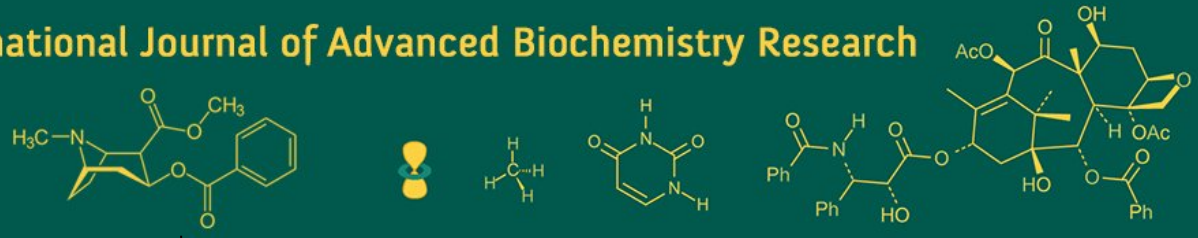
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2023; SP-7(2): 434-437
www.biochemjournal.com
Received: 07-08-2023
Accepted: 13-09-2023

Ashwini
Department of Agricultural Microbiology, College of Agriculture, Dharwad, Karnataka, India

Corresponding Author:
Ashwini
Department of Agricultural Microbiology, College of Agriculture, Dharwad, Karnataka, India

Effect of formulations of microbial consortia on the nutrient content in maize shoot (*Zea mays* L.)

Ashwini

DOI: https://doi.org/10.33545/26174693.2023.v7.i2Sf.250

Abstract
A pot experiment was conducted to study the effect of developed formulations of microbial consortia on the nutrient content in maize crop. A total of five formulations of two different microbial consortia were developed. Microbial consortia includes the agriculturally beneficial microorganisms such as nitrogen fixer (either of *Azospirillum* ACD-15 or *Gluconacetobacter* G_1), P-solubilizing (*Pseudomonas striata*) and K-solubilizing bacterium (KSB) in different formulations *viz*., kaolinite, bentonite, lignite, liquid and Na-alginate formulations. The results showed that the application of these microbial consortia in different formulations enhanced nutrient (N, P and K) content in maize shoot over uninoculated and recommended biofertilizer (*Azospirillum* ACD-15 and *Pseudomonas striata*) application. Among the five formulations used, liquid formulation showed the highest N, P and K content in maize shoot at 45 DAS and K content at 90 DAS. Na-alginate formulation showed the highest N and P at 90 DAS. This study identifies the importance of formulation of bioinoculants consortia as they increase the shelf life of bioinoculants in consortia.

Keywords: Maize shoot (*Zea mays* L.), *Pseudomonas striata*, kaolinite, bentonite, lignite

Introduction
In sustainable agriculture practices, bioinoculants called as plant growth promoting rhizobacteria (PGPR) which play a crucial part in enhancing crop yields by improving crop growth either through direct or indirect ways (Meena *et al*., 2015; Jha and Subramanian, 2016) [10, 9]. These bioinoculants are applied as biofertilizers and biopesticides. Biofertilizers are defined as the substances containing living microorganisms which when applied to seed, surfaces of plant or soil, they colonize the plant and promotes growth by increased nutrient availability due to their action (Vessey, 2003) [21]. Biofertilizers comprise mainly of N_2 fixing, phosphorus, zinc and K solubilizing bacteria, plant growth promoting rhizobacteria and consortium of N_2 fixing, phosphorus and potash solubilizing bacteria. Among biofertilizers, the crop production is enhanced by *Azotobacter* sp., *Azospirillum* sp., blue green algae, *Azolla* sp., *Psuedomonas* sp., *Bacillus* sp., mycorrhizae and *Rhizobium* spp. These biofertilizers help to solve problems such as chemical run off from the agricultural field and reduces the use of chemical fertilizers, boost the status of soil fertility and through their biological activity in the rhizosphere, increases crop productivity.
Soil microorganisms live in community exerting beneficial effects on the plants. Further, when these microorganisms are inoculated to seeds or rhizosphere soil, they should interact with each other and host crop and colonize developing roots without any negative effects (Harman, 1991) [6]. The positive interaction among microbes plays an important role in improving the status of nutrient in soil and enhances crop growth, nutrient uptake and ultimately increase yield of crops. But, these beneficial microorganisms are available in biofertilizers containing single microbial strains. To derive combined effect one has to use multi strains and to apply these biofertilizers as many times as number of microbial strains are required. This is time consuming, laborious, costly and may cause seed coat damage.
The concept of consortium of microbes has been developed using a number of microorganisms which are compatible with each other and when co-cultured, should excert synergistic effects without harming other beneficial microorganisms and enhanced uptake of nutrients, growth and crop yield (Akthar and Siddiqui, 2008) [2] and also suppressed diseases (Jain *et al*., 2012; Singh *et al*., 2013) [8, 18].

Many investigations have been documented that application of microbial consortium had resulted in enhanced nutrient content, growth and yield of crop (Raja *et al*., 2006; Rajasekar and Elango, 2011) [14, 15] and against *Fusarium udum* in *Cajanus cajan* (Dutta *et al*, 2008) [5].
Therefore, the objective of the current experiment was to determine the impact of different formulations of microbial consortia on the nutrient content in maize shoot under pot culture.

Materials and Methods

Collection of culture

The microbial strains used in this study such as local strain of *Azospirillum* ACD-15, *Gluconacetobacter* local strain G_1, *Pseudomonas striata* (PSB), and potassium solubilizing bacterium (KSB) were collected from the Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad. The cultures were purified by repeated four ways streaking and maintained as slants on respective specific medium.

Compatibility test

All four microbial strains were examined for their compatibility under *in-vitro* on a nutrient agar by cross streak method as described by Anandraj and Leema**,** (2010) [4].

Preparation of microbial consortium (MC)

Two different microbial consortia were prepared based on the equal population of microbial strains used. The consortia were prepared by mixing equal proportion (1:1:1) of microbial strains grown on their respective specific media.

- **Microbial consortium 1** (*Gluconacetobacter* G_1 + PSB + KSB)
- **Microbial consortium 2** (*Azospirillum* ACD-15 + PSB + KSB

Preparation of carrier based formulation

The carrier materials used in the experiment were lignite, kaolinite and bentonite. The pH of the lignite was adjusted to 7.0 by using calcium carbonate. The consortia were mixed thoroughly with sterilized lignite (neutralized), kaolinite and bentonite in the ratio of 1:3 and then packed in a polypropylene bag of 75 gauge for further use.

Preparation of liquid formulation

Liquid formulations of two microbial consortia were prepared by amending respective specific broth media with additives as given in Table. 1 (Nisarga and Patil, 2018; Prakash, 2018 and Sandesh, 2017) [11, 13, 16]. At the time of media preparation, additives were added separately to the respective media and sterilized at 121 ºC, 15 lbs/inch^2 for 15 min. After the sterilization, media were cooled and inoculated with 72 hr, 96 hr, 48 hr and 36 hr (time at which maximum population of microbial strains obtained) grown cultures of *Azospirillum* ACD-15, *Gluconacetobacter* G_1, PSB and KSB respectively at 5 per cent and incubated at 28 ± 2 ºC and used further.

Table 1: Cell protectants and their concentrations used in developing liquid formulations of microbial consortia

Amendments		Concentrations of amendments			
		G_1	ACD-15	PSB	KSB
Additives	Polyethylene Glycol (PEG) (%)	0.5	0.5	0.5	0.5
	Trehalose (mM)	5	5	-	-
	Glycerol (mM)	5	5	5	5
Adjuvant	Gum Arabica (%)	0.15	0.15	0.15	0.15
Surfactant	Polysorbate-20 (ppm)		250	250	250
Antioxidant	Ascorbic acid	0.02	-	-	-

Preparation of Na-alginate formulation

Microbial strains were Entrapment within Na-alginate beads under aseptic conditions as given by Saxena (2013) [17]. These beads were further used for pot culture experiment.

Note: Each consortium consists of a nitrogen fixer: PSB: KSB with 1:1:1 ratio was prepared by mixing individual microbial strains based on equal population.
Two microbial consortia in different formulations were treated as mentioned below in treatment detail. Pre-germinated maize seeds were treated with respective consortia diluted with 60 ml of sterile distilled water to distribute formulation uniformly on all seeds sown in respective pots.

Treatments details

MC1F1 = RDF + MC-1 Lignite based
MC1F2 = RDF + MC-1 Kaolinite based
MC1F3 = RDF + MC-1 Liquid based
MC1F4 = RDF + MC-1 Na- alginate based
MC1F5 = RDF + MC-1 Bentonite based
MC2F1 = RDF + MC-2 Lignite based
MC2F2 = RDF + MC-2 Kaolinite based
MC2F3 = RDF + MC-2 Liquid based
MC2F4 = RDF + MC-2 Na-alginate based
MC2F5 = RDF + MC-2 Bentonite based
F6 (Farmer's practice) = RDF + Recommended dose of *Azospirillum* ACD-15 + PSB
F7 (UIC) = RDF only

Note: MC = Microbial Consortia
*RDF = Recommended dose of fertilizers
UIC = Uninoculated control
Factor Microbial consortia (MC): 2 factors
Factor Formulation (F): 5 formulations

Nutrient content estimation in maize shoot

Nutrient content in maize shoot were estimated at 45 and 90 DAS. The micro Kjeldahl method, described by Jackson, (1973) [7] was used to estimate the N concentration in the shoot. Phosphorus content by Vanadomolybdo phosphoric yellow colour method (Tandon, 1998) [19]. And potassium content using flame photometer (Tandon, 1998) [19].

Statistical analysis

Factorial Completely Randomised Design (FCRD) was used in the statistical analysis of the study data. The data were interpreted in compliance with Panse and Sukhatme (1985)

[12]. The level of significance used in the 'F' and 't' test was $p<0.001$. The least significant differences method was used to compare the mean values between treatments (L. S. D). Duncan's Multiple Range Test was used to compare the treatment means (DMRT).

Results and Discussion

Inoculation of different formulations of microbial consortia was recorded to significantly improve nutrient content in maize shoot at 45 and 90 DAS.

The two different microbial consortia used significantly influenced the nitrogen content in maize shoot. The MC-2 recorded significantly higher nitrogen content (1.88 per cent) at 45 DAS and (2.66 per cent) at 90 DAS compared to MC-1 (1.82 per cent) at 45 DAS and (2.62 per cent) at 90 DAS. Among the five formulations used, significantly the highest nitrogen content in maize shoot was recorded in treatment receiving liquid formulation (2.19%) at 45 DAS and Na-alginate formulation (2.80 per cent) at 90 DAS.

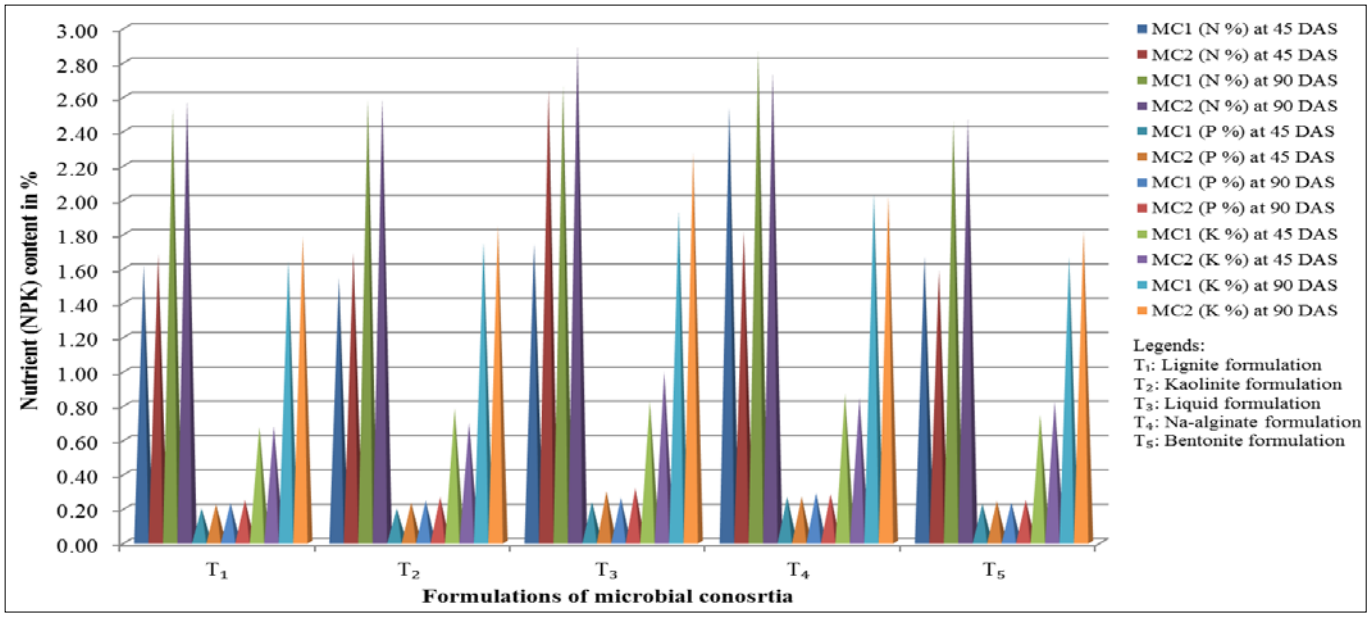


Fig 1: Maize shoot nutrient (N, P, and K) content as affected by microbial consortia formulations inoculated at 45 and 90 DAS.

Plant phosphorus content was also significantly enhanced by the microbial consortia treatment, where The MC-2 recorded significantly higher phosphorus content (0.25 per cent) at 45 DAS and (0.27 per cent) at 90 DAS compared to MC-1 (0.22 per cent) at 45 DAS and (0.25 per cent) at 90 DAS. Among the formulations used, treatment T_3 receiving liquid formulation and treatment T_4 receiving Na-alginate formulation (0.27 per cent respectively) at 45 DAS and treatment T_3 receiving liquid formulation and treatment T_4 receiving Na-alginate formulation (0.29 per cent respectively) at 90 DAS were recorded significantly highest plant nitrogen content.

Similarly, The MC-2 recorded significantly higher potassium content (0.81 per cent) at 45 DAS and (1.95 per cent) compared to MC-1 (0.78 per cent) at 45 DAS and (1.80 per cent) at 90 DAS. Among the formulations used, the potassium content of maize plant was recorded significantly higher in treatment T_3 receiving liquid formulation (0.99 per cent) at 45 DAS and (2.10 per cent) at 90 DAS compared to the other treatments.

The results of this study are in accordance with the findings of Thilagar *et al*., (2016) [20]; Abou and Abdel, (2012) [1] and they could be attributed to the multiple mechanisms of nutrient release by the microbial strains in microbial consortia. It is noticed that liquid formulation significantly enhanced N, P and K content in maize shoot at 45 DAS but at 90 DAS, Na-alginate formulation significantly enhanced N and P content except K content in maize shoots over other treatments including dual and uninoculated control. This is because Na-alginate formulation helps in slow release of bioinoculants compared to the liquid formulation (Amalraj *et al*. 2015) [3].

Conclusion

From this study, it was interesting to note that the formulations of microbial consortia significantly enhanced the nutrient content in maize shoot thereby growth of maize irrespective of microbial consortium used. Although the biofertilizer strains used in this study were exactly the same but the carrier materials used differed revealing the crucial role of the carrier material in this investigation.

Acknowledgment

The author is grateful to the Department of Microbiology, University of Agricultural Microbiology and All India Co-ordinated Research Project on Maize, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, India., for providing the facilities and financial support, to undertake the investigations. There are no conflicts of interest.

References

1. Abou II, Abdel MA. Impact of rock materials and bio-fertilization on P and K availability for maize (*Zea Mays*) under calcareous soil conditions. Saudi J Biol. Sci. 2012;19(1):55-63.
2. Akthar MS, Siddiqui ZA. Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp. and *Pseudomonas striata.* Crop Prot. 2008;27(3-5):410-417.

3. Amalraj ELD, Mohanty D, Kumar GP, Desai S, Ahmed SMH, Pradhan R, *et al*. Potential microbial consortium for plant growth promotion of sunflower (*Helianthus annuus* L.). Proc. Natl. Acad. Sci., India, Sect. B. Biol. Sci. 2015;85(2):635-642.
4. Anandaraj B, Leema R, Delapierre A. Studies on influence of bioinoculants *Pseudomonas fluorescens*, *Rhizobium* sp., *Bacillus megaterium* in green gram. J. Biosci. Technol. 2010;1(2):95-99.
5. Dutta S, Mishra AK, Kumar BSD. Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. Soil Biol. Biochem. 2008;40(2):452-461.
6. Harman GE. Deployment tactics for biocontrol agents in plant pathology, biological control: Alternatives for suppressing agricultural pests and diseases. Alan, R. Liss, New York; c1991. p. 779-792.
7. Jackson ML. Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi; c1973.
8. Jain A, Singh S, Sharma BK, Singh HB. Microbial consortium mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotina sclerotiorum*. J Appl. Microbiol. 2012;112(3):537-550.
9. Jha M, Subramanian S. Microbial consortium for sustainable rice production. Agroecol. Sustainable food systems. 2016;37(3):340-362.
10. Meena VS, Maurya BM, Verma JP, Abhinav A, Ashok K, Kangmin K, *et al*. Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica. Ecol. Engg. 2015;81(2):340-347.
11. Nisarga HS, Patil CR. Studies on developing mixed consortia of N_2-fixing and phosphate solubilizing liquid biofertilizers and population dynamics of biofertilizer strains in the consortia. J Farm Sci. 2018;31(2):156-161.
12. Panse VG, Sukhatme PV. Statistical Methods for Agricultural Workers, 4th Ed., ICAR, New Delhi (India); c1985, 347.
13. Prakash DA. Studies on developing liquid formulations of native isolate of gluconacetobacter. M. Sc. (Agri.). Thesis, Univ. Agric. Sci., Dharwad, Karnataka (India); c2018.
14. Raja P, Uma S, Gopal H, Govindarajan K. Impact of bio inoculants on rice root exudates, biological nitrogen fixation and plant growth. J Biol. Sci. 2006;6(5):815-823.
15. Rajasekar S, Elango R. Effect of microbial consortium on plant growth and improvement of alkaloid content in *Withania somnifera* (Ashwagandha). Curr. Bot. 2011;2(8):27-30.
16. Sandesh P. Optimization of cell protectants, adjuvant and additives to develop liquid formulation of *Azospirillum*. M. Sc. (Agri.). Thesis, Univ. Agric. Sci., Dharwad, Karnataka (India); c2017.
17. Saxena MJ, Chandra S, Nain L. Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. J Soil Sci. Plant Nutri. 2013;13(2):511-525.
18. Singh A, Sarma BK, Upadhyay RS, Singh HB. Compatible rhizosphere microbes mediated alleviation of biotech stress in chickpea through enhanced antioxidant and phenylpronoid activities. Microbiol. Res. 2013;168(2):33-40.
19. Tandon HLS. Methods of analysis of soils, plant, water and fertilizers. Fertilizer Development and Consultation Organization. 1998;31:9-16.
20. Thilagar G, Bagyaraj DJ, Rao MS. Selected microbial consortia developed for chilly reduces application of chemical fertilizers by 50% under field conditions. Scientia Hortic. 2016;198:27-25.
21. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255:571-586.