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Review Article

Development of Bio-Fertilizers and its Future Perspective

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Abstract: Global warming and climate change have resulted in unexpected drought, stormy rainfalls, extremely high temperature, cold damage and hurricanes in many places around the world where such disastrous tragedy had never occurred in the past decades. Establishing an environmental friendly co-existing mechanism on earth is of vital importance. In recent years, agrochemicals are extensively applied to obtain higher yield. Intensive application of agrochemicals leads to several agricultural problems and poor cropping systems. Farmers use more chemical fertilizers than the recommended levels for many crops. Excessive use of chemical nitrogen fertilizer not only accelerates soil acidification but also risks contaminating groundwater and the atmosphere. The extensive research program on beneficial bacteria and fungi has resulted in the development of a wide range of bio-fertilizers, which satisfied the nutrient requirements of crops and increased the crop yield as well. Many experiments in greenhouses and in field conditions have revealed that different crops responded positively to microbial inoculations. In particular, successful rhizobial inoculants were applied to leguminous plants and AM fungi for muskmelons in order to increase the yield and quality. Multifunctional bio-fertilizers were developed to reduce about 1/3- 1/2 of chemical fertilizer application. In the future, enhancement and maintenance of soil fertility through microorganisms will be a very significant concern.
Keywords: Bio-fertilizers, perspective, sustainable agriculture, inorganic fertilizers, inoculants.

INTRODUCTION

In past 50 years history, the chemical pesticides and fertilizers have played a crucial role in boosting the agricultural production, however they have a short history in modern agriculture. Their immediate action and low cost managed to bring them rapidly in to the center of attention. Their toxic effects on environment, plant, animal and human life diverted the focus on ecofriendly plant protection. Moreover, the development of resistance in insects against common pesticides has not been solved yet. Thus, practices such as Integrated Pest Management (IPM) have gained more importance. Biopesticides are important component of the IPM. The terminology 'Biopesticide' means use of beneficial microorganisms to control the pest. However, the availability of biopesticide is the major constraint; considering the total cropped area.

Indiscriminate use of chemical pesticides contributed in loss of soil productivity along with addition of salts to the soil [1].To revive the soil health and living on alternate source has become essential concept of biofertilizer came forward, which can be a good supplement for a chemical fertilizers, Biofertilizers are nutrient availability systems in which biological process are involved. The term biofertilizers includes selective micro-organism like bacteria, fungi and algae. Which are capable of fixing atmospheric nitrogen or convert soluble phosphate and potash in the soil into forms available to the plants? Biofertilizer is a cost effective, eco-friendly & renewable source of land nutrient they play a vital role in maintaining a long term soil fertility & sustainability. The biofertilizer with nitrogen fixer & phosphate solubilizer fixes 20-40 Kg of nitrogen per acre. The biofertilizer maintain the soil fertility cost by using in the yield is assured with biofertilizer & continuos use of biofertilizer makes the soil very fertile for good yield. The biofertilizer can be manufacture in soil form or in liquid form for spraying on the plants. Biopesticide and biofertilizer is a need of modern agriculture since demand for safe and residue free food is increasing [2]. Therefore, to cater the need, it is necessary to promote the efforts for production of biopesticides and biofertilizers in the state in private sector to encourage the entrepreneurs.

APPLICATION OF MICROBIAL BIOFERTILIZER

Application of the microbial biofertilizer is an important step in the BiofertilizerTechnology. If the microbial inoculant is not applied properly, the benefits from the biofertilizer may not be obtained. During application one should always remember that the most of the microbial biofertilizers are heterotropic, i.e. they cannot prepare their won food and depend upon the organic carbon of soil for their energy requirement and growth. So, they either colonise in rhizosphere zone or live symbiotically within the root of higher plants. The bacteria which are colonised in the rhizosphere zone obtain their organic carbon compounds from the root exudes of the higher plants. The symbiotic ones obtain organic carbon directly from the root. So, microbial inoculants must be applied in such a way that the bacteria will be adhered with the root surface. So, in case of transplanting crops, the inoculant are applied through roots, and in case of the crops in which seeds are sown directly in the field, the inoculants are applied through the seeds so that they can colonize in the rhizosphere region when the young roots are emerged after germination of seed [3].

On the basis of the above principal, the following inoculation methods has been developed:

1. Inoculation of the seeds by slurry inoculating technique
2. Inoculation of seeds by seed pelleting technique
3. Inoculation of the seedlings
4. Inoculation of the soil by solid inoculation technique

LABORATORY SETTING AND OPERATIONS

Aseptic techniques

Working in absence of contaminants is very important thing. Aseptic technique is essential for all pathology work and must be thoroughly practiced and mastered.Insect pathogenic fungi or bacterial cultures for insect pathology must be pure. This means that they must be free of any living microbes other than the one required. The presence of unwanted microorganism (fungi or bacteria) is known as contamination and the microbes responsible for contamination are referred to as contaminants. We use aseptic technique so that we can handle, or manipulate microorganisms without appearance of contaminants into the culture. Aseptic technique also helps to protect the operator from potential infection from pathogenic organisms [4].

Always use aseptic technique when handling microorganisms and also when preparing microbiological media in which to grow these organisms.

Sterilization

Elimination of all viable microbes from a material is known as sterilization. Sterilization is nonselective process. It is very important stage for any microbiological work. The success of proper sterilization ensures quality of final product. All equipments and medias to be used during the handling of the microorganism must be sterile.

Disinfection

Disinfection is a way to reduce the contaminant load. It removes potentially infective microbes, but does not render the object sterile. Many different methods of sterilization are being used. The sterilization method you use depends on the equipment you have and what it is you are sterilizing. As a general rule, the following methods are most appropriate.

Microbial growth media

Wet heat sterilization, usually using an autoclave although a domestic pressure cooker will do just as well

1. Raise the temperature to 121°C and the pressure in the closed chamber to 15 psi for 15-20 minutes.
2. Do not over fill vessels containing liquid; leave a large space at the top of all bottles to allow for expansion and boiling of the liquid on heating in the autoclave.
3. Loosen screw caps before autoclaving.

Laboratory growth media

Sterilize as above using wet heat sterilization or dry heat sterilization in an oven (see lab techniques) at 160°C for 1-2 hours. If you have no autoclave, pressure cooker or oven, you can use certain chemical agents such as strong acids or alkalines, phenols or ethylene oxide. All chemical methods are potentially hazardous to the operator and should be avoided where possible. Methods for chemical sterilization can be found in the Plant Pathologists Pocketbook.

Small pieces of equipments:

Sterilize glass rods and metal tools by dipping them in 70% ethanol (alcohol) and then flaming to burn off the alcohol. Sterilize inoculating loops and needles by holding in a flame until red-hot.

CURRENT STATUS OF BIO-FERTILIZERS DEVELOPMENT

The main and direct purposes of applying bio-fertilizers to soil are: to provide nutrient sources and good soil conditions for the growths of crops when used as a live body;, to partially substitute and enhance the function of chemical fertilizer and then subdue the application quantities of fertilizers and still maintain the same crop yields and the capital used for making bio-fertilizers is cheaper than that of chemical fertilizers and to lessen the negative effect aroused from applying chemical fertilizers to soil. On the other hand, the indirect purposes of using bio-fertilizers to soil are: to enhance the growth of root system to increase the water and nutrient absorption abilities of crops, extend the life of root, neutralize and degrade harmful materials accumulated in soil, promote survival efficiency of seedling after transplanting and get shorter time for the flower to come out.

The current researches and development of bio-fertilizers varies in different aspects such as follows:

Rhizobial Inoculants

In Taiwan, research work in the selection of efficient rhizobial strains for inoculation started in 1958. Collection, isolation and subsequent selection of effective rhizobial strains and its uses in agriculture have yielded fruitful results. Significant variations were observed among rhizobial strains [5]. Wu (1958) [15] selected a number of pure rhizobial strains from lupin, alfalfa, peanut, crotalaria and soybean and conducted a wide range of field experiments to select the effective inoculants. Yields were significantly increased when lupin, alfalfa, peanut and soybean were inoculated with selected rhizobial strains compared to those with non-inoculated plants.

After the 1980s, slow and fast-growing soybean rhizobial strains were isolated and selected from Taiwan soils for inoculation [6]. Several effective isolates were deposited in the Culture Collection and Research Center (CCRC) of the Food Industry Research and Development Institute in Taiwan (CCRC 1991) [1].

Few field experiments were conducted to determine the effects of single and mixed inoculations with rhizobium and Arbuscular-Mycorrhiza (AM) in six different tropical soils found in Taiwan [7]. The results indicated that inoculation with rhizobial strains alone increased N_2 fixation and soybean yield in three out of six fields. Inoculations with rhizobial strain singly, or in combination with AM, without any N_2 fertilizer applications, significantly increased soybean yield from 5% to 134% in the field experiments. The results from the other experimental site also showed that a mixed inoculant of rhizobium and AM can be an efficient biological fertilizer that maximizes soybean yields.

The effect of inoculating associative and free living nitrogen-fixing bacteria on the growth of crops

Corn seed blended with peat moss was mixed with Azospirillum sp. suspension (3.6 x 10^8 cfu ml^{-1}) at a ratio of 1:5 (w:w) and sowed in the field. The experimental result showed the growth of corn could be significantly promoted and that the flowering time of the corn became one week earlier than that of corn seed without Azospirillum sp. suspension [7]. The fresh weights of melon and papaya seedling inoculated with Azotobacter sp. suspension (10^7 cfu. ml^{-1}) once a week were 68% and 103% respectively, heavier than those of seedlings without inoculating suspension [7].

Phosphate-solubilizing microbial inoculants

Phosphate-solubilizing bacteria (PSB s) were isolated from various soils in Taiwan. Aliquots of soil diluted in sterile water (1:10, w:v) were placed on calcium phosphate medium (modified from Subba Rao, 1982 [11]) for the isolation of Phosphate-solubilizing bacteria.

The basic research on phosphate-solubilizing bio-fertilizers was successfully established during 1990s in Taiwan [8]. Crop plants such as peanut, various horticultural plants and vegetables were successfully inoculated with PSBs to obtain higher yields. Several field experiments concluded that PSBs do not only improved the growth and quality of crops but also drastically reduced the usage (by 1/3-1/2) of chemical or organic fertilizers.

Tomato and muskmelon seeds were immersed in PSB suspensions; diluted to 10^{-3} with Hoagland's solution for 1 minute and sowed in pot containing vermiculite to carry out the experiment. Experimental results showed that the suspensions of PSBs significantly promoted the height and fresh weight of tomato and muskmelon seedlings, respectively. The propagating stem length and growth tendency of muskmelon inoculated with TARIB 108 were larger than those of control treatment after field experiment was carried out for six weeks. The average fruit weight and volume of muskmelon inoculated with TARIB108 suspension was 50-g heavier and 24 cm^3 larger than that of non-inoculated treatment. The sweet index (Brix) of muskmelon inoculated PSB TARIB108 was 2 degrees higher than that of non-inoculated treatment. The propagating stem length of watermelon inoculated with PSB suspension was 60 cm longer than that of non-inoculated watermelon (Chien and Chang, 2004).

The suspensions (5 x 10^8 $cfu.ml^{-1}$) of Klebsiella sp., Bacillus sp. (PSBs) and Azospirillum sp. (associative nitrogen-fixing bacterium) were mixed at equal volume and then added to spent mushroom sawdust compost at a ratio of 1:100 to make corn crop have significantly higher and heavier shoot than those of control treatment in a pot cultural experiment [9].

AM Inoculants

The major AM fungi used for inoculation were Glomus spp. isolated from tropical soil of Taiwan (Young, 1986). Chlamydospores were borne terminally on single undifferentiated hyphae in soil. The mature spores were separated from the attached hyphae by a septum. The AM fungal inoculant was placed in pots containing sterilized mineral attapulgite $[(Mg.Al)_5Si_8O_{22}(OH)_4.4H_2O]$ with Zea mays as the host plant. The AM inoculant used in pot experiments contained approximately 50 spores per gram of soil together with infected roots [10].

Young et al. (1986) used two species of AM in a pot experiment to observe the effect of inoculation of AM fungi in the yield and mineral P utilization in soybean. The results showed that the AM fungi inoculation increased soybean yields over the uninoculated treatments to certain extent depending on the soil type. Moreover, the P uptake by soybean was significantly improved in the inoculated treatments. In a similar experiment, rhizosphere soil was used to assess the difference in P uptake by the soybean plants. Soybean in non-inoculated treatments took up minimum Al- P from acidic soils whereas, less Ca-P from calcareous soil and failed to absorb Fe-P from any soil types. Inoculation with either of the two mycorrhizal fungi improved the uptake of Al-P by soybean in acidic soils, and also increased the uptake of Ca-P in calcareous soils and significant amount of Fe-P uptake was evidenced. These results suggested that AM can enhance uptake of fixed soil P. The efficiency rate of utilizing various forms of mineral P by mycorrhizal plants depends on the species of mycorrhizal fungi inoculated and soil types. Further, Chang and Young (1992) showed that inoculation of tea cuttings (cv. TTES No. 12) inoculated with AM fungi or PSBs significantly enhanced the growth of tea seedlings [11].

River sand mixed with chicken compost at a ratio of 4:1 (v:v) filled in Jiffypot and added Glomus clarum inoculant to have 50 AM fungus spores and then planted with melon. The result showed the flowering time could be 7 days shorter than that of treatment without adding the inoculant. In green house experiment, it showed the quality of melon could be increased. The percentages of first class and second class melons were increased by 22% and 52%, respectively. After harvesting its fruit, the melon's stolon stem was removed and the new bud was successfully developed to have 70% survival percentage. Within 35 days, melon fruit attained its maturity to have another harvest [12]. The research result showed that such process could be used to lessen the cost of buying melon seedling.

Cheng and Chung, 2004 and Chiu et al. 2004 conducted an ecpreiment in papaya. Papaya seedling in pot medium containing 150 spores of AM fungi (Glomus aggregatum, Glomus etunicatum, Glomus clarum, Glomus mosseae, Acaulospora scrobiculata) grew faster and wider root system than those of control treatment. The shoot fresh weight and height of orange tree inoculated with AM fungus spore were 30.2-67.4% heavier and 17.6-39.4% higher than those of orange tree without inoculation after the experiment was carried out four months. Pot media planted with different flower crops added with AM inoculants have more than 100 spores has earlier flowering time, more numbers of flower, larger flower diameter, heavier fresh and dry flower weights than those of pot media without adding AM inoculants [13].

FARMERS' ACCEPTANCE AND UTILIZATION OF BIOFERTILIZERS

In order to promote sustainable agriculture, both central and local government agencies in Taiwan are supporting extensive application of bio-fertilizers. Major programs for the application of bio-fertilizer include production of rhizobial, P-solubilizing microbial inoculants for soybeans that can be used as vegetables and for other crops, and production of AM inoculants for melons and other horticultural crops. It also aims at improving biological nitrogen fixation in soybeans that are consumed as vegetables, peanuts, and in red bean. Similarly, emphasis in attaining higher yield and better quality horticultural crops were being given through three major programs- the production of inoculants, extension programs so that farmers can apply inoculants on to their farms and demonstration and awareness programs to show farmers the benefits of inoculated plots.

Soybeans for vegetable purpose are extensively produced in Taiwan and exported to Japan. Superior and constant maintenance of quality will be an important factor governing the export value of soybeans in the international market. But earlier, farmers were applying more chemical fertilizer than the recommended levels leading to inferior quality of beans. Since 1988, the Department of Soil and Environmental Sciences at National Chunghsing University in Taiwan actively started the production of efficient inoculants (liquid and solid bio-fertilizers) that can maintain yield and superior quality soybeans, which were exported and presently being consumed as vegetables in several countries. During last 20 years (from 1987 to 2006), enough inoculants were produced to inoculate approximately 65,091 ha of farmland. Over the years, farmer's economic gain also increased significantly (US$27 million) on using rhizobial inoculants. Moreover, a great deal of chemical fertilizer was saved and further groundwater pollution caused by N leaching was significantly reduced.

Healthy seedlings are one of the essential factors affecting the growth and yield of crops. Over the past decades, AM inoculants have been produced by the Agricultural Research Institute of COA, National Chunghsing University, National Pingtung University of Science and Technology in Taiwan, and the inoculants were distributed and technologically demonstrated to farmers by several Agricultural Experimental and Improvement District Stations for inoculating many crops, particularly horticultural and ornamental plants such as muskmelon, citrus, strawberry, lily, tomato, chrysanthemum, gerbera, tea, and fruit trees [14].

FUTURE PERSPECTIVE OF BIO-FERTILIZERS

Excess nutrients are accumulated in soils, particularly P as a result of over application of chemical fertilizers by farmers during intensive agricultural practices. Hence, major research focus should be on the production of efficient and sustainable bio-fertilizers for crop plants, wherein inorganic fertilizer application can be reduced significantly to avoid further pollution problems. In view of overcoming this bottleneck, it will be necessary to undertake short-term, medium, and long-term research, in which soil microbiologists, agronomists, plant breeders, plant pathologists, and even nutritionists and economists must work together [15].

The most important and specific research needs should highlight on following points:

1. Selection of effective and competitive multi-functional bio-fertilizers for a variety of crops.
2. Quality control system for the production of inoculants and their application in the field, to ensure and explore the benefits of plant-microorganism symbiosis.
3. Study of microbial persistence of bio-fertilizers in soil environments under stressful conditions
4. Agronomic, soil, and economic evaluation of bio-fertilizers for diverse agricultural production systems.
5. Transferring technological know-how on bio-fertilizer production to the industrial level and for optimum formulation.
6. Establishment of "Bio-fertilizer Act" and strict regulation for quality control in markets and application.

CONCLUSION

To improve and maintain the productivity of agricultural lands, the integrated approach to determine the most favorable plant-microorganism interaction is vital. The current trend of low input chemicals in sustainable agricultural systems will contribute to the goal. We have successfully shown the use of AM fungal inoculum in the field for many crops. Current inoculum development technologies are capable of producing sufficient quantities of effective inoculum with applausible shelf life for economical use in many horticultural systems (Safir 1994). Council of Agriculture (COA) held various seminars as well as workshops on the application of biofertilizers, so that farmers would have the opportunity to understand the effects of biofertilizers and are willing to use them. Farmers were invited to inspect the growth of AMF, Rhizobial or PSB inoculated crops in the fields and were encouraged to participate in workshops after viewing the successful outcomes of using biofertilizers. The application of inorganic chemical fertilizers was thus significantly reduced to 30-50%. This helps in the realization of environmental friendly and sustainable agriculture.

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