

**A DISSERTATION ON**  
**Effect of paddy straw mulch and VAM (Vesicular**  
**Arbuscular Mycorrhiza) inoculation on soil enzymatic**  
**activities of Rose-Scented Geranium (*Pelargonium***  
***graveolens*)**

**SUBMITTED TO THE DEPARTMENT OF BIOSCIENCES INTEGRAL**  
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**Degree of Master of Science**  
**In Microbiology**

**BY**

**ARPITA UPADHYAY**

**M.Sc. Microbiology (IV Semester)**  
**Department of Biosciences**



**Under the guidance of**  
**Dr. Priyanka Suryavanshi**  
**(Scientist)**

**Division of Crop Production and Protection**  
**CSIR- Central Institute of Medicinal and Aromatic Plants,**  
**Lucknow, UP**



**CSIR-CIMAP**  
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**Name of the Candidate** : Ms. Arpita Upadhyay  
**Institution** : Integral University, Lucknow  
**Category of Training** : Graduate Training 4 Months

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The institute wishes the candidate success in her future endeavors.

**Supervisor**

**Dr. Priyanka Suryavanshi**  
**Scientist**

**CSIR-CIMAP, Lucknow**



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### TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Ms. ARPITA UPADHYAY** a student of **M.Sc. Microbiology** (2nd year/4th semester), Integral University Lucknow, has completed her four-month dissertation work entitled “**Effect of paddy straw mulch and VAM inoculation on soil enzymatic activities of rose-scented Geranium (*Pelargonium graveolens*)**” Successfully. She has completed this work from CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plants) LUCKNOW under the guidance of **Dr. Priyanka suryavanshi**. The dissertation was a compulsory part of her M.Sc. Microbiology degree.

I wish her good luck and bright future.

**Dr. Snober S. Mir**

**Head of department of Bioscience**

**Integral University, Lucknow**

**Uttar pradesh-226026**

## DECLARATION

I, **ARPITA UPADHYAY**, a student of the “M.Sc. Microbiology” session: 2020-2022, Department of Bioscience, Integral University Lucknow, declare that I am solely responsible for all the work presented in the thesis **“Effect of paddy straw mulch and VAM (Vesicular Arbuscular mycorrhiza) inoculation on soil enzymatic activities of Rose-scented Geranium (*Pelargonium graveolens*)”** which is being submitted to Integral University, Lucknow, Uttar Pradesh, India for partial fulfilment for the award of the degree of Master of Science in Microbiology (2022), has been carried out by me under the supervision of **Dr. Priyanka Suryavanshi**, scientist , Crop Production and Protection Division, CSIR-CIMAP, Lucknow, U.P., India. I further declare that I take responsibility for the accuracy of this dissertation report.

Arpita Upadhyay

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### **List of Abbreviation**

VAM	Vascular Arbuscular Mycorrhiza
FID	Flame Ionization Detector
GC	Gas Chromatography
RCBD	Randomized complete block design
NPK	Nitrogen- phosphorous- potassium
EC	Electrical conductivity
SOC	Soil organic carbon
REDOX	Reduction – oxidation
Ppm	Parts per million
TTC	Triphenyl tetrazolium chloride
TPF	Triphenyl formazan
MUB	Modified universal buffer
SMBC	Soil microbial biomass carbon
FAS	Ferrous ammonium sulphate
DHA	Dehydrogenase activity
Rpm	Round per minute
P	Phosphorous
K	Potassium
Zn	Zinc
NCBI	National center for biotechnology information
IARI	Indian Agricultural Research Institute
%	Percent
Etc	Et cetera
ml	Milliliters
Nm	Nanometer
Min	Minute
µL	Microliter
Mm	Millimeter
NaoH	Sodium hydroxide
Fig	Figure
DAI	Days after inoculation
DAP	Days after planting

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## **1.INTRODUCTION**

Rose-scented geranium (*Pelargonium graveolens*) belongs to the family of Geraniaceae and is a multi-harvest plant, high value commercially important essential oil yielding aromatic plant (Shawl et al., 2006). It is cultivated for its dominating rose like sweet smell (Verma et al., 2011). Approximately only 200 species are important for commercial purposes. These are distributed throughout the world such as in southern Africa, Australia, Madagascar Middle East the other part of Africa, and Europe, Asia. Nowadays it is widely cultivated in Algeria, Egypt, China, France, Morocco, Russia, South Africa, Central America, Belgium, Spain, Madagascar, Reunion Islands, Congo, and India (Joy et al., 2001; Shawl et al., 2006; Charles, 2013). Geranium oil has been produced in several East and West African countries, principally in Kenya and Nigeria (Charles, 2013). The center of origin of this gene is in South Africa. The current international demand is of about 600 t geranium oil is largely met by China, Morocco, Egypt reunion island, and south Africa (Qinghai, 1993; Anon., 1996-1997). Here in India production of geranium essential oil of meager quantity of 5 t per year. However, in India yearly consumption of geranium oil is about 145 t. For that reason, most of the 145-t requirement is fulfilled through imports. However, successful efforts have been made to introduce the crop for its commercial cultivation, (Ram and Kumar, 1996a, b, 1997a, 1998a; Ram et al., 1995, 1997ab, 2001).

The main constituents of rose scented geranium oil are citronellol (19.28-40.23%), geraniol (6.45-18.40%), linalool (3.96-12.90%), citronellylformate (1.92-7.55%), Guaia-6,9-diene (0.15-4.40%) and traces of hundreds of compounds. (Joy et al., 2001; Boukhris et al., 2012).

Botanically rose-scented geranium is a male sterile erect and perennial aromatic shrub with a multi harvest high value commercially important essential oil (Weiss, 1997, Lis-Balchin, 2002). It is a highly fragrant shrub, about 1-1.3m in height with soft green to a grey-green stem which becomes



woody and dark with age. Leaves are always fragrant with rose essence, lobed with 5 to 7 palmates which grow opposite to each other from the stem. The inflorescence is pink and flowering is in spring and early autumn. The root system is extensively spread and it is believed to penetrate below 30cm, especially under stress conditions (Weiss,1997; Van Wyk& Gericke,2000; Demarne, 2002; Lis-Balchin,2002; Miller,2002). Rose-scented geranium is a hybrid flowering plant that suffers from some degree of male sterility making it difficult to propagate through seeds. Male sterile genes inhibit the development of viable pollen and prevent normal self-fertilization, resulting in infertile seeds. As a result, these plants are mainly propagated by stem cuttings from healthy mother plant material, but root cuttings and suckers are equally effective, although they require more time to produce. Application of tissue culture is possible, though more expensive than the current methods (Saxena et al., 2008).

The essential oil extracted from the herbage of the plant is widely used in cosmetics and fragrance industries and scenting of soaps. It is one of the top 20 essential oils of the world.

The essential oil is extracted by steam distillation (Rajeshwar Rao Singh and Bhattacharya, 1990a, b; Ram.Ram&Roy, 2003).

When tender shoots and abaxial and adaxial leaf surfaces of the plant are examined under the electron microscope, densely populate special structures called granular and agranular trichomes can be noticed. These granular trichomes are the ones responsible for the secretion and storage of the essential oil. The oil is yellowish to green, greenish-olive, brownish-green mobile liquid (Charles, 2013).

While the nongranular are responsible for creating discomfort to insects and other pathogens during feeding and to reduce moisture loss through evaporation under moisture limited conditions (Lis-Balchin, 2002). During the steam distillation process, membranes covering the glands (cuticles) get ruptured with heat and consequently the essential oil becomes released. The essential oil contents (%fresh mass basis) recovered during distillation is reported to vary within the range of 0.04 to 0.2% (Weiss 1997; Sabinna

Aiyana et al., Mosta et al 2006; Eiasu et al 2009).

It is not well defined when and at what growth stage the herbage should be harvested or the stage at which it can produce the greatest essential oil. However during harvest only the upper 10-15 cm length of shoot is required. Leaving sufficient biomass for re- growth for multiple harvest ( B.R.Rajeswara Rao,2002).

The variation in oil composition which is theorized to be influenced by environmental factors (Motsa, 2006). The first harvest is carried out three to six months after transplanting (Rao 2000); with good agricultural practices harvesting helps to avoid loss of oil yield due to leaf senescence.

The geranium crop requires well-drained porous soil which must be rich in organic matter. The use of chemical fertilizers has created lots of environmental problems. Modern and sustainable systems have increased crop productivity along with less damage to the environment (Martin et al. 2006). VAM and Mulch are used for better agricultural practices to produce the geranium crop.

Biodiversity in the soil is key to its stability. Biofertilizers are preferred alternatives to chemical fertilizers for improving the overall health status of plants. Besides being environment friendly, biofertilizers showcase other important features, including their sustainability within agricultural soils. Vesicular Arbuscular Mycorrhizae (VAM) is one such beneficial microorganism, which helps in enhancing agricultural production by benefitting the plants in many ways. (Ajaynair2002). The application of biofertilizers to reduce or eliminate the consumption of chemical resources is important to fertility and improves product quality (Pesakovica et al.2013). The application of organic mulch provides a crucial role in the growth of geranium. Mulching provides furtherance to soil and plants due to its property to decompose over time it is also useful in conserving soil water content. Organic mulch also suppresses annual weeds and offers various benefits to the crop. Mulch reduces soil evaporation, conserves soil moisture, suppresses weed growth, controls soil structure and temperature, influences soil micro-organisms, and is aesthetically pleasing. (Bansal et

al.,1971; Kaniviets and Fomin,1978; Patra et al.,1993; Ram and Kumar,1997b). Paddy straw mulch provides better production of geranium crops and protection from weeds retains water and provides nutrition as it decomposes.

Paddy is a major food crop and is abundantly available in India but has no effective use in farming communities (Muni Ram, D Ram, S. K Roy). Organic mulch proved better in terms of economizing to produce a yield of geranium oil from the harvest under subtropical conditions of the north Indian plains. Paddy straw mulch and VAM as biofertilizers had no adverse effect on the essential oil of geranium (Muni Ram, D Ram, and S.K Roy). Mulching enhanced the soil enzymatic activity compared to non-mulching which might be due to the availability of moisture in the mulched soil due to lesser evaporation of water. (Siczek and Franc.,2012)

## **2.Aim and Objectives**

This research was aimed to evaluate the study of soil enzymatic activities at different growth stages of rose scented geranium crop as influenced by biofertilizers (VAM) along with paddy straw mulch application with the following objectives.

1. To study the effect of paddy straw mulch and VAM inoculation on soil enzymatic activities at different growth stages of rose scented Geranium.
2. To study the effect of paddy straw mulch and VAM inoculation on soil microbial biomass carbon at different growth stages of rose scented Geranium.
3. To analyze the Influence of paddy straw mulch and VAM inoculation on soil health of rose scented Geranium.

### **3. Review of Literature**

#### **3.1 *Pelargonium graveolens* (Geranium)**

In today's world agricultural production does not only include edible, forage, timber and fiber producing plants but also herbal and medicinal plants. These medicinal plants, as the name suggests have medicinal and culinary value also have aroma and chemical importance in chemical and perfume industries. Rose-scented geranium crop ensures these characteristics hence the plants species were domesticated and cultivated for their essential oil. Essential oil falls under the secondary metabolite category and are stored in special structures (granular trichomes) located in one or more parts of plants (Taiz & Zeiger, 2002). Pure geranium oil is almost a perfume in and of itself, and it mixes beautifully with any other scent. It's commonly utilised in soap scenting and for isolating rhodinal, which is found in most high-end perfumes. In addition to an indigenous output of just approximately 20 t of oil per year, India imports more than 20 t of this oil from other nations to suit the local demands of the Indian fragrance industries.

The *Pelargonium* genus, which includes the rose-scented geranium (*Pelargonium graveolens*), is one of five genera in the Geraniaceae family (Miller, 2002; Rao, 2009). The chromosome number of *Pelargonium* is  $x=11$  and the somatic number for *P. graveolens* is  $2n=88$ . The Reunion cultivar is heptaploid ( $2n=77$ ) suffering to some degree of male sterility (Weiss, 1997). Geranium is a bushy, fragrant flowering plant. The stem is cylindrical, woody at the base, pubescent, green when young, and brown as it grows older. The leaves are alternating, stipulate, simple, and thickly pubescent, with 5 primary and secondary lobes. The leaves have a strong scent to them. The inflorescence is hairy and umbellate. The flower is bisexual, hypogenous, and has a pink corolla with reddish-purple patterns on the two posterior petals. There are ten stamens, each with subequal filaments that are joined at the base; the anthers are seven, ditheous, and easily shed. The ovary is hairy, superior, pentacarpellary, and syncarpous, while

the style is hairy, with five stigma distally.

The entire rose-scented geranium plant is fragrant, but the leaves, stalks, and flowers are the most cost-effective components to extract essential oils from. Hydro-and/or steam distillation are commonly used to extract essential oil from leaves, stalks, and flowers. The oil is a brownish green mobile liquid that ranges from yellow to green (Charles, 2013).

The major compounds of geranium essential oil are geraniol, citronellol, geranyl formate, citronellyl formate and 10-epi- $\gamma$ -eudosemol etc, which are dominating the quality of geranium oil (Mazeed et al., 2020). These compounds have been used in different medicine, religious ceremony and later in perfumery. The use of plants in perfumery is believed to have started in ancient China, India and Egypt. They were majorly used as fragrance by upper classes in many civilizations.

*Pelargonium graveolens* (rose-scented geranium) belongs to one of the five genera that are classified in the Geraniaceae family (Miller, 2002; Rao, 2009). Approximately 80% of the 270 distinct and so far discovered *Pelargonium* species are found in the Western Cape Provinces of South Africa (Lis-Balchin, 2002; Miller, 2002; Saraswathi et al., 2011). Taxonomically revised *Pelargonium* contains a total of 24 species and among these only *P. asperum*, *P. graveolens*, *P. radens*, *P. capitatum*, *P. roseus*, *P. tomentosum*, *P. zonale* and *P. roseum* are used in cultivation for geranium oil production (Verma et al., 2006; Lallic et al., 2008; Saraswathi et al., 2011).

Although South Africa is producing significant quantities of geranium oil, the commercial variety of rose scented geranium has undergone a lot of breeding such that it is no longer the same geranium which is originated in South Africa. Several researches have shown that seedling often take long to establish which results in high death rate and sometimes poor growth. Stunted growth and yellowing of leaves were also seen in some cases poor growth causes low herbage yield and consequently low total essential oil production per hectare. Poor growth is believed to be due to different factors including acid soil condition nutrient deficiencies. The oil yield and content

of aromatic plants are affected by a great many factors which are difficult to segregate since many of the factors are interdependent. Includes geographical conditions and genetic variation (Hussain,2009), harvesting time (Blank et al., Kumar et al,2013), plant spacing (Yasir et al.,2003); Khazaie et al.,2007), and post-harvest drying and storage (Hussain,2009). Reports also show the yield and quality of geranium were affected by harvesting frequency and plant shoot age (Motsa,2006), population density and seasonal changes (Demarne,2002), plant part distilled (Mallavarapu et al.,1997) temperature (Motsa et al 2006; Kumar et al.,2013), light and humidity, length of exposure of sunlight, availability of water altitude, and the presence of fungal diseases and insects (Ramakrishna and Ravishankar, 2011). The oil content and yield may also change as a result of the harvesting method used, the moisture content of the plants at the time of the harvest, and the prevailing steam distillation conditions (Hussain,2009). Rose-scented geranium grows well in a temperate, subtropical, and tropical climate with a long growing season without extreme weather conditions. A mild climate with low humidity is ideal for its growth (Joy et al., 2001; Kritika et al., 2012). The crop is grown in the northern Indian plains from October to June, though it may be grown in a variety of soils and climates. The crop planted during this season is cost-effective and produces a high yield per unit of time and area, as well as high-quality oil. It also works effectively in a cropping system without interfering with the production of a staple field crop (Nilofer et al., 2018).

The plant is evergreen when cultivated but dies back in nature during drought and winter season. The crop is grown as rain-fed in hilly areas and under irrigation in an altitude range of 1000-2100 m.a.s.l. (Joy *et al.*, 2001). The climatic conditions having warm winter and mild summer with well-distributed annual rain fall are ideal for growth; although the plant can survive even short night chills below 0° C without permanent physiological damage. The temperature in the range of 10-30° C during the growing season is indicated to give maximum leaf growth and high essential oil content (Weiss, 1997; DAFF, 2009). In medicinal and aromatic crops, it is

well indicated that the essential oil content and composition is related to the age of the; leaves, thus emphasizing the importance of the growth stage at which harvesting takes place (Motsa,2006). the right time age and frequency of harvesting rose-scented geranium plants has always been a controversy. It is however not clear at what age herbage should be harvested or the stage at which can produce great quality essential oil.

Members of the genus *Pelargonium* include annual and perennials of various aromatic and morphological features such as bulbs and tuber roots which could have contributed to the survival of the plants in harsh environmental conditions (Miller,2002; Lewu. et al.,2007). In addition, some pelargonium species are characterized by succulent stems that possibly enable them to undergo crassulacean acid metabolism (CAM) in water-stressed conditions (John et al.,2003), thereby improving their water use efficiency (Lumbers et al.,1998). Oils from the Moroccan, Algerian and Egyptian types ranked next to the bourbon type and presumably earn a premium and are considered qualitatively over the oil from the Chinese type which has a highly variable odor and is the cheapest in price (Weiss, 1997). Apart from the commercially renowned rose-scented geranium chemotypes, several essential oil-rich members of the genus *Pelargonium* and their hybrids have been reported. Essential oil of *Pelargonium graveolens* cv. Kunti (grown in India) is rich in geraniol (40-50%) whereas essential oil of soma clonal mutant of the same cultivar was found to contain iso menthone (71%) as its major constituent (Gupta et al., 2001). Rose-scented geranium oil is among the top 20 available plant volatile oils (Williams and Harborne, 2002). The hydro- and/or steam-distillation of the leaves generates pale yellow colored oil (yield of 0.19%, v/w), which possesses a tenacious rose-like odour such as citrus and minty undertones (Motsa, 2006). Rose scented geranium is grown in India on red soils that are often low in phosphorus and other nutrients, and efforts are being undertaken to boost essential oil output.

The chemical composition of the oil is complex in nature and comprises a wide array of compounds. The composition of these chemical compounds



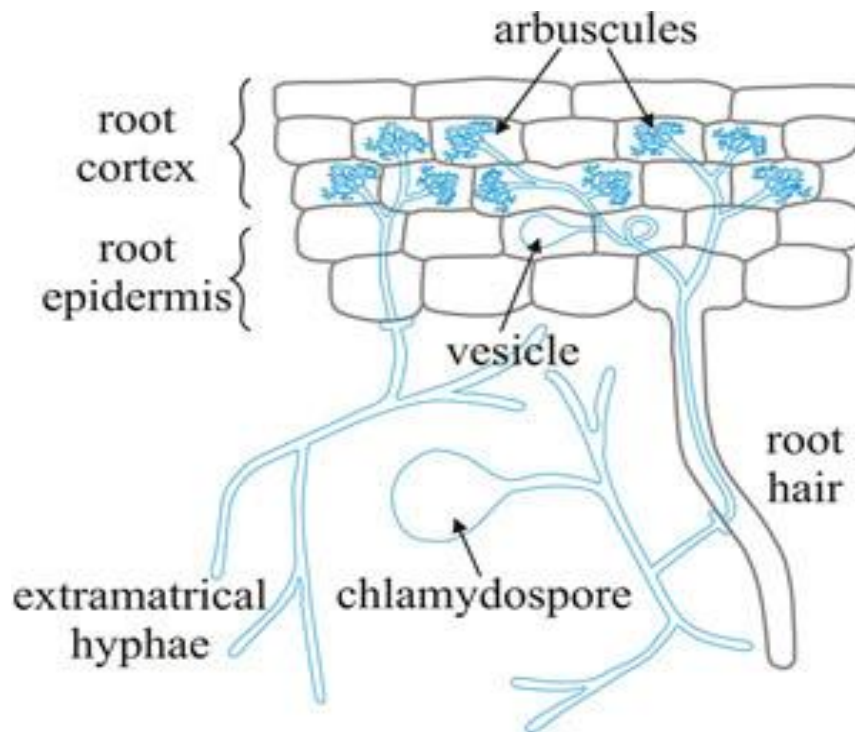
varies among geraniums that originated from different countries (Rana et al., 2002). The authors identified Geraniol ratio ranging from 1 to 3 is associated with a better odour quality of the oil (Saxena et al., 2000). Rose-scented geranium essential oil is used worldwide in the perfumery and cosmetic industries. It is considered among the best skin care oils because of its property of opening skin pores and cleaning oily complexions (Miller, 2002; Peterson et al., 2005). It is also used for treating dysentery, hemorrhoids, inflammation, heavy menstrual flows, and cancer. The French medicinal community is currently treating diabetes, gallbladder problems, gastric ulcers, liver problems, sterility, and urinary stones with this oil (Peterson et al., 2005). The leaves are used in form of herbal tea to de-stress, fight anxiety, improve circulation, and cure tonsillitis (Peterson et al., 2005). The leaves are used as a form of herbal tea to de-stress, fight anxiety, improve circulation, and cure tonsillitis (Peterson et al., 2005).



**Close-up view of rose scented Geranium flower**

### **3.2 VAM (Vesicular Arbuscular Mycorrhiza) Inoculation**

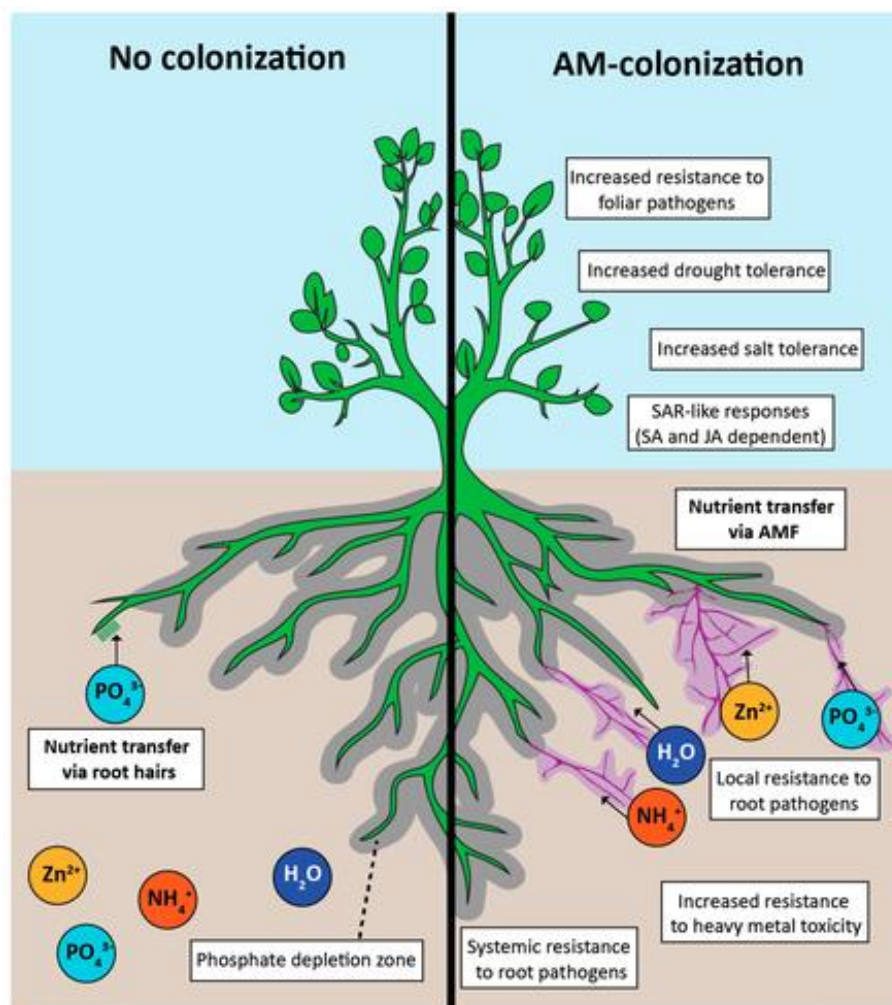
Vesicular Arbuscular Mycorrhiza is a symbiotic association between fungus and the root, in which fungus supplies the root nutrients and the root provides carbohydrates to the fungus. (Harley & Smith, 1983). Because of their wide relationship with plants and international distribution, arbuscular mycorrhizal (AM fungal) interactions are the most common symbioses discovered in nature (Harley and Smith, 1983; Verma, 2010). Arbuscular mycorrhizal (AM) fungi are known to speed up activities that can boost agricultural development, and as a result, these microorganisms have become a popular research topic for long-term sustainability (Johansson et al., 2004). Mycorrhiza is a mutualistic relationship between soil-borne fungus and the roots of higher plants (Sieverding, 1991). VAM enhances the uptake of immobile inorganic nutrients and increase resistance to diseases and other stress conditions. these fungi are not yet been cultured on synthetic media but can multiply in association with root of host plants. Mycorrhiza is a mutualistic relationship between soil-borne fungus and the roots of higher plants (Sieverding, 1991). The underground section of the plant, known as mycellium, is in contact with the plant's roots in this association, providing no harm to the plant. The name 'mycorrhiza' comes from a Greek word that means 'fungus root' (Friberg, 2001).



**Figure showing main cellular features of arbuscular mycorrhiza**

A German plant pathologist named A.B Frank (1855) created the word to characterise the symbiotic relationship between plant roots and fungi, and it is likely the world's oldest and most widespread plant symbiosis. The link between mycorrhizal fungus and plants improves nutrient uptake, creation of growth-promoting substances, drought tolerance, salinity tolerance, and also regulates plant defence mechanisms (Hause et al.,2007). Mycorrhizal fungi serve as a link between plants and the soil (Bethlenfalvay, 1992), and plants that lack these connections struggle to obtain the soil resources they require to thrive (Perry et al., 1987). AMFs (arbuscular mycorrhizal fungi) are symbiotic soil fungi that invade the roots of roughly 80% of vascular plants. Numerous plant species benefit from the mycorrhizal symbiosis, which promotes their growth and survival (Smith and Read, 1997). The formation of the highly complex mycorrhizal relationship necessitates a constant exchange of signals between the host roots and AMF, which has an impact on the host's entire metabolism (Smith and Read, 1997). According to studies, the usage of VAM in the agriculture ecosystem

increased crop yield. It was found that when maize seedlings were injected with VAM before sowing, biomass increased (Vamerali et al. 2003). The beneficial effects of AM fungi have been attributed to the direct effect of better plant nutrition through the acquisition of less mobile nutrients such as phosphorus (P), zinc (Zn), copper (Cu), and occasionally ammonium ( $\text{NH}_4^+$ ) from the soil by the hyphae of AM fungi and subsequent transfer to the host plant, as well as the indirect effect of altered plant morphology and/or physiology (George et al. 1994, Kothari et al. 1990).



\*Image showing difference between AM fungi colonized plant and non mycorrhizal plant.

VAM promote growth by acting as biofertilizers. Biofertilizers have shown

great potential as a renewable and environment-friendly source of plant nutrients. Biofertilizers are used as live formulation of beneficial microorganism which is applied to the seeds, root, or soil, mobilizes the availability of nutrients, particularly by their biological activity, and help to build up the lost microflora and in turn, improve soil health in general (Ismail et al., 2014). Their mode of action can vary and can be used alone or in combination. For easy application, biofertilizers are packed in suitable carriers such as peat or lignite. Carrier can play an important role in maintaining sufficient self-life (Singh et al. 1999).

Shape	(i.e., globular, spherical, irregular etc.)
Size	Globular: diameter (minimum-average-maximum) Irregular shape: length x width (minimum-average-maximum)
Color	(Compare with standard color chart)
Hyphal attachment	(i.e., sporiferrous saccule, bulbous, suspensor etc) Sporiferrous saccule= Acaulospora Bulbous suspensor=Gigaspora
Surface ornamentation	(i.e., smooth, rough, reticulate etc)
Vesicle	(Presence or absence in mycorrhizal roots)

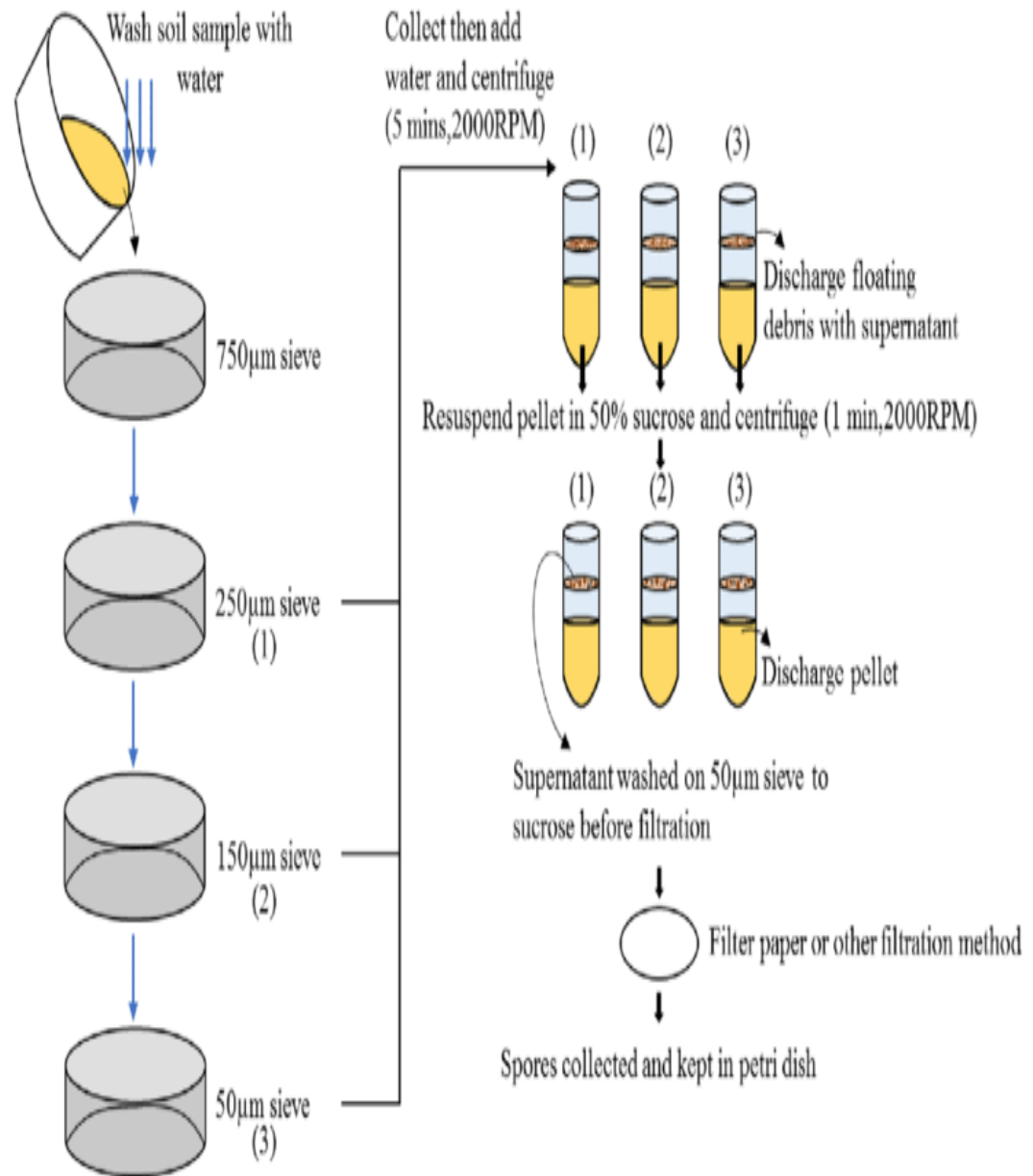
#### Culturing of VAM

AM fungus require a symbiotic relationship with plants in order to reproduce. Culturing AM fungus entails inoculating AM fungi into a host plant and

growing the infected plant. Spores gathered from soil can be used for the AM fungal inoculum. Soil spores, on the other hand, aren't always active in colonising plants.

As a result, trapping culture is frequently used. As an inoculum, dirt or soil sieving is utilised (Soil Trap Culture). Mycorrhizal plants taken from the field can be transplanted to potting medium as Plant Trap Culture to isolate AM fungus populating roots. (Murakoshi et al. 1998) Sterile soil or a soil-sand mixture is commonly used as a potting media. Various horticultural potting products can also be employed. However, potting medium ingredients should be low in accessible phosphate and, ideally, low in organic matter. Fungi isolated from specific soils may require specific soil qualities for growth in various circumstances. Leguminous species (i.e., *Trifolium* spp., *Medicago* spp., *Lotus japonicus*), grass species (i.e., *Lolium* spp., *Paspalum notatum*), and other herbaceous species (i.e., *Plantago* spp.) can all be utilised as hosts. *Allium* spp. (onion and leek) are also good hosts. Although AM fungi do not display host specificity in general, certain species do. As a result, the plant from which the target AM fungus was isolated can be employed as a host plant.

Single spore isolation: Single spore isolation is required to purify an isolated fungus. Even though the spores are morphologically identical, contaminants with comparable morphologies are frequently present. A series of pot cultures of such multispore isolates might result in an unforeseen contamination breakout. Additionally, even if the culture only contains one species, it may be made up of genetically varied groups. Purification via single spore isolation is required for such genetic research or population genetics. (Murakoshi et al. 1998).



**Diagrammatic representation of spore isolation.**

Some biofertilizers solubilizes phosphate from either organic or inorganic bound phosphates, thereby facilitating plant growth (Vassilev et al., 2006).

Soil microorganisms such as arbuscular mycorrhizal fungi (AMF or AM fungi) represent a key link between plant and soil mineral nutrients. Thus, they are collecting growing interest in natural biofertilizers. They provide the host with mineral nutrients and water in exchange for photosynthesis product ((Smith and Read, 2008). AMF can alleviate the limitation in plant

growth caused by inadequate nutrient supply (Nouri et al., 2014). Phosphate-solubilizing bacteria are microorganisms capable of converting soil insoluble phosphorous (P) into a soluble form making it available for plants (Tilak et al. 2005). Microorganisms stimulate plant growth with a variety of mechanisms, including the production of plant hormones, increasing plant P uptake, nitrogen fixation, production of antibiotics, secretion of enzymes that regulate the amount of ethylene in plants and control of pathogenic fungi (Pal et al. 2001). The fertility of the soil is also restored by biofertilizers so the plant gets well protected from getting any disease (Amna 2010). Reports have shown that the use of bio-organic fertilizers is associated with improvement of soil structure and organic and inorganic materials resulting in improved quality (Taha et al.2011). Biofertilizers have also emerged as an important component of integrated nutrient supply systems and have shown promise to improve crop yields and nutrient supply system and have shown promise to improve crop yields and nutrient supplies.

### **3.4 Influence of organic mulch**

Geranium is cultivated as a rainfed perennial crop in a few places in India, for its high value essential oil which is extensively used in perfumery and cosmetic industries. India produces the meager quantity of 5t per year on the contrary 145 t is consumed yearly .therefore most of the 145 t requirement is largely accomplished through imports. Under semi-arid subtropical circumstances, organic mulch has a critical role in soil moisture conservation, temperature management, microorganism population, soil structures, and nutrient dynamics, all of which benefit crop yield. (Muni Ram et al.,2002).

Mulch is most likely derived from the German word "molsch," which meaning "soft to decay," and reportedly refers to gardeners spreading straw and leaves over the ground as mulch (Jacks *et al.*,1955) Mulching is a horticultural and agricultural practise that involves the use of organic materials. This approach is highly useful for insulating plant roots from heat



and cold. Mulch is applied to the soil surface surrounding plants to produce a favourable environment for growth. This could involve temperature management, salinity reduction, and weed control. It has a significant impact on the crop's ripeness, yield, and quality. Mulching can also be used on most field crops. It is preferred in fruit orchards, flower and vegetable production, nurseries, and forests where crops do not require frequent cultivation (Bharadwaj.,2013). Mulches are used in agriculture for a variety of reasons, but the most essential goals are water saving and erosion control, especially in dry and semi-arid areas. Mulching is also used for a variety of reasons, including modifying soil temperature, weed control, soil conservation, and adding plant nutrients when organic mulch decomposes, improving soil structure, and increasing crop quality and yield. Mulching helps to avoid soil deterioration by preventing runoff and soil loss, as well as weed infestation and water evaporation. Mulch can help to prevent water loss, soil erosion, weed problems, and nutrient loss. (Van Derwerken and Wilcox, 1988). It improves the physical, chemical, and biological properties of soil, as well as the growth and yield of crops, by facilitating more soil moisture retention and helping to control temperature fluctuations. It also adds nutrients to the soil, which helps to improve the physical, chemical, and biological properties of soil. (Dilip Kumar *et al.*, 1990).

Plant and animal resources such as straw, hay, peanut hulls, leaf mould, compost, sawdust, wood chips, shavings, and animal manures are used to make organic mulches. Organic mulch reduces nitrate leaching, improves soil physical properties, prevents erosion, provides organic matter, regulates temperature and water retention, improves nitrogen balance, participates in the nutrient cycle, and boosts biological activity (Hooks and Johnson, 2003; Muhammad et al., 2009; Sarolia and Bhardwaj, 2012). Natural materials are difficult to distribute on growing crops and demand a lot of human effort (Bhardwaj, 2011).

### **The impact of mulching on the soil**

Conserve soil moisture: By modifying beneficial microclimatic conditions in the soil, soil moisture can be conserved through mulching. When organic

mulch is applied to the soil surface, it helps to minimise weed growth, reduce evaporation, and promote rainwater infiltration throughout the growing season. On the ground, crop leftovers or mulch (Rathore *et al.*, 1998). Mulch minimises evaporation and reduces the need for watering (Anonymous, 2003). Mulching improves the biological condition of the soil and prevents decreases in soil water levels, according to Chawla (2006), Khurshid *et al.* (2006), and Muhammad *et al.* (2009). Mulching cools the soil in the summer and warms it in the winter, preventing temperature extremes. The capacity of the mulching material to reflect and transmit solar radiation influences the effect of mulching on the temperature regime of the soil

Fertilizer loss due to leaching is decreased as surplus rainfall is shed drained the root zone. In sandy soils, this is especially true. This permits the grower to apply additional fertiliser to the row before planting the crop.

Chilli leaf N, P, and K content increased after coconut fronds were mulched (Hassan *et al.*, 1994). Vos and Sumarni (1997) found that plants grew quicker, ripened earlier, and had lower P and higher N concentrations in their leaves and fruits. Furthermore, they found that rice straw mulch raised K- content and lowered P- concentration in bell pepper leaves when compared to no-mulch leaves. Mulch protects the soil surface from harmful elements, minimises nutrient leaching, and enhances vegetable growing conditions (Baumann *et al.*, 2000; Kolota and Adamczewska Sowiska, 2004). Mulched treatments exhibit considerably increased overall uptake of nitrogen, phosphate, and potassium than similar unmulched treatments, according to Acharya and Sharma (1994) and Muhammad *et al.* (2009). Mulching creates a favourable environment for growth, resulting in plants that are more vigorous, healthier, and possibly pest-resistant. Root growth is stimulated by increased soil temperature and moisture content, which leads to increased plant growth.

Mulched plants, on the other hand, tend to grow and mature more uniformly than unmulched plants (Bhardwaj *et al.*, 2011; Sarolia and Bhardwaj 2012). Vegetables for the summer. Cucumbers, muskmelons, and watermelons are examples of this type of fruit. Mulching has a positive effect on eggplant

and peppers. Early maturity and higher yields are two advantages of this variety. During the growing season, the weather is favourable. Organic Mulches accelerated flowering and decreased the number of days it took for flowers to bloom. Organic mulches induced earliness in flowering, less days to fruit set and harvest in tomato crop over control (Ravinderkumar and Shrivastava, 1998).

Mulching lowers the germination and nutrition of many weeds by creating a physical barrier. Mulching aids in the reduction of weed seed germination, weed growth, and weed control (Vander Zaag et al., 1986). Weed seed germination can be prevented or physically suppressed by covering or mulching the soil surface. Weed management can be achieved with loose materials such as straw, bark, and composted municipal green waste (Merwin et al., 1995).

Mulching encourages soil microorganisms such as algae, mosses, fungi, bacteria, actinomycetes, and other creatures such as earth worms, resulting in a more rapid breakdown of organic materials in the soil and release of plant nutrients for crop growth. Earth worms thrive beneath the mulch layer, helping to improve soil aggregate stability, infiltration, and other factors. The impact of diverse organic mulches' microbial loads on bacterial populations could be attributed to differences in chemical composition and decomposition rates (Mukherjee et al., 1991; Wu et al., 1993). Over time, the soil biota grows in a mulched soil environment, enhancing nutrient cycling and organic matter accumulation (Holland, 2004). Organic mulching technology encourages a wide range of beneficial soil macroinvertebrates. Crop residue mulch provided a lot of food for soil macro invertebrates as well as nutrients to promote vegetation growth and established a good environment for them (Sugiyarto et al., 2009).

Mulch can promote plant growth and development, yield, soil evaporation and nutrient leaching, pest and weed incidence, and fruit cleanliness and quality yield (Lamont, 1993; Farias-Larios and Orozco-Santos, 1997; Walters, 2003; Decoteau, 2007; Diaz-Perez et al., 2007; Hutton and Handley, 2007). Finally, boost gross, net, and benefit returns.



**Paddy straw mulch application on field**

## **4. Materials and Methods**

**4.1-Site description and experimental design** – field experiments were carried out at the research farm, CSIR- CIMAP, Lucknow, and the microbiological and soil health parameters were investigated in the Division of Agronomy and Soil science CIMAP, Lucknow. These experiments were laid out in a randomized complete block design (RCBD) with 10 treatments replicated thrice on which 25 days old seedlings were planted. AM used for inoculation was acquired from IARI PUSA New Delhi.



**Image showing experimental site CSIR-CIMAP farm**

**4.2 Treatment combination** – four treatment combinations of two variables organic mulch paddy straw mulch t ha<sup>-1</sup> (M<sub>1</sub>), no mulch (M<sub>0</sub>), two variables of VAM [VAM inoculation (V<sub>1</sub>) and no VAM applied (V<sub>0</sub>)].

	V <sub>0</sub>	V <sub>1</sub>
M <sub>0</sub>	M <sub>0</sub> V <sub>0</sub>	M <sub>0</sub> V <sub>1</sub>
M <sub>1</sub>	M <sub>1</sub> V <sub>0</sub>	M <sub>1</sub> V <sub>1</sub>

The combinations of treatments are given below;

**T1 – M0 V0**

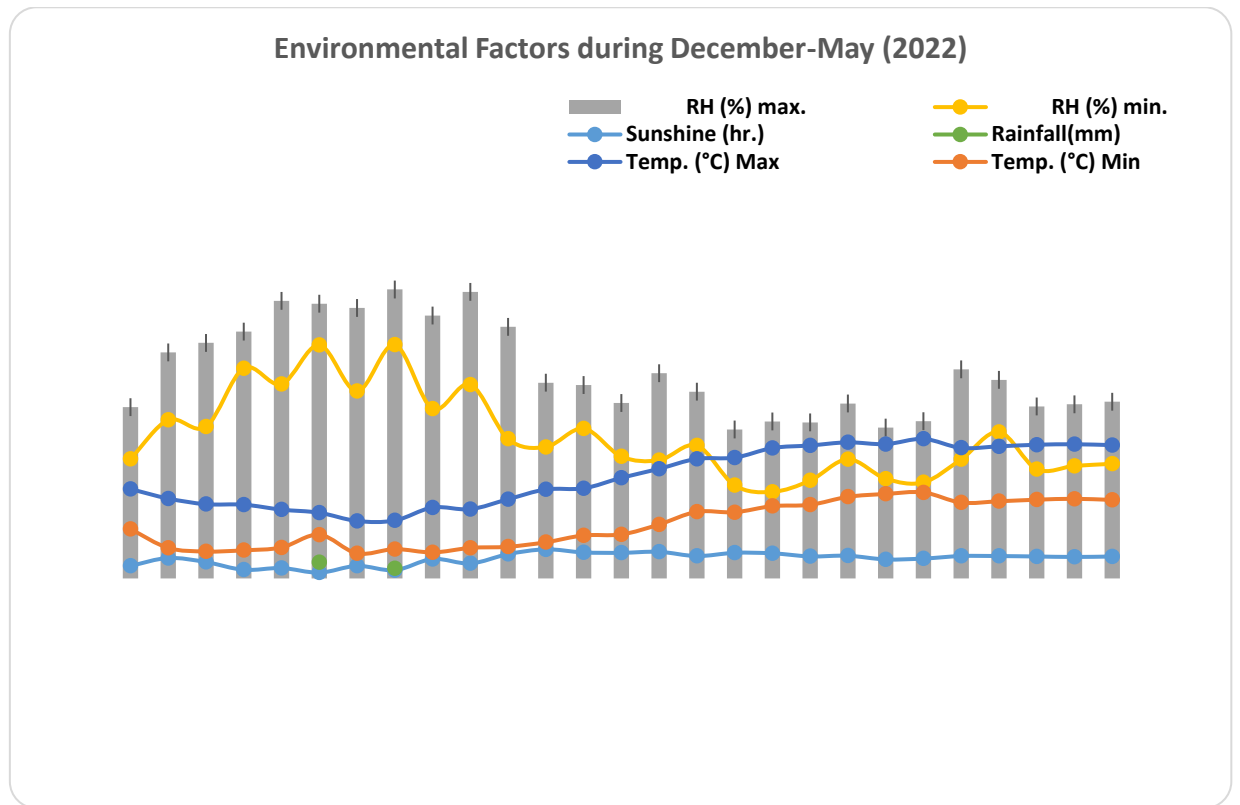
**T2 - M1 V0**

**T3 – M0 V1**

**T4 – M1 V1**

### **4.3 Weather Conditions**

The mean minimum and maximum temperature ranges from 7 °C to 42 °C respectively during the crop period and the relative humidity ranges from 26 to 87. All the necessary weather data is represented in the graph below:



**Graph 1. Showing weather condition during the crop period.**

**4.4 Biofertilizer and mulch material:** Biofertilizer used in our study was purchased from the Indian Council of Agricultural Research (ICAR)-Indian Agricultural Research Institute (IARI) PUSA New Delhi. Paddy straw mulch used as an organic mulch in the experiment was collected from Rajauli

district Barabanki which was transported by a vehicle provided by CIMAP.

## **4.5 Agronomical practices**

### **4.5.1. Nursery Raising**

Fresh cuttings of geranium cv-cim-bio 171 were collected by 90 days old plants of some variety from the gene bank of CSIR-CIMAP, Lucknow. Fresh cuttings were collected and planted in raised bed nursery for root setup. It took approximately 25 days for the plant cuttings to complete rooting.

### **4.5.2 Field preparation**

Initially 1 ploughing with disc plough was done and another two ploughing were done with the traditional cultivator and then planking with a wood planker after that a flood irrigation was done for maintaining moisture till planting. Just before planting a ploughing along with planking was done after that field layout was prepared and ready for planting.

### **4.5.3 Fertilizer Application**

In the experiment the recommended doses of fertilizer for geranium crop (150 kg N, 60 kg P<sub>2</sub>O<sub>5</sub> and 40kg K<sub>2</sub>O ha<sup>-1</sup>), were applied. N was applied through urea, P was applied through Di Ammonium Phosphate (DAP) and K was applied by the application of muriate of potash (MOP). All the nutrient resources or fertilizers were collected from the store of experimental farm of CSIR-CIMAP.

### **4.5.4 Planting**

30 days old rooted cuttings were collected from the nursery area at the day of planting. VAM inoculation as well as root dipping was done before planting as per the treatment requirement. Planting was done on a spacing of 50 cm X 50 cm with row to row and plant to plant spacing considered as suggested by Aus Ganya, (2022) a booklet of MAP's cultivation published by CSIR-CIMAP, Lucknow.



### **Planting of geranium cuttings**

#### **4.5.5 Gap filling**

The survival of the rooted cuttings of geranium variety CV-CIM-BIO 171 was excellent hence there was no need for gap filling.

#### **4.5.6 Weed control**

A common homogenous hand hoeing was performed 30 days after planting and before mulch application in the field. After mulch 3 hand weeding was done at the interval of 45,60,75 days except for mulch plots.

#### **4.5.7 Irrigation**

Initial irrigation was given just after planting. Total 8 irrigations were implemented at the interval of 10-15 days except for mulched plots, wherein mulched plots were irrigated 5 times at the interval of 15-20 days. Interval during irrigations generally depends on climatic conditions. The schedule for irrigation was adapted from Aus Ganya,2022 (CSIR-CIMAP).





**First irrigation after planting**

#### **4.5.8 Plant protection measures**

Chlorpyrifos 50% EC at the rate of 1000m/h<sup>-1</sup> was applied at the time of third irrigation or after mulching. There was no need for any other insecticide or pesticide in the geranium crop because no insect/pest attacked the geranium crop during whole crop period.

#### **4.5.9 Harvesting and Essential Oil Extraction**

Harvesting was done manually by cutting the above ground biomass at the base of the plant in the third week of May. From each plot fresh herb yield was recorded. For essential oil extraction 500 g of fresh plant samples were obtained from each plot prior to harvest for essential oil extraction through hydro-distillation process using a Clevenger apparatus (Clevenger, 1928). The isolated essential oil was dried over anhydrous sodium sulphate and packed tightly, stored in the dark, and kept in the freeze for determination of essential oil constituents (if required). The oil yield was calculated by multiplying the fresh herb yield with oil content.

#### **4.5.10 Quality analysis of essential oil**

The quality analysis of the essential oil was performed on Centurion

Scientific Gas Chromatograph (model CS-5800), equipped with a DBWax (30 m ×0.25 mm internal diameter, film thickness 0.50 µm) fused silica capillary column and flame ionization detector. The oven temperature was programmed from 70°C to 170°C with a ramp of 4°C/ min then programmed to 240°C with a ramp of 5°C/min with initial and final hold times of 5 and 15 min, respectively. Nitrogen was used as a carrier gas at 1.0 ml/min. The injector and detector temperatures were 240 °C and 250 °C, respectively. The sample of 0.2 µL (diluted in hexane, 1:1) was injected in the split mode (1:70). The constituents of the essential oil were identified based on retention index, determined using a homologous series of n-alkanes (C7-C30 hydrocarbons, Supelco Bellefonte, PA USA), and by comparing with the literature data. The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

#### **4.6 Estimation of soil microbial activities**

By this experiment we estimated soil enzymatic activities like dehydrogenase, acidic and alkaline phosphatase activities, and estimation of soil microbial biomass carbon was also performed, which are explained below;

##### **4.6.1 Soil enzyme activity –**

**1. Soil Dehydrogenase Activity-** DHA is one of the most requisite and most sensitive bioindicators, relating to soil fertility and quality. Dehydrogenase is a key enzyme in the biological oxidation of organic materials in soil.

**Principle-**(Casida et.al 1964) Dehydrogenase plays an crucial role in the biological oxidation of soil organic matter by transferring hydrogen from organic substances to inorganic acceptors. Under anaerobic condition 2,3,5 Triphenyl tetrazolium chloride (TTC) act as an electron acceptor and converts to the pink color compound Triphenyl formazan (TPF). Dehydrogenase can be analyzed by the rate of formation of TPF from TTC.

**Reagents-** 3% TTC (2,3,4 Triphenyl tetrazolium chloride), 1% glucose , Methanol, standard TPF( Triphenyl formazan).

**Procedure**

- 1-Take 1 gramme of fresh soil and place it in a 15 mL air-tight screw-capped test tube.
- 2-Add 0.2ml of 3% TTC solution to each tube.
- 3-To each tube, add 0.5ml of 1 percent glucose solution and shake horizontally.
- 4-Incubate all the tubes at  $28 \pm 0.5$  °C for 24 hours.
- 5-After incubation, pour 10 mL of methanol into each tube and shake horizontally for 6 hours.
- 6-After 6 hours, filter it and measure the absorbance at 485nm wavelength.
- 7- Extrapolate TPF forms the standard curve drawn.

**2. Acid and Alkaline phosphatase test** – Phosphate belongs to the hydrolase category of enzymes and catalyses the hydrolysis of the substrate. Acid phosphatase and alkaline phosphatase are the two forms of phosphatase based on pH.

**Principle-**(Tabatabai and Bremmer, 1969; Eivazi and Tabatabai, 1977). P-nitrophenyl phosphate works as a substrate for both acid and alkaline phosphatase in the acid and alkaline phosphates test, yielding p – Nitrophenol after hydrolysis by either enzyme.

**Reagents-** Toluene, Modified universal buffer (MUB), 0.025M p-Nitro phenyl phosphate, 0.5M Calcium chloride solution, 0.5M Sodium hydroxide solution, Standard p-Nitrophenol solution.

**Procedure-**

1. Take two sets of 1gm fresh soil in vials for each soil, one of which will be used as a control.
2. Add 0.2ml (200µl ) toluene.
3. Add 4ml of MUB (pH 6.5 or 11) to all the vials
4. In only one set of samples, add 1 mL of p-nitrophenyl phosphate solution.
5. Swirl all the vials together and place in incubator for 1 hour.

6. Add 1ml of 0.5 M CaCl<sub>2</sub>
7. Add 4ml of 0.5 NaOH
8. Swirl all the vials for a few seconds
9. Add 1ml of p- nitrophenyl phosphate to the control set sample.
10. Using Whatman filter paper No 2 all the suspension are filtered quickly.
11. Yellow color intensity is measured @ 440 nm.

**4.6.2 Soil microbial biomass carbon (SMBC)-** Soil microbial biomass carbon is the living component of soil organic matter.

**Principle-**A direct measurement of carbon and other nutrients contained in microbial biomass is carried out in the fumigation method. To destroy the organisms in the soil samples, chloroform is fumigated overnight.

**Reagents-** Distilled chloroform, Conc sulphuric acid, 0.5M potassium sulfate, 0.2N potassium dichromate, Orthophosphoric acid, 0.005 N ferrous ammonium sulfate (FAS), ferroin indicator

**Procedure-**

1. Take fresh soil and pass it through a 2mm sieve; do not dry the dirt before analysing it.
2. For each sample, take three 10gm sets of soil. One set, should be kept in the oven for 24 hours at 100°C.
3. Calculate the moisture content of the soil by weighing the dry soil.
4. Keep one set in a 50 ml beaker for fumigation, keep another set-in refrigerator.
5. Place 20ml of distilled chloroform in a 100ml beaker. Place some glass beads in it.
6. Fill the vacuum desiccator with all of the soil and the chloroform-filled beaker.
7. Turn on the vacuum pump for 5 minutes until the chloroform boils, then place the desiccator in the dark for 24 hours.
8. After 24 hours release the vacuum and take out the beaker containing distilled chloroform.
9. Transfer both fumigated and unfumigated soil in a 150ml conical flask.

10. Add 25ml of 0.5M potassium sulfate
11. Shake for half an hour.
12. Filter with Whatman filter paper no -1.
13. Transfer 10ml filtrate in a 250ml conical flask.
14. Add 2ml 0.2N potassium dichromate.
15. Add 10ml of concentrated sulphuric acid and 5ml of orthophosphoric acid.
16. Keep it for half an hour.
17. Add 200ml of distilled water and transfer it to 500ml of the conical flask.
18. Titrate against 0.005 ferrous ammonium sulfate until the green color develops.

#### **4.7 Soil Analysis**

**4.7.1 Electrical conductivity or EC** – The number of soluble salts in a sample is determined by electrical conductivity meter.

**Principle-** the electrical current pass-through salt ion in the same way it can through metal.as a result, as the number of soluble salts in the soil increases, so does the electrical conductivity of soil water content.

**4.7.2 pH-** pH is actually a estimate of hydrogen ion activity. It is defined as the negative logarithm of hydrogen ions.

**Principle-** pH indicates whether the soil is acidic, neutral, or alkaline in reaction. Because crop growth suffers greatly in both extremely low pH strongly acidic and very high pH alkaline situations, proper reclamation is required.

**Procedure-**(For EC and pH)

- 1- Weigh 10 gram dried and sieve soil.
- 2- Add 10 gram of soil in beaker and pour 25 ml of distilled water in it.
- 3- With the help of glass rod stir soil in distilled water for 5 minutes.
- 4- Let it stay for about half an hour.
- 5- Obtain readings by through the pH meter and conductivity meter

**4.7.3 Mineralizable nitrogen (N)-** The amount of mineralized nitrogen which available to the plant by the microbial activity for the consumption.

**Principle-** The fundamental principle is that alkaline potassium permanganate method, when a known weight of soil sample is digested in the presence of sulphuric acid and digested under pressure, the intricate structure is broken down to a simple structure, releasing nitrogen in a simple form of ammonia.

**Reagents –** 0.32%  $KMnO_4$ , 0.1N NaOH, 0.1N Sulphuric acid, 0.1 M oxalic acid, 2.5% NaOH, Mixed indicator.

**Procedure-**

- 1-Weigh 10 gm of soil (0.2mm) in 800 ml Kjeldahl flask.
- 2-Add 100ml  $KMnO_4$  and 100ml of 2.5%NaOH
- 3-Add 10 ml of sulphuric acid and 3-4 drops of mix indicator.
- 4-Titrate by 0.1N NaOH fill the burette with 0.1N NaOH
- 5-Titrate till green color develop and read the titrated value.

**4.7.4 Available potassium (K)-**

Principle- The principle underlying is that when a large number of elements when excited in a flame, emit radiation of a characteristic wavelength. The excitation causes one of the outer electrons of a neutral atom to move to the outer orbit of a higher energy level. When the excited atom returns to a lower energy level, light at a characteristic wavelength is emitted thus potassium gives 404 and 767m $\mu$ .

**Reagent –** 1N Ammonium acetate

Stock standard solution: Sodium- NaCl (ppm), potassium-KCl (ppm)

**Procedure-**

- 1-Take 5gm of soil in 100ml of the conical flask
- 2-Add 25ml ammonium acetate and shake for 5min
- 3-Filter through Whatman No1 filter paper and read the reading by flame photometer.

**4.7.5 Available phosphorus (P)-** Phosphorus (P) is one of the macronutrients required for plant growth it regulates protein synthesis. Therefore, phosphorus is important for cell division

**Principle-**Phosphorus is extracted from the soil with 0.5M  $NaHCO_3$  at a nearly constant pH of 8.5. The phosphate ion in the solution treated with

ascorbic acid in an acidic medium provides a blue color complex.

**Reagents-** Olsen's reagent(0.5N $\text{NaHCO}_3$ ), solution A (Ammonium molybdate), solution B ( Antimony potassium tartate ), 5N Sulphuric acid, Ascorbic acid solution.

**Procedure-** (Hanway and Heidel)

1-Weigh 5gm of sieved soil.

2-add 50 ml of $\text{NaHCO}_3$  and a pinch of activated charcoal

3-Shake for half an hour and then filtered with Whatman filter paper No 1

4- Keep the filtrate and transfer 5ml of an aliquot in a 25 ml volumetric flask and acidify with 5N sulphuric acid.

5-add 200ml of distilled water and then 4ml of ascorbic acid.

6-Take the reading by spectrophotometer at 660 nm wavelength.

**4.8 Micronutrients:** The DTPA (diethylenetriaminepentaacetic acid) micronutrient extraction method is a non-equilibrium extraction for estimating the potential soil availability of Zn, Cu, Mn, and Fe.

**Reagents:** DTPA (Diethylenetriamine pentaacetate)

**Procedure:**

1. 10 gram finely sieved soil sample was weighed
2. 20 ml extraction agent (DTPA) was added to the sample
3. Mixing was done through shaker for two and a half hours.
4. Filter through Whatman No1 filter paper was done.
5. Readings were taken by ICP OES.

#### **4.9 Isolation of spores from soils and their observation for identification**

The wet sieving method can be used to gather AM fungus spores in soil.

The gravity of spores is slightly lower than the gravity of soil particles. The spores from soil can be concentrated by successive decantation of soil suspension followed by screening with fine mesh. Because the spores are globular or sub globular with a diameter of 50–500  $\mu\text{m}$ , they can be identified in sievings using a dissecting microscope.

**Equipments:**

1) Sieve: Sieves having a variety of mesh sizes. 1 mm, 100  $\mu$ m, and 50  $\mu$ m mesh sizes are necessary at the very least. Other sizes are preferable, such as 500 $\mu$ m and 250 $\mu$ m. Commercial stainless-steel sieves are available. However, using PVP tubes and nylon mesh, you may create your own plastic sieve.

2) Fine glass pipettes: The tip of a disposable glass Pasteur pipette (1 ml) is softened and sharpened using the flame of a gas burner. Tips of various diameters can be made to match the sizes of the spores.

3) Forceps: Fine tweezers are preferable to heavy forceps.

4) Dissecting microscope: A stereoscopic zoom microscope with a bifurcated fibre arm illuminator is recommended. A method of transmitted illumination is also required.

5) Biological compound microscope: a biological compound microscope. The DIC illumination system from Nomarsky is recommended.

Procedure:

1. 10 to 50 g of freshly collected soil sample is put into 1 to 2 liters of plastic beakers. Usually, rhizosphere soils are rich in AM fungal spores. Beaker size can be changed depending on the soil sample size.

2. Soil is suspended with about 500 ml to 1 liter of tap water. Soil macro-aggregates should be crushed with hand.

3. After 10-30 seconds\* of settling down of soil particles, the upper layer of soil suspension is poured into the sieving.

4. The procedure should be repeated until the upper layer of soil suspension is transparent.

5. The sieving on the fine mesh is collected into a small beaker and dispersed with ultra-sonication.

6. Weak sonication (i.e., 30W 30 sec) is enough, and strong sonication may destroy fungal spores.

7. Then the dispersed sample is again passed through the sieve.

8. Depending on toughness of soil aggregate, the sonication process can be repeated.

9. Usually AM fungal spores are collected on 100  $\mu$ m. Some small spores



are on 50  $\mu\text{m}$ .

10. To collect large spores such as *Gigaspora margarita*, 250  $\mu\text{m}$  sieve is efficient.

(Because the spores of AM fungus have distinct forms and colours, it is easy to distinguish them from organic detritus collected on sieve).

## Instrument used



**INCUBATOR**



**WEIGHING  
BALANCE**



**PH METER**



**DESSICATOR**



**SPECTROPHOTOM**



**HOT AIR OVEN**



Vortex



Water bath



Centrifuge



**Inductively coupled plasma  
optical emission spectroscopy  
(ICP-OES)**

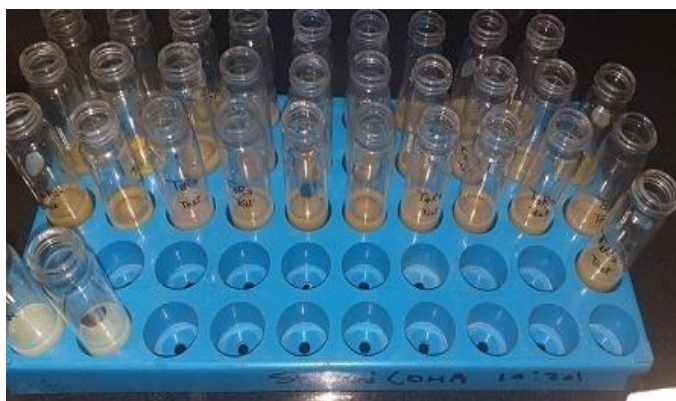
## **5. Results**

The results obtained in the present investigation entitled “Effect of Mulch and VAM inoculation on soil enzymatic activities at different stages of rose-scented geranium (*Pelargonium graveolens*) crop” has been presented and discussed here.

### **5.1 Acidic Phosphatase Activity:**

Acidic phosphatase activity at various stages of geranium crop are represented in table:1. The acidic phosphatase activity was recorded much lower than that of alkaline activity irrespective of the treatment which may be due to alkaline reaction of the soil. Previous study has revealed that phosphatase activity was strongly influenced by pH of soil (Eivazi and Tabatabai,1997; dick 1994). The maximum acidic phosphatase activity as influenced by the treatments was recorded in **T<sub>4</sub> (Mulch + VAM)** followed by **T<sub>3</sub> (Non Mulch + VAM)** and **T<sub>2</sub> (Mulch + Non VAM)** at early growth stage (60 DAP),at the same stage among the treatment minimum acidic phosphatase activity was found in **T<sub>1</sub> (No mulch + No VAM)**. Whereas during late growth phase the maximum acidic phosphatase activity was observed in **T<sub>3</sub>** followed by **T<sub>2</sub>** and **T<sub>4</sub>** however the minimum activity was observed in **T<sub>1</sub>** of late growth phase (90 DAP). Comparably, in flowering stage (120 DAP) the maximum acidic phosphatase activity was observed in **T<sub>1</sub>** followed by **T<sub>3</sub>** and **T<sub>2</sub>** and the minimum was observed in **T<sub>4</sub>**. During harvesting stage (150 DAP) The maximum activity was found in **T<sub>1</sub>**. Mean values for the treatments for acidic phosphatase activity was recorded maximum in **M<sub>0</sub>V<sub>1</sub>** followed by **M<sub>1</sub>V<sub>1</sub>** and minimum was seen in **M<sub>0</sub>V<sub>0</sub>** control.

With regards to the acid phosphatase activity in soil (Table 1) mean value ranged maximum (226.73 µg PNP g<sup>-1</sup> h<sup>-1</sup>) in harvest stage followed by early stage (228.20 µg PNP g<sup>-1</sup> h<sup>-1</sup>) and the minimum mean value was recorded during the late growth stage of 90 DAP (193.41 µg PNP g<sup>-1</sup> h<sup>-1</sup>) similar trend was previously recorded by (Mandal et al,2006).



1- Acid phosphatase test results (development of yellow color indicating presence of acid phosphatase enzyme).

**Table 1: Effect of paddy straw mulching along with VAM inoculation on acidic phosphatase activity in rhizospheric soil at different growth stages of rose scented geranium.**

Treatment	Acidic Phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ )				Mean
	Early growth stage (60 DAP)	Late growth stage (90 DAP)	Flowering Stage (120 DAP)	Harvesting stage (150 DAP)	
<b>M<sub>0</sub>V<sub>0</sub></b>	133.09	45.53	143.16	29.81	<b>87.89</b>
<b>M<sub>1</sub>V<sub>0</sub></b>	154.2	70.67	96.37	54.2	<b>93.86</b>
<b>M<sub>0</sub>V<sub>1</sub></b>	171.82	95.22	107.41	68.03	<b>110.62</b>
<b>M<sub>1</sub>V<sub>1</sub></b>	233.4	56.67	87.97	55.51	<b>108.39</b>
<b>Mean</b>	<b>173.13</b>	<b>67.05</b>	<b>108.73</b>	<b>51.89</b>	<b>100.2</b>

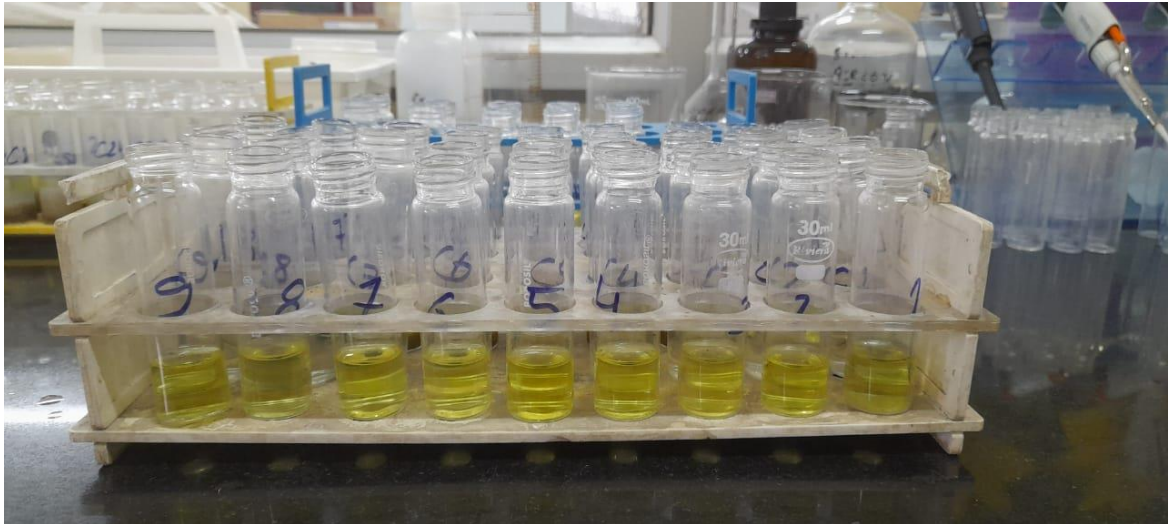
\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.



## **5.2 Alkaline Phosphatase Activity:**

The maximum alkaline phosphatase activity by the microbes was observed in **T<sub>2</sub> (Mulch + No VAM)** followed by **T<sub>4</sub> (VAM + Mulch)** and **T<sub>3</sub> (No Mulch + VAM)** while the minimum enzymatic activity was seen in **T<sub>1</sub> (No Mulch + No VAM)** during the early growth stage (**60 DAP**). Whereas during the **late growth phase (90 DAP)** maximum enzymatic activity was observed in **T<sub>3</sub> succeeded** by **T<sub>2</sub>** and **T<sub>1</sub>** while the minimum was observed in **T<sub>4</sub> (VAM + Mulch)**. Similarly at flowering stage the maximum acidic phosphatase activity was observed at **T<sub>3</sub> (No Mulch + VAM)** subsequently **T<sub>4</sub> (VAM + Mulch)** and **T<sub>1</sub> (No Mulch + No VAM)** while the minimum activity was recorded in **T<sub>2</sub> (Mulch + No VAM)**. Furthermore, during the harvesting stage (150 DAP) maximum enzymatic activity was recorded in **T<sub>2</sub>** accompanied by **T<sub>3</sub>, T<sub>1</sub>** to which the minimum activity was observed in **T<sub>4</sub>**. Mean values for the treatments for acidic phosphatase activity was recorded maximum in **M<sub>0</sub>V<sub>1</sub> (No Mulch + VAM)** followed by **M<sub>1</sub>V<sub>1</sub> (VAM + Mulch)** & **M<sub>1</sub>V<sub>0</sub> (Mulch + No VAM)** minimum was seen in **M<sub>0</sub>V<sub>0</sub>** control.

With respect to the alkaline phosphatase activity in soil (Table 2) mean value ranged maximum (226.73  $\mu\text{g PNP g}^{-1} \text{h}^{-1}$ ) in early growth stage followed by flowering stage (228.20  $\mu\text{g PNP g}^{-1} \text{h}^{-1}$ ) & harvesting stage (56.63  $\mu\text{g PNP g}^{-1} \text{h}^{-1}$ ) and the minimum mean value was recorded during the late growth stage (53.83  $\mu\text{g PNP g}^{-1} \text{h}^{-1}$ ) similar trend was previously recorded by (Mandal et al,2006).



## 2. Development of yellow color shows alkaline positive results

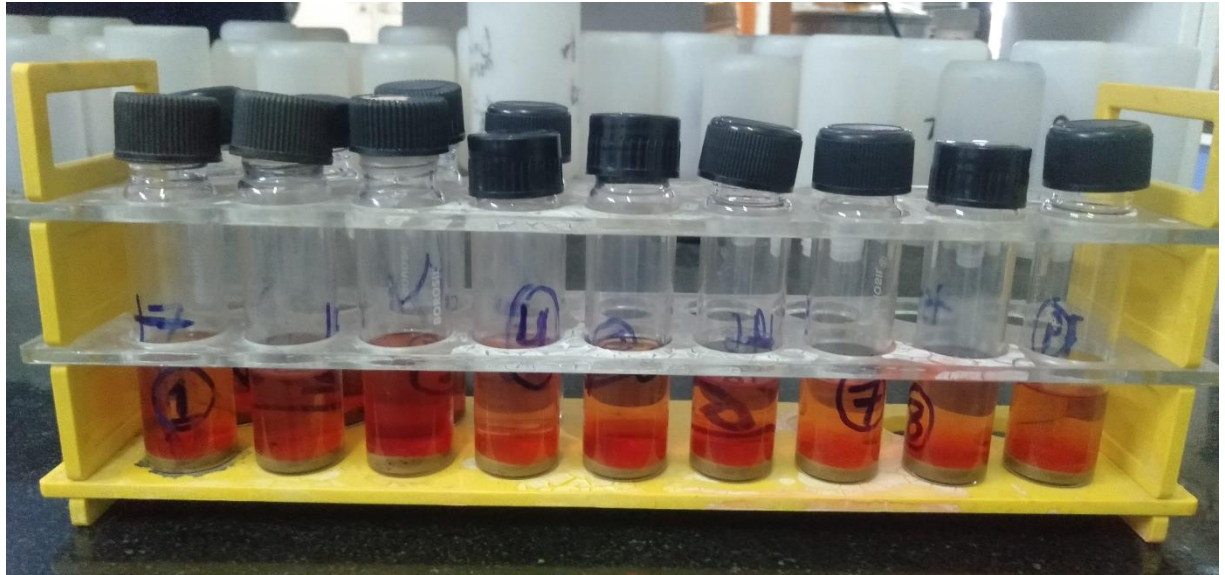
**Table 2: Effect of paddy straw mulching along with VAM inoculation on acidic phosphatase activity in rhizospheric soil at different growth stages of rose scented geranium.**

Treatment	Alkaline Phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ )				Mean
	Early growth stage (60 DAP)	Late growth stage (90 DAP)	Flowering Stage (120 DAP)	Harvesting stage (150 DAP)	
<b>M<sub>0</sub>V<sub>0</sub></b>	217.8	51.56	188.46	54.36	<b>128.04</b>
<b>M<sub>1</sub>V<sub>0</sub></b>	305.28	52.88	155.02	60.95	<b>143.53</b>
<b>M<sub>0</sub>V<sub>1</sub></b>	232.14	75.28	260.95	58.15	<b>156.63</b>
<b>M<sub>1</sub>V<sub>1</sub></b>	257.74	35.584	239.37	53.04	<b>146.63</b>
<b>Mean</b>	<b>253.24</b>	<b>53.83</b>	<b>210.95</b>	<b>56.63</b>	<b>143.66</b>

\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.

### **5.3 Dehydrogenase Activity;**

Data on Dehydrogenase activity in soil sample showed a significant effect of different fertilizer treatments and stages of wheat growth and their interactions (table 3). Biological and biochemical properties of the soil has revealed that early and sensitive indicators of the soil ecological stress or any other environment changes.(Dick,1994).since Dehydrogenase activity is only present in viable cells it is thought to reflect the total range of oxidative activity of soil microflora and consequently may be considered to be a good indicator of microbial activity (Nannipieri et al., 1990).generally the enzymatic activity of the soil is are closely related to the organic matter content (beyer et al., 1993).During the early growth stage (60 DAP) it was seen that the maximum Dehydrogenase activity was found in **T<sub>3</sub> ( No Mulch + VAM)** followed by **T<sub>4</sub> ( VAM + Mulch )** and **T<sub>2</sub>(Mulch + No VAM )** while the minimum DHA value was observed in control **T<sub>1</sub> ( No Mulch +No VAM )**.Similarly, In the late growth stage (90 DAP) increasing trend was observed among which the maximum DHA activity was recorded in **T<sub>4</sub>** followed by **T<sub>3</sub>** and **T<sub>2</sub>** and the lowest was found in **T<sub>1</sub>**. Further during the flowering stage (120 DAP) the maximum DHA activity was recorded in **T<sub>4</sub>** succeeded by **T<sub>3</sub>** and **T<sub>1</sub>** while the minimum was observed in **T<sub>2</sub>**. At the harvesting stage (150 DAP) a comparatively decreasing trend of DHA was observed the maximum activity was seen in **T<sub>2</sub>**.



**3. Development of pink color shows DHA positive result.**

**Table 3: Effect of paddy straw mulching along with VAM inoculation on Dehydrogenase activity in rhizospheric soil at different growth stages of rose scented geranium.**

Treatment	Dehydrogenase ( $\mu\text{g TPF g}^{-1} \text{ soil h}^{-1}$ )			
	Early growth stage	Late growth stage	Flowering Stage	Harvesting stage
	(60 DAP)	(90 DAP)	(120 DAP)	(150 DAP)
<b>M<sub>0</sub>V<sub>0</sub></b>	49.17	154.86	179.9	62.43
<b>M<sub>1</sub>V<sub>0</sub></b>	52.1417	179.9	154.86	75.61
<b>M<sub>0</sub>V<sub>1</sub></b>	66.47	203.45	203.45	63.42
<b>M<sub>1</sub>V<sub>1</sub></b>	53.13	224.54	224.54	67.54
<b>Mean</b>	<b>55.13</b>	<b>190.69</b>	<b>190.69</b>	<b>67.25</b>

\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.

#### **5.4 Soil Microbial Biomass Carbon (SMBC):**

Microbial biomass carbon (MBC) in soil samples showed a significant effect of different treatments at different stages of geranium crop and their interactions in (Table 4).

The maximum microbial biomass carbon during the early growth stage (60 DAP) was recorded in **T<sub>3</sub> (No Mulch + VAM)** followed by **T<sub>2</sub> (Mulch + No VAM)** and **T<sub>4</sub> (VAM + Mulch)** while the minimum was observed in **T<sub>1</sub> (No Mulch + No VAM)**. Similarly at late growth phase (90 DAP) the maximum microbial biomass carbon was observed in **T<sub>2</sub>** followed by **T<sub>4</sub>** and **T<sub>3</sub>** while the minimum was recorded in the **T<sub>1</sub>**. The observation on the effect of physiological stages clarifies that microbial biomass carbon was subject to time of sampling. Highest value is associated with **T<sub>2</sub>** and the lowest was **T<sub>1</sub>**. Microbial biomass was a small but very dynamic component of the soil organic matter fluctuating with weather, crop, input and seasons (Garcia and Rice, 1994). In this experiment, soil moisture was not significantly different from other treatments, temperature was important but in spite of high temperature during the harvesting stage (May 2022 40 degree Celsius) the microbial biomass were recorded. Thus, the interaction effect of treatments and rhizosphere conditions generated by plant growth phases might be indicated.

Treatment	Soil Microbial Biomass Carbon (SMBC) $\mu\text{g/gm}$ Soil				
	Early growth stage (60 DAP)	Late growth stage (90 DAP)	Flowering Stage (120 DAP)	Harvesting stage (150 DAP)	Mean
<b>M<sub>0</sub>V<sub>0</sub></b>	155.64	147.59	160.66	191.43	<b>163.83</b>
<b>M<sub>1</sub>V<sub>0</sub></b>	239.88	255.07	245.78	320.59	<b>265.33</b>
<b>M<sub>0</sub>V<sub>1</sub></b>	283.72	172.67	254.12	310	<b>255.13</b>
<b>M<sub>1</sub>V<sub>1</sub></b>	233.54	198.3	225.98	232.68	<b>222.62</b>
<b>Mean</b>	<b>228.2</b>	<b>193.41</b>	<b>221.63</b>	<b>263.68</b>	<b>226.73</b>

\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.

### **5.5 Physical properties of post-harvest soil.**

Application of paddy straw as an organic mulch and VAM inoculation caused a significant increase in herb and oil yield of geranium over no mulch (table 5). The increase in herb yield at the respective stages of harvests was observed to be 13.54%, 16.12% and 15.36% respectively over control. Whereas the corresponding oil yield was 34%, 74% and 12% respectively over control. The observed impact of mulch and VAM application could be attributed to maintaining a favorable environment in terms of increased soil moisture, lower soil temperature, and better nutrient availability, resulting in improved growth and vegetative biomass output. These favorable results were previously recorded by (Patra et al., 1993) and (Ram et al., 2002).

**Table 5: Physical properties of post-harvest soil as influenced by paddy straw mulching and VAM inoculation.**

<b>Treatment</b>	<b>Soil Moisture (%)</b>	<b>Bulk Density (g cm<sup>-3</sup>)</b>	<b>Water Holding Capacity (%)</b>
<b>M<sub>0</sub>V<sub>0</sub></b>	62.5	1.22	68.2
<b>M<sub>1</sub>V<sub>0</sub></b>	68.3	1.27	68.9
<b>M<sub>0</sub>V<sub>1</sub></b>	71.2	1.31	69.8
<b>M<sub>1</sub>V<sub>1</sub></b>	67.7	1.24	72.8
<b>Mean</b>	<b>67.43</b>	<b>1.26</b>	<b>70.13</b>

\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.

### **5.6a: Post harvest chemical properties of soil**

Nutrient imbalance is one of the major abiotic constraints limiting productivity of crops.

Mineralisable nitrogen at post-harvest soil is presented in (table 6a). Highest mineralisable nitrogen was recorded in **T<sub>4</sub> (Mulch + VAM)** followed by **T<sub>3</sub> (No Mulch + VAM)** and **T<sub>2</sub> (Mulch + No VAM)** as well as the minimum was recorded in **T<sub>1</sub> (No Mulch + No VAM)**. Recorded data revealed that the paddy straw mulching enhanced the nitrogen content over control. Treatment of mulch application showed a considerable increase of **6%**. Whereas application of VAM gave significant increase of **11.36%** in the post-harvest status of mineralisable nitrogen in soil and maximum increase of **30%** was seen in the treatment includes VAM along with mulch application over control. The method used for measuring mineralizable nitrogen in this study was chemical based, this is one of the most accepted methods use in the entire world. Mineralizable nitrogen is mediated by several enzymatic processes, such as amino acid, dehydrogenases, amino acid oxidase, amidinohydrolases (Nannipieri et al., 1990). In this study substantial association between MBC, and enzymatic activities indicates that the method represents the proportion mediated by enzymatic activities. Data of available phosphorus at post-harvest soil in represented in the same table 6a. Highest available phosphorus was observed in **T<sub>4</sub> (Mulch + VAM)** along with **T<sub>2</sub> (Mulch + No VAM)** followed by **T<sub>3</sub> (No Mulch + VAM)** while the lowest was observed **T<sub>1</sub> (No Mulch + No VAM)**. Available phosphorus was present in the range of 76.62 to 93.55. The percentage increase was **22.60%**, **14%**, **22.09%** respective to the treatments. VAM has the characteristics of enhancement of phosphorus availability and potentially stimulate soil P. hence increase is recorded among the treatments including VAM. It was revealed that application of mulch and VAM has considerable results on available phosphorus content. The activity of phosphatase may be the reason of P mineralization and plant uptake (Mandal et al., 2007). Soil K concentration at post-harvest are shown in the same table (6a).



Highest extractable potassium **observed** in **T<sub>2</sub> (Mulch + No VAM)** followed by **T<sub>4</sub> (Mulch + VAM)** and **T<sub>3</sub> (No Mulch + VAM)** and the minimum was observed in **T<sub>1</sub> (No Mulch + No VAM)** i.e, control. The percentage increase was recorded as 34.87%, 22.26% and 28.15% for **M<sub>1</sub>V<sub>0</sub>**, **M<sub>0</sub>V<sub>1</sub>**, **M<sub>1</sub>V<sub>1</sub>** respectively. It was observed that the significant increase is seen due to the application of VAM and mulch.

Content of Fe considerably improved due to the application of treatments which is mentioned in the table (6a). The highest Fe content was recorded in **T<sub>4</sub> (Mulch + VAM)** followed by **T<sub>3</sub> (No Mulch + VAM)** and **T<sub>2</sub> (Mulch + No VAM)** and the minimum was observed in **T<sub>1</sub> (No Mulch + No VAM)** i.e, control. The percentage increase was recorded maximum in the **M<sub>1</sub>V<sub>1</sub> (T<sub>4</sub>)** by 50% followed by 21.19% **M<sub>0</sub>V<sub>1</sub> T<sub>3</sub>** and **M<sub>1</sub>V<sub>0</sub> T<sub>2</sub>** 16% over control.

**Copper (Cu)** in the post-harvest soil is presented in (table 6a). Cu had notable increase during the treatments and the highest Cu content was observed in **T<sub>4</sub> (Mulch + VAM)**

followed by **T<sub>3</sub> (No Mulch + VAM)** and **T<sub>2</sub> (Mulch + No VAM)** and the minimum was observed in **T<sub>1</sub> (No Mulch + No VAM)**. It ranges from 0.97 to 1.94.

As for Manganese considerable and minimal increase is noted given in the table. The highest Mn content was recorded in **T<sub>4</sub> (Mulch + VAM)** followed by **T<sub>3</sub> (No Mulch + VAM)** and **T<sub>2</sub> (Mulch + No VAM)** and the minimum was observed in **T<sub>1</sub> (No Mulch + No VAM)**.

**Table:6a Effect of paddy straw mulching along with VAM inoculation on soil nutrient status of rose scented geranium.**

	N	P	k	Fe	Cu	Mn
Treatment						
M0V0	206.97	22.75	133.28	6.37	0.971	15.53
M1V0	219.52	93.94	179.76	13.22	1.317	15.65
M0V1	230.49	87.39	162.96	13.78	1.418	16.93
M1V1	269.69	93.55	170.8	17.1	1.946	17.92
Mean	231.67	74.4	161.71	12.62	1.41	16.48

**5.6b: Post harvest Organic carbon of soil**

Post-harvest soil organic carbon is represented in the table 6b. The highest amount of soil was recorded in **T<sub>2</sub> (Mulch + No VAM)** followed by **T<sub>3</sub> (No Mulch + VAM)** and the same was observed in **T<sub>4</sub> (Mulch + VAM)**. Highest percentage of organic carbon was recorded in **M<sub>1</sub>V<sub>0</sub> (T<sub>2</sub>)** by the increase of 25% followed by **M<sub>0</sub>V<sub>1</sub> (T<sub>3</sub>)** which has increase of 5% organic carbon and the same increase was observed in **M<sub>1</sub>V<sub>1</sub> (T<sub>4</sub>)** by 5% over control. This data concludes that remarkable effect mulch was observed which had a positive effect on soil organic carbon content. However, VAM along with mulch also shows a considerable increase in organic carbon.

Soil pH has an enormous influence on soil biogeochemical processes. Recent advances in research have made intriguing revelations about the important role of soil pH in many soil processes Soil pH is, therefore, described as the “master soil variable” that influences myriads of soil biological, chemical, and physical properties and processes that affect plant growth and biomass yield. (Neina., 2019) In this experiment the pH ranges from **7.2 to 7.9** in the respective treatments represented in table 6b. The maximum pH was recorded 7.9 in **M<sub>1</sub>V<sub>1</sub> (T<sub>4</sub>)** followed by **M<sub>1</sub>V<sub>0</sub> (T<sub>2</sub>)** and **M<sub>0</sub>V<sub>1</sub> (T<sub>3</sub>)**. pH and EC are important for monitoring the effects of agricultural management practices on the efficiency of nitrogen use and related

environmental impacts. Both pH and EC need to be measured since a good range for electrical conductivity could be found in an acid soil that was unsuitable for plant growth. The measurement of both pH and EC provides a more complete indication of the chemical nature of the soil. The parameters of EC and pH can be indicators of effects on biological activity where certain microbial mediated processes are affected by shifts in pH or EC. (Smith *et al.*,1997). The parameters of electrical conductivity (EC) and pH are easily measured in the field and can provide information on the nutrient condition and acidity of the soil. The maximum was observed EC in **T<sub>4</sub> (Mulch + VAM)** followed by **T<sub>3</sub> (No Mulch + VAM)** and **T<sub>2</sub> (Mulch + No VAM)** and the minimum was observed in **T<sub>1</sub> (No Mulch + No VAM)**.

**Table:6b Effect of paddy straw mulching along with VAM inoculation on soil chemical properties of rose scented geranium.**

<b>TREATMENT</b>	<b>Organic Carbon</b>	<b>EC</b>	<b>pH</b>
<b>M<sub>0</sub>V<sub>0</sub></b>	1.39	34.81	7.2
<b>M<sub>1</sub>V<sub>0</sub></b>	1.75	35.82	7.7
<b>M<sub>0</sub>V<sub>1</sub></b>	1.46	40.11	7.5
<b>M<sub>1</sub>V<sub>1</sub></b>	1.46	46.13	7.9
<b>Mean</b>	1.51	39.22	7.4

\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.

### **5.7 Fresh herb yield oil content and oil yield.**

The application of VAM and mulch has given considerable growth in its fresh herb yield which is represented in the table:7. The herb yield ranges from 358.79 to 413.93. The maximum yield was observed in **T<sub>3</sub> (No Mulch + VAM)** followed by **T<sub>4</sub> (Mulch + VAM)** and **T<sub>2</sub> (Mulch + No VAM)** over control. Considerable increase in percentage was recorded in **M<sub>1</sub>V<sub>0</sub> (T<sub>2</sub>)** by 18.78 % followed by **M<sub>0</sub>V<sub>1</sub> (T<sub>3</sub>)** 16.12% and **M<sub>1</sub>V<sub>1</sub> (T<sub>4</sub>)**.

The oil content ranged from 0.10 to 0.13 in the respective treatments. The data is represented in the table. The maximum oil content was observed in **T<sub>3</sub> (No Mulch + VAM)** followed by **T<sub>2</sub> (Mulch + No VAM)** and **T<sub>4</sub> (Mulch + VAM)** over control.

Oil yield was observed to be maximum in **T<sub>3</sub> (No Mulch + VAM)** followed by **T<sub>2</sub> (Mulch + No VAM)** and **T<sub>4</sub> (Mulch + VAM)** over control. There is significant increase in oil yield percentage, the maximum was observed in **M<sub>0</sub>V<sub>1</sub> (T<sub>3</sub>)** by 74% followed by **M<sub>1</sub>V<sub>0</sub> (T<sub>2</sub>)** and **M<sub>1</sub>V<sub>1</sub> (T<sub>4</sub>)** by 12%.

**Table 7: Effect of paddy straw mulching and VAM inoculation on fresh herb yield and oil content as well as essential oil yield of rose scented geranium.**

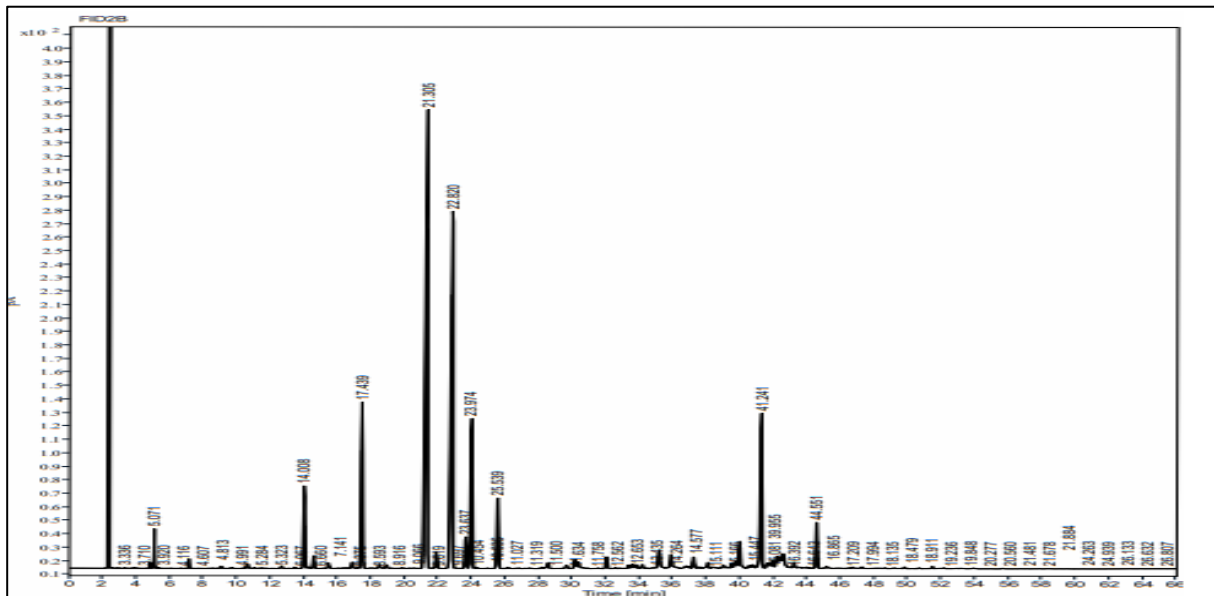
<b>TREATMENT</b>	<b>Fresh Herb Yield (q/h)</b>	<b>Oil Content (%)</b>	<b>Oil Yield (kg/h)</b>
<b>M<sub>0</sub>V<sub>0</sub></b>	358.79	0.1	50.54
<b>M<sub>1</sub>V<sub>0</sub></b>	407.4	0.16	67.9
<b>M<sub>0</sub>V<sub>1</sub></b>	416.66	0.2	88.19
<b>M<sub>1</sub>V<sub>1</sub></b>	413.93	0.13	56.8
<b>Mean</b>	<b>410.59</b>	<b>0.13</b>	<b>66.25</b>

\*Note: M<sub>1</sub>- Mulch application at the rate of 7.5 t ha<sup>-1</sup>; M<sub>0</sub>- No Mulching; V<sub>1</sub>- VAM inoculation at the rate of 5 kg ha<sup>-1</sup>; V<sub>0</sub>-No VAM application.

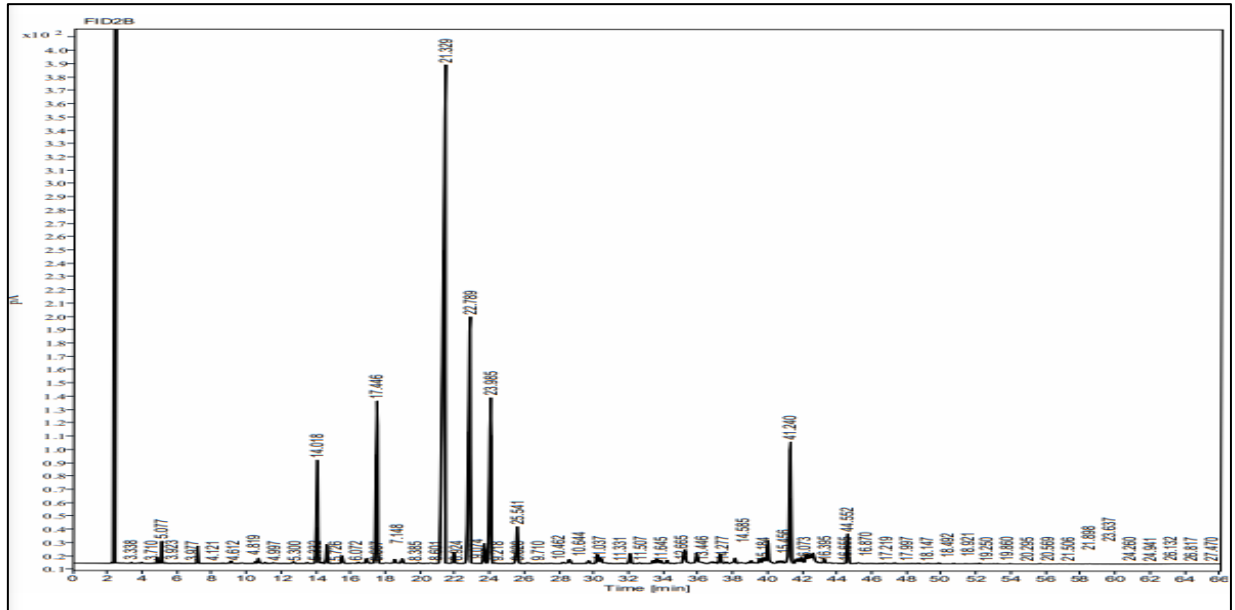
### 4.8 Essential Oil Quality of Rose-scented Geranium

The GC-FID analysis of geranium essential oil indicates that, there was no significant impact of the treatment on chemical composition of Geranium oil. The quality of produced oil is excellent and meets the standard of the world aroma industry. Moreover, paddy straw mulch & VAM inoculation be a possible combined be a possible strategy for quality production geranium essential oil. Chromatograms received from GC-FID analysis are shown below as graph-1,2,3 and 4.

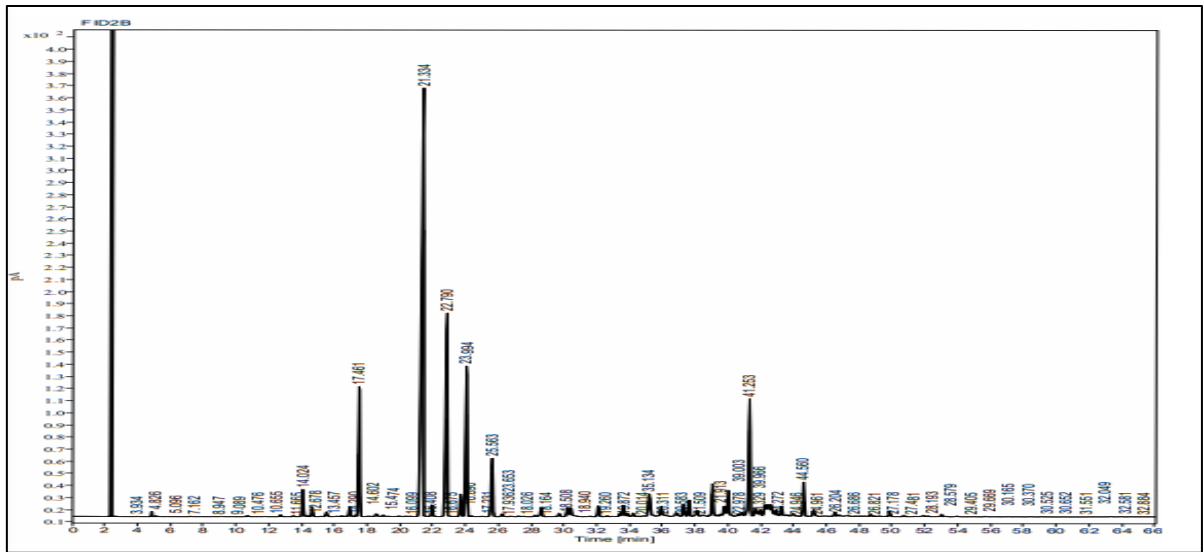
M0V0



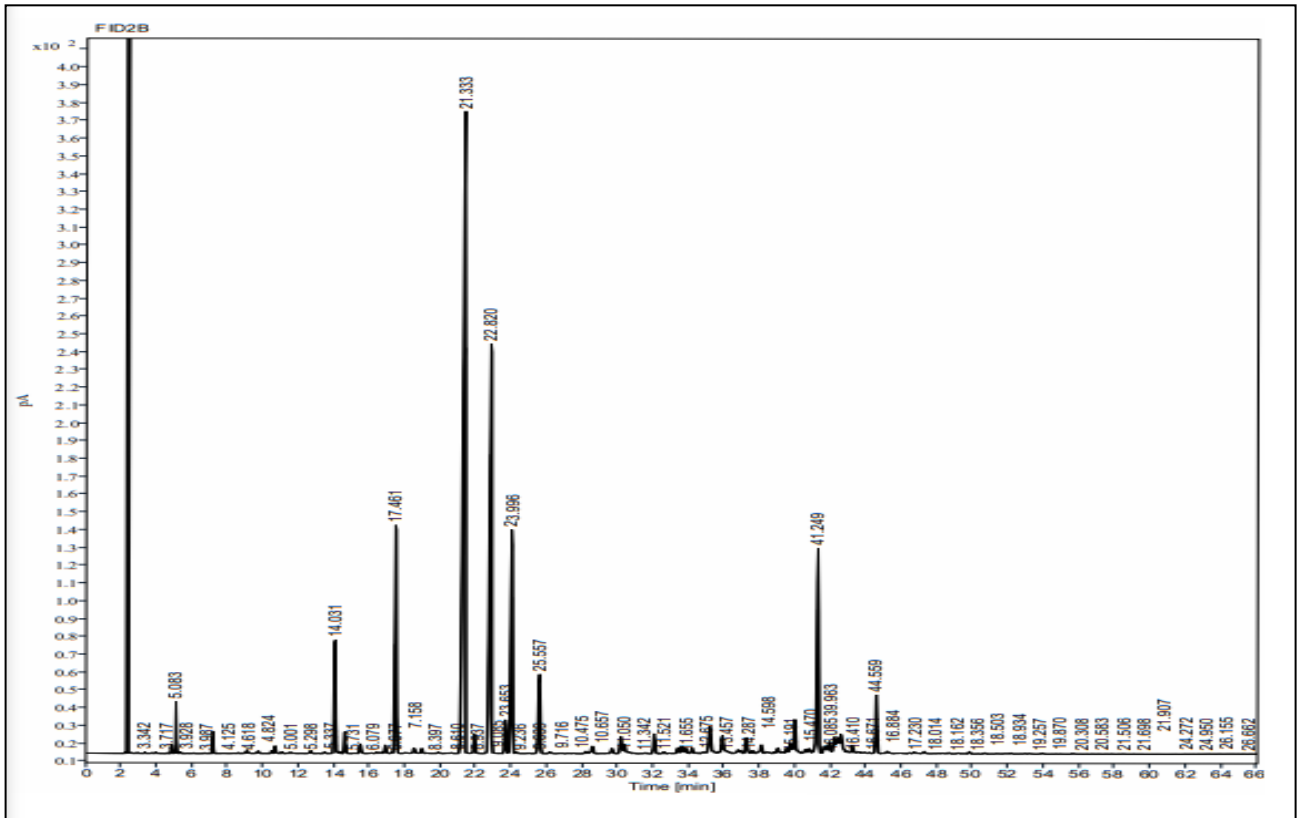
M0V1



M1V0



M1V1



## **6. Discussion**

Phosphorus (P) is an essential nutrient for all living organisms ([White and Hammond, 2008](#)). As weathering of minerals and the deposition of atmospheric dust are minor sources of P ([Wang et al., 2015](#)), the recycling of organic P from litter and soil organic matter is of utter importance for plant growth and microbial activity in terrestrial ecosystems. In P-poor ecosystems, limited P recycling may dampen the response of plant growth to elevated CO<sub>2</sub> concentration ([Ellsworth et al., 2017](#)). [Yang et al. \(2014\)](#) reported in a modeling study that the effect of elevated CO<sub>2</sub> on plant productivity in the Amazon Basin critically dependent on assumptions regarding the P-recycling efficiency within soils, which was strongly related to the parameterization of phosphatase production in their model.

The rate at which ecosystems can recycle P from litter and soil organic matter is poorly quantified by observation ([Gill and Finzi, 2016](#)). Soil phosphatases secreted by fungi, bacteria, and plant roots play an important but poorly quantified role in transforming complex and unavailable forms of organic P into assimilable phosphate ([Caldwell, 2005](#)). Potential phosphatase activity in soils, which can be measured in the lab from soil samples, is an indicator of the capacity of enzyme communities to cleave organic molecules containing P ([Krämer and Green, 2000](#)), and serves as a surrogate for the lacking measurements of P mineralization in the soil.

Potential phosphatase activity measured in the laboratory under optimal conditions (optimal temperature, well-mixed soil, no water limitation; [Eivazi and Tabatabai, 1977](#)) provides an upper limit of their actual activity in a soil ([Margalef et al., 2017](#)), which cannot be directly measured. Acid phosphatases (AP) are more widespread than alkaline phosphatases at soil pH values representative of most natural soils ([Margalef et al., 2017](#)), which justifies our focus on AP in this study.

Many experiments have investigated the responses of potential AP activity to fertilization ([Maistry et al., 2015](#)), temperature changes ([Sardans et al., 2006](#)), and water availability ([Zhou et al., 2013](#)) or to other disturbances under controlled conditions ([Sinsabaugh et al., 2008](#)). Gradients of AP



along transects have been measured in few regions ([Kitayama, 2013](#)). They found that warming, increasing soil water and nitrogen availability can enhance AP activity. However, these studies were limited to site or small region scale and only considering a subset of potential environmental factors. Recently, [Margalef et al. \(2017\)](#) addressed this gap, by compiling a global data set of phosphatase activity and using correlation analysis, regression analysis and structural equation models (SEMs) to provide insights of the drivers of phosphatase activity distribution on global scale. However, the approach used by [Margalef et al. \(2017\)](#) cannot account for non-linear responses to different variables ([Ma et al., 2017](#)) and omitted important variable like soil labile P.

The benchmarking of the growing number of land models which include phosphorus cycling is currently hampered by the lack of spatial explicit information on AP on regional to global scale. The limited understanding of the drivers responsible for differences in AP across different ecosystems and climatic and soils conditions further hampers the global efforts of including P cycles in the land surface models ([Reed et al., 2015](#)).

Machine learning (ML) is a family of approaches which has been increasingly used to identify patterns in complex ecological data sets and scale up site measurements ([Were et al., 2015](#)), but have not been used for upscaling the spatial patterns of AP.

We used two ML methods in combination with gridded fields of environmental factors to upscale site data of potential AP ([Margalef et al., 2017](#)) to gridded AP fields for continental Europe. Then we identify the main drivers behind the spatial variation of upscaled AP in Europe. Finally, we used the best ML model trained by European data in a first attempt to produce a global map of AP, and this map is cross-validated using non-European data.

Activity of phosphatases is important in studying the P cycle because this can provide a route for P mineralization and plant uptake. However, similarity in their activities was not persistent, and sometimes even contrasting: for example, at different stages of geranium given in (table 1-

2). The acid phosphatase activity was much lower than alkaline phosphatase activity, irrespective of the treatments, which may be due to the alkaline reaction of the soil. Earlier studies also proved that phosphatase activity was strongly influenced by soil pH (Eivazi and Tabatabai, 1977; Dick, 1994). The significantly greater activities of alkaline phosphatase activity in the VAM treated soils may be due to enhanced microbial activity and perhaps diversity of phosphate solubilizing bacteria due to manure input over the years. The phosphatase activity was also closely related to the microbial biomass C content (Table 4), thus consistent with the observations of (Parham et al., 2002) and (Mandal et al., 2007). Bacteria and fungus make up most of the microbial biomass, which breakdown crop wastes and organic matter in the soil. This process releases nutrients into the soil that are available for plant uptake, such as nitrogen (N). The top 10 cm of soil contains around half of the microbial biomass, as well as most of the nutrient release. In general, the microbial biomass component of soil organic matter contains up to 5% of the total organic C and organic N in the soil. When microbes die, these nutrients are released in plant-absorbable forms. Microbial biomass can be a considerable source of nitrogen, including more than 60 kg N per hectare in some situations. Microbial biomass can also be used as a predictor of total organic carbon changes. Microbial biomass C, unlike total organic C, reacts swiftly to changes in management. Biomass of microbes Carbon is a metric for the amount of carbon (C) in soil organic matter that is alive (i.e. bacteria and fungi). Microbes degrade organic materials in the soil, releasing carbon dioxide and nutrients that plants can use. Microbial biomass tends to increase in farming methods that maximize organic matter return to soil while minimizing soil disturbance. The size of the microbial biomass is influenced by soil parameters such as pH, clay, and the availability of organic carbon (Jennifer Carson, 2012).

**Results revealed that application of paddy straw mulch and VAM also increased the herb and essential oil yield in geranium crop over unmulched and non-inoculated plot which was taken as control.**

## **7.CONCLUSION**

The conclusion of our study suggests the use of organic mulch consider as natural organic resource for nutrient management and for sustaining microflora in soil. Furthermore, VAM inoculation has a positive impact on soil enzymatic activities as well as soil microbial biomass carbon at different stages of crop and shows a significant impact on chemical properties of post-harvest soil. The Second set of the study revealed that the VAM inoculation and paddy straw mulching separately and together, both sets had significant influence on fresh herb yield, oil content and oil yield and both the resources show no any negative impact on chemical composition of essential oil of rose geranium. Hence, finally we concluded that paddy straw mulching at the rate of 7.0 t ha<sup>-1</sup> along VAM biofertilizer inoculation at the rate of 6.0 kg ha<sup>-1</sup> seems to be a possible strategy for efficient nutrient management in rose-scented geranium.

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