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Potassium supplement for optimised culture medium for outdoor cultivation of *Nostoc commune* in the Philippines

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Abstract

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Nostoc commune is a nitrogen-fixing cyanobacterium. It could serve as an alternative source of biofertiliser that is both ecologically beneficial and sustainable. In this study, different concentrations of potassium (0, 3, 6, and 9 mg/kg) were added to an optimised culture medium containing 10% soil-water extract with 2.6 mg/kg of phosphorus. The soil-water extract solution was prepared using the Maligaya heavy clay soil series. The findings revealed that *Nostoc commune* grown on an optimised culture medium supplemented with 6 mg/kg or 9 mg/kg of potassium showed significant growth and development, as evidenced by increased fresh weight, persistent dark green colouring, and a high number of heterocysts for up to 28 days after inoculation. This optimised culture medium, supplemented with 6 mg/kg or 9 mg/kg of potassium, can be used to inoculate large-scale outdoor cultures.

Keywords: biofertiliser, environment-friendly, N-fixing cyanobacteria, nitrogen fertiliser.

INTRODUCTION

Nostoc commune Vaucher is a cyanobacterium used worldwide in traditional medicine and as a food source. *Nostoc commune* and its by-product extracts have been shown to strengthen the immune system and inhibit oxidation, inflammation and cancer (Li & Guo, 2018). *Nostoc commune* extract found in wetlands has antioxidant properties (Ninomiya et al., 2011).

In the Philippines, the cyanobacterium *Nostoc commune* was found in rice paddies and hilly areas of northern Luzon. *Nostoc commune* is an edible cyanobacterium that forms gelatinous colonies that can expand into visible blooms under favourable conditions, such as high moisture and adequate sunlight. Its protein content varies from 20% to 51% crude protein, and its standing crop can reach 673.3 g/m² of fresh weight (Matinez-Goss, 2018).

Conversely, the production of commercial fertilisers is expensive due to the high cost of the necessary technology (Kongshaug, 1998). The Philippines imports fertiliser, particularly from China, Indonesia, and Malaysia. The two most popular commercial nitrogen fertilisers in the Philippines are ammonium sulphate (21% nitrogen) and urea (46% nitrogen). The recommended usage of commercial fertilisers may be beyond the budget of most farmers due to the high cost of imported fertilisers (Briones, 2017). However, biofertilisers provide an alternative to expensive commercial fertilisers. Biofertilisers are made up of living or dormant microorganisms that are efficient at fixing nitrogen, solubilising phosphate or breaking down cellulose. These microorganisms are applied to seeds, soil or composting facilities (Sethi et al., 2018).

Furthermore, by absorbing nitrogen from the air and promoting plant growth, biofertilisers can improve soil health. Biofertilisers are defined as products derived from cyanobacteria, fungi and bacteria. Consequently, they are crucial for both environmental sustainability and agricultural productivity. They can be categorised in several ways, including improving soil microcultures, encouraging plant growth, phosphate solubilisation and mobilisation, and nitrogen fixation (Agarwal et al., 2018).

Since nitrogen is one of the most important nutrients for rice plants, cyanobacteria such as *Nostoc commune* are a promising source of biofertiliser and nitrogen (Rai et al., 2019). According to Roy et al. (2016), *Nostoc commune* can fix 30–100 kg of nitrogen per hectare from the atmosphere. Due to its role as a biofertiliser, *Nostoc commune* can be used to provide nitrogen for rice cultivation (Ito & Watanabe, 1985; Singh & Singh, 1987; Pereira et al., 2009; Mandal et al., 2011). Enriching the soil with 2.6 mg/kg of phosphorus encourages the growth and development of *Nostoc commune* colonies. It may improve the fertility of troubled soils, raising the productivity of rice-growing regions (Ramos et al., 2022).

The following reasons explain why potassium supplementation for *Nostoc commune* is not optimised in the Philippines' conditions: there are very few local studies investigating the effect of potassium; there is no standardised basis for comparison; soil potassium levels often fluctuate or are already sufficient; and nitrogen-based studies overshadow potassium optimisation. This study will propose a potassium supplementation regime for a low-cost, easily maintained culture medium for outdoor cultivation of *Nostoc commune*. A ten per cent soil-water extract with optimal levels of potassium and phosphorus (2.6 mg/kg) can create and improve a reliable supply of readily available nitrogen. The on-farm production of *Nostoc commune*, particularly in rice-producing regions, will increase productivity in culturally depleted areas.

This study aimed to compare different potassium concentrations in an optimised culture medium for the outdoor growth and development of *Nostoc commune*. The following questions were addressed in low-potassium culture medium for *Nostoc commune*: (1) What is the optimal medium for *Nostoc commune* with a potassium supplement? (2) What are the maximum growth and developmental rates of *Nostoc commune* using optimised culture medium with different concentrations of potassium? (3) What are the highest numbers of heterocysts using optimised culture medium with different concentrations of potassium?

MATERIALS AND METHODS

Biological material

The goal of the study was to determine the optimal potassium concentration for *Nostoc commune* growth and development using the optimised culture medium (10% soil-water extract solution with 2.3 mg/kg phosphorus). During the 2024 wet season, the starter (*Nostoc commune*) was purchased in Badoc, Ilocos Norte, Luzon Island, the Philippines. Before being employed in the study, starters were acclimated for 48 hours in a basin filled with tap water.

Medium preparation

There was no *Nostoc commune* in the soil collected at the Philippine Rice Research Institute Central Experimental Station. Analysis revealed that the soil was heavy clay (Maligaya series), with medium nitrogen levels, high phosphorus levels, low potassium levels, moderate acidity (pH 5.59), medium levels of organic matter (OM), and optimal levels of copper and iron.

Soil (100 g) and tap water (400 mL) were mixed for 10 minutes to prepare the soil-water extract. After preparation, the mixture was left for two days. For 20 minutes, the soil-water extract solution was autoclaved at 121°C and 15 psi. The control treatment (T1) consisted of a 10% soil-water extract solution without supplementation of phosphorus and potassium. The optimised culture medium was adopted from the study of Ramos et al. (2022), which is composed of a 10% soil-water extract solution with 2.6 mg/kg of phosphorus (T2). The other treatments are the following: optimised culture medium with 3 mg/kg of potassium (T3); optimised culture medium with 6 mg/kg of potassium (T4); and optimised culture medium with 9 mg/kg of potassium (T5). Phosphorus and potassium for the study were from commercial fertilisers such as muriate of potash (0–0–60) and solophos (0–18–0).

Outdoor culture set-up

The culture medium was placed in plastic containers measuring 25 cm × 17.5 cm × 8 cm. Four grams (4 g) of fresh *Nostoc commune* were inoculated in each plastic container with 750 mL of different culture media as treatments. Tap water was added to maintain the 750 mL amount of culture medium. The culture medium was stirred every day for 10 minutes. The outdoor experiment was conducted at the Philippine Rice Research Institute Central Experiment Station during the 2024 wet season, in a completely randomised design (CRD) with four replications per treatment. The light intensity, temperature, and rainfall data in the experimental area ranged from 4.2 to 4.9 kWh/m²/day, 27.5 to 28.6°C, and 210 mm to 410 mm, respectively (Fig. 1).

Fig. 1. *Nostoc commune* growing in optimised culture medium (10% soil-water extract with 2.3 mg/kg phosphorus) with different concentrations of potassium under outdoor conditions.

Per cent growth, fresh weight, and colour of *Nostoc commune*

The growth and development of *Nostoc commune* were evaluated by fresh weight, surface-dried weight, and growth percentage. The formula used to compute the per cent (%) growth was (actual fresh weight divided by total fresh weight) ×100. Furthermore, fresh weight growth was defined as the difference between actual fresh weight and initial fresh weight.

The colour of the colony of the *Nostoc commune* received the following ratings: 1 for yellowish, 2 for light green, 3 for moderate green, and 4 for dark green. For four weeks, data were collected every seven days. The heterocysts of the colony of *Nostoc commune* were inspected and counted once a week using a compound microscope in a high-power field in one area at 7, 14, 21, and 28 days after inoculation.

Statistical analysis

The Kruskal-Wallis H test was used to compare all treatments, and the interaction between treatments and times was analysed using a two-way permutational multivariate analysis of variance (9999 permutations) based on Euclidean distance. Because of the presence of null values in the data set for the per cent and fresh weight growth, the pairwise comparison of the treatments was performed using Dunn's post hoc test. At $p < 0.05$, the significance level was set. All data were analysed using RStudio software.

RESULTS

Per cent growth of *Nostoc commune* under outdoor conditions

The per cent growth of *Nostoc commune* in an optimised culture medium (10% soil-water extract solution with 2.3 mg/kg phosphorus) with different potassium concentrations was evaluated and quantified from 7 to 28 days after inoculation. The highest percentage growth of *Nostoc commune* was recorded in the optimised culture medium (without potassium) and in cultures supplemented with 3, 6, and 9 mg/kg of potassium, with 5.53%, 6.31%, 6.60%, and 6.57% growth at seven days after inoculation, respectively. Moreover, at 14 days after inoculation, the highest growth was recorded in the optimised culture medium supplemented with 3, 6, and 9 mg/kg of potassium with 24.86%, 28.94%, and 28.74% growth, respectively. Furthermore, at 21 and 28 days after inoculation, the highest growth of *Nostoc commune* was recorded in the optimised culture medium supplemented with 6 and 9 mg/kg potassium, with 58.55% and 92.95% growth, respectively. The per cent growth of *Nostoc commune* in optimised culture medium with 6 and 9 mg/kg potassium did not differ significantly, as determined by statistical analysis. The result of per cent growth of *Nostoc commune* in the different concentration of potassium in optimised culture medium were significant differences at 7 (H = 9.71, p = 0.046), 14 (H = 14.7, p = 0.0055), 21 (H = 13.33, p = 0.0099), and 28 (H = 16.62, p = 0.0025) days after inoculations (Table 1).

A two-way permutation analysis of variance also supported the impact of treatment and time on the per cent growth of *Nostoc commune* (Table 2). It demonstrated that both treatment (p = 0.001) and time (p = 0.001) had a significant effect on the percentage growth of *Nostoc commune*. Nevertheless, there was no significant interaction between the two variables (treatment and time).

Fresh weight growth of *Nostoc commune* under outdoor conditions

The fresh weight growth of *Nostoc commune* in an optimised culture medium (10% soil-water extract solution with 2.3 mg/kg phosphorus) with different potassium concentrations was evaluated and quantified from 7 to 28 days after inoculation. The fresh weight of *Nostoc commune* ranged from 0 to 195 mg at 7 (H = 9.79, p = 0.044) days after inoculation. The highest fresh weight of *Nostoc commune* was recorded in the optimised culture medium containing 6 and 9 mg/kg potassium, ranging from 859 mg to 2757 mg and 853 mg to 2710 mg, respectively, from 14 to 28 days after inoculation. The fresh weight of *Nostoc commune* in an optimised culture medium containing 6 and 9 mg/kg potassium did not differ significantly, as determined by statistical analysis. The results of the studies conducted on the 14th (H = 14.7, p = 0.0055), 21st (H = 19.52, p = 0.00065), and 28th (H = 9.96, p = 0.041) days after inoculation showed significant differences in the fresh weight growth of *Nostoc commune* in an optimised culture medium with varying potassium concentrations (Table 3).

Table 1. Per cent (%) growth of *Nostoc commune* in optimised culture medium supplemented with different concentrations of potassium under outdoor conditions at 7, 14, 21, and 28 days after inoculation. Values are means ± standard deviation (SD). Different letters indicate significant differences (p < 0.05) between the treatments based on Dunn's post hoc test. Abbreviations in treatment: SWE – soil-water extract, P – phosphorus, K – potassium

Treatment	Per cent (%) growth after inoculation			
	7 days	14 days	21 days	28 days
10% SWE	0.00 ± 0.0[a]	2.62 ± 3.0[a]	12.72 ± 6.6[a]	27.42 ± 16.0[a]
10% SWE + 2.3 mg/kg P	5.53 ± 3.1[b]	18.45 ± 4.5[b]	37.91 ± 14.7[ab]	51.26 ± 24.2[ab]
10% SWE + 2.3 mg/kg P + 3 mg/kg K	6.31 ± 4.1[b]	24.86 ± 5.9[c]	44.27 ± 7.2[ab]	63.56 ± 28.1[ab]
10% SWE + 2.3 mg/kg P + 6 mg/kg K	6.60 ± 1.5[b]	28.94 ± 3.8[c]	58.56 ± 3.0[b]	92.95 ± 47.9[b]
10% SWE + 2.3 mg/kg P + 9 mg/kg K	6.56 ± 1.8[b]	28.74 ± 3.5[c]	57.81 ± 5.2[b]	91.36 ± 46.6[b]

Table 2. Effect of treatments and times on per cent (%) growth of *Nostoc commune* in an optimised culture medium supplemented with different concentrations of potassium under outdoor conditions, as determined by a two-way permutation analysis of variance (Euclidean distance, 9999 permutations)

Factor	Sum of squares	df	Mean square	F	p-value
Treatment	108152.50	4	27038.13	20.64	0.001
Time	360653.10	3	120217.7	91.78	0.001
Interaction	10483.40	12	873.62	0.67	0.743
Total	479289.00	19			

Table 3. Fresh weight with dried surface growth of *Nostoc commune* in optimised culture medium supplemented with different concentrations of potassium under outdoor conditions at 7, 14, 21, and 28 days after inoculation. Values are means ± standard deviation (SD). Different letters indicate significant differences (p < 0.05) between the treatments based on Dunn's post hoc test. Abbreviations in treatment: SWE – soil-water extract, P – phosphorus, K – potassium, mg – milligram

Treatment	Fresh weight with dried surface growth (mg) after inoculation			
	7 days	14 days	21 days	28 days
10% SWE	0 ± 0.0[a]	78 ± 89.8[a]	377 ± 196.4[a]	813 ± 339.2[a]
10% SWE + 2.3 mg/kg P	164 ± 90.6[a]	547 ± 133.2[b]	1125 ± 434.6[b]	1520 ± 114.8[b]
10% SWE + 2.3 mg/kg P + 3 mg/kg K	187 ± 121.4[a]	737 ± 175.2[c]	1313 ± 212.5[b]	1885 ± 323.2[c]
10% SWE + 2.3 mg/kg P + 6 mg/kg K	196 ± 43.8[a]	859 ± 111.9[d]	1737 ± 89.0[c]	2757 ± 428.5[d]
10% SWE + 2.3 mg/kg P + 9 mg/kg K	195 ± 53.6[a]	853 ± 103.2[d]	1726 ± 153.4[c]	2710 ± 408.2[d]

Table 4 showed that a two-way permutation analysis of variance also confirmed the impact of treatment and time on the fresh weight growth of *Nostoc commune*. Treatment ($p = 0.001$) and time ($p = 0.001$) had a substantial impact on the fresh weight growth of the *Nostoc commune*. The two factors (treatment and time) did not, however, interact significantly.

Colour of *Nostoc commune* under outdoor conditions

The greenness of the *Nostoc commune* in an optimised culture medium (10% soil-water extract solution with 2.3 mg/kg phosphorus) with different potassium concentrations was evaluated from 7 to 28 days after inoculation to determine chlorophyll-a levels. There were no noticeable distinctions between the treatments in terms of the greenness rating until 28 days after inoculation, except for the *Nostoc commune* inoculated in 10% soil-water extract solution without phosphorus and potassium supplement, which became moderate green (rate 3) to light green (rate 2) from 21 to 28 days after inoculation (Table 5, Fig. 2).

Heterocyst count using the microscope

Heterocysts of *Nostoc commune,* together with the vegetative and dividing cells and mucilage sheath, were observed using a microscope (Fig. 3). The number of heterocysts observed in all treatments ranged from 21 to 23 and 22 to 23 at 7 and 14 days after inoculation, respectively. The number of heterocysts across all treatments showed no significant differences at 7 (H = 8.52, $p = 0.076$) and 14 (H = 0.63, $p = 0.096$) days after inoculation. The number of heterocysts in *Nostoc commune* is observed across various culture media, and it typically shows notable variations in optimised culture medium supplemented with different concentrations of potassium (3, 6, and 9 mg/kg), ranging from 23 and 23 to 24 heterocysts at 21 and 28 days after inoculation. The heterocyst count of *Nostoc commune* showed significant differences at 21 (H = 19.52, $p = 0.00065$) and 28 (H = 9.96, $p = 0.041$) days after inoculation (Table 6).

The effect of the treatment and time on the heterocyst count of the *Nostoc commune* was also supported by a two-way permutation analysis of vari-

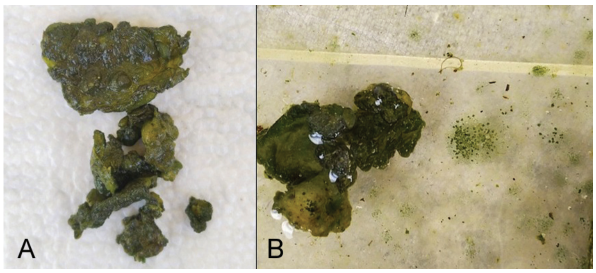

Fig. 2. The development of new colonies from a seriate colony of *Nostoc commune*. A seriate colony of *Nostoc commune* (A), and a bunch of new colonies from a seriate colony (B).

Table 4. Effect of the treatment and time on fresh weight growth of *Nostoc commune* in optimised culture medium supplemented with different concentrations of potassium under outdoor conditions, according to the results of a two-way permutation analysis of variance (Euclidean distance, 9999 permutations)

Factor	Sum of squares	df	Mean square	F	*p*-value
Treatment	182600000	4	45650000	22.71	0.001
Time	648600000	3	216200000	107.54	0.001
Interaction	10210000	12	851000	0.42	0.862
Total	961500000	19			

Table 5. Evaluated colours of the *Nostoc commune* in optimised culture medium supplemented with different concentrations of potassium under outdoor conditions at 7, 14, 21, and 28 days after inoculation. Colour rate: 1 – yellowish; 2 – light green; 3 – moderate green; 4 – dark green. Abbreviations in treatment: SWE – soil-water extract, P – phosphorus, K – potassium

Treatment	Colour rate after inoculation			
	7 days	14 days	21 days	28 days
10% SWE	4	4	3	2
10% SWE + 2.3 mg/kg P	4	4	4	4
10% SWE + 2.3 mg/kg P + 3 mg/kg K	4	4	4	4
10% SWE + 2.3 mg/kg P + 6 mg/kg K	4	4	4	4
10% SWE + 2.3 mg/kg P + 9 mg/kg K	4	4	4	4

Table 6. Heterocyst count of *Nostoc commune* in optimised culture medium supplemented with different concentrations of potassium under outdoor conditions at 7, 14, 21, and 28 days after inoculation. Values are means ± standard deviation (SD). Different letters indicate significant differences ($p < 0.05$) between the treatments based on Dunn's post hoc test. Abbreviations in treatment: SWE – soil-water extract, P – phosphorus, K – potassium

Treatment	Heterocyst count after inoculation			
	7 days	14 days	21 days	28 days
10% SWE	21 ± 1.3[a]	22 ± 2.1[a]	17 ± 1.5[a]	19 ± 1.0[a]
10% SWE + 2.3 mg/kg P	23 ± 2.1[a]	22 ± 1.5[a]	21 ± 2.2[b]	22 ± 1.7[b]
10% SWE + 2.3 mg/kg P + 3 mg/kg K	22 ± 1.8[a]	22 ± 1.5[a]	23 ± 2.2[c]	23 ± 1.5[c]
10% SWE + 2.3 mg/kg P + 6 mg/kg K	24 ± 3.7[a]	23 ± 2.4[a]	23 ± 2.2[c]	24 ± 1.5[c]
10% SWE + 2.3 mg/kg P + 9 mg/kg K	23 ± 1.7[a]	23 ± 3.1[a]	23 ± 1.5[c]	24 ± 2.4[c]

Table 7. Effect of the treatment and time on heterocyst count of *Nostoc commune* in optimised culture medium supplemented with different concentrations of potassium under outdoor conditions, according to the results of a two-way permutation analysis of variance (Euclidean distance, 9999 permutations)

Factor	Sum of Squares	df	Mean Square	F	*p*-value
Treatment	8.68	4	2.169	8.07	0.004
Time	8.33	3	2.775	10.32	0.002
Interaction	6.03	12	0.502	1.87	0.116
Total	33.05	19			

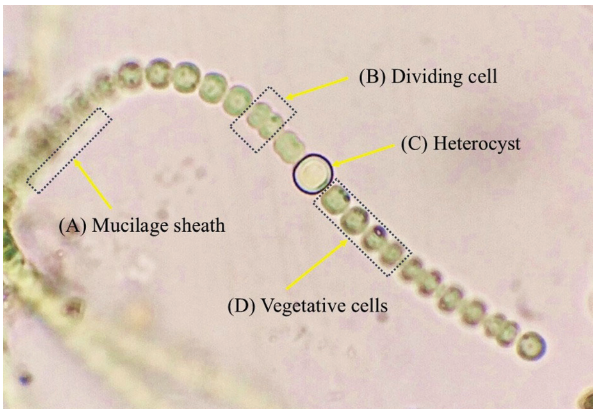

Fig. 3. Viscous extracellular matrix and development of *Nostoc commune* observed using a microscope with high power field (HPF): mucilage sheath (A), dividing cell (B), heterocyst (C), and vegetative cells (D). Original microscopic photo of the author.

ance (Table 7). It showed that heterocyst counts of the *Nostoc commune* were significantly influenced by both treatment ($p = 0.004$) and time ($p = 0.002$). However, the interaction between the two factors (treatment and time) was not significant.

DISCUSSION

The present study identified the optimal potassium concentration in an optimised culture medium (10% soil-water extract solution with 2.3 mg/kg phosphorus) for the development and growth of *Nostoc commune*. While potassium can promote the growth and development of *Nostoc commune*, phosphorus may have a major impact on the development and growth of *Nostoc* species. (Dong et al., 2019). It has also been demonstrated that adding phosphorus (P_2O_5) to cyanobacteria culture media increases growth and reproductive output of numerous cyanobacteria, including *Nostoc* species. Moreover, higher potassium levels result in greater production of *Nostoc* sheath and biomass (Kuffner & Paul, 2001). In the study by Pantastico & Gonzales (1976), *Nostoc* grown in an outdoor culture medium with phosphorus and potassium produced 214% more fresh biomass than in an outdoor culture medium without phosphorus and potassium.

The generation of chlorophyll a in *Nostoc commune* appeared to be enhanced by the optimised culture medium (10% soil-water extract solution with 2.6 mg/kg phosphorus) supplemented with 3, 6, and 9 mg/kg potassium, resulting in a shade of dark green. A green pigment called chlorophyll a is necessary for photosynthesis, which allows them to absorb light energy and turn it into food. Furthermore, the hydrological driver of cyanobacterial surface growth, which can aid their proliferation, was enhanced by soil-water extract solutions when supplemented with varying potassium and phosphorus rates (Rodríguez-Caballero et al., 2012; Mehdizadeh Allaf & Peerhossaini, 2022; Ramos et al., 2022).

Rich phosphorus and potassium culture media were shown to support a high number of heterocysts in *Nostoc commune*, thereby promoting optimal growth and development (Böhme, 1998). With their anaerobic interior, heterocysts are differentiating cells that enable the oxygen-sensitive process of atmospheric nitrogen fixation through respiration. In free-living *Nostoc* species, 7% of the cells differentiate into heterocysts, which constitute a component of the filament. The capacity of cyanobacteria to fix nitrogen has also been enhanced by increased potassium and phosphorus levels. Furthermore, nitrogenase is shielded from oxygen by heterocysts, which prevent oxygen that enters the cell from the outside and oxygen generated by photosystem II in nearby vegetative cells (Liengen, 1999).

Heterocysts and vegetative cells in the filament differ from one another in a composite; the process is controlled to alter the structure and function of cyanobacteria. Furthermore, culture media supplemented with potassium and phosphorus contained more heterocysts than culture media devoid of these nutrients (Wolk et al., 1994). Additionally, heterocysts enhance the fixing of ambient nitrogen molecules. The heterocyst of *Nostoc commune* is responsible for the resting spore. The protoplasm of a heterocyst, or living material, grows into a new filament and becomes useful. The presence of heterocysts within the colony of *Nostoc commune* indicates that phosphorus and potassium are functioning effectively (López-Igual et al., 2010).

The effects of treatments on per cent growth, fresh weight growth, and heterocyst count do not depend on time (7, 14, 21, and 28 days after inoculation). It indicates that the effect of treatments is consistent across all time points, and time does not alter the differences between treatments.

The creation of an optimistic, cost-effective, and sustainable cultural medium for the outdoor culture of *Nostoc commune* using a 10% soil-water extract solution, *Nostoc commune* was introduced with varying potassium concentrations (3, 6, and 9 mg/kg) and an optimal phosphorus concentration (2.6 mg/kg).

CONCLUSION

The growth and development of *Nostoc commune* are best stimulated by an optimal culture medium enriched with 6 mg/kg and 9 mg/kg potassium. For the growth and development of *Nostoc commune*, these two formulas work best. It can be used as an inoculum to start extensive outdoor farming next to rice paddies. Using an optimised culture medium can maximise *Nostoc commune* production. This medium will be used to improve the fertility of low-nitrogen soils and increase crop yield. It can also increase rice production without the need for large quantities of synthetic nitrogen fertiliser.

Author contribution. The research was conducted, the data analysed, and the text written by the author. The author read and approved the final version of the article.

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