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Effect of Jeevamrutha as a biodynamic solution against *Rhizoctoniasolani* of Maize (*Zea Mays L.*)

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Abstract

In traditional Indian organic agriculture, Jeevamrutha (JA) is a complex microbial bioformulation made from locally available materials such as cow dung, cow urine, jaggery, pulse flour, soil, and water. Farmers using JA often report that it enhances soil and plant health and is affordable, sustainable, and eco-friendly.Moreover, partial research studies shown on JA support the farmers' claim. To increase JA's reliability and suitability among the users, it must be scientifically validated in-depth. The success of JA was thought to be largely dependent on the structural and functional diversity of proteins, metabolites, and microorganisms. Until the 12-day incubation period, physico-chemical parameters and microbiological diversity were analyzed in order to understand the dynamics of JA during this time. Maize field trial was conducted to assess the impact of JA against *Rhizoctoniasolani*.The aim of this study was to provides information regarding the functional properties of JA, as well as their significance in enhancing soil health and encouraging farmers to use JA.

Keywords:
Jeevamrutha, Formulation, sustainability

INTRODUCTION

Maize (*Zea mays* L.), also known as corn, is the third most important cereal grain after rice and wheat in the world (Sandhu, Singh, &Malhi, 2007). It is a member of the grass family Poaceae, and is native to Mexico and Central America (PA Edde, 2021). Maize is a versatile crop that can be grown in various climates and soils, and is used as food, feed, fuel, and industrial products (Gul et al., 2021).

It is used as a staple food in India. Maize is an active ingredient in a variety of dishes, such as cornbread, tortillas, popcorn, and cornflakes. It is grown over a 9.26 million ha area in India, with major production in the states of Uttar Pradesh, Madhya Pradesh, Rajasthan, Andhra Pradesh, and Karnataka. (Kumar Malik *et al.*, 2018)

The maize crop covered 197 million ha globally (FAOStat, 2021). However, in order to meet the anticipated demands and ensure food security, it is imperative to augment production significantly. According to projections, the maize production target for the year 2050 is set to increase by a factor of 3.25. (Ramesh *et al.*, 2017).

Biotic and abiotic stresses are the major constraints to maize productivity (Mohamed Ahmed El-Esawi 2022). Biotic factors, viz., fungi, bacteria, viruses, and nematodes, and abiotic factors, such as low soil fertility, drought, cold, and heat stress, are known to decline maize productivity. The constraint of agricultural productivity is often attributed to several factors, and plant diseases constitute a significant concern. Plant diseases not only reduce plant productivity but also negatively impact livestock and human health, causing environmental harm. Biotic stress in major cereals results in a loss of over 23% of the estimated attainable yield. (Balodi*et al.*, 2017)

The crop raised during the Kharif season in the Indian subcontinent is susceptible to a variety of diseases, namely foliar diseases, stalk rots, banded leaf and sheath blight (BLSB), with maydis leaf blight (MLB) being the most significant foliar disease in maize growing areas. The major foliar risks to maize agricultural sustainability in India, where tropical maize is grown, are MLB and BLSB. (Kumar Malik *et al.*, 2018)

Banded leaf and sheath blight, a soil-borne disease caused by *Rhizoctoniasolani f. sp. sasakii*, has been observed in major Maize growing countries. It is more common in humid climates with temperatures of approximately 28 ^{o}C(Hooda*et al.*, 2017)

Rhizoctoniasolani is a soil-borne pathogen that lives on infected crop waste and in the soil as mycelium or sclerotia. The vegetative stage of the crop, where the weather is warm and humid, encourages the development of the disease. Rapid infection is favored by an ideal temperature of 28.2 °C and relative humidity of greater than 88% (H. Kaur *et al.*,2020) Under optimum environmental circumstances, the pathogen migrates from the basal sheath to the developing ear of crops. (Hooda*et al.*, 2017)

Farmers are unable to avoid the unexpected deleterious consequences of chemical-intensive agriculture. The difficulty of sustaining or improving agricultural yield while protecting the environment has given rise to several different sustainable agriculture narratives, with organic farming appearing to hold the most promise. Producing food crops through organic farming results in crops with a smaller ecological footprint, better soil organic carbon (SOC), soil fertility, and water quality (Schreefel*et al.,* 2020). Utilizing organic bio-fertilizers gained popularity in recent years due to a greater focus on the restoration of soil quality and its interaction with ecological concerns. Cow excreta-based bio-fertilizers are among the numerous alternative bio-fertilizers, and they show promise because of their quicker breakdown rate, comparatively low precursor needs, and increased microbial load (Anandan*et al.,* 2016)

Organic farming is vastly superior to chemical farming, as it provides adequate protection against soil degradation. This agriculture method prioritizes using natural processes to cultivate crops, rather than relying on synthetic chemicals and pesticides. As a result, organic farming is essential for promoting the long-term health and sustainability of soil, while simultaneously reducing the release of harmful toxins into the environment (Ali et al. 2011). The Indian government has recognized the significance of Zero Budget Natural Farming (ZBNF) in doubling farmer income while promoting sustainable farming practices. ZBNF involves utilizing natural resources for farming with no external inputs, thereby reducing production costs and benefiting the ecosystem. This approach has been incorporated into India's fiscal year 2019-20 budget (Pandia*et al.,* 2019) Beejamrutha and Jeevamrutha, two organic biofertilizers, are highly effective in enhancing crop yield by enriching soil nutrition. Beejamrutha comprises cow dung, cow urine, water, and lime, while Jeevamrutha comprises a mixture of cow dung, cow urine, jaggery, pulse flour, and water. These biofertilizers can be a valuable addition to crop management practices for those seeking to boost crop yields (Anandan*et al.,* 2016).

According to Palekar (2006), Jeevamrutha is a liquid biodynamic microbial organic formula that farmers in India have traditionally used for several years. Its origin can be traced back to southern India and is renowned for its efficacy in Andhra Pradesh, Tamil Nadu, and Karnataka. It is an eco-friendly and sustainable approach to farming, aimed at enriching the soil with essential nutrients and promoting overall plant health. The term "Jeevamrutha" is derived from two Sanskrit words: "Jeeva," which means life, and "Amrutha," which means nectar of immortality (*A. Kaur*, 2020). This name aptly reflects the philosophy behind the practice, as it seeks to enhance the vitality of the soil and create a thriving ecosystem for plants to flourish.

Jeevamrutha is produced by mixing 10 kg of cow dung, 10 L of cow urine, 2 kg of jaggery, 2 kg of pulse flour, and a handful of undisturbed soil, with 200 L of water (Palekar, 2006). In order to prepare JA, individual contents are combined with water and secured with a cloth covering to prevent the intrusion of dust while allowing for aeration. The formulation is then incubated in a shaded area without temperature regulation and stirred using a wooden log stick 20 times in a clockwise direction, followed by 20 times in an anticlockwise direction, twice daily. Our prior research has indicated that the incubation period for JA can range from 3 to 14 days. However, most farmers choose to incubate JA for 7-8 days before its application in the field (P *et al.,*

2022). It can be used alone or with organic amendments such as vermicompost or farmyard manure. Notably, JA is more widely accepted by small and medium landholding farmers and marginalized farmers communities as opposed to large landowning farmers (Research Project *et al.,*)

This overview led to the study's objectives, which were to: (i) better understand the dynamics of the bacterial population and physico-chemical properties during JA incubation; (ii) evaluate the phytotoxicity of the optimized jeevamrutha formulation; and (iii) determine how long-term field application of JA affects soil health parameters.

Hence, the present investigation has been conducted (i) to understand the presence of beneficial microbes in jeevamrutha, (ii) to check the antagonistic effect against *Rhizoctoniasolani*(iii)to examine the effect of field application of JA on soil application.

MATERIALS AND METHODS

Isolation of beneficial microorganisms from Jeevamrutha

Collection of biological materials

Fresh cow dung and cow urine were collected from the desi cow breed at Shree Rama Krishna Gaushala in Phagwara, Punjab. These cows were fed a natural diet without the use of hormones or antibiotics. The pulse flour and jaggery were purchased from the community market. A soil sample was taken from the rhizosphere zone of the Ficus tree (*Ficusreligiosa*).

Jeevamrutha preparation (JA)

JA, was prepared in accordance with the methodology described by **Palekar (2006)**. Jeevamrutha was prepared by mixing 10 kg of local cow dung with 10 liters of cow urine, 2 kg of local jaggery, 2 kg of corn flour, 200 liters of water, and a handful of garden soil. These ingredients were thoroughly mixed in a plastic drum and kept for seven days to foster fermentation. To improve aeration and mitigate the formation of maggots, the content was stirred thoroughly in a clockwise direction on a daily basis with a wooden stick (S. Palekar, 2006, Sutar et al., 2018).

Microbial analysis:

The sample was homogenized, at 10000 rpm for 10 minutes using a homogenizer (Harvest Innovative Machines LLP India) and serially diluted to 10^1-10^5 in Phosphate buffer saline (PBS). Beneficial microorganism *Trichoderma*, Bacillus, *Beauveria* and *Pseudomonas* were isolated from jeevamrut solution using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983), Nutrient Agar (NA) medium (Rangaswami, 1972), Sabouraud Dextrose Yeast Agar (SDYA) (Shimazu, M., & Sato, H. (1996)), and King's B medium (KMB) (King et al., 1954) respectively.Fromeach factor, 100µl quantity was poured on different selective medium as above mentioned,for their isolation/ enumeration (CFU/ml) (Chakraborty et al.2019). These plates were

incubated for 72 h at 28°C and observed on daily basis. Individual colonies of fungal and bacterial species were identified based on morphological characters ((Webster and Lomas, 1964) and biochemical test (Hildebrand et al., 1992), respectively.

Isolation of *Rhizoctoniasolani*

Root samples were obtained from maize infected plants showing the symptoms of sheath blight. Collected roots were cleaned by running tap water, chopped into small pieces (1-2 mm), sterilized with sodium hypochlorite (NaOCl 1%) solution for 10 seconds and washed with distilled water to remove any residues of the chemical. After removing any remaining water from the root pieces using filter papers, they were dried and placed in Petri dishes with potato dextrose agar (PDA) medium, added with 200 mg/L of the antibiotic chloramphenicol. Around four days, all Petri plates were incubated at a temperature of 25 ± 2 °C (Al-Fadhal et al. 2019).

Antagonistic activity of bio controls *againstRhizoctoniasolani*

Trichoderma spp. and Pseudomonas spp. isolated from the prepared Jeevamrutha. were tested for biocontrol ability against *R. solani.* Mycelial discs (5 mm dia) were cut from the active margins of seven-day-old antagonist and pathogen colonies for the dual culture experiment. On the PDA medium, a pathogen block and one of the antagonists were placed 3 cm apart. The control fungi were inoculated individually on one side of the Petri plates containing PDA media (Awad et al.2018).

The effectiveness of *Trichoderma spp.* culture filtrates was evaluated by inoculating 5 mm-diameter discs of the fungus, taken from the active margin of a 7-day-old colony, into 100 milliliters of sterilized potato dextrose broth (PDB) and incubated for ten days at 252°C without shaking. The culture broth was filtered through Whatman filter paper no. 1 and then again through a Millipore membrane filter (0.45 µ) to obtain cell-free culture filtrates. Each *Trichoderma sp.* culture filtrate (4 ml) was put into a sterile Petri plate, and 16 ml of PDA was added afterward to reach a 20% concentration. As a control, sterile water was added to PDA in place of culture filtrate.

Placing pathogen mycelial discs (5 mm in diameter) in the center of the solidified agar plates was done after separating them from cultures that were actively growing. After four days of incubation at 25±2°C, the percentage of inhibition in radial colony growth relative to the control was determined for the Petri plates.

In a randomized block design, the inhibitory effect of culture filtrate, volatile metabolites, and dual culture interaction were measured in triplicate and repeated twice. The formula provided by Singh et al., (2002) was used to measure the percent suppression of pathogen mycelial growth:

$$I = (C-T/C) \times 100$$

Where, I = inhibition (%), C = colony diameter in control plate and T = colony diameter in treated plate.

Antagonistic activity of bacteria.

Into the sterilized petri dishes, the same volume of sterilized King's B medium and potato dextrose agar (PDA) was mixed and transferred into sterilized petri dishes. The center of Petri plates was inoculated with a substantial inoculum derived from an active-growing fluorescent Pseudomonas, and the pathogens' mycelial disc was streaked out 1 cm from the plate's edges. Only phytopathogens were used to inoculate control plates. The Vincent (1947) formula was used to compute the percent suppression of pathogens by Pseudomonas isolates over control: [(Growth of pathogen in control - Growth of pathogen with Pseudomonas isolate)/ Growth of pathogen in control] x 100.

Estimation of total phenol content.

With a few minor modifications, the total phenolics of the extracts were calculated using the Folin and Ciocalteu reagents according to the methodology outlined by Singleton and Rossi. The sample and standard readings at 765 nm were obtained using a spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian) in comparison to the reagent blank. Into 0.6 mL water and 0.2 mL Folin-Ciocalteu's phenol reagent (1:1), 0.2 mL of test sample was added. After five minutes, the mixture was supplemented with one milliliter of saturated sodium carbonate solution (8% w/v in water), and distilled water was added to make the volume reach three milliliters. After a dark phase of 30 minutes, the procedure was centrifuged and the absorbance of blur from multiple samples was measured at 765 nm. The phenolic content of the dry plant material was determined using gallic acid equivalents (GAE)/g, which was derived using a standard curve of gallic acid (5–500 mg/L, $Y = 0.0027x - 0.0055$, $R^2 = 0.9999$). Each findings was done three times. (J. A. Rossi and V. L. Singleton, 1965).

Compatibility tests of *Trichoderma* and *Pseudomonas*

The cross-streak assay technique was applied to evaluate the compatibility of the two antagonistic fungal bacterial strains (Velho-Pereira, S., &Kamat, N. M. (2011). In a Petri dish filled with KMB media, two parallel lines of *Pseudomonas* was streaked, and two lines perpendicular were streaked to the isolate ISO1 with *P. aeruginosa* ISO2. For three to four days, the plates were incubated at 28°C. The experiment was repeated using three replicates. Bacterial growth at the interjection was noted (Ghafri et al., 2020).

Field Trial

To examine the effect of JA, on enhancing the beneficial properties of soil, a field experiment was conducted in the Department of Plant Pathology, Lovely Professional University, Punjab, India. Different observations were taken.

Results and discussion

In our study, on the basis of microscopic observations and cultural characteristics, the beneficial microorganisms were identified. The identified organisms were *Trichoderma, Beuvariabassiana, Pseudomonas fluorescens,* and *Bacillus subtilis*. The beneficial microorganisms were commonly found in natural preparations such as jeevamruth and Ghana jeevamruth. (Fig 1). Similar studies were done by several researchers (Tutika et al., 2018).

The results of the experiments revealed that prolonged incubation periods significantly influenced the microbial composition and nutrient dynamics of Jeevamrutha. Samples incubated for 10-14 days exhibited higher microbial diversity, increased levels of nitrogen, phosphorus, and potassium, and improved overall stability compared to shorter incubation periods. However, excessively long incubation periods beyond 14 days led to a decline in microbial activity and nutrient availability, indicating a point of diminishing returns. .

Jeevamrutha, a liquid organic product that is rich in antagonistic microorganisms and is widely employed as a pesticide (Ramanathan, 2006). *Trichoderma spp.* are the most common antimicrobial fungal species isolated from natural soils and root ecosystem. *Pseudomonas* is a soil bacterium that colonizes the plant root zone aggressively, boosts growth, and causes disease resistance. *B. bassiana* is a commonly used organism known to control both insects and pathogens in crops (Ownley et al., 2004).

When Jeevamrutha was first prepared, it had a somewhat green tint that deepened with time. When Jeevamrutha was first prepared, it had a slight odor that got stronger after 20 days and remained that way until the completion of the storage time. The presence of jaggery may be the cause of these. The decomposition of cow dung in Jeevumrutha was accelerated due to the proliferation of bacteria fostered by the presence of water and jaggery. As a result, a faintly unpleasant odor and a dark green color were generated (Ravindra et al., 2016).

According to our previous research, Jeevamrutha is usually incubated for 3-14 days before being applied to the field. Farmers, on the other hand, use an incubation period of 7-8 days. JA undergoes a succession of physical, chemical, and biological changes in an erratic pattern during incubation, which is similar to composting, anaerobic digestion and fermentation.

Jeevamrutha promoted the growth of all biocontrol agents, which might be attributed to its increased nutrient content and lower pH when compared to other natural media. Jeevamrutha, with a pH ranging from 4.8 to 5.2, has more beneficial microorganisms as well as higher levels of other metabolites that aid in the proliferation of microorganisms (Devakumar et al. 2008).

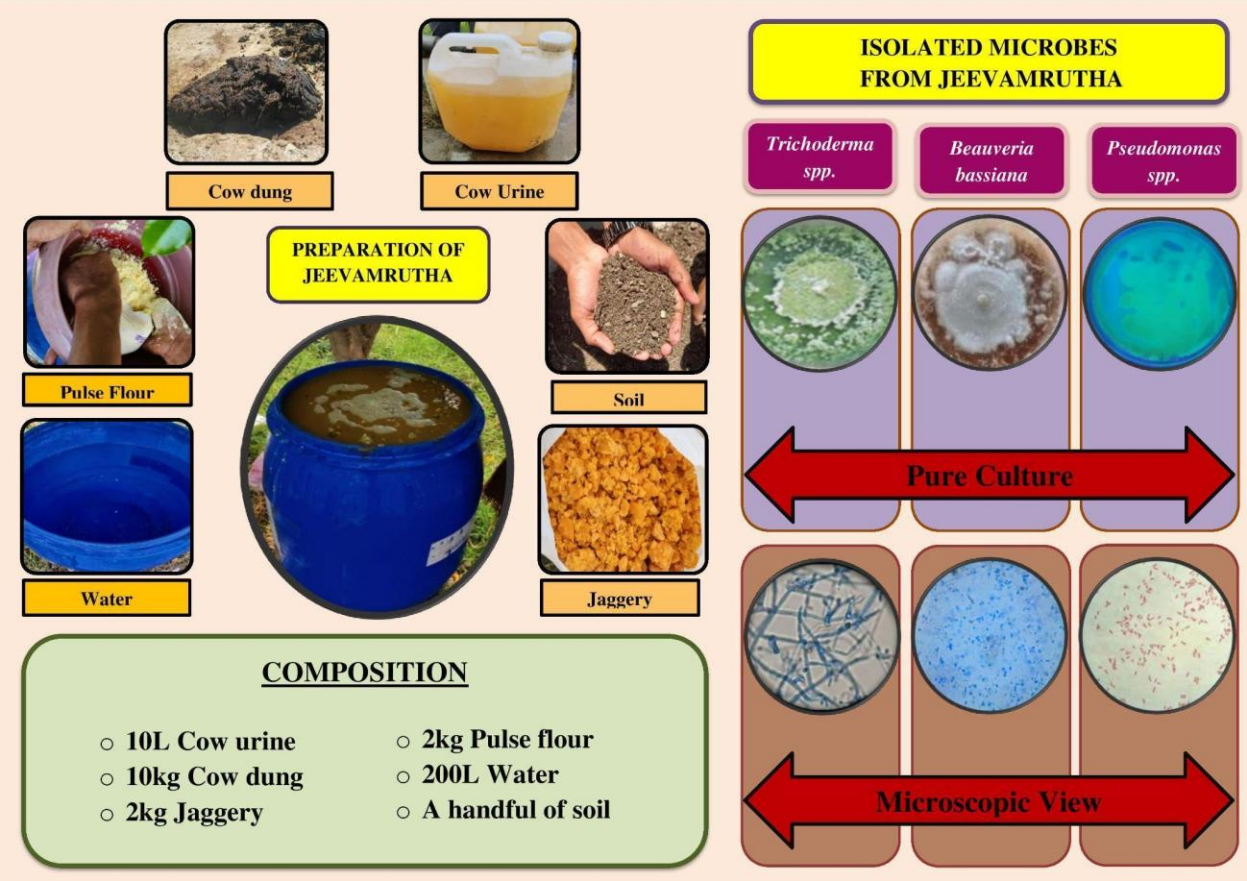


Fig: - Isolation and characterization of potential biocontrol agents from Jeevamurtha

Isolation of *Trichoderma*

A serial dilution of sample was performed under the laminar air flow, bringing up the dilution to 10^4 as(Shashikanth*et al.,* 2018) observed the microbial counts of the natural concoctions in the present study were significantly lower than the minimum requirements for commercial formulations, ranging from 10^2 to 10^4 CFU dilutions compared to 10^6 or 10^8 CFU dilutions, respectively.

- Isolation and identification of *Trichoderma spp.*

Trichoderma spp. was isolated from the natural soil and roots of ecosystem products. The findings were very comparable to the morphologic findings published by A. (1969). Prior studies carried out similar traits for *Trichoderma spp.* identification based on how phialides and conidia look (Samuelsels*et al.,* 2017).

- **Morphological and Microscopic characteristics of *Trichoderma spp*.**

Vegetative septate hyphae served as the key to identifying Trichoderma.

The secondary branches' tips were retained at 90 degrees to the axis from which they originated, with the longest branches growing closest to the base of the hyphae. The hyphae

ended in flask-shaped conidiogenous or phialides, which developed smooth-walled conidia with subglobose or ellipsoidal shapes. The characteristics observed here are similar to the findings by Tutika., (2018). For preliminary identification of the microorganisms, the isolates were examined under a microscope and gram reaction for identification (Mittwer et al.1950, Kiernan, J. A. 2017).

- Morphological characteristics of *Trichoderma spp.*

The morphological characteristics of *Trichoderma spp*. can be used to identify different species of this genus. The conidia of *Trichoderma spp*. are typically light white with various hues of green or yellow. The phialides are densely packed and relatively slender. The colonies of *Trichoderma spp*. can have a variety of colors, including yellow, brilliant green, dull to dark green, floccose, and conidiophore tufts that are compact. The colony's reverse is typically lacking in coloration. These morphological characteristics are important for identifying *Trichoderma* spp. because they can vary between different species. For example, the conidia of *Trichoderma harzianum* are typically green, while the conidia of *Trichoderma viride* are typically yellow. The colonies of *Trichoderma harzianum* are also typically more compact than the colonies of *Trichoderma viride*. The morphological characteristics of *Trichoderma spp.* can also be used to assess the quality of the cultures. These characteristics are similar to the phenotypic characteristics identified by Kumar., *et al* (2012). Overall, the morphological characteristics of *Trichoderma* spp. are an important tool for identifying and assessing the quality of cultures.

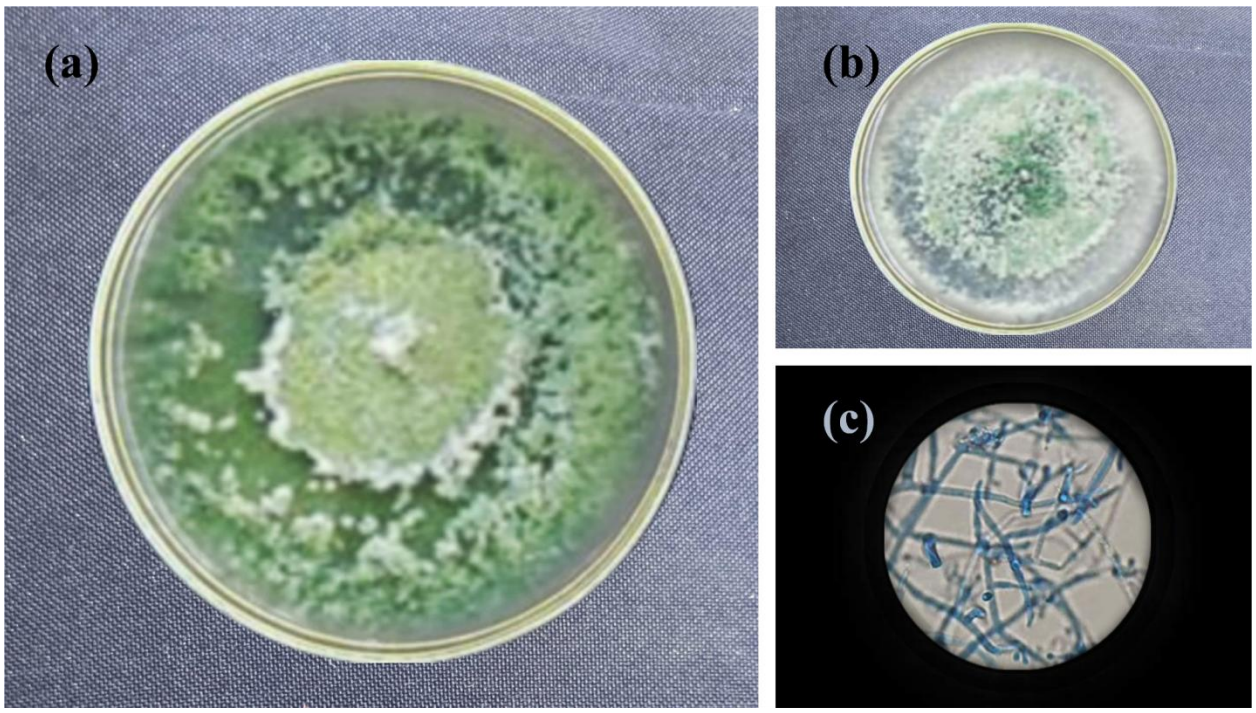


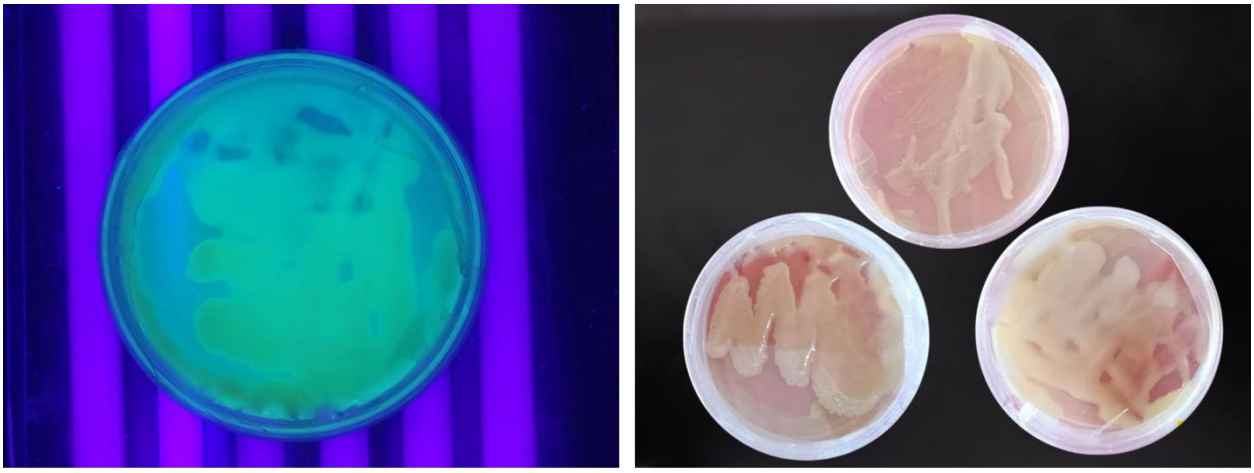
Fig. Pure culture of*Trichoderma* spp.; (a) and (b) morphological characteristics of *Trichoderma* spp., (c) microscopic image under 40X

Isolation and characterization of *Pseudomonas* spp.

Pseudomonas fluorescens is a gram negative, ubiquitous organism that is commonly found in agricultural soils. This bacteria contains several traits, such as colonizing the rhizosphere, enhancing plant growth (PGPR), and acting as a biocontrol agent. (Belkar and Gade et al. 2012, David et al. 2018). The presence of this beneficial bacteria in Jeevamrutha was confirmed by inoculating on King's B Medium (King *et al.*, 1954). It is a selective medium that is widely used to detect the fluorescent pigments produced by *Pseudomonas*.

Under ultraviolet (UV) light, the majority of bacteria in the genus Pseudomonas develop siderophores that fluoresce at 365 nm. The reason behind the colonies' green hue is the production of pyoverdine into the medium for iron absorption (Lamichhane&Varvaro, 2013). According to this investigation, Pseudomonas spp. were found in Jeevamrutha. The bacteria were recognized as convexly elevated, small, spherical, shiny colonies. Under UV light, the smooth, yellowish-green surface of the colonies took on a luminous appearance. These traits closely resemble those that Sodimalla (2015) observed when Pseudomonas spp. were isolated from rice fields in the state of Telangana. When Jayashree et al. (2000) isolated fluorescent Pseudomonas from the rhizosphere of black gram, carrot, banana, pepper, rice, and forest trees planted in various Tamil Nadu locations, they too noticed similar findings. By observing the luminous colonies of pseudomonas under UV light, their presence was confirmed.

The results of this study confirm that Jeevamrutha contains *Pseudomonas* spp., which are PGPR that can benefit plant growth and development. According to certain research, the soil type determines how often *P. fluorescens* occurs naturally, with suppressive soils acting as the natural niche for *Pseudomonas* (Shashikanth*et al.*, 2018)



a b

Fig.. Morphological characteristics of *Pseudomonas fluorescens* (a) Under the UV light (B) on King’s B medium.

Isolation of Entomopathogenic Fungi

B. bassiana is an entomopathogenic fungus that has been shown to infect over 100 insects of various orders and stages of development. It has been described as an organism that controls both diseases and insects in crops (Ownley et al., n.d.).

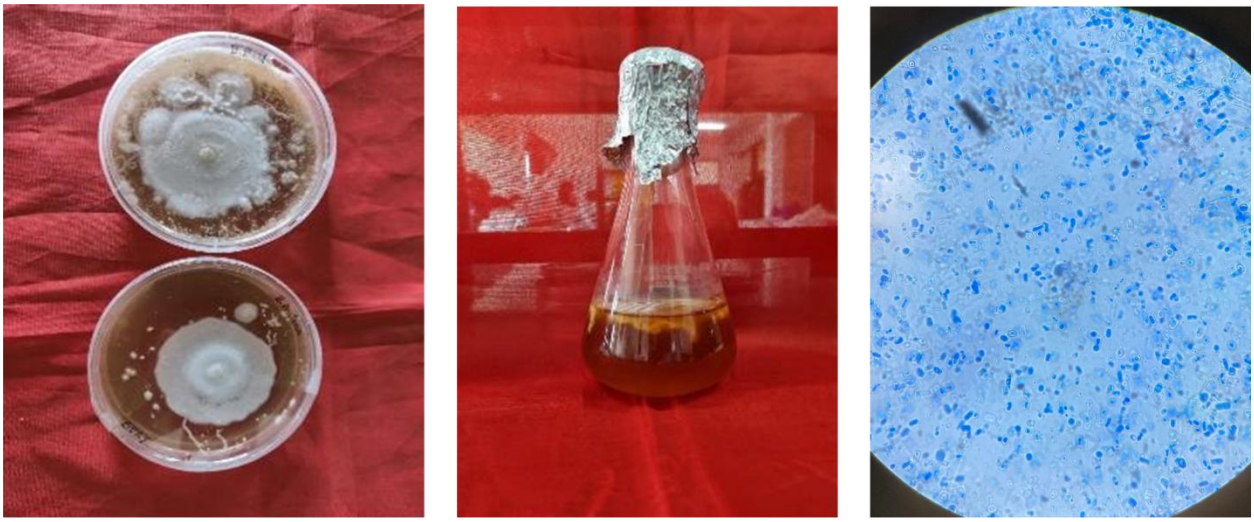
By plating on SDAY medium, the presence of these entomopathogenic fungi in the natural products was examined. Colony morphology was used to identify the B. bassiana colonies.

It was discovered that the colonies were evenly spaced, circular in form, smooth in texture, and white color with dense white conidiation. Under a microscope, colonies of B. bassiana were observed to have branched hyphae that formed conidiogenous cells. The conidiophores appeared flask-shaped and generated zigzag chains of conidia. The conidia had a spherical or subglobose form. These findings corresponded to the colony description given by Affandi et al. (2012) while separating the B. bassiana colonies from the surrounding soil and plants. According to their findings, B. bassiana grows as white mycelium on PDA medium, forming a layer of white powder on the plate.

According to Vänninen (1996), there may be a low presence of *B. bassiana* in extensively cultivated areas due to the shortage of hosts. It is hypothesized that *B. bassiana* necessitates frequent serial passage through the hosts to survive. According to recent research, *B. bassiana* may interact with other plants or fungi. On the other hand, *B. bassiana* functions in plant tissue as an endophytic fungus (White et al., 2002). According to Elliott et al. (n.d.), endophytic fungus are thought of as mutualistic creatures that protect plants, and the presence of *B. bassiana* in interior plant tissue is thought to be an adaptive defense mechanism against herbivorous insects.

Furthermore, *B. bassiana* is thought to function as a natural infection pathway of endophytic activity in temperate climates where it is linked to the phylloplanes of diverse plants in hedgerows as a result of deposition from surrounding environments (Meyling et al., n.d.).

The fungus *B. bassiana* may have been present in this herbal mixture because of its affinity with the leaf surface above soil level.



a b c

Fig.. Cultures of *Beauveria bassiana* isolated from jeevamrutha (a) Growth on SDAY medium(b) PDB medium(c) Microscopic examination under 10X magnification.

Collection, isolation, purification, identification of the pathogen

Banded leaf and sheath blight of maize caused by *Rhizoctoniasolani* comes under major diseases which deals considerable yield loss. Therefore, the present investigation on studying the "Effect of novel Jeevamrutha solution against *Rhizoctoniasolani* of Maize" was carried out in the field as well as in the laboratory during 2022- 2023, at the Agri research farm of Lovely Professional University, Phagwara (Punjab). From March to June, this disease starts to appear and becomes severe from May to June.

Collection of diseased plant materials

The plots where the maize trial was held were timely observed and samples of maize brought in paper bags for further investigation in the laboratory.

Fig. Effect of jeevamrutha on maize under in field conditions

Isolation and purification of *Rhizoctoniasolani*

The isolation of *R. solani* was done by diseased plant leaves which were collected from a trial held on the LPU research farm. The leaves were cut half part of healthy tissue and half were infected later the bits were transferred into Petri plates containing potato dextrose agar (PDA) medium. The Petri plates were incubated at 26±1℃ for seven days. Characterization was done of mycelium and conidiospores.

The mycelium of *R. solani* is a white, thread-like growth that can be seen on the surface of infected plants or in culture plates. The mycelium is made up of hyphae, which are long, branching filaments. The hyphae of *R. solani* can penetrate the tissues of plants and cause damage. The mycelium can also be identified by its ability to produce sclerotia, which are hard, black structures that can survive in the soil for long periods of time.

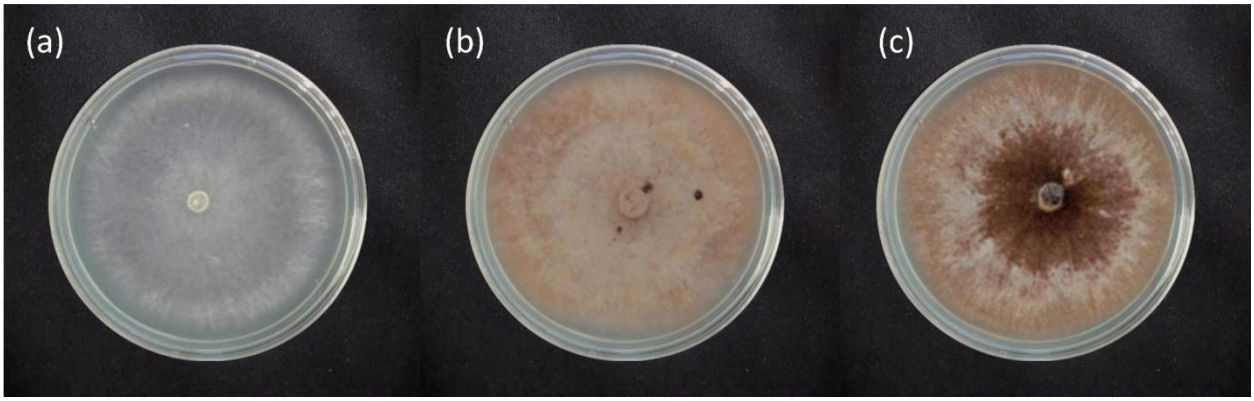


Fig. Growth of *Rhizoctoniasolani*upto 30 days of incubation; (a) 10th days of incubation, (b) 20th day of incubation, (c) 28th day of incubation

Nutrient composition

According to the findings presented in Table 3, the components that make up jeevamrutha suggest an acidic characteristic. Furthermore, it is worth noting that jeevamrutha is a particularly abundant source of both macro and micro -nutrients.

Table: Nutrient composition of jeevamrutha and their constituents

Sample	pH	N (unit)	P (unit)	K(unit)	Mg(ppm)	Cu (ppm)
Jeevamrutha	4.82	1.86	0.167	0.27	44	50
Local cow urine	8.13	1.64	0.11	2.455	6.5	18
Local cow dung	8.06	0.68	0.278	0.233	9.33	3.55
Corn flour	6.44	1.67	0.84	0.94	11.5	12.41
Jaggery	6.3	0.82	0.207	0.29	9.1	28.78

Table: Growth inhibition percentage of *R. solani* with Jeevamrutha treatments.

S. No.	Treatments	Colony Diameter (mm)	Percent growth inhibition (%)
1	Jeevamrutha 1%	12.47±0.18^{e}	81.39
2	Jeevamrutha 5%	10.68±0.03^{d}	84.12
3	Jeevamrutha 10%	8.47±0.23^{c}	90.91
4	Jeevamrutha + *Trichoderma* spp. 5%	7.69±0.23^{b}	91.86
5	Jeevamrutha+*Pseudomonas fluorescens* 5%	12.38±0.05^{e}	81.67
6	Jeevamrutha + *Trichoderma* spp.+ *Pseudomonas fluorescens* 5%	6.7±0.42^{a}	93.44
7	Control	89.92±0.52^{f}	0.9

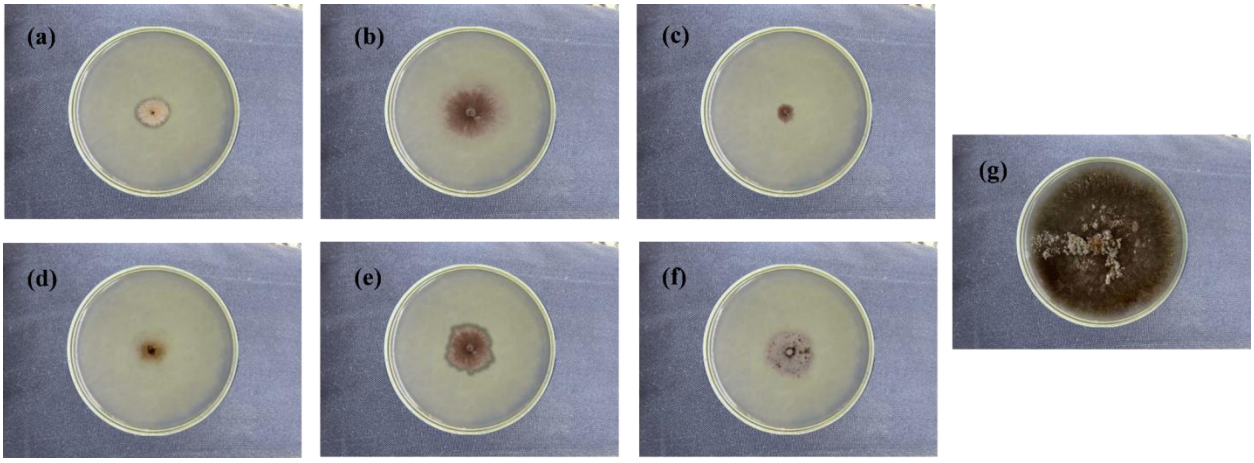


Fig. : Growth of *Rhizoctoniasolani*against different Jeevamrutha treatments.

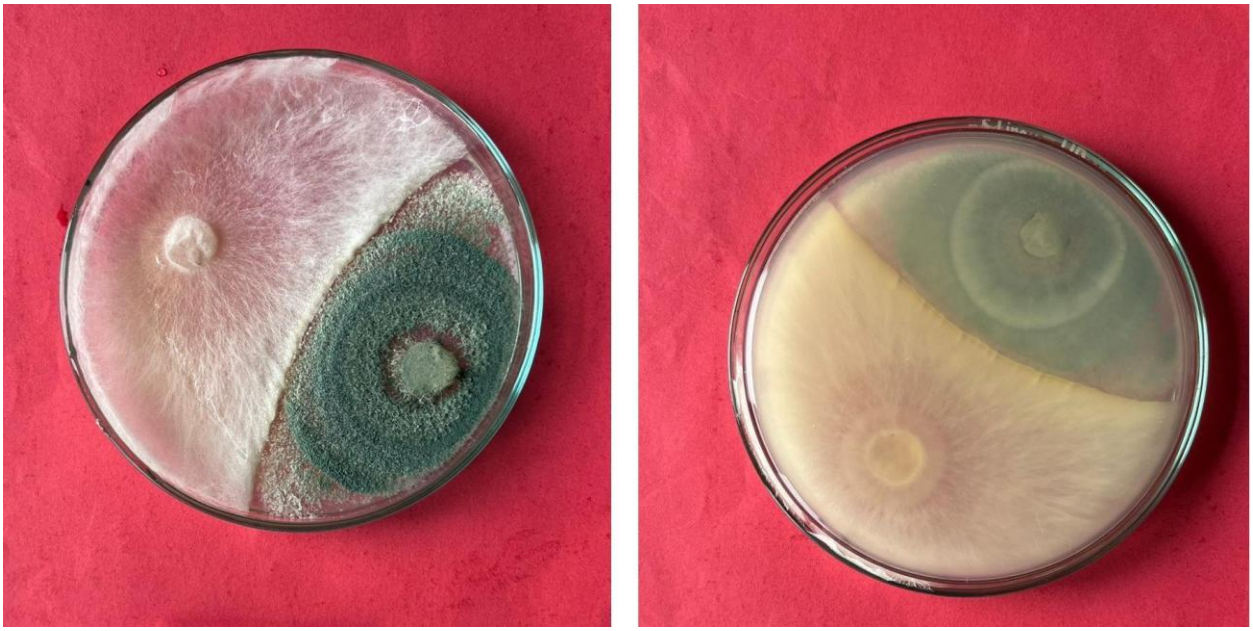


Fig. : Dual culture of *Trichoderma spp.*

Fig. : Compatibility of *Trichoderma spp.* with *Pseudomonas fluorescens*

Table: Disease incidence, shoot length, root mass and Phenol content.

Treatments	**Disease Incidence (%)**	**Shoot Mass (cm plant^{-1})**	**Root mass (g plant $^{-1}$)**	**Phenol (nm)**
Jeevamrutha	48.73±3.85	40.06±16.76	5.21±0.22	2.33±0.07

Jeevamrutha + *Trichoderma* spp.	46.47±4.1	38.2±15.71	5.06±0.07	8±0.07
Jeevamrutha + *Pseudomonas*	39.1±3.05	38.46±15.16	5.3±0.07	2.45±0.05
Trichoderma spp.	41.6±2.21	38.3±16.04	5.37±0.08	1.98±0.13
Pseudomonas fluorescens	38.76±8.58	38.26±16.73	5.1±0.06	2.4±0.03
Jeevamrutha + *Trichoderma* spp. + *Pseudomonas fluorescens*	37.16±5.15	40.1±16.66	5.52±0.08	2.52±0.04
Control	52.1±2.93	36.7±14.88	4.63±0.28	1.57±0.14

The data presented in the table illustrates the impact of Jeevamrutha treatments on R. solani /Fusarium disease occurrence, shoot and root mass, and phenol content in maize. These treatments involve the use of Jeevamrutha, *Trichoderma*spp., and. Pseudomonas fluorescens. All agents known to combat plant diseases caused by *Fusarium oxysporum 1*. The control group comprises plants withtreatmentst. The data showed varying rates of disease incidence amongst the different treatments. The control group had the highest incidence at 52.1%, while Jeevamrutha alone had a slightly lower incidence at 48.73%. Interestingly, the combination of Jeevamrutha, *Trichoderma* spp., and *Pseudomonas fluorescens* showed lowest incidence at 37.16%. This indicates the biological agents effectively reduced disease severity, with the combination being the most successful. It should be noted, however, that the standard deviations were quite large, indicating a considerable amount of variability among the plants. A thorough analysis, possibly through statistical testing, would be required to determine if the observed differences are significant.In terms of shoot mass, the control group demonstrated the lowest measurement at 36.7 cm. However, the most notable increase in shoot mass was observed in the group treated with Jeevamrutha, *Trichoderma spp.*, and *Pseudomonas fluorescens*, reaching 40.1 cm. This finding suggests that the use of these natural agents resulted in improved plant growth, with the combination being more effective than individual use. It's worth noting that there was significant variation among the plants, as seen in the large standard deviations. To confirm the significance of these differences, a statistical analysis would be necessary.The results of the experiment indicated a significant increase in root mass when Jeevamrutha, *Trichoderma spp*., and *Pseudomonas fluorescens* were combined, with a recorded mass of 5.52 g compared to the control group's 4.63 g. This suggests that the use of biological agents had a positive impact on root development, and the combined treatment proved to be more beneficial than individual agents. Additionally, the standard deviations were consistently small, indicating minimal variation among the plants. A statistical analysis would be necessary to determine the significance of these differences. Similar trends were observed in the phenol content, as the combination of Jeevamrutha, *Trichoderma spp*., and *Pseudomonas fluorescens* yielded the

highest concentration of 2.52 nm, while the control group had the lowest at 1.57 nm. These findings further support the positive effects of biological agents on plant growth and development. The phenol content of plants was significantly influenced by the use of biological agents. It was found that the group treated with Jeevamrutha, *Trichoderma spp*., and *Pseudomonas fluorescens* had the highest amount of phenols (2.52 nm), while the control group had the lowest (1.57 nm). This points to the effectiveness of these agents in increasing the plant's defense against pathogens, as phenols are known to play a crucial role in this function. Additionally, the combination of these agents proved to be more effective in increasing phenol content compared to using each agent alone. These results were also supported,by small standard deviations, indicating consistency among the plants. A statistical analysis would further validate the significance of these differences. Overall, the table clearly illustrates that Jeevamrutha, *Trichoderma spp*., and *Pseudomonas fluorescens* - three important biological agents - all had a positive impact on the plants' disease incidence, shoot mass, root mass, and phenol content. The most successful treatment was found to be a combination of all three agents, closely followed by a combination of Jeevamrutha and *Pseudomonas*, and then individual applications of *Pseudomonas fluorescens* or *Trichoderma spp*. It's worth noting, however, that caution should be exercised when interpreting these results due to the small sample size and high variability. Additional statistical testing is necessary to confirm the significance of these differences.

Phenols play a crucial role in plants as they are a diverse group of secondary metabolites with various functions that contribute to the plant's growth, development, and interaction with the environment. Phenols are a class of organic compounds that include phenolic acids, flavonoids, tannins, and other secondary metabolites found in plants. Phenolic compounds act as potent antioxidants, protecting plants against oxidative stress caused by factors such as UV radiation, pathogens, and pollutants. They also contribute to the plant's defense mechanisms by acting as chemical barriers against herbivores and pathogens. Additionally, phenols are involved in signaling pathways, regulating plant responses to environmental cues, such as stress and nutrient availability. Beyond their protective functions, phenols are known for their potential beneficial effects on human health, as many of these compounds possess antioxidant and anti-inflammatory properties, making them valuable components in herbal medicine and dietary supplements.

To determine the amount of phenols in plant extracts, there are a few methods available, such as spectrophotometric methods (like the Folin-Ciocalteu method) or high-performance liquid chromatography (HPLC). Spectrophotometric methods use particular reagents to create colored complexes with phenols, and the intensity of the color is directly proportional to the concentration of phenols. Meanwhile, HPLC is a highly accurate and selective method that separates phenolic compounds in the extract, allowing for exact quantification. To measure the phenol concentration, create calibration curves using appropriate standards with known amounts of phenol.

Conclusions.

Investigating the effect of JA during incubation leads to the conclusion that JA is a fermentative type of product. Farmers' practice of harvesting JA after 7–8 days of incubation is justified by the relatively high microbial load observed in our study. According toour fieldresults of our study enhance JA's reliability and promote its acceptance among the progressivefarmers.

REFERENCES

Devakumar, N., Shubha, S., Gowder, S. B., and Rao, G. G. E. 2014. Microbial analytical studies of traditional organic preparations beejamrutha and jeevamrutha. *Building organic bridges*. **2**:639-642.

Tutika, S. R. I. D. E. V. I., Dara, S. H. A. S. H. I. K. A. N. T. H., Saikia, N. I. R. M. A. L. I., and Cherukuri, S. R. 2018. Screening and isolation of beneficial microorganisms from natural and organic concoctions collected from various parts of Andhra Pradesh and Telangana. *Biopestic. Int*. **14**:101-108.

Duraivadivel, P., Kongkham, B., Satya, S., and Hariprasad, P. 2022. Untangling microbial diversity and functional properties of Jeevamrutha. *Journal of Cleaner Production*. *369*, 133218.

Seenivasagan, R., and Babalola, O. O. 2021. Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product. *Biology*, **10**(11):1111.

Yassin, M. T., Mostafa, A. A. F., Al-Askar, A. A., Sayed, S. R., and Rady, A. M. 2021. Antagonistic activity of Trichoderma harzianum and Trichoderma viride strains against some fusarial pathogens causing stalk rot disease of maize, in vitro. *Journal of King Saud University-Science*, **33**(3):101363.

Yassin, M. T., Mostafa, A. A. F., and Al-Askar, A. A. 2021. In vitro antagonistic activity of Trichoderma harzianum and T. viride strains compared to carbendazim fungicide against the fungal phytopathogens of Sorghum bicolor (L.) Moench. *Egyptian Journal of Biological Pest Control*, **31**(1):1-9.

Bokhari, N. A., and Perveen, K. 2012. Antagonistic action of Trichoderma harzianum and Trichoderma viride against Fusarium solani causing root rot of tomato. *African Journal of Microbiology Research*, **6**(44):7193-7197.

Kumar, K., Amaresan, N., Bhagat, S., Madhuri, K., and Srivastava, R. C. 2012. Isolation and characterization of Trichoderma spp. for antagonistic activity against root rot and foliar pathogens. *Indian Journal of Microbiology*, 52:137-144.

Saharan, B. S., Tyagi, S., Kumar, R., Vijay, Om, H., Mandal, B. S., and Duhan, J. S. 2023. Application of Jeevamrit improves soil properties in zero budget natural farming fields. *Agriculture*, **13**(1): 196.

Pandia, S., Trivedi, A., Sharma, S. K., and Yadav, S. 2019. Evaluation of Jeevamrut and its Constituents against Alternaria Leaf spot of Mungbean in vitro and under Cage House Condition in Rajasthan. *International Journal of Current Microbiology and Applied Science*, **8**:2240-51.

Rathore, G., Kaushal, R., Sharma, V., Sharma, G., Chaudhary, S., Dhaliwal, S. S., and Hossain, A. 2023. Evaluation of the Usefulness of Fermented Liquid Organic Formulations and Manures for Improving the Soil Fertility and Productivity of Brinjal (Solanum melongena L.). *Agriculture*, **13**(2):417.

Devakumar, N., Shubha, S., and Rao, G. G. E. 2014. Multiplication of bio-control agents on locally available organic media. *Building Organic Bridges*, **2**:643-646.

Gul, H., Rahman, S., Shahzad, A., Gul, S., Qian, M., Xiao, Q., et al. 2021. Maize (*Zea mays* L.) productivity in response to nitrogen management in Pakistan. *Am. J. Plant Sci.***12**:1173–1179. doi: 10.4236/ajps.2021.128081.

Sandhu, K. S., Singh, N., and Malhi, N. S. 2007. Some properties of corn grains and their flours I: Physicochemical, functional, and chapati-making properties of flours. *Food Chemistry*, **101**: 938–946.10.1016/j.foodchem.2006.02.040.

El-Esawi, M. A. (Ed.). 2022. Maize Genetic Resources: Breeding Strategies and Recent Advances.

Edde, P. A. (2021). *Field crop arthropod pests of economic importance*. Academic Press.

FAOStat. (2021). FAO Stat. FAO, Rome. http://www.fao.org/faostat

Palekar. 2006. Three days workshop on Philosophy and technology of Zero budget farming, organized by Karnataka Rajya Raitha Sangha (KRRS) HasiruSene

Chakraborty, B., and Sarkar, I. 2019. Quality analysis and characterization of Panchagavya, Jeevumrutha and Sasyamrutha. *Int. J. Curr. Microbiol. App. Sci*, **8**(5):2018-2026.

Awad, N. E., Kassem, H. A., Hamed, M. A., El-Feky, A. M., Elnaggar, M. A., Mahmoud, K., and Ali, M. A. 2018. Isolation and characterization of the bioactive metabolites from the soil derived fungus Trichoderma viride. *Mycology*, **9**(1):70-80.

Al-Ghafri, H. M., Velazhahan, R., Shahid, M. S., and Al-Sadi, A. M. 2020. Antagonistic activity of Pseudomonas aeruginosa from compost against Pythium aphanidermatum and Fusarium solani. *Biocontrol Science and Technology*, **30**(7):642-658.

Kiernan, J. A. 2017. Fibers, bacteria and things that look like fungi under the microscope. *Journal of Histotechnology*, **40**(2):29-30.

Mittwer, T., Bartholomew, J. W., and Kallman, B. J. 1950. The mechanism of the gram reaction. II. The function of iodine in the gram stain. *Stain Technology*, **25(**4):169-179.

Al-Fadhal, F. A., Al-Abedy, A. N., and Alkhafije, D. A. 2019. Isolation and molecular identification of Rhizoctoniasolani and Fusarium solani isolated from cucumber (Cucumis sativus L.) and their control feasibility by Pseudomonas fluorescens and Bacillus subtilis. *Egyptian Journal of Biological Pest Control*, **29**:1-11.

Haq, I., Kumari, V., Kumar, S., Raj, A., Lohani, M., and Bhargava, R. N. 2016. Evaluation of the phytotoxic and genotoxic potential of pulp and paper mill effluent using Vigna radiata and Allium cepa. *Advances in Biology*, *2016*.

Patel, K., and Patel, M. (2020). Improving bioremediation process of petroleum wastewater using biosurfactants producing Stenotrophomonas sp. S1VKR-26 and assessment of phytotoxicity. *Bioresource technology*, **315**:123861.

Shimazu, M., and Sato, H. 1996. Media for selective isolation of an entomogenous fungus, Beauveria bassiana (Deuteromycotina: Hyphomycetes). *Applied Entomology and Zoology*, **31(**2):291-298.

Velho-Pereira, S., and Kamat, N. M. 2011. Antimicrobial screening of actinobacteria using a modified cross-streak method. *Indian journal of pharmaceutical sciences*, **73(**2):223.

Elad, Y., Chet, R., 1983. Improved selective media for isolation of Trichoderma spp. and Fusarium spp. Phytoparasitica 11, 55–58

King, E.O., Ward, M.K., Raney, D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.***44**:301–307.

Rangaswami, G., 1972. Diseases of Crop Plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, India, p. 520

Hildebrand, D.C., Schroth, M.N., Sands, D.C., 1992. Pseudomonas. In: Schaad, N.W. (Ed.), Laboratory Guide for Identification of Plant Pathogenic Bacteria. , 2nd ed. International Book Distributing Co., Lucknow, India, pp. 60–80

Webster, J., Lomas, N., 1964. Does Trichoderma viride produce gliotoxin and viridin? *Trans. Br. Mycol. Soc*.**47**:535–540.

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, **16**(3):144-158.

David, B. V., Chandrasehar, G., and Selvam, P. N. 2018. Pseudomonas fluorescens: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In *Crop improvement through microbial biotechnology* (pp. 221-243). Elsevier.