Effect of waste tea powder infused bio formulation on the germination of seeds

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Abstract

Tea leaves after its utilization for the preparation of non-alcoholic beverage are thrown as it is in the environment. However, leftover tea leaves are good source of plant nutrients. Thus, waste tea leaves powder added with plant beneficial microbes could be useful for plant growth and development. In the present study, seeds treated with a potent phosphate solubilising strain combined with waste tea powder showed earlier germination of seeds and increased root and shoot length over untreated seeds. Thus, the application of tea powder with beneficial bacteria to seeds is an ecofriendly approach for plant development and hence for sustainable agriculture.

Keywords: Food waste, Microbial stimulant, Seed development, Organic fertilizer

1.0 Introduction

Seed germination is an important step in the life cycle of plants. Seeds that germinate quickly have good competence, increased plant growth, and thus increased crop yield over slower-germinating seeds. Several chemical substances are available in the market that can enhance seed germination. However, these chemicals are hazardous to ecosystem (Pan M. et al., 2022). The increasing demand for sustainable and ecofriendly agricultural practices has led to the exploration of novel bioformulations that can enhance seed germination and plant growth. Food waste is a major component of organic waste that has widespread issue in the food system. Globally each year approximately one third of the food produced for human consumption is lost or wasted which constitutes approximately 1.3 billion metric tons. Tea powder is also one component of food waste of organic origin. Tea which is prepared from *Camellia sinesis* leaves is one of the world's most popular non-alcoholic beverages (Tarashkar M. et al., 2023). Tea leaves powder contains nitrogen along with phosphorus and potassium as well as other micro nutrients that are beneficial for soil as well as plants (Mandal S. et al., 2024). These are high in tannic acid which helps to increase oxygenation and facilitates the growth of root system. Similarly, tea powder decomposes easily in landfills and do not cause harm to environment. Tea leaves are effective for fruit bearing plants, herbs, and flowering plants (Lazcano C. and Dońınguez J., 2011). The immense consumption of tea generates thousands of kilos of waste tea powder, most of which is tossed in dustbins, but this waste can actually be used as nutrient rich fertilizer (Debnath B. et al., 2021).

Hence, the application of waste tea in soil is an environment friendly approach over the application of chemicals. Moreover, application of waste tea powder in combination with plant beneficial microbes could be more advantageous for plant development. Soil contains an enormous number and variety of microbial flora which are beneficial for effective seed germination (Garg F., 2024). In context of this, a research study was designed to isolate and wrap a potent plant beneficial bacterial strain in waste tea powder and to study its effect on the germination of seeds.

2.0 Materials and Methods

2.1 Collection of samples

Soil samples were collected from banana field of Sangli district and sugarcane field of Kolhapur district. The soil samples were collected in sterile polythene bags and immediately brought to the laboratory.

Tea powder leftover after preparation of tea was collected in polythene bags and brought to the laboratory. The powder was washed repeatedly with water to remove residual sugar. The powder was dried under sun.

2.2 Isolation of bacteria from soil sample

Serial dilutions of soil samples were done from 10^{-1} to 10^{-6} in the sterile distilled water. 0.1 ml of each dilution was spread on the sterile nutrient agar plates. The plates were labelled properly. These plates were then incubated at room temperature for 24 hrs. After incubation, well isolated bacterial isolates were checked for purity of culture by Gram staining procedure. Pure cultures were labelled as Isolate 1, Isolate 2, and so on. Finally, all pure cultures were stored on nutrient agar slants in refrigerator.

2.3 Screening of bacteria (PSB) on the basis of phosphate solubilization

All pure cultures were spot inoculated on sterile Pikovskaya's agar medium (Pikovskaya R., 1948). Plates were incubated at room temperature for 72 hrs. After incubation plates were examined for development of clear zone around colonies which is the indication of phosphate solubilisation (Babu S. et al., 2017). The bacterial isolates showing phosphate solubilization were further examined for detection of potent phosphate solubilizer (PSB) by calculating solubilization index (Paul D. & Sinha S., 2017).

2.4 Identification of potent PSB isolate

The potent PSB isolate was identified by studying colony characteristics, morphological characteristics (Gram nature, motility, capsule and spore development), and biochemical characteristics (sugar fermentations and other characteristics).

2.5 Study of auxiliary plant beneficial characteristics of potent PSB isolate

2.5.1 Study of nitrogen fixation ability

The potent Isolate 6 was inoculated on nitrogen-free bromothymol blue (NFB) agar medium. The colour change of the medium from green to blue around growth confirms nitrogen fixation by bacteria (Nakbanpote W. et al., 2014).

2.5.2 Study of ammonia production

A loopful suspension of potent isolate was inoculated in peptone water broth. After incubation, 0.5 ml Nessler's reagent was added and ammonia production was identified on the basis of development of brown to yellow colour in the tube (Bhattacharyya C. et al., 2020).

2.5.3 Study of salt tolerance ability

A fresh suspension of the potent isolate no. 6 was inoculated in nutrient broth containing various concentrations of NaCl (1 to 10%) and KCl (1 to 10%) separately. All tubes were examined for turbidity after incubation at RT for 24 hrs (Sharma A. et al., 2021).

2.6 Antibiotic sensitivity testing

The potent isolate was examined for sensitivity towards different antibiotics like amoxicillin (AMx10), penicillin-G (P10), ofloxacin (OF5), ciprofloxacin (C15), chloramphenicol (C30), and tetracycline (TE30) by agar well diffusion method (Taoufiq K. et al., 2024).

2.7 Preparation of tea powder-based bio formulation

Dry leftover tea powder was sterilised by autoclaving. It was then added with 24 hr old nutrient broth culture of potent isolate 6. This bio formulation was incubated at RT for 24 hrs.

2.8 Seed germination studies

Seeds of *Vigna radiata* (Mung bean) were surface sterilised and subsequently washed repeatedly with sterile distilled water. Various treatments were given to seeds.

Treatment 1: Untreated seeds (control)

Treatment 2: Seeds were treated with Isolate 6 alone

Treatment 3: Seeds were treated with waste tea powder alone

Treatment 4: Seeds were treated with novel bio formulation containing waste tea powder and Isolate 6

All seeds were then placed on moist cotton bed in separate sterile Petri plates. Plates were labelled properly. Everyday germination of seeds of each treatment was examined.

3.0 Results and Discussion

3.1 Isolation of bacteria and screening of PSB

Table 1 Screening of bacterial isolates on the basis of phosphate solubilization

Isolate name	Phosphate solubilisation
Isolate 1	+
Isolate 2	+
Isolate 3	-
Isolate 4	+
Isolate 5	-
Isolate 6	+
Isolate 7	+
Isolate 8	+
Isolate 9	+
Isolate 10	-

Isolate 11	-
Isolate 12	-

+ Phosphate solubilisation; - No phosphate solubilization

More than 50 bacterial isolates were obtained from soil samples on nutrient agar media. Based on the morphological studies, 12 purified isolates with diverse morphology when studied for phosphate solubilisation, 7 isolates coded as Isolate 1, Isolate 2, Isolate 4, Isolate 6, Isolate 7, Isolate 8, and Isolate 9 showed phosphate solubilisation, while 5 isolates labelled as Isolate 3, Isolate 5, Isolate 10, Isolate 11, and Isolate 12 seen failed to solubilise phosphate (Table 1). The assessment of phosphate solubilization of all positive isolates revealed Isolate 6 as most effective phosphate solubilizer on the basis of solubilization index (Table 2)

Table 2 Screening of most potent phosphate solubilizer

Isolate code	Colony diameter (cm) (A)	Zone diameter (cm) (B)	Solubilization index SI = B/A
Isolate 1	0.5	1.2	2.40
Isolate 2	0.4	0.6	1.50
Isolate 3	0.4	No zone	-
Isolate 4	0.5	1.0	2.0
Isolate 5	0.3	No zone	-
Isolate 6	0.5	1.3	**2.60**
Isolate 7	0.5	0.7	1.40
Isolate 8	0.3	0.5	1.66
Isolate 9	0.5	0.6	1.20
Isolate 10	0.4	No zone	-
Isolate 11	0.2	No zone	-
Isolate 12	0.2	No zone	-

3.2 Identification of potent PSB isolate

Identification characteristics of Isolate 6 are represented in Table 3

Table 3 Identification of potent Isolate 6

Colony characteristics	
Size	Diameter = 1mm
Shape	Circular
Colour	White
Opacity	Opaque
Margin	Entire
Elevation	Convex
Surface	Smooth
Consistency	Sticky
Morphological characteristics	
Gram nature	Gram positive cocci arranged in clusters
Motility	Non motile

Spore formation	Non spore former
Capsule formation	No capsule
Biochemical characters	
Glucose	+
Lactose	-
Maltose	-
Sucrose	-
Ribose	+
Galactose	-
Mannitol	-
Catalase	+
Urease	-
Gelatinase	+

+ Utilization of sugar/Production of enzyme;

- No utilization of sugar/No production of enzyme

3.3 Study of auxiliary plant beneficial characteristics of potent PSB Isolate 6

Table 4 Plant beneficial characteristics of potent PSB Isolate 6

Characteristic	Result
Nitrogen fixation	+
Ammonia production	+
Amylase activity	+
NaCl tolerance	Turbidity up to 7% NaCl
KCl tolerance	Turbidity up to 8% KCl

The isolate showed growth on nitrogen-free bromothymol blue (NFB) agar medium with colour change of the medium from green to blue around growth indicating fixation of nitrogen. Similarly, change in the colour of peptone water broth culture after addition of Nessler's reagent confirmed ammonia production by isolate. The isolate also observed to have amylase activity (Table 4). Salt tolerance studies revealed that the isolate tolerate high percentage of salt (Table 4) indicating that the isolate can also be applied in saline soils.

3.4 Antibiotic sensitivity testing

The potent Isolate 6 when analysed for its sensitivity towards various antibiotics, it was observed that the isolate is sensitive to chloramphenicol, ciprofloxacin, tetracycline, and ofloxacin and resistant to penicillin-G and amoxicillin (Table 5). The sensitivity of isolate towards number of antibiotics suggests that the usage of isolate is safe for environment.

Table 5 Sensitivity of Isolate 6 to various antibiotics

Name of antibiotic	Short form	Inhibition zone (mm)
Chloramphenicol	C30	1.5

Ciprofloxacin	C15	2
Tetracycline	TE30	1
Penicillin-G	P10	-
Amoxicillin	AMX10	-
Ofloxacin	OF5	2

- No inhibition zone

3.5 Study of seed germination

Table 6 Effect of tea powder based bio formulation on germination of *Vigna radiata*

Parameters/ Treatments	% of germination (after 24 hrs.)	Radical length in cm (average of 10 seeds) After 5 days	Shoot length in cm (average of 10 seeds) After 5 days
Untreated seeds (Control)	0	0.51	1.72
Seeds treated with Isolate 6 only	53	1.84	4.32
Seeds treated with waste tea powder only	49	1.56	4.02
Seeds treated with bio formulation of tea powder and Isolate 6	60	2.68	6.66

The studies on seed germination indicated that seeds coated with waste tea powder based bio formulation of Isolate 6 have earlier germination than untreated seeds. Simultaneously, seed germination was more in case of seeds treated with bio formulation as compared to seeds treated with Isolate 6 only as well as seeds treated with waste tea powder only. Similarly, assessment of shoot length showed nearly 4 times increase in the average shoot length of bio formulation treated seeds over untreated seeds. The study of radical length of seeds indicated that bio formulated seeds are having nearly 2 fold increase over untreated seeds. The seeds germination research demonstrated that seeds treated with waste tea powder alone and Isolate 6 alone are benefited more as compared to untreated seeds. However, tea powder in combination with Isolate 6 seen more advantageous for seed germination than when applied alone (Table 6). The positive effect of earlier germination, and increased shoot and root length in bio formulated treated seeds may be contributed by nutrients present in waste tea powder as well as because of supply of various nutrients by Isolate 6.

4.0 Conclusion

The study demonstrates the potential of waste tea powder infused bioformulation as a sustainable and eco-friendly approach to enhance seed germination and promote plant growth. The findings suggest that waste tea powder can provide essential nutrients and bioactive compounds that improve seed germination rates and hence plant productivity. The usage of waste tea powder can also help to reduce waste disposal issues. Moreover, usage of bacterial culture with waste tea powder further improves seed germination. Thus,

bio formulations based on waste tea powder may offer a cost – effective alternative to synthetic fertilizers and growth promoters for plants.

5.0 References

1. Babu, S. V., Triveni, S., Reddy, R. S., & Sathyanarayana, J. (2017). Screening of maize rhizosperic phosphate solubilizing isolates for plant growth promoting characteristics. *Int J Curr Microbiol App Sci*, *6*(10), 2090-2101.

2. Bhattacharyya, C., Banerjee, S., Acharya, U., Mitra, A., Mallick, I., Haldar, A., ... & Ghosh, A. (2020). Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Scientific reports*, *10*(1), 15536.

3. Debnath, B., Haldar, D., & Purkait, M. K. (2021). Potential and sustainable utilization of tea waste: A review on present status and future trends. *Journal of Environmental Chemical Engineering*, *9*(5), 106179.

4. Garg, F. C. (2024). Role of Microorganisms in Seed Germination.In *New Perspectives on Seed Germination*. IntechOpen.

5. Lazcano, C., & Domínguez, J. (2011). The use of vermicompost in sustainable agriculture: impact on plant growth and soil fertility. *Soil nutrients*, *10*(1-23), 187.

6. Mandal, S., Yadav, A., Kumar, S., &Mura singh, S. (2024). An extensive review study on bioresources recovery from tea waste and its emerging applications. *Waste Management Bulletin*, *2*(4), 155-166.

7. Nakbanpote, W., Panitlurtumpai, N., Sangdee, A., Sakulpone, N., Sirisom, P., & Pimthong, A. (2014). Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd contaminated soil: identification and effect on rice under saline conditions. *Journal of Plant Interactions*, *9*(1), 379-387.

8. Pan, M., Yau, P. C., Lee, K. C., & Man, H. Y. (2022). Effects of different fertilizers on the germination of tomato and cucumber seeds. *Water, Air, & Soil Pollution*, *233*(1), 25.

9. Paul, D., & Sinha, S. N. (2017). Isolation and characterization of phosphate solubilizing bacterium Pseudomonas aeruginosa KUPSB12 with antibacterial potential from river Ganga, India. *Annals of Agrarian Science*, *15*(1), 130-136.

10. Pikovskaya, R. I. (1948). Phosphate mobilization in soils as related to life processes of some microorganisms. *Mikrobiologiya*, *17*, 362-370.

11. Sharma, A., Dev, K., Sourirajan, A., & Choudhary, M. (2021). Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 99.

12. Taoufiq, K., Aberchane, L., Amri, O., Oufdou, K., El Biari, K., Talbi, A., ... &Faghire, M. (2024). Antibiotics resistance and PGPR traits of endophytic bacteria isolated in arid region of Morocco. *International Journal of Plant Biology*, *15*(4), 1063-1076.

13. Tarashkar, M., Matloobi, M., Qureshi, S., & Rahimi, A. (2023). Assessing the growth-stimulating effect of tea waste compost in urban agriculture while identifying the benefits of household waste carbon dioxide. *Ecological Indicators*, *151*, 110292.