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Role and perspective of Azotobacter in crops production

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ABSTRACT

Low nitrogen content in soil is usually overcome by chemical fertilization. After long application period, high-dose and intensive use of N fertilizers can cause ammonia volatilization and nitrates accumulation in soil. In sustainable agriculture, the use of bacterial inoculant integrated with nutrient management system has a role in soil health and productivity. Azotobacter-based biofertilizer is suggested as a chemical nitrogen fertilizer substitute or addition in crop production to improve available nutrients in the soil, provide some metabolites during plant growth, and minimize fertilizer doses. The objective of this literature reviewed paper is to discuss the role of Azotobacter in agriculture; and the prospective of Azotobacter to increase yield and substitute the chemical fertilizer in food crops production. The results revealed that mechanisms by Azotobacter in plant growth enhancement are as biofertilizer, biostimulant, and bioprotectant. Nitrogen fixation by Azotobacter is the mechanism to provide available nitrogen for uptake by roots. Azotobacter stimulates plant growth through phytohormones synthesis; indole acetic acid, cytokinins, and gibberellins are detected in the liquid culture of Azotobacter. An indirect effect of Azotobacter is exopolysaccharide production and plant protection. Inoculation of Azotobacter in the field integrated with organic matter and reduced chemical fertilizer are reported to improve plant growth and yield.

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1. Introduction

Agriculture provides 80% of food security and engages over one-third of people worldwide (McGuire, 2015). In order to fulfill the increasing demand for agricultural products for food, for more than five decades the chemical fertilization has been adopted as an effective method to improve crops yield (Geng, Cao, Wang, & Wang, 2019; Yousaf et al., 2017) but high-dose and intensive use of fertilizer has taken its toll on the environment. Chemical fertilizers are also a significant source of greenhouse gas emissions and contribute greatly to climate change.

Topsoil compaction and significant loss of organic matter are some of the adverse effects of chemical fertilization (Massah & Azadegan, 2016). The use of nitrogen fertilizer such as urea evidently results in ammonia volatilization especially in tropics where the temperature is high (Fan, Li, & Alva, 2011; Jadon et al., 2018). Since the efficiency of N fertilizer is low, nitrate may leach from N fertilizer mainly in the rainy season and contaminates the groundwater (Sebilo, Mayer, Nicolardot, Pinay, & Mariotti, 2013; Wang, Gao, Li, Zhang, & Wang, 2015).

Agricultural production in the tropics is facing numerous challenges for future food production sustainability. Most of the soil in the tropics is low in nitrogen due to high rainfall and intensive organic matter decomposition (Moura et al., 2016). Maintaining soil health while maintaining production volume is one of the goals of sustainable agriculture. This target can be fulfilled using soil microbes especially a few plant growth-promoting rhizobacteria (PGPR). In soil, the PGPR may improve plant health and enhance plant growth rate in absence of environmental pollutants (Calvo, Nelson, & Kloepper, 2014).

Different kinds of PGPR have been studied and few of them have been commercialized as biofertilizer; and the highlighted genus include (Glick, 2012): Azotobacter,

Azosprillum, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, and Serratia (Table 1). The ability of Azotobacter enables to fix N non-symbiotically has been widely studied. The occurrence of this organism has been reported in the rhizosphere of several crops such as rice (*Oryza sativa* L.), maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), bajra (*Pennisetum glaucum* L.), vegetables, and plantation crops (Mazid & Khan, 2015). Recently Azotobacter is considered as an important fertilizing agent that contributes to the N availability and substitutes chemical fertilizer (Mohamed & Almaroai, 2016; Subedi, Khanal, Aryal, Chhetri, & Kandel, 2019) and produces secondary metabolites especially phytohormones; and exopolysaccharides that are not present in chemical fertilizers.

Numerous studies showed that Azotobacter is a PGPR with direct mechanisms as biofertilizer, biostimulants, and/or indirect mechanisms as bioprotectant. Azotobacter reduced the doses of chemical fertilizer and decreased early blight diseases in long beans (Hindersah et al., 2018). This is in line with Azotobacter's ability to degrade the cell wall of fungal pathogen with Jadhav & Sayyed (2016) which may be related to the production of hydrolytic enzymes (Romero-Perdomo et al., 2017).

The Azotobacter inoculants have been formulated as biofertilizers, especially in India, China, and Indonesia because it can increase agricultural output. Azotobacter inoculation is the application of biotechnology to support the development of agricultural practices that minimize pollution and decrease soil quality. Azotobacter inoculants might be important in supplementing the plant nutrient in remote areas outside the city or on the island. In such areas the supply of chemical fertilizer is limited and farmers there are mostly cannot afford the expense of chemical fertilizers.

In this review, our main goal is to highlight the role and perspective of nitrogen-fixing Azotobacter for sustainable agriculture, and here the mechanism of Azotobacter as PGPR and its role regarding biofertilizer, biostimulant, bioprotectant activity has been discussed. The objectives of this paper were to illustrate the important roles of Azotobacter to increase plant growth and productivity as well as reduce chemical fertilizer, and the prospective of Azotobacter to minimize the chemical fertilizer rates in food crop production.

2. Materials and Method

The research method is a literature review to find materials relevant to the Azotobacter. For writing this paper, identification and evaluation of the relevant literature within basic or applied research of Azotobacter has been carried out. This review paper is composed of various literature studies derived from research data mainly for the last 10 years. Less literature was collected from literatures published more than 10 years ago. The search engine utilized to find the materials (paper) was google.com and scholar.google.com. The literature study was mostly performed between 2018-2020.

Literatures were obtained from indexed journals with moderate to high reputation Indonesian indexed by SINTA and with moderate (Ebsco, PubMed, NCBI) and high reputation (Scopus, Science Direct, Web of Science). A Few articles in Scopus-indexed proceedings have also been cited. Journals that are used as references are those included in the category of soil science, agriculture, microbiology, biological science, and environment agriculture. The reference collection method was carried out by a collection of experimental result analysis and review papers related to Azotobacter as biofertilizer and PGPR.

3. Results

3.1. Azotobacter morphology

The first Azotobacter species characterized was A. beijerinckii in 1901. Phylogenetic analysis through the 16S rRNA gene has successfully indicated that the Azotobacter genus consists of seven species in both dry and wetlands: A. chroococcum, A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A. nigricans, and A. salinestris (Kennedy, Rudnick, MacDonald, & Melton, 2015; Mazinani & Asgharzadeh, 2014; Rubio et al., 2013; Zhengtao, Wenge, Di, Yuan, & Tingting, 2019).

Azotobacter colonies are 3-8 mm in diameter with smooth, irregular, clear, transparent, and sparkling surfaces without pigments and some form opaque white, brown, black-brown, black, and yellow-green pigments (Banerjee, Supakar, & Banerjee, 2014; Jiménez, Montaña, & Martínez, 2011). Colony characteristics depend on growth media composition (Kennedy et al., 2015). *Azotobacter chroococcum* colony on nitrogen-free media is slightly viscous, semi-transparent during initial growth, and then turns dark brown (Abdel-Hamid, Elbaz, Ragab, Hamza, & El Halafawy, 2010).

The cell morphology is pleomorphic (Upadhyay, Kumar, Singh, & Singh, 2015), usually straight Bacilli with rounded ends becoming more ellipsoidal or coccus (Figure 1). Azotobacter is a chemo-organo heterotrophic organism that forms cysts in under drought stress and produced capsules (Mukhtar, Bashir, & Nawaz, 2018) which structurally consisted of polysaccharide hence the name become exopolysaccharide (Gauri, Mandal, & Pati, 2012).

Table 1. The species of rhizobacteria have been studied and commercialized as single strain or mixed biofertilizer

Rhizobacteria	References
Azotobacter	Subedi, Khanal, Aryal, Chhetri & Kandel, 2019
Azospirillum	Zeffa et al., 2019
Bacillus	Akinrinlola, Yuen, Drijber & Adesemoye, 2018
Burkholderia	Paungfoo-Lonhienne et al., 2016
N-fixer Enterobacter	Uttari, Nyana & Astriningsih, 2016
N-fixer Klebsiella	Liu et al., 2018
Pseudomonas	Qessaoui et al., 2019
Variovorax	Jiang et al., 2012
Serratia	Helaly, Hassan, Craker & Mady, 2020

(a) (b) (c) (d)

Figure 1. Morphology of A. chroococcum (a) and Azotobacter sp. (b); Azotobacter colonies without pigment; Azotobacter colonies with melanin (d) (Image sources: Gospodaryov & Lushchak (2011) (a); Jiménez et al., (2011)(b-d))

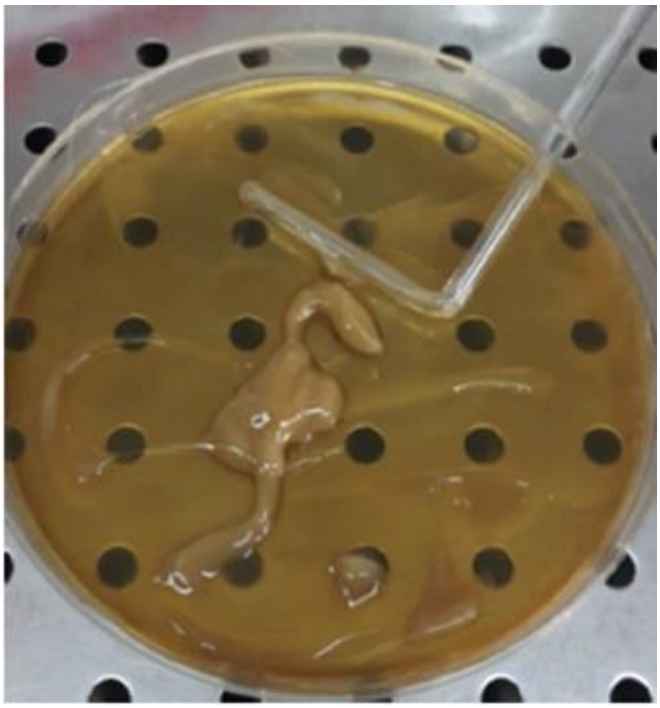

Figure 2. Collection of EPS produced by *Azotobacter chroococcum* 76A after 24h incubation at 30 °C (image source: (Ventorino et al., 2019)

Azotobacter species can produce cysts and the vegetative cell becomes immobile (Yoneyama, Yamamoto, Hashimoto, & Murata, 2015). A high rate of respiration, macromolecular synthesis, and N fixation will take place when cyst germinates and vegetative cells appeared (Loperfido & Sadoff, 1973). A dormant cell covered by a two-layered capsule; the exine and intine (Espín, 2016) tolerate and survive in a water-limited environment (Sivapriya & Priya, 2017). The polyhydroxy butyrate, alginate, and alkylresorcinols are a basic element of mature cysts of Azotobacter (Haroun & Abdel-Hamid, 2015; Yoneyama et al., 2015). The resistance of cysts is a prospect for better Azotobacter formulation as biofertilizer; A. chroococcum cysts in the liquid formulation will preserve for two years and still enable to enhance the growth of maize after germination (Abdel-Hamid S, Hamza, Elbaz, Ragab, & Halafawy, 2012).

The gram-negative Azotobacter live and proliferate in the rhizosphere and phyllosphere of agricultural plants (P. Kumar et al., 2018; Maurya, Kumar, Raghuwanshi, & Singh, 2012). In the soil, Azotobacter is found in slightly acidic, neutral, or slightly alkaline soils with an acidity of 4.8-8.5 (Singh, 2011), but they also grow in soils with a pH between 7.07-8.56 (Mazinani, Aminafshar, Asgharzadeh, & Chamani, 2012). The optimum acidity for self-propagation and nitrogen fixation is 7.0-7.5 (Singh, 2011) but *Azotobacter vinelandii* can grow at a pH range of 5-9 and show maximum growth at pH 8 (Mukhtar et al., 2018).

Most Azotobacter strain was sensitive to acidic pH, high salt concentration, and mesophilic temperatures (Sethi & Adhikary, 2012). Mukhtar et al. (2018) revealed that the optimal temperature for Azotobacter growth is 30°C although they can propagate at 25-40°C. However, their proliferation is greatly decreased above 30°C (Mukhtar et al., 2018; Sethi & Adhikary, 2012). The Azotobacter is aerobic (Jiménez et al., 2011) but García et al. (2020) recently explain the ability of Azotobacer to proliferate in microaerophilic conditions.

3.2 Mechanisms to promote plant growth

3.2.1 Nitrogen fixation

Direct mechanisms of Azotobacter as a biostimulant to induce plant growth and development is nitrogen fixation by which nitrogen gas (N_2) is reduced to NH_3 catalyzed by nitrogenase consists of Fe-protein and FeMo-protein (Sivasakthi, Saranraj, & Sivasakthivelan, 2017) Nitrogen reduction require both reducing equivalents and 16 ATP of energy for each N_2 fixation. All researchers agree that nitrogenase activity is destroyed by O_2 and sensitive to available nitrogen. In N-limited environment, Carbon to Nitrogen ratio will increase and induce the cell to synthesize nitrogenase and fix N_2; in such condition, maximum respiration decrease O_2 exposure to nitrogenase (Oelze, 2000).

Azotobacter strains have different N_2 fixation capacities. Murumkar et al. (2012) reported A. chroococcum isolates to have nitrogenase activity of 19.5–217.3 nmol C_2H_4 mg^{-1} protein h^{-1}. While Danapriatna (2016) verified the lower nitrogenase activity of some Azotobacter isolates from paddy rhizosphere; 24.63- 134.29 nmol C_2H_4 g^{-1} h^{-1}. Five isolates of

Azotobacter can fix 8.14-8.46 mg N g^{-1} glucose (Bag, Panda, Paramanik, Mahato, & Choudhury, 2017). More recently some studies showed that Azotobacter strains fix N_2 was up to 73.8 kg ha^{-1} $year^{-1}$ in soil (Mahato & Kafle, 2018).

3.2.2 Phytohormone production

The researchers in general reported the presence of three phytohormones in Azotobacter liquid culture, namely Indole acetic acid (IAA), Cytokinin (CK), and Giberreline (GA). Among 15 saline-resistant, A. salinestris AT19 produces IAA (18.2 µg mL^{-1} IAA), lowest GA_3, and average Zeatin; but A. chroococcum AT25 strain produces all three phytohormones in average concentrations at day five when they are in late logarithmic phase at day three (Rubio et al., 2013). The Azotobacter produced those phytohormones to function in rooting and simulating plant growth (Vikhe, 2014).

The ability of Azotobacter to synthesize IAA by Azotobacter brought more attention to the study. Six Azotobacter isolates produced 12-48.1 mg L^{-1} IAA in the medium with 5 mg mL^{-1} tryptophan, an inducer of IAA synthesis, at 3-5 days after incubation (Patil, 2011). 16 isolates of Azotobacter produced IAA in tryptophan-enriched media up to 42.80-82.00 µg mL^{-1} (A. Kumar et al., 2014). A similar effect of tryptophan enrichment on IAA production was showed by five Azotobacter isolates that synthesize 3.07-459 mg mL^{-1} IAA (Zulaika, Solikhah, Alami, Kuswytasari, & Shovitri, 2017).

3.2.3 Exopolysaccharides production

Azotobacter species produce capsules (Mukhtar et al., 2018; Vermani, Kelkar, & Kamat, 1997); an extracellular macromolecule polysaccharide layer outside the cell envelope that can be extracted from bacterial liquid culture (Figure 2). The exopolysaccharides EPS consist of simple sugars and organic acids (Hindersah, 2015). The concentration of EPS which is secreting outside the cell environment depends on the carbon source. Azotobacter excreted 0.84 mg L^{-1} – 7.5 g L^{-1} of EPS in liquid inorganic or organic media; and the concentration of EPS becomes higher in the presence of N (Emtiazi, Ethemadifar, & Habibi, 2004; Khanafari & Sepahei, 2007; Ventorino et al., 2019).

The natural role of EPS in Azotobacter is to protect cells from drying out and protect nitrogenase from oxygen (Sabra, Zeng, Lunsdorf, & Deckwer, 2000; San Yu & Ullrich, 2018). Secreting EPS to an outer cell is an Indirect mechanism by which Azotobacter improves plant growth and yield (Gauri et al., 2012) due to aggregate and pore composition improvement (Harahap, Dwi, & Gofar, 2018).

3.2.4 Plant protection

Some experiments showed that Azotobacter can induce resistance of food crops to certain soil-borne diseases. The antifungal activity of Azotobacter was detected for the fungus *Aspergillus flavus*, Cercospora sp., and *Fusarium oxysporum* with more intensive inhibition at high concentrations (Ponmurugan, Sankaranarayanan, & Al-Dharbi, 2012). Viscardi et al. (2016) reported the first-ever antimicrobial activity of A. chroococcum strains 67B and 76A against Sclerotinia minor CBS 112.17 tomato plants.

The Azotobater is reported to suppress plant diseases. Istifadaha et al. (2017) verified that A. chroococcum inhibits wilt diseases incidence of chili up to 40% compared to the control in the pot experiment. The A. chroococcum decreased damping-off disease incidence of 16.7 and 2.5% in cotton and rice plants respectively (Chauhan, Wadhwa, Vasudeva, & Narula, 2012). Field experiments proved that A. chroococcum reduced the intensity of leaf blight attacks on mustard plants caused by R. solani by 25.64% (Kalay, Hindersah, Talahaturuson, & Latupapua, 2017).

Table 2. The response of food plants to the application of *Azotobacter* biofertilizers experiments

Treatments	Response	Reference
Azotobacter AS4 and 75% chemical fertilizer	Increased soil nitrate, shoot dry weight, and N uptake of Sorghum (*Sorghum bicolor*)	Hindersah & Kamaluddin, 2014
Azotobacter sp	Increased cell viability in the rhizosphere of Chili (*Capsicum annum* L.), and nitrate & ammonium content in the soil	Hindersah, Priyanka, Rumahlengan & Kalay, 2016
Azotobacter and *Bradyrhizobium*	Increased N uptake, plant height, leaves number, and the shoot-root ratio of Soybean (*Glycine max)*	Rahmayani, Hindersah, Fitriatin, 2017
Azotobacter sp.	Increased germination, root and shoot length, and shoot dry weight of Garden cress (*Lepidium sativum*) in Cr and Cd contaminated soil	Sobariu et al., 2017
Azotobacter, vermicompost, and NPK fertilizer	Increased pod weight of Soybean	Setiawati, Sofyan, Nurbaity, Suryatmana & Marihot, 2018
A. chroococcum AC1 and AC10	Co-inoculation showed a greater positive effect on plant growth	Romero-Perdomo et al., 2017
Multi strains of *Azotobacter*	Better growth and higher yield of shallot bulbs compared to single *Azotobacter* in saline soil of 4.19 dS/m.	Widawati, 2017
Azotobacter sp., *Azospirillum* sp. and 75% dose NPK fertilizer	Increased in germination, plant height, leaf area, Branches per plant, and Leaf per branch of the tomato	Reddy et al., 2018

Table 3. The impact of *Azotobacter* on some important food crops in field application

Treatments	Response	Reference
Azotobacter, Farmyard manure, and NPK fertilizer	Increased 15-35% of dry shell of Corn (*Zea mays*) but no effect when applied with organic matter and NPK	B. Baral & Adhikari, 2014
Azotobacter sp. and manure	Hiked in maize grain yield by 35% over the non-inoculated plants	B. R. Baral & Adhikari, 2013
A. chroococcum	Increased tuber yield up to 20%-23% and crystal sugar beet (*Beta vulgaris*) rendement up to 21%-23%	Mrkovački et al., 2016
A. chrocoocum strain 5 and *Pseudomonas putida*	Improved phosphorous nutrition, grain yield, and root biomass of wheat	Seyed, Khalilzadeh & Jalilian, 2017
A. chroococcum, *Candida sake*, and some N fertilizer levels	Produced highest grain yield of wheat and replace 47,6 kg N/ha	Mohamed & Almaroai, 2016
Azotobacter sp. and PSB	Increased in head volume, head yield per plot, as well as ascorbic acid, protein, and nitrogen content of cabbage (*Brassica oleracea* var. capitata)	Devi, Choudhary, Jat, Singh & Rolaniya, 2017
Azotobacter, NPK fertilizer, and organic fertilizer	Increased yield of wheat (*Triticum aestivum*) up to 63,1%	Mahato & Kafle, 2018
Azotobacter sp.	onion plant exhibited higher dry weight of bulb and harvest index of onion	Kurrey et al., 2018
Azotobacter and NPK fertilizer	Substituted 50% chemical, increased yield, and improved morphological traits of	Subedi, Khanal, Aryal, Chhetri & Kandel, 2019
Azotobacter and Neem cake	Increased yield of Knol Khol (*Brassica caulorapa* L.) over the control and other N-fixer	Shah, Chaudhary, Rana & Singh, 2019
Azotobacter sp. and Phosphate Solubilizing Bacteria (PSB)	Increased plant height, fruit length, but did not affect the number of Okra (*Abelmoschus esculentus*) fruit; Increased yield parameters of calabash (*Lagenaria siceraria*)	Din et al., 2019
Azotobacter and 50% N fertilizer	Increased the 1,000 rice grain weight by 17 and 23%	Banik et al., 2019

3.2.5 Role of Azotobacter in crops production

Azotobacter inoculation not only increases plant growth and yield but also changes the plant quality and decreases the dose of chemical fertilizer. Bhattacharjee & Dey (2014) recorded the 5-24% yield increment in Azotobacter inoculation of vegetable, cerealia as well as estate crops over yield obtained with chemical fertilizers.

The application of Azotobacter has a role in the production of amino acids since the supply more N to the plant (Nosheen et al., 2016). Kurrey et al. (2018) reported that the presence of chlorophyll a and b, as well as carotenoids in onion leaves, was much higher in Azotobacter inoculated plants compare to uninoculated plants. Azotobacter has been accepted to replace chemical fertilizers due to its natural ability to fix atmospheric nitrogen (Bageshwar et al., 2017; Mohamed & Almaroai, 2016; Subedi et al., 2019). An Increase in crop productivity or yield has been achieved through soil dressing and seed inoculation of Azotobacter by supplying more nitrogen to the crops (Arjun, Roshan, & Sushma, 2015). In Table 2 and Table 3, we are trying to provide the glimpses of impact on greenhouse and vast field application areas of Azotobacter, respectively.

4. Discussion

The morphology and physiology of Azotobacter have been studied intensively for more than four decades. Recent researches reconfirmed that the Azotobacter is pleomorphic, capsule- and cyst-forming heterotrophic, aerobic, and mesophilic bacteria that proliferates mainly in aerobic conditions. Recent findings also verified that Azotobacter is microaerophilic.

Azotobacter contributes to plant growth through four known mechanisms: nitrogen fixation, phytohormone synthesis, EPS production, and plant protection. The nitrogen fixation and phytohormone production are the direct mechanisms by which plants benefit from available nitrogen and exogenous phytohormone as nutrients. Although the nitrogenase is sensitive to O_2, the capacity of Azotobacter to fix N is mainly demonstrated by the strains isolated under aerobic environments and some isolates from irrigated paddy fields showed lower nitrogenase activities.

The first quantitative study of phytohormones production by Azotobacter was reported decades ago (Taller & Wong, 1989) which described some species of cytokinins in *Azotobacter vinelandii* culture medium when the bacteria reached the late logarithmic phase. Recent researches also showed that phytohormones in liquid culture were collected at the end exponential phase or between 3-5 days after inoculation.

EPS production and plant protection are the way Azotobacter to influence plant growth indirectly. Azotobacter mainly produces EPS to facilitate soil particle aggregation and hence nutrient uptake. Reports indicated that the role of Azotobacter on plant growth not only by providing plant nutrients but also protecting plants from soil-borne diseases. However, the effect of EPS and bioprotectant traits of Azotobacter on food crop production has not been deeply studied.

Azotobacter inoculation either in the pot (greenhouse) or field experiment demonstrated the different plant responses including plant growth, as well as quantity and quality of yield. Co-inoculation of Azotobacter with other rhizobacteria such as phosphate solubilizing bacteria (PSB) leads to positive plant response. The ability of mixed inoculant is reasonably more effective to enhance plant growth compared with single species of rhizobacteria. Multi-strains and mixed inoculants with other rhizobacteria have been performed to strengthen the impact on plant performance by the synergistic interaction between different species. In this case, the Azotobacter and PSB contribute to providing N and P respectively (Santana, Marques, & Dias, 2016; Sharma, Verma, & Kaur, 2017).

In order to increase or improve plant growth of yield of important crops, almost all references explained the usage of Azotobacter is integrated with the organic matter either as basic fertilizer or the treatments. The purpose of organic matter amendment following Azotobacter inoculation is obtained to provide carbon, nitrogen, and electron acceptor for Azotobacter heterotrophic metabolisms that become essential for ensuring the Azotobacter functions to promote growth and hence yield. Organic matter amendment has a significant role to improve or maintain soil quality and hence ensure crop quantity and quality.

In Table 3, B. R. Baral & Adhikari (2013) verified that Azotobacter inoculation with organic matter and NPK fertilizer application did not affect the corn yield. They applied the recommended dose of organic matter and NPK fertilizer. Soil rich in nitrogen suppresses the nitrogen fixation since nitrogenase is shut down in the presence of excess N. This disagrees with another field trial (Table 3) that utilized reduced N fertilizer to have an optimal function of Azotobacter.

According to researchers, Azotobacter inoculation increased the yield. However, the true mechanism or exact activity through which the Azotobacter influences the crop growth and production or morphology are yet to be fully discovered. In general, measuring the yield trait is not followed by determining the availability and fate of fixed N in soil and plant, or tracing the fate of phytohormones produced by bacteria. So, it remains unknown whether the yield increment is caused by nitrogen fixation or phytohormone production. Nevertheless, the reports of the Azotobacter effects on plant yield is convincing enough for its use in crop production.

The role of Azotobater as biofertilizer is not only to increase plant growth and yield but also to reduce the chemical fertilizer level. In the relation to climate change issues, Azotobacter as biofertilizer is a potential bioagent to reduce ammonia and nitrous oxide emission; and nitrate leaching. However, without appropriate biofertilizer as well as nitrogen fertilizer application, the goal to reduce greenhouse gas emissions and increase fertilizer efficiency might not be successful. In order to increase the yield and reduce N volatilization and N leaching, some steps may be taken as follows:

a. Reducing level nitrogen fertilizer by Azotobacter Biofertilizer
b. Organic matter application to ensure Azotobacter proliferation and increase soil quality.
c. Azotobacter liquid biofertilizer application by seed coating before sowing, multiple applications by foliar application or soil dressing.

As excessive usage of fertilizer is a problem in many regions, the governments of many Asian Countries are thinking to phase out chemical fertilizer subsidies and implement fertilizer reduction policies. The biofertilizer, include Azotobacter-based fertilizer will take an important part of chemical fertilizer policy.

5. Conclusion

Optimal growth requirements for heterotrophic-aerobic Azotobacter cell multiplication such as temperature, acidity, oxygen availability is in accordance with the agro-climatic conditions of dry land in tropics. The role of Azotobacter in providing N will be optimal because the soil quality in the tropics is limited by the low level of N; Limited N induces the N fixation. For decades, researchers agree that the main mechanisms by which Azotobacter enhances crop production are nitrogen fixation and phytohormone synthesis. The IAA, CK, and GA were synthesized by Azotobacter and excreted to the liquid cultures and stimulate plant growth.

More recently, exopolysaccharide production and plant protection are believed to have a positive impact indirectly to plant growth and might be yield. Based on their mechanisms to affect plant growth and yield, the Azotobacter has a role as biofertilizer, biostimulant, and bioprotectant. However, the use of Azotobacter related to their EPS and bioprotectant substances have not been widely elaborated. Azotobacter is the alternative of chemical fertilizers, pesticides, and artificial growth regulators which shows many side-effects to sustainable agriculture. For future usage of Azotobacter and increase their effectiveness in the field, a better formulation of Azotobacter-based biofertilizer is needed. Formulation of liquid and carrier-based Azotobacter inoculants should consider the morphological and physiological properties of Azotobacter to enhance the quality and shelf-life of Azotobacter-based biofertilizer as well as their function to boost crop production.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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