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Influence of Microbial Biofertilizers on Germination of Millet Varieties and Millet use in Biocontrol

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Abstract:- Influence on germination of Dansalka and Bahaushe millet (*Pennisetum glaucum* L. Br.) varieties by biofertilizers produced from *Azotobacter vinelandi* and *Rhizobium phaseoli* along with bacterial control abilities of millet grains extract were studied using laboratory facilities at Usmanu Danfodiyo University, Sokoto and Center for Microbiology and Biotechnology, Bhopal. Biofertilizers were produced from both microorganisms using upstream and downstream fermentation method, with Ashby and yeast extract mannitol broths as media. Three working concentrations of 10, 15 and 20mg/ml were obtained from each biofertilizer. Standard methods were adopted for the germination studies, treatments were replicated three times. Millet extracts of 60:40 water and alcohol, were tested for *in-vitro* bacterial control by plate disc method. Second day after sowing revealed no germination from controls, but biofertilizer treatments recorded highest germination rate from 20ml/l treatments resulting in 63% for Dansalka in *Azotobacter*, 57% for Dansalka and Bahaushe in *Rhizobium* and 53% for Bahaushe in *Rhizobium*. The 15ml/l treatments followed with 37% Bahaushe in *Rhizobium* and 33% for other millets in both fertilizers. Full (100%) germination was in 20ml/l from 3rd day and by 4th day, all 20ml/l and 15ml/l treated millets fully germinated. Dansalka in 10ml/l *Azotobacter* germinated 100% on day 4, while the control had 87% as final germination. Highest extract inhibition was 15.33mm against *A. vinelandi* followed by 14.33mm against *R. phaseoli* by 400mg/l. The lowest inhibition was 3.67mm on *Xanthomonas axonopodis* and *X. campestris* by 300mg/l. The 200mg/l showed no inhibition. Both fertilizers positively influenced millet germination while extract at highest concentration had mild inhibition of the microorganisms. Therefore, *Azotobacter* and *Rhizobium* biofertilizers are recommended especially for organic millet cultivation.

Keywords:- *Millet Microorganisms Biofertilizers Germination Inhibition.*

I. INTRODUCTION

Bio-fertilizer is a biotic constituent of specific microbial cells which when applied stimulates plant growth by accelerating the rate of nutrient release through nitrogen fixation, phosphorus cycle and other processes [1]. It is a modernized form of organic fertilizer to which beneficial microorganisms have been incorporated [2]. Cultures of specific microbes are selected *in vitro* for the biofertilizer production in order to fulfill specific plant nutrient requirements [3]. Bio-fertilizers have been in use a long time ago, because the knowledge of applied microbial inoculum is a long history which passes from generation to generation of farmers which started with culture of small scale compost production that has evidently proved the suitability of bio-fertilizer [4]. Several benefits have been associated with fertilizers. These include fixing nutrient availability in the soli through improving soil fertility, as complex organic compounds are converted to simple forms [5]. Their modes of action include; Nitrogen fixation; which include symbiotic *Rhizobium, anabonema,* associate symbiotic *Azosprillium* and water-free living *Azolla* in association with cyanobacteria used in wetland rice [6]. Phosphorus mobilization and solubilization; which include ecto mycorrhizae intracellular obligate endosymbiotic fungi with vesicles for nutrient storage and abuscules for directing phosphorus, zinc and Sulphur into the plant root system [7]. Also includes plant growth promoting bacteria that either serve as bio-protectants, bio-fertilizers or bio-stimulants, such as *Pseudomonas* sp [8]. Many factors combine to cause limitations to use of bio-fertilizers which include; Technical; the use of less effective strains, lack of competent technical staff [9], poor quality inoculant synthesis [10] and short shelf life of produced inoculants [11] make up this group. Infrastructural; these include appropriate production facility deficiency, absence of crucial equipment for production [12], lack of production or storage space and absence of cold storage facility for inoculants [13]. Economic and quality factors are unavailability of required funds because there is low profit generation in small scale units. Also, the manufacturers are sometimes ignorant of quality management and control methods [14] coupled with the absence of strict regulations (Bagyaraj and Aparna, 2009[15]). There is also inadequate presence of channels or markets for producers of bio-fertilizers plus the right inoculants are usually unavailable [16]. Bio-fertilizers have been observed to be environment friendly and do not cause pollution unlike inorganic fertilizers which often 'run off' into water bodies causing eutrophication and 'blue baby syndrome' (acquired methemoglobinemia) at high nitrate level is above 10 mg/L [17]. Excessive application does not arise in the use of bio-fertilizer and special skills are not required for its application [18]. They also act as a soil conditioners adding organic matter to the soil helping to bind the soil particles together and preventing soil eructing, desertification, and erosion while at the same tine increasing the water retention capacity of the soil [2]. *Pennisetum glaucum* (L) R.Br, commonly called millet, belongs to the Class Liliopsida (Monocotyledons), Subclass Commelinidae, Order Cyperales, Family Gramminae (Grass family) and the Genus *Pennisetum* (fountaingrass) [19]. It is thought to have basically originated from Africa or India, and is one of the major crops of Nigeria, China, Russia, India, South – East Asia, Sudan, Pakistan, and Arabia. It is one of the most

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drought – resistant grains in commercial production which tolerates sandy and acidic soils than other summer grain crops. It is an erect annual grass reaching up to 3m high, with profuse root system [20]. It is deep rooted and can use residual nitrogen phosphorus and potassium which make it need level of fertility required by other summer grains. These characteristics enhance its desirability in lower input dry land production systems. It grows well at temperatures 75 – 90ºF with emergence at 2 – 4 days under favorable conditions [21]. Millets are tall grasses with heads of small seeds grown in harsh environments where other crops generally fail. For centuries, millet has been a prized crop in China, India, Greece, Egypt and Africa where it is used in everything from bread to couscous and as cereal grain. Pearl millet (*Panicum glaucum* L.), finger millet (*Eleucine corcana* L.), and foxtail millet (*Setariaitalica* L.) are the most important millets. Across Africa and Asia, it is grown mainly for food and feeds for livestock. In non-traditional areas such as Southern United states, Brazil, Australia and Korea, it is grown for forage and silage production for dairy [22]. The crop is tolerant to cold, salt, alkali and drought conditions as such, can be cultivated in various soil types, under poor growing conditions [23]. It has been observed by Chandrasekara (2010), [24] that there are phenolics in millet whole grains. In African countries, the national average grain yield is generally low range of 400 – 600 kg/ha [25] and for Uganda, yields between 0 – 900kg/ha have been reported [26]. Millet is important in treating stomach ulcers; beneficial for heart health, bone growth, development and repairs, diabetes control; helps weight loss, and reduces cholesterol and risk of cancer [27].

II. MATERIALS AND METHODS

The study was conducted in Aliero, the Headquarters of Aliero Local Government in Kebbi State Nigeria. Located on latitude 113'S, 12º44'N and longitude 36ºW, 4ºE, Aliero Local government has a flat, slightly undulating topography with compact and brown soil [28]. It has a population of 125,785 inhabitants [29]. The major crops Aliero are Onions, Millet, Groundnuts and Sorghum.

Seed treatment was according to procedures of Center for Microbiology and Biotechnology (CMBT) research and training institute, Bhopal. Seeds were poured into a beaker and washed with distilled water, then with detergent. It was then rinsed with tap water, followed by distilled water. The seeds were then washed with salt solution in the ratio of 39:1 then washed with distilled water. They were finally washed with 95% alcohol before being rinsed thoroughly with distilled water. The organisms, *Rhizobium* and *Azotobacter* were used to produce 25ml of biofertilizers in two replicate laboratory bottles following which the biofertilizers were then bioassayed and three different concentrations of 10mg/ml, 15mg/l and 20mg ml were attained from each biofertilizer replicate. These concentrations were then used in application on the *Pennisetum glaucum* pots in three triplicates for each, while leaving three pots as uninoculated controls. The organisms were isolated from the soil and cultured in the laboratory to obtain pure cultures before the biofertilizers were produced.

Sowing of millet was performed adopting the procedure as reported by Iwuagwu *et al.* (2013), [30] as modified. Ten seeds of each millet variety were sown in each pot containing 2kg of soil. The physical and chemical properties of the soil prior to planting were analysed. Inoculation was carried out 2 days before planting. The treatments included: control (No application), 10, 15 and 20ml/l. For the treatment combinations, 2ml of the individual treatments (*Azotobacter* and *Rhizobium*) was mixed in each soil to form the inoculums and sufficient water added. The millet was planted during the rainy season at 2cm depth in triplicate pots for each treatment. The water supply was from rain. Seed germination was observed for six days. The mean rate and percentage seed germination was recorded.

III. RESULTS AND DISCUSSIONS

A. *Effect of Biofertilizers in Millet Germination*

a) Bahaushe and Dansalka Millet Germination in *Azotobacter* Biofertilizer

Table 1 shows the percentage means of germination for Bahaushe and Dansalka varieties, where in day one after sowing, none of the pots containing the different biofertilizers treatments had germination. At the second day, however germination was recorded from all three treatments, except control with 20ml/l showing greater germination of 53% for Bahaushe. The 15ml/l treatment showed 33%, while the 10ml/l germination was 17%. Day three germination recorded 100% germination of seedlings in 20ml/l treatment, while control had 27%. The 15 ml/l 80%, 10ml/l had 83% and control recorded 63%. Final germination for control was 87%, revealing that some of the seeds could not germinate, without the treatment. Copeland *et al.* (2015), [31] observed that the effects of soil-derived communities on plant undergo continuous succession in above-ground, while the below-ground fractions of the plant were reported by Shade *et al.* (2013), [32].

The percentage for Dansalka millet also in Table 1 revealed highest germination in 20ml/l treatment. All the seedlings in pots 2 and 3 had emerged as at the third day after sowing, but mean germination was 97%. Prior to this, second day, mean germination was 63%. No seedling germination was observed on the first day after planting the seeds in this treatment. From day 4, all 100% germinated in 20ml/l. The second rate of germination was from 15ml/l. Under this treatment total seedling emergence was also on the fourth day post planting but at the third day mean germination was 90%. Mean germination by the second day post sowing was 37%. No germination was observed in control on day 2. Like all other treatments in this category, there was also no emergence observed on the first day post planting. The lowest rate of germination with *Azotobacter* biofertilizer happened in the 10ml/l treatment where 83% emergence on day 4 was recorded.. The prolonged activity of biofertilizers may not be guaranteed as Finkel *et al.* (2017), [33] observed that even if plant growth promoting inoculants colonize plants at the initial stage, their presence over time is not guaranteed [34]. Haney *et al.* (2015) [35] reported that heterologous bacterial inoculants can persist in soil for up to seven weeks. The germination of the two millet varieties in biofertilizers is represented in Plates 1 and 2. in the

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Rhizobium biofertilizer, the Bahaushe is presented in Plates 1A, 20ml/l; 1B, 15ml/l and 1C the control. The Dansalka germination is presented in Plates 1D, 20ml/l; 1E, 15ml/l and 1F the control. Also, *Azotobacter* biofertilizer germination is presented in Plate 2.

		10ml/l		15ml/l		20ml/l	
Day	Control	BAZO	DAZO	BAZO	DAZO	BAZO	DAZO
1	0	0	0	0	0	0	0
2	0	17	17	33	37	53	63
3	27	60	57	80	90	100	97
4	63	83	100	100	100	100	100
5	83	100	100	100	100	100	100
6	87	100	100	100	100	100	100

Table 1: Percentage Germination of Bahaushe and Dansalka Millet in *Azotobacter* Biofertilizer

Key: BAZO: Bahaushe in *Azotobacter*; DAZO: Dansalka in *Azotobacter*

		10ml/l		15ml/l		20ml/l	
Day	Control	BARZ	DARZ	BARZ	DARZ	BARZ	DARZ
1	0	0	0	0	0	0	0
2	0	13	17	37	33	57	67
3	27	47	37	100	100	100	100
4	63	80	70	100	100	100	100
5	83	93	83	100	100	100	100
6	87	93	90	100	100	100	100

Table 2: Percentage Germination of Bahaushe and Dansalka Millet in *Rhizobium* Biofertilizer

Key: BARZ Bahaushe in *Rhizobium*; DARZ: Dansalka in *Rhizobium*

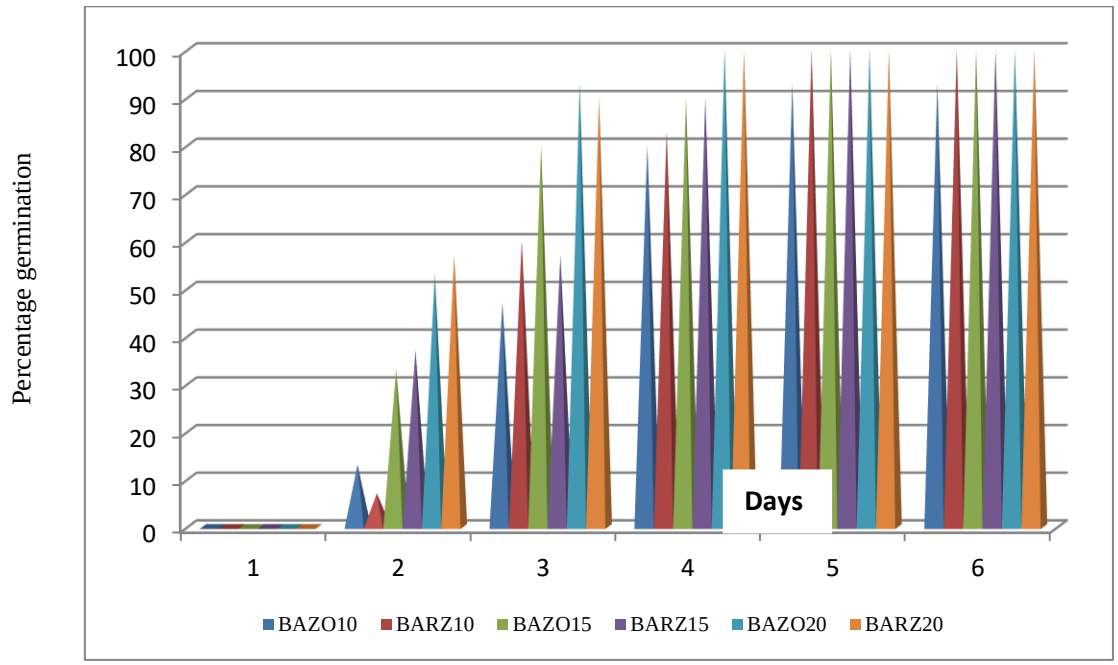

Fig. 1: Percentage Bahaushe Millet Germination in Azotobacter and Rhizobium Biofertilizers

Key: BAZO10 = Azotobacter 10ml/l; BARZ10 = Rhizobiumin 10ml/l; BAZO = Azotobacter 15ml/l; BARZ15 = Rhizobiumin 15ml/l; BAZO20 = Azotobacter 20ml/l; BARZ20 = Rhizobiumin 20ml/l

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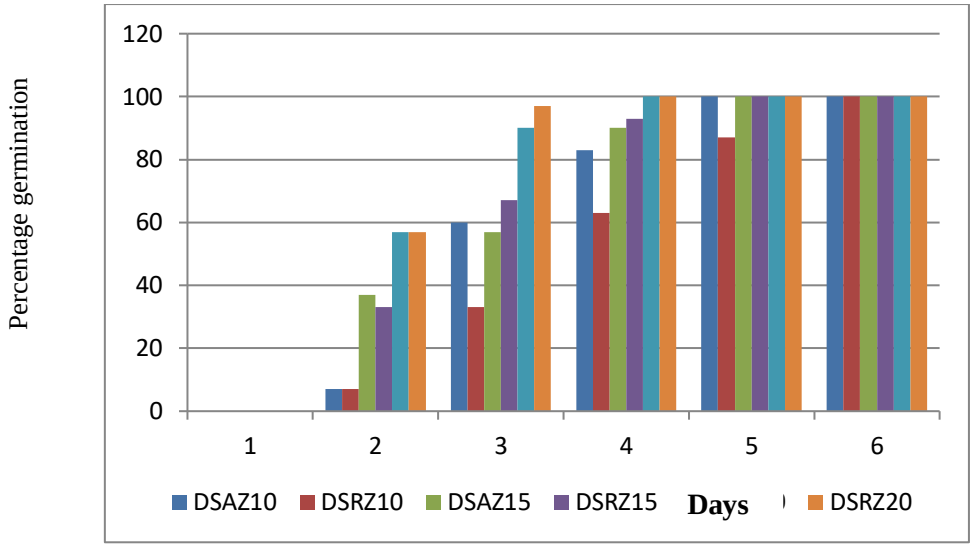

Fig. 2: Percentage Dansalka Millet Germination in *Azotobacter* and *Rhizobium* Biofertilizers

KEY: DSAZ10 = *Azotobacter* 10ml/l; DSRZ10 = *Rhizobium* 10ml/l; DSAZ15 = *Azotobacter* 15ml/l; DSRZ15 = *Rhizobium* 15ml/l; DSAZ20 = *Azotobacter* 20ml/l; DSRZ20 = *Rhizobium* 20ml/l

b) Bahaushe and Dansalka Millet Germination in *Rhizobium* Biofertilizer

Zero germination was recorded in all pots with all treatments at the first day after planting the seeds, as shown by the percentage mean of the results in Table 2. The same was also recorded at the second day after planting from control, but 10ml/l treatments recorded 13% while 15 and 20ml/l had 37 and 57% germination respectively for Bahaushe. The third day recorded the highest germination from 20ml/l and 15ml/l with full germination of all seeds. The 10ml/l had 47% germination. The fifth and sixth day, however maintained 93%for 10ml/l, against the control values of 83 and 87% for the respective days. The pictorial presentation of germination results are presented on the Figure 2. The bar chart showed that the 20ml/l concentration a peaked at day three while control did not reach peak even at the last experimental day. Tan *et al.*, (2014) [36] observed a positive effect of *Rhizobium* application on rice.

The rate of germination of Dansalka variety had the highest germination in both 20ml/l and 15ml/l biofertilizer. The results also shown in Table 2 had total germination on the third day for 20ml/l and 15ml/l treatments while 10ml/l had 37% and the control recorded 27%. A day before however, being day 2, total germination from 20ml/l was recorded from pot 2 but the mean germination was 67%. Day two post sowing revealed five seedlings from pot 1 of 15ml/l treatment but with mean germination of 57%. The 10ml/l treated pots had 17% against the control values of zero on day 2. Anubrata and Rajendra (2014) reported overall increment in growth of *Capsicum annum* using biofertilizers containing *Rhizobium* [37].

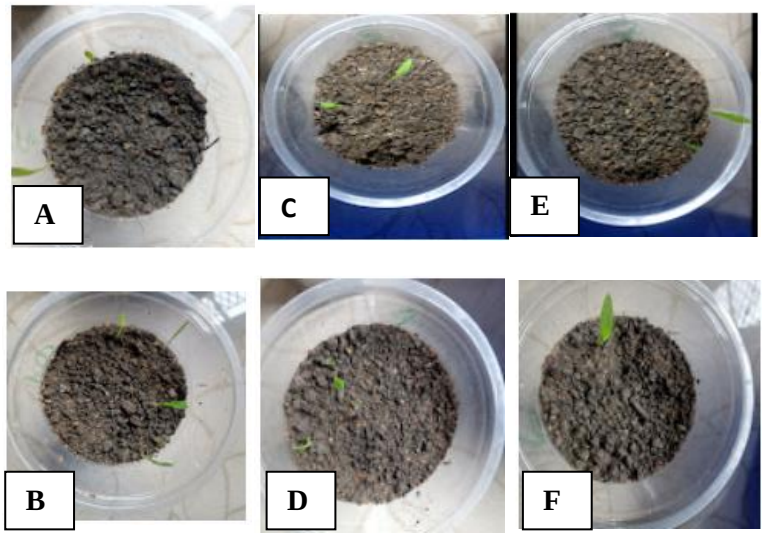

Plate 1: Germination of Millet seeds in *Rhizobium* Biofertilizer

KEY: A,B: 20 20ml/l C,D: 15ml/l E: 10ml/l F: Control

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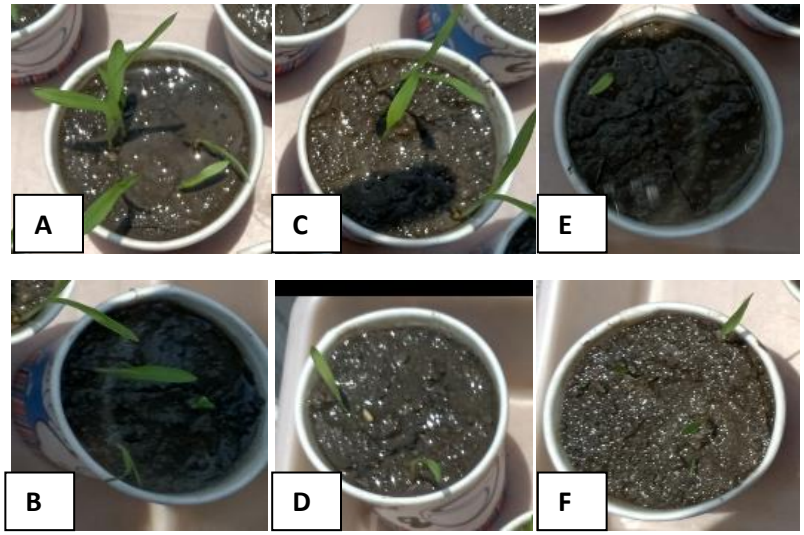

Plate 2: Germination of Millet Seeds in *Azotobacter* Biofertilizer

KEY: A,B: 20ml/l C,D: 15ml/l E: 10ml/l F: Control

B. Antimicrobial activity of Millet

The control ability of the extract on the bacteria as presented in Table 3 the highest value of 15.33 was observed from the highest concentration of 400ml/l on *X, campestris*, and *A. vinelandi*, 15.00mm against *X. axonopodis* and *R. phaseoli*.. The lowest extract concentration of 200ml/l was mostly ineffective revealing 3.67mm. Inhibition by 300ml/l recorded 12.33mm against *A. vinelandi* and 12.00mm for *X. axonopodis* and *R. phaseoli*, against the control values of 18.00mm. Inhibition values from 200ml/l revealed inhibition of 7.00mm against *R. phaseoli*, 4.00mm against *X. campestris* and 3.67mm against both *X. axonopodis* and *A. vinelandi*. Only one of the triplicate plates in the 10ml/l showed any sign of activity. The analysis of variance for the antibacterial and antifungal activity of the millet extract on the various isolated organisms. The effect on the bacteria showed no significant difference within the same concentration in all organisms, but the change in concentration demonstrated significant difference as a result of effect on each organism. *Rhizobium* sp revealed the greater difference between all concentrations. The observed inhibition activity is due to the presence of antioxidants and phenolics present in millets. The antioxidant capacity of millets has been reported, in a study, where it was found that the bound phytochemicals of grain prevents colon, prostate, breast and other digestive cancers (Florence and Asna, 2012) [38]. Phytochemicals and phenolic compounds in millets enhance its antioxidant activity and make it nutritionally superior to other cereals (Prabha and Selvi, 2016) [39]. Little significant difference was observed at $p<0.05$, in the antibacterial capabilities, with the difference only reflecting in the concentrations, but not between organisms.

Conc (ml/l)	*X. axonopodis*	*X. campestris*	*A. vinelandi*	*R. phaseoli*
0.00	18.00±0.20a	18.00±0.20a	17.00±0.43a	18.00±0.20a
200.00	3.67±0.63c	4.00±0.00c	3.67±0.00c	7.00±0.00c
300.00	12.00±0.63b	11.67±0.63b	12.33±0.63b	12.00±0.05b
400.00	15.00±0.05ab	15.33±0.05ab	15.33±0.a04b	15.00±0.04ab

Table 3: Antimicrobial Activity of Millet Extract on Bacteria

IV. CONCLUSIONS AND RECOMMENDATIONS

The study revealed that microbial biofertilizers from *Azotobacter* and *Rhizobium* have positive influences on the germination of both Bahaushe and Dansalka varieties and the *Azotobacter* biofertilizer promoted faster seed germination than the *Rhizobium* biofertilizer. The Bahaushe variety had a faster germination rate than the Dansalka when treated with the two biofertilizers. The extract displayed inhibition activity against the bacteria at high concentrations, but was not effective at low concentrations.

Use of the biofertilizers is recommended but with cautionary measures including using appropriate protective gears during application, sticking to proper care of the fertilizers during storage so as to avoid mutation of the organisms. The combined use of these bacterial biofertilizers with organic amendments and cover crops could be used as an emerging tool for restoring degraded soils. the need to increase farmers and other users' awareness on the use of biofertilizers as safer crop production components It is mandatory however to guide the farmers on the consequences of misuse of microbe-based biofertilizers so as to avoid actions that can result in the mutation of such organisms which might give rise to unwanted consequences.

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