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Eco-friendly Cultivation of Annual Chrysanthemum for Enhancing Seed Quality and Soil Properties

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Authors' contributions

This work was carried out in collaboration among all authors. All authors contributed to this research. Material preparation, conceptualization and layout of work was planned by author BSD. Data collection and analysis was done by author SL. The formal analysis and data curation was carried out by author NK. The first draft of the manuscript was written by author SL and all authors commented on previous versions of the manuscript. Final approval was made by all the authors after proofreading.

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ABSTRACT

Sustainable agriculture is the cultivation of healthy soil and seedlings which are essential for producing high-quality yields that meet the nutritional needs of living organisms. The soil and its microenvironment are crucial for significantly improving plant growth and promotion of sustainable agriculture practices. This focus on sustainability aims for long-term crop and livestock production while minimizing negative environmental impacts. Therefore, for eco-friendly cultivation of annual

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chrysanthemum a study was carried out in the open field of the Department of Seed Science and Technology at Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P) India for two years (2019-2021). The field experiment consisted of nine different formulations of *jeevamrit* arranged in Randomized Complete Block Design (RCBD) with three replications for each treatment. Notably, the use of T_6 (Drenching with *jeevamrit* @30% at 15 days interval) and T_7 (Drenching with *jeevamrit* @35% at 15 days interval) significantly enhanced the production while also enriching the soil with the considerable increase in beneficial microbial count like bacteria, fungi and actinomycetes. Hence, this seeks to identify an environmentally friendly method to improve seed production quality in annual chrysanthemum and enhance soil nutrients.

Keywords: Bioformulations; jeevamrit; annual chrysanthemum; floriculture.

1. INTRODUCTION

In India, there is a substantial demand for annual flowers and their seeds for export, cultivation, aesthetic purposes and gardening. Among these the annual chrysanthemum (*Glebionis coronaria* (L) Spach) ranks as one of the most sought-after winter annual flower crops following the traditional chrysanthemum (Desai, 1962). This flower belongs to the Asteraceae family and is originally from the Mediterranean region. It has also been cultivated and naturalized in East Asia and certain areas of North America. Its significant for its short growth cycle and its ability to be cultivated year-round (Thakur et al., 2022). It is commercially propagated from seeds and thrives well in mid or slightly cool climates. However, it tends to flower prematurely when grown in warm summer conditions. Traditionally, its cultivation has relied on chemical-based fertilizers and plant protection products which can negatively impact the environment and the health of living organisms in the surrounding area (Meena, 2016). The widespread use of chemical sources for macro and micronutrient along with plant protection within a rigid crop production system has resulted in an imbalance in nutrient availability in the soil. The imbalance has significantly affected plant growth, flowering patterns, seed yield and quality as well as health of the soil. To produce high-quality annual chrysanthemum flowers and seeds it is essential to have healthy and well-nourished soil (Azeezahmed et al., 2016).

Therefore, adopting farming systems that utilize natural products could serve as viable alternatives to address these issues. Palekar (2006) and Sreenivasa et al., (2010a) noted that formulations such as *Panchagavya*, *Jeevanruth* and *Beejamruth* contains a high microbial load including *Azotobacter*, *Azospirillum*, Phosphobacteria, lactic acid bacteria, Pseudomonas, Methylotrophs, Actinomycetes and fungi. These micro-organisms multiply in the soil and act as stimulants to enhance microbial activity thereby improving plant health (Adhikari, 2020). The cultivation of various flowers for loose and cut flower production along with production of high-quality seeds in annual chrysanthemum using natural farming methods appear to be highly viable alternatives to traditional farming practices that rely on chemical fertilizers and pesticides. This approach also helps reduce production costs (Sreenivasa et al., 2010b; Devvrat 2017). Hence, the current study was aimed to evaluate and compare between the effects of different formulations of *jeevamrit* and chemical fertilizers on seedling quality parameters of annual chrysanthemum (*Glebionis coronaria* (L.) Spach) and soil nutrients in the western Himalayas of India.

2. MATERIALS AND METHODS

Experimental site: The experiment was conducted in the open field conditions of Department of Seed Science and Technology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh, India) from 2019 to 2021. This location is situated in the hilly regions of the Western Himalayas at an altitude of 1,060 meters above mean sea level with a latitude of 30°51'0" N and longitude of 77°11'30" E. The area's climate is predominantly semi-temperate characterized by average temperatures ranging from 9 to 25^{o}C, with humidity levels at 67 % and rainfall of 75 mm.

Experimental design: The field experiment consisted of nine different treatments arranged in Randomized Complete Block Design (RCBD) with three replications for each treatment. The specific details of the treatments applied in the field can be found in (Table 1). Each treatment was carefully selected to assess its effects on the parameters being studied allowing for a comprehensive evaluation of their impact on the growth and quality of annual chrysanthemum. The replication of each treatment helps to validate the findings and ensures that the results are statistically significant:

Planting material: The healthy, bold, disease free and uniformly sized seeds of annual chrysanthemum cv. open pollinated 'Mix' were selected and sown in nursery beds. They were then covered with sieved well-rotten farmyard manure and properly irrigated. The experimental field was prepared by thoroughly ploughing the soil to a depth of 30-35 cm a few days before transplanting.

The field was levelled properly and raised beds of size 1.0 m × 1.0 m × 15 cm (L × B × H) were prepared for the planting. The recommended dose of fertilizers (RDF) (i.e. 40g N/m² + 20g P/m² + 30g K/m² along with 5Kg of FYM/m²) was applied. Healthy, disease free and robust seedlings of uniform size and vigor at the 5-6 leaf stage were selected and transplanted into 1.0 × 1.0 m beds. The seedlings were spaced 30 × 30 cm allowing for a total of nine plants in each plot.

Soil sampling: Prior to setting up the experiment the random soil samples were collected using an auger from various locations across the experimental field at a depth of 0-15 cm to assess the nutrient status of the soil. A composite sample was then created from these individual samples and analyzed to determine the soil's physical and chemical properties.

Methodology for the preparation of natural farming inputs:

Jeevamrit: The fresh dung (10 kg) and urine (10 L) from desi cow were thoroughly mixed with 200 liters of water (200 L). Then carefully (jaggery) (2 kg), pulse flour (2 kg) and a handful of undisturbed soil (handful) was added to the mixture. The ingredients were mixed well and the solution was stirred thoroughly twice daily for 4-5 days. On the fifth day, the mixture was filtered using muslin cloth and the r esulting filtrate was prepared for use in soil drenching.

Drekastra: To prepare the solution fresh cow dung (1Kg) and cow urine (5 liter) from a desi cow were added in a tub containing 100 liters of water. These natural inputs provided a nutrient-rich base for the preparation. Next, drek leaves (5 Kg) were manually crushed to release their active compounds and added to the mixture ensuring the leaves were fully integrated with the cow dung, urine and water. The solution was then stirred thoroughly three times a day in a clockwise direction using a wooden stick. The mixture was left undisturbed for 48 hours to allow fermentation to take place. Once the fermentation process was complete the solution was carefully filtered using muslin cloth to remove solid residues and ensure a smooth refined liquid. This filtrate was then ready for use according to the specified treatment protocol providing a natural and effective option for soil or crop management.

Brahmastra: To prepare the solution neem leaves (5 kg), custard apple leaves (2 kg), papaya leaves (2 kg), pomegranate leaves (2 kg) and guava leaves (2 kg) were first crushed to release their natural extracts. These crushed leaves were then placed in a tub containing 10 litres of cow urine. The mixture was thoroughly stirred to ensure all the ingredients were evenly blended. The ingredients were mixed thorughly and boiled 4 times until it reduced to half of the previous quantity. Boiling in stages allowed better integration of the leaf extracts with cow urine and ensured the solution have been reached the desired potency. After boiling, the mixture was left to rest for 48 hours. Once the settling process was complete the liquid portion of the mixture was carefully filtered using muslin cloth to remove impurities and achieve a refined usable solution. This filtrate was then ready for application in agricultural practices.

Application of *Jeevamrit*, *Drekastra* and *Brahmastra:* The application of *Drekastra* @ 2.5%, *Brahmastra* @ 2.5% was initiated seven days after transplanting following the prescribed treatment schedule. Additionally, *jeevamrit* was applied at 15-days intervals. These treatments were consistently administered as per the protocol and were discontinued once the seeds began the maturation phase, ensuring the growth and development processes were supported during the critical early stages but not during seed ripening. This strategic timing aimed to optimize plant health and productivity while aligning with the natural progression of the crop's lifecycles.

Table 1. Details of treatments implemented in the experimental field are as follows

Treatments	Treatment details
T_1	Drenching with *jeevamrit* @ 5% at 15 days interval
T_2	Drenching with *jeevamrit* @ 10% at 15 days interval
T_3	Drenching with *jeevamrit* @ 15% at 15 days interval
T_4	Drenching with *jeevamrit* @ 20% at 15 days interval
T_5	Drenching with *jeevamrit* @ 25% at 15 days interval
T_6	Drenching with *jeevamrit* @ 30% at 15 days interval
T_7	Drenching with *jeevamrit* @ 35% at 15 days interval
T_8	RDF (40g N/m² + 20g P/m²+ 30 g K/m² with 5Kg of FYM/m²)
T_9	Absolute control

**All other element of natural farming were consistent across all treatments including mulching (Achhadan), Seed treatment with Beejamrit, moisture conservation (Whaapsa), intercropping with sweet pea and the application of Drekastra (2.5%), Brahamastra (2.5%) and sour buttermilk (0.25%) at seven-day intervals*

Table 2. Physico-chemical properties of soil before trans-planting

Sr. No	Contents	Values obtained	Soil status
1.	Soil pH	6.75	Normal
2.	Electrical conductivity (dS/m)	0.289	Normal
3.	Organic carbon (%)	1.46	High
4.	Available Nitrogen (kg/ha)	440.67	Medium
5.	Available Phosphorus (kg/ha)	39.15	High
6.	Available Potassium (kg/ha)	242.41	Medium

Seedling quality and soil observations: Seed and seedling quality parameters such as seed germination (%), speed of germination, seedling length (cm), seedling dry weight (mg), seed vigour index-I and seed vigour Index- II and seed electrical conductivity (dS/m) were measured. Seed Vigour Index-I and Seed Vigour Index-II was determined by using the formula established by Abdul-Baki and Anderson (1973).

- ✓ Seed vigour index-I = Germination (%) × Seedling length (cm)
- ✓ Seed vigour index-II = Germination (%) × Seedling dry weight (mg)

The Seed vigour index-I and Seed vigour index-II provides a comprehensive metric that combines germination efficiency with seedling robustness offering valuable insights into the seed's potential for vigorous and uniform growth under field conditions.

To measure seed electrical conductivity four replicates of 0.5 g seeds from each treatment were randomly selected and weighed. These seed samples were then soaked in 25 ml of distilled water and incubated for 24 hours at 25°C in a dark environment. Initially the electrical conductivity of the distilled water was recorded. Following the incubation period the electrical conductivity of the soak solution also known as the leachate was measured using an electrical conductivity meter. The actual electrical conductivity value was calculated by accounting for the baseline conductivity of the distilled water. This process evaluates the membrane integrity of the seeds based on the leachates leached into the soaked solution.

The viable microbial count (cfu/g) in soil samples was determined using the serial dilution and standard spread plate method as outlined by SubbaRao (1999). Nutrient agar (NA) medium was used to culture bacteria, potato dextrose agar (PDA) medium was utilized for fungi and Kenknight & Munaier's medium was employed for actinomycetes. This approach facilitated the enumeration and differentiation of microbial populations in the soil samples.

Similar to the pre-experiment soil sampling the post-experiment soil samples were collected from a 0-15 cm depth for each treatment plot at the end of both years. The samples were then shade-dried grinded by using pestle and mortar, sieved through a 2 mm sieve and stored in polythene bags for subsequent analysis. These samples were examined for various physico-chemical properties and microbial populations by using the specified methods.

Table 3. Methods for the determination of various properties of soil:

Particular	Method employed	References
pH	Potentiometric (1:2 soil-water suspension)	Jackson (1973)
EC	Wheat stone bridge circuit method (1:2 soil-water suspension)	Jackson (1973)
Organic carbon	Rapid titration method	Walkley and Black (1934)
Available N	Micro Kjeldahl method	Subbiah and Asija (1956)
Available P	Olsen's method	Olsen et al. (1954)
Available K	Flame photometric method	Merwin and Peech (1951)

Statistical analysis: The statistical analysis for Randomized Complete Block Design (RCBD) was conducted as per experimental framework as outlined by Gomez and Gomez (1984). The data were analyzed by analysis of variance (ANOVA) and degree of significance between treatments was determined by using Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

The data analyzed for the studied parameters in Table 4 clearly exhibit the significant influence of natural farming on seed quality and soil nutrients. In different applications of *jeevamrit* solutions on annual chrysanthemum, treatment T_7 (i.e. Drenching with *jeevamrit* @ 35% at 15 days interval) attained the highest seed germination percentage (84.25 %), speed of germination of seeds (21.78), longest seedlings (9.57 cm), seedling dry weight (7.49 mg), seed vigour index-I (789.34), seed vigour index-II (618.23) followed by T_6 (i.e. Drenching with *jeevamrit* @ 30%) and T_5 (i.e. Drenching with *jeevamrit* @ 25%). The *jeevamrit* treatment resulted in seedlings of superior quality leading to the highest germination percentage and faster germination rates. This improvement can be attributed due to the enhanced microbial population in soil (Kirar, 2014; Sharma et al., 2021) which convert macronutrients from non-soluble forms to the readily absorbable form (Fig 2 and Fig 3). The plant receiving the treatment T_8 (i.e. RDF) (as shown in Table 4) also showed statistically similar results with T_7 for seed germination percentage (82.50 %), speed of germination of seeds (20.73), longest seedlings (9.33 cm), seedling dry weight (7.43 mg), seed vigour index-I (767.83), seed vigour index-II (611.05) which may be due to high amount of NPK supplied to plants (Fig. 1).

The minimum value (0.226 dS/m) for seed electrical conductivity (Table 4) was found in treatment T_7 (i.e. Drenching with *jeevamrit* @ 35%) and it is statistically similar to the T_6 (0.232 dS/m), T_5 (0.236 dS/m), as well as the maximum seed electrical conductivity (0.284 dS/m) is recorded in the treatment T_9 i.e. absolute control.

The most favorable values for electrical conductivity have been recorded in seeds harvested from plants treated with T_7 and T_6. These seeds exhibit the lowest EC levels likely due to their robust and well-developed structure indicating the superior health and quality. The bold and healthy nature of these seeds minimized the leakage of the solutes during the testing because their cellular integrity and membrane stability were better preserved. This reduced solute leakage reflects their enhanced physiological and biochemical properties making them ideal for agricultural purposes (Chadha, 2012).

The seeds subjected to the treatment did not exhibit substantial leakage of accumulated solutes which can likely be attributed to the presence of a highly compact seed coat. This compact structure may have acted as a strong barrier limiting the movement of solutes out of the seed. Additionally, the membrane surrounding the embryos is presumed to have been composed of exceptionally rigid and durable tissues. This tough tissue likely resisted disintegration during hydration even when the seeds were exposed to conditions designed to test their electrical conductivity. As a result, the structural integrity of the seed coat and embryonic membrane effectively prevented significant solute leakage during the hydration process. Consequently, the seeds exhibited a minimal electrical conductivity value. These findings align closely with the research outcomes reported by Sanas (2016) for annual chrysanthemum and Kumari (2016) for pansy.

An analysis of the data presented in Fig. 1 reveals that the application of various treatments had a significant on the available NPK content in the soil. The maximum values for available N, P and K (370.85 kg/ha, 85.35 kg/ha and 424.85 kg/ha, respectively) were found in T_8 i.e. RDF and they were statistically comparable with the treatments T_7 (364.54 kg/ha, 75.73 kg/ha and 413.13 kg/ha NPK). However, lowest availability of N, P and K content (291.93 kg/ha, 53.56 kg/ha & 306.90 kg/ha, respectively) was recorded in T_9 (absolute control).

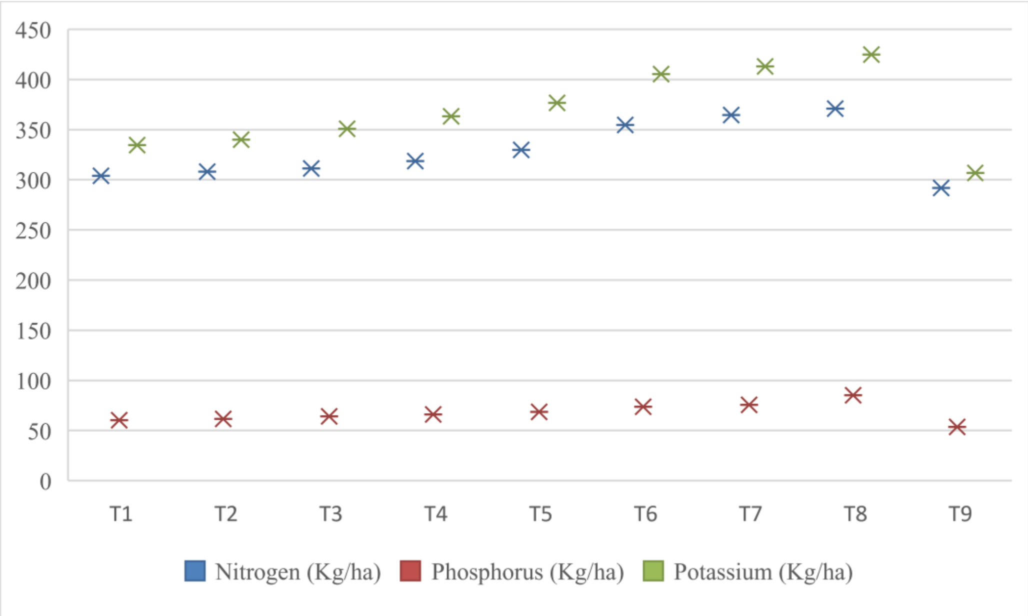

Fig. 1. Effect of bioprospecting in annual chrysanthemum for enriching available soil nutrients

T_1: Drenching with jeevamrit @ 5%, T_2: Drenching with jeevamrit @ 10%, T_3: Drenching with jeevamrit @ 15%, T_4: Drenching with jeevamrit @ 20%, T_5: Drenching with jeevamrit @ 25%, T_6: Drenching with jeevamrit @ 30%, T_7: Drenching with jeevamrit @ 35%, T_8: RDF, T_9: Absolute control

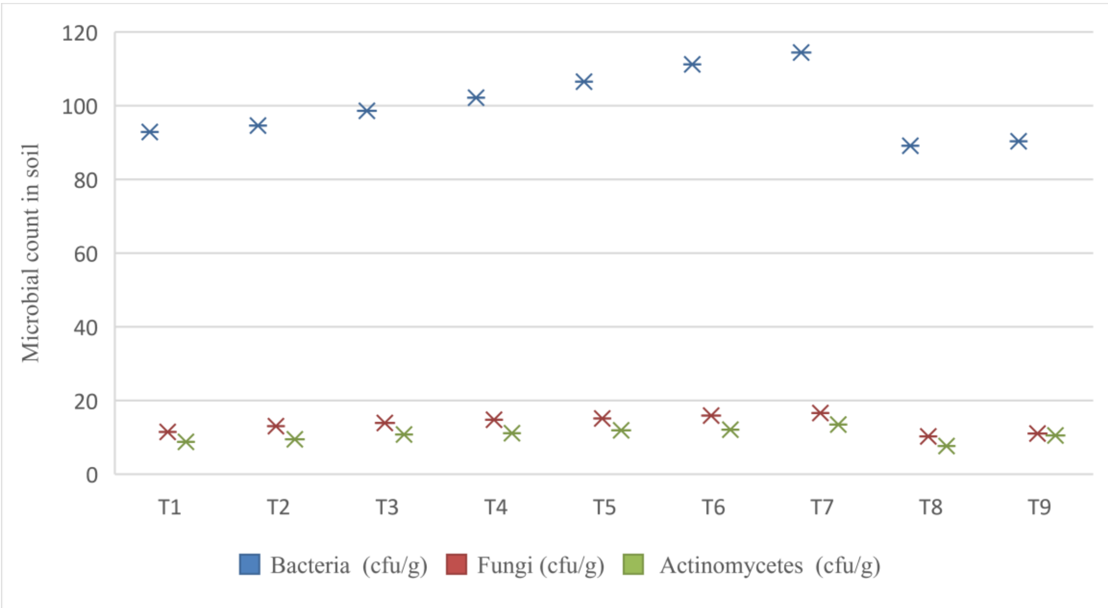

Fig. 2. Effect of bioprospecting for available total microbial count in soil samples

T_1: Drenching with jeevamrit @ 5%, T_2: Drenching with jeevamrit @ 10%, T_3: Drenching with jeevamrit @ 15%, T_4: Drenching with jeevamrit @ 20%, T_5: Drenching with jeevamrit @ 25%, T_6: Drenching with jeevamrit @ 30%, T_7: Drenching with jeevamrit @ 35%, T_8: RDF, T_9: Absolute control

Table 4. Comparison between different doses of *jeevamrit* with RDF on seed and seedling quality parameters of annual chrysanthemum

	Treatment details	Germination (%)	Speed of germination	Seedling length (cm)	Seedling dry weight (mg)	Seed Vigour Index-I	Seed Vigour Index-II	Seed electrical conductivity (dS/m)
T_1	Drenching with *jeevamrit* @ 5% at 15 days interval	77.50^{c} (8.86)	15.59^{ef}	9.03^{bcd}	6.94^{de}	699.66^{cd}	537.67^{de}	0.261^{a}
T_2	Drenching with *jeevamrit* @ 10% at 15 days interval	78.75^{b} (8.93)	15.96^{e}	9.11^{bcd}	7.09^{cde}	717.76^{bcd}	558.62^{bcd}	0.257^{a}
T_3	Drenching with *jeevamrit* @ 15% at 15 days interval	79.25^{b} (8.96)	16.45^{de}	9.16^{bcd}	7.17^{abcd}	726.15^{abcd}	568.07^{abcd}	0.248^{a}
T_4	Drenching with *jeevamrit* @ 20% at 15 days interval	80.75^{b} (9.04)	18^{cd}	9.21^{abcd}	7.23^{abcd}	743.6^{abcd}	584.16^{abcd}	0.239^{a}
T_5	Drenching with *jeevamrit* @ 25% at 15 days interval	81.50^{b} (9.08)	18.87^{bc}	9.22^{abcd}	7.27^{abcd}	751.27^{abc}	592.81^{abc}	0.236^{a}
T_6	Drenching with *jeevamrit* @ 30% at 15 days interval	82.00^{ab} (9.11)	20.13^{ab}	9.27^{abc}	7.41^{abc}	760.41^{abc}	606.69^{ab}	0.232^{a}
T_7	Drenching with *jeevamrit* @ 35% at 15 days interval	84.25^{a} (9.31)	21.78^{a}	9.57^{a}	7.49^{a}	789.34^{a}	618.23^{a}	0.226^{a}
T_8	RDF	82.50^{ab} (9.14)	20.73^{ab}	9.33^{ab}	7.43^{ab}	767.83^{ab}	611.05^{a}	0.23^{a}
T_9	Absolute control	71.75^{d} (8.53)	13.48^{f}	8.37^{e}	6.81^{e}	601.02^{e}	488.59^{e}	0.284^{a}
Mean		**79.81 (8.99)**	**18.03**	**9.1**	**7.21**	727.42	**575.9**	**0.242**
C.D.		**0.26**	**1.75**	**0.3**	**0.21**	54.08	**39**	**0.008**

**The values in the parenthesis are square root transformed. In a column, means followed by same letters do not differ significantly at the 5% level by Duncan's Multiple Range Test*

Bacteria **Fungi** **Actinomycetes**

T_7 (Best treatment)

Bacteria **Fungi** **Actinomycetes**

T_8 (RDF treatment)

Fig. 3. Comparison between Jeevamrit @35% application and RDF on viable microbial count in soil (cfu/g)

The more content of available NPK in the soil with the application T_8 might be due to the incorporation of recommended doses of NPK. Similar values for NPK content had also been reported in marigold (Kumar et al. 2010) and in broccoli (Sharma 2008).

The effect of all the treatments (T_1 to T_7) in enriching the soil with the population of beneficial microbes revealed that count of viable beneficial bacteria (114.41 × 10^5 cfu/g soil) and fungi (16.59 × 10^3 cfu/g soil) was reported to be significantly maximum in T_7 (i.e drenching of *jeevamrit* @ 35% at 15 days intervals) (Fig. 2). However, the minimum count of beneficial living bacteria (90.28 × 10^5 cfu/g soil) and fungi (14.28 × 10^3 cfu/g soil) as well as load of actinomycetes (11.43 × 10^2 cfu/g soil) was tested in the plots incorporated with T_8 (i.e. RDF).

The use of *jeevamrit* which includes high-carbon ingredients such as dung and jaggery along with nitrogen rich components like cow urine, gram flour and milk promotes the growth of bacteria within the soil microflora. These cow-based products are rich in beneficial microorganisms that efficiently break down complex organic compounds into soluble minerals thereby enhancing soil fertility (Sharma et al., 2021). These findings are quite matchable to the earlier results obtained by Chaitra, 2007) in China aster and Bisht (2023) in marigold.

4. CONCLUSION

The various formulations of *jeevamrit* were found to be statistically comparable to RDF for seedling quality parameters and NPK content. Moreover, they outer performed RDF and the control in enhancing the microbial count in the soil. Therefore, for quality seedling production treatment T_7 (i.e. Drenching with *jeevamrit* @ 35% at 15 days interval) proved most effective. It resulted in the highest seed germination percentage, speed of germination of seeds, longest seedlings, seedling dry weight, seed vigour index-I, seed vigour index-II and the lowest seed electrical conductivity. The T_7 treatment (i.e. Drenching with *jeevamrit* @ 35% at 15 days interval) significantly enhanced the NPK content of the soil and increased the viable beneficial bacteria and fungi. Thses improvements contributed to better soil fertility and the microbial activity which are essential for sustainable agricultural practices. Therefore, T_7 emerges as an optimal bioprospecting formulation not only for producing high quality seedling but also for promoting better soil health and long-term productivity. The results of this study can serve as a foundation for developing eco-friendly and cost-effective bio fertilization technology tailored for annual chrysanthemum cultivation. By reducing reliance on synthetic fertilizers this approach not only promotes sustainable agriculture but also minimize the consumption and production of chemical fertilizers. This technology has the potential to enhance soil health, support environmental conservation and provide economically viable solution for growers seeking sustainable alternatives in floriculture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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