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MASTER'S THESIS

The use of beneficial microorganisms for biological seed coating of selected crops

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Declaration

I declare that I am the author of this master's thesis and that I used only sources and literature displayed in the list of references in its preparation.

In České Budějovice on ....

Signature

Abstrakt

Cílem diplomové práce byla zaměřena na biologické ošetření osiva kukuřice, sóji a okurky pomocí entomopatogenní houby *Beauveria bassiana,* mykoparazitické houby *Trichoderma virens* a antagonistické bakterie *Bacillus mycoides*. Pro obalení semen byl použit 0.5% roztok karboxymethylcelulóza, ve kterém byla v 1 ml koncentrace spor $1x10^6$. Po namoření osiva byl stanoven počet spor vláknitých hub na jednom semeni a zároveň byl sledován vliv mikroorganismů na klíčivost semen a vývoj sazenic. Z výsledků vyplývá, že pozitivní efekt na růst a vývoj sazenic všech testovaných plodin měla antagonistická bakterie *B. mycoides* a entomopatogenní houby *B. bassiana*. Naopak mykoparazitická houba *T. virens* slabě retardovala během klíčení vývoj kořínků a klíčků u kukuřice a sóji. Ošetřená semena okurek pomocí *T. virens* vykázala rychlejší vývoj obou hodnocených parametrů. Setím mořeného osiva pomocí užitečných mikroorganismů lze snadno vnést tyto mikroorganismy do půdy a tím zvýšit supresivitu půd. Klíční rostlinky tak mohou být chráněny proti půdním škůdcům nebo původcům onemocnění rostlin.

Klíčová slova: moření osiva, kukuřice, sója, okurka, *Beauveria bassiana, Trichoderma virens, Bacillus mycoides,* biologická ochrana rostlin

Abstract

The aim of the thesis was focused on the biological treatment of corn, soybean and cucumber seeds using the entomopathogenic fungus *Beauveria bassiana*, mycoparasitic fungus *Trichoderma virens* and antagonistic bacteria *Bacillus mycoides*. A 0.5% solution of carboxymethylcellulose was used for seed coating, in which the concentration of spores in 1 ml was 1x106. After seed coating, the number of spores of filamentous per one seed was determined and also the influence of microorganisms on seed germination and seedling development was evaluated. The results show that the antagonistic bacterium *B. mycoides* and entomopathogenic fungi *B. bassiana* had a positive effect on the growth and development of seedlings of all tested crops. The mycoparasitic fungus *T. virens* slightly retarded the development of roots and shoots in corn and soybean during germination. On the other hand, cucumber seeds coated by *T. virens* showed a faster development on both parameters evaluated. By sowing coated seeds with useful microorganisms, which can be easily introduced into the soil in addition increasing soil suppressiveness. Thus, seedlings can be protected against soil-dwelling pests or plant pathogens.

Key words: seed coating, corn, soybean, cucumber, *Beauveria bassiana*, *Trichoderma virens, Bacillus mycoides*, biological control

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Content

1 PREFACE

In today's modern agriculture, chemical applications against diseases and pests are widely used. The reason and advantage of this treatment is its ease of application and immediate effect. However, as a modern and educated society, we should think about the effects and consequences of conventional (chemical) agriculture. Residues of pesticides and other chemicals used in conventional agriculture effect and will affect future generations and their health. The soil will continue to be contaminated with residues on a larger scale, affecting the natural biological cycle. The problem with today's society is that it is short-sighted and comfortable with cutting back in any way it can at the expense of its personal needs, thereby mitigating the effects of industrial actions on our planet. Unfortunately, the actions of people, especially monopolistic corporations, are already having an impact on the state of the planet, on a global scale.

In agriculture, the use of biological pest management seems to be a suitable way to mitigate these disastrous effects. This method of protection is not new; on the contrary, it was among the first attempts of plant protection in history. The Integrated Pest Management Regulation of European Union, in force since 1 January 2014, obliges all professional users, including farmers, to comply with the IOR principles established by law. The regulation includes a preference for the use of biological protection over chemical plant treatments. Modern biological control agents are highly and long-term effects while being friendly to human health and the environment. They are low or non-toxic to non-target species. The bioagents used thus increase the richness, diversity and stability of natural systems in the agricultural landscape, enabling quality production. In addition to being non-toxic, bioagents are suitable for alternative cropping systems. Biological agents can also be used to treat seeds as effectively as pesticides.

This thesis begins by describing the biocontrol to the use of microorganisms, the different seed coating methods, and agents, and discusses how these microorganisms are utilized in biological seed coating. Additionally, the seed coating with microorganism effect on seed germination and plant growth are examined. All stages of the process are considered where possible, including isolation of microorganisms.

This thesis will share in detail knowledge on the use of beneficial microorganisms especially *B. bassiana*, *T. virens* and *B. mycoides* as biological control agents to seed

coating and also about adhesive carboxymethylcellulose (CMC). Most of the research papers often reported their positive effects on seed germination, nodulation (in legumes) and plant growth. An attempt has been made to draw not only upon scientific literature, but also practical experience, which contains much of the current expertise. One difficulty in reviewing this area is that much of the work is done by commercial companies but not in Myanmar and is not available for publication. Finally, more research needs and future prospects for biological control by coating seed with microorganisms are summarized.

The aim of this thesis was to study the efficacy of the selected strain of entomopathogenic fungus *Beauveria bassiana,* mycoparasitic fungus *Trichoderma virens* and antagonistic bacterium *Bacillus mycoides* on biological seed coating of selected crops.

2 LITERATURE REVIEW

2.1 Integrated pest management (IPM)

Integrated pest management (IPM) as a means of crop protection has been successfully used in agricultural practice for a long time. It includes biological, physical, cultural, and targeted chemical control, as well as the use of resistant spe-cies. It uses, whenever practical and possible, all available information on the appli-cation and a combination of all gentle control methods. Index of Refraction (IOR) consists of careful monitoring of the abundance of pests and their natural enemies, which involves continuous adjust-ment of control methods to current needs. The con-trol methods work together in a complex, they are neither antagonistic nor mutually exclusive (Navrátilová 2015).

The classic definition of FAO (Food and Agriculture Organization) from 1967 char-acterizes IOR as "a complex system of measures aimed at regulating the abun-dance of pest populations, taking into account ecological, economic, toxicological and hy-gienic requirements, to maintain the abundance of pest populations at tolera-ble levels, deliberately favouring and using natural methods of controlling pest pop-ulations" (Landa 2002).

Complementary methods of IOR include agrotechnical, genetic, mechanical, physical, biotechnical, and chemical methods that control pest populations in the long term, con-sidering the economic situation and without undesirable ecological and toxicological side effects on the environment. These are efficient control of pathogens, pests, and weeds (Bailey et al. 2010).

2.2 Biological control by microorganisms

The term of biological control or biocontrol refers to the use of organisms and micro-organisms, consisting fungi, bacteria, viruses, and nematodes, in order to improve the prevention of diseases, pests, and weeds (Fravel, 2005). In spite of the biological con-trol has been used for at least 2000 years, modern biological control technology was use started at the end of the 19th century (van Lenteren and Godfray 2005). There are different mechanisms by which microorganisms act in biological control, which are generally classified as; competition, antibiosis, suppression, parasitism/predation, in-duced systemic resistance, and hypovirulence. The antagonistic activity was generally correlated with the production of secondary metabolites (Hoitink and Boehm, 1999).

There are several commercial products for biocontrol based on various species of microorganisms, for instance, Beauveria bassiana, Metarhizium anisopliae, Trichoderma spp. and Bacillus spp., which are used in order to control generally 48 families of insects from the following orders: Lepidoptera, Coleoptera, Hemiptera, Thysanoptera, and Orthoptera (Ortiz-Urquiza et al., 2014; Wang et al., 2014; Rezende et al., 2015).

The use of microorganisms in biological control can play as a crucial role in many complicated visions of crop protection, as it can maintain the agroecosystem health and productivity, to reduce the populations of pests and diseases without causing damage to crops, in order to produce higher-quality food and better nutritional content (Wall et al., 2015; Etesami and Maheshwari, 2018; Gouda et al., 2018). Biocontrol agents can be used together with other chemical products and also physical methods such as solarization or steam sterilization (Ma et al., 2016).

The chemical pesticides have negative impact to environment and their efficiency can lessen as time goes by (Wang et al., 2015). Berg, 2009 has been reviewed that compared to chemical control, using biological control by microorganisms have several advantages, which are more safe, reduced environmental pollutions and potentially risk of chemical residual effect on human health, show targeted multi activity, although they multiply themselves, they are controlled by the plant and by the original microbial populations as well, decompose rapidly than conventional chemical pesticides, limited the improvement of resistence by several mechanisms, and can be also used in conventional or integrated pest management systems.

Most of the biological seed treatments available in the market are used to control fungi, and also available others specific against nematodes and bacteria (Stockwell & Duffy, 2012). For some instances, Serenade is effective to control Rhizoctonia, Fusarium, Pythium, and some variants of Phytophthora, which is based on Bacillus subtilis from Bayer Crop Science, and Clariva is useable as a nematicide for soybean crop, which is based on the bacterium Pasteuria nishizawae from Syngenta (Schmidt et al., 2010; Syngenta, 2018). Biocontrol agents are very sensitive to environmental stresses such as temperature, humidity, and UV radiation. For this reason, biocontrol agents are often coated with carriers or binders to protect and stabilize (Anal & Singh, 2007, Malik et al., 2017).

2.3 Seed coating

Jeffs (1986) reviewed that many coating technologies for improved agricultural productivity were developed during the mid-20th century. Seed coating technology has continually improved through the 1970s to 1990s (Taylor and Harman, 1990; Scott, 1989 and Hill, 1999). Recently reviews focus on seed enhancements and seed coating agents in the 21st century by Taylor (2003), Pedrini et al. (2017), Halmer (2000), and Pedrini et al., (2020). The extensive commercial utilization of seed coating for field scale agriculture started in 1960s and is continually increasing (Hazra and Patanjali, 2016). Seed coating is one of the reliable methods to apply exogenous materials (such as biopolymers, colorants, biocontrol agents, microbes, and other additives), in these coating materials around the germinating seeds offers great potential to enhance in seed quality (viability and vigor) and yield through enhancing the seed situation and execution (Adak et al., 2016). Previously, the impacts of seed coatings have been evaluated mainly in terms of their effect on phytopathogenic microorganisms. Nevertheless, their impact on potentially beneficial, plant-related microorganisms has only been analyzed indirectly by examining rhizosphere communities in plants derived from coated seeds (Nettles et al., 2016). The coating is able to convey the seeds in a bigger, rounder, heavier, and more uniform structure. Seed coating is considered as a biological apparatus for early plant establishment and also as a stimulant of seed quality (Hazra and Patanjali 2016, Ma 2019). Generally, there are five seed coating methods: dry powder, seed dressing, film coating, pelleting, and encrusting, dependent on size, shape, weight, and utilization properties of the coated seeds (Pedrini et al., 2017).

a) Dry Powder Coating

Dry powder seed coating method is used for mixing seeds with a dry powder and previously called “planter box” treatment. Dry powders are used to control fungi or bacteria and seeds can have a shorter shelf-life (Taylor, 2003). This technology can be performed on a farm to apply labeled pest control treatments (Jeffs, 1986). The most common dry powders are talc and graphite (Anderson, 2014). Recent research has shown that soy-based protein is an environmentally friendly and the use of soy-based proteins has the potential to reduce the risk of negative impacts on pollinators and humans (Badua et al., 2019). The dosage of dry coating powders applied to seeds is

inversely relative to seed size, and as seed size decreases the amount of powder retained increases because of the increment in seed surface area of smaller seeds (Anderson, 2014).

b) Seed Dressing

Seed dressing is the most widely used method for low dosages of active ingredients onto seeds (Kimmelshue et al., 2019). This method can be used to apply a wide range of active materials, especially chemical plant protectants. For better result of chemical seed treatment, in particular insecticides, finishing powders or fluency powders are added immediately after the liquid application to absorb excess liquid (Anderson, 2014).

c) Film coating

The film coating originally developed for the pharmaceutical and confectionery industries has been modified as a seed coating method (Taylor, 2003). Film coating is the technique by using rotating drum machines of encapsulating seeds with a thin layer of comprising of a mixture of polymers, plasticizers, pigments, and colourants (Taylor et al., 1998). Film coating provides an ideal technique for the application of chemical and biological seed treatments (Taylor and Harman, 1990, McGee, 1995). The thin coating layer does not significantly change the size and shape of seeds but improves the handling properties of the seeds and at the same time eliminating or minimizing products (such as pesticides, biological and micronutrients) dust-off (Taylor et al., 2001). The formulations are commercially available that are ready-to-use as liquids or as prepared dry powders. Recently, film coating has acquired widespread acceptance in the seed industry as a reliable approach to deal with improving the productivity of many important crop species, such as rapeseed (*Brassica napus)*, soybean (*Glycine* max), *Gossypium sp*., sunflower (*Helianthus annuus)*, alfafa (*Medicago sativa)*, wheat (*Triticum aestivum*) and corn (*Zea mays*) (Oliveira et al., 2016; Zhou et al., 2017).

d) Pelleting

Pelleting is the technique of coating seeds with inert materials (for instance, calcium peroxide, talc, bentonite, sand, and diatomaceous earth) in order to enable precise metering and enhance plantability by modifying their shape, size, and weight. This helps make planting easily and exactly for such crops grow from very small or irregularly shaped seeds, such as onion (*Allium cepa*), carrot (*Daucus carota*), cotton (*Gossypium hirsutum*), Lettuce (*Lactuca sativa*), and rice (*Oryza sativa*) (Zhang et al., 2009; Mei et al., 2017). The pelleting process and formulations made by the seed industry have been improved. Pellets should not slow the germination rate or reduce the percentage compared to the non-pelleted control. The pellet can act as a barrier against oxygen diffusion, which affects germination (Sachs et al, 1981).

e) Encrusting

Encrusting is a method of coating seeds with the addition of liquids and solid particles, which leads to a coated seed that is completely covered, but the original shape of the seed is preserved (Taylor, 2020). Encrusting is the process of coating seeds by using a small amount of adhesive and inert material which cause to be a smoother surface, more uniform shape and increased the size and weight of seeds that can be used not only in the greenhouse but also in the field, consequntly attempting seed planting productivity (Szemruch and Ferrari, 2013). This technique is mostly used for plants that get benefit from seed singulation and during the time of post-emergence do not require for thinning (Oliveira et al., 2016). Coating with encrusting method result more weight to seeds than film coating and significantly less weight than pelleting, which is more economical compared to pelleting (Ma, 2019).

2.4 Seed coating agents

2.4.1 Protectants

A wide range of active components may serve as plant protectants including natural products, biologicals, and synthetic chemicals such as pesticides, insecti-cides, fungicides, bactericides, nematicides, and herbicides (Ehsanfar and Modarres-Sanavy, 2005; Elzein et al., 2010). When the conditions are favourable for the path-ogens to

infect and predate the plant, generally seed coating with protectants can improve plant establishment, increase the germination rate, enhance the plant growth, and yield (Yang et al., 2014; Ryu et al., 2006). However, some coating pro-tectants such as fungicides and insecticides can be harmful to the environment and could lead to defiling the agroecosystem (Smith et al., 2016).

2.4.2 Micronutrients

Seed coating with polymer micronutrients [for examples, phosphorus (P), po-tassium (K), copper (Cu), manganese (Mn) and zinc (Zn)] or plant nutrient-rich sub-stance (e.g., biochar) can provide plant host available nutrients during plant growth, therefore achieving optimum crop production (Williams et al., 2016). Wiatrak (2013) found that the effect of polymer seed coating with a mixture of Cu, Mn and Zn on wheat and soybean crops and discovered a cost-effective technique to in-crease the plant growth and quality yield of both crops. Despite its widespread use, the critical state of micronutrients in soils and plants should be evaluated before making recommendations for fertilizers (Anthony et al., 2012). All fertilizers were synthetic chemicals, but several are available as organically approved. Zinc oxide and zinc sulphate are the most encouraging micronutrients used in the seed coating of cereal crops and pulses (Acha et al., 2006; Shivay et al., 2008; Masuthi et al., 2009; Adhikari et al., 2016).

2.4.3 Biostimulants

The combination of the properties of growth stimulants and their application through seed coating has remarkable potential for enhancing plant performance (Madsen et al., 2016). There are various categories of plant biostimulants applied as seed coatings are as followed; beneficial bacteria and fungi (Ben-Jabeur et al., 2019, and Rocha et al., 2019), plant and animal-derived proteins, protein hydrolysates and amino acids (Amirkhani et al., 2016, Wilson et al., 2018, Amirkhani et al., 2019, Qiu et al., 2020), carbohydrate derivatives (Chookhongkha et al., 2012, Ziani et al., 2010), seaweed (Michalak et al., 2017) and herbal extracts (Ben-Jabeur et al., 2019). The application of biostimulant components is not widely integrated as a seed treatment in agriculture. Biostimulants applied as seed coatings may serve as sus-tainable, inexpensive and contribute to great potential for improving plant estab-lishment compared to foliar and soil

application methods (Ma et al., 2019 and Amirkhani et al., 2019). The beneficial effects of biostimulants applied as seed coat-ings on germination increment and growth stimulation on several types of crop spe-cies. For instance, seed coating with plant-derived protein-enhanced germination index and seedling uniformity, as well as broccoli vitality index, compared to un-coated seeds under optimum conditions (Amirkhani et al. 2016, Amirkhani et al. 2019). In another study has been reported in response to biostimulant seed coating, enhancement in the percentage and the rate of germination in red clover, and root enhancement in ryegrass (Qiu et al. 2020). However, the type of biostimulant coat-ings can be varied to inhibit root growth, delay germination, biocontrol, and fertili-zation functions, and also bind seeds to surround soils (Gorim and Asch, 2012).

2.4.4 Markers

Different marker substances (such as visible or invisible dyes, fluorescent tracers, sound, and magnetic powders) have been developed into coatings to trace the seed in the supply chain and protect the true seeds from fake seeds in the market (Amirkhani et al. 2016, Pedrini et al., 2017). The colour marker is the most used marker in coating processes to help users as identification of a specific variety of seed treatment (Ma et al., 2019). Additionally, researchers have also evaluated dou-ble marking with fluorescent dyes and magnetic powder while in the coating can improve the technology against counterfeiting and seed security in the supply chain, consequently ensuring the use of high-quality seeds in crop production (Guan et al., 2013). Riboflavin which is a natural fluorescent compound was used for marking cucumber seeds for authentication and it was not phytotoxic after application com-pared to non-treated seeds (Sikhao et al., 2014). Coloured seed indicated to the seed coat treatment with a suitable fungicide or pesticide and is used to lessen the risk of livestock or human consumption (Pedrini et al., 2017).

2.4.5 Microorganisms

Beneficial microorganisms are widely used as biofertilizers and biocontrol inocula. Coating the seeds is considered a useful and convenient tool for introduces beneficial microorganisms into the soil and the rhizosphere of plant tissues (Bennett et al., 2009).

The application of microorganisms to seeds is an appealing suggestion because it combines a specific effect and a limited impact on the environment (McQuilken et al., 1998). There are three main groups of microorganisms that are considered beneficial for plant nutrition: arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and nitrogen-fixing rhizobia, which are not usually considered as PGPR. Microbial inoculants based on these microorganisms can be divided into different categories depending on their use (Jeffries et al., 2003; Podile and Kishore, 2006; Franche et al., 2009). The procedure of coating seeds with PGPM (especially PGPB, AMF, and rhizobia) with an active ingredient as a coating agent is a microbial seed coating (Oliveira et al., 2016; Ma et al., 2019; Rocha et al., 2019). Nevertheless, commercialization and execution of microbial inoculants still encounter limiting elements, especially because of poor microbial survival and in-adequate colonization of plant host (Ma et al., 2016).

De Gregorio et al. (2017) proposed that coating seeds with nanofiber immobi-lized (PGPB) may serve as a beneficial environmentally friendly approach to sup-port the production of soybean. Besides, a method for inoculating seeds with PGPB using alginate beads as a substrate for applying PGPB to crops (Bashan et al., 2002). Likewise, the encapsulation of PGPB Bacillus subtilis with alginate beads enriched with humic acid was also tested to protect the PGPB inoculum from poor soil condi-tions for their successful introduction in the rhizosphere (Young et al., 2006).

2.5 Carriers and binders used in microbial seed coating

2.5.1 Carriers

Carrier is defined as the important component of the inoculants that helps to deliver an appropriate number of microbes in good physiological conditions (Pacheco-Aguirre et al., 2017). The carriers used for microbial seed coating should have the following properties; easily stick to seeds and make certain of seed germi-nation and seedling growth, the adequate shelf life in addition to the survival of mi-crobes on the seeds. According to their function carriers can be generally classified as nutrients (for examples: P, K, Cu, Mn, and Zn) or fillers (for examples: biochar, cellulose, chitosan, peat, talc, lime, and vermiculite) (Zhang et al., 2009; Mastouri et al., 2010; Berninger et al., 2016; Głodowska et al., 2016; Głodowska et al., 2017; Ruiz-de-La-Cruz et al., 2017; Padhi

and Pattanayak, 2018). The most common carriers for microbial coating include silica and biochar.

Several properties of biochar are suggested to be suitable as an inoculant car-rier and seed-coating material. The physicochemical properties of biochar such as highly permeable structure and surface area can be inhabited by bacteria, protect microorganisms from predation, high water holding capacity can prevent bacterial desiccation, reduced carbon is a potential source of energy that can provide bacteri-al life, moreover, biochar can provide some mineral nutrients.

2.5.2 Binders

Binders according to Elzein et al., (2010). are commonly used to provide structural support and retention of active ingredients, even to prolong the survival of microbial inoculants. Some of the binders that can be used in seed coating are listed below:

Xanthan gum (E415) - a substance of natural origin, a polysaccharide which is a product of a certain bacterium Xanthomonas campestris (Anonymous1). This sub-stance has hydrocolloidal properties and therefore binds water well, which is partic-ularly useful in the substitution of gluten (Anonymous2).

Guar gum (E412) - Galactomannan found in the seeds of the plant Cyamopsis tetragonolobus, grown mainly in India. It is the ground endosperm of the seeds of this plant; guar gum is obtained by peeling and grinding these seeds (Anonymous3). In Europe, guar gum is used as an additive in the food industry.

Gum Arabic (E414) - a resin extracted from the sap of certain species of acacia (Acacia senegal and Acacia seyal) that grow in North Africa. It is a highly digestible mixture of carbohydrates and glycoproteins used as a stabiliser in the food industry (Anonymous4).

Polyvinyl acetate (PVAC) - a synthetic polymer. It is produced by polymerization of vinyl acetate (VAM). Polyvinyl alcohol is produced by partial or complete hy-drolysis of the polymer.

Polyvinyl alcohol (PVA) - a synthetic polymer that is soluble in water. This polymer is produced by alkaline hydrolysis of polyvinyl acetate in e.g., methanol (Fikr and Kahovec, 2008).

The binder that I used during my experiments was Carboxymethylcellulose (CMC).

Carboxymethylcellulose (CMC) - novel modified carboxymethylcellulose (CMC) composite films were prepared by a casting method. The effects of CMC addition on some physical properties of the resulting blend films were investigated. The moisture absorption and water-solubility properties of the blend films indicate simi-lar trends (Ghanbarzadeh et al., 2010).

Reports on CMC-modified starch biocomposite films are scarce. CMC is a cellulose ether that exhibits thermal gelation and forms excellent films due to its polymeric structure and high molecular weight chains. It would be expected that CMC could improve the mechanical and barrier properties of the starch-based films due to the chemical similarity of starch and CMC, which enables good compatibility between them (Ma et al., 2008). For example, CMC has been used as an adhesive binder and thus as a coating polymer in seeds (De Camargo et al., 2017). Microbial survival is critical not only during the storage period of biological products but also after intro-duction into the soil, where inoculants must compete with native soil mi-crobes for both nutrients and habitable niches (Malusá et al., 2012).

2.6 Entomopathogenic fungi

In plant pathology, biological control most often refers to the use of entomopathogenic fungi (EPF). Entomopathogenic fungi (EPF) are playing an important role in natural ecosystems and are being developed as alternative control agents for insect pests (Gao et al. 2011). EPFs are divided into differentGroups I.e. *Zygomycota, Ascomycota, Basidiomycota, Deuteromycota, Chytridiomycota* and *Oomycota* (Shah and Pell 2003). *Beauvaria, Metarhizium, Lecanicillium* and *Isaria* species were commercially produced as major EPF (Schrank and Vainstein 2010, Lacey et al. 2015).

EPFs are usually isolated from soil or insects (Meyling and Eilenberg 2007; Quesada-Moraga et al. 2007). Similarly in some other studies, the larvae of highly susceptible insect species, such as the mealworm, *Tenebrio molitor L.* and the wax moth, *Galleria mellonella* L. are used as baiting insect for the EPF recovery from soil (Zimmermann 1986).

EPF can directly penetrate when conidia (asexual) land on the cuticle of a suitable host and the life cycle of EPF begins after the conidia land on the cuticle of the host, they attach and start germination (Gillespie et al. 2000, Shah and Pell 2003, Schrank and Vainstein 2010). The next step in the life cycle is hyphal differentiation into blastospores / hyphal bodies in host hemolymph (Gillespie et al. 2000). The number of blastospores spread inside the hemolymph of the host producing toxins that eventually kills the host (Shah and Pell 2003). The hyphae come out from the host cadaver under suitable environmental conditions (Shahid et al. 2012) The EPF then produces conidia on the surface of the cadaver that is dispersed in the environment and the life cycle may start again if the conidia come in contact with a suitable host (Schrank and Vainstein 2010).

EPF infect to the insect of almost all orders; most common are *Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera* and *Hymenoptera* (Ramanujam et al. 2014). EPF has been much focused on their role as pathogens, although there have additional roles in nature as plant endophytes, rhizosphere colonists and plant growth promoters (Lacey et al. 2015). Enhanced plant growth mediated by the endophytic colonization with various genera of fungal entomopathogens, has been established after inoculation with fungi by different methods such as seed treatment, foliar spray, and root drench (Gurulingappa et al. 2010; Lopez and Sword 2015; Jaber and Enkerli 2016, 2017; Jaber and Araj 2018). The endophytic abilities of EPF have the potential to increase the biological control of soil-dwelling pests or plant pathogens that cannot be easily controlled by chemical insecticides (Moonjely et al., 2016).

EPF has recently been discovered that they have the potential to be developed as versatile microbial agents with multiple uses in sustainable agriculture, for example as dual-biocontrol agents of insect and pathogen pests and also as biofertilizers (Vega et al. 2009; Lacey et al. 2015; Jaber and Ownley 2018).

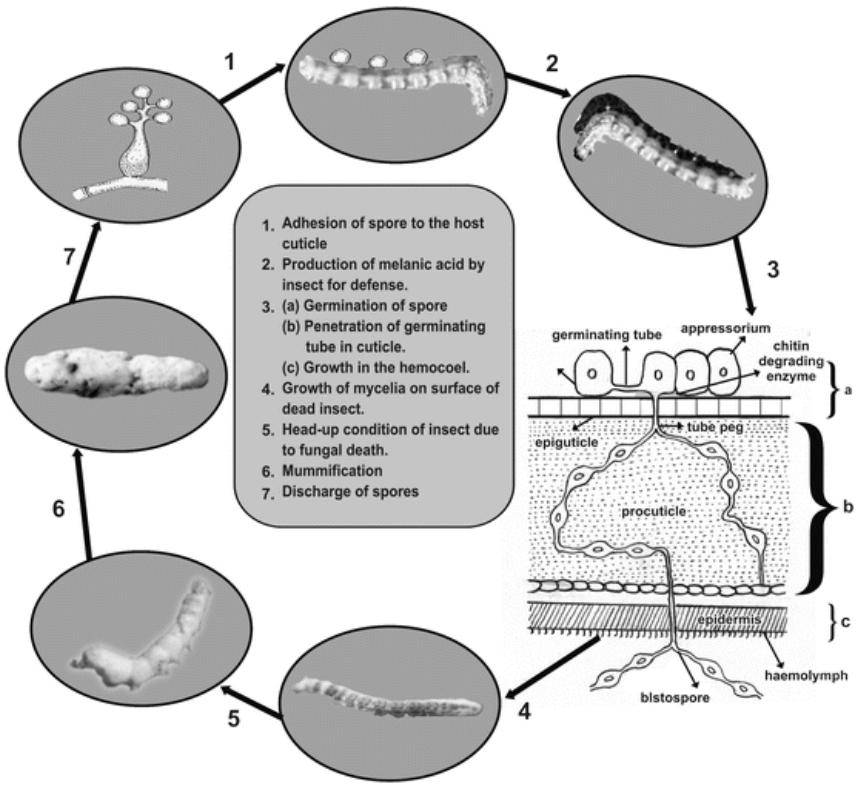


Fig 1: Mechanism of action of entomopathogenic fungi (Sandhu et al., 2017)

2.6.1 Beauvaria bassiana

Beauveria bassiana belongs to the class deuteromycete which is the most widely circulated species of the genus. Agostino Bassi was described to *B. bassiana* as an entomopathogen since 1835. However, Bing and Lewis were not recognized *B. bassiana* as an endophytic fungus until 1991, but later they discovered again its endophytic ability to colonize corn plants (Zea mays L.) (Bing and Lewis 1991, 1992; Wagner and Lewis 2000).

B. bassiana has been recorded as endophyte corn, bean plants and cucumber (Mutune et al. 2016, Shaalan and Ibrahim 2018). Endophyte *B. bassiana* grows within the tissues of corn plants (*Zea mays* L.) and they enter through the plant cuticle after germination of spores and hyphal growth across the leaf surface (Wagner & Lewis, 2000; Bruck, 2010). Also, endophytic isolates of *B. bassiana* has been effectively controlled European corn borer, *Ostrinia nubilalis* (*Lepidoptera: Crambiadea*) while being non-pathogenic to *Z. mays* (Bing & Lewis, 1991; Lewis et al., 2002; Vidal, 2015).

B. bassiana produces secondary metabolites such as non-peptide pigments and polyketides (*oosporein, bassianin and tenellin*), nonribosomally synthesized peptides (*beauvericin, bassianolides and beauveriolides*) and secreted metabolites that have roles in the pathogenesis and virulence (oxalic acid) (Xiao et al. 2012). These metabolites have insecticidal properties and also able to inhibit the growth of other microorganisms (van der Weerden et al. 2013).

The biological control with *B. bassiana* for insect pests has been the subject of numerous articles and reviews (Roy et al. 2005). *B. bassiana* play as a pathogen to a number of insects including hosts of agricultural distinction such as the Colorado potato beetle and codling moth (Sandhu et al. 2012). *B. bassiana* infects a wide range of insect species (over 700 species) and is used to control of plant pests (Vilcinskas and Matha 1997, Glare et al. 2008, Xiao et al. 2012). *B. bassiana* has many genetically significant species with different geographical locations and hosts. It is used as an insecticide by spraying spores on crops as suspensions or wettable powder (Sandhu et al. 2012).

B. bassiana have the ability to promote plant growth, transmit the nutrients to plants or activate plant resistance mechanisms as an endophyte (Greenfield et al., 2016; Moonjely et al., 2016; Vega, 2018). Endophytic *B. bassiana* has been experimentally proved to suppress damping off caused by the soil-borne pathogens *Rhizoctonia solani*and *Pythium myriotylum* in tomato (Ownley et al. 2004) and cotton (Ownley et al. 2008), bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum* in cotton (Ownley et al. 2008), the Zucchini yellow mosaic virus in squash (Jaber and Salem 2014), and downy mildew caused by *Plasmopara viticola* in grapevines (Jaber 2015).

Seed coating with entomopathogenic fungi promoted the fungal persistence in the system of the rhizosphere or even roots endophytically after entomopathogenic fungal colonization (Rivas-Franco et al., 2019). Dash et al. (2018) found that the bean plant heights and biomass were increased after seed inoculation with *B. bassiana*. Also, in cotton seedlings get results after pre-treatment with *B. bassiana* reduced severity baterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum* (Griffin, Ownley, Klingeman, & Pereira, 2006; Ownley et al., 2008).

Some species of *B. bassiana* are able to colonize in the tissues of plants, also above and below of soil that was the different to *Metarhizium* which only has been found associated with roots (Greenfield et al., 2016; Meyling et al., 2011; Moonjely et al., 2016).

2.7 Mycoparasitic fungus

Mycoparasitism means the interaction between mycoparasitic fungi, which have the ability to parasitize on other fungi, and mycohost, which means the fungus acts as a host to be parasitized. (Barnett, 1963). Mycoparasitic fungi include *Trichoderma, Gliocladium,* and *Pythium spp.* (Chelkowski, 1998). Mycoparasitism interactions can be divided into two groups, necrotrophic and biotrophic parasitism (Boosalis, 1964).

Necrotrophic parasites usually have a broader host range than biotrophic parasites because necrotrophic parasites produce non-specific toxic compounds. The famous necrotrophic mycoparasites include *Trichoderma spp.* which play a crutial role in biological control (Barnett 1963; Boosalis 1964). Normally, biotrophic mycoparasites get nutrients from living host cells (Boosalis 1964). Biotrophic mycoparasites are *Cosmospora, Melanospora, Nitschkia,* and *Anthostomella.* Jeffries and Young (1994) reported that the effects caused by necrotrophic mycoparasites are more harmful than biotrophic mycoparasites.

Mycoparasitic fungi of *Trichoderma* spp. directly attack some plant pathogenic fungi as biofungicides due to lytic activity of cell wall-degrading enzymes (Mukhopadhyay et al. 1992; Viterbo et al., 2002). Chet et al. 1998 was reported that lytic activity involved chemotropism, recognition, attachment and coiling, and cell wall penetration and digestion of host cell content consecutively. Significantly increase the growth parameters in bitter gourd, loofah and cucumber were discovered by using several strains of T*richoderma spp*., under greenhouse conditions (Lo and Lin 2002).

Generally, *Trichoderma* can be found around the root, soil, plant debris, forest humus and orchids (Howell 2003; Jash et al., 2008). *Trichoderma spp.* has also been evaluated frequently as a seed treatment and can control pathogenic ascomycetes, basidiomycetes, oomycetes and may even be against nematodes. Not only that *Trichoderma*

spp. can also enhance plant growth by several other mechanisms, including enhancement of systemic plant resistance and enhanced root proliferation (Schuster and Schmoll 2010) and show a great potential for agricultural use.

2.7.1 Trichoderma virens

Trichoderma virens (formerly Gliocladium virens GL-21) has been reported as an aggressive mycoparasite and they are able to parasitize not only hyphae but also fungal resistance structures (Aluko and Herring 1970, Tu 1980, Howell 1982). *Trichoderma virens* is one of the commercially formulated biocontrol agent that effectively protects from soilborne plant pathogens, such as *Rhizoctonia solani*, *Sclerotium rolfsii* , and *Pythium* spp. (Lumsden et al., 1996, Viterbo et al., 2007).

Among the mycoparasitic fungi, *T. virens* is distinct beacause it can produce extracellular chitinase and several structurally complex antibiotics such as viridin, gliotoxin, and peptaibols, which have ability to inhibit the growth of pathogens in an artificial environment and also have capacity to synthesize with lytic enzymes (Howell et al., 1993; Baek et al., 1999; Wiest et al., 2002).

Treatment of seeds of vulnerable cultivars with *T. virens* prevents seed infection by disrupting the process (Howell., 2002). If pathogenic propagules are stimulated to germinate, seed treatment with *T. virens* may not prevent early disease in seedling. Lastly, the ability of seed treatment with *T. virens* as fungicides has considerably expanded the range of activity of the fungus as a biocontrol agent. However, seed treatment with *T. virens* may provide longer-lasting protection to the developing root system than seed treatment with chemical only (Howell et al., 1997). The death cells of *T. virens* are ineffective as biocontrol agents (Howell., 2003). *T. virens* has been reckoned with versatile and effective biocontrol agent. In addition, *T. virens* has been reported as a plant endophyte, capable of asymptomatically colonizing a host plant and occupying the same place as phytopathogens (Tsavkelova et al., 2005).

2.8 Antagonist bacteria

The term antagonism is used to describe a generalized mechanism that reduces the viability or disease-causing activity of a pathogen. Several antagonistic mechanisms

against plant pathogens have been discovered which involve production of various antibiotics, enzymes, and bioactive volatile compounds such as ammonia, hydrogen cyanide, alkyl pyrones, alcohols, acids, esters, ketones and lipids. And other mechanisms are hypovirulence, induced systemic resistance and enhanced plant growth response and competition parasitism for niche or infection site, carbon, nitrogen, or various minerals (Ownley and Windham, 2007).

The use of antagonistic microorganisms as biocontrol agents have proved to successfully prevent various plant diseases in many countries (Sivan 1987). Several antagonistic bacteria species such as *Pseudomonas* spp. and *Bacillus* spp. have been successfully used to control plant pathogens and they have been often related with the plant growth, yield and crop quality (Ahmed et al., 2014, Aeron et al., 2011., Orhan et al., 2006). In addition, they are aggressive colonizers of the rhizosphere of several crops and show a wide range of antagonistic activities against many pathogens (Schippers et al., 1987). *Bacillus* species have a highly antagonistic effect against certain pathogenic fungi including *F. solani* (Sunick et al., 1997).

The used of antagonistic microorganisms in seed treatment to protect soil-borne pathogens is an ideal method as bring it to rhizosphere where plant pathogens such as Pythium and Rhizoctonia are active. A spectrum of antagonistic microorganisms has been used experimentally and commercially for this purpose nevertheless it has been used rarely as seed treatments (McQuilken et al. 1998, Butt and Copping 2000; Berg 2009). On the other hand, microbial antagonists can provide plant protection in conditions where chemical treatments are unaccessible, for example use of micrioorganisms in oilseed rape crop to delivery for inhibition of pathogen *Verticillum dahlia* (Muller and Berg 2008).

2.8.1 Bacillus mycoides

Bacillus mycoides was first described by Fliigge in 1886 and it is characterized by the formation of rhizoid or mycoid colonies when its spreading rhizoidal colony structure was observed in soil cultures (Fliigge., 1886, Logan and De Vos, 2009). In some studies, has already found that strains of *B. mycoides* can be isolated from the rhizospheres of plants such as *Elaeagnus angustifolia* L., tea, corn and grapevine (Benizri et al., 2002, Karagöz et al., 2012). Among the *Bacillus* group *B. mycoides* and *B. subtilis*

were able to secrete both indole acetic acid (IAA) and high antifungal enzymes, such as chitinase, protease, laminarinase, gluconase and peroxidase. These bacteria strains may be good candidate for effectively used as biocontrol agents to prevent from various fungal pathogens such as *Fusarium, Rhizoctonia, Phytophthora, Sclerotinia, Nectria, Pythium* and *Gaeumanomyces* (Bargabus et al., 2002 and Essghaier et al., 2009).

Nakamura and Jackson, 1995 considered that *B. mycoides* is as a saprophytic organism. *B. mycoides* is ubiquitous around the rhizosphere and in the soil. Some *B. mycoides* isolates have beneficial plant growth and biocontrol activity in various plants, including sugar beet, cucumber, and sunflower (Bargabus et al., 2002, Neher et al., 2009, Ambrosini et al., 2016). Moreover, *B. mycoides* is capable to reduce the severity of several important diseases such as the bacterial vascular necrosis of sugar beet, angular leaf spot of cucumber, early blight (*Alternaria solani*) of potato and bacterial spot of both pepper and tomato in both glasshouse and field experiments (Bargabus et al., 2003, Zietlow et al., 2004, Jacobsen et al., 2007). In addition, Diaz et al., 2009 have shown that *B. mycoides* effectively stimulated root development and increased root biomass on cloned plants of *Eucalyptus* spp. reproduced by vegetative propagation.

3 MATERIALS AND METHODS

3.1 Source of organisms

The experiments were aimed at testing the influence of two species of fungi and one species of bacterium on the growth and development of roots or sprouts of selected seeds.

The entomopathogenic fungus used in experiments was *Beauveria bassiana*, strain CCM 8382, and was isolated from the adult of bark beetle Ips typographus in Sumava National Park in 2007. This strain is patented in Czech Republic under the number PV 308112 and deposited at the Culture Collection of Microorganisms in Brno, Masaryk University, Faculty of Science, Brno, Czech Republic. The mycoparasitic fungus *Trichoderma virens*, isolate GL-21 was re-isolated from the bioproduct SoilGard 12G of the company Certis USA Llc., USA. The antagonistic bacterium *Bacillus mycoides*, isolate J was re-isolated from the bioproduct LifeGard WG of the company Certis USA Llc., USA.

Mature culture of all the strains was maintained at the laboratory of Department of Special Plant Production, Faculty of Agriculture, the University of South Bohemia in Ceske Budejovice.

3.2 Plants

In the experiments, seeds of selected crops were used, corn (*Zea mays* L.) variety Perrero (Z230), soybean (*Glycine max* L.) variety Abelina and cucumber (*Cucumis sativus* L.) variety Stela F1. The seeds of all selected crops were obtained from the company SAATBAU ČESKÁ REPUBLIKA s.r.o., Czech Republic.

3.3 Insect

In experiments, the larvae of the mealworm *Tenebrio molitor* and the death beetles of *T. molitor* were used. The population was reared by continuous breeding under constant conditions (thermostat, 25±1°C, photoperiod 16/8). The individual was feeding wheat bran, peeled barley and flour. All the stages of *T. molitor* were read separately because of the cannibalism of this species of insect.

3.4 Other material

Alginate pellets were used for re-isolation of fungi and bacterium from the soil as a nutritive trap. Alginate pellets are formed from the bran mixed with alginate. The mass was dripped into the solution of CaCl2. After forming the pellets, the pellets were drained through a sieve and were dried during the nights in the Laminar flow-box. Dry pellets were stored in the refrigerator until using for experiment.

3.5 Adhesive used in experiments

For seed coating was used Carboxymethyl cellulose (CMC) from the company Sigma Aldrich, Germany. The adhesive served as binding materials to attach spores on the surface of seeds.

3.6 Preparation of suspension

The culture of fungi was cultivated on potato dextrose agar (PDA) and incubated at 25 °C for 5 days (*T. virens*) and 10 days (*B. bassiana*). The suspension from the full sporulated cultures of *B. bassiana* strain CCM 8382 and *T. virens* strain GL-21 was prepared. The suspension was obtained by spilling the surface of fully sporulated strains GL-21 and CCM 8382 with 0.05% sterile solution of Tween 80. The obtained suspension of each fungus was filtered through a sterile gauze and a titer was determined using improve Neubauer's counting chamber. Based on the observed value, the suspension was adjusted to a final concentration of $2x10^7$ spores in 1 ml.

Suspension of *B. mycoides* was prepared directly from the bioproduct LifeGard WG. The 1 g of powder was diluted in 100 ml in 0.05% sterile solution of Tween 80 and properly mixed. Next, one dilution (1:10) was done, the final concentration was $3.0x10^7$ spores per 1 ml.

Adhesive served as a sticky agent, with the help of which spores of fungi or bacterium were more easily caught on the surface of the seeds. A basic 1.0% solution before the final "tank-mix" was prepared, which, in a 1:1 (v/v) ratio, was mixed with a suspension spore of strains of either entomopathogenic fungus *B. bassiana* (CCM 8382) or mycoparasitic fungus *T. virens* (strain GL 201) adjusted to a titer of 2×10^7 spores in 1 ml suspension. Mixing produced a 0.5% adhesive solution with a concentration of 1×10^7 spores in 1 ml. For bacterium B. mycoides, the 1% solution of CMC was mixed with

the suspension in concentration $3.0x10^7$ spores per 1 ml. Final 0.5% solution contain $1.5\ x10^7$ spores of bacterium in 1 ml.

After the final mixture of each microorganism was formed, the "tank-mix" was left for 30 minutes, and then the mixture was used for seed coating.

3.7 Biological treatment of seed of selected crops

3.7.1 Seed coating

Four times of 300 seeds of soyabean, 300 seeds of corn and 400 seed of cucumber were separately placed into the plastic box. The seed of each crop were coated by solution of each microorganism.

1. Corn:

a) 30 ml of *B. bassiana* solution was poured on the seeds in the box.

b) 30 ml of *T. virens* solution was poured on the seeds in the box.

c) 30 ml of *B. mycoides* solution was poured on the seeds in the box.

2. Soyabean:

a) 30 ml of B. bassiana solution was poured on the seeds in the box.

b) 30 ml of T. virens solution was poured on the seeds in the box.

c) 30 ml of B. mycoides solution was poured on the seeds in the box.

3. Cucumber

a) 15 ml of B. bassiana solution was poured on the seeds in the box.

b) 15 ml of T. virens solution was poured on the seeds in the box.

c) 15 ml of B. mycoides solution was poured on the seeds in the box.

Each variant of the seeds was thoroughly mixed with the solution to obtain uniform covering of the surface of each seed. After coating, the seeds were transferred on the sieve for drying by using an active air flow in the flow-box (Biohazard, KRD, s.r.o.). After drying, the seeds were placed into the new sterile plastic box and cover by lid. The boxes with seeds were stored in the refrigerator to obtained suitable condition for microorganisms. The exposition prevents rapid spore inactivation by higher room temperature which is around 23 to 25 °C. The spores on the seeds kept in the refrigerator

at 4 °C can stay viable for at least 1 year. The seeds were stored until they were used for experiment.

3.7.2 Yield spore of beneficial fungi

The objective of the test aimed at setting up the number of spores attached on the surface of the seed. The 15 seeds of each crop of each variant were transferred into the glass tube. 5 ml of 0.05% solution of Tween 80 was added to glass tube contained corn and soybean seeds. 3 ml of solution was added to cucumber seeds.

The content of each glass tubes was left for 60 minutes to allow to soak the layer of CMC on the surface of the seed for better releasing and spreading out of spores into the solutions. After 60 minutes, the glass tubes were also shaken every by using vortex (Heidolph, Reax top, German) to ensure that the spore really releases into the solution. In the solution, the concentration of spores in 1 ml was counting using improved Neubauer's counting chamber using the light microscope (Nicon). Spores were counted 2 days after inoculation. The results were times by amount of ml (corn 5 ml, soybean 5 ml and cucumber 3 ml). The number of spores per one seed was determined.

The test serves as an indicator of the quality of seed coating. The quantity of spores per seed is an indicator that helps to understand how many spores can be entered by means of seeds into the soil during sowing. And it can also give the information to what extent the fungus or bacterium species can colonize the environment close to the seeds.

3.7.3 Laboratory germination test

The aim of the test was to verify the biological value of the seed after adhesion in combination with the entomopathogenic fungus *B. bassiana*, mycoparasitic fungus *T. virens* and antagonistic bacterium *B. mycoides*.

First experiment was realized on Petri dishes. The 9 seeds of corn and soybean and 25 seeds of cucumbers were placed on the surface of 2 % water agar in Petri dish (90 mm) in the arrangement 3 x 3, respectively 5 x 5 seeds.

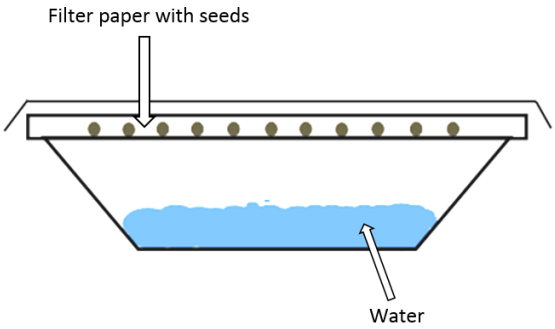


Fig 2. The laboratory germination box

For germination test in the germinated boxes, 100 seeds of corn, 100 seeds of soybean, and 100 seeds of cucumber were used. The 500 ml of the water was put into the lower part of germination box. On the desk, the filter paper was placed and wetted by water. On the soaked filter paper, the seeds were arranged in the square 50 x 50 seeds separately for each crop. Two replications for each variant were prepared.

For both experiments, the seed germination was evaluated after 4 and 7 days. The percentage of germination was established. The growth and development of roots and shoots were measured by ruler and indexes were determined.

3.7.4 Index scales

A more detailed evaluation of all parameters, including the development of sprouts and seed roots, took place after 4 days according to the following scale:

Table 1. Evaluation scale of growth and development for corn

Characteristics of the coleoptile length index	Index	Characteristics of the root length index
not germinated	0	not germinated
coleoptile grows upwards to 0.9 cm	1	the radicle grows downwards to 1.9 cm
coleoptile grows upwards 1 to 1.9 cm	2	the radicle grows downwards to 2-3.9 cm
coleoptile grows upwards 2 to 2.9cm	3	the root grows downwards to 4-4.9 cm
emergence of the foliage leaves, longer than 3 cm	4	the length of the root is longer than 5 cm

Table 2. Evaluation scale of growth and development for soybean

Characteristics of the coleoptile length index	Index	Characteristics of the root length index
not germinated	0	not germinated
the appearance of germ 0.3 cm	1	the radicle grows downwards to 1 cm
The hypocotyl elongates to 1 cm	2	the radicle grows downwards to 3 cm
The hypocotyl elongates to 1.5 cm	3	the root grows downwards to 4.5 cm
Becomes easier to see the cotyledon (>1.5)	4	the length of the root is longer than 4.5 cm

Table 3. Evaluation scale of growth and development for cucumber

Characteristics of the coleoptile length index	Index	Characteristics of the root length index
the seed does not germinate, or the tip is cracked 0.1	0	the seed does not germinate, or the tip is cracked 0.1-3
the appearance of the cotyledon 0.2-4	1	radicle grows (white tip) to 1 cm
cotyledons up to seed size, cotyledons in the hook 0.5-1	2	root size 1 cm up to seed length, 2 cm
Release of cotyledons, plantlets length 2 cm	3	root length to 2 to 3 cm
plant erect, longer than 2 cm	4	the length of the root is longer than 3 cm

3.8 Re-isolation of beneficial microorganisms from soil after sowing the seed into the sterile substrate

The aim of this experiment was established to verify the occurrence of beneficial organisms which were transferred via seed into the substrate. For experiment, the 700 ml of sterile soil substrate was added to clear plastic boxes (10x7x3 cm) for each variant.

The 48 seeds of corn (arrangement, 6 x 8), 48 seeds of soybean (6 x 8) and 60 seeds of cucumber (6 x 10) were sowed in the substrate. The seeds in the box were watering and covered by lid to keep high humidity for seed germination. The boxes were placed into the growing chamber and the plant were planted at 25 °C and photoperiod was 12:12 (light: dark).

The seedling germination was evaluated after 3, 6 and 7 days. After 23 days, the above-ground part was cut with disinfected scissors. The soil substrate and plant residues were used for detection of microorganisms.

Isolation of beneficial fungi from the variant where the seeds coated by *B. bassiana* and *T. virens* was done by using selective medium and by using the traps. For isolation of *B. bassiana* was used artificial medium PDA and selective medium based on a.i. dodine. The receipt is: distilled water 1 000 ml, PDA 39 g, cycloheximide 0.25 g, chloramphenicol 0.5 g, dodine 0.05 g.

For isolation of *T. virens* was used also PDA and selective medium based on Rose Bengal. The receipt is: distilled water 1 000 ml, PDA 39 g, Bengal rose 0.15 g, chloramphenicol 0.25 g, streptomycin 0.05 g, propamocarb 1.2 ml.

For the *B. mycoides* nonselective medium was used because it does not contain the antibiotics, it was used only PDA.

For each variant, 20 ml of soil substrate was placed into the Erlenmeyer flask (250 ml) and 100 ml od 0.05% solution of Tween 80 was added. The Erlenmeyer flasks were placed on the shake for 30 minutes. The soil elution was diluted one times (1:10). The 0.5 ml of elution and 0.5 ml of first dilution were separately poured on the surface of PDA and selective mediums and spread by spatula around the surface of the medium. The Petri dishes with treated suspension into the medium were placed into the plastic bags and incubated in the thermostat at 25 °C. The CFU (Colony forming units) were counting on PDA after 2 days for *T. virens*, after 4 days for *B. bassiana* and *B. mycoides*. On selective medium, the counting of CFU was done later, 5-7 days for both fungi.

For trap, the larvae of mealworm *T. molitor* were used as an alive trap except for *T. virens* which given information about efficacy of entomopathogenic fungus *B. bassiana*. The dead beetle of *T. molitor* and alginate pellets were also used as a trap and these traps were used for all three microorganisms. If the object is overgrowing by fungi or bacteria, it gives information that the microorganisms had very good saprotrophic effect.

The remaining soil substrate was taken from the large plastic boxes of each variants and transferred into 66 small plastic boxes (10x7x3 cm). 10 larvae of T. molitor, 10 dead beetles and 10 alginate pellets were inserted into prepared small boxes according to their variants. All boxes were closed with the lids and incubated at room temperature of 23±1°C. Boxes were frequently inverted, shaken gently and kept upside down for the total incubation period. The efficacy of microorganisms on the larvae, dead beetles and alginate pellets were evaluated on the 40th day after the larvae were inserted.

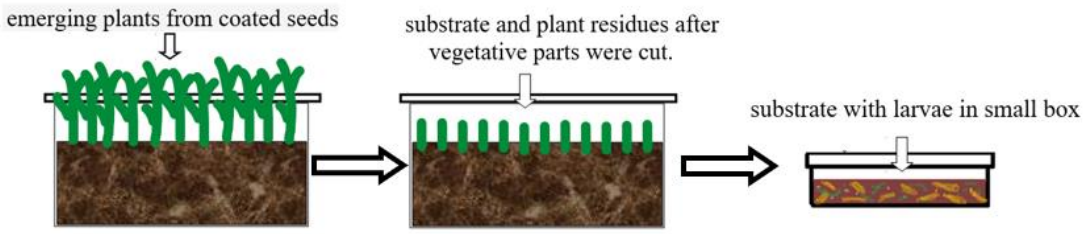


Fig 3. Evaluation of the efficacy of beneficial microorganisms

3.9 Statistical analysis

For evaluation of number of corn roots, the variation test one-way ANOVA was used, the means were compared, using the LSD test at $p \leq 0.05$. When a significant *F* test was obtained at $P = 0.05$, separation of treatment means was performed using Tukey's test. The root length index and shoot length index were evaluated using the Kruskal-Wallis test. In addition, descriptive statistics were used to evaluate means and standard deviations. The Statistica software Version 13 (StatSoft Inc.) was used for conducting all statistical analyses.

4 RESULTS

4.1 Number of spores of entomopathogenic fungus *Beauveria bassiana* and mycoparasitic fungus *Trichoderma virens* on the surface of selected seed crops

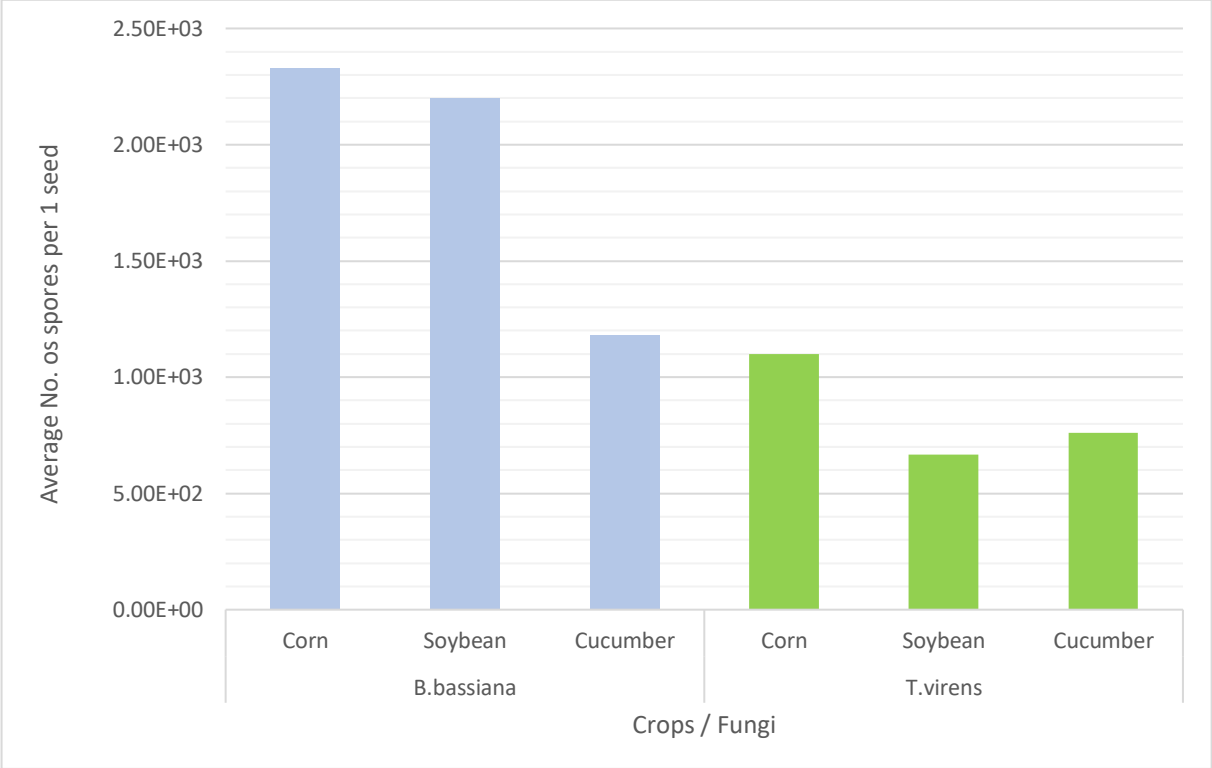
The number of spores attached to the seeds correlate with the seed size. The quantity of spores per seed is an indicator that helps to understand how many spores can be entered by means of seeds into the soil during sowing. The highest number of spores of *B. bassiana* were attached during the corn seed coating process. The second value was determined after elution of soybean seeds. The average of spores per 1 soybean seed was 2,200. Cucumber is smaller than other crops, so the number of spores per 1 seed was almost two times lower (1,180).

After seed coating by *T. virens*, the biggest number of spores were again determined on the corn seeds. Almost the same number per one seed was obtained from the soybean and cucumber seed. The reason can be, that the spores from soybean seed were not well eluted.

Table 4: Average spore number per 1 seed of entomopathogenic fungus *B. bassiana* and mycoparasitic fungus *T. virens* on selected seed crops

Fungi	**Crops**	**Spores per seed**
B.bassiana	Corn	2.33E+03
	Soybean	2.20E+03
	Cucumber	1.18E+03
T. virens	Corn	1.10E+03
	Soybean	6.67E+02
	Cucumber	7.60E+02

Graph 1: Comparison of the number of spores of entomopathogenic fungus *B.bassiana* and mycoparazitic fungus *T. virens* from one seed of selected crop species



4.2 The effect of seed coating of selected crops by beneficial microorganisms on the seed germination

4.2.1 Germination of seeds (%) on Petri dish

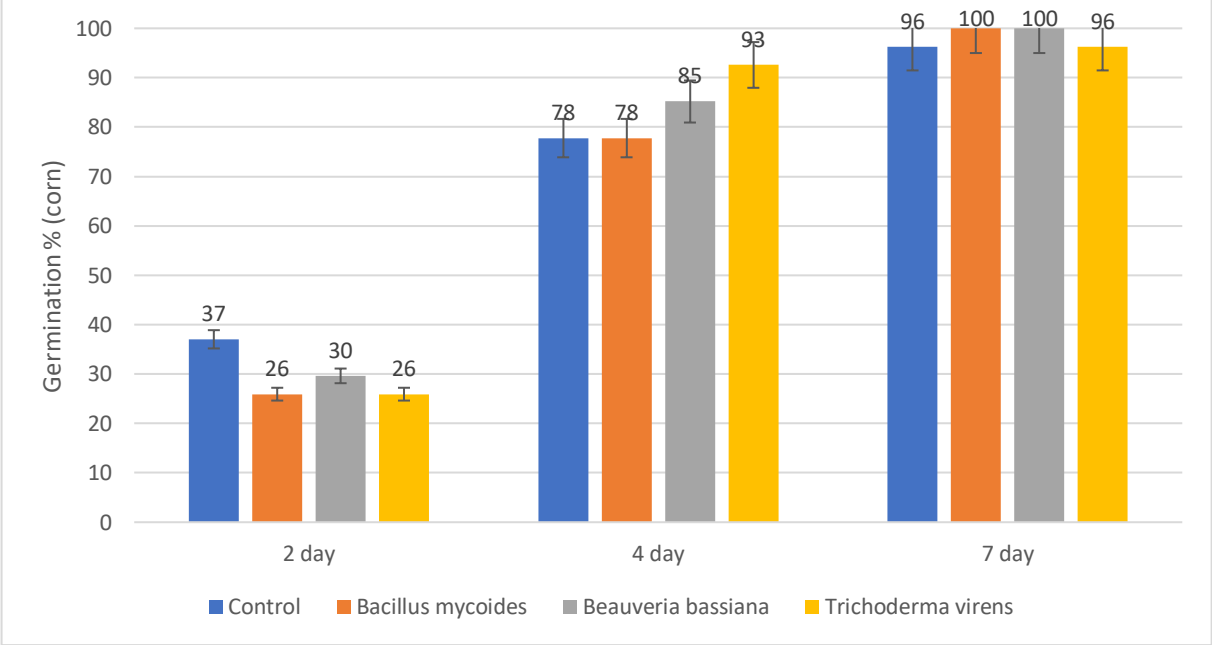
The treated seed with different microorganisms had significant effects on the final germination percentage. In this experiment the germination of all crops was started from 2 days after sowing. The germination rate of corn seeds 2 days after sowing, control gave better performance than other treatments. Even though after 7 days of sowing, *B. bassiana* and *B. mycoides* had significantly higher than control, but no significant differences were observed on the seed germination coated with *T. virens*.

From the beginning, the germination rate of soybean seeds was high compared to corn and cucumber. The data presented in Graph. 3 revealed that the percentage of seed germination for all the treatments were 100% after 4 days of sowing.

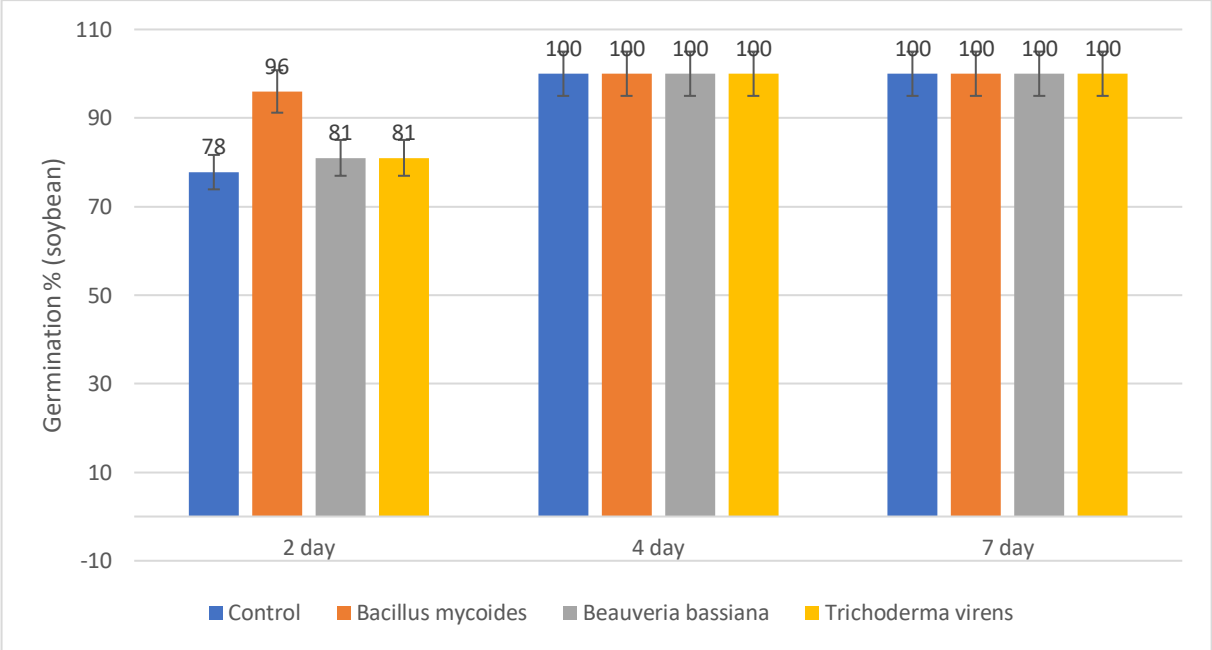
Among the treatments of cucumber seeds, *B. bassiana* stimulated the seed germination which was significantly higher than control. *T. virens* and *B. mycoides* also gave better

performance than control but percentage value of *T. virens* was the same as control 7 days after sowing (Graph 4).

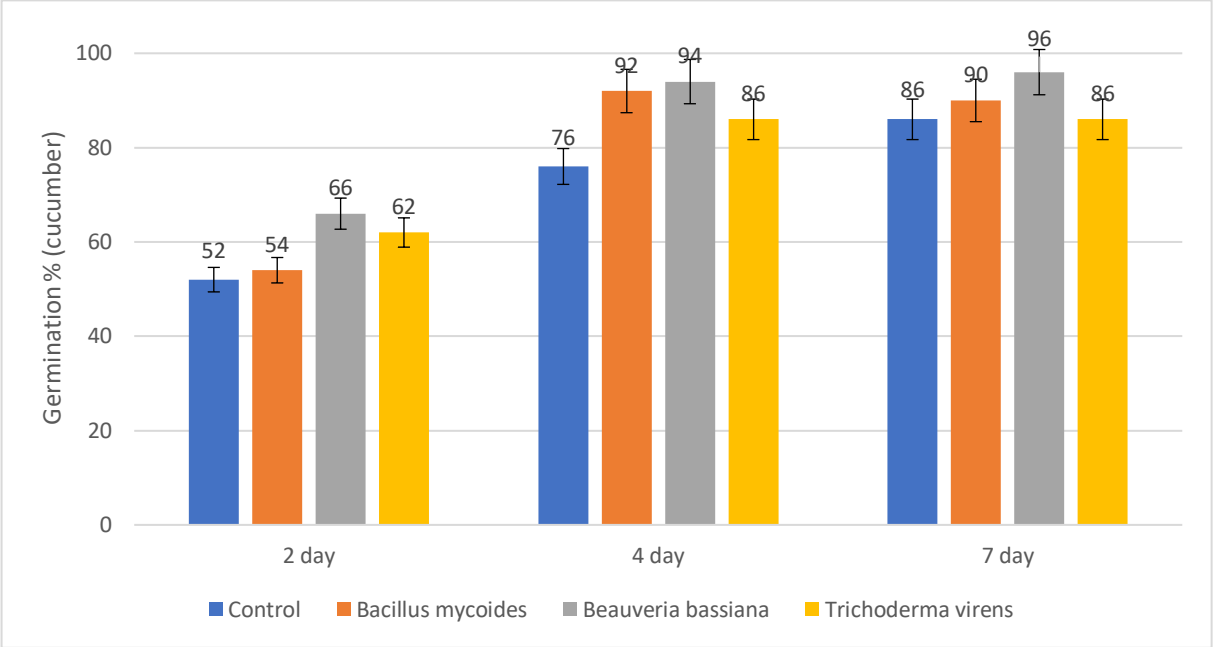
Graph 2: Showing the effect of corn seed germination on Petri dish



Graph 3: Showing the effect of soybean seed germination on Petri dish



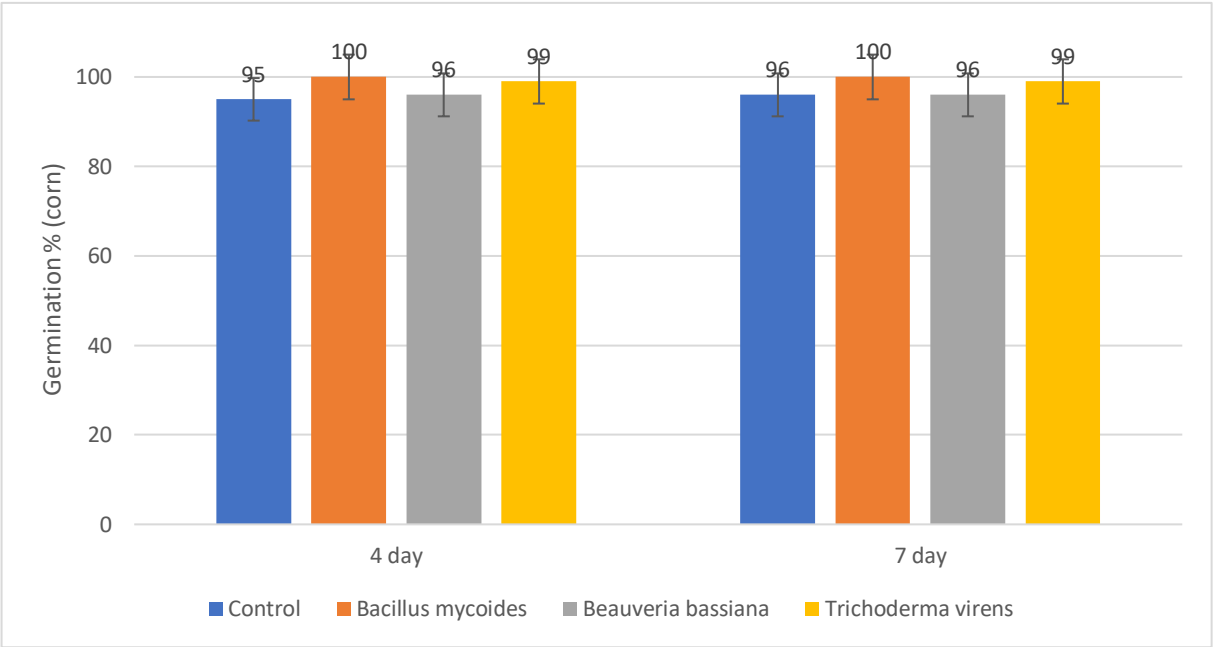
Graph 4: Showing the effect of cucumber seed germination on Petri dish



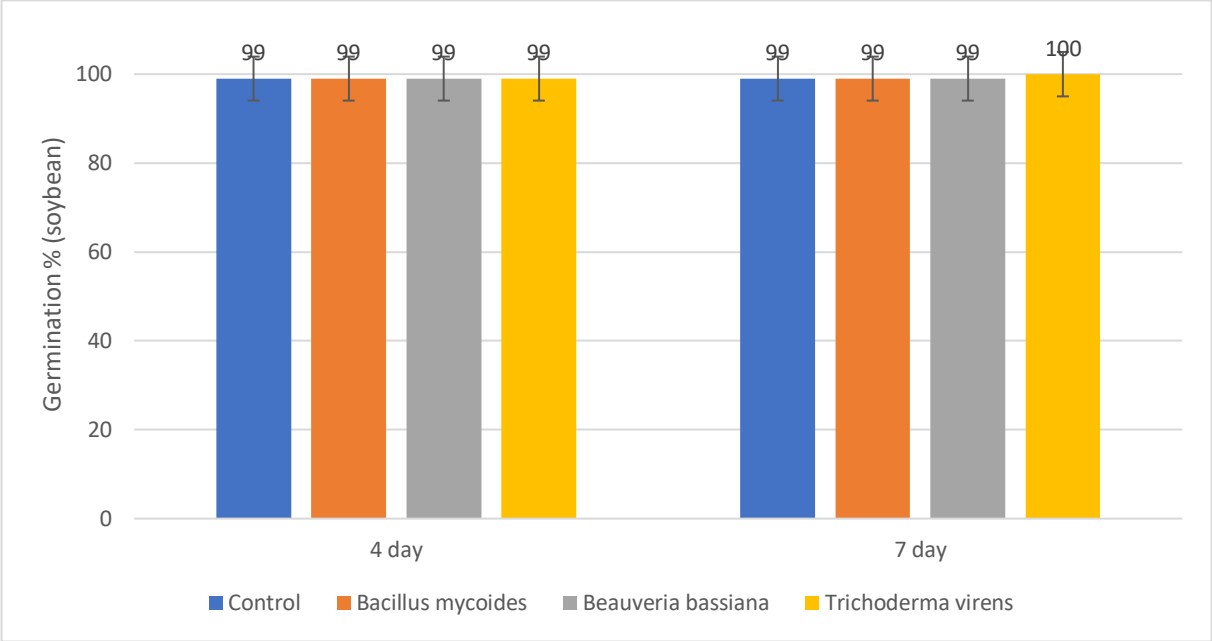
4.2.2 Germination of seeds (%) on filter paper

As can be seen from the Graph no. 5 and 6, no significant differences were observed on the effects of seed treatments with microorganisms on the germination percentage of corn and soybean seeds. In cucumber, the germination percentage of all treatments with microorganisms were slightly higher than control.

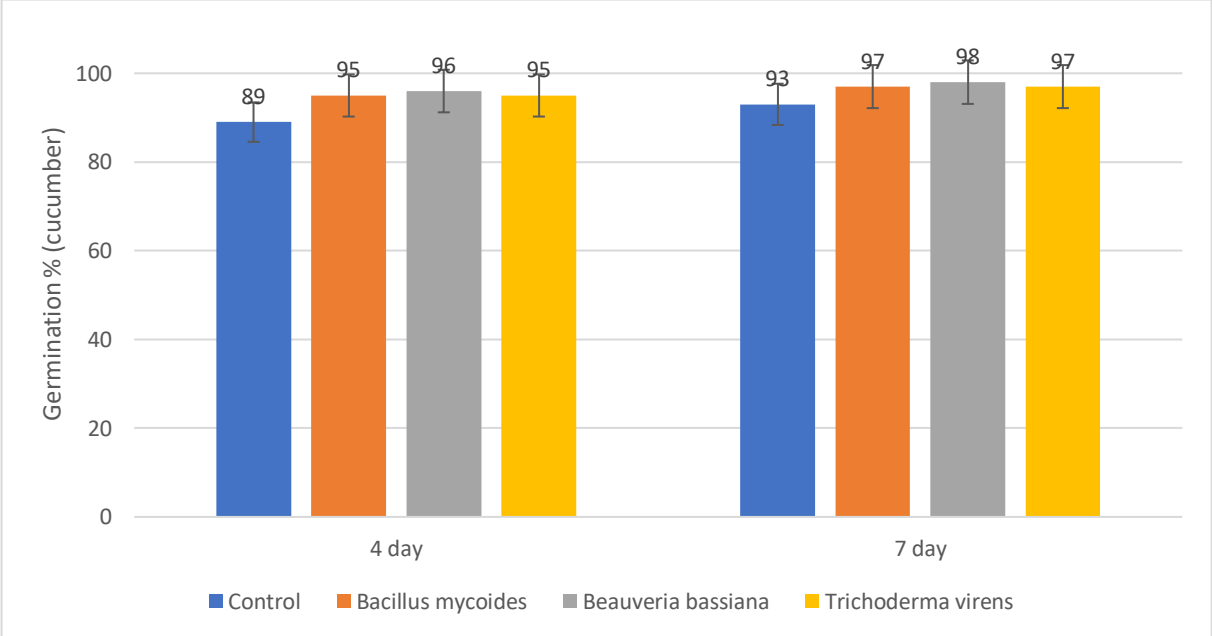
Graph 5: Showing the effect of corn seed germination on filter paper



Graph 6: Showing the effect of soybean seed germination on filter paper



Graph 7: Showing the effect of cucumber seed germination on filter paper

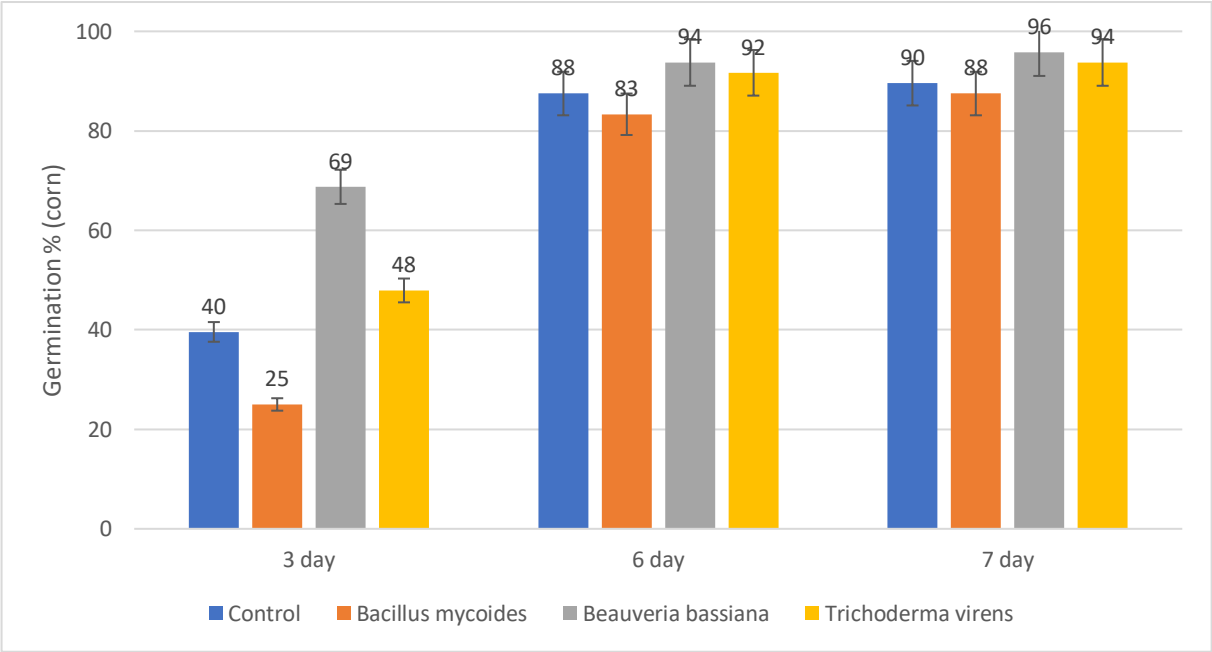


4.2.3 Germination of seeds (%) in the soil substrate

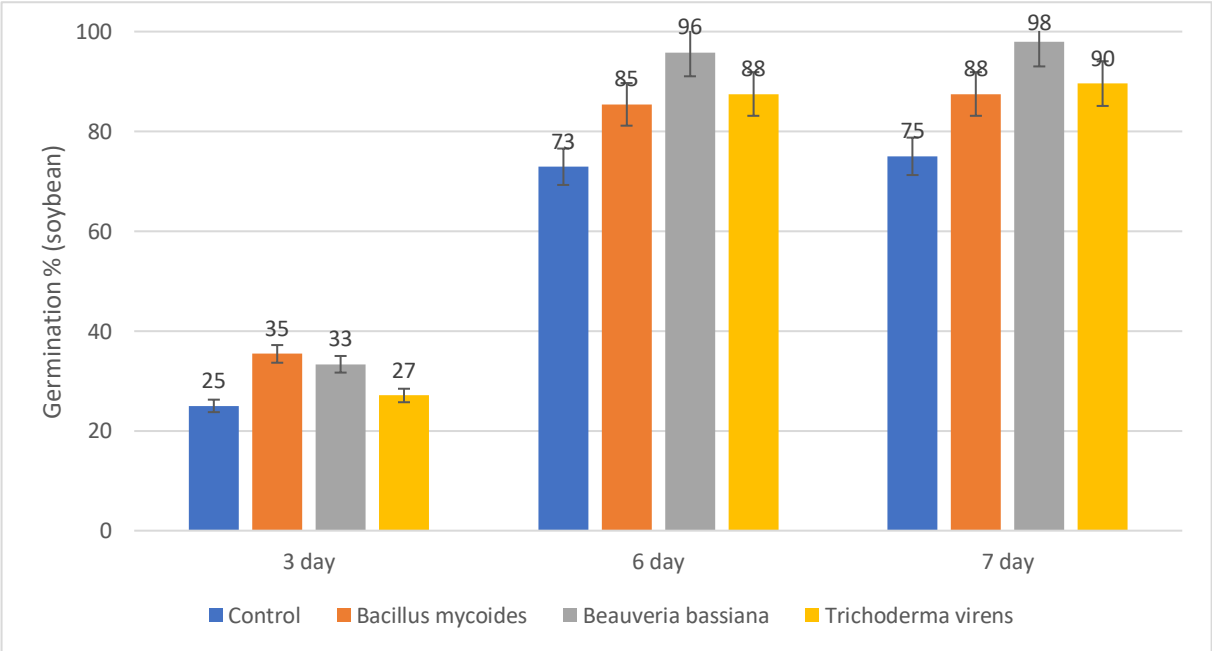
Effect on seed germination percentage of corn in soil, soybean and cucumber seeds were observed differently by microorganisms. In corn, *B. bassiana* enhanced seed germination, which was significantly higher than control and different treatments. *T. virens* also enhanced germination percentage over control and *B.mycoides*. The seed

germination percentage in *B.mycoides* was 88% after 7 days of sowing, which was the lowest one.

Graph 8: Showing the effect of corn seed germination in soil

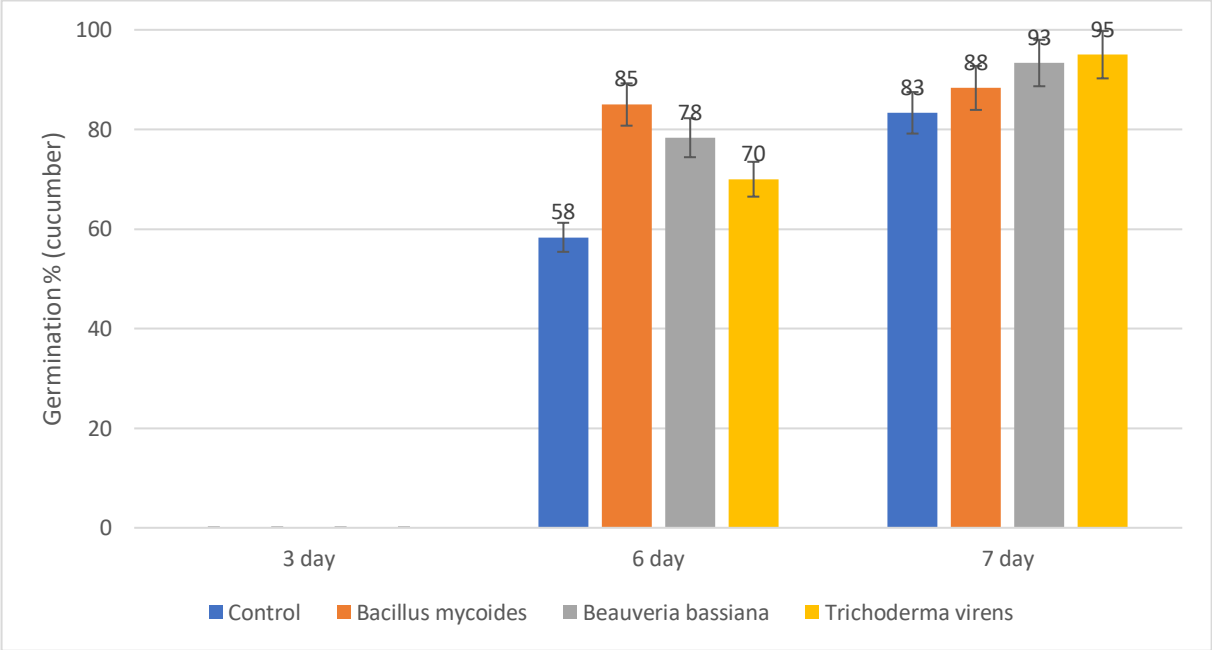


Graph 9: Showing the effect of soybean seed germination in soil



No germination of cucumber seeds in soil were observed within 3 days after sowing. After 7 days, germination percentage of all the treated seed with microorganisms gave better performance than control. The highest germination percentage was obtained in cucumber by treating with *T. virens*. Second highest seed germination was obtained at the treatment with *B. bassiana*. The rate of germination for *B. mycoides* was steady up to 6 days after sowing.

Graph 10: Showing the effect of cucumber seed germination in soil



For soybean, *B. bassiana* also showed the best performance among other treatments in seed germination. At beginning, rate of germination in *T. virens* was low compared to the treatment with *B.mycoides.* After 6 days of sowing, *T. virens* was gradually increase. The final germination percentage of all treatments with microorganisms were significantly higher than control.

4.3 Effect of seed coating by beneficial microorganisms on the growth and development of selected crops

4.3.1 Effect on shoot and root length of selected crops on Petri dish

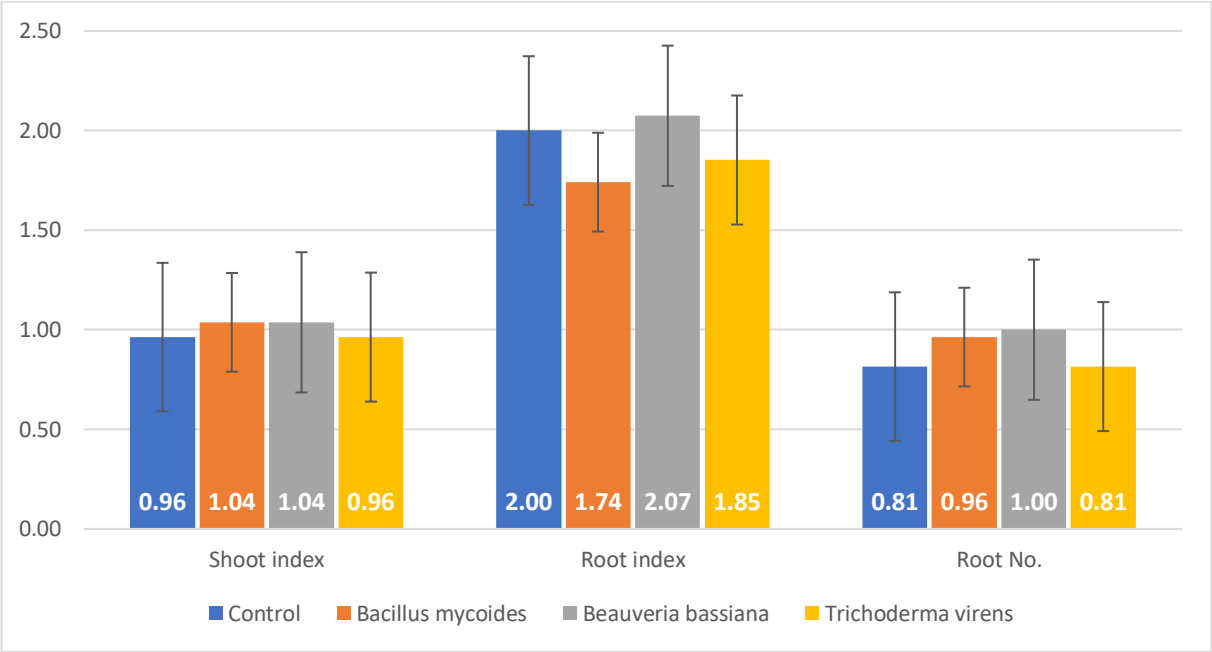
Corn seeds

The evaluation of shoot and root length of corn seedling on Petri dish were observed no significant differences for all treatments after 4 days of sowing. The length of seeds shoots and roots was expressed as an index. The shoot and root length of corn seedlings were gradually increased with proceeding of time after sowing. At 7 DAS, the highest shoot and root length (3.62 and 3.96) was examined at the treatment with *B. mycoides*. The seedling of *T. virens* had the shortest shoot and root length (2.81 and 3.44 respectively). However, the maximum number of roots were observed at *T. virens* coated seedlings (Table 5).

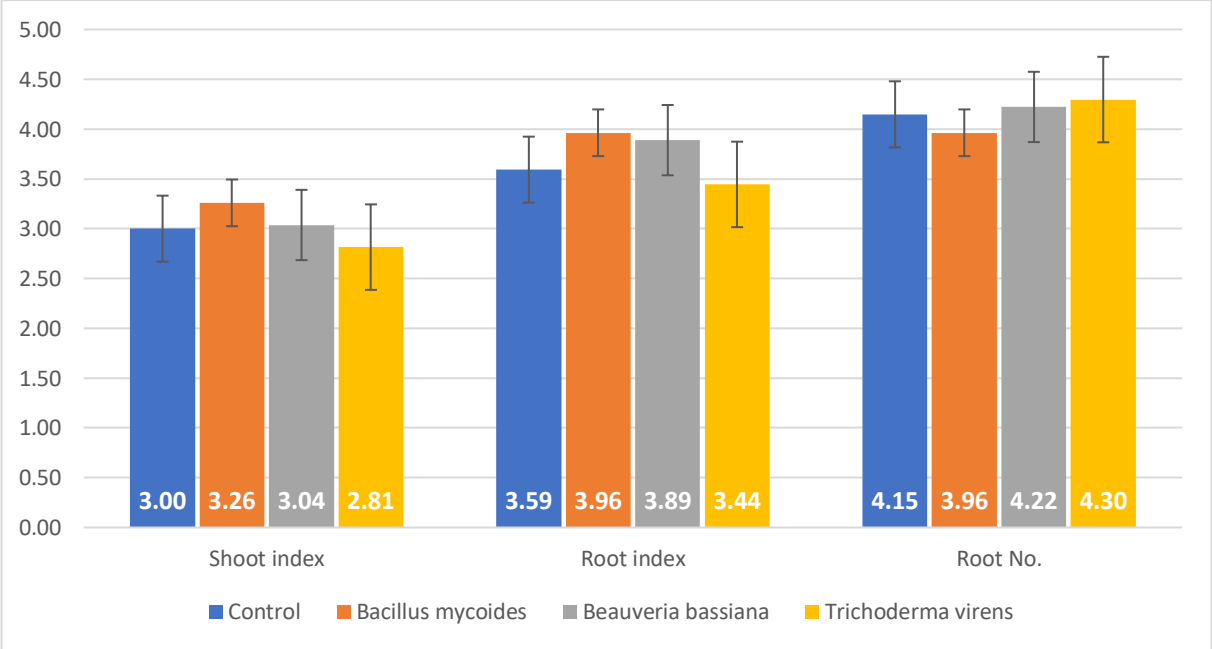
Table 5. Effect of treatments with microorganisms on seedling growth of corn on Petri dish

Treatments	Shoot index of length (Mean±SE)		Root index of length (Mean±SE)		Number of roots (Mean±SE)	
	Day 4	Day 7	Day 4	Day 7	Day 4	Day 7
Control	0.96±0.19	3.00±1.09	2.00±0.72	3.59±0.91	0.81±1.09 a	4.15±1.11 a
B. mycoides	1.04±0.19	3.26±0.75	1.74±0.58	3.96±0.19	0.96±1.20 a	3.96±0.84 a
B. bassiana	1.04±0.19	3.04±0.88	2.07±0.66	3.89±0.31	1.00±1.15 a	4.22±0.79 a
T. virens	0.96±0.19	2.81±0.94	1.85±0.52	3.44±0.92	0.81±1.09 a	4.30±1.05 a
	H (3,N=108) = 3.962963 p =0.2655	H (3,N=108) = 2.947029 p =0.3999	H(3,N=108) = 4.895175 p=0.1796	H (3, N=108) = 8.555660 p=0.0358	F=0.19, df= 3.104, p=0.9019	F= 0.582, df= 3.104, p= 0.6279

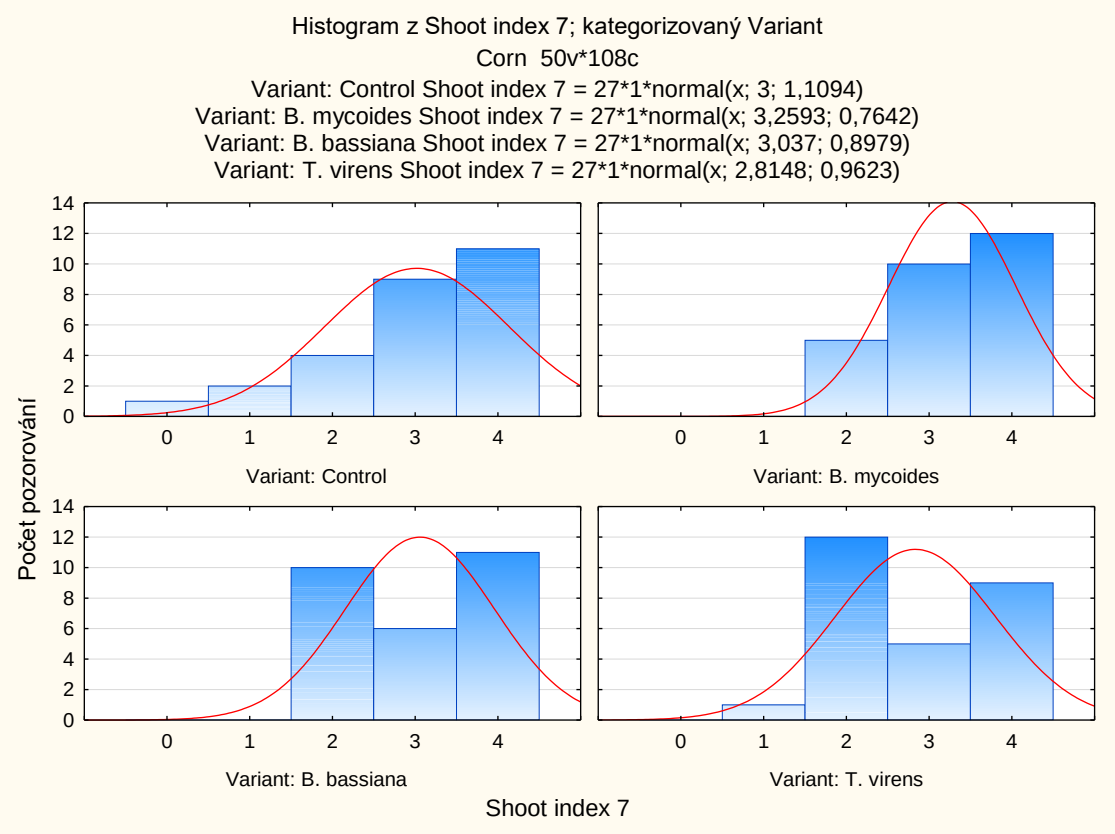
Graph 11: Effects of microorganisms on seedling root numbers, shoot and root index of corn after 4 days of sowing on Petri dish



Graph 12: Effects of microorganisms on seedling root numbers, shoot and root index of corn after 7 days of sowing on Petri dish

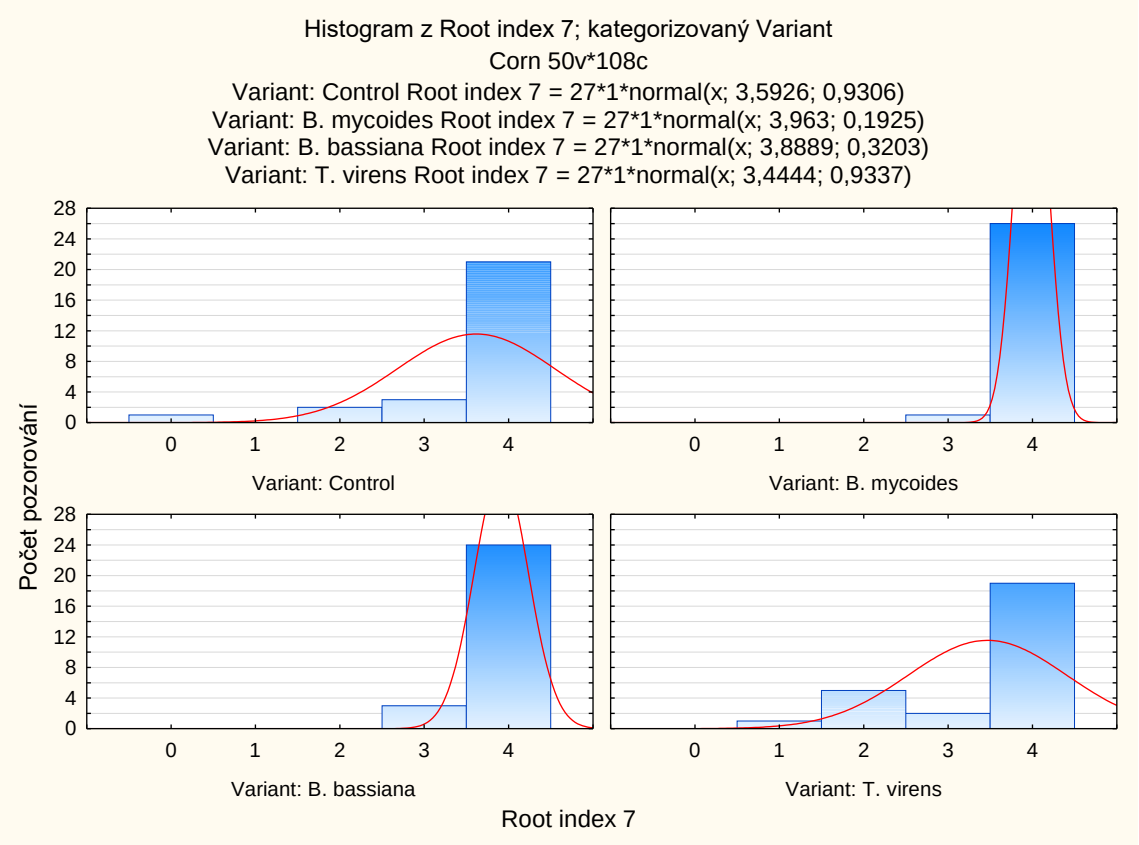


Histogram 1: Effect of treatments with microorganisms on frequency of shoot index distribution of corn after 7 days



The histogram statistically shows the frequencies of corn shoot length index after 7 days of germination test on 2% agar in Petri dishes. It is evident from the histogram that all indices from 1 to 4 occurred in the control variant. Index 0, which means that the seed does not germinate, was the most represented in the control variant. Indexes from 2 to 4 were the most presented in the seed treated with all beneficial microorganisms. The best results of the shoot length index were shown by the variant where maize seeds were coated by species of antagonistic bacteria *B. mycoides*. Entomopathogenic fungus *B. bassiana* also had significant efficacy on shoot length index. Similar results were observed for the evaluation of the root length index. Again, *B. mycoides* and *B. bassiana* had a significant effect on the development of maize roots. The effect of *T. virens* on root formation showed weak retardation in maize compared to the control variant.

Histogram 2: Effect of treatments with microorganisms on frequency of root index distribution of corn after 7 days



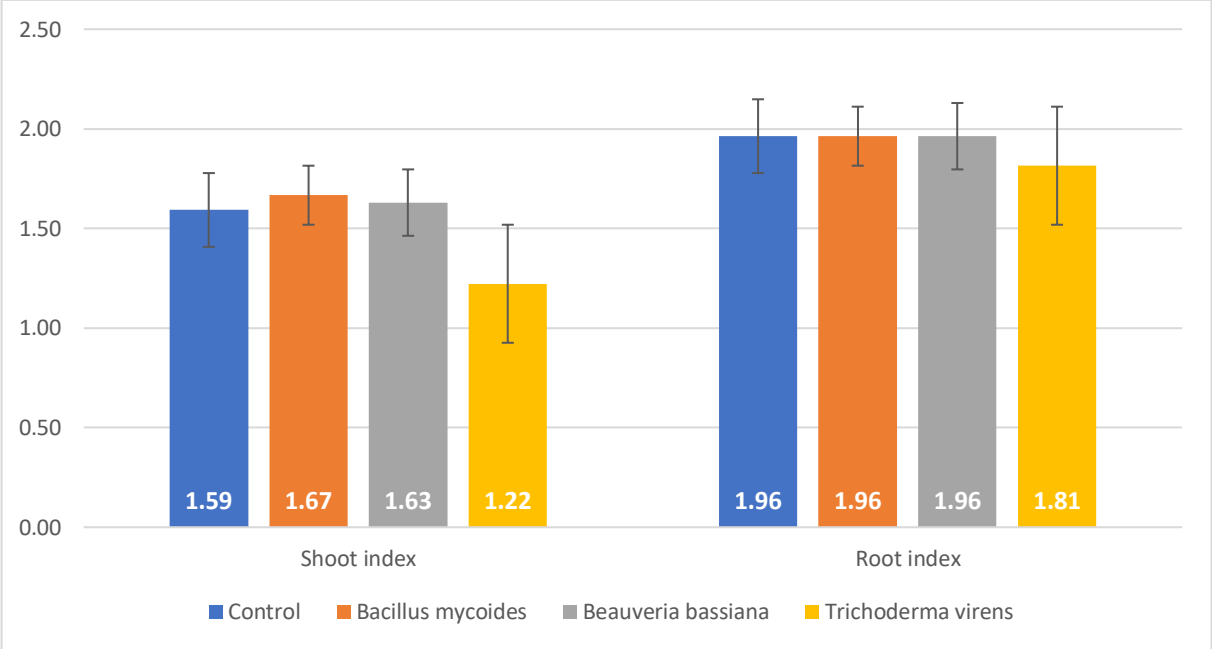
Soybean seeds

In soybean, the data on the *B. mycoides* at 4 and 7 DAYS produced the highest shoot length among other treatments, and these were index 1.67 and 3.59 respectively (Table 6). The minimum shoot length was recorded in *T. virens* coated seedlings. The root length of soybean seedling with beneficial microorganisms were significantly higher over control except *T. virens*. Up to 4 DAS, the shoot and root length was similarly increased, and the values were significantly higher over *T. virens*.

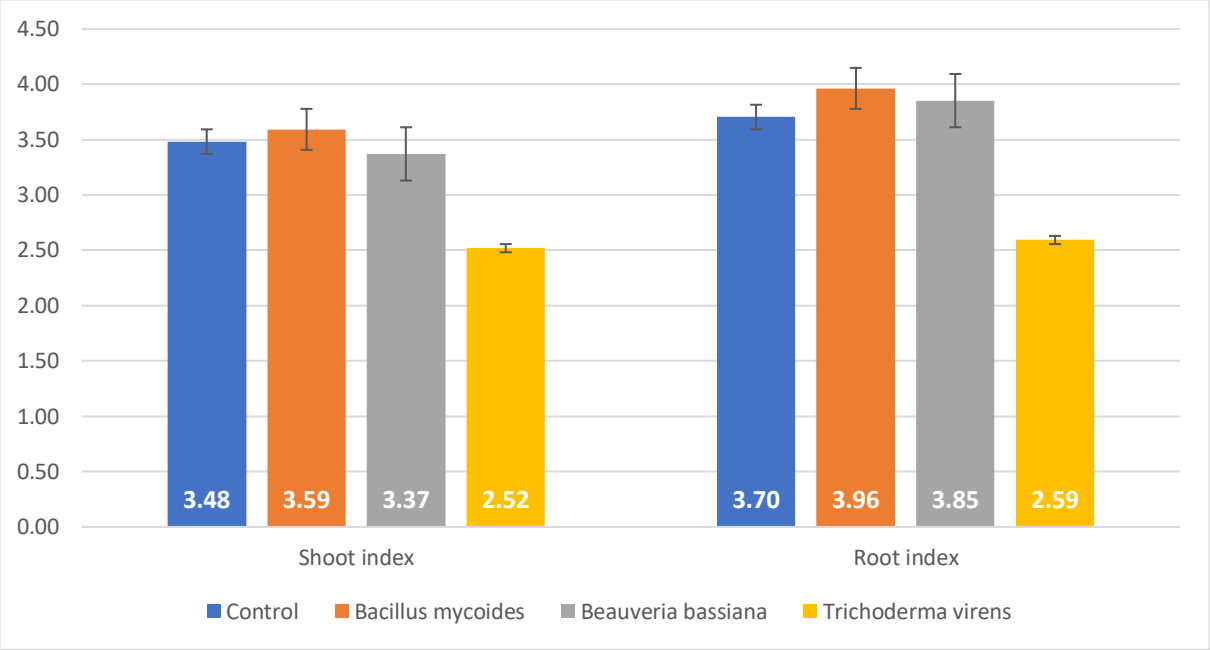
Table 6: Effect of treatments with microorganisms on seedling growth of soybean on Petri dish

Treatments	Shoot index of length (Mean±SE)		Root index of length (Mean±SE)	
	Day 4	Day 7	Day 4	Day 7
Control	1.59±0.49	3.48±0.67	1.96±0.64	3.70±0.81
B. mycoides	1.67±0.47	3.59±0.68	1.96±0.54	3.96±0.19
B. bassiana	1.63±0.48	3.37±0.87	1.96±0.33	3.85±0.52
T. virens	1.22±0.42	2.52±0.57	1.81±0.55	2.59±0.68
	H (3,N=108) =13.65566 p =0.0034	H (3,N=108) =29.51769 p =0.0000	H (3,N=108) =1.664998 p=0.6447	H (3,N=108) =60.46716 p =0.0000

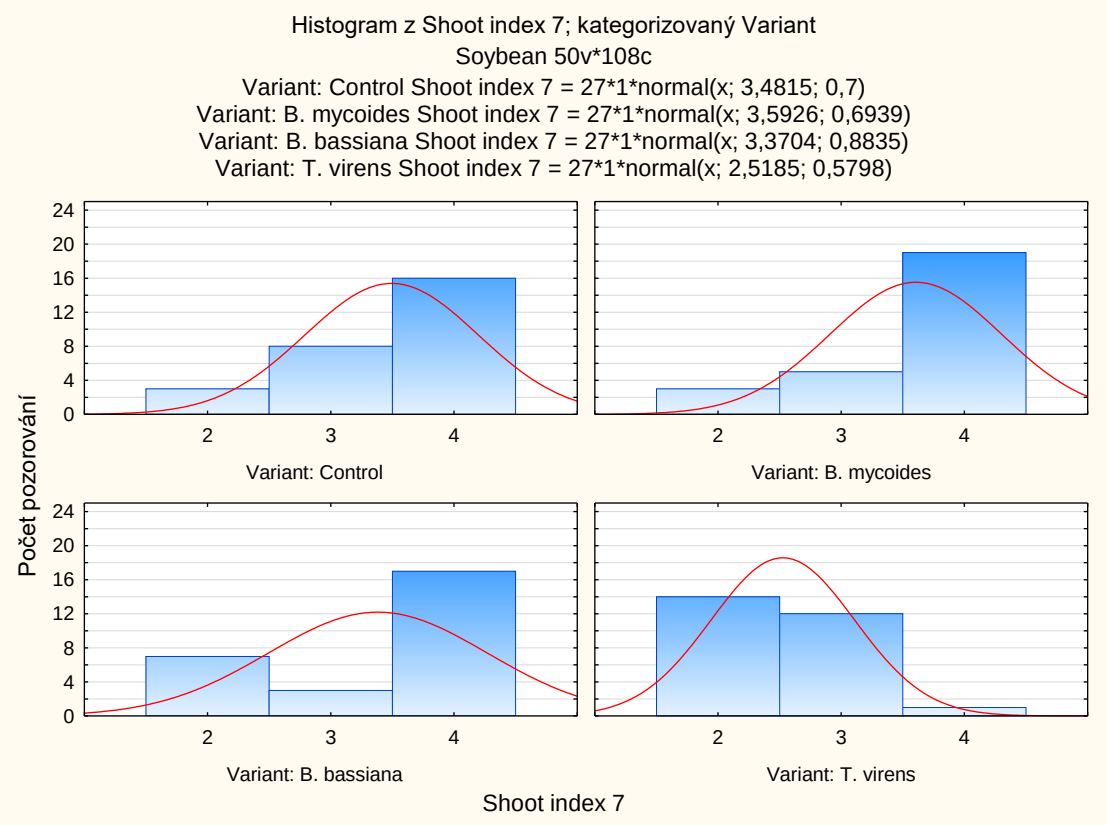
Graph 13: Effects of microorganisms on seedling shoot and root index of soybean after 4 days of sowing on Petri dish



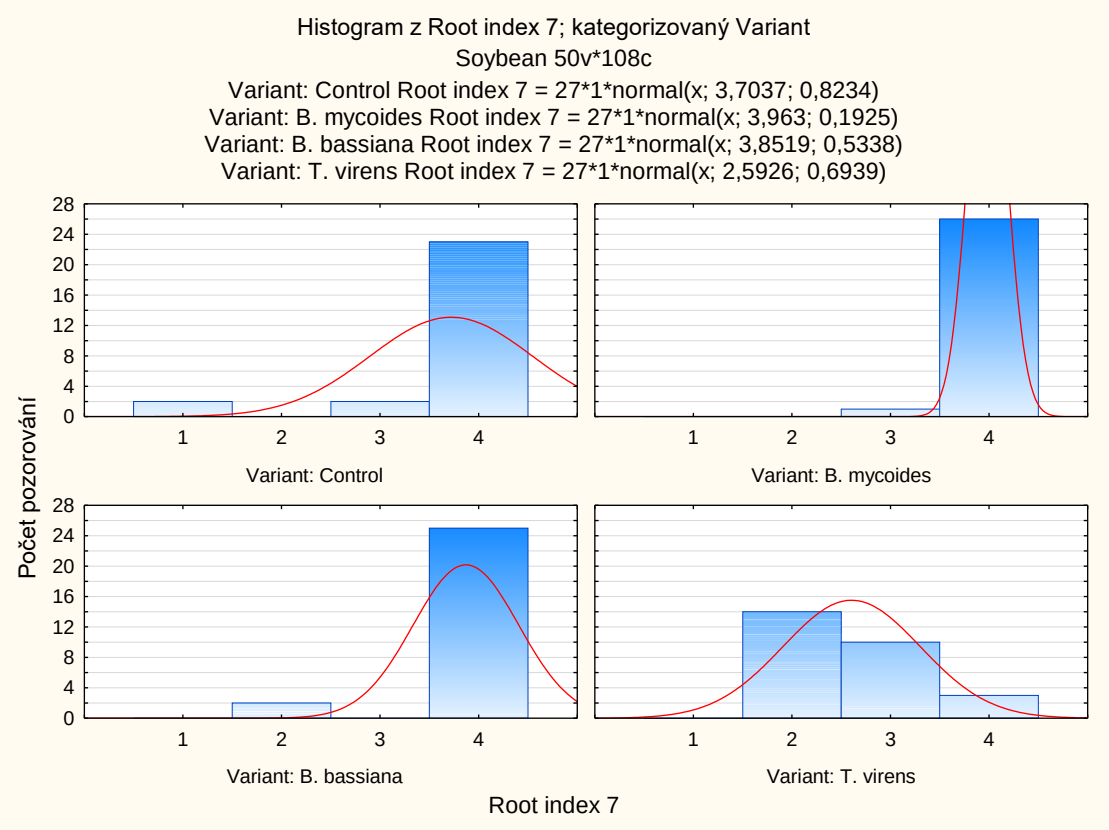
Graph 14: Effects of microorganisms on seedling shoot and root index of soybean after 7 days of sowing on Petri dish



Histogram 3: Effect of treatments with microorganisms on shoot index distribution of soybean after 7 days



Histogram 4: Effect of treatments with microorganisms on root index distribution of soybean after 7 days



The histogram shows differences in shoot length index of soybean among the beneficial microorganisms. In comparison to control variants, the better results were determined in *B. mycoides* variant. Antagonistic bacteria *B. mycoides* have a positive effect on soybean shoot development. Index 4 was the most frequently observed. Entomopathogenic fungus *B. bassiana* was almost comparable with the control variant. However, the shoots were shorter. It is shown the frequency of index 2 is higher than in the control variant. Mycoparasitic fungus *T. virens* has negatively effect on the shoot development of soybean after 7 days. The soybean shoots developed slowly.

The same observation was determined in the root length indexes. Again, the beneficial microorganisms B. mycoides and B. bassiana had positive effect on the roots development. The roots of the soybean seeds coated by these microorganisms were longer than roots in *T. virens* variant.

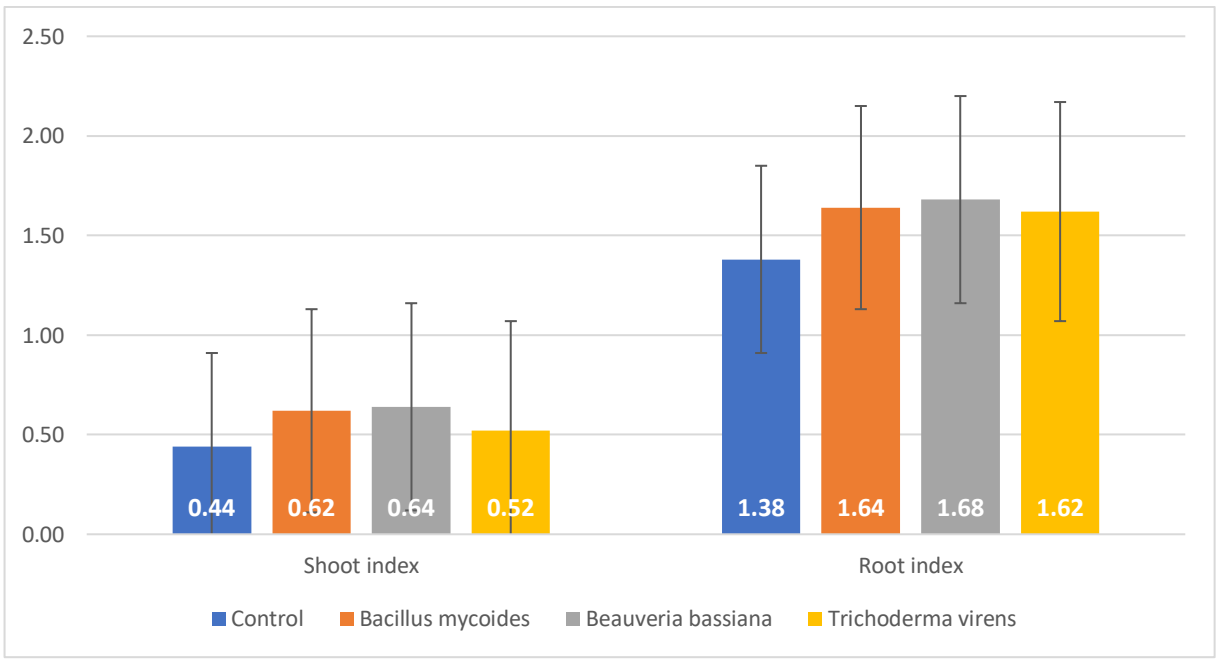
Cucumber seeds

For cucumber, the shoot length at 4 DAS and root length for both recorded days showed no remarkable differences by the interaction of microorganisms on this Petri dish experiment (Table 7). The shoot length of seedlings at 7 DAS was significantly higher affected by treatment with *B. bassiana* compared to control and other treatments. However, no significant differences were observed in root length between treatments.

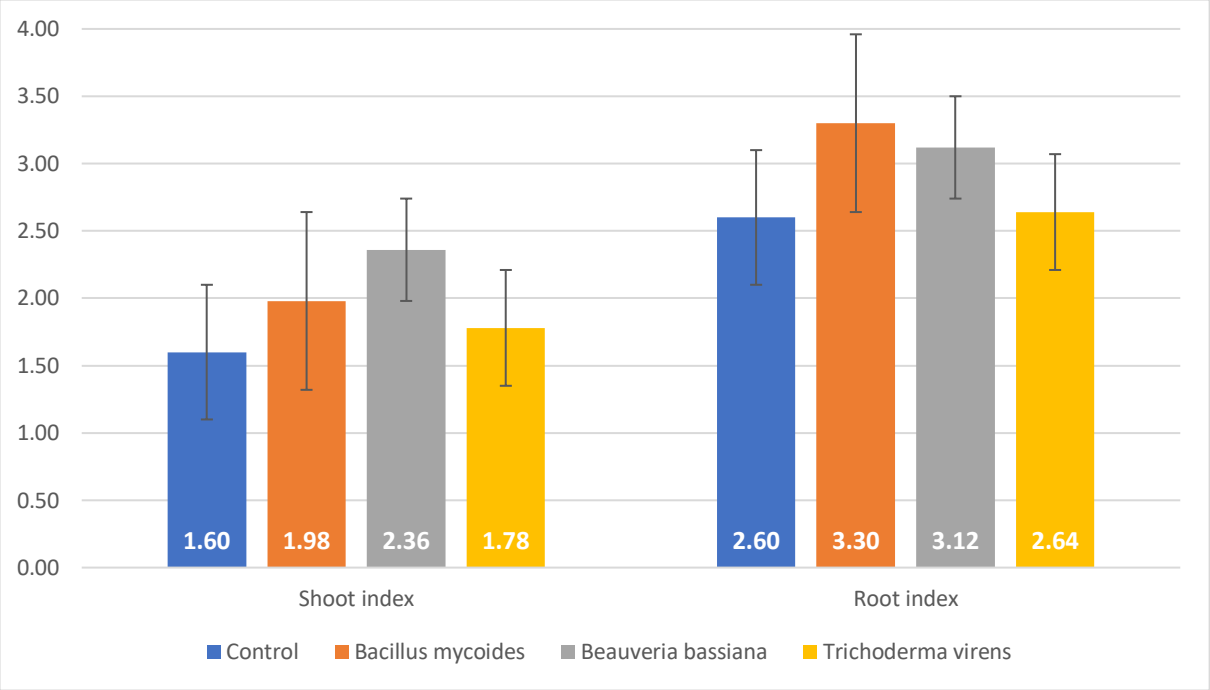
Table 7: Effect of treatments with microorganisms on seedling growth of cucumber on Petri dish

Treatments	Shoot index of length (Mean±SE)		Root index of length (Mean±SE)	
	Day 4	Day 7	Day 4	Day 7
Control	0.44±0.57	1.60±1.08	1.38±1.20	2.60±1.59
B. mycoides	0.62±0.56	1.98±1.17	1.64±1.16	3.30±1.33
B. bassiana	0.64±0.56	2.36±1.29	1.68±1.21	3.12±1.31
T. virens	0.52±0.50	1.78±0.99	1.62±1.34	2.64±1.44
	H (3,N= 200) =4.526425 p=0.2099	H (3,N= 200) =10.56460 p =0.0143	H (3,N= 200) =1.513183 p=0.6792	H (3,N= 200) =10.52150 p=0.0146

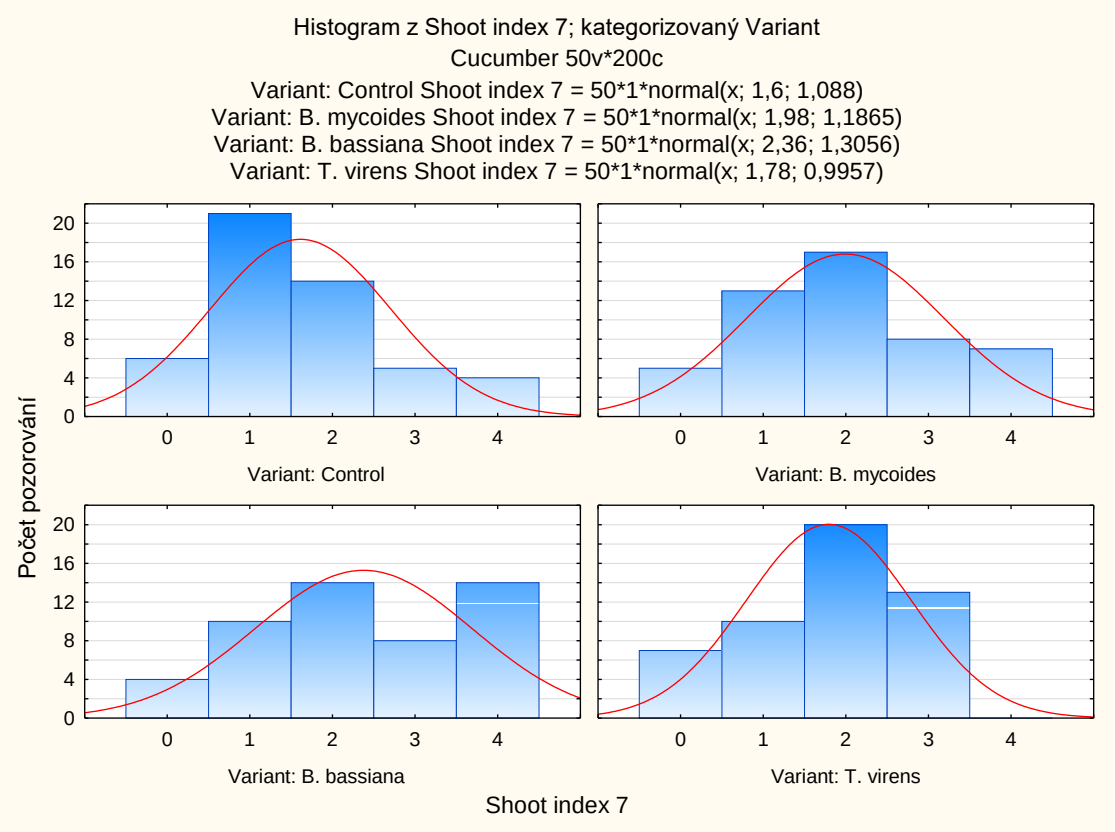
Graph 15: Effects of microorganisms on seedling shoot and root index of cucumber after 4 days of sowing on Petri dish



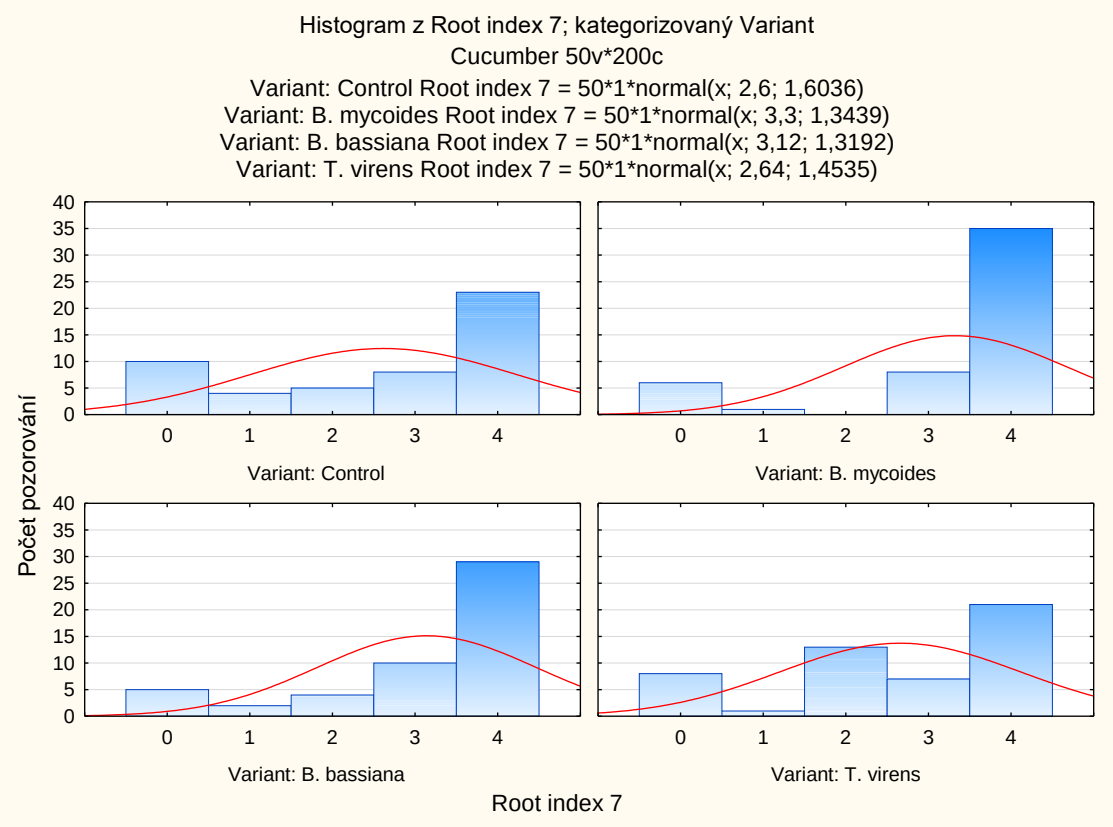
Graph 16: Effects of microorganisms on seedling shoot and root index of cucumber after 7 days of sowing on Petri dish



Histogram 5: Effect of treatments with microorganisms on shoot index distribution of cucumber after 7 days



Histogram 6: Effect of treatments with microorganisms on root index distribution of cucumber after 7 days



Entomopathogenic fungus *B. bassiana* had positive effect on the cucumber seedlings development on 2 % water agar in Petri dishes. The most frequented indexes of seeds treated by *B. bassiana* were 2 and 4. The seeds in control variant germinate and developed slowly in compare with beneficial microorganisms, expect variant where the seeds were coated by *T. virens*. However, *T. virens* has positive effect against plant pathogens, so after sowing the seedlings can be protected with this mycoparasitic fungus. Both *B. mycoides* and *B. bassiana* positively affected cucumber root development. Also, *T. virens* show better results in root length index in compared to control variant after 7 days of germination.

4.3.2 Effect on shoot and root length of selected crops on filter paper

Corn seeds

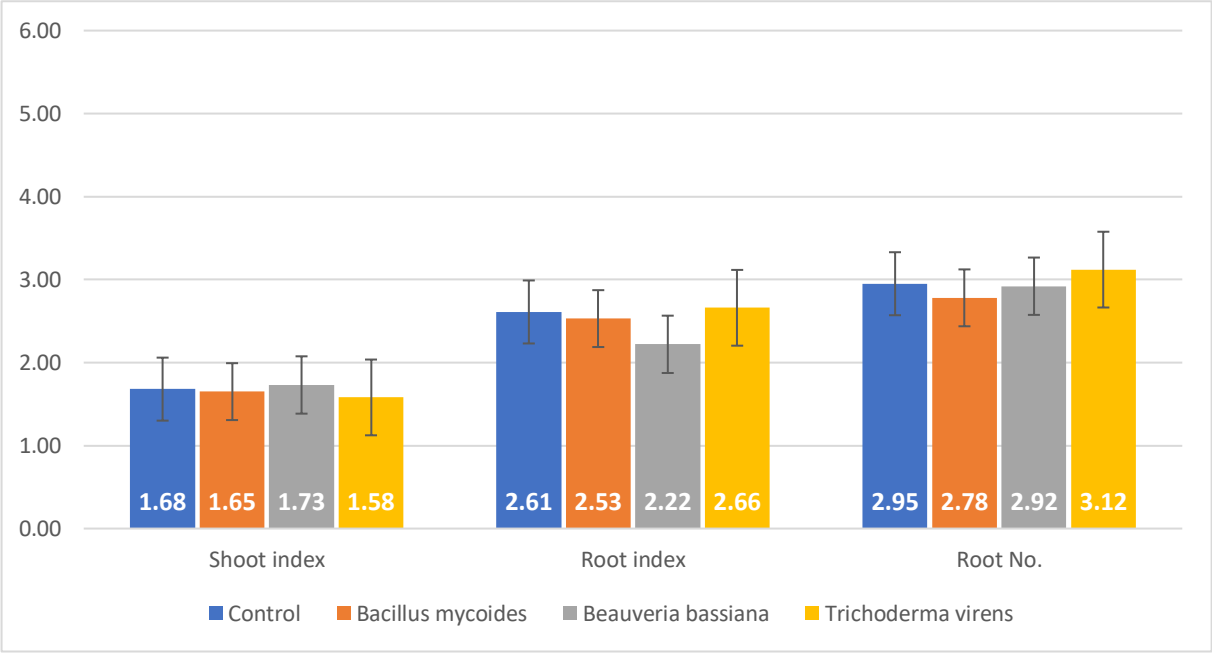
The evaluation of entomopathogenic fungi (*B. bassiana*), mycoparasitic fungi (*T. virens*) and antagonistic bacteria (*B. mycoides*) on the growth parameters of corn seedling on filter paper is described in Table 8. After 4 DAS, there was no significant effect on the shoot length for all treatments. The highest root length at 4 DAS was examined

in *T. virens* (index 2.66). Among the treatments of microorganisms, *B. mycoides* gave the highest shoot and root length (index 3.98 and 3.97 respectively**)** and the lowest shoot and root length (3.67 and 3.75) was obtained in *B. bassiana* at 7 DAYS.

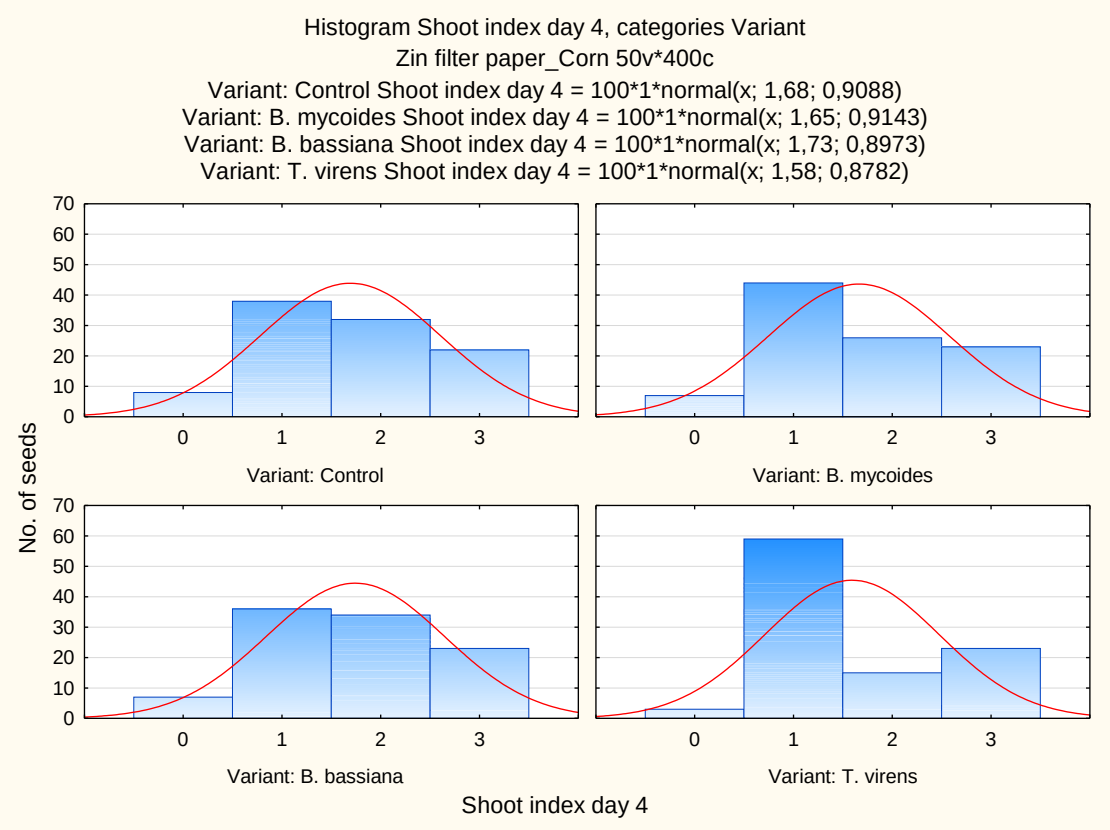
Table 8: Effect of treatments with microorganisms on seedling growth of corn on filter paper

Treatments	Shoot index of length (Mean±SE)		Root index of length (Mean±SE)		Number of roots (Mean±SE)	
	Day 4	Day 7	Day 4	Day 7	Day 4	Day 7
Control	1.68±0.90	3.82±0.80	2.61±0.79	3.77±0.88	2.95±1.42	5.58±1.86 a
B. mycoides	1.65±0.91	3.98±0.14	2.53±0.64	3.97±0.17	2.78±1.27	5.28±1.33 a
B. bassiana	1.73±0.89	3.67±0.98	2.22±0.89	3.75±0.82	2.92±1.50	4.41±1.59 b
T. virens	1.58±0.87	3.84±0.60	2.66±0.64	3.91±0.45	3.12±1.42	4.70±1.51 b
	H (3,N= 400) =2.401630 p =0.4933	H (3,N= 400) =11.58916 p=0.0089	H (3,N= 400) =21.99130 p=0.0001	H (3,N=400) =7.731231 p=0.0519	F=0.4045, df=3.396, p= 0.4045	F=11.169, df=3.396, p= 0.0000

Graph 17: Effects of microorganisms on seedling root numbers, shoot and root index of corn after 4 days of sowing on filter paper

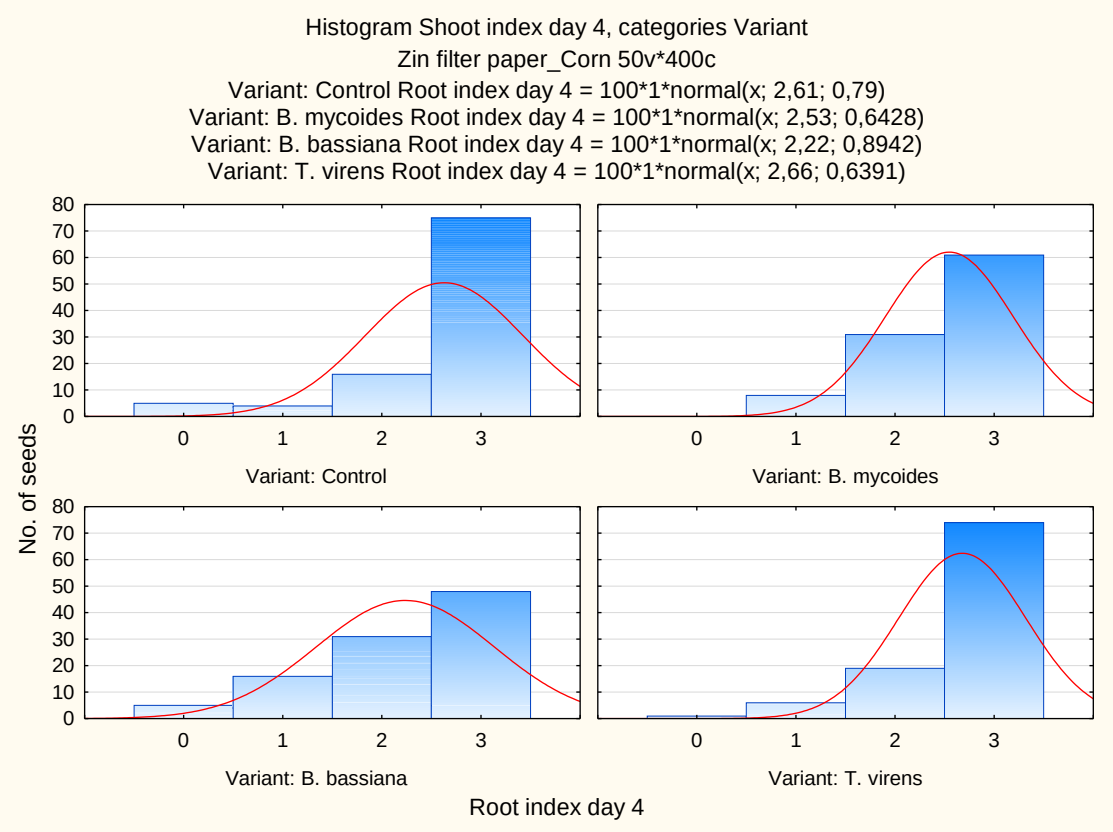


Histogram 7: Effect of treatments with microorganisms on shoot index distribution of corn on filter paper after 4 days

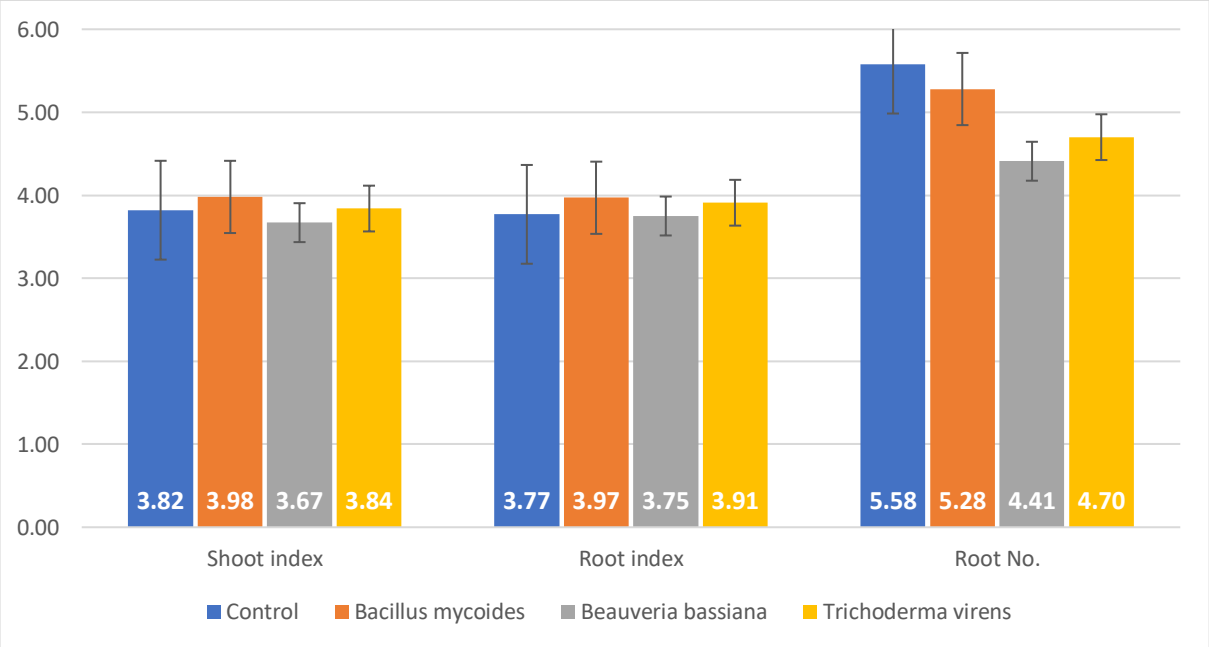


The influence of corn seed coating on the shoot development was equable in all variants after 4 day of germination except seeds which were coated by *T. virens*. In each variant, not germinated seeds were observed. In the *T. virens* variant, the index 1 was dominant. The roots were well developed in control variant and T. virens variant. Mycoparasitic fungus *T. virens* had a positive effect on the corn roots development in compared to antagonistic bacteria *B. mycoides* and entomopathogenic fungus *B. bassiana*.

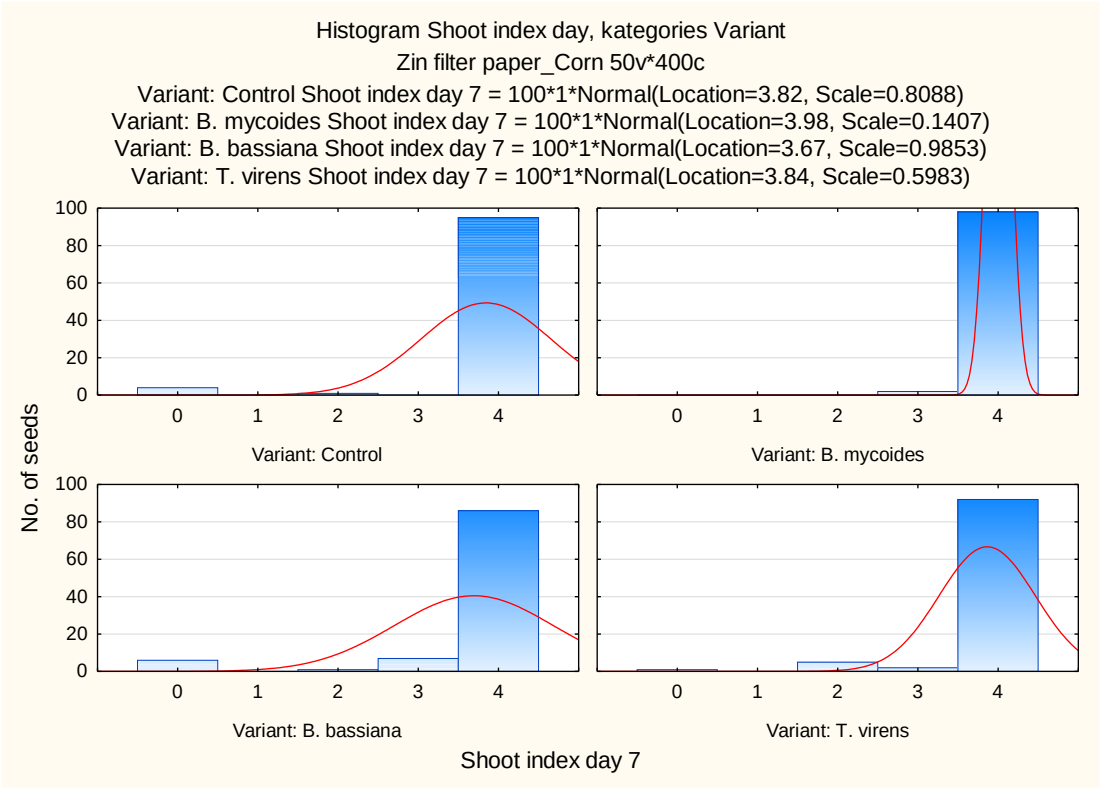
Histogram 8: Effect of treatments with microorganisms on root index distribution of corn on filter paper after 4 days



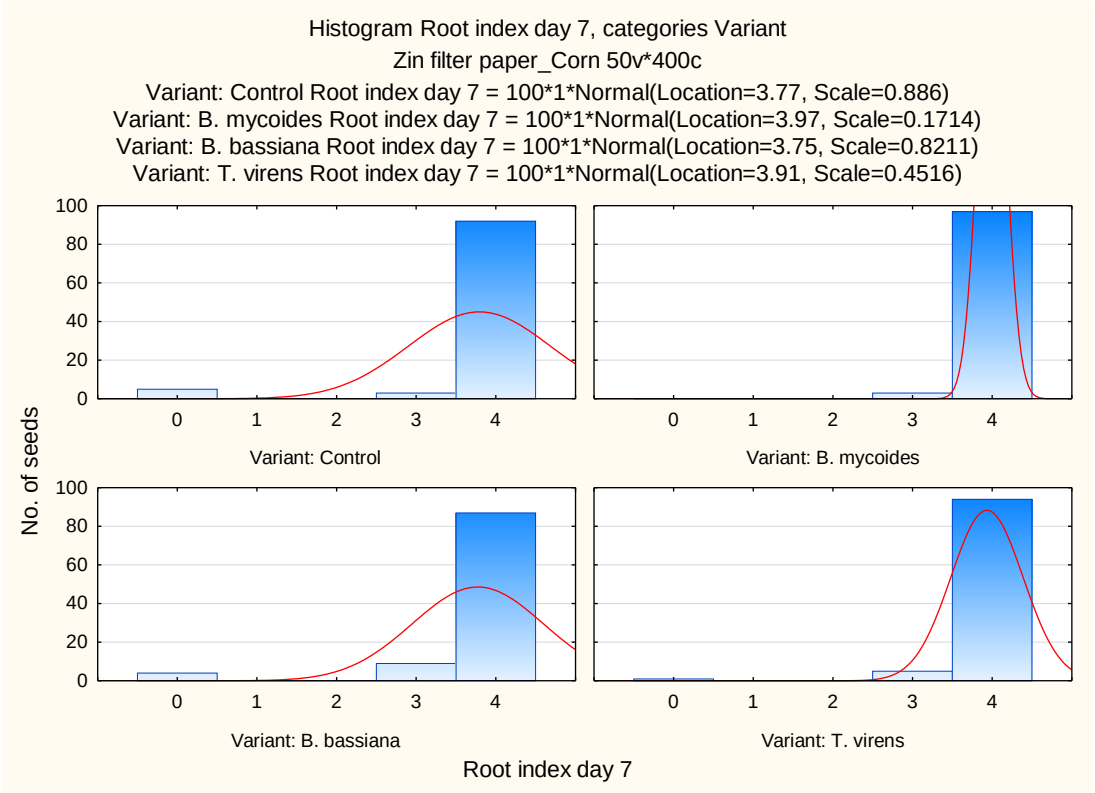
Graph 18: Effects of microorganisms on seedling root numbers, shoot and root index of corn after 7 days of sowing on filter paper



Histogram 9: Effect of treatments with microorganisms on shoot index distribution of corn on filter paper after 7 days



Histogram 10: Effect of treatments with microorganisms on root index distribution of corn on filter paper after 7 days



After 7 days, all the variants show almost the same results in the histograms. All beneficial organisms had positive effect on the shoot development and also root development of corn seedlings. The influence of beneficial organisms is almost uniform. It is evident from the histograms that index 4 was the most frequent in all variants of both determined indexes.

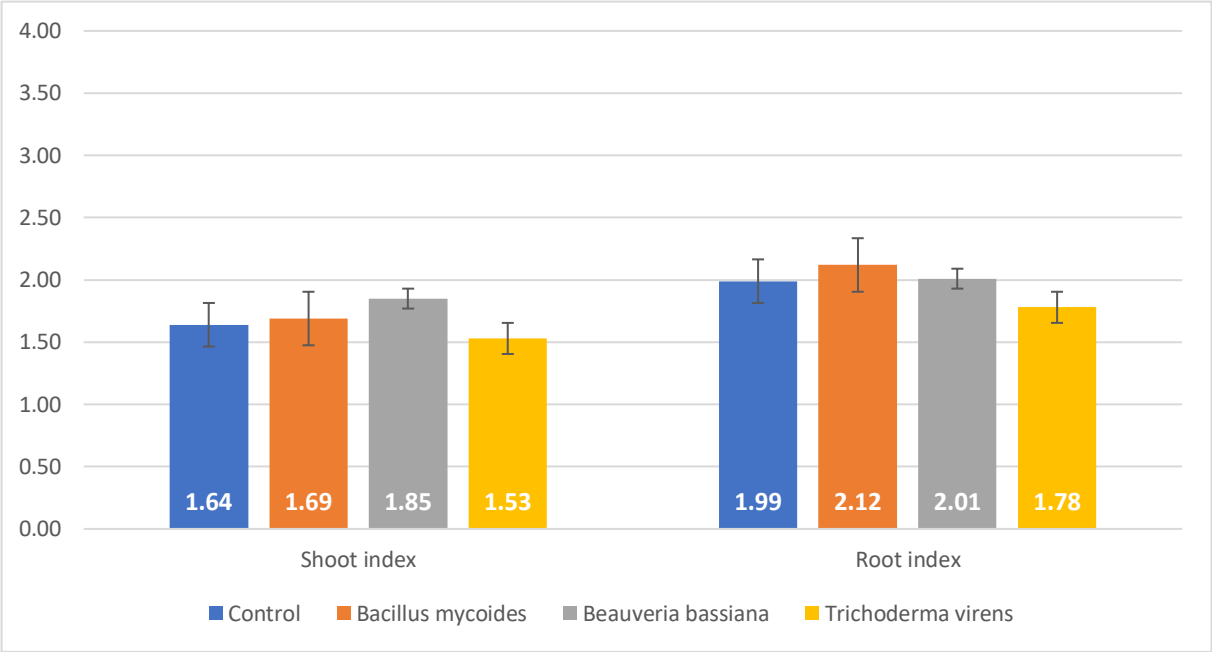
Soybean seeds

Shoot length of soybean with *B. bassiana* at 4 DAS was significantly higher than control and other microorganisms. *B. mycoides* at 4 DAS enhanced root length significantly higher over other treatments. After 7 DAS, there was no significantly differences in the shoot and root length among treatments. However, the lowest shoot and root length was obtained in *T. virens* condition.

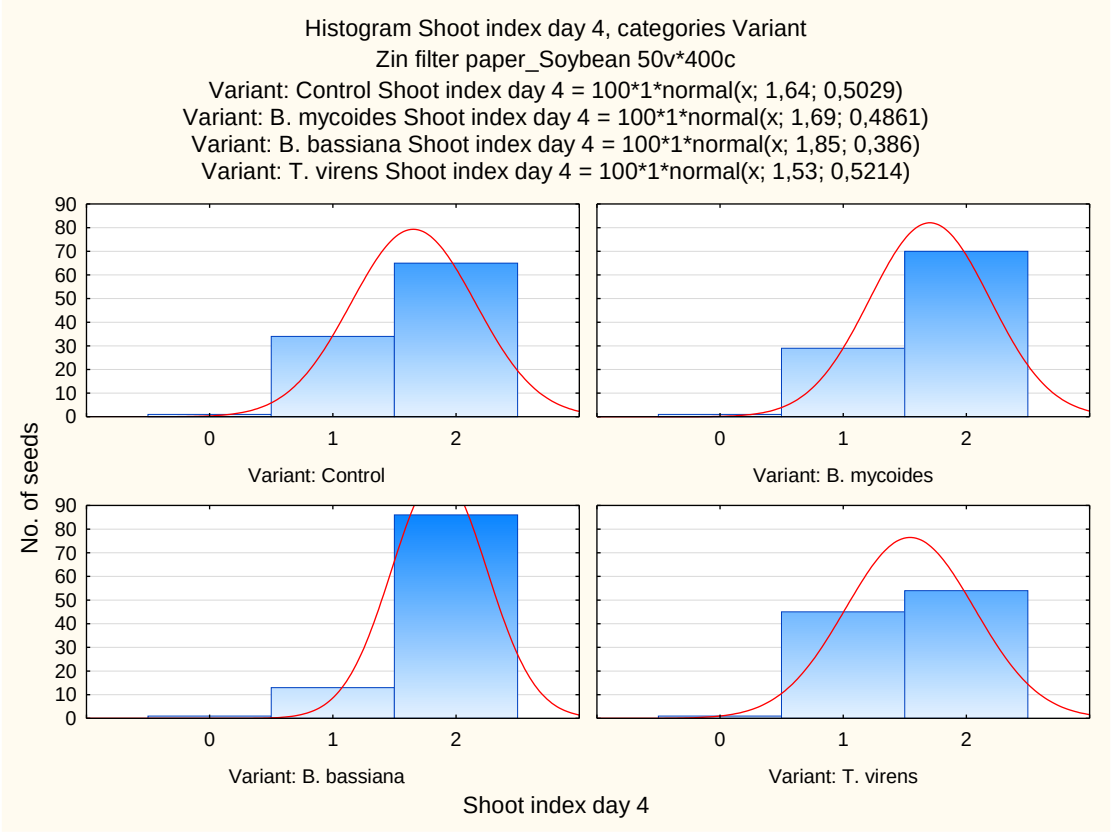
Table 9: Effect of treatments with microorganisms on seedling growth of soybean on filter paper

Treatments	Shoot index of length (Mean±SE)		Root index of length (Mean±SE)	
	Day 4	Day 7	Day 4	Day 7
Control	1.64±0.50	3.56±0.89	1.99±0.78	3.83±0.51
B. mycoides	1.69±0.48	3.75±0.55	2.12±0.77	3.81±0.52
B. bassiana	1.85±0.38	3.64±0.82	2.01±0.87	3.86±0.49
T. virens	1.53±0.53	3.50±0.84	1.78±0.69	3.75±0.43
	H (3,N= 400) =24.04606 p=0.0000	H (3,N= 400) =3.386497 p=0.3358	H (3,N= 400) =10.76559 p =0.0131	H (3,N= 400) =7.562959 p=0.0560

Graph 19: Effects of microorganisms on seedling shoot and root index of soybean after 4 days of sowing on filter paper



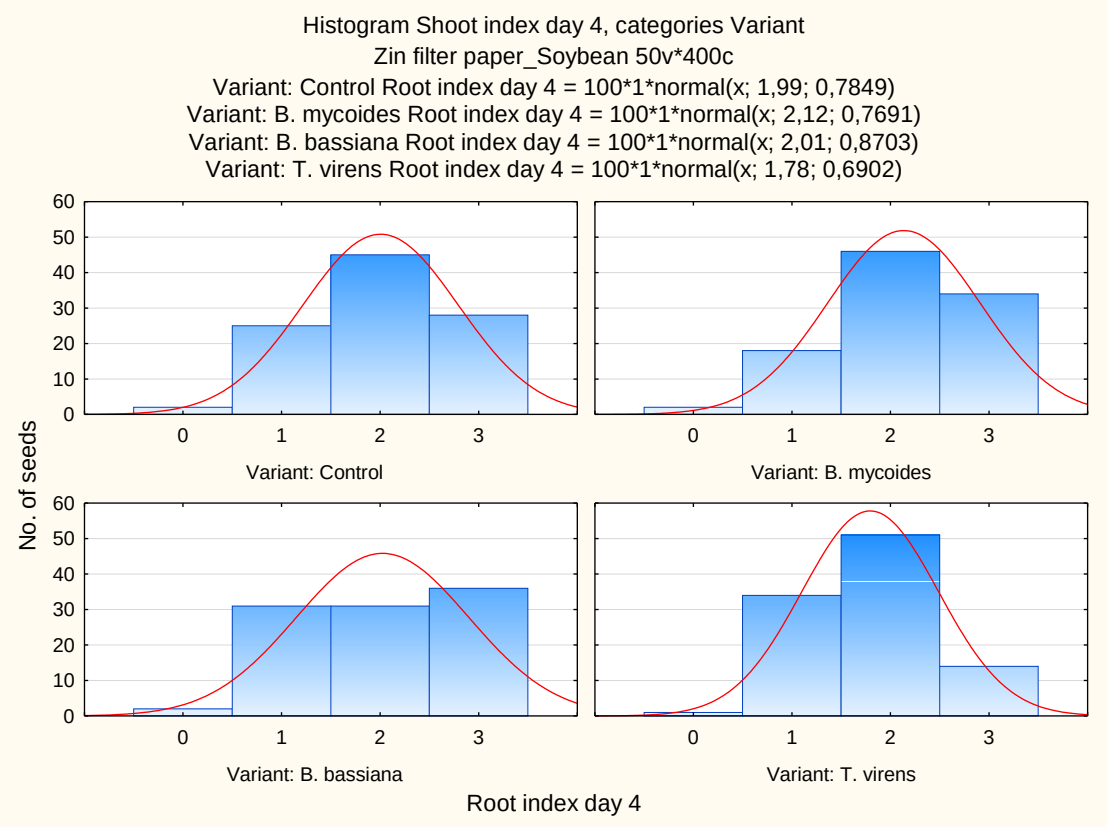
Histogram 11: Effect of treatments with microorganisms on shoot index distribution of soybean on filter paper after 4 days



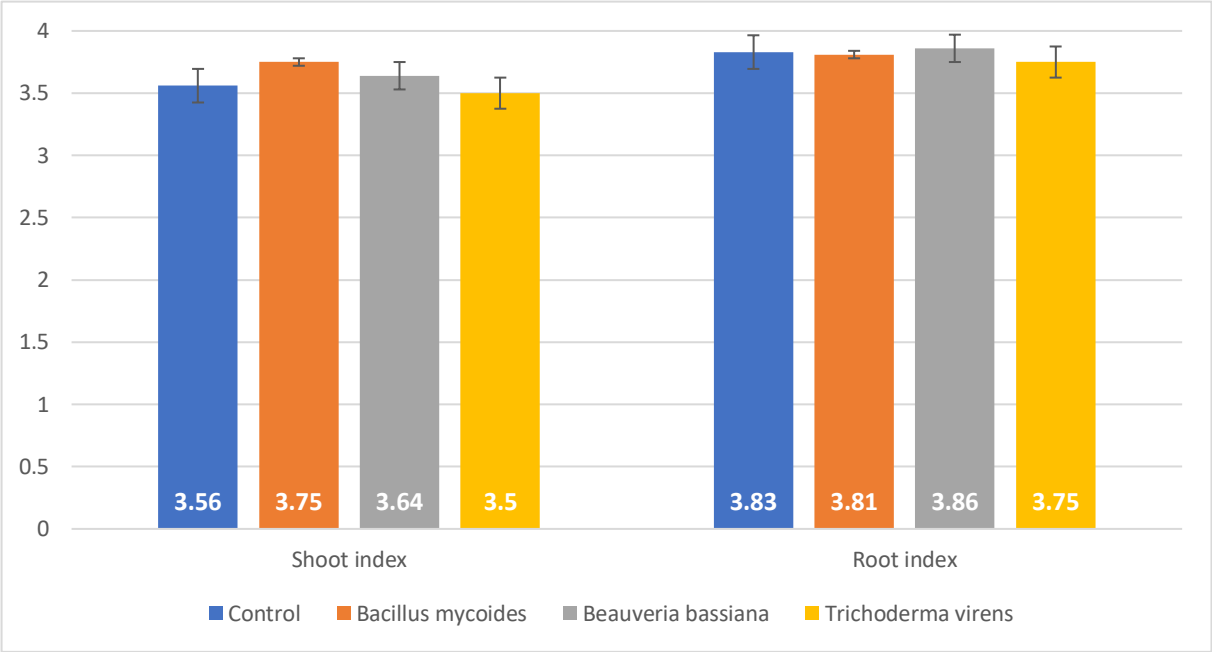
The histogram shows the frequencies of the shoot length indexes that were found during the evaluation of the tests after 4 days of seeds of soybean germination. Index 2 represents the most numerous of all indexes observed after soybean seeds germination on the filter paper. The best variant was that where the seeds were coated by entomopathogenic fungus *B. bassiana* but it was similar to variant where the seeds were coated by bycteria *B. mycoides* and control variant. The almost similar frequency of index 1 and index 2 of shoot length was observed in the variant where the soybean seeds were coated by *T. virens*.

The bacteria *B. mycoides* and entomopathogenic fungus B. bassiana postively influenced the root development of soybean after 4 days of germination. The most frequent index was 2. However, both variants had higher frequent index 3 in compare to control variant. The root development was slower in variant *T. virens*.

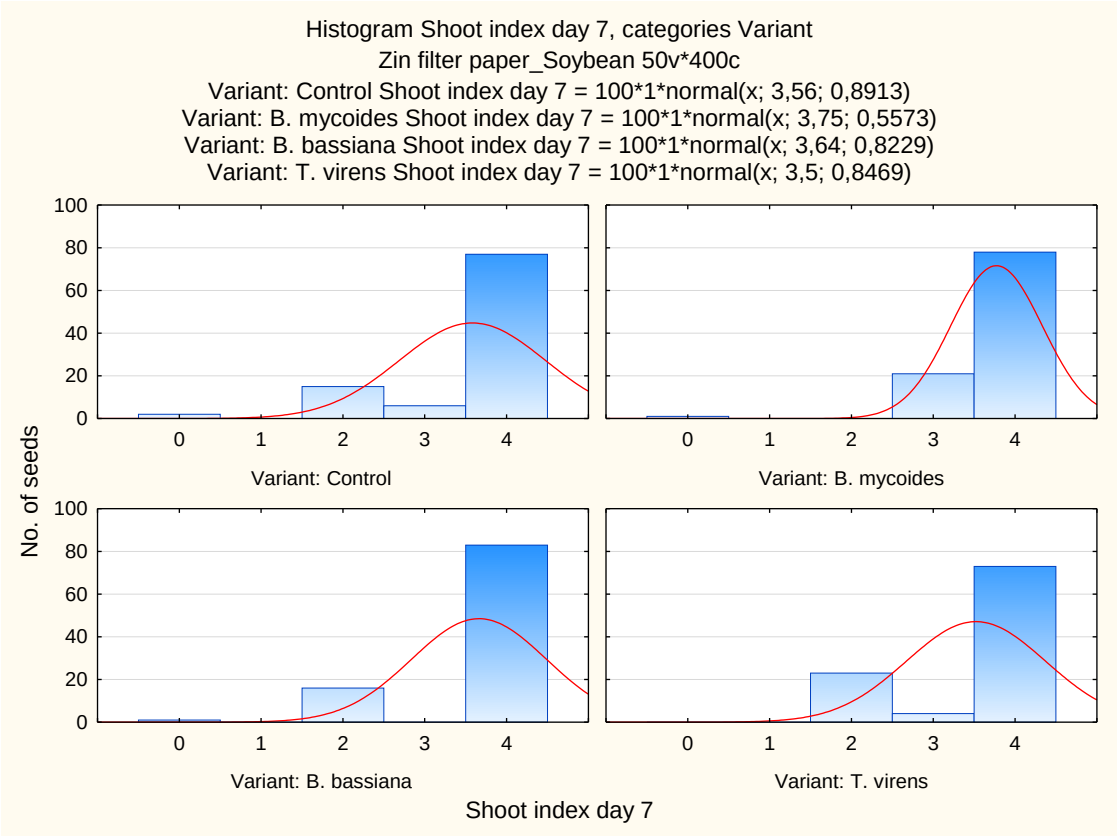
Histogram 12: Effect of treatments with microorganisms on root index distribution of soybean on filter paper after 4 days



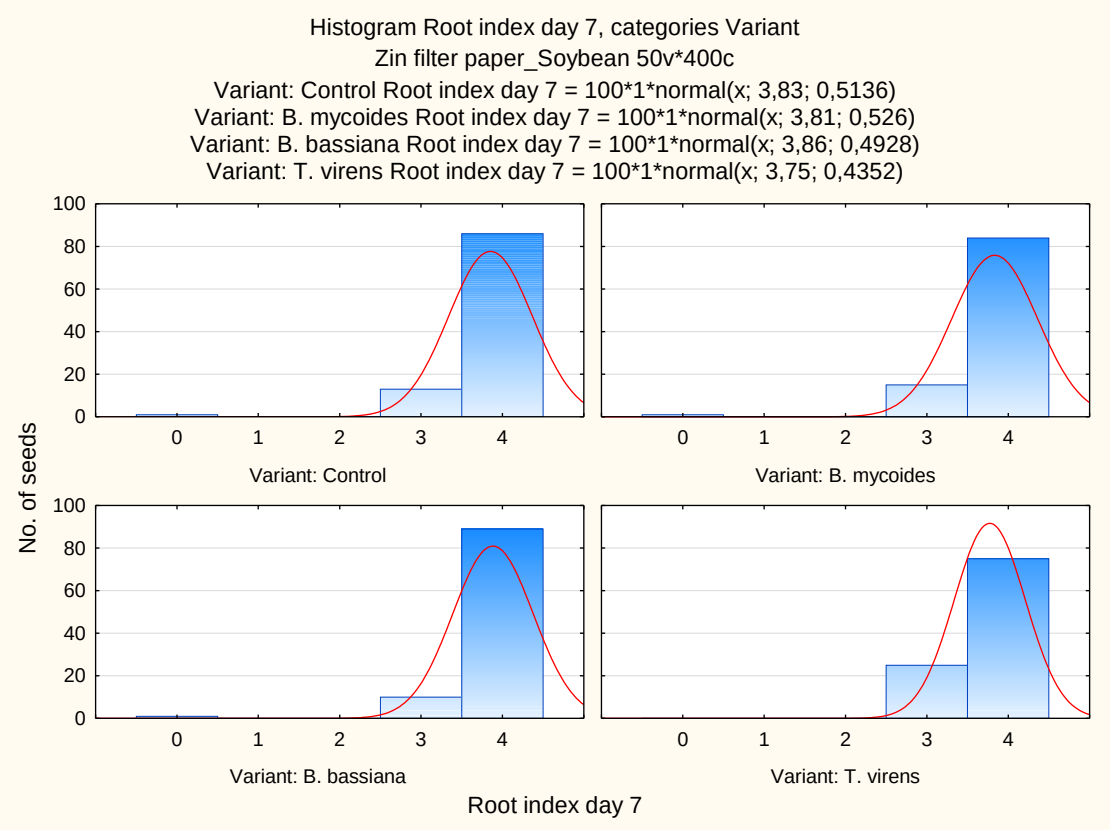
Graph 20: Effects of microorganisms on seedling shoot and root index of soybean after 7 days of sowing on filter paper



Histogram 13: Effect of treatments with microorganisms on shoot index distribution of soybean on filter paper after 7 days



Histogram 14: Effect of treatments with microorganisms on root index distribution of soybean on filter paper after 7 days



Histograms show that all the variants did not affect the sprout's development in time. The index 4 of soybean shoots is dominant in all variants after 7 days. The variant B. mycoides shown better results in shoot observation from all the beneficial microorganisms. The roots were developed well in all variants. Only the seeds treated by T. virens developed shorter roots in comparison to other variants.

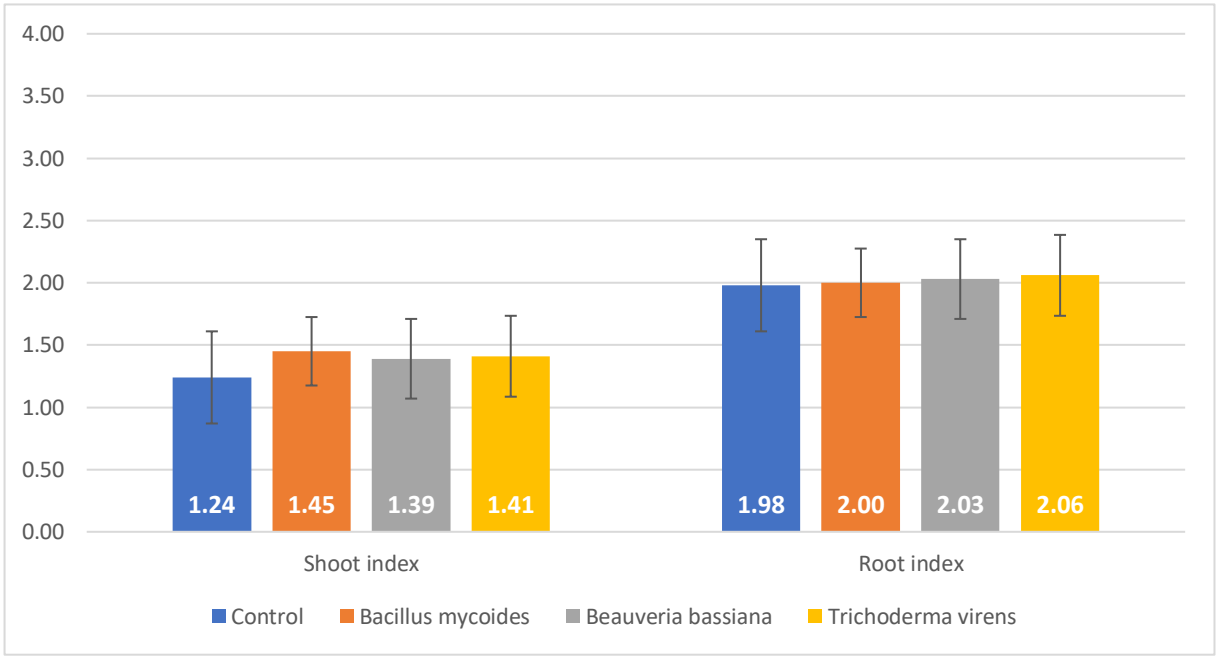
Cucumber seeds

In cucumber, significantly differences between treatments were not observed for all parameters tested. However, after 4 DAS, all treatments of microorganisms found similar in increasing shoot and root length over control.

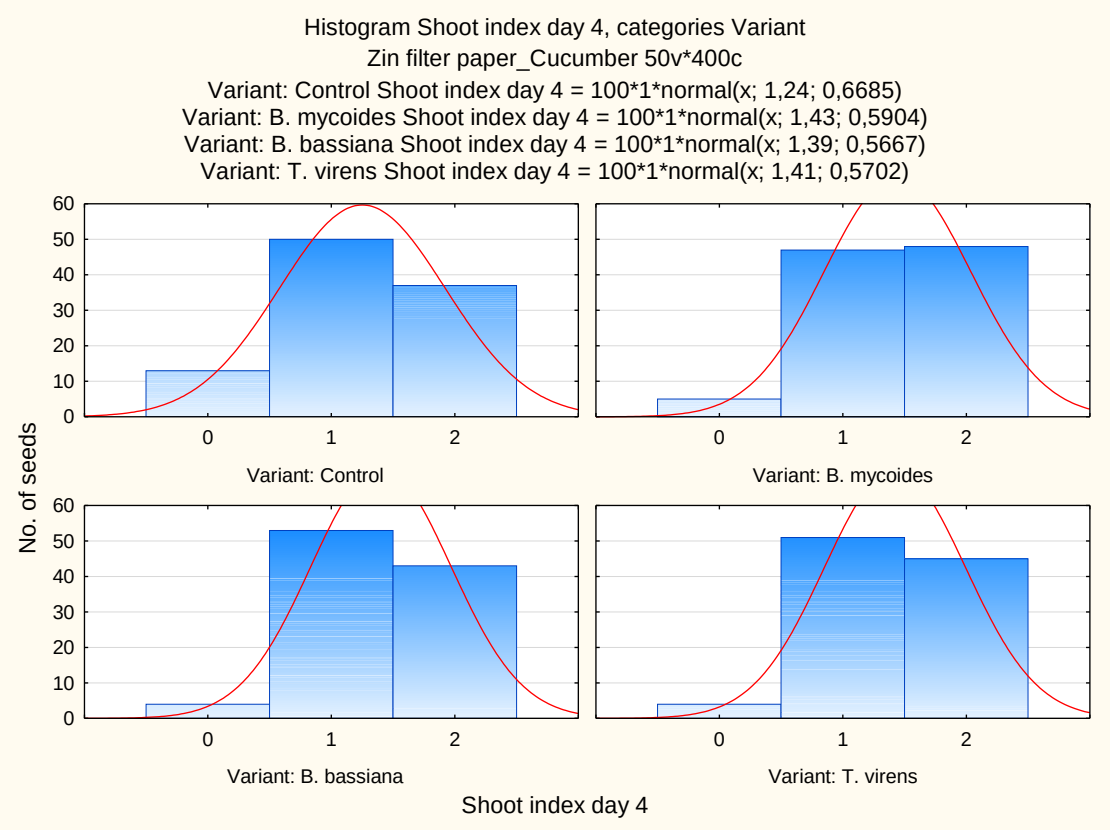
Table 10: Effect of treatments with microorganisms on seedling growth of cucumber on filter paper

Treatments	Shoot index of length (Mean±SE)		Root index of length (Mean±SE)	
	Day 4	Day 7	Day 4	Day 7
Control	1.24±0.67	3.62±1.06	1.98±1.03	3.56±1.10
B. mycoides	1.45±0.55	3.79±0.82	2.00±0.97	3.81±0.74
B. bassiana	1.39±0.56	3.78±0.72	2.03±0.93	3.72±0.87
T. virens	1.41±0.57	3.86±0.69	2.06±0.85	3.85±0.71
	H (3,N= 400) =4.897813 p=0.1794	H (3,N= 400) =6.121033 p=0.1059	H (3,N= 400) =0.0663529 p=0.9955	H (3,N= 400) =8.458583 p=0.0374

Graph 21: Effects of microorganisms on seedling shoot and root index of cucumber after 4 days of sowing on filter paper

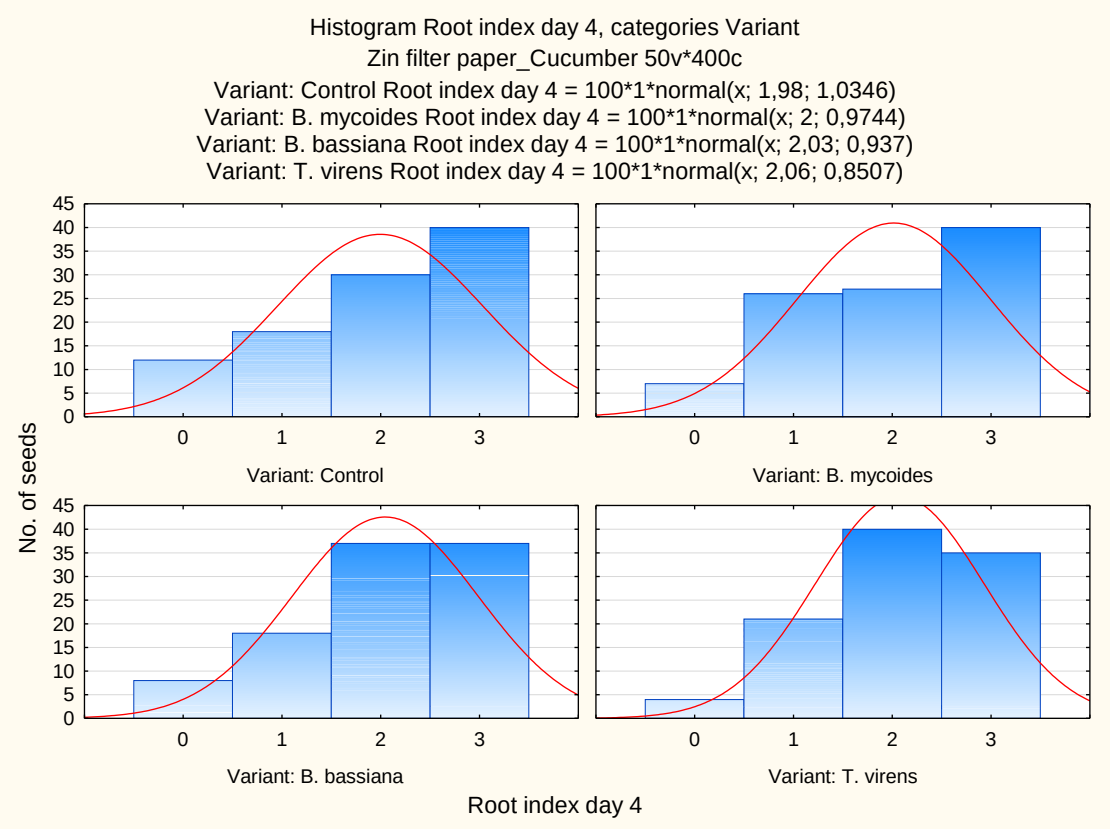


Histogram 15: Effect of treatments with microorganisms on shoot index distribution of cucumber on filter paper after 4 days

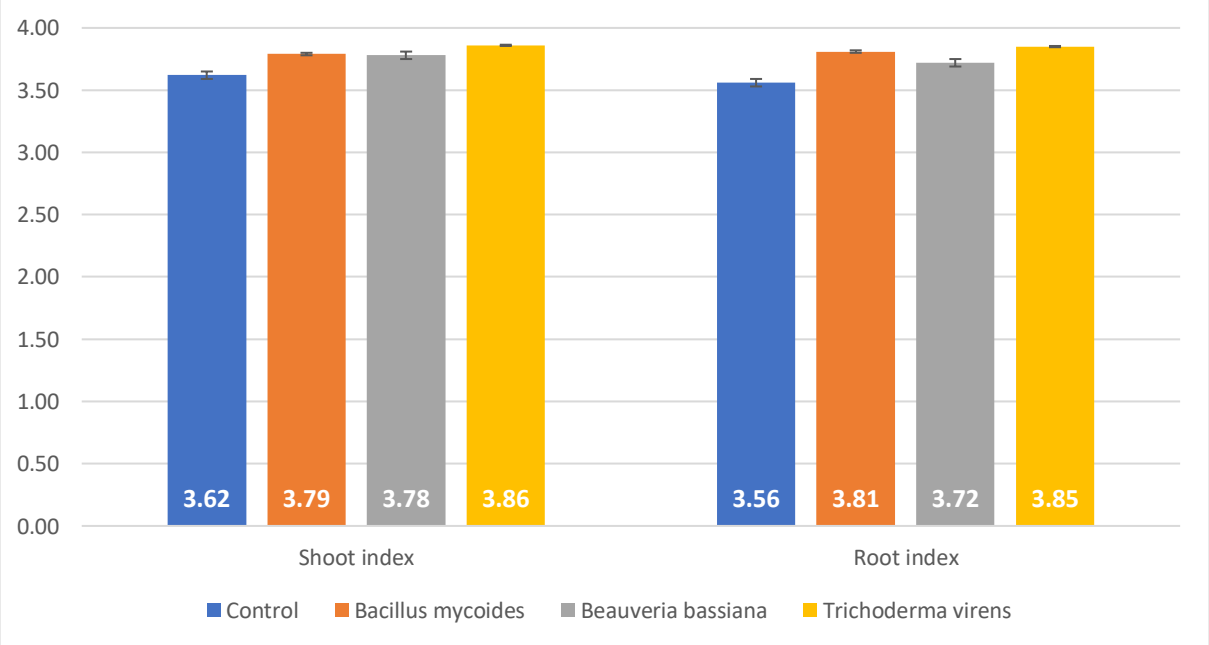


All the variants showed almost the same development of cucumber shoots after 4 days of germination on the filter paper. Antagonistic bacteria *B. mycoides* developed longer shoots than cucumber seeds in the next variants. Beneficial microorganisms have a positive effect on cucumber seedling's development. The cucumber roots development was also not affected by beneficial microorganisms. However, it seems that beneficial microorganisms a little bit retard the development of the root in comparison to the control variant. The distribution of indexes ranged from 0 to 3. The most dominant indexes were 2 and 3. From all beneficial microorganisms, the seeds coated by myco-parasitic fungus *T. virens* developed shorter roots.

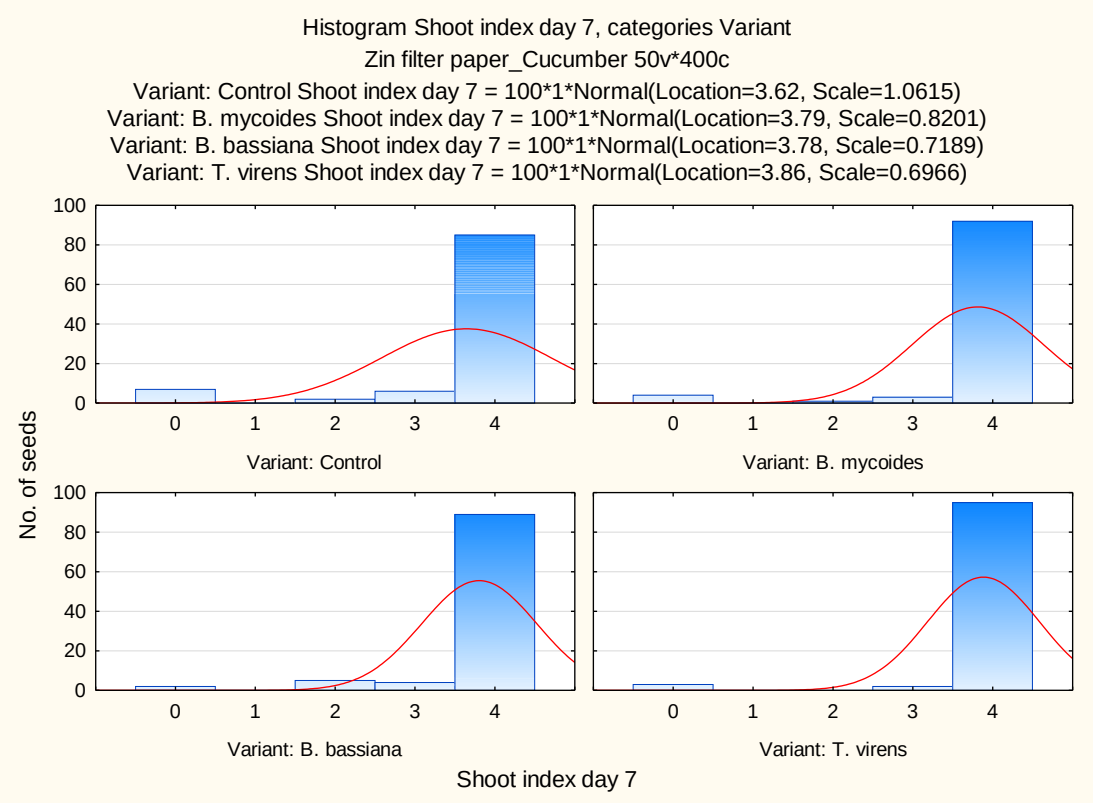
Histogram 16: Effect of treatments with microorganisms on root index distribution of cucumber on filter paper after 4 days



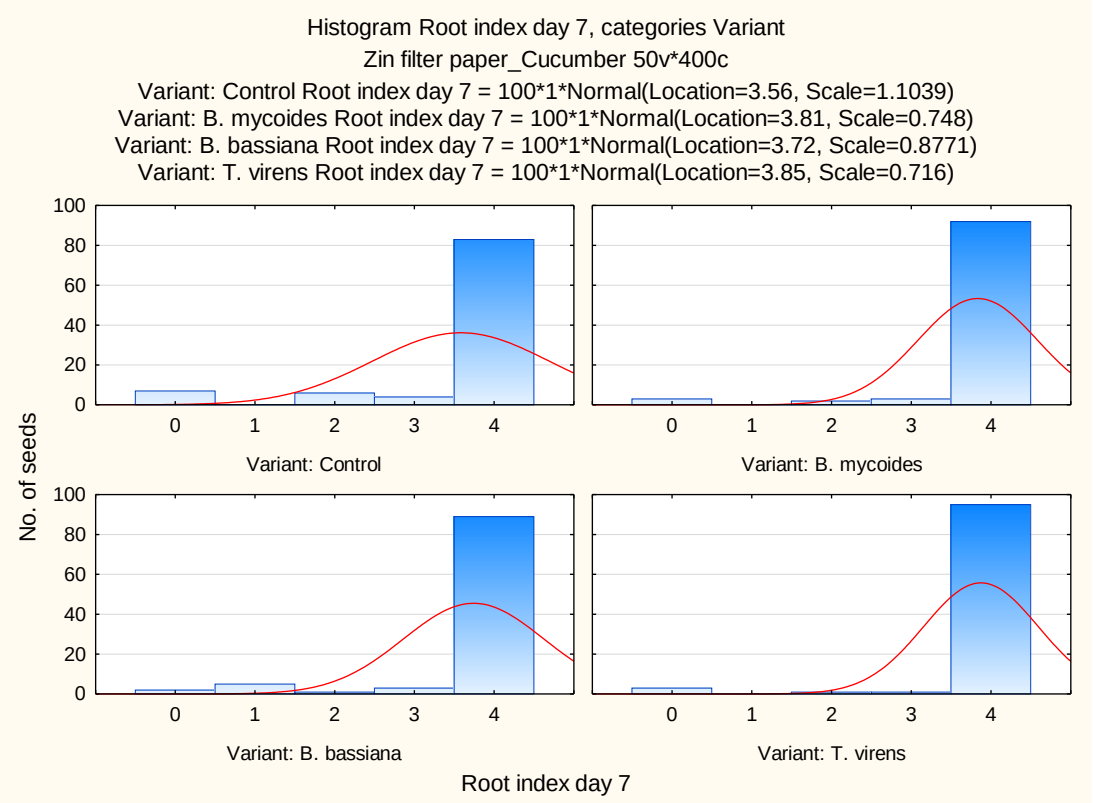
Graph 22: Effects of microorganisms on seedling shoot and root index of cucumber after 7 days of sowing on filter paper



Histogram 17: Effect of treatments with microorganisms on shoot index distribution of cucumber on filter paper after 7 days



Histogram 18: Effect of treatments with microorganisms on root index distribution of cucumber on filter paper after 7 days



After 7 days, all the variants show almost the same results. The beneficial microorganisms support cucumber seedlings development. The shoot length index 4 is dominant in all variants, the better results are observed in the variant where the cucumber seeds were treated by mycoparasitic fungus *T. virens*. The shoots and also the roots were supported by this beneficial fungus. The shorter shoots were observed in the control variant but the differences among variant are not statistically significant. The week significant differences are observed in root indexes (α=0.05).

4.4 Re-isolation of beneficial microorganisms from the soil substrate where the seedlings were growing

Table 11: Parasitic and saprotrophic abilities of beneficial microorganism

		TBM* infect./dead/alive	Dead beetles**	Alginate pellets**
Control	Corn	0/6/4	0/9	1/9
	Soybean	0/5/5	0/9	1/9
	Cucumber	n/a	n/a	n/a
Bacillus mycoides	Corn	0/4/6	0/10	2/8
	Soybean	0/1/9	1/9	2/8
	Cucumber	1/3/6	3/9	3/7
Beauveria bassiana	Corn	4/6/0	3/7	5/5
	Soybean	6/4/0	7/3	4/6
	Cucumber	4/6/0	5/5	3/7
Trichoderma virens	Corn	-	3/7	8/2
	Soybean	-	4/6	6/4
	Cucumber	-	6/4	5/5

*Tenebrio bait method – parasitic effect of microorganisms (infected/dead/alive)

** saphrothrophic effect (overgrowering/non-overgrowering)

The beneficial microorganisms transferred with seeds into the soil substrate were re-isolated after the end of the experiment. The coated seeds of all variants were sown into the sterile soil substrates. The seedling germination was observed in the previous experiment and after 1 month of growing, the plants were cut and the larvae of *T. molitor* were transferred to the variants where the seeds were coated by entomopathogenic fungus *B. bassiana* and also to the variant where the seeds were coated by *B.*

mycoides. The parasitic and saprotrophic effect of entomopathogenic fungus was observed. Alive larvae were not observed. Fungus *B. bassiana* infected the larvae in all the variants. The best variant was that one where the soybean seeds were coated.

Dead beetles were put into all variants. Dead beetles can show the saprotrophic effect of all microorganisms. The best saprotrophic effect showed the beneficial fungi, entomopathogenic fungus *B. bassiana*, and also mycoparasitic fungus *T. virens*. The alginate pellets are also substrate which is used for the re-isolation of beneficial microorganisms from the soil. Some alginate pellets were mostly overgrown by fungus *B. bassiana* and *T. virens*. Also, bacteria *B. mycoides* was caught by alginate pellets from the soil.

5 DISCUSSION

This study showed the effect of biological seed coating by using beneficial microorganisms in selected crops: corn (*Zea mays* L.), soybean (*Glycine max* L.), and cucumber (*Cucumis sativus* L.). The reason for choosing these crops is that corn is the second most crucial cereal after rice, soybean is a major grain legume crop, and cucumber is an important vegetable belonging to the family Cucurbitaceae in Myanmar. However, the yield of Myanmar is only about one-third of the world average yield (USDA 2018) because of insufficient agricultural inputs and problems with pests and diseases. For biological seed coating, two types of filamented fungi (*Beauveria bassiana* and *Trichoderma virens*) and one antagonistic bacterium (*Bacillus mycoides*) were used.

The results of the diploma thesis experiments showed that beneficial microorganisms when introduced through seed coating, have a positive effect on the growth of plants, and the germination of plants in all selected crops. Biological seed coating with microorganisms is potentially the most effective method of integrated pest management and it is also suitable for use instead of chemicals. Moonjely et al., 2016 found that biological seed coating of entomopathogenic fungi has the potential to increase the biological control of soil-dwelling pests or plant pathogens that cannot be easily controlled by chemical insecticides. *B. bassiana* is one of the entomopathogenic fungus (EPF) that was used due to its ability to cause insect diseases on most of the common pests and plant pathogen. Sandhu et al. 2012 found that *B. bassiana* play as a pathogen to several insects including hosts of agricultural distinction such as the Colorado potato beetle and codling moth. Furthermore, *B. bassiana* has been experimentally proved to suppress damping-off caused by the soil-borne pathogens *Rhizoctonia solani* and *Pythium myriotylum* in tomato (Ownley et al. 2004) and bacterial blight caused by *Xanthomonas axonopodis pv. Malvacearum* in cotton (Ownley et al. 2008), the Zucchini yellow mosaic virus in squash (Jaber and Salem 2014), and downy mildew caused by *Plasmopara viticola* in grapevines (Jaber 2015).

The second fungus was the mycoparasitic fungus *T. virens* that can parasite on the plant pathogens which occurred around the seed in many types of soils that suppresses plant pathogens such as *Rhizoctonia solani*, *Fusarium spp.*, *Sclerotinia spp*., *Pythium spp.*, *Verticillium spp.*, and *Botrytis spp*. (Howell et al., 2000; Howell, 2002,

Viterbo et al., 2007). On the other hand, *B. mycoides* as a representative of antagonistic microorganisms, have beneficial plant growth and biocontrol activity in various plants, including sugar beet, cucumber, and sunflower (Bargabus et al., 2002, Neher et al., 2009, Ambrosini et al., 2016). Moreover, *B. mycoides* isolates BmJ is capable to reduce the severity of several important diseases such as the bacterial vascular necrosis of sugar beet, angular leaf spot of cucumber, early blight (*Alternaria solani*) of potato and bacterial spot of both pepper and tomato in both glasshouse and field experiments (Bargabus et al., 2003, Zietlow et al., 2004, Jacobsen et al., 2007). Therefore, this bacteria strain BmJ may provide control of other cucurbit diseases. For all the above reasons, the use of biocontrol in agriculture has grown continuously, especially biocontrol of filamentous fungi such as *B. bassiana, T. virens* and mycoparasitic bacterium *B. mycoides.*

In the present study, we used CMC as an adhesive binder in seed coating to aim for effective colonization of seeds, to maintain the viability and perhaps the virulence of spores of microorganisms. Seed coating with conidia of entomopathogenic fungi can be efficiently delivered to the rhizosphere of seedlings through seed coating because, after conidia germination, the developing hyphae would survive on the ooze produced by the plant roots and at the same time provide the plant with nutrients and protect against pests and diseases (Bruck, 2005; Ownley, Gwinn, & Vega, 2010).

Part of the diploma thesis was the evaluation of the number of spores attached to the seeds which are an important value to recognize how many spores can be spread into the soil after sowing. The spores spread into the soil can protect the seedlings against pests or diseases. The number of spores per seed was recorded that coating with *B.bassiana* produces more spores than *T. virens* in all selected crops (Table 1). The present results were per the findings of Petlamul et al. (2017) who reported that *B. bassiana* had the efficacy to release cellulolytic enzymes on CMC for cellulose reduction to carbon source led to their growth. The seeds of corn and soybean had significant differences in the attachment of spores to 1 seed compared to cucumber seeds. This is logical, since the seeds of these crops have a different morphological structure, and smaller size, therefore not as many spores have been caught on the seed surface of cucumber as on corn and soybean seeds. However, no different number of spores were observed between cucumber and soybean in the variant of *T.virens*. The reason can be,

that the spores from soybean seed were not well eluted. During the soybean seed coating process, the seed coat layer getting separated from the embryo.

The microorganism isolates used in this study had no impact on seed germination percentage in all treatments including controls. Nevertheless, the germination percentage of seed coating with microorganism in the present study did not show many differences under laboratory conditions. Jaber and Enkerli (2016) observed similar results that neither *B. bassiana* nor *M. brunneum* was affected on broad bean (*Vicia faba*) seed germination. However, Russo et al. (2019) reported that seed treatment with *B. bassiana* increased the germination of corn (*Zea mays)* seeds. Additionally, *B. bassiana* and methylcellulose do not have inhibitory effects on corn seed germination in laboratory condition (Kuzhuppillymyal et al., 2020).

Plant growth parameters such as primary root length, shoot height and root numbers were determined after 4 days and 7 days of sowing. There were some interactions between microorganisms isolates and seedling age for the measured plant growth parameters. So far, much research has been done on the microbial seed coating with vegetables (such as onion, beet, artichoke, carrot, tomato, and cucumber), cereals (such as Sorghum, wheat, and corn), oilseeds (such as rapeseed, and Sesame) and legumes (such as soybean and cowpea). Most of these research papers often reported their positive effects on seed germination, nodulation (in legumes), plant growth and yield after seed coating with microorganisms to crops (Ying, 2019).

In this study, the microorganism variants through the seed coat from tested soil were re-isolated by using larvae of *T. molitor,* dead beetles and alginate pellets. The parasitic and saprotrophic effect of all microorganisms was observed. This result was per the findings of Torres et al. (2009) who reported that entomopathogenic fungus *B. bassiana* infects a wide range of insects and they are also able to survive as saprophytes in the soil.

On the other hand, agriculture is the major economic sector of Myanmar, responsible for approx. 36% of the national GDP and food security for the people are dependent on the agricultural sector. At the same time, Peeters et al., (2015) reported that Myanmar lacks an effective and fully operational system for pesticide regulation and control and support to farmers regarding best practices in sustainable pest management and

pesticide use. The present study can contribute to sustainable agricultural production in Myanmar through improving plant health, promote food safety and reducing risks of pesticide use, enhancing the economic performance of the agricultural sector and protect the health of growers, the surrounding community and consumers as well. For all the above reasons, Myanmar's agriculture is a strong incentive to find alternative strategies to replace the use of chemical fertilizers and pesticides. The present study encourages reducing the utilization of chemical pesticides in Myanmar.

6 CONCLUSIONS

The germination and the growth parameters of the corn seed was positively influenced by the combination of carboxymethylcellulose (CMC) with conidia of the antagonistic bacterium *B. mycoides* and entomopathogenic fungus *B. bassiana*. Seed coating of mycoparasitic fungus *T. virens* strain GL-21 with the CMC adhesive has been found slowly on the growth of shoot and root length, however *T. virens* had the highest number of roots on petri dish.

Spores of *B. mycoides* and *B. bassiana* have synergistic action with the CMC adhesive, also provided positive stimulation of the seed germination and plant growth for soybean seed. Nevertheless, seed coating of *T. virens* resulted the negative effect on soybean seed germination and on growth parameters comparable to the effects of *B. bassiana* and *B. mycoides* in all substrates.

On the cucumber seed of the selected variety Stela F1 has the positive effect of the *B. bassiana* was clearly visible, especially after 7 days of germination. The combination of this CMC with the *T. virens* fungus gave also positive effect on the length of shoots and roots on filter paper.

As mentioned in the discussion, many scientific studies had already confirmed that these fungi and bacterium have the positive effect on plant growth and germination. Moreover, the findings conclude that CMC could be used in seed coating of microorganisms which serve as a source of nutrients for germinating spores and helps to enhance its shelf life. This study is considered as a preliminary research to the utilization of beneficial microorganisms especially entomopathogenic fungus *B. bassiana*, antagonistic bacterium *B. mycoides* and mycoparasitic fungus *T. virens* as biological seed coating in plants to protect from insect pest and pathogens.

The number of spores per seed indicated that helps to understand how many spores can be entered by means of seeds into the soil during sowing. The highest number of spores of the fungi *B. bassiana* and *T. virens* were observed on corn seeds, average $2.33x10^3$ and $1.10\ x10^3$ spores per 1 seed, respectively. Based on the results of this work, it is possible to recommend reducing the dose of suspension by half, however, the solution should contain a higher concentration of spores.

This study confirms that coating of selected seeds with microorganisms are suitable to either control plant disease and insect pests or promote seed germination and plant growth. Additionally, this has led to a steadily increasing interest in biological control for pests and diseases. The results may bring a new perspective on the use of beneficial microorganisms as seed coating agents, may be a promising plant protection strategy in Myanmar. Moreover, field research is needed to determine how to enhance seed germination and plant growth parameters in field condition.

7 REFERENCES

Acha A. J., Vieira H. D., Freitas M. S. M. (2016) Perennial soybean seeds coated with high doses of boron and zinc. African *Journal of Biotechnology*, **15**(37), 1998-2005.

Adak S., Banerjee R. (2016) A green approach for starch modification: Esterification by lipase and novel imidazolium surfactant. *Carbohydrate Polymers*, **150**: 359-368.

Adhikari T., Kundu S., Rao A. S. (2016) Zinc delivery to plants through seed coating with nano-zinc oxide particles. *Journal of Plant Nutrition*, **39**(1): 136-146.

Aeron A., Dubey R.C., Maheshwari D.K., Pandey P., Bajpai V.K., Kang S.C. (2011) Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and bio-control of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L.) *Eur. J. Plant Pathol.* **131** (1):81-93

Ahmed E. A., Hassan E. A., El Tobgy K. M. K., Ramadan E. M. (2014) Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. *Annals of Agricultural Sciences*, **59**(2): 273-280.

Aluko M. O., Hering T. F. (1970) The mechanisms associated with the antagonistic relationship between *Corticium solani* and *Gliocladium virens*. *Transactions of the British Mycological Society*, **55**(2): 173-179.

Ambrosini A., Stefanski T., Lisboa B. B., Beneduzi A., Vargas L. K., Passaglia L. M. P. (2016) Diazotrophic bacilli isolated from the sunflower rhizosphere and the potential of *Bacillus mycoides* B38V as biofertiliser. *Annals of Applied Biology*, **168**(1): 93-110.

Amirkhani M., Mayton H. S., Netravali A. N., Taylor A. G. (2019) A seed coating delivery system for bio-based biostimulants to enhance plant growth. *Sustainability*, **11**(19): 5304.

Amirkhani M., Netravali A. N., Huang W., Taylor A. G. (2016) Investigation of soy protein–based biostimulant seed coating for broccoli seedling and plant growth enhancement. *HortScience*, **51**(9): 1121-1126.

Anderson D. (2014) Talc and Graphite: What You Need to Know before you Plant Machinery. AgWeb J.

Anonymous 1 [cit.2016-03-4] Dostupné z: http://www.paleo-doupe.eu/sortiment/xantanova-guma-100-g

Anonymous 2 [cit. 2016- 03- 4] Dostupné z: http://www.wolfberry.eu/produkt/xanta-nova-guma-100-g/318/

Anonymous 3 [cit. 2016- 03- 4] Dostupné z: http://www.zdravapotravina.cz/seznam-ecek/E412

Anonymous 4 [cit.2016-03-4]Dostupné z:http://ecka.pedie.cz/wiki/E412 (Guarov%C3%A1_guma)

Anthony P., Malzer G., Sparrow S., Zhang M. (2012) Soybean yield and quality in relation to soil properties. *Agronomy Journal*, ***104***(5), 1443-1458.

Badua S. A., Sharda A., Strasser R., Cockerline K., Ciampitti I. A. (2019) Comparison of soy protein based and commercially available seed lubricants for seed flowability in row crop planters. *Applied Engineering in Agriculture*, ***35***(4): 593-600.

Baek J. M., Howell C. R., Kenerley C. M. (1999). The role of extracellular chitinase from *Trichoderma virens* Gv29-8 in the biocontrol of *Rhizoctonia solani*. Curr. *Genet*. **35**:41-50. 3.

Bailey A., Chandler D., Grant W.P., Greaves J., Prince G., Tatchell M. (2010): Biopesticides: pest management and regulation. *CAB International, Wallingford*, UK, 88-90.

Bargabus R. L., Zidack N. K., Sherwood J. E., Jacobsen B. J. (2002) Characterisation of systemic resistance in sugar beet elicited by a non-pathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. *Physiological and molecular plant pathology*, **61**(5): 289-298.

Bargabus R. L., Zidack N. K., Sherwood J. E., Jacobsen, B. J. (2003) Oxidative burst elicited by *Bacillus mycoides* isolate Bac J, a biological control agent, occurs independently of hypersensitive cell death in sugar beet. *Molecular plant-microbe interactions*, **16**(12): 1145-1153.

Barnett H.L. (1963) The nature of mycoparasitism by Fungi. Annu. *Rev. Microbiol*, **17**: 1-14.

Bashan Y., Hernandez J. P., Leyva L. A., Bacilio M. (2002) Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. *Biology and Fertility of Soils*, **35**(5): 359-368.

Benizri E., Dedourge O., Dibattista-Leboeuf C., Piutti S., Nguyen, C., Guckert A. (2002) Effect of maize rhizodeposits on soil microbial community structure. *Applied Soil Ecology*, **21**(3): 261-265.

Ben-Jabeur M., Kthiri Z., Harbaoui K., Belguesmi K., Serret M. D., Araus J. L., Hamada, W. (2019) Seed coating with thyme essential oil or Paraburkholderia phytofirmans PsJN strain: Conferring Septoria leaf blotch resistance and promotion of yield and grain isotopic composition in wheat. *Agronomy*, **9**(10): 586.

Bennett A. J., Mead A., Whipps J. M. (2009) Performance of carrot and onion seed primed with beneficial microorganisms in glasshouse and field trials. *Biological Control*, **51**(3): 417-426.

Berg G. (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Appl Microbiol Biotechnol*, **84**:11–18.

Berninger T., Mitter B., Preininger C. (2016) The smaller, the better? The size effect of alginate beads carrying plant growth-promoting bacteria for seed coating. *Journal of microencapsulation*, **33**(2): 127-136.

Bing L., Lewis L.C. (1991) Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) *Vuill*. *Environmental Entomology*, **20**:1207–1211.

Bing L.A., Lewis L.C. (1992) Endophytic *Beauveria bassiana* (Balsamo) *Vuillemin* in corn: the influence of the plant growth stage and *Ostrinia nubilalis* (Hübner). *Biocontrol Sci Technol*, **2**:39–47.

Boosalis M.G. (1964) Hyperparasitism. Annu. *Rev. Phytopathol*, **2**: 363-376.

Bruck D. J. (2005) Ecology of Metarhizium anisopliae in soilless potting media and the rhizosphere: implications for pest management. *Biological control*, **32**(1): 155-163.

Bruck D.J., (2010) Fungal entomopathogens in the rhizosphere. *BioControl*, **55**(1).

Butt T. M., Copping L. G. (2000) Fungal biological control agents. *Pesticide Outlook*, **11**(5): 186-191.

Chelkowski J. (1998) Distribution of *Fusarium* species and their mycotoxins in cereal grains. In: Sinha, K.K., and Bhatnagar, D. Mycotoxins in agriculture and food safety. *CRC Press*, 44-64.

Chet I., Benhamou N., Haran S. (1998) Mycoparasitism and lytic enzymes. In: Harman GE, Kubicek CP (eds) *Trichoderma* and *Gliocladium*, vol **2**. Enzymes, biological control and com-mercial applications. Taylor and Francis London, 153-171

Chookhongkha N., Sopondilok T., Photchanachai S. (2012) Effect of chitosan and chitosan nanoparticles on fungal growth and chilli seed quality. In International

Conference on Postharvest Pest and Disease Management in Exporting Horticultural Crops, **973**: 231-237.

Dash C. K., Bamisile B. S., Keppanan R., Qasim M., Lin Y., Islam S. U., Wang L. (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**: 385-392.

De Bach P. (1964) Biological control of insect pests and weeds. Biological control of insect pests and weeds.

de Camargo F. R. T., Silva I. L., Barros P. J. R., Ascheri D. P. R., Rodovalho R. S., Bellizzi N. C., de Campos A. J. (2017) Physiological quality of soybean seeds treated with carboxymethyl cellulose and fungicide. *American Journal of Plant Sciences*, **8**(11): 2748.

De Gregorio P. R., Michavila G., Ricciardi Muller L., de Souza Borges C., Pomares M. F., Saccol de Sá E. L., Vincent P. A. (2017) Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. *PloS one*, **12**(5): 0176930.

Díaz K., Valiente C., Martínez M., Castillo M., Sanfuentes E. (2009) Root-promoting rhizobacteria in *Eucalyptus globulus* cuttings. *World Journal of Microbiology and Biotechnology*, **25**(5): 867-873.

Ehsanfar S., Modarres-Sanavy S. A. (2005) Crop protection by seed coating. *Communications in Agricultural and Applied Biological Sciences*, **70**(3): 225-229.

Elzein A., Heller A., Ndambi B., De Mol M., Kroschel J., Cadisch G. (2010) Cytological investigations on colonization of sorghum roots by the mycoherbicide *Fusarium oxysporum* f. sp. strigae and its implications for Striga control using a seed treatment delivery system. *Biological Control*, **53**(3): 249-257.

Essghaier B., Fardeau M.L., Cayol J.L., Hajlaoui M.R., Boudabous A., Jijakli H., Sadfi-Zouaoui N. (2009) Biological control of grey mould in strawberry fruits by halophilic bacteria.

Etesami H., Maheshwari D. K. (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and environmental safety*, **156**: 225-246.

Fikr J. a J. Kahovec. Názvosloví organické chemie. Vydání 2. ISBN 80-7346-017-**3**, 2008

Flügge C. (1886) Die Mikroorganismen: mit besonderer Berücksichtigung der Aetiologie der Infectionskrankheiten. Vogel.

Franche C., Lindström K., Elmerich C. (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and soil*, ***321***(1), 35-59.

Fravel, D.R. (2005). Commercialization and implementation of biocontrol. *Annu. Rev. Phytopathol*, **43**: 337–359.

Gao Q., Jin K., Ying S.H., Zhang Y., Xiao G., Shang Y., Wang C. (2011) Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLoS Genet*, **7(**1).

Ghanbarzadeh B., Almasi H., Entezami A. A. (2010) Physical properties of edible modified starch/carboxymethyl cellulose films. *Innovative food science & emerging technologies*, **11**(4): 697-702.

Gillespie J. P., Burnett C., Charnley A. K. (2000) The immune response of the desert locust *Schistocerca gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae var acridum*. *Journal of Insect Physiology*, **46**(4): 429-437.

Głodowska M., Husk B., Schwinghamer T., Smith, D. (2016) Biochar is a growth-promoting alternative to peat moss for the inoculation of corn with a pseudomonad. *Agronomy for sustainable development*, **36**(1): 21.

Głodowska M., Schwinghamer T., Husk B., Smith D. (2017) Biochar based inoculants improve soybean growth and nodulation. *Agricultural Sciences*, **8**(9): 1048-1064.

Gorim L., Asch F. (2012) Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. *Journal of Agronomy and Crop Science*, **198(**2): 81-91.

Gorim L., Asch F. (2012) Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. *Journal of Agronomy and Crop Science*, **198(**2): 81-91.

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological research*, **206**: 131-140.

Greenfield M., Gómez-Jiménez M. I., Ortiz V., Vega F. E., Kramer M., Parsa, S. (2016) *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. *Biological Control*, **95**: 40-48.

Griffin M.R. (2007) *Beauveria bassiana*, a cotton endophyte with biocontrol activity against seedling disease. Dissertation. University of Tennessee.

Guan Y. J., Wang J. C., Hu J., Tian Y. X., Hu W. M., Zhu S. J. (2013) A novel fluorescent dual-labeling method for anti-counterfeiting pelleted tobacco seeds. *Seed Science and Technology*, **41**(1): 158-163.

Guan Y., Wang J., Tian Y., Hu W., Zhu L., Zhu S., Hu J. (2013) The novel approach to enhance seed security: dual anti-counterfeiting methods applied on tobacco pelleted seeds. *Plos one*, **8**(2): e57274.

Gurulingappa P., Sword G.A., Murdoch G., McGee P.A. (2010) Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. *Biol Control*, **55**:34–41

Halmer P. (2000) Commercial seed treatment technology. Seed Technology and Its Biological Basis. Sheffield Academic Press, Sheffield, England, 257-286.

Hazra D. K., Patanjali, P. K. (2016) Seed coating formulation technologies: an environmental biology friendly approaches for sustainable agriculture. *Bioscience Methods*, 7.

Hill H.J. (1999) Recent developments in seed technology, **1**: 105–112.

Hoitink, H.A.J.; Boehm, M.J. (1999). Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. *Annu. Rev. Phytopathol*, **37:** 427–446.

Howell C.R. (1982) Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani*, and damping-off of cotton seedlings. *Phytopathology*.**72**: 496–498.

Howell C.R. (2002) Cotton seedling preemergence damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. Phytopathology **92**:177-180.

Howell C.R. (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Dis*. **87**:4-10. 12.

Howell C.R., DeVay J.E., Garber R.H., Batson W.E. (1997) Field control of cotton seedling diseases with *Trichoderma virens* in combination with fungicide seed treatments. J. Cotton, Sci. **1**:15-20

Howell C.R., Stipanovic R.D., Lumsden R.D. (1993) Antibiotic production by strains of *Gliocladium virens Biocontrol Sci. Tech*, **3**:435-441

Jaber L.R. (2015) Grapevine leaf tissue colonization by the fungal entomopathogen *Beauveria bassiana* s.l. and its effect against downy mildew. *BioControl*, **60**:103–112.

Jaber L.R., Araj S.E. (2018) Interactions among endophytic fungal entomopathogens (Ascomycota: *Hypocreales*), the green peach aphid Myzus persicae Sulzer (Homoptera: *Aphididae*), and the aphid endoparasitoid Aphidius colemani Viereck (Hymenoptera: *Braconidae*). *Biol Control*, **116**:53–61.

Jaber L.R., Enkerli J. (2016) Effect of seed treatment duration on growth and colonization of Vicia faba by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. *Biol Control*, **103**:187–195.

Jaber L.R., Enkerli J. (2017) Fungal entomopathogens as endophytes: can they promote plant growth? *Biocontrol Sci Technol*, **27**:28–41.

Jaber L.R., Salem N.M. (2014) Endophytic colonisation of squash by the fungal entomopathogen *Beauveria bassiana* (Ascomycota: *Hypocreales*) for managing Zucchini yellow mosaic virus in cucurbits. *Biocontrol Sci Technol*, **24**:1096–1109.

Jacobsen F., Mohammadi-Tabrisi A., Hirsch T., Mittler D., Mygind P. H., Sonksen C. P., Steinstraesser L. (2007) Antimicrobial activity of the recombinant designer host defence peptide P-novispirin G10 in infected full-thickness wounds of porcine skin. *Journal of Antimicrobial Chemotherapy*, **59**(3): 493-498.

Jash S., Pan S. (2008) Variability in antagonistic activity and root colonizing behaviour of *Trichoderma* isolates. *Journal of Tropical Agriculture*, **45**(1), 29-35.

Jeffries P., Gianinazzi S., Perotto S., Turnau K., Barea J. M. (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and fertility of soils*, **37**(1): 1-16.

Jeffries P., Young T. W. (1994) Interfungal parasitic relationships. *Cab International*.

Jeffs K.A. (1986) Seed Treatment, 2nd ed.; The British Crop Protection Council (BCPC) Publication: Surrey, p. 332.

Karagöz K., Ateş F., Karagöz H., Kotan R., Çakmakçı R. (2012) Characterization of plant growth-promoting traits of bacteria isolated from the rhizosphere of grapevine grown in alkaline and acidic soils. *European Journal of Soil Biology*, **50**: 144-150.

Kimmelshue C., Goggi A. S., Cademartiri, R. (2019) The use of biological seed coatings based on bacteriophages and polymers against *Clavibacter michiganensis* subsp. nebraskensis in maize seeds. *Scientific reports*, ***9***(1), 1-11.

Kuzhuppillymyal-Prabhakarankutty L., Tamez-Guerra P., Gomez-Flores R., Rodriguez-Padilla M. C., Ek-Ramos M. J. (2020) Endophytic *Beauveria bassiana* promotes drought tolerance and early flowering in corn. *World Journal of Microbiology and Biotechnology*, **36**(3): 1-10.

Lacey L.A., Grzywacz D., Shapiro-Ilan D.I., Frutos R., Brownbridge M. and Goettel M. S. (2015) 'Insect pathogens as biological control agents: back to the future', Journal of invertebrate pathology, **132**: 1- 41.

Landa Z. (2002) Biologická ochrana zahradních rostlin proti chorobám a škůdcům v polních podmínkách, ve sklenících a fóliovnících. In: Demo M., Hričovský I. (Eds.): Trvalo udržateľné technológie v záhradnictve. Slovenská poľnohospodárska univerzita v Nitre, 225-280.

Lewis L.C., Bruck D.J., Gunnarson R.D., (2002) On-farm evaluation of *Beauveria bassiana* for control of *Ostrinia nubilalis* in Iowa, USA. *BioControl,* **47,** 167-176.

Lo C.T., Lin C.Y. (2002) Screening strains of Trichoderma spp for plant growth enhancement in Taiwan. *Plant Pathology Bulletin*, **11**(4), 215-220.

Logan N.A., De Vos P. (2009) Genus I. Bacillus Cohn 1872, 174AL. Bergey's Manual of Systematic Bacteriology, **3**: 21–128.

Lopez D. C., Sword G. A. (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*). *Biol Control*, **89**:53–60.

Lumsden R. D., Walter J. F., C. P. Baker**.** (1996) Development of *Gliocladium virens* for damping-off disease control. Can. J. *Plant Pathol*.**18:**463-468.

Ma X., Chang P. R., Yu, J. (2008) Properties of biodegradable thermoplastic pea starch/carboxymethyl cellulose and pea starch/microcrystalline cellulose composites. Carbohydrate Polymers, **72**(3): 369-375.

Ma Y., Oliveira R.S., Freitas H., Zhang C. (2016) Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation, **7**: 918.

Ma, Y. (2019). Seed coating with beneficial microorganisms for precision agriculture. *Biotechnology advances*, **37**(7): 107423.

Ma, Y., Látr, A., Rocha, I., Freitas, H., Vosátka, M., & Oliveira, R. S. (2019). Delivery of inoculum of Rhizophagus irregularis via seed coating in combination with *Pseudomonas libanensis* for cowpea production. *Agronomy*, *9*(1), 33.

Madsen M. D., Davies K. W., Boyd C. S., Kerby J. D., Svejcar T. J. (2016) Emerging seed enhancement technologies for overcoming barriers to restoration. *Restoration Ecology*, **24**: S77-S84.

Malik, R., Gupta, R., Srivastava, S., Choudhary, B. S., & Sharma, M. (2017) Design, synthesis and biological evaluation of selected 3-[3-(amino) propoxy] benzenamines as acetylcholinesterase inhibitors. *Journal of Biomolecular Structure and Dynamics*, ***35***(11), 2382-2394.

Malusá E., Sas-Paszt L., Ciesielska, J. (2012) Technologies for beneficial microorganisms inocula used as biofertilizers. *The scientific world journal*, 2012.

Masuthi D. A., Vyakaranahal B. S., & Deshpande V. K. (2009) Influence of pelleting with micronutrients and botanical on growth, seed yield and quality of vegetable cowpea. *Karnataka Journal of Agricultural Sciences*, **22**(4): 898-900.

McGee D. C. (1995) Epidemiological approach to disease management through seed technology. *Annual review of phytopathology*, ***33***, 445-466.

McQuilken, M. P., Halmer, P., & Rhodes, D. J. (1998). Application of microorganisms to seeds. In *Formulation of microbial biopesticides*, pp. 255-285.

Mei J., Wang W., Peng S., Nie, L. (2017) Seed pelleting with calcium peroxide improves crop establishment of direct-seeded rice under waterlogging conditions. *Scientific reports*, **7**(1): 1-12.

Meyling N. V., Eilenberg J. (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biological control*, **43**(2): 145-155.

Michalak I., Dmytryk A., Schroeder G., Chojnacka K. (2017) The application of homogenate and filtrate from Baltic seaweeds in seedling growth tests. *Applied Sciences*, ***7***(3), 230.

Moonjely S., Barelli L., Bidochka M. J. (2016) Insect pathogenic fungi as endophytes. *Advances in genetics*, **94**: 107-135.

Mukhopadhyay A.N., Shrestha S.M., Mukherjee P.K. (1992) Biological seed treatment for control of soil borne plant pathogens FAO. *Plant Prot Bull* **40**:21-30.

Muller H., Berg G. (2008) Impact of formulation procedures on the effect of the biocontrol agent *Serratia entomophila* HRO-C48 on Verticillium wilt in oilseed rape. *BioControl*, **53**: 905–916.

Mutune B., Ekesi S., Niassy S., Matiru V., Bii C., Maniania N. K. (2016) Fungal endophytes as promising tools for the management of bean stem maggot *Ophiomyia phaseoli* on beans *Phaseolus vulgaris*. *J pest sci*, **89**:993–1001.

Nakamura L., Jackson M. (1995) Clarification of the taxonomy of *Bacillus mycoides. International journal of systematic bacteriology*, **45**(1): 4–7.

Navrátilová M. (2015) Uplatňování systému integrované ochrany rostlin v souvislosti se změnou legislativy (27). Biologická ochrana rostlin jako nedílná součást principů IOR (1). Agromanuál, **7**: 42-46.

Neher O.T., Johnston, M.R., Zidack N.K., Jacobsen, B.J. (2009) Evaluation of *Bacillus mycoides* isolate BmJ and *B. mojavensis* isolate 203-7 for the control of anthracnose of cucurbits caused by *Glomerella cingulata* var. *orbiculare*. *Biological Control*, **48**(2), 140-146.

Nettles R., Watkins J., Ricks K., Boyer M., Licht M., Atwood L. W., Koide R. T. (2016) Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. *Applied Soil Ecology*, **102**: 61-69.

Oliveira R. S., Rocha I., Ma Y., Vosátka M., Freitas H. (2016) Seed coating with arbuscular mycorrhizal fungi as an eco-technological approach for sustainable agricultural production of common wheat (Triticum aestivum L.). *Journal of Toxicology and Environmental Health, Part A,* **79**(7): 329-337.

Oliveira A. F. D., Soldi V., Coelho C. M. M., Miqueloto A., Coimbra J. L. (2009) Preparation, characterization, and properties of polymeric films with potential application in seed coatings. *Química Nova*, ***32***(7), 1845-1849.

Orhan E., Esitken A., Ercisli S., Turan M., Sahin F. (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry Sci. Hortic, **111** (1): 38-43

Ortiz-Urquiza, A., Luo Z., Keyhani N.O. (2014) Improving mycoinsecticides for insect biological control. *Appl. Microbiol. Biotechnol*, **99:** 1057–1068.

Ownley B. H., Griffin M. R., Klingeman W. E., Gwinn K. D., Moulton J. K., Pereira R. M. (2008) *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of invertebrate pathology*, **98**(3): 267-270.

Ownley B. H., Gwinn K. D., Vega F. E. (2010) Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. *BioControl*, **55**(1): 113-128.

Ownley B. H., Pereira R. M., Klingeman W. E., Quigley N. B., Leckie B. M. (2004) Beauveria bassiana, a dual-purpose biocontrol organism, with activity against insect pests and plant pathogens. 255-269.

Ownley B. H., Windham M. T. (2007) Biological Control of Plant Pathogens. Plant pathology concepts and laboratory exercises, 37.

Pacheco-Aguirre J. A., Ruíz-Sánchez E., Ballina-Gómez H. S., Alvarado-López C. J. (2017) Does polymer-based encapsulation enhance performance of plant growth promoting microorganisms? A meta-analysis views. *Agrociencia*, **51**(2): 174-187.

Padhi P. P., Pattanayak S. K. (2018) Effect of lime coating and molybdenum seed treatment on productivity and nutrient uptake of different pulses grown in Alfisols. IJCMAS, **7**:1417-1426.

Pedrini S., Balestrazzi A., Madsen M. D., Bhalsing K., Hardegree S. P., Dixon K. W., Kildisheva O. A. (2020) Seed enhancement: getting seeds restoration ready. *Restoration Ecology*, **28**: S266-S275.

Pedrini S., Merritt D. J., Stevens J., Dixon K. (2017) Seed coating: science or marketing spin? *Trends in plant science*, **22**(2), 106-116.

Peeters F. M., van Meggelen J., Schepers H. T. A. M. (2015) Crop protection and pesticide risk assessment Myanmar: towards sustainable agricultural and export of high value crops (No. 2621).

Petlamul W., Sripornngam T., Buakwan N., Buakan S., Mahamad K (2017) The capability of *Beauveria bassiana* for çellulase enzyme production. Proceedings of 7th International Conference on Bioscience, Biochemistry and Bioinformatics, 65–66.

Podile A. R., Kishore G. K. (2007) Plant growth-promoting rhizobacteria. In Plant-associated bacteria, 195-230.

Qiu, Y., Amirkhani, M., Mayton, H., Chen, Z., & Taylor, A. G. (2020). Biostimulant seed coating treatments to improve cover crop germination and seedling growth. Agronomy, **10**(2): 154.

Quesada-Moraga E., Landa B. B., Muñoz-Ledesma J., Jiménez-Diáz R. M., Santiago-Alvarez C. (2006). Endophytic colonisation of opium poppy, Papaver somniferum, by an entomopathogenic *Beauveria bassiana* strain. *Mycopathologia*, **161**(5): 323-329.

Quesada-Moraga E., Navas-Cortés J. A., Maranhao E. A., Ortiz-Urquiza A., Santiago-Álvarez C. (2007) Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. *Mycological research*, **111**(8): 947-966.

Ramanujam B., Rangeshwaran R., Sivakmar G., Mohan M., Yandigeri M.S. (2014) Management of Insect Pests by Microorganisms. Proceedings of Indian National Science *Academy*, **80**(2):455-471.

Rezende J.M., Zanardo A.B.R., da Silva Lopes M., Delalibera I., Rehner S.A. (2015) Phylogenetic diversity of Brazilian Metarhizium associated with sugarcane agriculture, **60**: 495–505.

Rivas-Franco F., Hampton J.G., Morán-Diez M.E., Narciso J., Rostás M., Wessman P., Glare T.R. (2019) Effect of coating maize seed with entomopathogenic fungi on plant growth and resistance against *Fusarium graminearum* and *Costelytra giveni*. *Biocontrol Science and Technology*, **29**(9):877-900.

Rocha I., Duarte I., Ma Y., Souza-Alonso P., Látr A., Vosátka M., Oliveira, R. S. (2019) Seed coating with arbuscular mycorrhizal fungi for improved field production of chickpea. *Agronomy*, **9**(8): 471.

Ruiz-de-La-Cruz G., Aguirre-Mancilla C. L., Godínez-Garrido N. A., Osornio-Flores N. M., Torres-Castillo J. A. (2017) Chitosan mixed with beneficial fungal conidia or fungicide for bean (*Phaseolus vulgaris* l.) seed coating. *Interciencia*, **42**(5): 307-312.

Russo M. L., Scorsetti A. C., Vianna M. F., Cabello M., Ferreri N., Pelizza, S. (2019) Endophytic effects of *Beauveria bassiana* on Corn (*Zea mays*) and its herbivore, Rachiplusia nu (Lepidoptera: Noctuidae). *Insects*, **10**(4): 110.

Ryu C. M., Kim J., Choi O., Kim S. H., Park C. S. (2006) Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biological Control*, **39**(3): 282-289.

Sandhu S.S., Sharma A.K., Beniwal V., Goel G., Batra P., Kumar A., Jaglan S., Sharma A.K., Malhotra S. (2012): Myco-biocontrol of insect pests: Factors involved, mechanism, and regulation. *Journal of Pathogens*, **1**:1-10.

Sandhu S.S., Shukla H., Aharwal R.P., Kumar S., Shukla S. (2017) Efficacy of Entomopathogenic Fungi as Green Pesticides.

Sachs M., Cantliffe D. J., Nell T. A. (1981) Germination studies of clay-coated sweet pepper seeds (No. RESEARCH).

Schmidt, E. C., Nunes, B. G., Maraschin, M., Bouzon, Z. L. (2010) Effect of ultraviolet-B radiation on growth, photosynthetic pigments, and cell biology of Kappaphycus alvarezii (Rhodophyta, Gigartinales) macroalgae brown strain. *Photosynthetica*, ***48***(2), 161-172.

Schippers B., Bakker A. W., Bakker P. A. (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annual review of Phytopathology*, **25**(1): 339-358.

Schrank A., Vainstein M.H. (2010) *'Metarhizium anisopliae* enzymes and toxins', Toxicon, **56**(7): 1267-1274.

Schuster A., Schmoll M. (2010) Biology and biotechnology of *Trichoderma*. *Appl Microbiol Biotechnol*, **87**:787–799.

Scott J.M. (1989) Seed coatings and treatments and their effects on plant establishment, **42**: 43–83.

Shaalan R., Ibrahim L. (2018) Entomopathogenic fungal endophytes: can they colonize cucumber plants? In: IX International Scientific Agriculture Symposium "agrosym 2018". University of East Sarajevo, *Jahorina, Bosnia and Herzegovina*,4-7

Shah P.A., Pell J.K. (2003) 'Entomopathogenic fungi as biological control agents', *Applied microbiology and biotechnology*, **61**(5-6), 413- 423.

Shahid A.A., Rao Q.A., Bakhsh A., Husnain T. (2012) Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. *Archives of Biological Sciences*, **64**(1), 21-42.

Shivay Y. S., Kumar D., Prasad R., Ahlawat I. P. S. (2008) Relative yield and zinc uptake by rice from zinc sulphate and zinc oxide coatings onto urea. Nutrient Cycling in *Agroecosystems*, **80**(2): 181-188.

Sikhao P., Teerapornchaisit P., Taylor A. G., Siri B. (2014) Seed coating with riboflavin, a natural fluorescent compound, for authentication of cucumber seeds. *Seed Science and Technology*, **42**(2), 171-179.

Sivan A., Ucko O., Chet I. (1987) Biological control of *Fusarium* crown rot of tomato by *Trichoderma harzianum* under field conditions. *Plant Disease*, **71**(7): 587-592.

Smith R. G., Atwood L. W., Morris M. B., Mortensen D. A., Koide R. T. (2016) Evidence for indirect effects of pesticide seed treatments on weed seed banks in maize and soybean. *Agriculture, Ecosystems & Environment*, **216**: 269-273.

Stockwell V. O., Duffy B. (2012) Use of antibiotics in plant agriculture. *Revue Scientifique Et Technique-Office International Des Epizooties*, ***31***(1).

Sunick K., Sungjoon Y., Honggi K. (1997) Selection of antagonistic bacteria for biological control of ginseng diseases. *Korean Journal of Plant Pathology*, **13**: 342-348.

Syngenta (2018). Syngenta Crop Challenge In Analytics.

Szemruch C. L., Ferrari L. (2013) Encrusting offers protection against phytotoxic chemicals and maintains the physiological quality of sunflower (Helianthus annuus) seeds. *Seed Science and Technology*, **41**(1): 125-132.

Taylor A. G. (2020) Seed storage, germination, quality, and enhancements. The Physiology of Vegetable Crops, 2nd ed.; *Wien, HC, Stutzel, H., Eds*, 1-30.

Taylor A. G., Allen P. S., Bennett M. A., Bradford K. J., Burris J. S., Misra, M. K. (1998) Seed enhancements. *Seed science research*, **8**(2): 245-256.

Taylor A.G. (2003) Seed treatments. In Encyclopedia of Applied Plant Sciences; Thomas, B.D.J., Murphy, B.G., Eds.; Elsevier Academic Press: Cambridge, 1291–1298.

Taylor A.G., Eckenrode C.J., Straub R.W. (2001) Seed treatments for onions: Challenges and progress. *HortTechnology*, **36**: 199–205.

Taylor A.G., Harman G.E. (1990) Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathol*, **28**: 321–339.

Torres M. S., White J. F. (2009) Clavicipitaceae: free-living and saprotrophs to plant endophytes. *Encyclopedia of Microbiology*, ***1***: 422-430.

Tsavkelova E.A., Aleksandrova A.V., Cherdyntsera T.A., Kolomeitseva G.L. (2005) Netrusov AI. Fungi associated with the Vietnamian tropical orchids. *Mikol Fitopatol*, **39**: 46–52.

Tu J.C. (1980) *Gliocladium virens*, a destructive mycoparasite of *Sclerotinia sclerotiorum*. *Phytopathology*, **70**: 670–674.

USDA (2018) World agricultural production: crop production tables. Production, Supply and Distribution, Office of Global Analysis, Foreign Agricultural Service/USDA, Washington.

Van der Weerden N. L., Bleackley M. R., Anderson M. A. (2013) Properties and mechanisms of action of naturally occurring antifungal peptides. *Cellular and molecular life sciences*, **70**(19): 3545-3570.

van Lenteren J.C, Godfray H.C.J. (2005) European science in the enlightenment and the discovery of the insect parasitoid life cycle in The Netherlands and Great Britain. *Biol Control*, **32**:12–24.

Vega F. E., Goettel M. S., Blackwell M., Chandler D., Jackson M. A., Keller S., Roy H. E. (2009) Fungal entomopathogens: new insights on their ecology. fungal ecology, **2**(4): 149-159.

Vidal S., (2015) Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. *Current Science*, **108**: 1 – 9.

Vilcinskas A., Matha V., Götz P. (1997) Effects of the entomopathogenic fungus *Metarhizium anisopliae* and its secondary metabolites on morphology and cytoskeleton of plasmatocytes isolated from the greater wax moth, *Galleria mellonella*. *Journal of Insect Physiology*, **43**(12): 1149-1159.

Viterbo A., Inbar J., Hadar Y., Chet I. (2007) Plant disease biocontrol and induced resistance via fungal mycoparasites. The Mycota IV: environmental and microbial relationships. Springer, Heidelberg, Germany, 127-146.

Viterbo A., Ramot O., Chernin L., Chet I. (2002) Significance of lytic enzymes from *Trichoderma* spp. in the biocontrol of fungal plant pathogens. *Antonie Van Leeuwenhoek*, **81**(1), 549-556.

Wagner B. L., Lewis L. C. (2000) Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. *Applied and Environmental Microbiology*, **66**(8), 3468-3473.

Wall D.H., Nielsen U.N., Six, J. (2015) Soil biodiversity and human health. Nature, **528:** 69–76.

Wang C., Feng M.G. (2014) Advances in fundamental and applied studies in China of fungal biocontrol agents for use against arthropod pests, **68**: 129–135.

Wang Q., Zuo J.H., Wang Q., Na Y., Gao L.P. (2015) Inhibitory effect of chitosan on growth of the fungal phytopathogen, *Sclerotinia sclerotiorum*, and sclerotinia rot of carrot, **14** (4): 691-697

Wiatrak P. (2013) Effect of polymer seed coating with micronutrients on soybeans in Southeastern Coastal Plains. *American Journal of Agricultural and Biological Sciences*, **8**(4): 302-308.

Wiest A., Grzegorski D., Xu B.W., Goulard C., Rebuffat S., Ebbole D.J., Bodo B., Kenerley C.M. (2002) Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase J. *Biol. Chem*, **277**:20862-20868

Williams M. I., Dumroese R. K., Page-Dumroese D. S., Hardegree S. P. (2016) Can biochar be used as a seed coating to improve native plant germination and growth in arid conditions? *Journal of Arid Environments*, **125**: 8-15.

Wilson H. T., Amirkhani M., Taylor, A. G. (2018) Evaluation of gelatin as a biostimulant seed treatment to improve plant performance. *Frontiers in plant science*, **9**: 1006.

Xiao G., Ying S. H., Zheng P., Wang Z. L., Zhang S., Xie X. Q., Feng M. G. (2012) Genomic perspectives on the evolution of fungal entomopathogenic in *Beauveria bassiana*. *Scientific reports*, **2**(1): 1-10.

Yang D., Wang N., Yan X., Shi J., Zhang M., Wang Z., Yuan H. (2014) Microencapsulation of seed-coating tebuconazole and its effects on physiology and biochemistry of maize seedlings. *Colloids and Surfaces B: Biointerfaces*, **114**: 241-246.

Young C. C., Rekha P. D., Lai W. A., Arun A. B. (2006) Encapsulation of plant growth-promoting bacteria in alginate beads enriched with humic acid. *Biotechnology and bioengineering*, **95**(1): 76-83.

Zhang J. X., Xue A. G., Tambong J. T. (2009) Evaluation of seed and soil treatments with novel *Bacillus subtilis* strains for control of soybean root rot caused by *Fusarium oxysporum* and *F. graminearum*. *Plant Disease*, **93**(12): 1317-1323.

Zhou J., Deng B., Zhang Y., Cobb A. B., Zhang Z. (2017) Molybdate in rhizobial seed-coat formulations improves the production and nodulation of Alfalfa. *PloS one*, **12**(1): e0170179.

Ziani K., Ursúa B., Maté J. I. (2010) Application of bioactive coatings based on chitosan for artichoke seed protection. *Crop Protection*, **29**(8): 853-859.

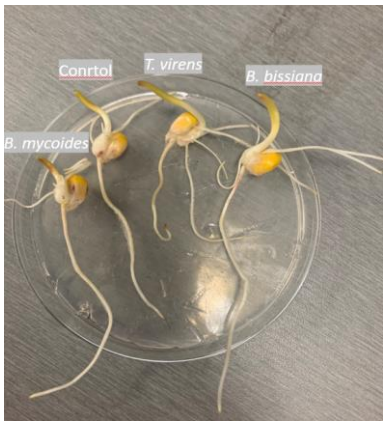
Zietlow O. T., Johnston M. R., Zidack N. K., Jacobsen B. J. (2004) Induced systemic resistance in cucumber to Glomerella cingulata var. orbiculare and Pseudomonas syringae pv. lachrymans by *Bacillus mycoides*, isolate BacJ and *Bacillus pumilus*, isolate MSU 203. *Phytopathology*, **94**(6).

Zimmermann G. (1986) The ‘Galleria bait method’ for detection of entomopathogenic fungi in soil. *Journal of Applied Entomology*, **102**: 213–215.

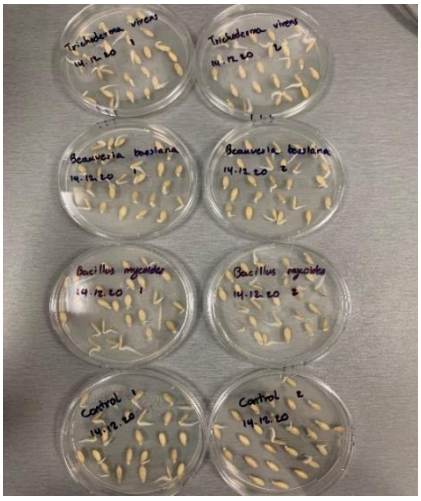
8 ANNEXES

Picture sheet 1 Influence of beneficial microorganisms on seed germination and seedling growth of selected crops in laboratory (Photo: Zin Moe Wai Aung)

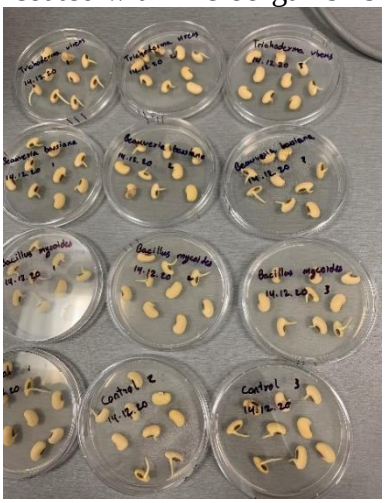
Germination of corn seeds on Petri dishes 4 DAS



Development of roots and shoots in corn after seed coated with microorganisms



Germination of cucumber seeds on Petri dishes 4 DAS



Germination of soybean seeds on Petri dishes 4 DAS

Germination of cucumber seeds on filter paper 7 DAS

Germination of corn seeds on filter paper 7 DAS

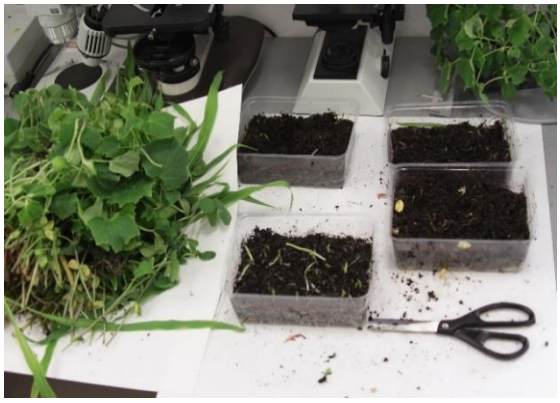
Picture sheet 2 Influence of beneficial microorganisms on seed germination in soil substrate and the procedure for evaluating the effectiveness of these microorganisms in soil substrate (Photo: Zin Moe Wai Aung)

Sowing selected crops into soil substrate

Germination of selected crops in soil substrate 7 DAS

Germination of selected crops in soil substrate 7 DAS

After 23 days, before cutting the above-ground part of plants

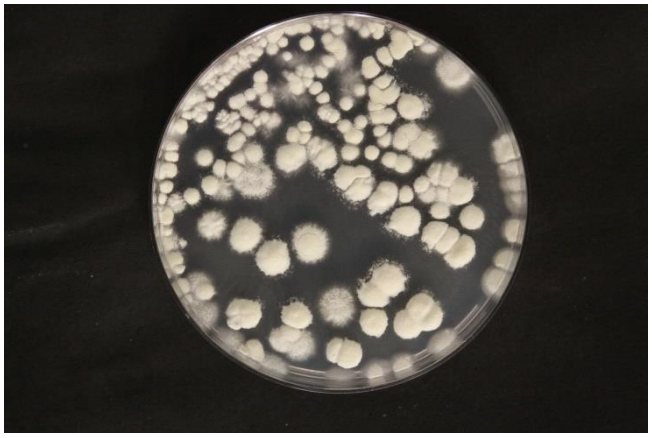


The above-ground part was cut with disinfected scissors

Picture sheet 3 The procedure for evaluating the effectiveness of these microorganisms in soil substrate (Photo: Zin Moe Wai Aung)

The remaining soil substrate was taken from the large plastic boxes transferred into small plastic boxes

T. virens growing on the selective medium based on a.i. dodine



B. bassiana growing on the artificial medium PDA

8.1 List of abbreviations

AMF	Arbuscular mycorrhizal fungi
CFU	Colony forming units
CMC	Carboxymethylcellulose
DAS	Days after sowing
EPF	Entomopathogenic fungi
PDA	Potato dextrose agar
PGPR	plant growth-promoting rhizobacteria