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; Microorganisms; 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 dependence 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, Pseudomonas, Paenibacillus and Rhizopogon 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 spp.: 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, molluscsides, 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]. Buyssens 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 to be 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 pea 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 up to 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

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Table 1: BEs as promoting fungi 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, soy, legumes and alfalfa. 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 Kuilack [25] demonstrated that the major acidic metabolites produced by *P. oxalic* 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] 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. Karanam 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 microorganisms 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

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| **Table 2:** BEs promoting bacteria of crop production. |
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 increased 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 Kille 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. Yuruan 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. Eltibany 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, nitrates, esculin and arbutin, phosphatases, adenosine, cellulose, guanine, hypoxanthine, pectin, testosterone, tyrosine, and many types of antibiotics (e.g. 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, in bacteria. B. 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]. Bruttì 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 storage but 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
toxicology. 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. irregularus. 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 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,
cytokins, 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]. Bioux 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 both seaweeds 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 polysaccharides 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 microalgae Spirulina platensis, 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 increased 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 several 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 different 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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References


promoting *Paenibacillus mucilaginosus* isolated from vegetable fields in Zhejiang. Annal Microbiol 64: 1745-1756.  