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Benefit from integrating zinc oxide nanoparticles with probiotic bacteria to produce strong Biofertilizer for increasing the production of agricultural crops, vegetables and fruits

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Abstract

In this research, new methods were found to produce a strong, In this study, zinc oxide useful and usable biofertilizer in any agricultural soil and with any agricultural crop, whether fruit or vegetables, which is Nanoparticles is combined and it is incubated and prepared with some important nutrients for the growth and reproduction of the probiotic bacteria microorganisms present together to produce a biofertilizer and organic fertilizer rich in important nutrients for plant growth and increased production of agricultural crops, A mixed and combined nutritional medium of many materials and vegetables and fruits. nutrients suitable for the purpose of growing many living microorganisms such as bacteria and fungi and controlling the growth rates of microorganisms with the percentage of food present in the biofertilizer (F/M Ratio).It was reached to increase the efficiency of agricultural fertilizer production, the work of biological fertilizers, and the production of a new strong and effective biological product in increasing the production of agricultural crops and supplying the plant with what it needs from important nutrients to increase growth, early production and improve the quality of agricultural soil. The main goal of adding many food media is the multiplicity and diversity of the carbon source, the multiplication of the growth of microbial isolates, the increase in their numbers, the reduction of growth, reproduction and regeneration quickly, and to reach an increase in the efficiency and effectiveness of biological fertilizer for soil and plants together, as it is considered an integrated nutrient medium. Biofertilizers, a sustainable ecofriendly agricultural approach to crop improvement is used to supplement chemical fertilizers mainly to maintain soil fertility. Continuous application of expensive chemical fertilizers causes reduction of organic matter content in soil and also microbial activity drastically. Biofertilizers are organic, bio- degradable. They contain micro-organisms, provide nutrients viz., N, P, K and other nutrients, antibiotics, hormones like auxins, cytokinin, vitamins which enrich root rhizosphere. The present article highlights biofertilizer mediated crop functional such as plant growth and productivity, nutrient profile, plant protection and there by crop improvement. The knowledge gained from the literature appraised here in will help us to understand the physiological bases of biofertilizers towards sustainable agriculture in reducing problems associated with the use of chemicals fertilizers. Therefore, there is an urgent need to adapt biological sciences applications in agriculture field. Biotechnology is an amalgamation of variety of disciplines- molecular biology, bioinformatics, biochemistry, genetics and microbiology. The usage of combinations of these disciplines in agricultural field leads to generation of biotech crops with increased yield and enhanced quality. Agriculture biotechnology not only upgrades the quality but also utilizes the resources and livestock for the well-being of animals and wild plants. Phosphorus, Probiotic bacteria with yoghurt and sugar Charcoal sodium chloride and some other nutrients such as flour and starch are mixed with zinc oxide nano particles to produce a bio-fertilizer fully of nutrients necessary for plant growth, increase the production of agricultural crops, which improve the quality characteristics of agricultural soil, treat stress and poor production and some agricultural pests that may negatively affect plant growth and work to reduce the rate of increase of agricultural crops, vegetables and fruits , 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 mobilization 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.

Keywords:

Bio-fertilization, biological control, Biocidal and fungicide, ZnO nanoparticles, plant growth hormone.

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1. Introduction:

The agricultural applications of probiotics with regard to animal, fish, and plants production have increased gradually. However, a number of uncertainties concerning technological, microbiological, and regulatory aspects exist [3]. Probiotics are live microbes that can be formulated into many different types of products, including foods, drugs, and dietary supplements. Probiotic is a relatively new word that is used to name the bacteria associated with the beneficial effects for the humans and animals. The term probiotic means ‘‘for life’’ and it was defined by an Expert Committee as ‘‘live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition’’ [4]. FAO/WHO Expert Consultation believes that general guidelines need to provide to how these microorganisms can be tested and proven for safety and potential health benefits when administered to humans. Lactobacillus and Bifidobacterium are most commonly used probiotics in food and feed (Table 1). Other microorganisms such as yeast Saccharomyces cerevisiae and some Escherichia coli and Bacillus species are also used as probiotics. Lactic acid bacteria (LAB) which have been used for food fermentation since the ancient time, can serve a dual function by acting as food fermenting agent and potentially health benefits provider. LAB are GRAS (general recognized as safe) with no pathogenic, or virulence properties have been reported. For the use of LAB as probiotics, some desirable characteristics such as low cost, maintaining its viability during the processing and storage, facility of the application in the products, resistance to the physicochemical processing must be considered. Several studies and experiments are focused on impact of bio effectors’ 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. The study begins with collecting samples, then isolating and purifying some microbes that can be cultured from the samples that have been collected, followed by a survey of all the isolates obtained to find out their ability to synthesize some nanoparticles, and then selecting and identifying isolates with promising positive results after that. Determining the optimal conditions for improving the production process, along with characterizing the nanoparticles that have been produced by some spectroscopy, and finally, some medical and environmental applications expected for these nanoparticles Zinc oxide has been used because of its importance in important applications agricultural field When it is mixed with some important microorganisms such as probiotic bacteria, carbon source and important food media for the growth and reproduction of microorganisms useful in plant growth and increase the production of agricultural crops.

Table 1 : probiotic microorganism. Adapted from 5,6

Lactobacillus species	Bifidobacterium species	Others
L. acidophilus	*B. adolescentis*	*Bacillus cereus*
L. amylovorus	*B. animalis*	*Clostridium botyricum*
L. brevis	*B. breve*	*Enterococcus faecalis*[a]
L. casei	*B. bifidum*	*Enterococcus faecium*[a]
L. rhamnosus	*B. infantis*	*Escherichia coli*
L. crispatus	*B. lactis*	*Lactococcus lactis* subsp. *cremoriss*
L. delbrueckii subsp. *bulgaricus*	*B. longum*	*Lactococcus lactis* subsp. *lactis*
L. fermentum		*Leuconostoc mesenteroides* subsp. *dextranicum*
L. gasseri		*Pediococcus acidilactici*
L. helveticus		*Propionibacterium freudenreichii*[a]
L. johnsonii		*Saccharomyces boulardii*
L. lactis		*Streptococcus salivarius* subsp. *thermophilus*
L. paracasei		*Sporolactobacillus inulinus* [a]
L. plantarum		
L. reuteri		
L. salivarius		
L. gallinarum[a]		

Characteristics of probiotics

Characteristics of probiotics will determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period. Probiotics are safe for human consumption and no reports have found on any harmfulness or production of any specific toxins by these strains [7, 8]. In addition, some probiotics could produce antimicrobial substances like bacteriocins. Therefore, the potential health benefit will depend on the characteristic profile of the probiotics. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes [9], and The most common temporarily increase the rate of mitosis in enterocytes [10, 11]. probiotics are Lactobacillus and Bifidobacterium. In general, most probiotics are gram- positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains [7]. They are non-flagellated, non-motile and non-spore-forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as L. casei prefer 30 °C and the optimum pH for initial growth is 6.5-7.0 [7]. L. acidophilus is microaerophilic with anaerobic referencing and capability of aerobic growth. Bifidobacterium are anaerobic but some species are aero-tolerant. Most probiotics bacteria are fastidious in their nutritional requirements [12, 13]. With regard to fermentation probiotics are either obligate homofermentative (ex. L. acidophilus, L. helvelicas), obligate heterofermentative (ex. L. brevis, L. reuteri), or facultative heterofermentative (ex. L. casei, L. plantarum) [14]. Additionally, probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide, and a variety of bacteriocins [15, 16]. Probiotics should have the ability to interact with the host microflora and competitive with

Probiotic .microbial pathogens, bacterial, viral, and fungal [16]. Probiotics health benefits research suggests a range of potential health benefits to the host organism. The potential effects can only be attributed to tested strains but not to the whole group of probiotics. Probiotics have shown to provide a diverse variety of health benefits to human, animal, and plans. However, viability of the microorganisms throughout the processing and storage play an important role in transferring the claimed health effects. Therefore, the health benefits must be documented with the specific strain and specific dosage [17].

Plant health:

The more beneficial the bacteria and fungi are, the more "fertile" the soil is. These microorganisms break down organic matter in the soil into small, usable parts that plants can uptake through their roots. The healthier the soil, the lower the need for synthetic herb/pesticides and fertilizers.The concept that certain microorganisms 'probiotics' may confer direct benefits to the plant acting as biocontrol agents for plants. The plant probiotic bacteria have been isolated and commercially developed for use in the biological control of plant diseases or biofertilization [38]. These microorganisms have fulfilled important functions for plant as they antagonize various plant pathogens, induce immunity, or promote growth [38-40]. The interaction between bacteria and fungi with their host plants has shown their ability to promote plant growth and to suppress plant pathogens in several Milk and its products is good vehicle of probiotic strains due to its inherent studies [41-44]. properties and due to the fact that most milk and milk products are stored at refrigerated temperatures. Probiotics can be found in a wide variety of commercial dairy products including sour and fresh milk, yogurt, cheese, etc. Dairy products play important role in delivering probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability [45-48]. Several factors need to be addressed for applying probiotics in dairy products such as viability of probiotics in dairy [19, 48], the physical, chemical and organoleptic properties of final products [49-51], the probiotic health effect [52, 53], and the regulations and labeling issues [4, 54]. Yogurt is one of the original sources of probiotics and continues to remain a popular probiotic product today. Yogurt is known for its nutritional value and health benefits. Yogurt is produced using Streptococcus salivarius subsp. thermophilus a culture of L. delbrueckii subsp. bulgaricus and bacteria. In addition, other lactobacilli and bifidobacteria are also sometimes added during or after culturing yogurt. The probiotic characteristics of these bacterial strains that form the yogurt culture are still debatable. The viability of probiotics and their proteolytic activities in yoghurt must be considered. Numerous factors may affect the survival of Lactobacillus and Bifidobacterium spp. in yogurt. These include strains of probiotic bacteria, pH, presence of hydrogen peroxide and dissolved oxygen, concentration of metabolites such as lactic acid and acetic acids, buffering capacity of the media as well as the storage temperature [19, 66, Although yogurt has been widely used as probiotics vehicle, most commercial yogurt 67]. products have low viable cells at the consumption time [19, 68]. Viability of probiotics in yogurt depends on the availability of nutrients, growth promoters and inhibitors, concentration of solutes, inoculation level, incubation temperature, fermentation time and storage temperature. Survival and viability of probiotic in yogurt was found to be strain dependant. The main factors for loss of viability of probiotic organisms have been attributed to the decrease in the pH of the medium and accumulation of organic acids as a result of growth and fermentation. Among the factors, ultimate pH reached at the end of yogurt fermentation appears to be the most important factor affecting the growth and viability of probiotics. Metabolic products of organic acids during storage may further affect cell viability of probiotics [66]. The addition of fruit in yogurt may have negative effect on the viability of probiotics, since fruit and berries might have antimicrobial activities. Inoculation with very high level of probiotics with attempts to compensate the potential viability loss, might result in an inferior quality of the product. The present of probiotic was found to affect some characteristics of yogurt including: acidity, texture, flavor, and appearance [69]. However, encapsulation in plain alginate beads, in chitosancoated alginate, alginate-starch, alginate-prebiotic, alginate-pectin, in whey protein-based matrix, or by adding prebiotics or cysteine into yogurt, could improve the viability and stability of probiotics in yogurt [70-79].

Plant Probiotic Microorganisms:

Today, world population increase, soil degradation, environmental contamination, and climate change affect agriculture and forestry, which are crucial activities for human and animal survival [1]. This has led to plant probiotic microorganism (PPM)-based product development, which is an alternative to biofertilizers, biopesticides, and phytoremediation [3]. Additional new sustainable agriculture concepts, using available environmental resources [4], are being developed. PPM are beneficial microorganisms that co-evolved with plants in either a symbiotic or free-living association. This association mainly occurs in the soil, but there are other association types, such as microalgae-associated bacteria [5]. Root system soil environments have a high microbial presence due to rhizodeposits and root exudates. Some of these microbes can support their hosts, which stimulates plant growth, reduces pathogen infection, increases yield, and reduces biotic or abiotic plant stresses such as salt stress [3,5–9]. Soil microbial populations consist of plant growth-promoting rhizobacteria, plant disease-suppressive bacteria and fungi, N2-fixing cyanobacteria, actinomycetes, and soil toxicant-degrading microbes, among others [4]. Fungi are another highly studied PPM group with important functions. For example, endophytic fungi like Exophiala sp. are phytohormone secretors and can improve plant growth under abiotic stresses [12,13,14]. Trichoderma strains have also been studied; Palma et al [15] identified molecular mechanisms that are activated during the in vitro interaction between tomatoes (Solanum lycopersicum L.) and the strain Trichoderma longibrachiatum MK1. The results reveal the enrichment of cell defense/stress and primary metabolism categories and promoted changes on secondary metabolism and transport.

Agricultural applications of probiotics:

Probiotics applications have been extended from human applications to diversity of A strong growing agricultural application. Agricultural applications include animal and plants. market for plant probiotics for the use in agricultural biotechnology has been shown worldwide with an annual growth rate of approximately 10%. Based on the mode of action and effects, the plant probiotics products can be used as biofertilizers, plant strengtheners, phytostimulators, and biopesticides [38]. Berg has reported several advantages of using plant probiotics over chemical pesticides and fertilizers including: more safe, reduced environmental damage, less risk to human health, much more targeted activity, effective in small quantities, multiply themselves but are controlled by the plant as well as by the indigenous microbial populations, decompose more quickly than conventional chemical pesticides, reduced resistance development due to several mechanisms, and can be also used in conventional or integrated pest management systems [38]. Plant growth promotion can be achieved by the direct interaction between beneficial microbes and their host plant and also indirectly due to their antagonistic activity against plant pathogens. Several model organisms for plant growth promotion and plant disease inhibition are well-studied including: the bacterial genera Azospirillum [44, 135], Rhizobium [136], Serratia [137], Bacillus [138, 139], Pseudomonas [140, 141], Stenotrophomonas [142], and Streptomyces Several [143] and the fungal genera Ampelomyces, Coniothyrium, and Trichoderma [144].

mechanisms are involved in the probiotics-plant interaction. It is important to specify the mechanism and to colonize plant habitats for successful application. Steps of colonization include recognition, adherence, invasion, colonization and growth, and several strategies to establish interactions. Plant roots initiate crosstalk with soil microbes by producing signals that are recognized by the microbes, which in turn produce signals that initiate colonization [43, 51]. Colonizing bacteria can penetrate the plant roots or move to aerial plant parts causing a decreasing in bacterial density in comparison to rhizosphere or root colonizing populations [43]. Furthermore, in the processes of plant growth, probiotic bacteria can influence the hormonal balance of the plant whereas phytohormones can be synthesized by Besides these the plant themselves and also by their associated microorganisms [38]. mechanisms, probiotic bacteria can supply macronutrients and micronutrients. They metabolize root exudates and release various carbohydrates, amino acids, organic acids, and other compounds in the rhizosphere [43]. Bacteria may contribute to plant nutrition by liberating phosphorous from organic compounds such as phytates and thus indirectly promote plant growth [145]. Furthermore, probiotic can reduce the activity of pathogenic microorganisms through microbial antagonisms and by activating the plant to better defend itself, a phenomenon termed “induced systemic resistance” [146, 147]. Microbial antagonism includes the inhibition of microbial growth, competition for colonization sites and nutrients, competition for minerals, and degradation of pathogenicity factors [38, 43]. In Japanese composting, at least three groups of compositing bacteria were used individually, or in combination. The following species were used: Bacillus bacteria groups, Lactic acid bacteria groups and Actinomycetous groups. These bacteria species can protect plant products from cropping hazards. They do this by expelling against various bad worms and insects, such as nematodes with potatoes and some types of insects with soybeans and maize. They are also effective in controlling fungi such as powdery mildew, downy mildew, phythium (damping off with many plants), plasmodipophora brosscae (club-root with the cabbage Jamily); Crucijert1e (plants. and fusarium of wilt with tomato and banana) [148].

The word “nano” comes from the Greek for .Nanotechnology, encapsulation, and probiotics "dwarf". A nanometer is a thousandth of a thousandth of a thousandth of a meter (10-9 m). Nanoparticles are usually sized below 100 nanometers which will enable novel applications and benefits. Nanotechnology of probiotics is an area of emerging interest and opens up whole new possibilities for the probiotic's applications. Their applications to the agriculture and food sector are relatively recent compared with their use in drug delivery and pharmaceuticals. The basic of probiotic nanotechnology applications is currently in the development of nano-encapsulated probiotics. The nanostructured food ingredients are being developed with the claims that they offer improved taste, texture and consistency. Applications of nanotechnology in organic food production require precaution, as little is known about their impact on environment and human health. Some recent food applications of nanotechnology, safety and risk problems of nanomaterials, routes for nanoparticles entering the body, existing regulations of nanotechnology in several countries, and a certification system of nanoproducts were reported [168, 169]. Currently, no regulations exist that specifically control or limit the production of nanosized particles and this is mainly owing to a lack of knowledge about the risks [169]. Nanoencapsulation is defined as a technology to pack substances in miniature using techniques such as nanocomposite, Nano emulsification, and nano structuration and provides final product functionality and control the release of the core [170]. Encapsulation of food ingredients may extend the shelf life of the product. Nanoencapsulation of probiotic is desirable technique that could deliver the probiotic bacteria to certain parts of the gastrointestinal tract where they interact with specific receptors [170]. These nanoencapsulated probiotic bacterial may also act as de novo vaccines, with the capability of modulating immune Microencapsulation with alginate can be applied to many different responses [171]. probiotic strains and results show better survival than free cells at low pH of 2.0, high bile salt concentrations, and moderate heat treatment of up to 65 ◦C [156]. Microencapsulation may prove to be an important method of improving the viability of probiotic bacteria in acidic food products and help deliver viable bacteria to the host's gastrointestinal tract. Furthermore, microencapsulation appeared to be effective in protecting cells from mild heat treatment and thus could stimulate research in functional food products that receive a mild heat treatment [156]. The microencapsulation allows the probiotic bacteria to be separated from its environment by a protective coating. Several studies have reported the technique of the microencapsulation by using gelatin, or vegetable gum to provide protection to acid-sensitive Bifidobacterium and Lactobacillus [172-176].

2. Experimental:

2.1 Materials and methods: .

Soil and banana leaf samples used for experiments were taken from the same location to estimate some elements. The experience of using the microbial solution (biological fertilizer) on the crop of watching live fertilization of the biological fertilizer and its effect on soil and plant (Banana plant and its leaves).

2.2 ZnO nanoparticle synthesis

0.6 M aqueous solution of Zn (NO3)2·6H2O and 1 M of NaOH were prepared. After the zinc nitrate hexahydrate was completely dissolved, 1 M NaOH was slowly adding drop by drop for 25 minutes with high magnetic stirring. The process was permitted to continue for 1 hour after the aqueous solution of NaOH was added, and the container was sealed at this temperature for 1 hour. Afterward, the sample was transferred to settle for an overnight period before the resultant liquid was carefully separated. The precipitate was removed after 15 minutes of centrifugation. ZnO NPs were precipitated and rinsed four times with the double distilled water and ethanol before being dried in an air environment at roughly 90° C. Zn (OH)2 was totally oxidized to produce ZnO NPs. The existence of nanoparticles and other functional groups was determined by Fourier transform infrared spectroscopy (FT-IR). The size, shape, optical, and structural properties of the produced ZnO NPs were all measured. An X-ray diffractometer (analytical) was used to record the X-ray diffraction (XRD) pattern of manufactured ZnO NPs using Cu-K radiation with a wavelength of (λ = 0:1541 nm) in the scan range of 2theta = 10° –80° . A scanning electron microscope (SEM) with (EDXA, SIRION) for the morphology of the specimen was examined using compositional analysis of generated ZnO NPs.

2.3. Preparation of ZnO with microbial solution (As a food medium) for growth of microorganisms.

1.0 g of ZnO nanoparticles were refined by 10 L deionizing water dispersion and ultrasonication for 30 min and mixing with different microbial types17. In the presence of many important nutrients for the growth of probiotic bacteria and Saccharomyces cerevisiae such as sugar - yogurt - sodium chloride - ground charcoal - flour - citric acid - sodium carbonate - agricultural sulfur - mineral salts used to feed poultry and sheep – vinegar.

2.4 The effects biofertilizer priming on in increasing crop production and improving soil quality.

Biofertilizer was used for in increasing the production of agricultural crops, vegetables and by the following method. The recommended dose of fruits and improving soil quality biofertilizer is delivered to work efficiently, 4 litters per acre in each irrigation for all types of agricultural crops, whether by spraying or dripping with irrigation water every time for all of the crops.

2.5 The effects biofertilizer priming Effects of Biofertilizer Preparation on the Production of Growth Regulators and Treatment of Plant Diseases, Nematodes:

Biofertilizer was used for Secretion of growth regulators that increase the production of agricultural crops, enlarge the size of the fruit, increase weight, speed of maturity and increase flowering in plants and vegetable and fruit crops It also has the ability to treat some plant diseases, root rot, nematodes, downy mildew, powdery mildew, stress treatment and nutrient deficiency.

3. Results and discussion:

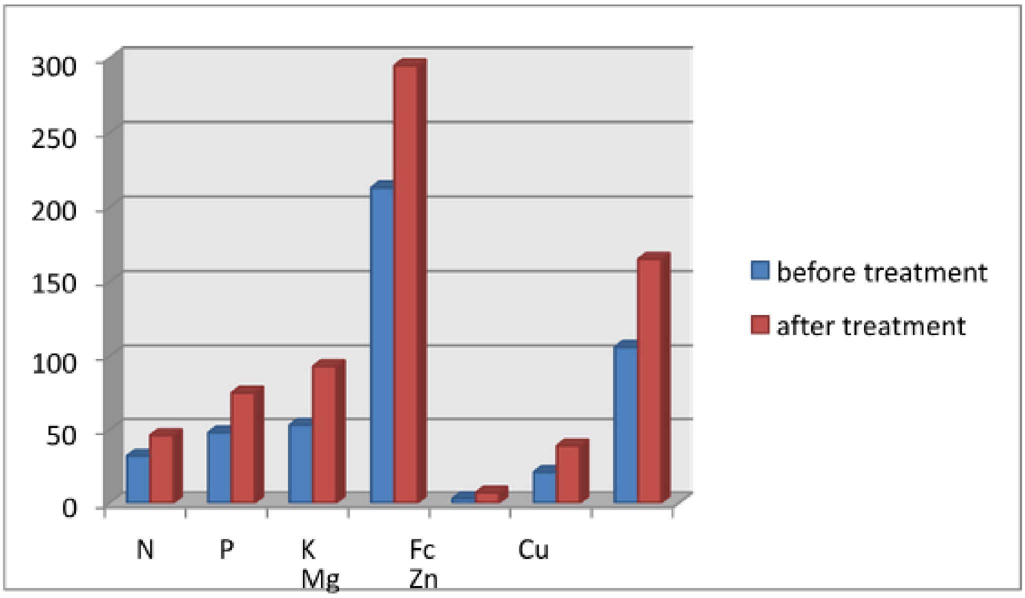
3.1 : Sample No. (1) - the experience of using the compound (biological fertilizer - nematode) on the crop of watching live fertilization of the biological fertilizer and its effect on soil and plants:

Estimation of soft and large elements in soil mg / sq.m Before treatment

Sample	Zn	Mg	Cu	Fc	K	P	N
Existing	106	21	3	213	53	48	32

Estimation of soft and large elements in soil mg / sq.m After treatment

Sample	Zn	Mg	Cu	Fc	K	P	N
Existing	165	39	7.0	295	93	75	46

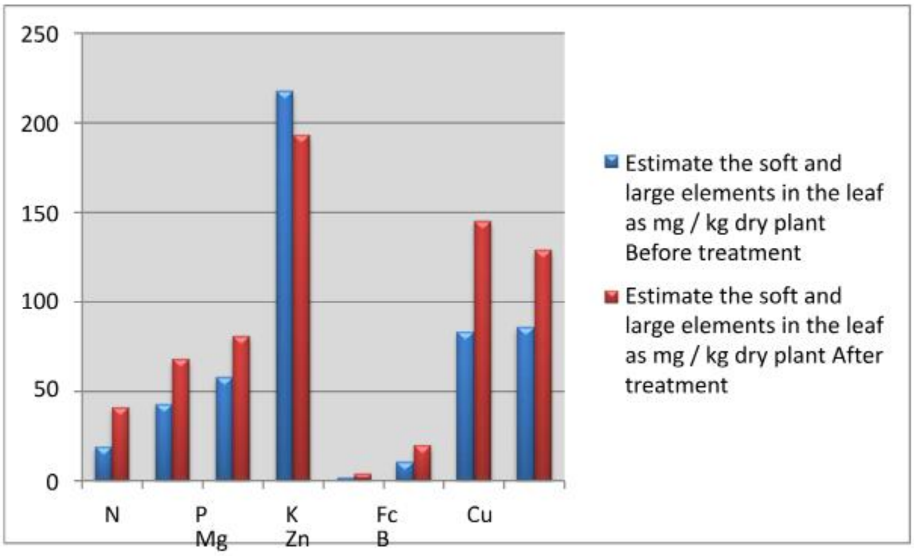


Estimate the soft and large elements in the leaf as mg / kg dry plant Before treatment

Sample	B	Zn	Mg	Cu	Fc	K	P	N
Existing	86	83	11.0	1.8	218	58	43	19

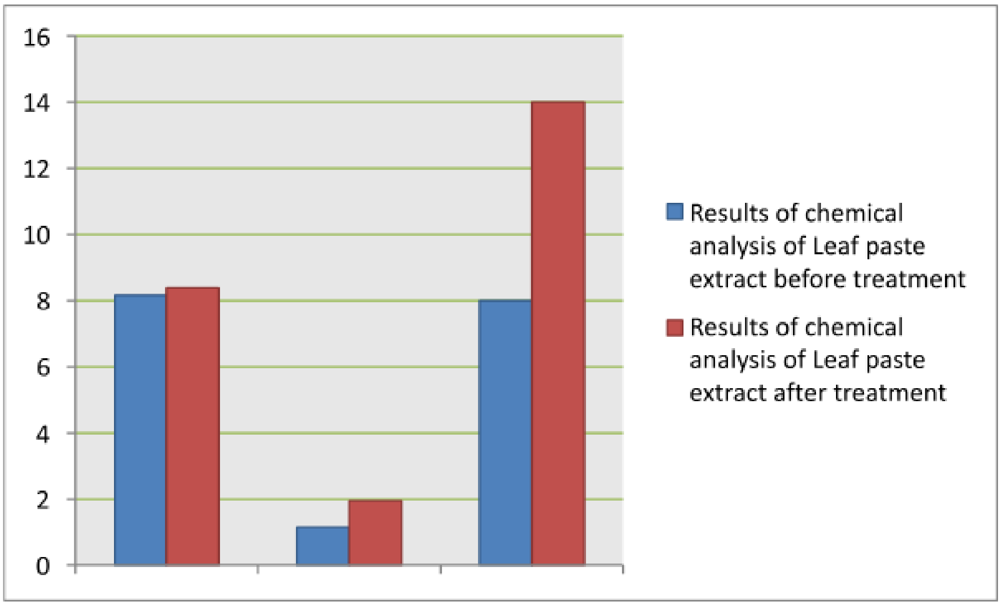
Determination of macro- and micro-facilitator elements in the leaf mg/kg dry plant After the transaction:

Sample	B	Zn	Mg	Cu	Fc	K	P	N
Existing	129	145	19.6	3.6	193	81	68	41



Results of chemical analysis of soil paste extract before treatment (before sample application)

Sp		Ec		PH		Sample
Existent	Optimal	Existent	Optimal	Existent	Optimal	
8.0	32	3.21	5.28	8.38	7.57	Soil
9.3	24	1.14	2.26	8.16	7.7	leaf



Results of chemical analysis of soil paste extract after treatment:

Sp		Ec		PH		Sample
Existent	Optimal	Existent	Optimal	Existent	Optimal	
14	32	4.12	5.28	8.38	7.57	Soil
11.8	24	1.93	2.26	8.0	7.7	leaf

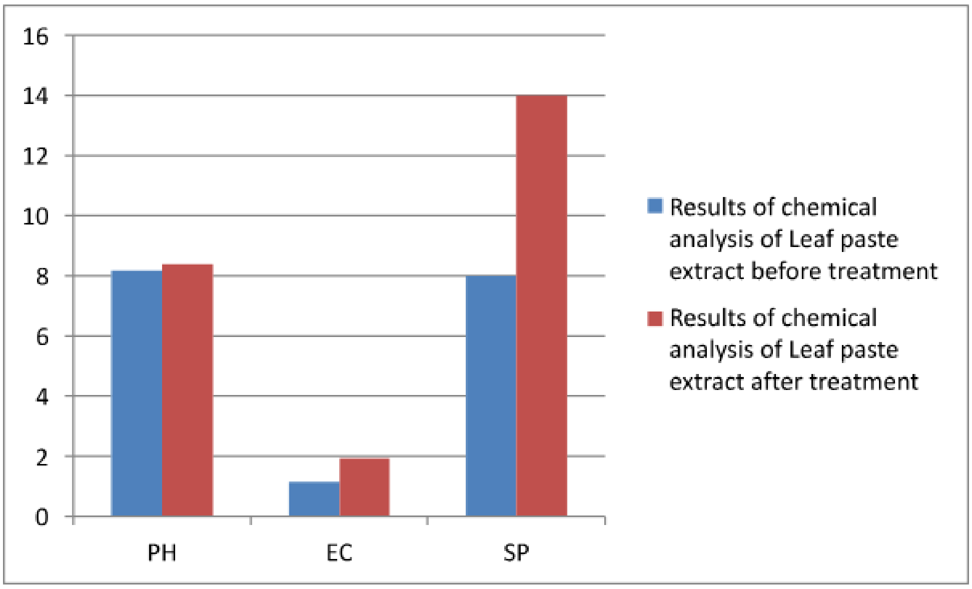
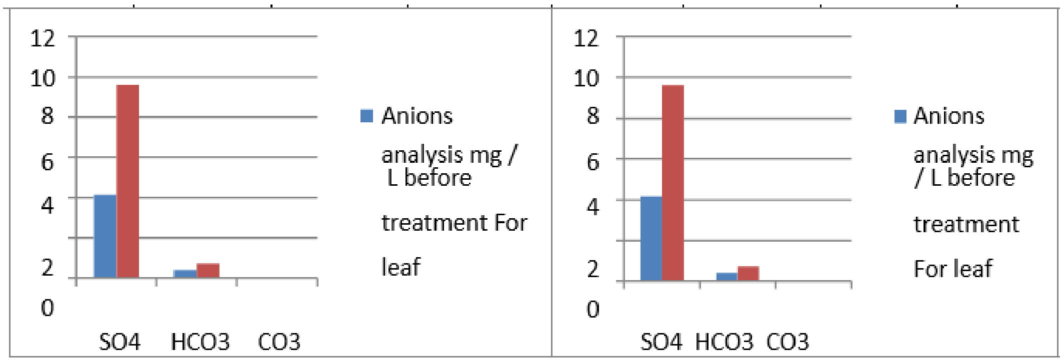


Table of anions analysis mg / L before treatment:

SO4		HCO3		CO3		Sample
Existent	Optimal	Existent	Optimal	Existent	Optimal	
8.9	32.50	0.65	1.65	3.5	0	Soil
4.16	22.50	0.43	1.15	-	0	leaf

Table of anions analysis mg / L after treatment:

SO4		HCO3		CO3		Sample
Existent	Optimal	Existent	Optimal	Existent	Optimal	
14.3	32.50	0.68	1.65	2.96	0	Soil
9.6	22.50	0.72	1.15	-	0	leaf



Cation analysis table, mg / l before treatment:

K		NA		Mg		CA		Sample
Existent	Optimal	Existent	Optimal	Existent	Optimal	Existent	Optimal	
3.2	5.0	11.2	16.50	7.2	10	21	29	Soil
2.0	5.15	2.3	4.6	3.6	7.8	7.3	12	leaf

Cation analysis table, mg / l after treatment:

K		NA		Mg		CA		Sample
Existent	Optimal	Existent	Optimal	Existent	Optimal	Existent	Optimal	
2.1	5.0	9.4	16.50	4.3	10	18	29	Soil
1.4	5.15	1.8	4.6	2.1	7.8	5.6	12	leaf

Estimation of soft and large elements in soil mg / sq.m.Before the transaction:

B	Zn	Mg	Cu	Fc	K	P	N	Sample
215	222	42	12	808	266	103	64	Optimal
95	118	29	3.2	306	118	73	53	Optimal

Estimation of soft and large elements in soil mg / sq.m. After the transaction

B	Zn	Mg	Cu	Fc	K	P	N	Sample
215	222	42	12	808	266	103	64	Optimal
99	118	33	3.2	366	146	73	55	Optimal

Estimate the soft and large elements in the leaf as mg / kg dry planBefore the transaction:

B	Zn	Mg	Cu	Fc	K	P	N	Sample
200-180	180-150	18-16	14-12	750-650	210-200	100-97	80-60	Optimal
71	96	11.5	2.6	108	96	61	35	Optimal

Estimate the soft and large elements in the leaf as mg / kg dry plantAfter the transaction:

B	Zn	Mg	Cu	Fc	K	P	N	Sample
200-180	180-150	18-16	14-12	750-650	210-200	100-97	80-60	Optimal
71	96	11.5	2.8	108	103	66	43	Optimal

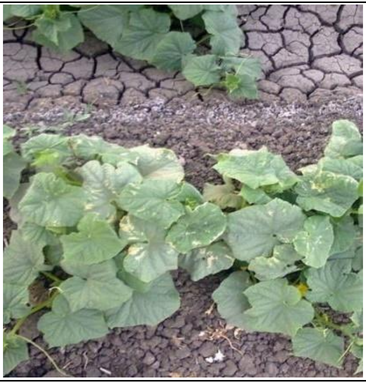
3.1 The use of biofertilizer to increase production, enlarge the size of the fruit and increase flowering:

The study begins with collecting samples from the surrounding environment of the plant and the laboratories of the Faculty of Agriculture, Minia University, then isolating and purifying some microbes that can be cultured from the samples that have been collected, followed by a survey of all the isolates obtained to find out their ability to synthesize some nanoparticles, and then selecting and identifying isolates with promising positive results after that. Determining the optimal conditions for improving the production process, along with characterizing the nanoparticles that

have been produced by some spectroscopy, and finally, some medical and Zinc oxide has been used environmental applications expected for these nanoparticles because of its importance in important applications, whether in the agricultural field or wastewater treatment. Among the most important actual and field observations also of the biological fertilizer when designing experiments in a scientific way on the basis of scientific research and the use of agricultural repeaters after the successful use of random methods on a wider scale in different areas and multiple agricultural areas with different types of seeds and agricultural crops to obtain useful agricultural results and more accurate and important and present research Distinguished scientific and realistic study through actual field and practical application. The main goal of adding many food media is the multiplicity and diversity of the carbon source, the multiplication of the growth of microbial isolates, the increase in their numbers, the reduction of growth, reproduction and regeneration quickly, and to reach an increase in the efficiency and effectiveness of biological fertilizer for soil and plants together, as it is considered an integrated nutrient medium. Biofertilizers, a sustainable eco- friendly agricultural approach to crop improvement are used to supplement chemical fertilizers mainly to maintain soil fertility. Continuous application of expensive chemical fertilizers causes reduction of organic matter content in soil and also microbial activity drastical- ly. Biofertilizers are organic, bio-degradable. They contain micro-organisms, provide nutrients viz., N, P, K and other nutrients, antibiotics, hormones like auxins, cytokinins, vitamins which enrich root rhi- zosphere. The present article highlights biofertilizer mediated crop functional such as plant growth and productivity, nutrient profile, plant protection and there by crop improvement. The knowledge gained from the literature appraised here in will help us to understand the physiological bases of biofertilizers towards sustainable agriculture in reducing problems associated with the use of chemicals fertilizers .Therefore, there is an urgent need to adapt biological sciences applications in agriculture field. Biotechnology is an amalgamation of variety of disciplines- molecular biology, bioinformatics, biochemistry, genetics and microbiology. The usage of combinations of these disciplines in agricultural field leads to generation of biotech crops with increased yield and enhanced quality. Agriculture biotechnology not only upgrades the quality but also utilizes the resources and livestock for the well-being of animals and wild plants. Some of the application of agriculture biotechnology encompasses genetic engineering, plant and animal tissue culture technology, hybridization, bioprocess and fermentation technology, gene selection through mutagenesis and biosensors for biological monitoring. New information technologies such as electronic communication systems, data processing and automation are gaining tremendous attention in order to improve the quality and efficiency of the farm work. This study mainly focuses on the improvisation and development of new varieties of crop plants through biofertilizers included in agriculture biotechnology And the use of nanotechnology technology in field experiments, the expansion of the use of biological fertilizers and new applications using nanotechnology compounds, and the importance of biotechnology in increasing agricultural crops on different agricultural crops in different regions and different governorates .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 mobilization 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.

Figures for the effect of bio-enriching use:

Dates	Pomegranate plant	Pomegranate plant
		
Cucumber plant Treatment downy mildew and powdery mildew		**Cucumber plant increased flowering**



Grape plant increase size, weight and cluster length



Microbial isolation and preparation of bio control agent

Biofertilizer preparation

Microbial inoculation

Microbial inoculation



Some types of nematodes and root roots diseases

Study of the effect of biological fertilizer on nematodes, plant pathogens and root rot

Conclusion:

This study mainly focuses on the derivation and development of new varieties of biofertilizers and agricultural fertilizers by mixing some beneficial microorganisms and zinc oxide nanoparticles to produce biofertilizers included in agricultural biotechnology and using this product in field experiments, expanding the use of biofertilizers and clarifying the role and importance of biotechnology in increasing the production of agricultural crops, vegetables and fruits on various agricultural crops in different regions and different agricultural soils .The uses of probiotics and their applications have shown tremendous increase in the last two decades. Probiotics can turn many health benefits to the human, animals, and plants. Applications of probiotics hold many challenges. In addition to the viability and sensory acceptance, it must be kept in mind that strain selection, processing, and inoculation of starter cultures must be considered. Probiotics industry also faces challenges when claiming the health benefits. It cannot be assumed that simply adding a given number of probiotic bacteria to a food product will transfer health to the subject. Indeed, it has been shown that viability of probiotics throughout the storage period in addition to the recovery levels in the gastrointestinal tract are important factors [3, 48, 83]. For this purpose, new studies must be carried out to: test ingredients, explore more options of media that have not yet been industrially utilized, reengineer products and processes, and show that lactose-intolerant and vegetarian consumers demand new nourishing and palatable probiotic products.

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