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Use of Active Microrganisms in Crop Production – A Review

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Abstract

Phosphorus, other elements and natural resources are scarce, and so it is necessary to find alternative strategy to increase availability of nutrients for plants. One possible way could be application of so-called bioeffectors (BE) which should improve the mobilisation of nutrients (especially phosphorus) from less available forms in soil, improve plant growth and contribute to mycorrhiza development. BEs are commercially supplied products which contain active substances (live microorganisms and active natural compounds). BEs can be used in organic agriculture, because their application represents no risk for the environment. Several studies and experiments are focused on impact of bioeffectors' application and their active compounds on plants. Experiments were performed under different conditions (field, pot, greenhouse), on various testing plants and on various bioeffectors. These BEs have been used as a fertilizer, fungicide or molluscicide and they were applied either to soil, seed or leaf. Application should increase growth of root system and above-ground part of plants and also nutrient uptake. These products are developed for a wide variety of crops (e.g. maize, wheat, tomatoes, rape, spinach, grass, ornamentals). This review summarizes the most recent knowledge in this scientific field.

Keywords: Bioeffector; Microoragnisms; Soil; Nutrients; Crop production

Introduction

Most of the nutrients found in soil are in for plants inaccessible forms, therefore our society and crop production depend on commercially produced fertilizers. Even commercially produced fertilizers used in agriculture are produced from natural nutrient resources and as such are limited in availability. The most limited nutrient for plant production and agriculture is phosphorus with its natural reserves estimated for fifty years. For these reasons, it is necessary to find an alternative strategy for future generations that would help in better availability and use of plant nutrients in the application of lower input of commercially/industrially supplied products and would also be environmentally friendly.

Phosphorus (P) is an essential, non-renewable nutrient for plant development and growth. Plants acquire P from soil solution as orthophosphate anions. However, orthophosphate is very reactive and may be immobilised through precipitation or adsorption, making P highly insoluble and unavailable to plants. The majority of P fertilizers are currently derived from rock phosphate, which is predicted to become increasingly scarce in the future. Research and development on the efficient use of other available sources of P is therefore crucial [1-3]. Phosphorus deficiency is one of the major limiting factors for decreased agricultural production [4]. Due to a growing world population it is expected that demand for food and feed will increase. Limited availability of productive agricultural land and increasing dependance on mineral fertilizers make it necessary to develop alternative strategies for plant nutrition [5,6]. BEs can contribute, depending on soil and climate conditions, to overcome limitations in the availability of nutrients. These compounds contain microorganisms such as bacteria or fungi and active natural substances (extracts from soil, compost or seaweeds, microbial residues, plant extracts). These products are developed for a wide variety of crops (e.g. crops, grass, ornamentals, grass). Their effective use should cause the mobilisation of nutrients from less bioavailable forms in soil [5] and further support root growth [7,8] and mycorrhiza development [9]. Microorganisms may play an important role in enhancing availability of P to plants and have been proven to enhance uptake directly by extending the root system (e.g. mycorrhizal associations), increasing mobilisation of orthophosphate from soil organic and inorganic phosphorus, and stimulating root growth [1].

Mycorrhiza is highly effective in absorbing nutrients from the soil, especially for nitrogen and phosphorus. Nitrogen and phosphorus are often limited in supply and fungal hyphae are able to absorb these nutrients more efficiently and from greater area of soil than the roots, which leads to increased plant growth. This causes mutually beneficial linkage between plants and fungi, the sugars (organic carbon) formed during photosynthesis are transported to the roots and the fungi are taken and the nutrients are absorbed by fungal hyphae from the soil and are transported into plants [10,11]. Arbuscular mycorrhizal fungi colonise most agricultural species (exceptions include *Brassica* spp., and *Lupinus* spp.) and play an important role in the phosphorus nutrition of many farming systems worldwide, especially on soils with low available phosphorus [3].

Literature Review

Examples of plant strategies for phosphorus obtaining:

a) Growth of roots

b) Root exudates (acidic phenolics)

c) Mycorrhiza

d) Cooperation with microorganisms (P-solubilization).

One alternative strategy in plant production can be use of unmycorrhizal organism's P mobilizing nutrients, which should help to increased nutrient availability for plants. These substances are so-called bioeffectors.

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Bioeffectors

In the last two decades, increased interest in sustainable agricultural practices has seen the growing development and use of commercial microbial inoculants for increasing crop productivity and resource use efficiency. Microbial inoculants mainly include free-living bacteria, fungi and arbuscular mycorrhizal fungi [12]. Development of the BEs increases due to the potential use of these substances in organic farming and also because of the limited natural resources of nutrients [13].

These products are divided into three main groups, according to which of active substances or microorganisms they contain. BEs addressed comprise fungal strains of *Trichoderma*, *Penicillium* as well as bacterial strains of *Bacillus*, *Pseudomonades, PaeniBacillus* and *Rhizophagus* with well-characterized root growth promoting and nutrient-solubilising potential. Natural extraction products of seaweed, compost and plant extracts, as well as their purified active compounds with protective potential against biotic and abiotic stresses are also tested in various combinations [5].

Fungal bioeffectors: As mentioned above BEs can be divided into two main groups namely fungal and bacterial. Several fungal representatives have been selected and described further in this section and in Table 1. There are selected bacteria and their impact on crop production.

***Trichoderma ssp.*:** The genus *Trichoderma* spp. are wild filamentous fungi occurring in most soil types and different habitats. *Trichoderma* is a fungal genus that includes species that are currently being used as BEs or as biofertilizers [14,15]. The *Trichoderma* is known for producing enzymes and antibiotics. These species are attributed to a variety of physiological, antifungal and insecticidal effects. It acts against a broad spectrum of plant pathogens. These fungi increase plant growth and development, but also development of root system [7,8,13,16,17]. It has also been observed that selected *Trichoderma* strains can improve plant nutrients' uptake [18]. Increased growth occurs due to its strong anti-pathogenic activity, biosynthesis of hormones, improving nutrient uptake from the soil, root development by increasing metabolism rate of carbohydrates and increased photosynthesis [13]. The main hydrolytic enzymes secreted by the fungus are proteases, chitinases and endochitinases [16]. Chitinase are produced by e.g. bacteria, algae, fungi, plants, insects, nematodes, molluscides, vertebrates, including man and certain viruses [19].

***Trichoderma harzianum*:** *T. harzianum* is wild filamentous fungus that occurs in soil. *Trichoderma* belongs to fungi that includes species that are currently being used as biological control agents or as biofertilizers [14,15]. It has also been observed that selected *Trichoderma* strains can improve plant nutrient uptake [18]. Buysens et al. [20] used *T. harzianum* in study on potato were conducted in a greenhouse or *in vitro* conditions. Experiments were conducted at two sites in Belgium 2009-2012. The objective of this study was to investigate the impact on potato yield of the co-inoculation of *R. irregularis* (strain MUCL 41833) and *T. harzianum* (strain MUCL 29707) applied to a cover crop (*Medicago sativa*) preceding potato planting or to potato at planting. In both trials we observed that the most advantageous agricultural practice to increase potato yield was the inoculation of a preceding cover crop with both microorganisms. Inoculation with beneficial microorganisms increased potato tuber weight in both trials compared to the non-inoculated treatments. This was mainly attributed to improved arbuscular mycorrhizal fungi colonization of potato plants. The inoculation via cover crop seems a more efficient strategy as compared to the direct inoculation at potato plantation. However, difference between these strategies on potato production may not be solely attributed to Arbuscular mycorrhizal fungi colonization rates but could also be due to higher N availability, but it was not tested. Gupta et al. [21] conducted a study and pots experiment focused on the non-target effects of a microbial consortium comprising three selected bioinoculants: *Bacillus megaterium* (strain MTCC 453), *Pseudomonas fluorescens* (strain MTCC 9768) and *Trichoderma harzianum* (strain MTCC 801), on the resident as well as active microbial community structure in pigeon pea (*Cajanus cajan*) rhizosphere. The treatment was found to result in a significant increase in shoot length (1.2-fold), root length (1.3-fold), dry mass (2.4-fold) and grain yield (2.5-fold) of pigeon pear plants with the application of microbial consortium over control plants. The use of chemical fertilizers also led to improvement in plant parameters over control but upto a lesser extent than that with the microbial consortium. The performance of the consortium was found to be about 1.2-fold better than the recommended dose of chemical fertilizers in terms of grain yield. Ahmad et al. [22] conducted a pot experiment with *Brassica juncea* (var. Varuna) respectively focused on influence of soil salinity on brassica after application of *T. harzianum*. Stress caused by soil salinity causes the plants smaller and slower growth, change of plant physical and biochemical properties and decrease in yields of biomass. Results showed that the seedling plants were treated with *T. harzianum* were significantly more resistant to stress conditions caused by salinity, compared to untreated plants.

***Penicillium bilaii*:** Microorganism *P. bilaii* is a soil fungus that lives in symbiosis with plant roots and has been shown to increase the dissolution and absorption of phosphorus in certain crops [1,23]. Some *Penicillium* species can also release fixed phosphorus (P) in the soil and make it available to growing plants. Compared with other nutrients, P is the least mobile and available to plants in most soils. P-solubilizing fungi play an important role in the global phosphorus cycle and can supply P to plants in an environmentally friendly and sustainable

Fungi	Experimental conditions	Effect on the plant	References
Trichoderma spp.	Laboratory conditions	Improve growth and seed production of soybean	Paradiso et al. [79]
	Laboratory conditions	Growth promoter of cowpea	Chagas et al. [74]
Trichoderma harzianum	Pot experiment	Improve germination and seedling growth of wheat	El-Gremi et al. [13]
	Greenhouse and laboratory conditions	Increase potato yield	Buysens et al. [20]
	Pot experiment	Increase shoot and root length, dry mass and grain yield of Pigeon pea	Gupta et al. [21]
	Pot experiment	Increase growth of *Brassica juncea*	Ahmad et al. [22]
	Pot experiment	Increased root length, growth and shoot dry weight in *Brassica nigra* and melon	Galletti et al. [7]
Penicillium bilaii	Rhizobox experiment	Increase root length of maize	Gomez-Munoz et al. [1]
	Field conditions	Increase grain yield of wheat	Ram et al. [2]
	Field conditions	Increase root length and P-content in root of pea	Vessey and Heisinger [26]
	Plot experiment	Increase yield of alfalfa	Beckie et al. [28]

Table 1: BEs as promoting fungi of crop production.

Bacteria	Experimental conditions	Effect on the plant	References
Pseudomonas spp.	Laboratory, greenhouse and field conditions	Increased germination, shoot and root length, grain yield of maize	Kifle and Laing [30]
	Field conditions	Increased grain yield and straw weight of barley	Fröhlich et al. [34]
	Pot experiment and field conditions	Improves germination, growthh parameters and yield of maize	Gholami et al. [75], Nezarat and Gholami [78]
	Laboratory conditions	Growth stimulation of tomato plants	Gravel at al. [76]
Pseudomonas jessenii	Greenhouse and field conditions	Increase yield and shoot dry weight of chickpea	Valverde et al. [35]
	Greenhouse conditions	Increase growth of tomato	
Bacillus amyloliquesfaciens	Laboratory conditions	Increase root and shoot growth of rice	He et al. [41]
Bacillus subtilis	Field conditions	Increase macro and micro nutrient absorption, growth and plant production	Altuhaish et al. [54]
	Field conditions	Increase fresh and dry shoot and root weight	Turan et al. [55]
Paenibacillus mucilaginosus	Pot experiment	Improve growth of trifoliate orange seedlings	Wang et al. [56]
Rhizophagus intraradices	Greenhouse conditions	Increase the plant growth, number of leaves, plant height, shoot and root length and weight of tea	Sharma and Kayang [80]
	Field conditions	Increase growth of tomato	Mohamed et al. [62]

Table 2: BEs promoting bacteria of crop production.

manner. *P. bilaii* is used as a seed inoculant to improve P efficiency in a variety of crops such as wheat, maize, rape, bean, soya, legumes and alfalafa. This soil fungus is able to solubilize mineral phosphates and enhance plant uptake of phosphate [1,3,24]. Three mechanisms are involved by P-solubilising microorganisms: acidification of the soil, release of organic acid anions and release of phosphomonoesterase and phytase [1]. Cunningham and Kuiack [25] demonstrated that the major acidic metabolites produced by *P. bilaii* are oxalic and citric acid and so *P. bilaii* may increase the availability of phosphate to the plant by releasing organic acids. Gomez-Munoz et al. [1] conducted rhizobox experiments with maize, which was grown for 27 days in rhizoboxes enabling studies of root growth in addition to plant and soil parameters. In this experiment inoculated *P. bilaii* (strain ATCC 20851) either at the seed or the sewage sludge patch. At early growth stages, *P. bilaii* inoculation of seeds increased maize shoot length. However, at the end of experiment, the effect had ceased. Root growth was increased by seed *P. bilaii* inoculation alone and in combination with sewage sludge, whereas patch inoculation was less effective. Colonization studies performed at harvest showed that *P. bilaii* could not be detected in the maize rhizosphere but stayed at the place of inoculation. *P. bilaii* did not colonise the rhizosphere extensively but merely stayed at the place of inoculation. At the end of this experiment inoculation of *P. bilaii* showed no effect on shoot length or shoot biomass. Inoculation of sewage sludge with *P. bilaii* did not result in an increase in phosphorus uptake and thus proved to be less effective than seed inoculation. These findings confirm that *P. bilaii* application can promote root growth, increasing potential plant adsorptive capacity. While, in this study, the higher root development did not result in an increased P uptake, presumably due to severe limitations in the soil nutrient content, it remains an open question. Ram et al. [2] were conducted field experiments during 2009-2011 to evaluate the effect of seed inoculation with Penicillium bilaii on wheat at different rates of phosphorus fertilizer on P content in leaves and grain yield of irrigated wheat in India. The study showed potential of using *P. bilaii* as bio-inoculants along with 50% of recommended P fertilizer dose that produced wheat yield similar to 100% P when no *P. bilaii* was used. However, more such long-term studies are needed on different soil types varying in P availability, pH and P fixation capacity. Karamanos et al. [23] conducted a serie of 47 experiments with spring wheat. Experiments were carried out in the three prairie provinces in 1989 and 1995 and included the application of *P. bilaii*. Of the 47 trials was found the reaction to the P-fertilizers in 33 cases. These effects can not be attributed to the concentration of P in the soil, soil organic matter, texture or weather conditions and are considered a random event. Effect on the intake of phosphorus was only P-fertilizers. Vessey and Heisinger [26] describes experiments on pea (*Pisum sativum*) that were established at two locations in Canada. Inoculation of this organism in combination with a phosphorus fertilizer caused a prolongation of root length and increased the phosphorus content in the roots compared to the control which has been performed by phosphorus fertilizer. Gulden and Vessey [27] mainly focused on observation of formation of root hairs in pea after inoculation *P. bilaii*. The experiment was based on the application of the microorganism and P-fertilizer. In this experiment, the effects were investigated by *P. bilaii* on growth and morphology of the root of the pea grown in three different quantities delivered phosphorus (0, 1, 10 mg l^{-1}). The proportion of root hair was significantly higher in pea inoculated *P. bilaii* compared with control plants. Different quantities of supplied phosphorus did not affect the proportion of root hairs or their length. Root hairs in pea, which were inoculated *P. bilaii* were on average 33.3% higher than for uninoculated plants. Beckie et al. [28] used the *P. bilaii* for inoculation alfalfa in combination with P-fertilizers and the results of the experiments show that the greatest response to inoculation occurred at the beginning of the growing season. In the year following vaccination yield of vaccinated alfalfa grown on average by 3% compared to uninoculated plants (Table 1).

Bacterial bioeffectors: Several promising bacterial representatives have been selected and described further in this section and in Table 2. There are selected fungi and their impact on crop production.

Pseudomonas spp.: *Pseudomonas* sp. is ubiquitous in agricultural soils, well adapted to growing in the rhizosphere. *Pseudomonas* well suited as biocontrol and growth-promoting agents [29]. These bacteria are a component biofertilizers, which use along with mineral fertilizers may serve as an effective approach for enhancing the crop nutrient requirements, thereby leading to the sustainable crop production. Biofertilizers consist of beneficial microbes, which form colonies in soils and promote plant growth by increasing nutrient availability when applied as a seed dressing or on plant surfaces. These microorganisms can enhance the availability of deficient or immobile nutrients in soils after solubilizing their mineral forms. For example, *Pseudomonas putida* can promote plant growth by P-solubilization, biological nitrogen fixation, availability of trace elements such as Fe and Zn and the production of plant growth regulators. Use of *P. putida* has improved the growth and yield of various crops such as bean, pea, rice, tomato and wheat. Therefore, use of this bacteria has been suggested as a sustainable solution for improving crop production. Factor *P. putida* either alone or in combination with addition of phosphorus improved the plant growth, plant uptake (N, P, K) and antioxidative activity [4]. Laboratory, greenhouse, and field experiments were conducted at University of KwaZulu-Natal, Pietermaritzburg, in the 2010/2012 seasons to study the effects of eight strains of diazotrophic bacteria on the growth and yield of maize. Maize seeds were treated with *Bacillus megaterium*, *Pseudomonas* sp. (strains B5, A3, A6, A61), *Burkholderia ambifaria*, *Enterobacter cloacae* and *Pantoea ananatis*, aiming to stimulate plant growth, and maintain or increase yields while reducing the need for N fertilization. All the diazotrophic bacteria increased germination of maize seed, and *Pseudomonas* sp. (B5) and *B. megaterium* significantly increased shoot length. *Pseudomonas* sp. (B5) and *Pseudomonas* sp. (A3) very significantly increased root length and seed vigor index. Seed treatments with selected diazotrophs resulted in increases in seed germination, but they caused no significant increases in grain yield, dry weight, plant height and chlorophyll content when compared to the untreated control. This may have been due to high competition from the indigenous soil microflora, given that success of microbial inoculation depends on the colonization and competitive ability of the inoculants. Plant roots exudates, colonization of roots by other bacteria, and soil health may also influence the efficiency of bacterial inoculations [30-32] conducted the positive effect of seed inoculation with diazotrophic bacteria on shoot dry weight and yield of maize has been reported by many researchers, for example Kifle and Laing [30]. The most closely related bacteria are *Pseudomonas fluorescens*. Knot et al. [33] reported the fact that application of *Pseudomonas* sp. increases germination of *Poa pratensis* seeds in laboratory conditions, especially 2-4 years old seeds. Also Fröhlich et al. [34] researched the positive effects of this product in growing barley. When *Pseudomonas* used in field conditions grain yield and weight of the straw increased. Also in the greenhouse conditions plants showed greater yield and better growth. Yusran et al. [9] reported that application of Proradix and RhizoVital (individually or in combination) into soil in pot trial led to improved state of tomato roots. They were healthy and showed significantly higher colonization by arbuscular mycorrhizal fungi.

***Pseudomonas jessenii*:** *P. jessenii* is a fluorescent, gram-negative bacteria and this bacteria was applied in two regions of Spain, Castilla y Leon and Andalucia was conducted study by Valverde et al. [35] with aim to find useful biofertilizers for staple grain-legumes, chickpea. In this study were made pot, greenhouse and field experiments, where was tested single and dual inoculations or in combination with phosphate fertilizer on chickpea growth. Under greenhouse conditions, plants inoculated with *P. jessenii* (strain PS06) yielded a shoot dry weight 14% greater than the uninoculated control treatment, but it was not correlated with shoot P contents. Dual inoculation of *P. jessenii* with *Mesorhizobium ciceri* resulted in a decrease in shoot dry weight with respect to the single M. ciceri inoculation. Under field conditions, plants inoculated with *M. ciceri*, in single or dual inoculation, produced higher nodule fresh weight, nodule number and shoot N content than the other treatments. Inoculation with *P. jessenii* had no significant effect on plant growth. However, the co- inoculation treatment ranked the highest in seed yield (52% greater than the uninoculated control treatment) and nodule fresh weight. These data suggest that *P. jessenii* can act synergistically with *M. ciceri* in promoting chickpea growth. Eltlbany and Smalla [36] conducted a study in which the effect was observed adding *Pseudomonas jessenii* (strain RU 47) and *Bacillus amyloliquefaciens* (strain FZB42) on the growth of plants in an environment of naturally occurring bacteria and fungal colonies on rhizosphere as well as in the surrounding soil with tomato and corn plants. A greenhouse experiment was conducted with two different kinds of plants (tomato and maize). The experiment consisted of three variants (control, *P. jessenii* and *B. amyloliquefaciens*), and each variant had four repetitions. Parameters evaluated were plant growth. *P. jessenii* increased the growth of tomato plants compared to control, while *B. amyloliquefaciens* increased the growth of maize plants. It was found that the both microorganisms was clearly influenced by rhizosphere bacterial composition.

***Bacillus amyloliquefaciens*:** *B. amyloliquefaciens* is gram-positive, aerobic, and endospore-forming bacteria, which have been both widely used as producers of commercial chemicals in industry [37-39], and beneficial agents for plant growth promotion and suppression of soil-borne diseases in agriculture. *B. amyloliquefaciens* produces many metabolites such as e.g. enzymes (chitinase, peroxidases and proteases), casein, elastin, gelatin, starch, nitrites, esculin and arbutin, phosphatases, adenine, cellulose, guanine, hypoxanthine, pectin, testosterone, tyrosine, and many types of antibiotics (eg. bacillomycins, fengycin, difficidin) and other substances [39-42]. Production of antibiotic inhibiting growth of fungal pathogens [13]. Proteins secreted by *B. amyloliquefaciens* (strain FZB42) protects plants against disease by eliciting innate immunity [43]. Furthermore Lagerlöf et al. [40], Talboys et al. [44], Fan et al. [45], Burkett-Cadena et al. [46] report that the *B. amyloliquefaciens* promotes plant growth, based primarily on the production of secondary metabolites suppressing competing microbial pathogens and the diseases occurring in the rhizosphere of plants. It also encourages root development and improves seed germination. It was found that lactic acid is the main component of maize root exudates, and that these acid and other root exudates are a source of carbon and energy for the *B. amyloliquefaciens*. Due to these properties, are often *B. amyloliquefaciens* used as a "bio-fertilizer" and as means of biological protection in agriculture. The bacteria also reduce the influence of abiotic stress conditions at the plant, such as drought, salinity or lack of nutrients in the plant [39-41,47,48]. He et al. [41] dealt in their study with influence of *B. amyloliquefaciens* inoculation on the growth of rice plants under stress conditions caused by salinity for 30 days. This study was based on the assumption that the use of microorganisms provides an alternative technology to improve the ability of stress tolerance in plants. Results of laboratory experiments have shown that the inoculated plants in comparison with the control plants, better growth of the above-ground parts of plants, but also parts of the root. Stimulating root growth and the effective root surface is important for a better water and nutrients uptake, which is the most important tool for coping with stress. Healthy, strong and large enough root system plays an important role in maintaining optimum growth and development under stress conditions. Analysis of this study showed, besides other things, that the presence of deaminase in bacteria mitigates the effect of salt on chlorophyll, thus supporting the growth of plants under stressful conditions caused by salinity was largely credited deaminase activity, which bacteria produce.

***Bacillus subtilis*:** *B. subtilis* is a ubiquitous gram-positive bacterium commonly found in water, soil, air, and decaying plant residues. However, the primary occurrence of bacteria found in soil [49,50]. The bacteria produce endospores, which enable it to endure and overcome extreme temperatures and dry periods. *B. subtilis* produces a series of proteases and another enzyme. This bacterium is considered a benign organism, as it has no properties that cause disease nor is pathogenic or toxigenic for humans, animals or plants [50,51]. *B. subtilis* can be used as part of a fertilizer usable in organic farming which is applied to a crop seed or directly into soil where colonize the rhizosphere. Although reports on extensive positive effects of this bacteria to the plant (growth, yield, disease resistance) have been published, these positive effects are not yet sufficiently verified [52]. Brutti et al. [53]

conducted study and used of plant growth-promoting rhizobacteria in tomato production. Before sowing, the micro-organisms were inoculated into the substrate. Tomato seedlings were grown using two different substrates. The first substrate was composed of 70% peat and 30% perlite by volume. And a second substrate with 20% peat, 20% perlite and 60% compost by volume, both inoculated with *Bacillus subtilis* or *Pseudomonas fluorescens* or Bioroot, which is a commercial product containing *B. subtilis*, *P. fluorescens*, *Trichoderma harzianum*, yeast, algae and Nocardia. Inoculation improved the leaf area, shoot dry weight, root dry weight, radical contact area, volume of roots and root forks compared with the control without inoculation. And so, inoculation can be recommended as an alternative to tomato seedling growers' dependence on synthetic agrochemicals. Because of low soil fertility is caused by continue crop and using chemical fertilizer. Altuhaish et al. [54] conducted field experiment and the aim of this research was to investigate the effect of biofertilizer, which contain *B. subtilis* dried by different methods and exposed to different period of storage on nutrient, growth and productivity of tomato plant grown under the field conditions. The result showed that viability of bacterium tended to decline during storagebut did not significantly reduce the effect on growth and production of plant. Application of biofertilizer increased total macro and micro nutrient absorption, vegetative growth and plant production. This research suggested that application of biofertilizer improve growth and production and there was no different effect between 0 and 3 months storage of biofertilizers on plant growth. A greenhouse experiment was conducted by Turan et al. [55] to observe the effects of *Bacillus megaterium* (strain TV-91C), *Pantoea agglomerans* (strain RK-92), and *B. subtilis* (strain TV-17C) inoculation on the growth, nutrient, and hormone content of cabbage seedlings. The seeds of cabbage were incubated two hours at 28 degrees C. The highest concentrations for N and P were recorded in *B. megaterium*, while in *B. subtilis* for Ca, Na, and Fe and in *P. agglomerans* for K, Mg, and Mn. The hormone content of cabbage seedlings was significantly affected by application of microorganisms treatments. *B. subtilis* decreased the abscisic acid content compared to the other treatments. Inoculation increased fresh and dry shoot and root weight, stem diameter, seedling height, chlorophyll reading values, and leaf area of cabbage seedlings compared with the control. Highest fresh and dry shoot and root dry weight, stem diameter, seedling height, and chlorophyll reading values of cabbage seedlings were obtained from *B. megaterium* and following *P. agglomerans* and *B. subtilis*.

***PaeniBacillus mucilaginosus*:** *P. mucilaginosus* is a bacterium which has been widely used in agriculture since 1990 as a biological fertilizer. These bacteria take part on the biogeochemical cycle of potassium, phosphorus and other elements. It is able to degrade insoluble soil minerals releasing nutrient ions (potassium and water-soluble phosphorus), useful for plants [55-59]. *P. mucilaginosus* is typical silicate bacteria, has long been used as a biofertilizer in agriculture and has recently shown potential in bioleaching and wastewater engineering [60]. *P. mucilaginosus* is often used in biological fertilizers for its ability of phosphorus and potassium mineralization, and also for the ability of nitrogen fixation [61]. Wang et al. [56] researched the effects of combined inoculation with arbuscular mycorrhizal fungi (*Rhizophagus intraradices*) and plant growth promoting rhizobacteria (*PaeniBacillus mucilaginosus*) on the growth of citrus seedlings under phosphorus deficient conditions have not been extensively studied. A pot experiment was performed to compare growth, root morphology, and other physiological variables in trifoliate orange (*Poncirus trifoliata*) seedlings that had been inoculated with *Rhizophagus intraradices*, *PaeniBacillus mucilaginosus* or both. Root length were also considerably improved by inoculation with dual inoculation however, taproot length was notably reduced by mycorrhizal inoculation. At treatment with zero phosphorus level, seedlings inoculated with a combination of *R. intraradices* and *P. mucilaginosus* yielded the greatest leaf chlorophyll concentrations and fine root activity, in comparison to those had either not been inoculated at all, or inoculated with just one of them. Combined inoculation increased plant height, stem diameter, shoot dry weight, and root dry weight. In addition, total N and P concentrations and uptake in seedlings were substantially improved both by individual and combined inoculation.

***Rhizophagus intraradices*:** *R. intraradices* is an arbuscular mycorrhizal fungus used as a soil inoculant in agriculture and horticulture. Mohamed et al. [62] realized project, which has investigated the early growth rate and establishment of cherry tomato plants as a model system inoculated with *R. irregularis*. After one month of growth, the number of leaves of mycorrhizal tomato seedlings was significantly increased and the height was approximately doubled in response to inoculation compared with non-inoculatedtomatoes. Colonna et al. [12] realized experiment, which had the aim was to assess the effect of two commercial inoculants containing arbuscular mycorrhizal fungi alone or arbuscular mycorrhizal fungi in combination with plant growth promoting bacteria (*Rhizophagus intraradices*) on yield components and quality of artichoke (*Cynara cardunculus* subsp. scolymus). Overall, inoculation of arbuscular mycorrhizal fungi or dual inoculation arbuscular mycorrhizal fungi and *R.intraradices* could be considered an effective and sustainable tool to improve yield components with less pronounced positive effects on quality of artichoke. Very often various plant components and extracts are added to the active microorganisms. One of the most widely used ingredients is seaweed. Next chapter describes in detail most commonly used seaweed species.

***Algae extracts*:** Algae extracts are used in crop production as an alternative to conventionally use fertilizers and plant protection. These components have several functions for plant: protection against a broad spectrum of plant diseases and pests, support of plant metabolism, enzyme production, food for positive organisms.

Extracts from seaweed can be a component of the so-called biostimulants, which can enhance the growth, yield, and quality of crops. Algal biostimulant provide added benefit to plants when applied by foliar spray or drenching. Seaweed extracts have been widely used as amendments in crop production systems due to the presence of a number of plant-growth-stimulating compounds. Extract is rich for many nutrients and other substances such as amino acids, vitamins, cytokinins, and auxin and abscisic acid like growth promoting substances and have been reported to stimulate the growth and yield of plants [63], enhance tolerance to environment stress [64], increase nutrient uptake from soil [65], enhance antioxidant properties, and increase activity against broad range of pathogenic viral, bacterial, and fungal diseases and enhanced resistance to insect attack [65,66]. The most known and used algae is *Ascophyllum nodosum*.

***Ascophyllum nodosum*:** *A. nodosum* is a brown seaweed, which is a rich source of phenolic compounds with antioxidant and antimicrobial properties. Algae is a good source of bioactive agents such as laminarin, sulfated polysaccharides, carotenoids, vitamins, minerals and polyphenols [67]. Extracts from seaweed *Ascophyllum nodosum* are intended for the specific plant organs (leaves and roots). Utilization is actual in food production in different regions of the world through their positive effect when applied into the soil, if necessary reduction of harmful bacteria, fungi, insects and parasites [68].

Discussion

From the agricultural industry perspective, they are considered as alternative organic fertilizers to conventional agrochemicals, new generation of competitive fertilizers and growth stimulants [69]. Rioux et al. [70] reported that extract from the seaweed *A. nodosum* and the chemical composition of these algae includes a high percentage of ash, proteins, lipids, polysaccharides, antioxidants, minerals and inorganic salts absorbed from seawater. Furthermore Michalak et al. [71] and Rayirath et al. [72] published that the extract of brown seaweed *A. nodosum* increases the resistance of plants against environmental influences (stress factors), such as drought, salinity and frost. Furthermore Kadam et al. [67] conducted, that *A. nodosum* is also used as fertilizer in the agriculture. Brown algae is a rich source of biologically active compounds, such as polysaccharides, peptides, omega-3 fatty acids, carotenoids, phenolic compounds, vitamins and minerals. One of many important polysacharides is laminarin, which is contained at 0% to 35% in Algae dry matter [67,69,71]. *A. nodosum* enhanced the growth of field crops, fruit crops and vegetable crops. These studies reported also an improved vegetative growth, chlorophyll content, fruit yield, sugar content and resistance against leaf and soil borne pathogens [69]. Michalak et al. [71] researched the influence of supercritical algal extracts on the growth and development of winter wheat (variety Akteur). As a raw material for the supercritical fluid extraction, the biomass of microalga *Spirulina plantensis*, brown seaweed - *Ascophyllum nodosum* and *Baltic green macroalgae* was used. It was found that the tested biostimulants did not influence statistically significantly the plant height, length of ear, and shank length. Crop height was similar in all the treated and the untreated plots. There were no significant differences in ear-bearing culms' and barren culms' number between the treated and the untreated plots. Tandon and Dubey [65] conducted study, where used formulation with is extracted from *A. nodosum* in soybean under field conditions. They investigated the appropriate dose of formulation in combination with NPK fertilizers and its effects on chlorophyll content, number of trifoliate leaves, number of pods, number of nodules, root length, yield, and other parameters under field conditions in soybean. Biozyme application greatly influenced number of trifoliate leaves, leaf area, and leaf area index. Also total chlorophyll content and total number of nodules per plant was significantly influenced after application. Conclusion of this study was, that use of biostimulants extracted from *A. nodosum* may optimize the use of chemical fertilizers, thereby reducing the impact of environment pollution and increasing the soil fertility. The use of such biostimulants must be combined with all available modern agronomic practices and it is one of the possible alternative strategies in agriculture, in the future with aim at maximizing the potential of a crop plant to boost crop production, crop quality. Sen et al. [73] used *A. nodosum* (granule or liquid sprays) in field experiments with wheat in combination NPK fertilizers. The application of two liquid sprayings in combination with fertilizers increases in the grain and straw yields, respectively, compared to the control more than 10% [74-80]. Liquid spraying of the seaweed extract stimulates metabolic processes in the leaf and helps the plant exploit nutrients in the leaf. Considerable proportion of photosynthesis is carried out by bacteria on the leaf surface and application of liquid sprays is activated by the liquid spray and the rate of photosynthesis increases as a consequence.

Conclusion

There have been severals studies conducted in research of lack of nutrients and bioeffectors application. Some authors report positive impact of bioeffectors application on plant. Other authors do not identify with it because they do not have enough results and confirming conclusions. Studies and experiments were performed under different conditions, with diferent preparation and their active ingredients with also different parameters observed. It is therefore important to further develop these alternative plant nutrition strategies in the future.

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