiplantae*
Class:	*Liliopsida*
Order:	*Poales*
Family:	*Poaceae*
Genus:	*Zea*
Species:	*Z. mays*

Drought

Plants require light, water, carbon and mineral nutrients for their optimal growth, progress, and reproduction. Because of their immobile conditions, plants are prone to extensive environmental stresses (abiotic) and stresses induced by living entities (biotic). Major abiotic stresses are drought, high or low temperature, salt, acidic conditions, heavy metals, nutrients, and starvation whereas, bacteria, viruses, fungi, parasites and harmful insects are the major biotic stresses (Lata et al. 2018).

High salinity and drought are considered to be the major abiotic stress because, extended water stress induce a drop in leaf water potential and stomatal opening, diminishes leaf size, restrain root length, decrease seed number, size and viability, prolong flowering and fruiting period. (Lata et al. 2018).

Due to global warming, the frequency and intensity of drought are predicted to rise in future. Plants have to develop many strategies like reprograming their metabolic cycles in response to different stresses to cope up with these conditions (Ghaffari et al. 2019). Scientists have been working on crop varieties which are able to remain productive under different stresses, which has been slow largely due to polygenic inheritance of tolerance. Another suggestion to improve plants capacity to cope up with different stresses the usage of beneficial microbes that can interact with the plants to improve their tolerance mechanisms. This plant-microbe interaction has found to benefit many plants against both abiotic and biotic stresses (Ghaffari et al. 2019). These beneficial microbes perhaps acts as a type of biological trigger activating host defense system to enhance plants tolerance through osmoprotection cell wall elasticity improvement of antioxidant system up-regulation (Ferus, Barta, and Konôpková 2019a).

The interaction of mechanical and drought stresses has a significant effect on wheat plant water status and physiological responses (Hosseini, Mosaddeghi, and Dexter 2017). During a drought stress, the plants that have interaction with beneficial endophytes use significantly less water, increase biomass, reduce leaf conductance and slow transpiration (Lata et al. 2018). In 2017 Hosseini and collaborators (Hosseini et al. 2018) reported that *Piriformospora indica* enhanced maize water status and physiological trains under combined drought and mechanical stresses. Another study from 2019 (Ghaffari et al. 2019) reported that *P. indica* improves drought stress adaptation through metabolic and proteomic reprogramming in barley. *B. bassiana* enhanced drought tolerance in red oak seedlings (Ferus, Barta, and Konôpková 2019a).

Spodoptera frugiperda

Worldwide about 18 to 26 percentage reduction in crop production is due to insect pests; out of which a great part of it occurs in the fields before harvesting (Mantzoukas and Eliopoulos 2020). The Fall Armyworm, (FAW) *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), from the tropical and subtropical zones of America (Sisay et al. 2019) is a catastrophic insect pest of economic importance (Bhusal and Bhattarai 2019). This voracious insect pest has a polyphagous

feeding nature in more than 80 host species including many commercial crops like maize, cotton, rice, soy bean, bean and other crops from Gramineae family (Bhusal and Bhattarai 2019; Jaramillo-Barrios, Barragán-Quijano, and Monje-Andrade 2019; Kalleshwaraswamy, Poorani, and Maruthi 2019) until 2015, damages due to *S. frugiperda* were reported only from America (Kannidi Siddhartha 2019). But in the last few years *S. frugiperda* attack has been reported from various parts of the world (Kalleshwaraswamy, Poorani, and Maruthi 2019) in late 2016 it had been reported in Southern, Eastern and Northern parts of Africa (Siddhartha 2019) which briskly expanded across the country and by late 2018, it had been confirmed in almost 44 African countries (Bhusal and Bhattarai 2019). By 2018, intrusion of this insect pest was confirmed in Yemen and India and by 2019, devastation due to them were established in five more Asian countries including China (Hruska 2019). The destruction generated by this insect pest relies upon the geographic region, seed variety, planting time and the fundamental cultural habits in and around the field, although abiotic factors have an effect on egg and initial larval stage mortality during a rainy season and with various predators during dry season (Jaramillo-Barrios, Barragan-Quijano, and Monje-Andrade 2019).

S. frugiperda is treated as a crucial insect pest of maize, the third most essential cereal crop worldwide with a highest economic value in terms of production, potential and nutrition (Siddhartha 2019). This insect pest causes extensive damage to maize plants by feeding on young leaf whorls, corn cob and tassel (Bhusal and Bhattarai 2019). Younger larvae prefer epidermal leaf tissue and make holes on them, which is the damage symptom by these insect pests. Dead heart is formed by feeding on young plants through the whorls. Older larvae in the whorls of grown-up plants feed on cobs or kernels can reduce the quality and quantity of the yield (Sisay et al. 2019).

Scientific classification of *S. frugiperda*
https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=117472#null revised on June 25th, 2020.

Kingdom: *Animalia*
Phylum: *Arthropoda*
Class: *Insecta*
Order: *Lepidoptera*

Family: *Noctuidae*
Genus: *Spodoptera*
Species: *S. frugiperda*

Synthetic or chemical insecticides or genetically modified crops has been using to control insect pests (Sisay et al. 2019). Chemical control is the practice most often used to control this insect pest; however, this method has been inefficient due to incorrect and indiscriminate use, thus causing acute and chronic poisoning to farm workers, and inducing development of resistance, elimination of native natural enemies and pollution of soil. An alternative to the use of insecticides to control these pests used native natural enemies. The parasitoids of FAW families *Ichneumonidae, Braconidae, Eulophidae* and *Tachinidae* have been found in many Mexican states. Also, entomopathogenic bacteria, viruses, nematodes, and fungi have been reported (Ordóñez-García et al. 2015).

Even though, these control measures are very efficient, their extensive usage has provoked ecological problems, environmental contamination, development of resistance and ultimately negative effects on human health (Russo et al. 2019). Since the insects have gained resistance to various chemical insecticides, farmers are compelled for recurrent application of large amount of them, which will lead the accumulation of chemicals in the agricultural fields (Sisay et al. 2019). Taking together, scientists in different parts of the world are forced to develop more environmentally safe, cost-effective and reliable strategies to control insect pests (Mantzoukas and Eliopoulos 2020).

Integrated Pest Management (IPM) a global idea for agriculture is a holistic concept of approaching the crop production system as a whole process rather than only pest elimination. This approach combines various techniques like using resistant varieties, cultural manipulation, trap companion cropping and biological control (Mantzoukas and Eliopoulos 2020). Biological control is one of the technique to control insect pests with slightest environmental impact (De Silva et al. 2019). Cost-effectiveness, high yield, not harmful to beneficial insects and less chemical residues in the agricultural fields make entomopathogenic microorganisms as potential alternative to chemical pesticides (Mantzoukas and Eliopoulos 2020). At present, different species of bacteria (*Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Paenibacillus* spp., etc.) and fungi (*Beauveria*, *Metarhizium*, *Paecilomyces*, *Isaria*, *Lecanicillium*, *Hirsutella* etc.) are being applied

as biocontrol agents (Mantzoukas and Eliopoulos 2020; De Silva et al. 2019). Entomopathogenic fungus capacity to accommodate and sustain in external habitats other than their ancestry made them very efficient and adequate candidates for biological control measures (Bamisile et al. 2019; Dash et al. 2018). Considering as facultative microorganism that do not require arthropods as a host to complete their life cycles, *Metarhizium anisopliae* and *Beauveria bassiana* are the best characterized and most employed entomopathogenic fungi in biological control programs (Baron, Rigobelo, and Zied 2019)

As for other destructive *Spodoptera* species, many studies in chemical ecology have focused on the elucidation of the sex pheromone of this species with the aim of developing pest management strategies(Pinto-Zevallos, Strapasson, and Zarbin 2016) In certain areas, the control of *Spodoptera* relies on the use of transgenic crops, expressing the Cry toxin of *Bacillus thuringiensis* (Arias et al. 2015). However, several cases of resistance development have been reported. Cry1F resistant *S. frugiperda* was likely developed in 2006, in Puerto Rico; and although Cry1F resistant populations of *S. frugiperda* were not present in the U.S mainland during 2012, a 2013 study documented the first known Cry1F resistance in this (Li et al. 2016).

Biological control of pests

Biological control makes use of living organisms or their products, which manage the insect pest populations thereby minimizing the damage to crop yield, in terms of quality and quantity (Sree and Varma 2015). Biological control agents are considered suitable alternatives to the use of chemical pesticides as these organisms are likely specific to host insects, besides being safe to environment and mankind. Therefore, biological control is defined as the use of living organisms to suppress the population density or impact of a specific pest organism making it less abundant or less damaging. Thus, the aim of biological control is to reduce pest population below the economic threshold (Khan and Ahmed 2015).

In recent years, biological control of crop pests with entomopathogenic bacteria, viruses, fungi, and nematodes has been recognized as a valuable tool in pest management. Microbial control includes all aspects of utilization of microorganisms or their by-products for the control of pests. Microbial control agents are relatively host specific and do not upset other biotic systems. They are safe to humans, vertebrates, and beneficial organisms; do not cause environmental pollution; and are compatible with most other control methods. They are ideal for both short- and long-term

pest suppression. Unlike chemical pesticides, they do not leave chemical residues on crops, are easy and safe to dispose of, and do not contaminate water systems (Khan and Ahmed 2015).

Adoption of biopesticides available from plant products is now emerging as one of the important means to be used in protection of crops and the environment from pesticidal pollution, which is a global problem. Neem is regarded as most effective and ecofriendly. Derived from the Neem tree (*Azadirachta indica*), this contains several chemicals, including 'azadirachtin', which affects the reproductive and digestive process of several important pests (Gupta and Dikshit 2010). Nuñez-Mejía and collaborators worked with *A. indica* on *Trichoplusia ni* survival immune response and gut bacteria changes after the exposure to the *A. indica* volatiles (Nuñez-Mejía et al. 2016). Besides, the two primary effects of azadirachtin seem to be the blockage of calcium channels, and induction of mitochondria-mediated apoptosis. These two actions induce a cascade of effects, such as blockage of mitosis and a reduction in protein synthesis (Siegwart et al. 2015). Very few studies have highlighted the resistance to neem oil. Studies have demonstrated that the possible development of resistance may be due to the repeated treatments of a species (Siegwart et al. 2015).

Baculoviruses are target specific viruses which can infect and destroy a few important plant pests. They are particularly effective against the lepidopterous pests of cotton, rice, and vegetables. Their large-scale production poses certain difficulties, so their use has been limited to small areas (Dutta 2015). Lepidoptera larvae become increasingly resistant to NPV infections as they age (Siegwart et al. 2015).

Trichoderma is a fungicide effective against soil born disease such as root rot. It is particularly relevant for dry land crops such as groundnut, black gram, green gram, and chickpea, which are susceptible to these diseases. Preparations of *Trichoderma* biopesticide is cheap and require only basic knowledge of microbiology (Gupta and Dikshit 2010).

Trichogramma are minute wasps, which are exclusively egg parasites. They lay eggs in the eggs of various lepidopteran pests. After hatching, the *Trichogramma* larvae feed on and destroy the host egg. *Trichogramma* is particularly effective against lepidopteran pests like the sugarcane interned borer, pink bollworm and spotted bollworms in cotton and stem borers in rice. They are also used against vegetable and fruit pests (Gupta and Dikshit 2010)

Compared to other microorganisms, entomopathogenic fungi have received considerable attention as they are exceptionally virulent and function as lethal parasite of insect pests (Khan and Ahmed 2015). To date there are more than 700 species listed. Among these fungi, nine species are

commercialized or regularly studied: *B. bassiana, B. brongniartii, Metarhizium anisopliae, Aschersonia aleyrodis, Lecanicillium lecanii, Paecilomyces fumosoroseus, Entomophaga maimaiga, Hirsutella thompsonii* and *Lagenidium giganteum.* These fungi act as hyper parasites and penetrate their host through natural breaches in the cuticle or by creating breaches with enzymes such as chitinases. Other insect-wall-degrading enzymes are synthesized by these entomopathogenic fungi for example, cuticle-degrading protease, which is classified into two families, Pr1 and Pr2. In *M. anisopliae*, a Pr1 causes the melanization of the insect, which is a normal immune response in insects, but in extreme cases, it can lead to insect death (Siegwart et al. 2015).

Biological control using endophytes

Endophytes are microorganisms living in the internal tissues of the plants without causing any overt symptoms. The term "Endophyte" was introduced by De Bary in 1866 and was initially applied to any organism found within plant tissues that cause asymptomatic infections entirely within plant tissues without any symptoms of disease. An endophytic fungus lives in mycelial form in biological association with living plant at least for some time. Therefore, minimal requirement before a fungus to be termed as an endophyte should be the demonstration of its hyphae in the living tissue (Nisa et al. 2015).

The plant interacts and intimately associates with an array of taxa that is organ-specific to roots, stems, leaves, flowers, fruits, and seeds with some of them having beneficial effects on plants. How the endophyte microbiota is able to penetrate plant tissues and establish an intimate symptomatic partnership with their hosts in the appropriate plant organs, how some might switch to a virulent form while others could be beneficial for their hosts, are some lingering questions requiring in-depth insight to obtain satisfactory answers (Compant et al. 2016).

Every plant examined to date harbors at least one species of endophytic fungus and many plants, especially woody plants, may contain literally hundreds or thousands of species. With the discovery of "Taxol" from an endophytic fungi *Taxomyces andreanae* of pacific yew plant by Stierle and colleagues, a new era of research in endophytic biology opened, since before that pacific yew plant was the only source of "Taxol" known. Therefore, it is gradually established that certain endophytes during their long mutualistic symbiosis, somehow acquires potential to produce the phytochemicals mimetic to those as their host have (Verma et al. 2014).

Endophytes may contribute to their host plant by producing a plethora of compounds that provide protection and survival value to plants. Colonization of host plant by endophytic fungi is believed to contribute to host plant adaptation to biotic and abiotic stress factors. It is of special interest that in many cases, host plant tolerance to biotic stress has been correlated with fungal natural products. The nature and biological role of endophytic fungi with their plant host is variable. Endophytic fungi are known to have mutualistic relations to their hosts, often protecting plant against herbivory, insect attack or tissue invading pathogens and in some instances, endophytes may survive as a latent pathogen, causing quiescent infection for a long period and symptoms only when physiological or ecological conditions favors virulence (Nisa et al. 2015).

In terms of ecological interactions and evolutionary history, fungi that infect and kill insects are plainly fascinating. Most infect the host insect by transgressing the cuticle, that is, they do not have to be ingested to cause infection in the insect. This allows infection of insects with sucking mouthparts such as aphids. The breadth of variety of insect pathogenic fungi is significant, a group that comprises, over 100 fungal species. There are examples of insect pathogenic fungi found in most major fungal taxonomic groups from Chytridiomycota to basidiomycetes. Some of the insect pathogenic fungi are obligate pathogen whereas many are facultative. Adding further intrigue into their ecology is a subset of insect pathogenic fungi that additionally functions as endophytic symbionts of plants. Two genera of insect pathogenic fungi that fall within the category of endophytes are *Metarhizium* and *Beauveria*. The potential of these endophytes to control insect pests in agro ecosystems has been known since the early 20th century and numerous formulations of *Metarhizium* and *Beauveria* have been approved for use in crop protection (Barelli et al. 2016).

Metarhizium kills insect hosts within 3-7 days by producing toxins and absorbing nutrients. Once hemocoel nutrients are depleted, hyphae emerge from the insect cadaver and conidiate, resulting in the mummification of the insect host. *Metarhizium* is an excellent example of a fungus with a multifunctional lifestyle. It is an insect pathogen, a saprobe, and an endophyte. *Metarhizium* displays genotypic plasticity when exposed to dissimilar environments, thereby potentiating fungus effectiveness to saprophobically persist or as a colonizer of plant or insect hosts. It uses two different proteins, MAD1 and MAD2 to facilitate adherence on insect and plant surfaces respectively (Barelli et al. 2016).

More understanding is currently provided through mechanism-based studies, and using ecological, genetic and '-omics' approaches. Despite this effort currently carried out by an

increasing number of research teams, several questions and hypothesis should still be put on the table and contrasted. Several studies related to endophytes are, moreover, mostly biased by experimental models evaluated under gnotobiotic conditions that are far away from natural conditions. We need to move beyond and to analyze how the whole plant and its associated microbiota are working together under multitrophic scenarios (Compant et al. 2016).

Proteins

Plant pathogen interaction is a multifaceted process. At the beginning of the interaction, plants develop two pathways to recognize and resist pathogen attack. One pathway involves the formation of danger-associated molecular patterns (DAMPs) and Pathogen associated molecular patterns (PAMPs), whereas other ones result in effector-triggered immunity (ETI), and PAMPs-triggered immunity (PTI) after recognition by specific pathogen effector molecules. Accordingly, downstream signaling cascades are activated, producing antimicrobial compounds that kill the pathogen and thereby maintain the homeostasis. Numerous proteins and signaling pathways are engaged in this precisely controlled multifaceted process. Currently, proteomics provides a comprehensive insight to understand the intricacy of plant-pathogen interactions. Proteome is defined as the total protein components of the cell that are specified by the genome at specified condition and study the structure and function of all these proteins present in a cell, organ or organism at a particular time is known as the proteomics. Proteomics aim to find out the identity and amount of each protein present in a cell and actual function mediating specific cellular processes (Lodha, Hembram, and Basak 2013).

Acclimation to stress is mediated through profound changes in the gene expression, which results in the changes in composition of plant transcriptome, proteome, and metabolome (Kosová et al. 2011). Studies by different investigators like Gygi and collaborators (Gygi et al. 1999) and Brosche and collaborators (Brosche et al. 2007) demonstrated that changes in gene expression at transcriptional level often do not correspond to changes at protein level. Thus, plant proteomic investigation is important since proteins unlike transcripts are direct effectors of plant stress response. Apart from the enzymes that catalyze changes in metabolite level, proteins also are components of transcription and translation machinery, which means, the proteins also regulate plant stress response at transcript and protein level. Proteins have direct stress acclimation functions, leading to changes in plasma membrane, cell cytoplasm, cytoskeleton as well as

intracellular compartment composition which involve changes in their properties (Brosche et al. 2007).

Proteomics thus lead to identification of potential protein markers, whose changes in abundance can be associated with quantitative changes in some physiological parameters used for a description of genotype's level of stress (Kosová et al. 2011).

A study done by Alikhani and collaborators in 2013 (Alikhani et al. 2013) analyzed the proteome pattern of barley leaf sheaths in *Piriformospora indica*, colonized plants, which then compared with control plants under different salt stress conditions. Their results showed that *P. indica* reprogrammed the host physiology by altering the ion content and proteome pattern of barley leaf to cope with salt stress. Analyzing and integrating the physiological and proteomics data, Ghabooli and collaborators in 2013, (Ghabooli et al. 2013) showed that *P. indica* promoted growth of barley and altered the proteome pattern of *P. indica* colonized barley leaves under drought stress conditions.

In 2012, Sarhadi et al. (2012) used proteomic analysis to investigate the protein expression profiles of anther tissues in rice under salt stress. They compared the proteomic patterns of two rice genotypes, the salt sensitive and the salt resistant, under salt stress condition, and observed that there is a possible involvement of carbohydrate/energy metabolism and anther and pollen wall remodeling/metabolism in the adaptation of rice to salt stress at the reproductive stage.

Guttation

Plants need continuous water stream to supply stem and leaves with organic substances and inorganic ions which passes through xylem vessels powered by root pressure or sometimes by transpiration stream. On high humid atmospheric conditions, the difference in the water potential between ground and air will be zero, so no transpiration occurs, which happens normally in the night or in rainy seasons. To maintain the transport of solutes under this conditions, plants secrete water through an opening in the leaves known as hydathodes (Grunwald et al. 2003). The process of exudation of liquid droplets, loss of water from the tips, edges, adaxial and abaxial surfaces of uninjured leaves of a wide range plants is known as guttation (Singh 2016). Several transduction and transport proteins originating from both shoot and roots are found in the guttation fluid are also transported to the sites of active vegetative and reproductive growth where they are required for the formation and development of fruits and seeds in plants (Singh 2016). Some studies reports

the presence of peroxidase and catalases in maize and oats, reductase in timothy-grass, and peroxidases in strawberry, tomato and cucumber and recombinant proteins in tobacco (Komarnytsky et al. 2000). Schmolke and collaborators in 2018 reported plant guttation fluid as a potential passage of pesticide exposure to honey bees (Schmolke, Kearns, and O'Neill 2018).

Some of the Different Techniques Used in Proteomics.

1. **One-dimensional SDS gel electrophoresis**

Electrophoresis is used to separate complex mixtures of proteins from cells, subcellular fractions, column fractions or immunoprecipitants to investigate subunit compositions and to verify homogeneity of protein samples. Proteins migrate in response to an electrical field through pores in a polyacrylamide gel matrix, pore size decreases with increasing acrylamide concentration. The combination of pore size and protein charge, size and shape determine the migration rate of the protein. In sodium dodecyl sulfate (SDS) electrophoresis, standard Laemmli method is described for discontinuous gel electrophoresis under denaturing conditions. (Gallagher 2006).

2. **Fluorescent Two-Dimensional "Difference Gel Electrophoresis (2D-DIGE)**

DIGE proteomics uses 2D gel electrophoresis to analyze differential protein regulation between control and target samples. In this technique, the two samples to be compared are each treated with one of the two different but structurally similar fluorescent dyes (cy2, cy3 and cy5 etc.). Each dye reacts with amino groups, so that each protein is fluorescently labeled by the dye binding to lysine residues and the N-terminal amino group. The two protein mixtures to be compared are then mixed and run on a single 2D gel. Thus, every protein in one sample superimposes with its differentially labeled identical counterpart in other sample. Scanning of the gel at two different wavelengths that excite two dye molecules reveal whether any individual spot is associated with only one dye molecule rather than two. The resulting images are then analyzed by software which are specifically designed for 2D-DIGE analysis (Lodha et al. 2013).

3. **Mass Spectrometry**

Mass spectrometry (MS) is an analytic technique which plays central role in the field of proteomics. Proteins of peptides are fragmented by using the trypsin. The fragmented proteins are

then separated using the liquid chromatography. The samples are then ionized and converted into the gaseous phase. Two soft ionization techniques, namely, Matrix–Assisted Laser Desorption/Ionization (MALDI) and Electron Spray Ionization (ESI) are used for the ionization. Peptides having a specific mass are fragmented using collision-induced dissociation and sent through a second mass spectrometry which generates a set of fragment peaks from which the amino acid sequence of the peptide are inferred. Protein identification algorithms are used to compare the results obtained with the known standards. Such algorithms fall into two categories: database search algorithms and de novo search algorithms (Lodha et al. 2013).

Definition of the problem

Food production needs to be increased to meet the needs of the rapidly expanding world population. Unfortunately, several cereal losses are still inevitable owing to insect pests. Humans often face problems of the tremendous increase in the incidence of insect pests in agriculture and many urban areas. Chemical synthetic drugs with many side effects are being used to cope with these agriculture and medical problems. But insect pests are prone to develop “drug” resistances to decrease substantially the effectiveness of often used insecticides. Accordingly, there is an urgent need to work towards the production of safer insecticidal agents which are expected to be renewable, non-petrochemical, naturally, eco-friendly, and easily obtainable (Yu et al. 2010).

Since the late 1940’s, insect pest control has relied mostly on chemical insecticides, although in many industrialized nations, pest management strategies have been shifting to the use of transgenic plants expressing particular traits such as resistance to insects. However, the replacement of chemicals with transgenic plants does not represent a fundamental change in approach. A true paradigm shift would be a change from dependence on chemicals to a total system approach or to ecological engineering. A basic component of both approaches is a better understanding of the various ecological components in an ecosystem, including biological control agents. Among these, entomopathogenic fungi have been traditionally considered as important mortality factor for insects. Understanding the nature of these interactions could facilitate more effective exploitation of entomopathogenic fungi for pest biocontrol strategies throughout the world, including countries where the use of other strategies might not be affordable (Vega et al. 2009).

Many entomopathogenic fungi play additional roles in nature, including as plant endophytes, antagonists of plant pathogens, beneficial rhizosphere-associates and possibly even plant growth promoters (Vega et al. 2009). Some entomopathogenic taxa have been identified as natural endophytes in more than one host plant species i.e., *Beauveria bassiana* and, *Lecanicillium lecanii,* indicating that they are multi-host endophytes infect several plant species (Quesada et al. 2014).

Some studies showed that the entomopathogen *B. bassiana* could be artificially inoculated into leaves of corn plants and behave as an endophyte (Quesada et al. 2014). Compared with conventional biopesticides, the use of *B. bassiana* as inoculated endophytes have the advantage of targeting the pest within the plant at reduced application cost because little inoculum is required in cases where colonization is systemic. Furthermore, the endophytic fungus is protected inside the plant from abiotic and biotic factors. Most of these studies have only completed the first stage, that is, inoculation into the plant, although some of them have gone further, stating that the endophytic colonization of the plant by an entomopathogenic fungus affects the survivorship and development of insects, while reducing plant damage (Quesada et al. 2014).

Although the role of fungal endophytes as biological control agents of insect pests is recognized, the role of endophytic entomopathogens in the biological control of them is poorly studied. At the present time, reports of insect pests' control *in planta* are scarce, but occur in different systems and deserve attention. Many studies indicate that the mechanisms by which endophytic entomopathogens induce plant tolerance to insect pests are varied, and hypothetically, the following may be involved: (1) production of antifungal compounds, (2) competition for space and nutrients, (3) parasitism on the insect or (4) induction of host defense responses (Quesada et al. 2014).

B. bassiana produces an array of chemically diverse secondary metabolites which nominate it as superior entomopathogenic fungi. Beauvericin, bassianolide, bassianin, tenelllin, and cyclosporine A are the key secondary metabolites produced by *B. bassiana*. Investigation on Beauvericin has demonstrated that this metabolite has insecticidal, antibiotic, cytotoxic, and ionophoric properties. Recent research has shown that there are various tri-partite interactions between plant, pest insect and entomopathogenic fungi. Most interesting interactions are summarized as (a) Plant may affect the infection by the entomopathogen, (b) Plant may affect the

persistence of the entomopathogen, (c) *B. bassiana* can persist as an endophyte within plants. Another important aspect of this tri-trophic interaction is the fact that toxic metabolites of *Beauveria* spp. may enter the plants, though such reports validating the hazardous effects of its toxins on environmental health are still unreported (Keswani, Singh and Singh 2013)

Fungal entomopathogens, including *Beauveria bassiana* Vuillemin and *Metarhizium anisopliae* Sorokin, have been tested as biocontrol agents in laboratory and greenhouse trials against many pests. However, when fungi are sprayed onto plants, pests can be difficult to target because of their location on the underside of leaves such as the whitefly *Aleurotrachelus socialis* Bondar or because they are subterranean, such as the burrower bug *Cyrtomenus bergi* Froeschner. The efficacy of fungal entomopathogens is also limited by abiotic factors that reduce viability of fungal conidia. An alternative application method is to inoculate plants with fungal entomopathogens that become established as endophytes, thereby possibly providing the plant with protection against pests from within, lowering the volume of inoculum required, protecting the fungus against abiotic factors (Greenfield et al. 2016).

In addition, on sub-culturing the fungi in axenic medium, the endophytes tend to lose their ability to produce secondary metabolites. This process is referred to as attenuation, which has become a serious impediment to use of endophytic fungi as alternative source of plant secondary metabolites. Among the various reasons, it is hypothesized that the attenuation could be due a lack of host specific stimuli when the fungi are cultured in culture media, and/or due to silencing of genes in the culture media (Mohana Kumara et al. 2014). So, it is so important to grow these endophytes within the seed naturally, where they get the host specific stimuli to produce the secondary metabolites which have insecticidal (in the case of endophytic fungal entomopathogens) or growth promoting properties.

HYPOTHESIS

The use of *B. bassiana* in the treatments of plant seeds enhances its establishment as an endophyte, thus acting as a growth promoter, inducer of drought tolerance, and to control the population of *S. frugiperda* in corn.

GENERAL OBJECTIVE

The general objective of this study was to establish *B. bassiana* as an endophyte in *Z. mays* plant and evaluate its effects on plant growth and tolerance against drought and *S. frugiperda* damage. In addition, to analyze the proteins involved in this beneficial plant-fungus interaction.

PARTICULAR OBJECTIVES

1. To determine a method of inoculation for endophytic establishment of the three strains (GHA, PTG4 and PTG6) of *B. bassiana* in *Z mays* and *A. thaliana*.
2. To evaluate the positive endophytic effects of *B. bassiana* on *Z. mays* and *A. thaliana* plant growth and yield.
3. To evaluate the effect of endophytic *B. bassiana* in drought tolerance in *Z. mays* and *A. thaliana*
4. To evaluate the endophytic effects of *B. bassiana* on *S. frugiperda* tolerance in *Z. mays* plant under laboratory and field conditions.
5. To analyze the proteins involved in the *Z. mays-B. bassiana* interaction using plant guttation liquid.

MATERIALS AND METHODS

1. Method of inoculation for endophytic establishment of the three strains (GHA, PTG4 and PTG6) of *B. bassiana* in *Z. mays* and *A. thaliana*.

Arabidopsis thaliana seeds (Seeds from previous studies of Dr. Ek Ramos were planted and second generation seeds were used in the present study) were collected, and surface sterilized with a sterile solution prepared with 5 mL Tween, 3.5 mL 70% ethanol and sterile 1.5 mL distilled water. The sterilizing procedure was as follows: The *Arabidopsis* seeds were washed in 500µL 70% ethanol for two minutes, followed by 500µL sterile solution for five minutes. Then the seeds were washed five times with sterile distilled water. After that, the seeds were kept in 1 mL sterile distilled water for 1 week in 4° C to induce germination by scarification.

Candidate endophyte spores of *B. bassiana* GHA strain, commercially obtained as Botanigard® 22WP (BioWorks Inc., Victor NY), and PTG4 (GenBank accession number KC759730.1) and PTG6 (GenBank accession number KC759731.1) strains, isolated from *Periplaneta americana* [kindly provided by Dr. Patricia Tamez, from Autonomous University of Nuevo Leon (UANL), México] were stored at -80 °C in a So-Low Ultra freezer (Environmental Equipment, Cincinnati, OH). All *B. bassiana* strains were activated by plating stock cultures onto potato dextrose agar (PDA, BD Difco, CDMX, Mexico) and incubated in darkness at 25°C ± 2 °C for a week. To obtain a monosporic culture, a single selected colony was inoculated into a 500 mL Erlenmeyer flask, containing 200 mL of potato dextrose broth (PDB, BD Difco, CDMX, Mexico) and kept at 25 °C ±2 in an automatic rotary shaker (Orbit1900, Labnet, CDMX, Mexico) at 120 rpm, for five days or until blastospores production. Blastospores were counted in a Neubauer chamber and each treatment was adjusted to a final concentration of 1 x 10^6 spores/mL. Methyl cellulose (MC) (Sigma-Aldrich, St. Louis, MO) and cornstarch (CS) (Unilever Manufacturera, S. de R.L. de C.V., CDMX, Mexico) were mixed with blastospores, for adequate attachment to the seeds. Blastospores + MC (2% MC final concentration) was prepared by first dissolving the reagent in warm distilled water at 35-40 °C to a pre-gelatinized state. Blastospores + CS (4% CS final concentration) was prepared in the same way but using boiling distilled water. Blastospores were then mixed with

each adherent material at room temperature, until a homogeneous suspension was obtained; next, seeds [15 seeds/treatment (MC or CS)/strain] were added and dried at 25 °C ± 2°C for 2 h. Controls included seeds without any treatment (CC) and seeds without fungi, but with MC (CMC) or CS (CCS). Fifteen seeds per treatment are planted in vermiculate soil and black soil mixed 1:1proportion and previously sterilized, in seedling trays under growth chamber conditions; (25°C ± 2°C) kept humid by covering with plastic lids until germination.

Seedlings were daily irrigated with 5mL distilled water. Endophytic colonization was microbiologically determined in seedlings which plant height, fresh, and dry weights were recorded as mentioned below. Plants were surface sterilized, by first rinsing plants under tap water for five minutes, then submerged into 2% ethanol for two minutes, then five minutes in sterile water, and washed in sterile distilled water for five minutes; sterilization efficiency was determined by plating 100 µL of the last washing on PDA plates. Endophytic fungal inoculation efficiency was evaluated by plating 1-2 cm length fragments of leaves, shoots, and roots on PDA plates, under sterile conditions. Typical *B. bassiana* colonies were isolated and confirmed by PDA culturing and observed hyphal and conidial structures under a compound microscope (Zeiss Primo star, Carl Zeiss Microscopy Gmblt, Gottingen, Germany).

Zea mays seeds Chalqueño seeds were obtained from *Centro Internacional de Mejoramiento de Maíz y Trigo* (CIMMYT), Mexico, and from Dr. Verónica Garrocho-Villegas, from Autonomous National University of Mexico (UNAM), Mexico. The same procedures were used as with *A. thaliana*, with the only difference that the inoculated seed were dried for 24 hours at 25°C ± 2°C. Endophytic colonization of a subset of 15 plants/per treatment was tested at 14 days old *Z. mays* plants. They were analyzed for endophyte presence in root, stems and leaves following the same procedure as mentioned above for *A. thaliana*.

2. Potential endophytic effects of *B. bassiana* on *Z. mays* and *A. thaliana* plant growth and yield.

In the case of *Z. mays* plants, fourteen days after germination, plant height, fresh, and dry weights were recorded from half of the plants. Remaining plants were transplanted into individual

pots and maintained for observing plant performance and flowering time under greenhouse conditions. For *A. thaliana*, the rosette formation and flowering time were recorded.

3. Endophytic effect of *B. bassiana* in drought tolerance in *Z. mays*

At day 14 after germination, seedlings were left without watering for 10 days. On day 11, plants were watered and stored in the growth chamber to observe their vigor recovery after 24 h.

4. Endophytic effect of *B. bassiana* on *S. frugiperda* tolerance in *Z. mays* plants under laboratory and field trial.

S. frugiperda eggs were kindly donated by Dr. José Refugio Lomelí-Flores, [Posgrado en Fito sanidad, Entomología y Acarología, Colegio de Postgraduados, Montecillo, Texcoco, Estado de México, México] carefully placed in 700 ml plastic bottles and kept in the breeding room under controlled conditions (temperature of 27°C ± 3°C, humidity 60% ± 5, photoperiod 14 light: 10 dark) until hatched and then were transferred to individual diet cups with 5 ml modified artificial wheat germ diet as their food source (McGuire et al. 1997). This diet was replaced when necessary to prevent desiccation. To perform bioassay, *S. frugiperda* larvae belonging to the second laboratory generation were used.

Each third instar *S. frugiperda* larvae (Fig. 1) was carefully transferred onto 10 days old *Z. mays- B. bassiana* treated*, Z. mays*-no treated and *Z. mays* plants with only methyl cellulose plants (larva/plant/treatment) and then covered with mesh bags to prevent escapes (Fig. 2).

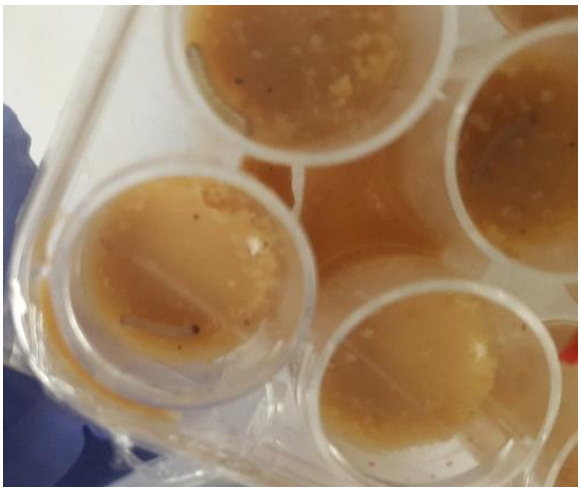

Fig. 1. 3rd instar *S. frugiperda* larva in the artificial diet

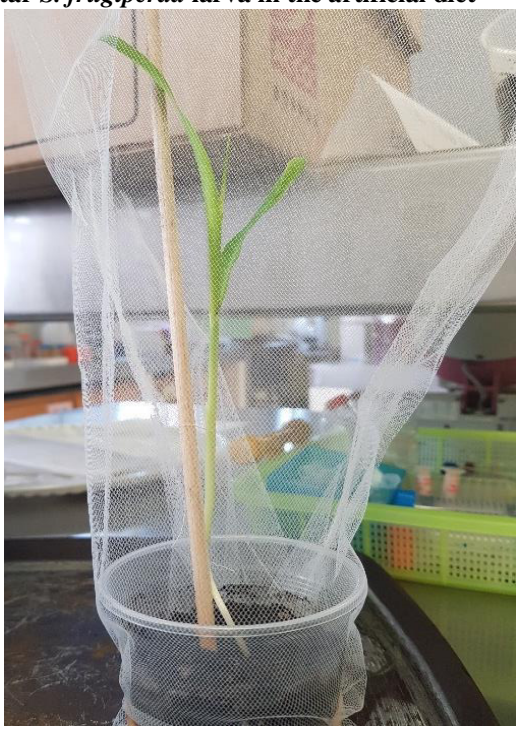

Fig. 2. *S. frugiperda* larva on *Z. mays* plant covered with a mesh bag.

Plants were replaced into the cages every 24 hours. When the larvae reached their 6th instar, were returned to the artificial diet to monitor pupal stage. Each pupa was examined under a stereoscope (Labomed Stereomicroscope, Luxeo2S, CA, USA) to determine their sex, weighed in a weighing balance (AND, A&D Company Limited, N-92, Korea) and their length was measured with a normal scholastic ruler. After that, pupae were transferred to individual plastic containers (7 cm diameter x 16 cm height) separating male and female, (4-5 pupae in one bottle), bottles were

covered with mesh bags. In the lower part of the bottle, a small piece of cotton embedded in sugar syrup was provided as food source for the adult flies. Bottles were analyzed every day to check adult emergence. The pupae were maintained under the most suitable laboratory conditions (25 °C ± 2 °C temperature, 60% ± 5 relative humidity and 14:12 h light and dark photoperiod). During this experiment parameters like a) initial larval numbers, b) number of larvae dead during the experiment, c) number of larvae remained as larvae even after 6th instar of larval developmental stage, d) number of larvae remained as prepupa, e) number of larvae reached the pupal stage in reasonable time, f) larval weight before transferring back to the artificial diet, g) pupal weight, h) pupal length and i) pupal sex ratio were recorded.

Field trial: Experiment 1

The first field trial was done in an agricultural field situated in General Teran, Nuevo Leon with geographical distribution 25°16'00''N99°41'00''0. The main agricultural products of this region include citric products, corn, sorghum, wheat, livestock products, etc. In this zone, maize crops are generally affected with *S. frugiperda* and farmers normally use *Bacillus thuringiensis* to control them. One-hectare field was prepared as follows: with the help of a tractor, the field soil was mixed and 38 furrows of 100 meters and 25 furrows of 80 meters, with a furrow to furrow distance 80 cm, were prepared manually. With the above-mentioned seed inoculation procedure, 3300 *Z. mays* seeds criollo (kindly donated by the field owner, without any insecticidal or fungicidal application) variety were inoculated with *B. bassiana* strain GHA with a concentration of 1x10^{6} blastospores/mL and methyl cellulose as adherent. A total of 2500 seeds were used as no-treated plants without any fungal or adherent treatment. The seeds were planted during mid-February 2019 on furrows with 25 cm between each seed, distance between each furrow was 80 cm and, in each furrow, 100 seeds were planted which were monitored every week. First 33 furrows were used to plant *B. bassiana* treated seeds, then left 5 furrows and the left 25 furrows were used for the negative control treatments (Fig. 3) In this experiment, we recorded the germination percentage at 3rd week after planting them and observed the presence of *S. frugiperda* larva between 5th true leaf

and 10th true leaf time period. Other than watering every day, neither any fertilizer, nor pesticides were applied in the fields during the whole experiment.

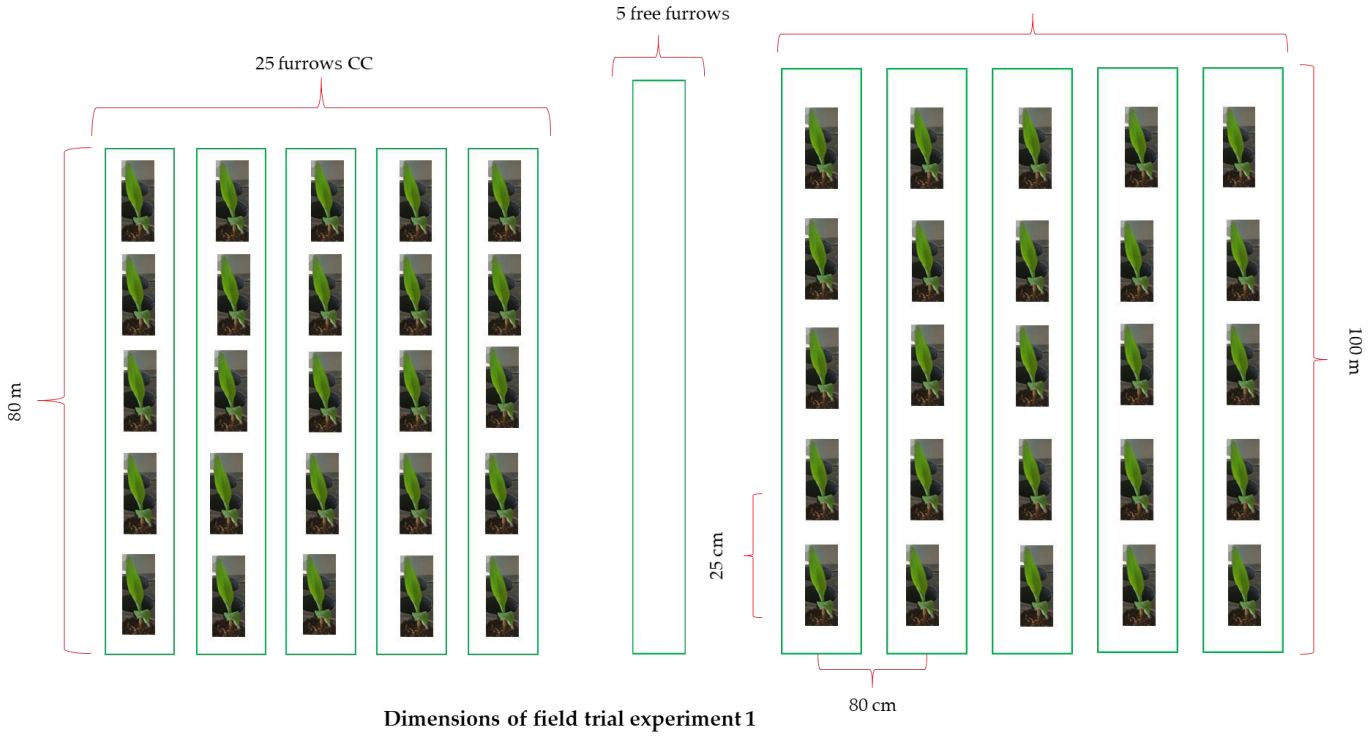

Fig. 3. Dimensions applied in the field trial experiment 1

Field trial: Experiment 2

The second field trial was conducted in the same way as the first trial, during mid-April, removing only no-treated plants of the previous experiment, but leaving aside *B. bassiana*- maize plants of the first trial. We inoculated 500 maize seeds with *B. bassiana* GHA strain with a concentration of $1x10^8$ blastospores/ml and methyl cellulose as adherent and kept 500 seeds as negative control without any fungal or adherent treatment. The seeds were planted as same manner as the first field experiment (Fig. 4). Plant height and number of leaves of second and fourth furrow were recorded on 3rd week after planting seeds. Five corn cobs from each treatment were collected,

weighed with a weighing balance (AND, A&D Company Limited, N-92, Korea) and measured its length using a normal scholastic ruler.

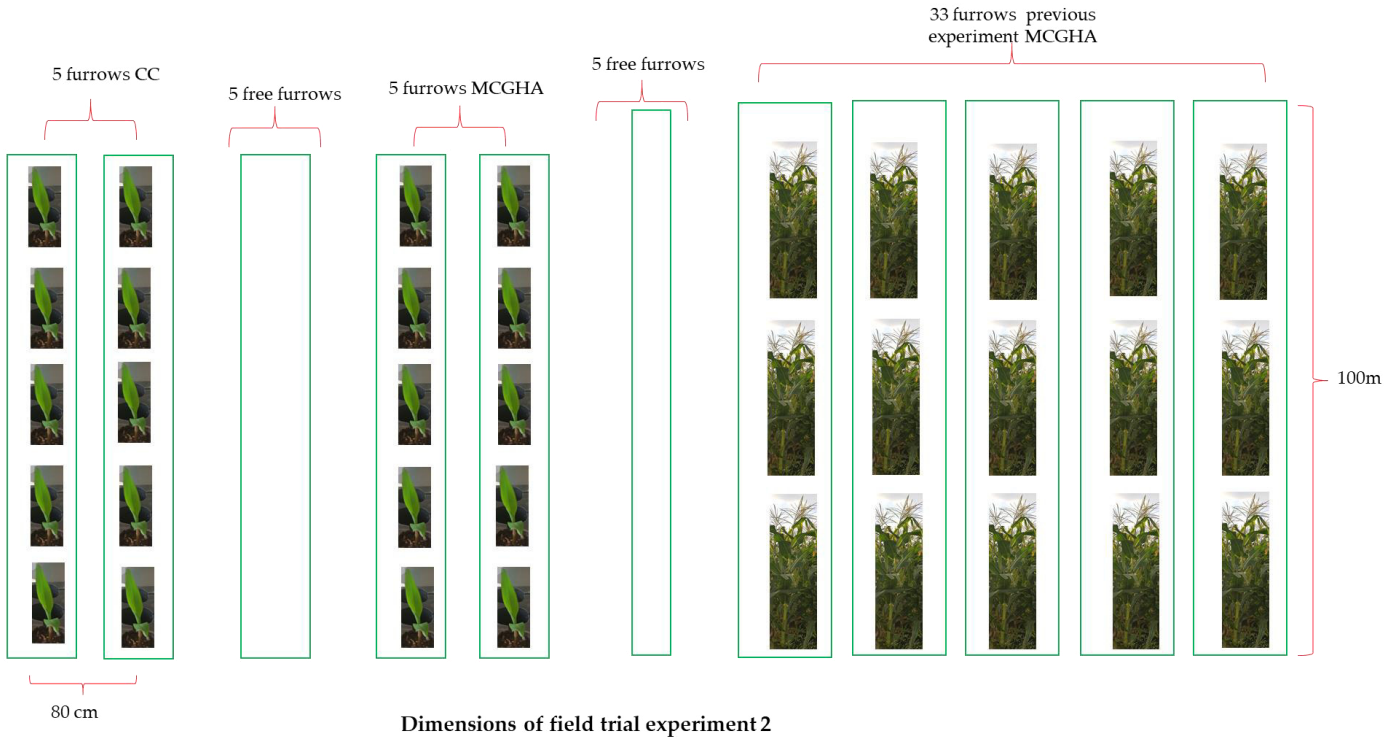

Fig. 4. Dimensions applied for the field trial experiment 2

5. Proteins present in the *Z. mays-B. bassiana* interaction using plant guttation liquid.

Z. mays seeds were treated as mentioned above. Once they were germinated, guttation fluids were collected into Eppendorf tubes, every day until 14th day, from negative control without any fungal or adherent treatments plants and from with only methyl cellulose and *B. bassiana* treated plants and stored at -20 °C freezer, until further use. Proteins were precipitated with acetone. For 1mL guttation liquid, 10 drops of acetone were added to each tube, mixed by inversion, and stored at -80 °C in a So-Low Ultra freezer (Environmental Equipment, Cincinnati, OH) overnight. Then they were centrifuged at 4°C in a climatized centrifuge (Eppendorf AG 22331 Hamburg, Centrifuge 5424R, 20BB Laboratory Equipment, Germany) for 20 min. Decanted the supernatant

liquid and kept open the lid for 5 minutes to evaporate rest of the acetone. To the precipitate added 10 µl of sterile distilled water, mixed thoroughly and read in a nanodrop (Thermoscientific Nanodrop Lite Spectrophotometer, Accesso Lab, Mexico) at 280 nm using water as blank. Took the lowest value of the readings and calculated total proteins present in the final volume and adjusted the volume of each reading to get the same quantity of proteins. These were run in one-dimensional vertical gel electrophoresis (Mini-PROTEAN Tetra cell, Bio-Rad, Mexico) according to the manufacturer's instructions.

Statistical Analysis

Effects of treatments on seed germination were analyzed and reported with mean values and standard errors from three different biological replicates. Furthermore, results of *B. bassiana* re-isolation experiments were pooled per treatment and reported as the total percentage of re-isolation of *B. bassiana* from different tissues of each treatment. This was taken as an estimate of the overall establishment of each strain of *B. bassiana* into *Zea mays* tissues, as these plants were a representative sample of the total plants treated. The remaining half of the total plants treated were transplanted and plant performance was recorded under greenhouse conditions. Data from plant performance were subjected to one-way ANOVA, and when a significant F test was obtained, separation of treatment means was performed using Duncan's multiple range test. Data were analyzed using the software IBM SPSS Statistics Version 21. Prior to the statistical analysis, the values for the effects of *B. bassiana*-treated plants on the developmental stages of *S. frugiperda* were arcsine transformed. Before ANOVA, all data were tested for homogeneity of variance using Levene's statistics. Considering there were only two groups to analyze in the germination data of the field trial experiment 1 and corn cob data from field trial experiment 2, Independent Sample T-test was used. Significance levels were calculated by Levene's test for equality of variance. All values were graphed using the software Origin 50 Version 2. Different letters indicated statistically significant differences between treatments at a confidence level of 95%. These data were tested for normality and homogeneity of the variance.

RESULTS

1. Method of inoculation for endophytic establishment of the three strain (GHA, PTG4 and PTG6) of *B. bassiana* in *Z. mays* and *A. thaliana*.

Effect of *B. bassiana* and adherents on *Z. mays* germination

Germination percentage depended on strain, adherent type, and time, MCPTG4 was the best treatment. (Table 1).

Table 1. *Zea mays* germination percentage under *B. bassiana-Z. mays* seed treatment

Treatments	Germination at day 5 (%)	Germination at day 14 (%)
CC	56 ± 4	63 ± 4
CMC	23 ± 4	50 ± 3
CCS	23 ± 4	47 ± 0
MCGHA	7 ± 7	53 ± 0
CSGHA	17 ± 4	73 ± 8
MCPTG4	46 ± 2	87 ± 7
CSPTG4	27 ± 0	53 ± 0
MCPTG6	14 ± 7	23 ± 10
CSPTG6	40 ± 7	63 ± 10

CC: Negative control (Untreated seeds), CMC: Negative control with the adherent methyl cellulose, CCS: Negative control with the adherent corn starch, MCGHA: *B. bassiana* strain GHA with methyl cellulose, CSGHA: *B. bassiana* strain GHA with corn starch, MCPTG4: *B. bassiana* strain PTG4 with methyl cellulose, CSPTG4: *B. bassiana* strain PTG4 with corn starch, MCTG6: *B. bassiana* strain PTG6 with methyl cellulose, and CSPTG6: *B. bassiana* strain PTG6 with corn starch.

Some *B. bassiana*-treated seeds germinated slower than untreated ones at 5-day post sowing; interestingly, overall germination at day 14 significantly ($p < 0.05$) increased in almost all treatments, as compared with the controls. MCPTG4 showed consistent positive effects on germination percentage (Day-5 = 46 ± 2%; day 14 = 87 ± 7%), as compared with the controls (day 5: CC = 56 ± 4%, CMC = 23 ± 4%, CCS = 23 ± 4%. Day 14: CC = 63 ± 4%, CMC = 50 ± 3%, CCS = 47 ± 0%), whereas the other treatments showed low germination percentages at day 5, which was recovered at day 14, except for MCPTG6 treatment (23 ±10%). GHA strain showed significant inhibitory effects on germination at day 5, which was higher using MC (MCGHA = 53 ± 0%, CSGHA = 73 ± 8%), indicating that GHA strain could overcome early inhibitory effects and adherent type synergistic effects, but depended on which one was used, CS was the best. PTG4 strain did not show early inhibitory effects, treated seeds germinated well from day 5 (day 5: MCPTG4 = 46 ± 2%, CSPTG4 = 27 ± 0%), as compared with negative controls. However, it was noticed that MC potentiated positive strains effect on germination at day 14 (day 14: MCPTG4 = 87 ± 7%), which was higher than that of negative controls. PTG6 strain showed early inhibitory effects only with MC (day 5: MCPTG6 = 14 ± 7%), which was not recovered at day 14 (MCPTG6 = 23 ± 10%). However, with CS there were no inhibitory effects observed (day 5: CSPTG6 = 40 ± 7%, day 14: CSPTG6 = 63 ± 10%), indicating that adherent type was important for PTG4 and PTG6 positive effects on germination.

Effect of *B. bassiana* and adherents on *A. thaliana* germination

Germination percentage depended on strain, PTG6 was better, and depending on the adherent methyl cellulose was the best treatment. (Table 2).

Table 2 Effect of *B. bassiana* and adherents on *A. thaliana* germination

Treatments	% of germination
CC	59 ± 0.07
CMC	62 ± 0.16
CCS	48 ± 0.186
MCPTG4	61 ± 0.127
MCPTG6	67 ± 0.037
CSPTG4	51 ± 0.117
CSPTG6	60 ± 0.04
*MCGHA	33%
*CSGHA	80%

CC: Negative control (Untreated seeds), CMC: Negative control with the adherent methyl cellulose, CCS*:* Negative control with the adherent corn starch, MCGHA: *B. bassiana* strain GHA with methyl cellulose, CSGHA: *B. bassiana* strain GHA with corn starch, MCPTG4: *B. bassiana* strain PTG4 with methyl cellulose, CSPTG4: *B. bassiana* strain PTG4 with corn starch, MCTG6: *B. bassiana* strain PTG6 with methyl cellulose, and CSPTG6: *B. bassiana* strain PTG6 with corn starch.*MCGHA and CSGHA, did not have repetition.

Even though, there were a slight difference in the germination percentage in different treatments and the negative controls, statistical analysis with one-way ANOVA, did not show any significant difference among them ($F_{(6,24)} = 0.301$, $P = 0.928$).

***B. bassiana* re-isolation percentage in *Z. mays* plants**.

There were no evidence of *B. bassiana* recovery from any of the negative control (CC, CMC, CCS) treatments from any of the plant tissues (roots, stems, leaves), whereas, each one of the three stains were recovered from roots regardless of which adherent was used. (MCGHA = 100%, CSGHA = 100%, MCPTG4 = 100%, MCPTG4 = 100%, CSPTG4 = 100%, MCPTG6 = 100%, CSPTG6 = 100%). However, in stems and leaves recovery percentage was different depending on the strain and adherent used. In stems, GHA had 88% of *B. bassiana* recovery, which did not depend on adherents (MCGHA = 88%, CSGHA = 88%). PTG4 had the highest recovery

percentage of 100 when MC was the adherent, but it decreased when CS was used (63%) and in the case of PTG6 71% of recovery observed with CS as the adherent, but recovered only 25% with MC was used as the adherent. In leaves, GHA had 50% recovery, which did not depend on adherents (MCGHA = 50%, CSGHA = 50%); PTG4 had the highest recovery (100%) with MC, but with CS only 25% was recovered, and PTG6 had 75% of recovery which did not depend on adherent type. Therefore, results indicated high endophytic inoculation efficiency, particularly with MCPTG4 treatment. In addition, recovery percentage was depended on the strains and adherents used. But overall, maize plants were successfully colonized by all *B. bassiana* strains. Results are showed in the Table 3.

Table 3 *B. bassiana* re-isolation percentage in *Z. mays* seedlings.

Treatments	% of *B. bassiana* re-isolation from roots	% of *B. bassiana* re-isolation from stem	% of *B. bassiana* re-isolation from leaves
CC	0%	0%	0%
CMC	0%	0%	0%
CCS	0%	0%	0%
MCGHA	100%	88%	50%
CSGHA	100%	88%	50%
MCPTG4	**100%**	**100%**	**100%**
CSPTG4	100%	63%	25%
MCPTG6	100%	25%	75%
CSPTG6	100%	71%	75%

CC: Negative control (Untreated seeds), CMC: Negative control with methyl cellulose, CCS: Negative control with corn starch, MCGHA: *B. bassiana* strain GHA with methyl cellulose, CSGHA: *B. bassiana* strain GHA with corn starch, MCPTG4: *B. bassiana* strain PTG4 with methyl cellulose, CSPTG4: *B. bassiana* strain PTG4 with corn starch, MCPTG6: *B. bassiana* strain PTG6 with methyl cellulose, CSPTG6: *B. bassiana* strain PTG6 with corn starch.

B. bassiana re-isolation percentage in *A. thaliana* plants

Because of the small size of the plant, here we divided the plant into root and shoot only. Due to practical difficulty in handling this plant, we do not have 3 biological repetitions for this experiment. With all the available data, the fungal strain GHA could be re-isolated from both the root and shoot part of the plant. Results are shown in the Table 4.

Table 4 *B. bassiana* re-isolation percentage in *A. thaliana* plants

Treatments	% of *B. bassiana* re-isolation from roots	% of *B. bassiana* re-isolation from shoots
CC	0	0
CMC	0	0
CCS	0	0
MCGHA	**71%**	**100%**
CSGHA	0	100%
MCPTG6	0	67%
CSPTG6	0	0

CC: Negative control (Untreated seeds), CMC: Negative control with methyl cellulose, CCS: Negative control with corn starch, *MCGHA*: *B. bassiana* strain GHA with methyl cellulose, *CSGHA*: *B. bassiana* strain GHA with corn starch, *MCPTG6*: *B. bassiana* strain PTG6 with methyl cellulose, *CSPTG6*: *B. bassiana* strain PTG6 with corn starch.

B. bassiana was not recovered from any of the negative control treatments, (CC, CMC, CCS) from root or shoot of *A. thaliana* plants. Whereas, with the fungal strain GHA, using adherent methyl cellulose it was re-isolated from both root and shoot (MCGHA root = 71%, MCGHA shoot = 100%). However, with the adherent corn starch it was re-isolated only from the shoot (CSGHA shoot = 100%). With the fungal strain PTG6, it could be re-isolated only from the shoot part of the plant with methyl cellulose (MCPTG6 67%). With adherent cornstarch and the fungal strain PTG6, it could not be re-isolated from roots of shoot.

2. Potential endophytic effects of *B. bassiana* on *Z. mays* and *A. thaliana* plant growth and yield.

PTG6-treated plants (MCPTG6 and CSPTG6) showed a faster growth rate in plant height for the first two weeks after their transplantation (Figs. 5A and B; Figs. 6A and B), but there was no significant difference at week 6 (Fig. 5F and Fig. 6F), independently of the adherent used. For other treatments, no significant differences in plant height were observed. By the end of the growing season, 37% of the flowering plants were negative controls (CC, CMC, CCS) and the remaining 63% were MCGHA, MCPTG4, CSPTG4 and MCPTG6 treated plants indicating positive effects on flowering percentage after using *B. bassiana* strains, which did not depend on the adherents.

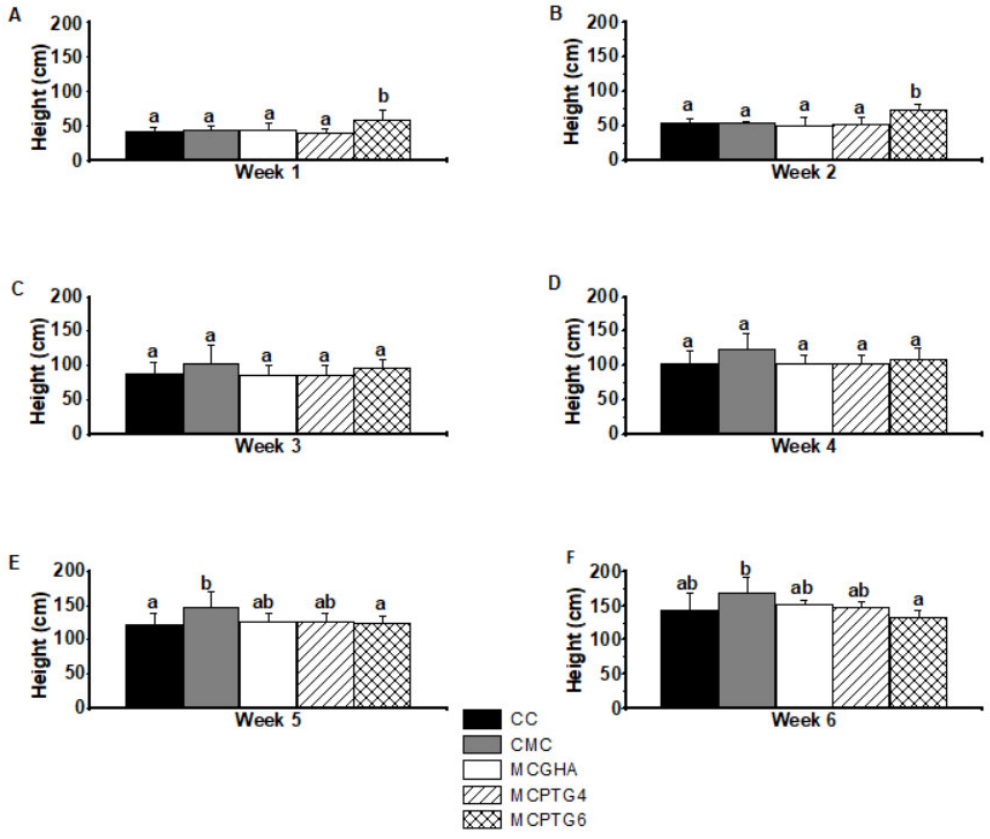

Fig. 5. Effect of *B. bassiana* seed treatments with methyl cellulose as adherent on the height of *Z. mays* plants. CC: Negative control, CMC: Negative control with methyl cellulose, CCS*:* Negative control with cornstarch, MCGHA: Fungal strain GHA with methyl cellulose, MCPTG4: Fungal strain PTG4 with methyl cellulose and MCPTG6: Fungal strain PTG4 with methyl cellulose. Post hoc analysis was done by Duncan multiple range test (α 0/05) after one-way ANOVA. Graphical bars with same letters indicate that there are no significant differences among them.

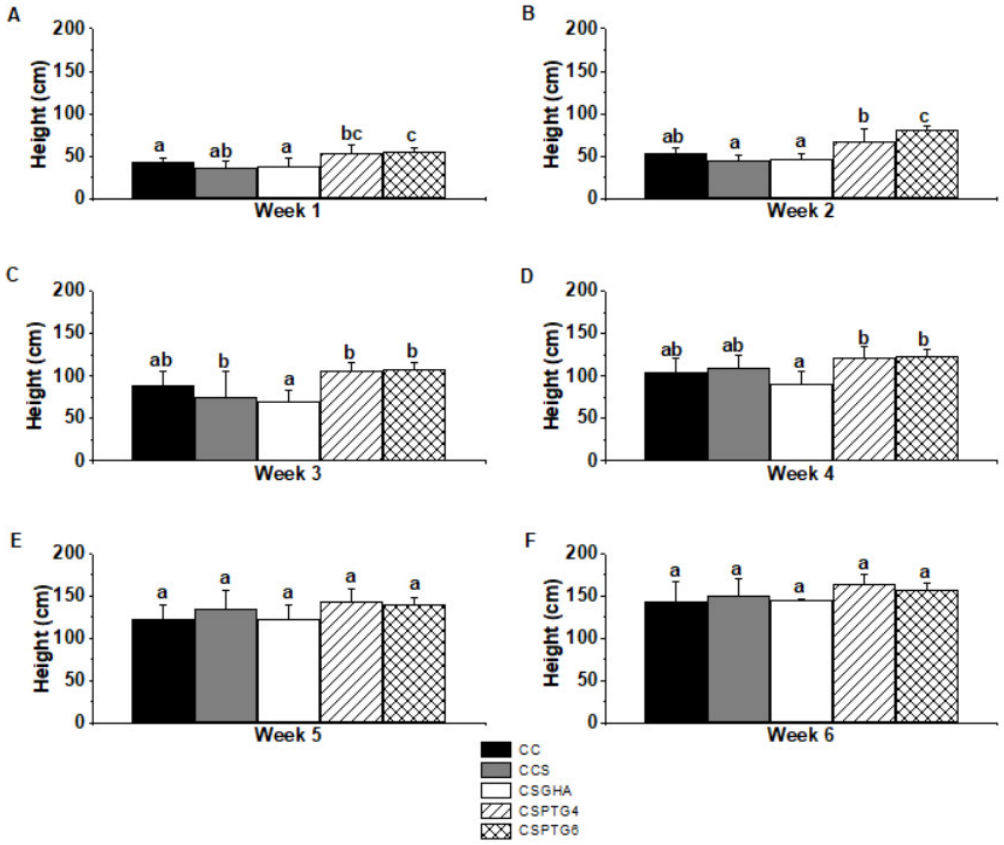

Fig. 6. Effect of *B. bassiana* seed treatments with corn starch as adherent on the height of *Z. mays* plants. CC: Negative control, CCS: Negative control with corn starch, CSGHA: Fungal strain GHA with corn starch, CSPTG4: Fungal strain PTG4 with corn starch and CSPTG6: Fungal strain PTG6 with corn starch. Post hoc analysis was done by Duncan multiple range test (α 0.05), after one-way ANOVA. Graphical bars with same letters indicate that there is no significant difference among them.

MCPTG4-treated plants flowered one to two weeks earlier than the rest of the plants. Furthermore, *B. bassiana* was re-isolated from corn cobs obtained from MCPTG4-treated plants. Figs. 7 A and B.

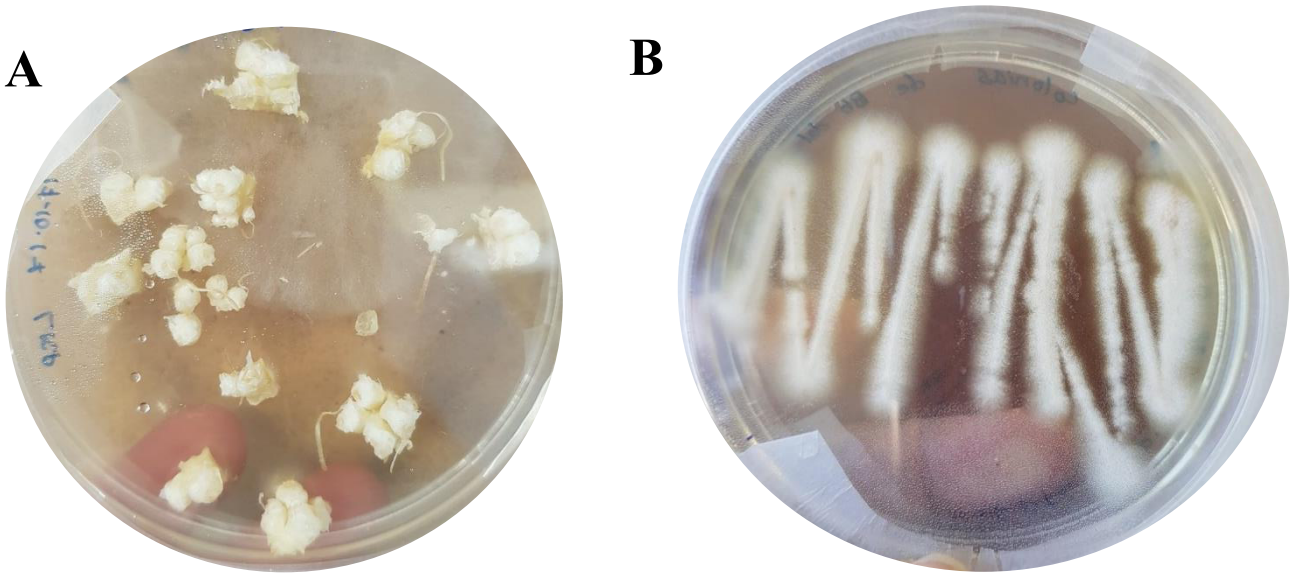

Fig. 7. Re-isolation of *B. bassiana* from corn cobs. A) Fragments of corn cobs on PDA plates without surface sterilization indicated fungus presence. B) *B. bassiana*-similar colonies were separated to another PDA plates to confirm the presence of the fungus.

Therefore, MC was selected as the best adherent type because of its convenient preparation procedure, its synergetic effects on germination (Table 1), endophytic inoculation efficiency (Table 2), plant height (Fig. 5) and flowering time.

We also analyzed plants fresh and dry weight from CC, CMC, MCGHA, MCPTG4 and MCPTG6 plants measuring at day 14. MCPTG4 and MCPTG6- treated plants fresh and dry weights were significantly higher as compared with those of MCGHA and all the negative control treatments (Figs. 8 A and B). Thus, indicating positive effects of both strain son plant biomass gain during the first two weeks after germination.

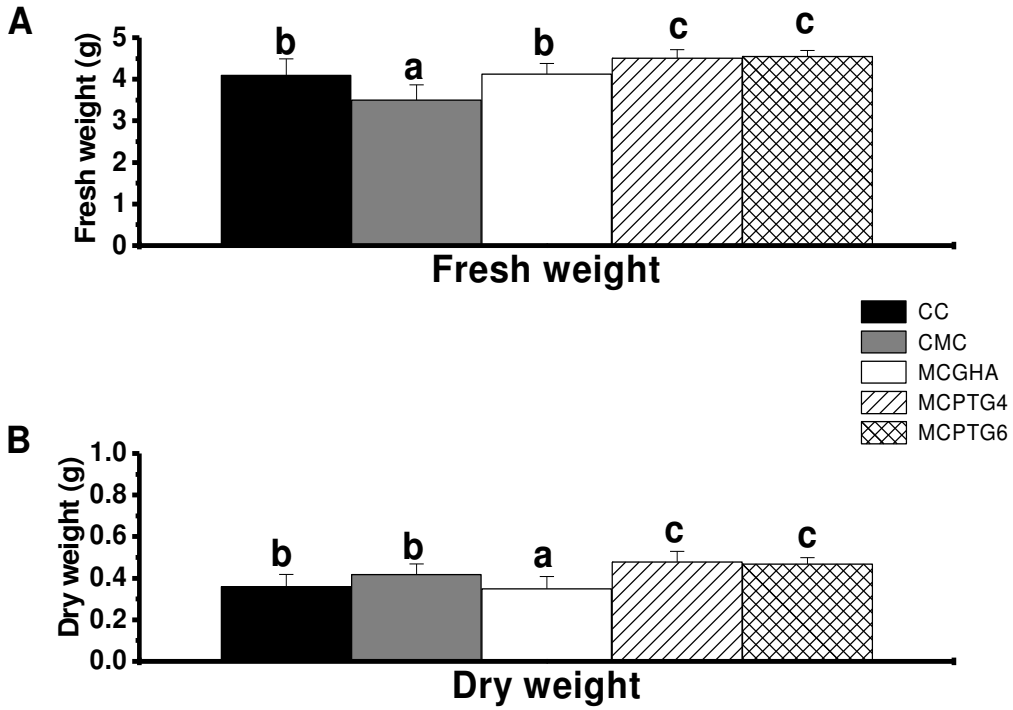

Fig. 8. Effect of *B. bassiana* seed treatments with methyl cellulose as adherent on the fresh (A) and dry (B) weights of 14 days old *Z. mays* plants. CC: Negative control, CMC: Negative control with methyl cellulose, MCGHA: *B. bassiana* strain GHA with methyl cellulose MCPTG4: *B. bassiana* strain PTG4 with methyl cellulose, MCPTG6: *B. bassiana* strain PTG6 with methyl cellulose. Post hoc analysis was done by Duncan multiple range test, after one-way ANOVA. Graphical bars with same letters indicate that there are no significant differences among them.

In the case of *A. thaliana*, the plants treated with *B. bassiana* strain GHA, showed the inflorescence 1 week before the negative control plants.

3. Endophytic effects of B. *bassiana* in drought resistance in *Z. mays*.

Because of the positive effects observed on plant growth and flowering time, tolerance against abiotic stressor drought was selected to evaluate. Vigor recovery was assessed 24 h after watering 10 days drought-stressed both untreated and *B. bassiana*-treated seedlings. Results showed a significant difference between untreated plants and *B. bassiana*-treated plants, as shown in Fig. 9. CC (5.63%) and CMC (28%) negative controls showed a slight vigor increment, but MCGHA

(63%), MCPTG4 (54%), MCPTG6 (55%) were much higher. These results showed vigor percentage recovery in *B. bassiana*-treated plants indicating induction of drought tolerance. GHA was significantly different to the other two fungal strains, showed the highest vigor percentage recovery effect.

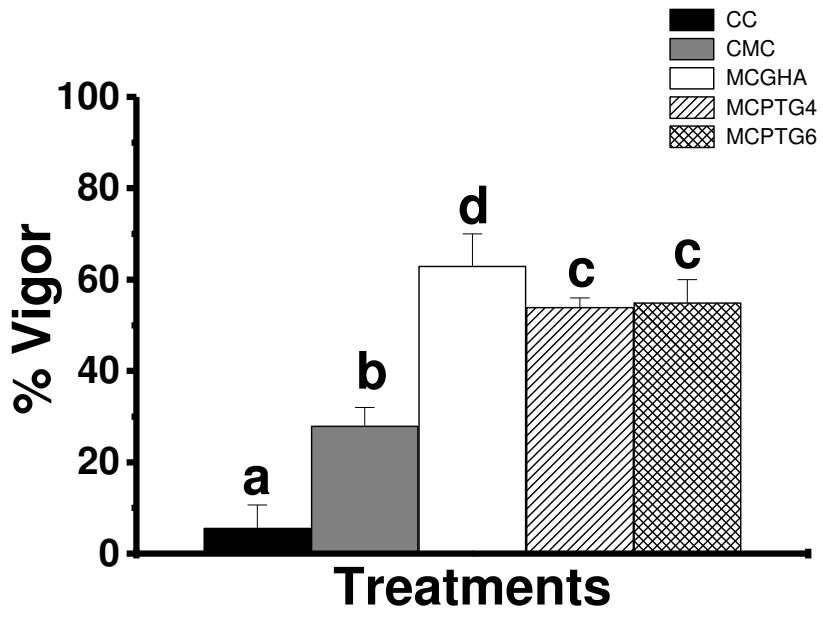

Fig. 9. Recovery percentage of vigor after 10 days gradual drought on 14 days old *Z. mays* plants. CC*:* Negative control*,* CMC: Negative control with methyl cellulose, MCGHA: *B. bassiana* strain GHA with methyl cellulose, MCPTG4: *B. bassiana* strain PTG4 with methyl cellulose, MCPTG6: *B. bassiana* strain PTG6 with methyl cellulose. Post hoc analysis was done by Duncan multiple range test, after one-way ANOVA. Graphical bars with same letters indicate that there is no significant difference among them.

4. Endophytic effect of *B. bassiana* on *S. frugiperda* tolerance in *Z. mays* plants under laboratory and field trial.

Effect of *B. bassiana* treated plants on the developmental stages of *S. frugiperda*

The development, survival, and mortality of *S. frugiperda* fed on untreated *Z. mays* plants and *B. bassiana*-treated *Z. mays* plants are shown in table 5. *S. frugiperda* larvae fed on *B. bassiana* strain PTG4-treated plants markedly troubled with their life cycle. (Table 5). Statistical analysis with

one- way ANOVA showed significant difference in the mean percentage values ($F_{(3, 11)} = 20.657$, $P < 0.001$) of larvae remained as larva during the experiment and pupa ($F_{(3,11)} = 5.170$, $P = 0.028$). However, there were no significant difference among the number of dead larvae ($F_{(3,11)} = 0.88$, $P = 0.491$) and prepupa ($F_{(3,11)} = 1.381$, $P = 0.317$).

Table 5. Effect of *B. bassiana* treated plants on the developmental stages of *S. frugiperda*

Stages	CC	CMC	MCPTG4	MCGHA
Initial pupa	100% [a]	100% [a]	100% [a]	100% [a]
Dead larva	6.67% [a]	3% [a]	3.67% [a]	0 [a]
Still larva	3.33% [a]	3% [a]	22% [b]	0 [a]
Prepupa	6.67% [a]	3% [a]	3.67% [a]	0 [a]
Pupa	83.33% [ab]	91% [ab]	63.33% [a]	100% [b]

Values followed by the same letters in a row are nor significantly different and with different letters in a row are significantly different after running post hoc Duncan multiple range test ($P = 0.05$). CC: Negative control, CMC: Negative control with methyl cellulose, MCGHA: *B. bassiana* strain GHA with methyl cellulose, MCPTG4: *B. bassiana* strain PTG4 with methyl cellulose.

S. frugiperda sixth instar larvae fed on the plants (both *B. bassiana* treated and no-treated) weighed before transferring them back to the artificial diet showed that those larvae fed on plants treated with *B. bassiana* strain PTG4 was significantly different from other treatments with $F_{(3,48)} = 4.813$, $P = 0.005$ (Fig. 10).

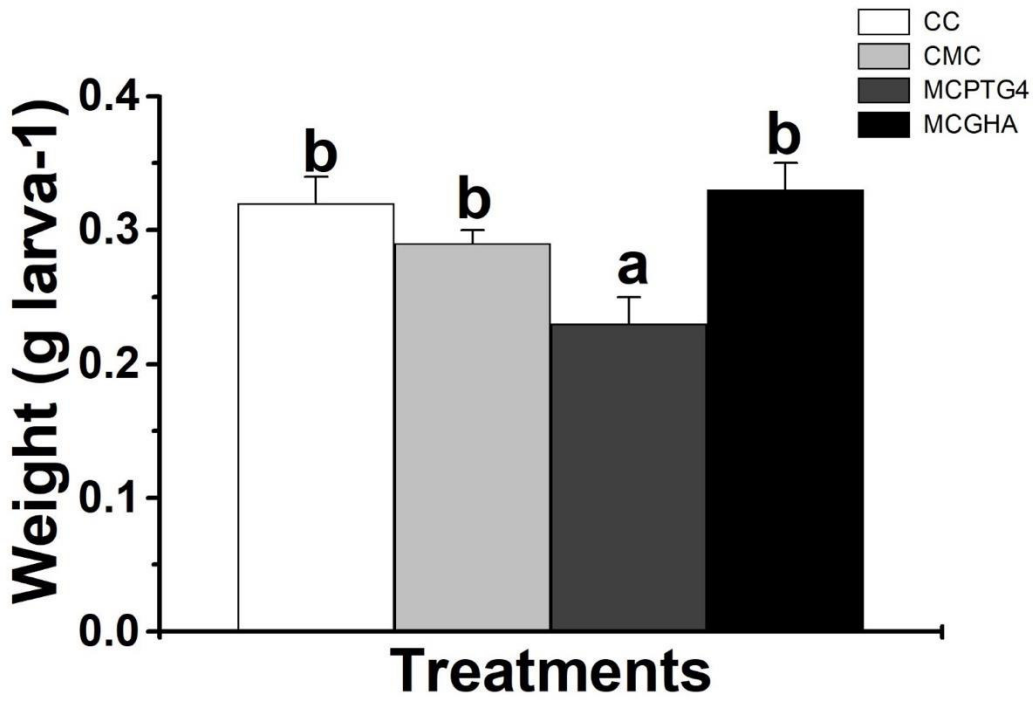

Fig. 10. *S. frugiperda* larval weight on 6th instar with different treatments. CC: Negative control, CMC: Negative control with methyl cellulose, *MCPTG4*: *B. bassiana* fungal strain PTG4 with methyl cellulose, MCGHA: *B. bassiana* fungal strain GHA with methyl cellulose. Post hoc analysis done with Duncan multiple range test (α = 0.05), after one-way ANOVA. Graphical bars with same letters indicate that there is no significant difference among them.

S. frugiperda pupal length showed a significant difference with the larvae fed on *B. bassiana* GHA treated plants when compared with other treatments with $F_{(3, 98)} = 4.491$ $P = 0.005$ (Fig. 11).

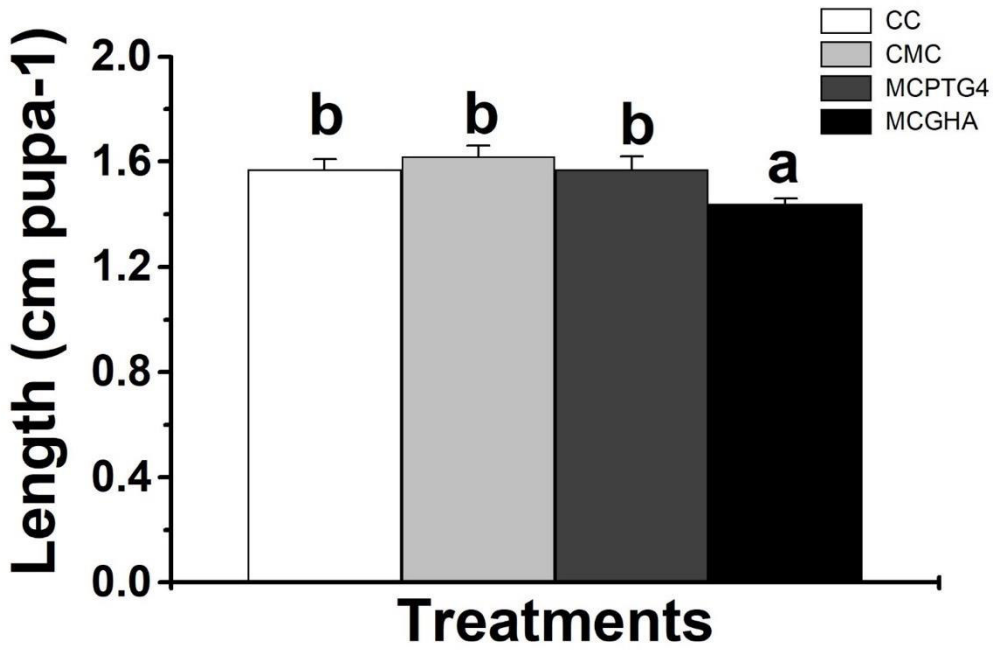

Fig. 11. *S. frugiperda* pupal length with different treatments. CC: Negative control. CMC: Negative control with methyl cellulose, MCPTG4: *B. bassiana* fungal strain PTG4 with methyl cellulose, MCGHA: *B. bassiana* fungal strain GHA with methyl cellulose. Post hoc analysis done with Duncan multiple range test ($\alpha = 0.05$) after one-way ANOVA. Graphical bars with same letters indicate that there is no significant difference among them.

S. frugiperda pupal weight showed a significant difference with the larvae fed on *B. bassiana* strain GHA treated plants when compared with negative controls and *B. bassiana* PTG4 strain fed larvae with $F_{(3, 97)} = 3.753$, $P = 0.014$ (Fig. 12).

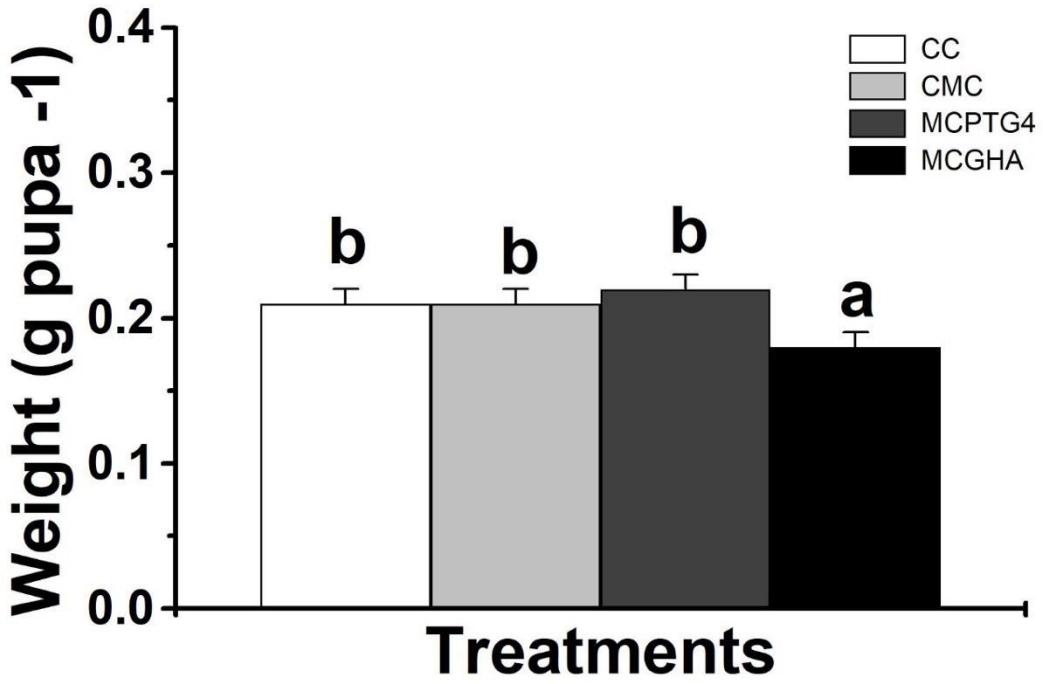

Fig. 12. *S. frugiperda* pupal weight with different treatments. CC: Negative control, CMC: Negative control with methyl cellulose, MCPTH4: *B. bassiana* fungal strain PTG4 with methyl cellulose, MCGHA: *B. bassiana* fungal strain GHA with methyl cellulose. Post hoc analysis done with Duncan multiple range test ($\alpha = 0.05$) after one-way ANOVA. Graphical bars with same letter indicate that there is no significant difference among them.

When compared the population percentage of the male and female pupae, observed significant difference among different treatments with $F_{(3,11)} = 7.033$, $P = 0.012$ for male and $F_{(3,11)} = 6.088$, $P = 0.018$ for female for pupae developed from larvae fed on both strains of *B. bassiana* and showed a lesser number of male than female pupae (Fig. 13).

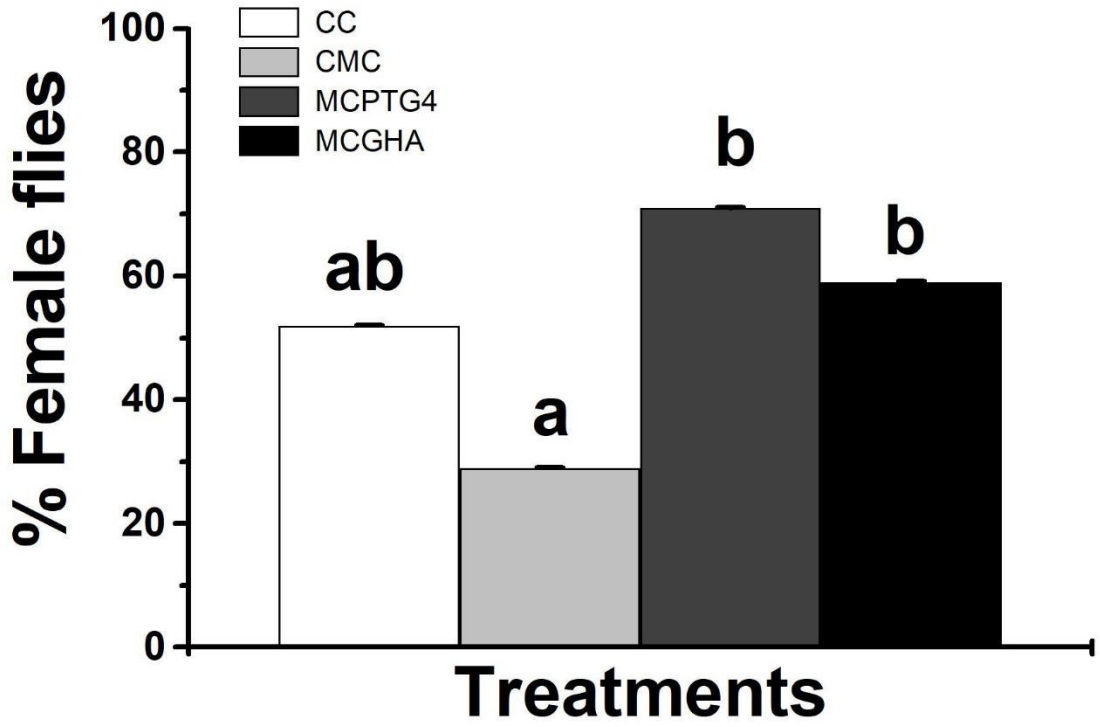

Fig. 13. Male and female population percentage of *S. frugiperda* pupa with different treatments. CC: Negative control, CMC: Negative control with methyl cellulose, MCPTG4: *B. bassiana* fungal strain PTG4 with methyl cellulose, MCGHA: *B. bassiana* fungal strain with methyl cellulose. Post hoc analysis done with Duncan multiple range test ($\alpha = 0.05$) after one-way ANOVA. Graphical bars with same letters indicate that is no significant difference among them.

Nevertheless, female adults emerged with wing deformities. We also observed parthenogenesis among the female pupae (Fig. 14).

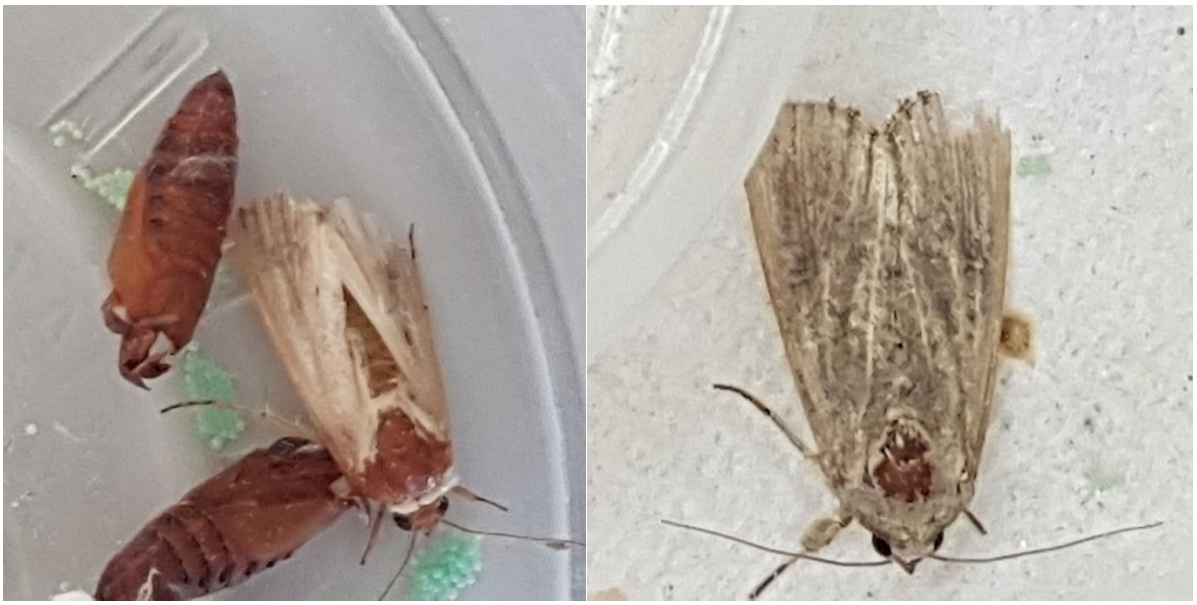

Fig. 14. Adult female flies with wing deformity and parthenogenesis.

Field trial: Experiment 1

Maize plant germination percentage was less in comparison with laboratory results, nevertheless, there was not any significant difference among negative control and *B. bassiana* treated plants in the field with $F_{(5, 57)} = 1.002$ and $P = 0.426$. The average germination percentage on 3rd week after planting the seeds for the no-treated plants were71.5% ± 5.4 and for the plants treated with *B. bassiana* were80.33% ± 3.49 Number of leaves per plant on 4th week after planting the seeds also showed no significant difference among negative control plants and *B. bassiana* treated plants with $F_{(5, 57)} = 0.928$ and $P = 0.471$. Average number of leaves per plant on 4th week after planting the seeds for the no-treated plants were 3.12 ± 0.22 and for the *B. bassiana*-treated plants were 3.49 ± 0.09.

To compare prevalence of *S. frugiperda* in fields, we divided the field into three section for both negative control and *B. bassiana*-treated plants. (Fig. 15). We did observe that *S. frugiperda* larvae between 2nd and 3rd instar were found in almost all furrows of negative control plants, whereas in *B. bassiana* treated plants they were present in those furrows near to the negative control plants which were reduced going away from negative control plants and none were present

in the last 12 furrows of *B. bassiana* treated plants (Fig. 16). We also observed the presence of various pathogenic and beneficiary insects during the experiments.

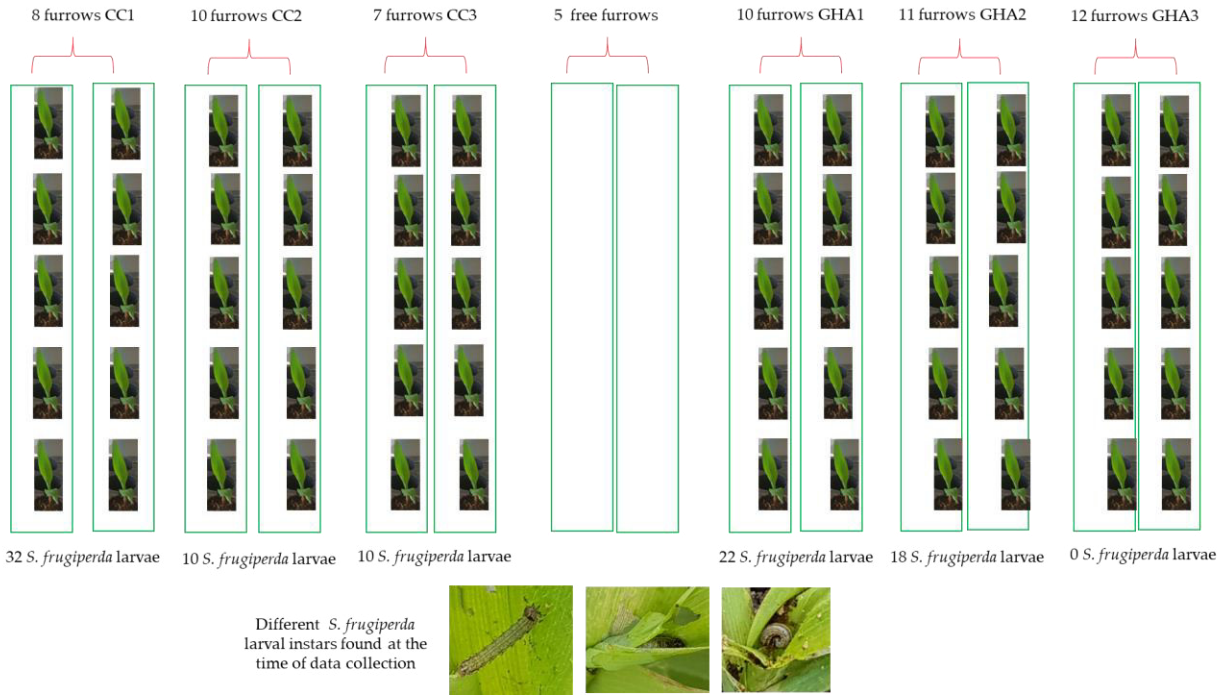

Fig. 15. Tendency of the presence of *S. frugiperda* larva in the treatments and different larval instars observed.

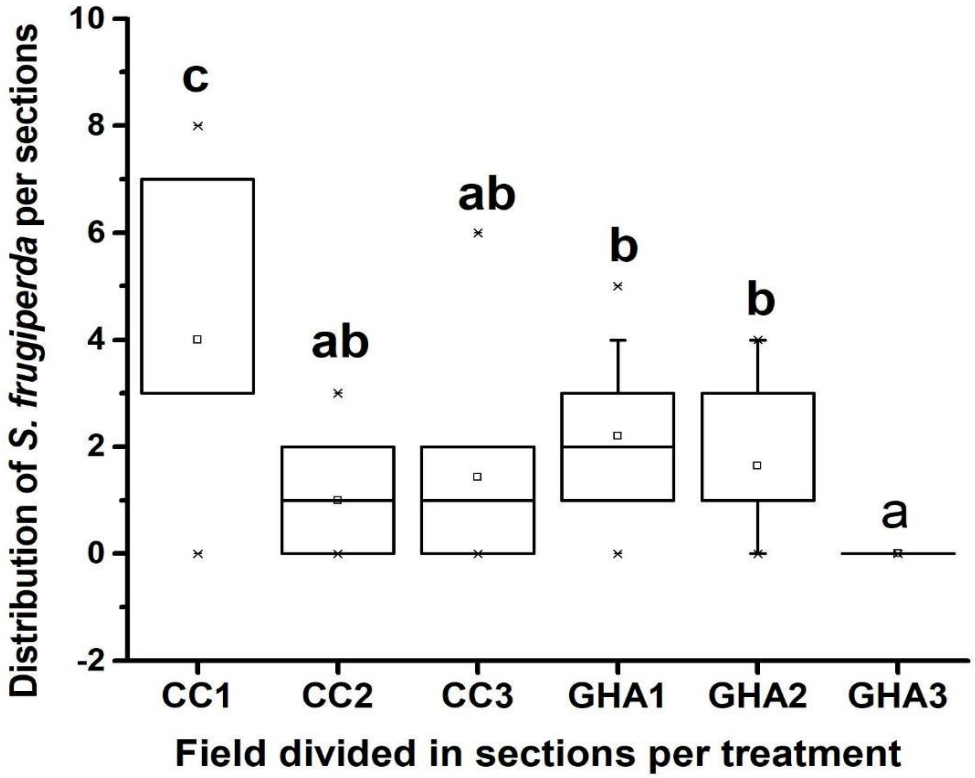

Fig. 16. Appearance of *S. frugiperda* in the field trial experiment 1 CC1, CC2, CC3: Negative control plants, GHA1, GHA2, GHA3: *B. bassiana* strain GHA with methyl cellulose. Post hoc analysis is done with Duncan multiple range test ($\alpha = 0.05$) after one-way ANOVA. Graphical bars with different letters indicate that there is no significant difference among them.

Field trial: Experiment 2

Independent-Sample T test analysis showed no significant difference in the percentage of germination in all five furrows of negative control (Mean 91.20 ± 2.8) and five furrows of *B. bassiana* treated plants (mean 87.20 ± 3.5) with $F = 0.225$ and $P = 0.648$.

Independent-Sample T test analysis showed no significant difference in the number of leaves and plant height. Average number of leaves in no treated plants on 3rd week after planting the seeds was 5.20 ± 0.055, whereas for the *B. bassiana*-treated plants it was 5.23 ± 0.054. The average plant height for the no treated plants were 12.04 ± 0.16 and for the *B. bassiana*-treated plants it

was 12.36 ± 0.15. In this trial, there were no *S. frugiperda* larvae detected neither in no treated plants nor in *B. bassiana* treated plants during the time of data collection.

Independent sample T-test analysis showed a significant difference between negative control and *B. bassiana* treated plants in corn cob length with $F = 0.006$ and $P = 0.937$, whereas, no significant difference in corn cob weight with $F = 0.048$ and $P = 0.831$. Results are shown in the table 6.

Table 6: Average weight and length of corn cobs.

Parameters	**CC** Average	**MCGHA** Average
Weight of fresh corn cob in grams	209.15 ± 26.11	183.43 ± 24.65
Length of fresh corn cob in centimeters	18.20 ± 0.74 *	16.71 ± 0.75**

Values followed by the same letters in a row are not significantly different and with different letters in a row are significantly different after independent sample T-test. CC*:* Negative control without any treatments or adherents, MCGHA: *B. bassiana* strain GHA with methyl cellulose.

5. Proteins present in the *Z mays-B. bassiana* interaction using plant guttation liquid.

One-dimensional electrophoresis with the guttation liquid from untreated maize plants and *B. bassiana* treated maize plants showed the presence of proteins between 100 kDa-150 kDa. Fig. 17.

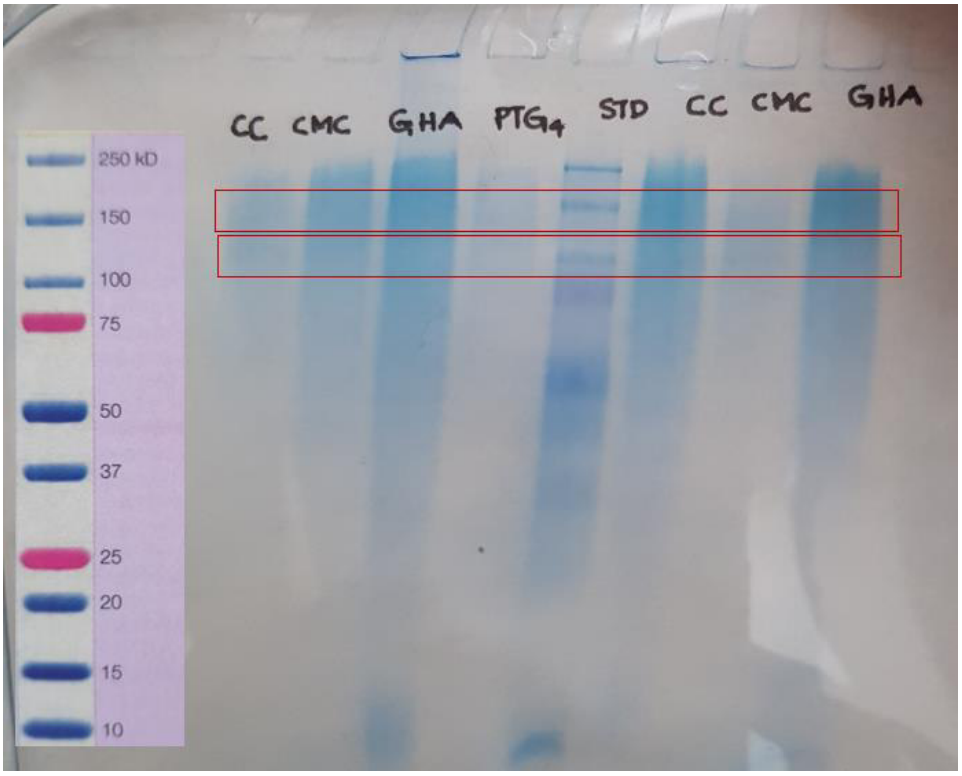

Fig. 17. Detected proteins between 150-100 kDa of size in the guttation liquid of maize plants.

DISCUSSION

All experiments were performed using fresh cultures of *B. bassiana* strain from frozen stocks on account of former reports on the correlation between subcultures and the genetic and physiological parameters (Eivazian-Kary and Alizadeh 2017) of *B. bassiana* in germination, conidiation and virulence (Jirakkakul et al. 2018).

Manufacturing and formulation are the decisive elements of the success of an entomopathogenic fungi as a commercial biocontrol agent. Solid substrate fermentation for aerial-conidia and liquid fermentation for blastospores are typical methods for their massive production. Though aerial conidia contain the main active ingredient as a biocontrol agent, it requires weeks for its sporulation and fermentation which can be decreased using blastospores. In addition, blastospores have got the capability to sustain drying and continue to be viable on long term storage circumstances (Mascarin et al. 2015), thus we used blastospores in our study.

At present exists a lot of methods like foliar spraying, plant dipping, stem injection, seed coating and root or soil drenching to inoculate artificially entomopathogenic fungi into different plants. In a review by McKinnon and collaborators (McKinnon et al. 2017) reported that seed coating and foliar treatments were used in most published bioassays for artificial inoculation. Some studies demonstrate that leaf is a feeble passage for entomopathogenic fungus entry to colonize in plants (Posada et al. 2007; Qayyum et al. 2015). However, an effective endophytic colonization of this fungus depends on factors like plant age, fungal species, inoculation methods and exposure to direct sunlight, rain and among others. Diverse studies show that *B. bassiana* does not maintain their survival and viability on exposure to direct sunlight or ultraviolet radiation (Inglis, Goettel, and Johnson 1995; Kaiser et al. 2019). Nevertheless, various studies reported that formulation with natural substances can overcome this obstacle (Fernandes et al. 2015; Kaiser et al. 2019; Kim et al. 2019; Lohse et al. 2015). In the present study, to make sure blastospores maintain viability and stability during exposure to direct sunlight or ultraviolet radiation, we used the seed coating method for an effective colonization; and to ensure the efficiency and stability of *B. bassiana* blastospores we used methyl cellulose or cornstarch for blastospore formulations (Kaiser et al. 2019; Rondot and Reineke 2018).

1. Method of inoculation for endophytic establishment of the three *B. bassiana* strains (GHA, PTG4 and PTG6) in *Z. mays* and *A. thaliana*

The first parameters were to determine the effects of strains and/or adherent types on germination percentages. There are reports indicating germination augments when using endophytes either bacteria or fungi. Zhu and collaborators in 2017 (Zhu et al. 2017) showed *in vitro* increases on *Ammodendron bifolium* (Pall.) germination, using *Bacillus* sp. Cohn Strain AG18, *Kocuria* sp. (Stack Brandt) strain AY9, and *Staphylococcus* sp. Rosenbach strain AY3. Furthermore, Russo and collaborators in 2019 (Russo et al. 2019) observed an improvement in *B. bassiana*-treated *Z. mays* seeds germination in soil. Jaber and Ownley in 2018 (Jaber and Ownley 2018) found that *B. bassiana* or *M. brunneum* did not alter *V. faba* seed germination in vitro. In this study, MCPTG4-treated plants showed the highest germination percentage at day 14. The other two strains also had high germination percentage, but the adherent type used had either inhibitory or enhancing effects. Therefore, high germination depended on strains and adherent types used. Among adherent's methyl cellulose (MC)showed higher performance than corn starch (CS). However, at day 5, *B. bassiana* treated plants germination was delayed, probably due to the type of seed coating used, which could have been rinsed off during watering, and then allowed germination to increase on day 14. Lata and collaborators in 2018 (Lata et al. 2018) reported similar delays in seedling emergence, with endophytic bacteria wheat seed treatments. Another explanation could be the later production of phytohormones by the fungus, one it had colonized the plant. (Arora and Ramawat 2017; Nisa et al. 2015). A plant is considered to be endophytically colonized based on the detection of the target fungal strain from any of its tissues (Sword, Tessnow, and Ek-Ramos 2017).

Re-isolation of all *B. bassiana* strains from *Z. mays* roots, stems and leaves indicated systemic plant colonization of these fungi as endophytes. Nonetheless, results showed root colonization in all treatments, followed by stems and leaves colonization ranging from 100% to 25%. These percentage differences were probably due to plant tissue size, all root tissues were plated on PDA plates, whereas only selected pieces of stems or leaves could be placed on agar plates. Guo, Huang, and Wang 2008 reported that endophytic fungi tissue specificity is a result of their adaptation to

conditions present in each plant tissue. In this regards, recent reports have shown some microscopic analysis of stems and leaves of cross sections indicating hyphal growth of *B. bassiana* (McKinnon et al. 2017). In 2009, Tefera and Vidal (Tefera and Vidal 2009) showed that the inoculation method used could alter colonization percentage in different plant tissues. In sorghum, they reported higher colonization in *B. bassiana* leaves using the leaf inoculation method, whereas seed inoculation resulted in higher root and stem colonization. Greenfield and collaborators in 2019 (Greenfield et al. 2016) reported 84% of endophytic root colonization into cassava roots, 7-9 days after soil drench inoculation. In addition, Sword and collaborators (Sword, Tessnow, and Ek-Ramos 2017) reported endophytic *Phialemomium inflatum* (Burnside) colonization in cotton plants after seed inoclation as follows: 50-80% in roots, 0-62.5% in stems, and 0-10% in leaves, whereas *B. bassiana* colonization was found to be 0-8.3% in roots or stems and 33-60% in leaves. Lata and collaborators in 2018 (Lata et al. 2018) re-isolated *B. bassiana* and *M. brunneum* from roots and shoots of wheat plants, 14 and 24 days post inoculation, using seed treatment and the highest colonization percentage was in root tissues. In a study by Moloinyane and Nchu (Moloinyane and Nchu 2019), after soil drenching of *B. bassiana* on grapevine plants, reported 48% of re-isolation from leaf tissues. All these reports demonstrated that the inoculation method is a key factor on fungal colonization in different plant tissues. Taken together, factor like soil microorganisms, temperature, relative humidity, growth media, plant age and species, inoculum density, and fungal species can affect successful colonization of fungi in different plant tissues. Kasambala and collaboratos (Kasambala Donga, Vega, and Klingen 2018) reported that *B. bassiana* does not display any tissue preferences in endophytic colonization in plants. Therefore, in the presnt study it was demonstrated that MCPTG4 treatment had a very high *B. bassiana* endophytic colonization efficiency in *Z. mays*, indicating this strain and adherent type mixture is a feasible seed coating method for further biotechnological applications.

2. Potential endophytic effects of *B. bassiana* on *Z. mays* and *A. thaliana* plant growth and yield

In addition to effects on germination and endophytic establishments, putative effects on plant overall performance were evaluated. Results shown in Figs. 5 and 6 demonstrated that during the first 2-3 weeks, *B. bassiana* strain PTG6 stimulated plant growth, whereas GHA and PTG4 -treated

plants did not show significant differences, compared with untreated plants. One explanation could be the activation of plant adaptation processes after transplantation. Afterwards, there was no significant difference between *B. bassiana*-treated and untreated plants. However, in Fig. 8, at day 14 there were significant differences in fresh and dry weight of *B. bassiana*-treated plants, indicating early positive effects. These observations have been also previously reported, after using other endophytes and inoculation treatments. Dash in 2018 (Dash et al. 2018) reported that *B. bassiana* enhanced *Phaseolus vulgaris* height, fresh shoot and root weight compared with untreated plants on day 7 after inoculation. Jaber and Ownley (Jaber and Ownley 2018) reported that, on day 14 post inoculation of wheat seeds with *B. bassiana* or *M. brunneum*, plants showed higher shoot height, root length and fresh root and shoot weights when compared with untreated plants. Russo and collaborators in 2019 (Russo et al. 2019) reported similar effects after spraying *B. bassiana* on corn plant leaves, with enhanced plant height, number of leaves, height, and node number where the first cob emerged. In contrast Moloinyane and Nchu in 2019 (Moloinyane and Nchu 2019) did not find any significant difference between *B. bassiana* treated and untreated grapevine plants on plant height, number of roots and leaves, and fresh and dry weights, four weeks after soil drenching treatment. Furthermore, Tall and Meyling (Tall and Meyling 2018) reported that *B. bassiana*-treated maize plants did not show any growth improvement during their experiment and suggested that fungus functioned as a link between plant and soil when nutrient scarcity occurred. Indeed, in the present study, GHA- and PTG4-treated plants flowered prior to PTG6-treated plants and controls, indicating a physiological adaptation to reproduce earlier despite biomass increase was not obvious in these treatments. This is relatively new, as only few previous reports indicated these effects can be induced by a fungal endophyte. *P. indica* promoted early flowering by regulating photoperiod and gibberellin pathways in *Arabidopsis* (Pan et al. 2017). In *Coleus forskohlii* (also known as *Plectranthus barbatus* Andrews), *P. indica* as root endophyte also induced early flowering and higher aerial biomass production (Das et al. 2012).

3. Effect of endophytic *B. bassiana* in drought resistance in *Z. Mays*

Abiotic stress drought was evaluated aiming for plant tolerance induction. As shown in Fig. 9, more than 50% of all *B. bassiana*-treated plants recovered their vigor upon watering, after a gradual drought for 10 days. MCPTG4 treatment showed high vigor performance compared with controls. However, the best treatment to induce tolerance was MCGHA. In a recent report, authors

demonstrated that under combined drought and mechanical stress, *P. indica* maintained maize water status and physiological traits by increasing root volume, leaf area, relative water content, leaf water potential, and proline content, whereas untreated plants showed less catalase and ascorbate peroxidase activities. The mechanism proposed was that inoculated plants might have done osmotic adjustments by accumulating proline, or by reducing oxidative damage system activation, or by maintaining cell wall elasticity (Hosseini et al. 2018). Llorens and collaborators in 2019 (Llorens et al. 2019) reported similar results indicating *Aegilops sharonensis* and *Triticum diccocoides* protected wheat plants from drought, by reducing stress response and improving plant physiological status. They found higher relative water content, proline, abscisic acid, and jasmonic isoleucine compound in endophyte-treated plants, in comparison with untreated plants, On the contrary, Ferus and collaborators (Ferus, Barta, and Konôpková 2019a) reported that *B. bassiana* relieved drought stress in red oak seedlings, but recorded small decreases in leaf relative water content and stomatal conductance, low free proline concentration but higher root growth, in comparison with untreated plants. They suggested that endophyte-treated plants response to drought was due to species-species interaction and concrete environmental conditions thus discrepancies regarding these studies may be due to plant species difference. The former two studies were done on monocotyledons whereas the later study was on a dicotyledon. Interestingly, in the present study, plant physiological status was improved and related to a recovery of vigor after drought stress. Further studies are needed to elucidate *B. bassiana*-induced drought tolerance in seed-treated plants to fully characterize the mechanism involved.

4. Endophytic effect of *B. bassiana* on *S. frugiperda* tolerance in *Z. mays* plants under laboratory and field trials

In this study, we did not monitor mortality rate of larvae separately for each larval instar. But we observed that a small percentage of larvae were dead, without any significant difference between larvae fed *Z. mays* no treated plants and *Z. mays-B. bassiana* treated plants. We did not observe any fungal outgrowth from *S. frugiperda* cadavers. This may be because larvae were not in direct contact with colonized *B. bassiana*. Mortality level of target insect pests with entomopathogens depends on larval developmental stage (Qayyum et al. 2015), inoculation method (Sánchez-Rodríguez et al. 2018), or fungal strains (Vidal and Jaber 2015). *B. bassiana*

mostly does not induce direct mortality in insect pests, but often show reduced larval growth rate, weight or longevity (Klieber and Reineke 2016; Lefort et al. 2016). In our study we observed that *S. frugiperda* larvae fed on *B. bassiana* strain PTG4 treated *Z. mays* plants were considerable affected their development prolonging their larval stage, declined larval weight and smaller number of pupas. Review notes by Vega in 2018 (Vega 2018) recorded that by blending liquid cultured *B. bassiana* after removing mycelia in the diet happened to reduce the percentage of population and prolonged the pupation time. Lopez and Sword in 2015 (Lopez and Sword 2015) did not find any differences in cotton boll worm larvae and pupal weight when they were fed on *B. bassiana* and *Purpureocillium lilacinum* inoculated cotton plants. In this present study we observed that larvae fed on *B. bassiana* strain GHA-treated plants, showed a decline in pupal length and weight. Since this was a no-choice experiment there were not enough left-over plant materials to quantify if there were any feeding preference in larvae between no-treated plants and *B. bassiana* treated plants. Another important observation of this study was that adult male-female ratio of those fed on *B. bassiana*-treated *Z. mays* plants. We observed a lesser number of adult male flies and female flies had some deformities in their wings. This result is agreement with Hassan and collaborators (Hassan, Abdullah, and Assaf 2019) where reported a malformation occurred with *B. bassiana* treated squash beetle. Whereas, Vianna and collaborators in 2018 (Vianna et al. 2018) did not find any significant difference in the sex ratio of *Helicoverpa gelotopoeon* when they were fed on *B. bassiana* treated soy bean plants. Akutse and collaborators in 2013 (Akutse et al. 2013) observed higher number of males were emerged in their study with different fungal strains on *Vicia faba* and *Phaseolus vulgaris* against *Liriomyza huidobrensis*. We also observed parthenogenesis in female adults, which might be because that there were lesser number of male, and female adults were born with wing deformities, which urged the necessity for this feature. Mahmood and collaborators in 2019 (Mahmood et al. 2019) reported a reduced survival and fecundity of *Sitobion avenae* feed on maize plants inoculated with *B. bassiana*. Insect immunity can be influenced by successive exposures of the same pathogen and can have a long-term effect on their survival (Jensen, Enkegaard, and Steenberg 2019). We need to perform additional studies to evaluate whether *B. bassiana* has some effects on *S. frugiperda*'s successive generations. Bamisile and collaborators in 2019 (Bamisile et al. 2019) reported endophytic *B. bassiana* in

foliar treated citrus limon plants acted as growth suppressor to three successive generations of *Diaphorina citri*.

In the field trial experiment 1, there were no significant differences among the no treated and *B. bassiana*-treated plants germination, whereas there was a small decline in comparison with laboratory results. This decrease might be due to the uncontrolled environmental conditions and seed variety we used for field trials, which made us clear that germination percentage of *Z. mays* plants were not affected with *B. bassiana*. This conclusion is on concordance with the work of Russo and collaborators (Russo et al. 2019) where they reported a 77%of negative control and 89% of *B. bassiana*-treated *Z. mays* plant germination. Average number of leaves per plant also did not show any significant difference among the no-treated and *B. bassiana*-treated plants. We divided the fields into three section of both the treatments to find out the tendency of larval appearance in different parts of the field. We did observe that there are significant differences between different sections. Even though there was a slight hike in the number of larvae in the initial furrows of *B. bassiana*-treated plants immediately after the no-treated plants, it declined eventually and became absolutely zero in the final 12 furrows of *B. bassiana*-treated plants. Ramírez-Rodríguez and Sánchez-Peña in 2016 observed that when *B. bassiana* was applied as an endophyte in *Z. mays* plants and let *S. frugiperda* larvae feed on them, it was not as pathogenic as in direct application.

In the second field trial, same results were obtained in terms of germination percentage, plant height, and average number of leaves of *Z. mays* plants in both treatments. But there was an increase in both germination percentage and average number of leaves in the second trial, that was 71.5% of no treated plants to 91.20% and from 80.33% to 87.20% in *B. bassiana*-treated plants in the case of germination and in the case of the average number of leaves per plant we did observe an increase from 3.12 to 5.20 leaves per plant in no treated plants and from 3.49 to 5.23 leaves per plant in *B. bassiana*-treated plants. This difference might be due to the higher concentration of blastospores we used in our second trial, that was from 1 x 10^6 blastospores /ml to 1 x 10^8 blastospores/mL. Our studies agreed with Lopez and Sword in 2015 (Lopez and Sword 2015) reports, who showed that *Gossypium hirsutum* height increased by establishing *B. bassiana* as an endophyte in the plants. Dash and collaborators in 2018

(Dash et al., 2018) reported an increase in the number of leaves of *P. vulgaris* after treating them with *B. bassiana*. The most interesting factor in the second experiment is that we did not observe any *S. frugiperda* larvae neither in no treated plants no in *B. bassiana*-treated plants during the period of our study. Hernandez-Trejo and collaborators in 2019 (Hernandez-Trejo et al. 2019) reported that *Metarhizium robertsii* decreased *S. frugiperda* incidence from 41.3 to 2.8% in the first application and 17.4 to 8.3% in the second application on maize plants. Our finding needs more field trials to understand the mechanisms underlined to these results. One of the possible reasons might be due to volatiles that may be produced by the *B. bassiana*-treated plants which may function as insect repellents. Plants can dispense an array of volatile compounds from different plant parts including flowers, leaves, stems, fruits, and roots which can perform crucial roles in behavior of insects in feeding, mating, egg-laying, and aggregation of conspecifics. These volatiles can function in contrasting activities like insect attractants or repellents and can also activate neighboring plants defense system (Veloz-Badillo et al. 2019). For example, naphthalene used as a potential insect repellent has been found to be produced by *B. bassiana* and *Muscodor vitigenus* in various studies (Crespo et al. 2008; Daisy et al. 2002). Another mechanism explained by Vega in his review on 2018 (Vega 2018) is the production of kairomones, chemical signals produced by the plants which are used by the insects to localize them. They probably can be interrupted by the endophytic entomopathogens in the plants.

We did not find any significant difference in the corn cob weight between the treatments, which is in concordance with the findings of Hernandez-Trejo in 2019 where application of *M. robertsii* on maize plants did not showed any significant difference in grain yield per hectare between treatments. Whereas Russo et al., (2019) found an increase in maize corn yield after applying *B. bassiana* under field conditions. Qayyum et al., (2015) did find a decrease in tomato size after colonizing the plants with *B. bassiana*. All these contradictory results might be due to the difference in the fungal strains, host plant species varieties or even may be geographical regions. More investigations are required to confirm all these factors. We did find that corn cob length was little greater in no treated plants than the *B. bassiana*-treated plants. They yielded more than 2 corn cob per plant. In this study we did not damage any pollinators or any other insects.

5. Proteins present in the *Z. mays-B. bassiana* interactions using plant guttation liquid.

Guttation is a physiological process which refers the exudation of liquid droplets from the edges, adaxial and abaxial surfaces of an uninjured leaf through small openings in the leaves called hydathodes and has got a very important role in soil-plant environment system (Singh 2016). Transpiration is induced through the gradient between low air water potential and high soil water potential which occurs normally in night time when air cools down or in rainy days, where, the plants maintain the solute transport mainly through hydathodes (Grunwald et al. 2003). Several root and shoot signal transduction, transport proteins found in guttation fluid are transported to active vegetative and reproductive growth sites for fruits and seed growth and formation (Singh 2016). Guttation fluid can be recollected continuously during the whole life period of a plant without stressing out the plant (Komarnytsky et al. 2000). Also, huge expenses in protein extraction and purification from biochemically complex plant tissues can overcome through this procedure (Komarnytsky et al. 2000). In our study, we could find out a lot of proteins from both no-treated and *B. bassiana* treated plants, with 100-150 kDa size, which can be compared with the results of Grunwald and collaborators in 2003. (Grunwald et al. 2003) where they found out more than 200 proteins using one- and two-dimensional electrophoresis. Further studies are needed to sequence these proteins and identify them.

CONCLUSIONS

- Inoculation of *B. bassiana* in the seeds of *Z. mays* using methyl cellulose or corn starch, protected the fungus from adverse abiotic factors.

- Improved flowering time of plants.

- *B. bassiana* can be used as a biofertilizer and growth promoter.

- Drought tolerance can be induced.

- *S. frugiperda* can be controlled.

- Xylem sap drop (guttation liquid) contain proteins of 100-150 kDa in size.

PERSPECTIVES

- To do more studies in the field to check the results obtained in the laboratory.

- Use other corn varieties and races.

- Follow up on the study of *S. frugiperda* larvae that were born from parthenogenesis, to evaluate the possible effects on the following generations.

- To analyze the microbiota of the excrement of *S. frugiperda* larvae after they were fed with plants treated with *B. bassiana.*

- Analyze the protein profile and bioactive peptides present in plants treated with *B. bassiana*.

BIBLIOGRAPHY

Ahlholm JU, Helander M, Henriksson J, Metzler M, Saikkonen K. Environmental conditions, and host genotype direct genetic diversity of Venturia ditricha, a fungal endophyte of birch trees. Evolution. 2002;56(8):1566-1573. doi:10.1111/j.0014-3820.2002.tb01468.x

Akutse, K. S., N. K. Maniania, K. K. M. Fiaboe, J. Van den Berg, and S. Ekesi. 2013. "Endophytic Colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae)." *Fungal Ecology* 6(4):293–301.

Alikhani, Mehdi, Behnam Khatabi, Mozhgan Sepehri, Mojtaba Khayam Nekouei, Mohsen Mardi, and Ghasem Hosseini Salekdeh. 2013. "A proteomics approach to study the molecular basis of enhanced salt tolerance in Barley (*Hordeum vulgare* L.) conferred by the root mutualistic fungus *Piriformospora indica*." *Molecular BioSystems* 9(6):1498–1510.

Ansari, M. W., S. S. Gill, and N. Tuteja. 2014. "*Piriformospora indica* a powerful tool for crop improvement." *Proceedings of the Indian National Science Academy* 80(2):317–24.

Arias Renee S., Portilla Maribel, Ray J.D., Blanco Carlos A. and Simpson S.A 2015: Ecology, behavior and bionomics first genotyping of *Spodoptera frugiperda* progeny from crosses between Bt-resistant and Bt-susceptible populations and 65-locus discrimination of isofamilies. Research and Reviews: Journal of Botanical Sciences 2015, 4(1) pg 18-29.

Arora, Jaya and Kishan Gopal Ramawat. 2017. *Endophytes: Biology and Biotechnology*. Vol. 15. edited by D. K. Maheshwari. Cham: Springer International Publishing.

Bamisile, Bamisope Steve, Chandra Kanta Dash, Komivi Senyo Akutse, Muhammad Qasim, Luis Carlos Ramos Aguila, Fangfei Wang, Ravindran Keppanan, and Liande Wang. 2019. "Endophytic *Beauveria bassiana* in foliar-treated citrus limon plants acting as a growth suppressor to three successive generations of *Diaphorina citri* kuwayama (Hemiptera: Liviidae)." *Insects* 10(6).

Barelli, Larissa, Soumya Moonjely, Scott W. Behie, and Michael J. Bidochka. 2016. "Fungi with multifunctional lifestyles: Endophytic insect pathogenic ungi." *Plant Molecular Biology* 90(6):657–64.

Baron, Noemi Carla, Everlon Cid Rigobelo, and Diego Cunha Zied. 2019. "Filamentous fungi in

biological control: Current status and future perspectives." *Chilean Journal of Agricultural Research* 79(2):307–15.

Bhusal, Kiran and Kamana Bhattarai. 2019. "A review on fall armyworm (*Spodoptera frugiperda*) and its possible management options in Nepal a review on fall armyworm (*Spodoptera frugiperda*) and its possible management options in Nepal." (August).

Brosche, Mikael, Payam Fayyaz, Basia Vinocur, Erwin Witters, Kris Laukens, Thomas Teichmann, Arie Altman, Erwin Dreyer, and Henri Poincare. 2007. "Gradual soil water depletion results in reversible changes of gene expression , protein profiles , ecophysiology , and growth performance in *Populus euphratica* , a poplar growing in arid regions." *Plant Physiology* 143(February):876–92.

Cannon, P. F., U. Damm, P. R. Johnston, and B. S. Weir. 2012. "Colletotrichum - current status and future directions." *Studies in Mycology* 73:181–213.

Castillo Lopez, Diana, Keyan Zhu-Salzman, Maria J. ulissa Ek-Ramos, and Gregory A. Sword. 2014. "The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (Formerly *Paecilomyces lilacinus)* and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions." *PloS One* 9(8):e103891.

Compant, Stéphane, Kari Saikkonen, Birgit Mitter, Andrea Campisano, and Jesús Mercado-Blanco. 2016. "Editorial special issue: Soil, plants and endophytes." *Plant and Soil* 1–11.

Crespo, R., N. Pedrini, M. P. Juárez, and G. M. Dal Bello. 2008. "Volatile organic compounds released by the entomopathogenic fungus *Beauveria bassiana*." *Microbiological Research* 163(2):148–51.

Daisy, Bryn H., Gary A. Strobel, Uvidelio Castillo, David Ezra, Joe Sears, David K. Weaver, and Justin B. Runyon. 2002. "Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus." *Microbiology* 148(11):3737–41.

Das, Aparajita, Shwet Kamal, Najam Akhtar Shakil, Irena Sherameti, Meenakshi Dua, Narendra Tuteja, Atul Kumar Johri, Ajit Varma, Aparajita Das, Shwet Kamal, Najam Akhtar Shakil, Irena Sherameti, Ralf Oelmüller, Meenakshi Dua, and Narendra Tuteja. 2012. "the root endophyte fungus *Piriformospora indica* leads to early flowering , higher biomass and altered secondary metabolites of the medicinal plant , *Coleus forsko*." 2324.

Dash, Chandra Kanta, Bamisope Steve Bamisile, Keppanan Ravindran, Muhammad Qasim, Yongwen Lin, Saif Ul Islam, Mubasher Hussain, and Liande Wang. 2018. "Endophytic

entomopathogenic fungi enhance the growth of *Phaseolus vulgaris* L. (Fabaceae) and negatively affect the development and reproduction of *Tetranychus urticae* koch (Acari: Tetranychidae)." *Microbial Pathogenesis* 125:385–92.

Dutta Srijita 2015 Biopesticides : An ecofriendly approach for pest control. World Journal of Pharmacy and Pharmaceutical Science, 2015, 4(6) pg 250-265.

Eivazian-Kary, Naser and Zhila Alizadeh. 2017. "Effects of sub-culturing on genetic and physiological parameters in different *Beauveria bassiana* isolates." *Journal of Invertebrate Pathology* 145(March):62–67.

Ek-Ramos, María J., Wenqing Zhou, César U. Valencia, Josephine B. Antwi, Lauren L. Kalns, Gaylon D. Morgan, David L. Kerns, and Gregory A. Sword. 2013. "Spatial and temporal variation in fungal endophyte communities isolated from cultivated cotton *(Gossypium hirsutum*)." *PLoS ONE* 8(6).

Fernandes, Éverton K. K., Drauzio E. N. Rangel, Gilberto U. L. Braga, and Donald W. Roberts. 2015. "Tolerance of entomopathogenic fungi to ultraviolet radiation: A review on screening of strains and their formulation." *Current Genetics* 61(3):427–40.

Ferus, Peter, Marek Barta, and Jana Konôpková. 2019a. "Endophytic fungus *Beauveria bassiana* can enhance drought tolerance in red oak seedlings." *Trees - Structure and Function* (1):1–8.

Ferus, Peter, Marek Barta, and Jana Konôpková. 2019b. "Endophytic fungus *Beauveria bassiana* can enhance drought tolerance in red oak seedlings." *Trees - Structure and Function* (1):1–8.

Gallagher, S.R. (2006), One-dimensional SDS gel electrophoresis of proteins. Current Protocols in Molecular Biology, 75: 10.2.1-10.2A.37. doi:10.1002/0471142727.mb1002as75

Ghabooli, Mehdi, Behnam Khatabi, Farajolah Shahriary Ahmadi, Mozhgan Sepehri, Mehdi Mirzaei, Ardeshir Amirkhani, Jesus V. Jorrin-Novo, and Ghasem Hosseini Salekdeh. 2013. "Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by *Piriformospora indica* in barley." *Journal of Proteomics* 94:289–301.

Ghaffari, Mohammad Reza, Mehdi Mirzaei, Mehdi Ghabooli, Behnam Khatabi, Yunqi Wu, Masoud Zabet-Moghaddam, Ghasem Mohammadi-Nejad, Paul A. Haynes, Mohammad Reza Hajirezaei, Mozhgan Sepehri, and Ghasem Hosseini Salekdeh. 2019. "Root endophytic fungus *Piriformospora indica* improves drought stress adaptation inbBarley by

metabolic and proteomic reprogramming." *Environmental and Experimental Botany* 157(May 2018):197–210.

Greenfield, Melinda, María I. Gómez-Jiménez, Viviana Ortiz, Fernando E. Vega, Matthew Kramer, and Soroush Parsa. 2016. "*Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation." *Biological Control* 95:40–48.

Grunwald, Ingo, Ines Rupprecht, Gadi Schuster, and Klaus Kloppstech. 2003. "Identification of guttation fluid proteins: The presence of pathogenesis-related proteins in non-infected barley plants." *Physiologia Plantarum* 119(2):192–202.

Guo, Liang Dong, Guo Rui Huang, and Yu Wang. 2008. "Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing." *Journal of Integrative Plant Biology* 50(8):997–1003.

Gupta, Suman and A. K. Dikshit. 2010. "Biopesticides: An ecofriendly approach for pest control." *Journal of Biopesticides* 3(1 SPEC.ISSUE):186–88.

Gygi, S. P., Y. Rochon, B. R. Franza, and R. Aebersold. 1999. "Correlation between protein and MRNA abundance in yeast." *Molecular and Cellular Biology* 19(3):1720–30.

Hassan, Feyroz Ramadan, Samir Khalaf Abdullah, and Lazgeen Haji Assaf. 2019. "Pathogenicity of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. endophytic and a soil isolate against the squash beetle, *Epilachna chrysomelina* (F.) (Coleoptera: Coccinellidae)." *Egyptian Journal of Biological Pest Control* 29(1).

Hernandez-Trejo, A., B. Estrada-Drouaillet, J. A. López-Santillán, C. Rios-Velasco, R. Rodríguez-Herrera, and E. Osorio-Hernández. 2019. "Effects of native entomopathogenic fungal strains and neem extract on *Spodoptera frugiperda* on maize." *Southwestern Entomologist* 44(1):117.

Hiruma, Kei, Nina Gerlach, Soledad Sacristán, Ryohei Thomas Nakano, Stéphane Hacquard, Barbara Kracher, Ulla Neumann, Diana Ramírez, Marcel Bucher, Richard J. O'Connell, and Paul Schulze-Lefert. 2016. "Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent." *Cell* 165(2):464–74.

Hosseini, Fatemeh, Mohammad Reza Mosaddeghi, and Anthony Roger Dexter. 2017. "Effect of the fungus *Piriformospora indica* on physiological characteristics and root morphology of wheat under combined drought and mechanical stresses." *Plant Physiology and*

Biochemistry 118(June):107–20.

Hosseini, Fatemeh, Mohammad Reza Mosaddeghi, Anthony Roger Dexter, and Mozhgan Sepehri. 2018. "Maize water status and physiological traits as affected by root endophytic fungus *Piriformospora indica* under combined drought and mechanical stresses." *Planta* 247(5):1229–45.

Hruska, Allan J. 2019. "Fall armyworm (*Spodoptera frugiperda*) Management by mmallholders." *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 14(September).

Inglis, G. Douglas, Mark S. Goettel, and Dan L. Johnson. 1995. "Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana.*" *Biological Control* 5(4):581–90.

Jaber, Lara R. and Bonnie H. Ownley. 2018. "Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens?" *Biological Control* 116:36–45.

Jaramillo Barrios, Camilo Ignacio, Eduardo Barragán Quijano, and Buenaventura Monje Andrade. 2019. "Populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) cause significant damage to genetically modified corn crops." *Revista Facultad Nacional de Agronomía Medellín* 72(3):8953–62.

Jensen, Rasmus Emil, Annie Enkegaard, and Tove Steenberg. 2019. "Increased fecundity of *Aphis fabae* on *Vicia faba* plants following seed or leaf inoculation with the entomopathogenic fungus *Beauveria bassiana.*" *PLoS ONE* 14(10):1–12.

Jirakkakul, Jiraporn, Sittiruk Roytrakul, Chettida Srisuksam, Pratchya Swangmaneecharern, Suthathip Kittisenachai, Janthima Jaresitthikunchai, Juntira Punya, Peerada Prommeenate, Jittisak Senachak, Laihong So, Anuwat Tachaleat, Morakot Tanticharoen, Supapon Cheevadhanarak, Songsak Wattanachaisaereekul, and Alongkorn Amnuaykanjanasin. 2018. "Culture degeneration in conidia of *Beauveria bassiana* and virulence determinants by proteomics." *Fungal Biology* 122(2–3):156–71.

Johnston-Monje, David and Manish N. Raizada. 2011. "Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology." *PLoS ONE* 6(6).

Kaiser, Deborah, Sven Bacher, Laurent Mène-Saffrané, and Giselher Grabenweger. 2019.

"Efficiency of natural substances to protect *Beauveria bassiana* conidia from UV radiation." *Pest Management Science* 75(2):556–63.

Kalleshwaraswamy, C. M., J. Poorani, and M. S. Maruthi. 2019. "Natural enemies of *Spodoptera frugiperda* (J . E . Smith) (Lepidptera: Noctuidae), a recent invasive pest on maize in South India. Scientific Notes." 102(3):619–23.

Kannidi Siddhartha, C. Chinniah and M. Shanthi. 2019. "In vitro bioassay of certain botanical oils for their efficacy against maize fall armyworm (J.E. Smith) *Spodoptera frugiperd*a (Noctuidae: Lepidoptera)." *Journal of Entomology and Zoology Studies 2* 7(5):606–9.

Kasambala Donga, Trust, Fernando E. Vega, and Ingeborg Klingen. 2018. "Establishment of the fungal entomopathogen *Beauveria bassiana* as an endophyte in sugarcane, *Saccharum officinarum*." *Fungal Ecology* 35:70–77.

Keswani, Chetan, Surya Pratap Singh, and Harikesh Bahadur Singh. 2013. "*Beauveria Bassiana:* Status, mode of action, applications and safety issues." *Biotech Today* 3(1):16.

Khan, Sehroon, Lihua Guo, Yushanjiang Maimaiti, Mahmut Mijit, and Dewen Qiu. 2012. "Entomopathogenic fungi as microbial biocontrol agent." *Molecular Plant Breeding* (July).

Kim, Jong Cheol, Mi Rong Lee, Sihyeon Kim, Se Jin Lee, So Eun Park, Sehyeon Baek, Leila Gasmi, Tae Young Shin, and Jae Su Kim. 2019. "Long-term storage stability of *Beauveria bassiana* ERL836 granules as fungal biopesticide." *Journal of Asia-Pacific Entomology* 22(2):537–42.

Klieber, J. and A. Reineke. 2016. "The entomopathogen *Beauveria bassiana* has epiphytic and endophytic activity against the tomato leaf miner *Tuta absoluta*." *Journal of Applied Entomology* 140(8):580–89.

Komarnytsky, S., N. V. Borisjuk, L. G. Borisjuk, M. Z. Alam, and I. Raskin. 2000. "Production of recombinant proteins in tobacco guttation fluid." *Plant Physiology* 124(3):927–33.

Kosová, Klára, Pavel Vítámvás, Ilja Tom Prášil, and Jenny Renaut. 2011. "Plant proteome changes under abiotic stress - Contribution of proteomics studies to understanding plant stress response." *Journal of Proteomics* 74(8):1301–22.

Lata, R., S. Chowdhury, S. K. Gond, and J. F. White. 2018. "Induction of abiotic stress tolerance in plants by endophytic microbes." *Letters in Applied Microbiology* 66(4):268–76.

Lefort, M. C., A. C. McKinnon, T. L. Nelson, and T. R. Glare. 2016. "Natural occurrence of the entomopathogenic fungi *Beauveria bassiana* as a vertically transmitted endophyte of *Pinus*

radiata and its effect on above- and below-ground insect pests." *New Zealand Plant Protection* 69(January):68–77.

Li, Guoping, Dominic Reisig, Jin Miao, Fred Gould, Fangneng Huang, and Hongqiang Feng. 2016. "Frequency of Cry1F non-recessive resistance alleles in North Carolina field populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)." *Plos One* 11(4):e0154492.

Llorens, Eugenio, Or Sharon, Gemma Camañes, Pilar García-Agustín, and Amir Sharon. 2019. "Endophytes from wild cereals protect wheat plants from drought by alteration of physiological responses of the plants to water stress." *Environmental Microbiology* 00.

Lodha, Tushar Dilipchand, Padmalochan Hembram, and Nitile Tep, Jolly Basak. 2013. "Proteomics: A successful approach to understand the molecular mechanism of plant-pathogen interaction." *American Journal of Plant Sciences* 04(06):1212–26.

Lohse, Rieke, Desiree Jakobs-Schönwandt, Stefan Vidal, and Anant V. Patel. 2015. "Evaluation of new fermentation and formulation strategies for a high endophytic Eetablishment of *Beauveria bassiana* in oilseed rape plants." *Biological Control* 88.

Lopez, Diana Castillo and Gregory A. Sword. 2015. "The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*)." *Biological Control* 89:53–60.

Mahmood, Zahid, Tove Steenberg, Khalid Mahmood, Rodrigo Labouriau, and Michael Kristensen. 2019. "Endophytic *Beauveria bassiana* in maize affects survival and fecundity of the aphid *Sitobion avenae*." *Biological Control* 137(May):104017.

Mantzoukas, Spiridon and Panagiotis A. Eliopoulos. 2020. " Endophytic entomopathogenic fungi : A valuable biological control tool against plant pests.". Applied sciences 10(1), 360

Martinuz, Alfonso, Getaneh Zewdu, Nicole Ludwig, Florian Grundler, Richard A. Sikora, and Alexander Schouten. 2015. "The application of *Arabidopsis thaliana* in studying tripartite interactions among plants, beneficial fungal endophytes and biotrophic plant-parasitic nematodes." *Planta* 241(4):1015–25.

Mascarin, Gabriel Moura, Mark Alan Jackson, Nilce Naomi Kobori, Robert Warren Behle, and Italo Delalibera Junior. 2015. "Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* Strains."

Journal of Invertebrate Pathology 127:11–20.

McKinnon, Aimee C., Susanna Saari, Maria E. Moran-Diez, Nicolai V. Meyling, Maya Raad, and Travis R. Glare. 2017. "*Beauveria bassiana* as an endophyte: A critical review on associated methodology and biocontrol potential." *BioControl* 62(1).

Meinke, David W., J. Michael Cherry, Caroline Dean, Steven D. Rounsley, and Maarten Koornneef. 1998. "*Arabidopsis thaliana*: A model plant for genome analysis." *Science* 282(5389):662–82.

Moloinyane, Siphokazi and Felix Nchu. 2019. "The effects of endophytic B*eauveria bassiana* inoculation on infestation level of *Planococcus ficus*, growth and volatile constituents of potted greenhouse grapevine (*Vitis vinifera* L.)." *Toxins* 11(2):72.

Mousa, Walaa K., Charles R. Shearer, Victor Limay-Rios, Ting Zhou, and Manish N. Raizada. 2015. "Bacterial endophytes from wild maize suppress *Fusarium graminearum* in modern maize and inhibit mycotoxin accumulation." *Frontiers in Plant Science* 6(October):805.

Nannas, Natalie J. and R. Kelly Dawe. 2015. "Genetic and genomic toolbox of *Zea mays*." *Genetics* 199(3):655–69.

Nisa, Humeera, Azra N. Kamili, Irshad A. Nawchoo, Sana Shafi, Nowsheen Shameem, and Suhaib A. Bandh. 2015. "Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: A review." *Microbial Pathogenesis* 82:50–59.

Nobuta, K. and B. C. Meyers. 2005. "Pseudomonas versus Arabidopsis: Models for genomic research into plant disease resistance." *Bioscience* 55(8):679–86.

Nuñez-Mejía, Gricelda, José A. Valadez-Lira, Ricardo Gomez-Flores, Cristina Rodríguez-Padilla, and Patricia Tamez-Guerra. 2016. "*Trichoplusia ni* (Lepidoptera: Noctuidae) Survival, immune response, and gut bacteria changes after exposure to *Azadirachta indica* (Sapindales: Meliaceae) Volatiles ." *Florida Entomologist* 99(1):12–20.

O'Connell, Richard J., Michael R. Thon, Stéphane Hacquard, Stefan G. Amyotte, Jochen Kleemann, et al.. 2012. "Lifestyle transitions in plant pathogenic Colletotrichum fungi deciphered by genome and transcriptome analyses." *Nature Genetics* 44(9):1060–65.

Ordóñez-García, Magali, Claudio Rios-Velasco, David I. Berlanga-Reyes, Carlos H. Acosta-Muñiz, Miguel Ángel Salas-Marina, and O. Jhonathan Cambero-Campos. 2015. "Occurrence of Natural enemies of *Spodoptera Frugiperda* (Lepidoptera: Noctuidae) in Chihuahua, Mexico." *Florida Entomologist* 98(3):843–47.

Ortiz-Urquiza, A. and N. O. Keyhani. 2016. “Molecular genetics of *Beauveria bassiana* infection of insects.” *Advances in Genetics* 94:165–249.

Ownley, Bonnie H., Kimberly D. Gwinn, and Fernando E. Vega. 2010. “Endophytic fungal entomopathogens with activity against plant pathogens: Ecology and evolution.” *BioControl* 55(1):113–28.

Pan, Rui, Le Xu, Qiao Wei, Chu Wu, Wenlin Tang, and Ralf Oelmu. 2017. “*Piriformospora indica* promotes early flowering in arabidopsis through regulation of the photoperiod and gibberellin pathways.” 1–15.

Pinto-Zevallos, Delia M., Priscila Strapasson, and Paulo H. G. Zarbin. 2016. “Herbivore-induced volatile organic compounds emitted by maize: Electrophysiological responses in *Spodoptera frugiperda* females.” *Phytochemistry Letters* 16:70–74.

Posada, Francisco, M. Catherine Aime, Stephen W. Peterson, Stephen A. Rehner, and Fernando E. Vega. 2007. “Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales).” *Mycological Research* 111(6):748–57.

Qayyum, Mirza Abdul, Waqas Wakil, Muhammad Jalal Arif, Shahbaz Talib Sahi, and Christopher A. Dunlap. 2015. “Infection of *Helicoverpa armigera* by endophytic *Beauveria bassiana* colonizing tomato plants.” *Biological Control* 90:200–207.

Rai, D., V. Updhyay, P. Mehra, M. Rana, and A. K. Pandey. 2014. “Potential of Entomopathogenic Fungi As Biopesticides.” *Ind. J. Sci. Res. and Tech* 2(5):7–13.

Ramirez-Rodriguez, Denisse and Sergio R. Sánchez-Peña. 2016. “ Endophytic *Beauveria bassiana* in *Zea May*s : Pathogenicity against larvae of fall Armyworm, *Spodoptera frugiperda* .” *Southwestern Entomologist* 41(3):875–78.

Rondot, Yvonne and Annette Reineke. 2018. “Endophytic *Beauveria bassiana* in grapevine *Vitis vinifera* (L.) reduces infestation with piercing-sucking insects.” *Biological Control* 116:82–89.

Russo, María Leticia, Ana Clara Scorsetti, María Florencia Vianna, Marta Cabello, Natalia Ferreri, and Sebastian Pelizza. 2019. “Endophytic effects of *Beauveria bassiana* on corn (*Zea mays*) and its herbivore, *Rachiplusia nu* (Lepidoptera: Noctuidae).” *Insects* 10(4):110.

Sánchez-Rodríguez, Antonio R., Silvia Raya-Díaz, Ángel María Zamarreño, José María García-Mina, María Carmen del Campillo, and Enrique Quesada-Moraga. 2018. “An endophytic *Beauveria bassiana* strain increases spike production in bread and durum wheat plants and

effectively controls cotton leafworm (*Spodoptera littoralis*) Larvae." *Biological Control* 116:90–102.

Sarhadi, Elham, Mitra Mohammadi Bazargani, Andres Godwin Sajise, Shapour Abdolahi, Naireen Aiza Vispo, Marydee Arceta, Ghasem Mohammadi Nejad, Rakesh Kumar Singh, and Ghasem Hosseini Salekdeh. 2012. "Proteomic analysis of rice anthers under salt stress." *Plant Physiology and Biochemistry* 58:280–87.

Schmolke, Amelie, Brian Kearns, and Bridget O'Neill. 2018. "Plant guttation water as a potential route for pesticide exposure in honey bees: A review of recent literature." *Apidologie* 49(5):637–46.

Shrivastava, Smriti and Ajit Varma. 2014. "From *Piriformospora indica* to rootonic : A review." *African Journal of Microbiology Research* 8(32):2984–92.

Siegwart, Myriam, Benoit Graillot, Christine Blachere Lopez, Samantha Besse, Marc Bardin, Philippe C. Nicot, and Miguel Lopez-Ferber. 2015. "Resistance to bio-insecticides or how to enhance their sustainability: A review." *Frontiers in Plant Science* 6(June):1–19.

De Silva, Nimali I., Siraprapa Brooks, Saisamorn Lumyong, and Kevin D. Hyde. 2019. "Use of endophytes as biocontrol agents." *Fungal Biology Reviews* 33(2):133–48.

Singh, Sanjay. 2016. "Guttation: Mechanism, momentum and modulation." *Botanical Review* 82(2):149–82.

Sisay, Birhanu, Tadele Tefera, Mulatu Wakgari, Gashawbeza Ayalew, and Esayas Mendesil. 2019. "The efficacy of selected synthetic insecticides and botanicals against fall armyworm, *Spodoptera frugiperda*, in maize." *Insects* 10(2).

Sword, Gregory A., Ashley Tessnow, and Maria Julissa Ek-Ramos. 2017. "Endophytic fungi alter sucking bug responses to cotton reproductive structures." *Insect Science* 24(6):1003–14.

Tall, Susanna and Nicolai V. Meyling. 2018. "Probiotics for plants? Growth promotion by the entomopathogenic fungus *Beauveria bassiana* depends on nutrient availability." *Microbial Ecology* 1–7.

Tefera, Tadele and Stefan Vidal. 2009. "Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*." *BioControl* 54(5):663–69.

The Arabidopsis Genome Initiative 2000: Analysis of the genome sequence of the flowering

plant *Arabidopsis thalinana*. Nature, 2000, 408(6814) Pgs 769-815.

Trivedi, Dipesh Kumar, Amit Srivastava, Praveen Kumar Verma, Narendra Tuteja, and Sarvajeet Singh Gill. 2016. "*Piriformospora indica* : A friend in need is a friend in deed." *Journal of Botanical Sciences* 5(1):3–6.

Valero-Jiménez, Claudio A., Harm Wiegers, Bas J. Zwaan, Constantianus J. M. Koenraadt, and Jan A. L. van Kan. 2016. "Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*." *Journal of Invertebrate Pathology* 133:41–49.

Vega, Fernando E. 2018. "The use of fungal entomopathogens as endophytes in biological control: A review." *Mycologia* 110(1):4–30.

Vega, Fernando E., Mark S. Goettel, Meredith Blackwell, David Chandler, Mark A. Jackson, Siegfried Keller, Masanori Koike, Nguya K. Maniania, Arnulfo Monz??n, Bonnie H. Ownley, Judith K. Pell, Drauzio E. N. Rangel, and Helen E. Roy. 2009. "Fungal entomopathogens: New insights on their ecology." *Fungal Ecology* 2(4):149–59.

Vega, Fernando E., Francisco Posada, M. Catherine Aime, Monica Pava-Ripoll, Francisco Infante, and Stephen A. Rehner. 2008. "Entomopathogenic fungal endophytes." *Biological Control* 46(1):72–82.

Veloz-Badillo, Guadalupe Monserrat, Jessica Riveros-Ramírez, Alejandro Angel-Cuapio, Oscar Arce-Cervantes, Benito Flores-Chávez, Josefa Espitia-López, Octavio Loera, and Paul Misael Garza-López. 2019. "The endophytic capacity of the entomopathogenic fungus *Beauveria bassiana* caused inherent physiological response in two barley (*Hordeum vulgare*) varieties." *3 Biotech* 9(1):1–6.

Venugopalan, Aarthi and Smita Srivastava. 2015. "Endophytes as in vitro production platforms of high value plant secondary metabolites." *Biotechnology Advances* 33(6):873–87.

Vianna, Florencia, Sebastian Pelizza, Leticia Russo, Natalia Allegrucci, and Ana Scorsetti. 2018. "Endophytic *Beauveria bassiana* (Ascomycota: Hypocreales) alters *Helicoverpa gelotopoeon*'s (D.) (Lepidoptera: Noctuidae) life cycle and reproductive parameters." *Journal of Plant Protection Research* 58(4):321–27.

Vidal, Stefan and Lara R. Jaber. 2015. "Entomopathogenic fungi as endophytes: Plant-endophyte-herbivore interactions and prospects for use in biological control." *Current Science* 109(1):46–54.

Yu, Hongsheng, Lei Zhang, Lin Li, Chengjian Zheng, Lei Guo, Wenchao Li, Peixin Sun, and

Luping Qin. 2010. “Recent developments and future prospects of antimicrobial metabolites produced by endophytes.” *Microbiological Research* 165(6):437–49.

Zhu, Yan Lei, Xiao Ping She, Jian She Wang, and Hai Ying Lv. 2017. “Endophytic bacterial effects on seed germination and mobilization of reserves in *Ammodendron biofolium*.” *Pakistan Journal of Botany* 49(5):2029–35.

Books

Currie Armanda F., Wearn James, Hodgson Su, Wendt Hilary, Broughton Sue and Jin Liang 2014. Advance in Endophytic Research (Eds) Verma Vijay C, Gange Alan C. Chapter 3. Foliar fungal endophytes in herbaceous plants : A marriage of convenience? Pgs. 61-81.

Khan Md. Aslam and Ahmed Wasim 2015. Bio control of Lepidopteran Pests: Use of soil microbes and their metabolites (Eds.) Sree, K. Sowjanya and Varma Ajit. Chapter 6. The management of Spodoptera pests using fungal pathogens. Pgs 123-160.

Mohana Kumara P. Swwetha S., Vasanthakumari M.M., Sachin N., Manjunatha B.L., Jadhav S. Sagar, Ravikanth G., Ganeshaiah K.N. and Uma Shaanker R. 2014. Advances in Endophytic Research (Eds) Verma Vijay C, Gange Alan C. Chapter 9 Endophytes and plant secondary metabolite synthesis: Molecular and Evolutionary perspective. Pgs 177-190.

Ortiz-Urquiza Almudena and Keyhani Nemat O. 2016 Genetics and Molecular Biology of entomopathogenic fungi.Edition 1 Volume 94 (Eds) Lovett Brian and St. Leger Raymond. J. Chapter 6 Molecular Genetics of *Beauveria bassiana* infection of insects. Pgs 165-249.

Quesada-Moraga Enrique, Herrero Noemi and Zabalgogeazcoa Inigo 2014. Advances in Endophytic Research. (Eds) Verma Vijay C and Gange Alan C, Chapter 4 Entomopathogenic and nematophagous fungal endophytes. Pgs 85-99.

Rao Srinivasa K.Y., Pattanayak Debasis and Sreevathsa Roshini 2015. Bio control of Lepidopteran Pests: Use of Soil Microbes and Their Metabolites (Eds.) Sree, K. Sowjanya and Varma Ajit, Chapter 3, Bt. Insecticidal Crystal Proteins: Role in Insect Management and Crop Improvement.. Pgs 53- 70.

Singh H.B., Keswani Chetan, Ray Shatrupa, Yadav S.K., Singh S.P., Sigh S., and Sarma B.K., 2015. Biocontrol of Lepidopteran Pests: Use of Soil Microbes and their Metabolites. (Eds) Sree, K., Sowjanya and Varma Ajit. Chapter 10 *Beauveria bassiana*: Biocontrol beyond Lepidopteran Pests. Pgs 219.-235.

Vijay C. Verma and Alan C. Gange Advances in Endophytic Research (2014) Chapter 4 Entomopathogenic and Nematophagous Fungal Endophytes Pgs 86-96

Sree Sowjanya K. and Varma Ajith 2015. Bio control of Lepidopteran Pests: Use of Soil Microbes and Their Metabolites (Eds.) Sree, K. Sowjanya and Varma Ajit, Chapter 1 An Introduction to Entomopathogenic Microorganisms. Pgs 1-10.

Verma Vijay Chandra, Prakash Satya, Singh Rana Gopal and Gange Alan Christopher 2014. Advances in Endophytic Research (Eds) Verma Vijay C and Gange Alan C. Chapter 11 Host-memtic Metabolomics of endophytes: Looking back into the future. Pgs 203-218.

Electronic materials

http://eol.org/pages/16092/names?all= .Cited on 22nd August 2016.

https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=23041#null Cited on 22nd June 2020

https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=42269#null Cited on 22nd June 2020

https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=117472#null Cited on 22nd June 2020

BIOGRAPHY

M.C. Laiju Kuzhuppillymyal Prabhakarankutty
Candidate for Ph.D in Science with the Orientation in Microbiology

Thesis: Entomopathogenic endophyte *Beauveria bassiana* promotes early flowering and drought survival in *Arabidopsis thaliana* and *Zea mays* and control *Spodoptera frugiperda* population in *Zea mays*.

Field of study: Biological Control

Biography:

Personal Data's: Born in Kerala, India on April 14th, 1976 as the daughter of Mr. (Late) K.A Prabhakarankutty and Smt. K.S. Prasanna. Wife of Mr. Bharathan Vellayikodath Soumyan since 2002 and Mother of Kalyani (2007) and Bhavani (2010)

Educational qualifications: Bachelor's degree in Science (Botany) 1996 (India)
Bachelor's degree in Education 2002 (India)
Master's Degree in Science (Biochemistry) 1999 (India)
Master's Degree in Science (Microbiology) 2016 (Mexico)

Work Experience: As teacher in Cecytebc 2010-2011 (Mexicali)
Assistant chemist in Laboratorio Santa Maria (Mexicali)
Biochemist in MOSCMM Hospital (India)