DIPLOMA THESIS

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OPTIMAL VERMICOMPOST RATIOS FOR LETTUCE SEEDLING GROWTH

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TABLE OF CONTENTS

1.INTRODUCTION AND OBJECTIVES

Agriculture involves cultivating soil, crops, and animal livestock. The agricultural industry has shifted towards sustainability. As per Brundtland's 1987 definition, sustainability ensures the well-being of humans and the planet, creating a peaceful and enduring future for all. Organic farming is a sustainable agricultural production method that has positive impacts on resources, finance, and society. It's increasingly popular among environmentally conscious consumers and can help developing countries achieve sustainable development goals. It's a promising way to achieve sustainability. (Hammas and Ahlem, 2017).

There are different types of waste, but non-hazardous waste is the largest. Finding an eco-friendly, rapid, and financially viable waste management technique is crucial for sustainable development. (Vuković et al., 2021). According to the literature, annual solid waste production is estimated to increase from 2.01 billion metric tons to 3.40 billion by 2050 (Vuković et al., 2021). India, for instance, produces around 3000 million tons of organic waste annually, which comes from agriculture, urban and industrial sources, and domestic activities. It is necessary to use waste material for economic and environmental reasons. As the population and urbanization increase, waste management has become a significant concern for environmental health (Singh et al., 2011).

Composting is a highly effective waste management technique that yields a stable nutrient-rich product that plants can easily access. Research has indicated that the stability of compost plays a critical role in the germination, growth, and development of seedlings. However, further investigation is needed to determine how compost composition specifically affects these factors (Rodríguez et al. et al., 2017). Compost is a versatile soil enhancer that can be used in a variety of productive systems, including intensive container-based vegetable and ornamental crop production. The European Union produced over 34.6 million m^3 of substrates for both professional and hobby markets in 2013. While peat still makes up the majority of growing media at 75.1%, organic materials like compost (7.9%) and other organic matter (10.8%) are increasingly being recognized for their benefits (Atzori et al., 2021) Overall, compost is an excellent resource for enhancing soil quality across various agricultural, horticultural, and gardening applications.(Singh et al., 2011).

In addition to composting, an alternative waste management technique is called vermicomposting (Pilli, M and Sridhar Durgam, 2019). Vermicomposting and composting differ significantly. According to Vuković et al. (2021), vermicomposting lacks the thermophilic phase that reduces pathogens. Vermicomposting needs more moisture content than composting. Vermicomposting is a crucial process for transforming waste into a valuable resource through inexpensive and environmentally friendly methods. It involves both physical and biochemical processes driven by earthworm activity. This physical process involves substrate aeration, mixing, and grinding, while the biochemical process is driven by the microbial decomposition of the substrate within the earthworm's intestinal tract. (Bharadwaj, 2010). Earthworms play a critical role in maintaining soil quality by stabilizing soil aggregates, improving infiltration, and promoting optimal plant growth conditions. Using vermicompost as a soil amendment has been found to enhance soil structure and reduce plant mortality (Lee, 1992) Researcher (Pilli, M and Sridhar

Durgam, 2019) have found that vermicompost is a nutrient-rich biofertilizer with higher nutrient content than traditional compost and manure that can significantly improve crop growth and yields.

Despite peat moss being a popular organic substrate, it has several drawbacks, such as being a non-renewable resource, a source of greenhouse gas emissions, and expensive to import (Rodríguez et al., 2017) Given these drawbacks, researchers are encouraged to substitute peat with vermicompost (Kinigopoulou et al., 2022) However, there is no exact solution for replacement, and several studies have investigated the combined use of vermicompost and peatmoss for different crops, such as lettuce and tomato (Rodríguez et al., 2017). Many researchers are also encouraging finding the ratio of vermicompost amendment for seedling production and the effectiveness and quality of vermicompost along storage (Kumar, Chandra and Viswavidyalaya, 2020) .

There is still a lot to discover about the optimal application ratio of vermicompost,and its effectivness in seedling production. In light of the growing importance of sustainable agricultural practices of vermicomposting in waste management and soil health enhancement, this research aims to address specific gaps in knowledge and explore practical applications. The following objectives will guide this study.

Objectives:

This research aim is to analyze the effectiveness of different ratios of two types of vermicompost, categorized by their age, in promoting optimal growth of organic lettuce seedlings. Specifically, we aim to evaluate the suitability of vermicompost combined with peat as an organic substrate for producing high quality organic lettuce seedlings, with the ultimate goal of reducing or substituting the usage of peat moss in seedling production practices. Through this analysis, we aim to identify the most effective vermicompost ratios for optimal organic lettuce seedling growth, thereby contributing to sustainable and environmentally friendly practices in seedling production.

Hypothetical questions

1. Does the germination test with Lepidium sativum show the same result in lettuce seedling production?
2. How does the age of the vermicompost affect the organic seedling growth, in terms of shoot and root?
3.Are there discernible changes in the maturity and phytotoxicity levels of vermicompost corresponding to its age, how do these variations impact the growth and quality of organic lettuce seedlings?
4. If there is a difference in shoot height and root height in specific treatments, how can that dissimilarity influence the overall health and adaptability of lettuce seedlings?
5. Which optimal ratio might be recommended in combination with peat for lettuce seedling production?

2.LITERATURE REVIEW

2.1. Vermicomposting as a Sustainable Waste Management Technique

Vermicomposting is a method that can effectively convert organic waste into soil conditioners without requiring high temperatures, making it a low-cost technology (El-Haddad et al., 2014) This technique is not only used to minimize environmental pollution it maintains the homeostatic state of soil. This method is considered to be the best solution to the immediate problem of declining soil fertility, soil degradation, and the indiscriminate use of chemical fertilizers.(Pilli, M and Sridhar Durgam, 2019) This process is facilitated by the close interaction between microbes and earthworms, which helps to stabilize the compost. (Vuković et al., 2021)

However, it's important to note that the duration of the vermicomposting process can vary depending on the specific conditions, ranging from 75 to 100 days (Kauser and Khwairakpam, 2022)

In 1881, Darwin was the first to acknowledge that in regions where earthworms are prevalent, the A-horizons of soils, which he referred to as vegetable mould, are persistently disrupted and reorganized by these creatures. It is a logical inference that the mineral and organic components that comprise the A- and B-horizons of soils are subject to this disturbance. Unstable initially, casts become essential and stable soil components, improving its structural stability, when left to dry. These stable aggregates play a crucial role in promoting plant growth. (Lee, 1992)

Vermicompost has the potential to activate photosynthesis, improve plant metabolism, and increase the presence of growth-regulatory substances, thereby promoting plant growth and development. Vermicompost has been found to secrete plant hormone-like substances, such as auxin and cytokinin, that can have a positive impact on plant growth. (Ievinsh, 2011) The end product of vermicomposting is rich in diverse microbial communities, such as phosphate solubilizers, N2 fixers, enzyme-producers, and plant growth-promoting bacteria. (Vuković et al., 2021)

Encouraging vermicompost instead of inorganic fertilizers is vital for sustainable agriculture. It helps improve soil health and fertility while reducing dependence on synthetic inputs.(Vuković et al., 2021)

2.1.1. Worms

Notably, earthworms, often referred to as "friends of farmers," play a vital role in vermicomposting processes. The lifestyle of earthworms involves burrowing and their body structure is simple. As a result, it is commonly believed that there is only one type of this soil creature, which may not be considered very attractive. Darwin identified 3000 species, Rhonda Sherman (2019) noted that out of over 9,000 earthworm species, only seven are suitable for vermicomposting. But according to Prajapati (2023) there are at least 4400 species of earthworm. Based on their feeding habits and the specific part of the soil profile they inhabit, earthworms have been categorized into three primary ecological groups, namely Epigeic, Anecic, and Endogeic (Domínguez, 2018).

Epigeic species are typically small, pigmented creatures that reside in the litter layer. They possess a high metabolic and reproductive rate, which enables them to adapt to the dynamic environmental conditions of the soil surface. These creatures do not create permanent burrows and primarily feed on decaying organic matter and litter materials. Furthermore, they exhibit impressive rates of consumption, digestion, and assimilation of organic matter Epigeic lumbricids include *Dendrobaena veneta, Dendrobaena hortensis, Dendrobaena octaedra, Eisenia fetida, Eisenia andrei, Dendrodrilus rubidus, Eiseniella tetraedra, and Allolobophoridella eiseni*. (Domínguez, 2018)

Endogeic species live in horizontal burrows approximately 10-15 cm deep in the soil. They feed on the organic matter present in the soil and have lower reproduction rates and longer life cycles compared to epigeic earthworms. Endogeic earthworms are more resilient to unfavorable conditions such as drought and lack of food. Some common endogeic earthworms include *Aporrectodea caliginosa, Aporrectodea rosea, and Octolasion lacteum*. (Domínguez, 2018; Prajapati *et al.*, 2023).

Anecic species are known for their substantial size and vertical burrows. They emerge at night to scavenge for deceased organic matter, leaving behind noticeable earthworm casts on the surface near their galleries. These earthworms are distinguishable by their dark brown hue and slow reproductive rates, as well as their lengthy life cycles. A prime example of an anecic worm would be the *Lumbricus terrestris*. (Domínguez, 2018)

There are differences between the earthworm species used for vermicomposting and the natural earthworms that cannot be used in a worm bin (Vuković et al., 2021). Importantly only epigeic species are suitable for vermicomposting (Sherman, 2019). The red earthworm (*Eisenia foetida*) is a popular choice due to its ability to multiply quickly and efficiently convert organic matter into vermicompost.(Prajapati *et al.*, 2023). Refer to Table 1 for their characteristic features.

Table 1: Characteristics of Red earthworm (*Eisenia foetida*) (Prajapati *et al.*, 2023)

Sl.No	Characters	*Eisenia foetida*
1.	**Body length**	**3-10cm**
2.	**Body weight**	**0.4-0.6 g**
3.	**Conversion rate**	**2.0 g/1500 worms/2 months**
4.	**Maturity**	**50-55 days**
5.	**Cocoon production**	**Every 3 months**
6.	**Incubation of cocoon**	**20 to 23 days**

These earthworms are capable of consuming 25% to 35% of their body weight per day. Earthworms possess chemoreceptors in the anterior region that respond to chemicals, allowing them to identify and react to smells

(Sherman, 2019). Earthworms can eat almost any organic matter and a kilogram of worms can consume a kilogram of residue daily. (Prajapati et al., 2023)

According to (Ranga.nr, 2020) it is recommended to have around 1000 to 2000 earthworms per square meter. Optimal vermicompost application rates vary depending on crop type and production system. Field crops require 5-6 tonnes/ha of vermicompost, while horticultural crops need an equal mix of manure and vermicompost. For fruit crops, apply 4-5 kg of vermicompost per plant in the tree basin. Potted plants require 100-200 gm/pot of vermicompost (Ranga.nr, 2020).

2.2 Vermicompost composition and nutrient dynamic

Vermicompost, also called worm casting or worm humus, is a nutrient-rich mixture that varies in composition based on the feedstock used for vermicomposting. Typically, it contains a blend of organic matter, microbial biomass, earthworm secretions, and mineral elements. The organic matter in vermicompost comprises partially decomposed plant material, animal manure, food scraps, and other biodegradable waste. Microbial biomass includes bacteria, fungi, protozoa, and other microorganisms crucial for nutrient cycling and soil health. Earthworm secretions contain enzymes, hormones, and beneficial microbes that further enhance the fertility of vermicompost (Domínguez and Gómez-Brandón, 2013; Filipović et al., 2023)

The research by Paul &and Metzger, (2005) shows that vermicomposting is a process that converts the nutrients in the original waste into readily available forms for plant uptake, such as nitrate, ammonium, exchangeable calcium and magnesium, and soluble phosphorus. Furthermore, Vuković et al., (2021) explain the various features that make vermicompost an effective fertilizer, including its homogeneity, high porosity, high water-holding capacity, stability, low C:N ratio, and eco-friendliness. Additionally, the presence of humic acids and various micro- and macronutrients in vermicompost can enhance plant growth and yield.

It is worth noting that vermicompost is enriched with essential plant nutrients such as nitrogen (N), phosphorus (P), and potassium (K), in addition to micronutrients including magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), boron (Bo), and copper (Cu), as reported by (Pilli, M and Sridhar Durgam, 2019) The pH range of vermicompost promotes the availability of nutrients for uptake, leading to better plant growth. Phytohormones found in water extracts can affect the ability of plants to differentiate cells and tissues into plant organs. Root plasma membrane H+-ATPase activity and root abscisic acid (ABA) plays a crucial role in the root growth-promoting action. Vermicompost is also an effective biocontrol agent against certain plant diseases. (Vuković *et al.*, 2021). Refer Table 2 for further comparison of nutrients from different feeds.

Table 2: Comparing nutrients of different feed ingredients of vermicompost. (Lim et al., 2015)

Nutrient content	Kitchen Waste	Green and pruning residues	Cane trash	Weeds	Vegetable market waste	Market waste	Floral waste
Organic carbon (%)	10.30	-	24.62	23.88	23.92	~22.0-36.0	~13.0-20.0
Nitrogen (%)	0.85	2.05	1.14	1.88	2.11	~1.1-1.9	~0.6-1.0
Phosphorus (%)	0.15	0.42	0.46	1.01	1.22	~0.5-0.7	~0.8-1.2
Potassium (%)	-	1.21	1.61	1.31	1.45	~0.7-0.9	~0.2-0.4
Calcium (%)	1.96	2.02	-	--	-	~4.5-6.0	~1.0-2.5
Magnesium (%)	0.80	0.51	-	-	-	~0.7-1.0	~0.4-0.6
Zinc (mg/kg)	-	57	61	81	89	-	-
Copper (mg/kg)	-	-	28	36	57	-	-
Iron (mg/kg)	-	6549	294	294	412	-	-
Manganese mg/kg)	-	286	32	32	98	-	-
References	26	27	28	28	28	29	29

Earthworm activity can also affect the pH and C:N ratio in manure. However, some research suggests that it is best to maintain a pH range of 6-7 for vermicomposting. This is because lower pH levels can decrease bacterial activity, which may cause worms to migrate or perish. For vermicomposting in the A-class category, a pH range of 6.5-8.4 is recommended. (Filipović et al., 2023)

The C:N ratio is an important indicator of compost maturity. A ratio of less than 20 confirms the mineralization of organic waste, indicating compost maturity. For agricultural purposes, a C:N ratio less than 12 is preferred. It is suggested that the vermicomposting process should begin with a C:N ratio of around 25-30 and decrease as the process continues. During the process, carbon is reduced as heterotrophic bacteria use organic material as a source of electrons, which is oxidized to CO2 and released into the atmosphere. Research has shown that the reduction in organic carbon during vermicomposting is mainly due to the respiratory activity of microorganisms and earthworms. Worms add nitrogen through their mucus and nitrogenous excretion.(Filipović *et al.*, 2001)

The research (Filipović et al., 2023) highlights EC (electrical conductivity) as a crucial factor in vermicompost phytotoxicity, with compost having an EC of 3.5–4.7 mS/cm not inhibiting germination of cabbage and spinach seed and root elongation. Nonetheless, the EC (electrical conductivity) value can vary depending on the substrate used for composting. A reliable verification of phytotoxicity can be obtained through vermicompost-based biological testing on excessive salinity.

The total potassium (K) content in vermicompost increases significantly with earthworm activity and depends on the waste type. It is higher in cattle manure compared to sewage sludge. No changes are observed in the total K content during the vermicomposting process over time. Studies have also shown that higher total K content is present in the presence of earthworms during vermicomposting trials with sewage sludge or cattle manure. The available potassium (K) levels in vermicompost vary greatly depending on the time. Slightly lower levels are observed in sewage sludge than manure. (Domínguez &and Gómez-Brandón, 2013).

Sewage sludge has a higher phosphorus (P) content than cattle manure. The total P content increases significantly throughout the vermicomposting process. Earthworm activity increases available P, which is also higher in sewage sludge compared to manure. The availability of P shows an opposite trend in both wastes over time. Earthworms have been found to increase the availability of P through their casts and burrow-linings, ultimately increasing the availability of P in vermicompost (Domínguez &and Gómez-Brandón, 2013).

Studies have indicated that vermicompost can improve germination initiation and seedling growth due to increased enzyme activity and the rich chemical composition (Ceritoğlu *et al.*, 2021). Additionally, the processed materials obtained from vermicomposting have been linked to better plant growth and productivity because of their physical and chemical properties. The concentration of vermicompost is also an important consideration (Atakli *et al.*, 2022). The concentration of vermicompost is also factor to notice the higher concentration may inhibit germination therefore considering the concentration is important (Ceritoğlu et al., 2021) while using vermicompost as an organic substrate.

Vermicompost is rich in nutrient content, growth promoters and beneficial microbes.(Yatoo et al., 2021). The activity of microorganisms is key to the process of decomposition, which plays a critical role in nutrient cycling in ecosystems. Earthworms, along with other organisms such as protozoa, nematodes, and microarthropods, also play a significant role in this process. In fact, due to their interactions with soil microorganisms, earthworms are particularly important in the decomposition of organic matter (Aira and Domínguez, 2011). vermicompost showing the lower values of microbial activity is the indication of high degree of stabilization. (Domínguez &and Gómez-Brandón, 2013)

2.3. Maturity and phytotoxicity of vermicompost

As vermicomposting significantly impacts nutrient dynamics and microbial activity, it also plays a crucial role in determining vermicompost's maturity and phytotoxicity, which are crucial factors that directly impact its suitability and effectiveness as a soil amendment in agriculture. However, the use of compost in agriculture has been hindered due to problems associated with compost or vermicompost phytotoxicity. This refers to the poisoning of living plants by substances present or produced in the growth medium, which are taken up and accumulated in plant tissue. The age of vermicompost is a crucial factor in promoting crop growth, as immature compost can negatively impact plant growth due to nitrogen deficiency and the presence of phytotoxic substances. To evaluate the maturity of vermicompost, experts use a range of stability indices such as the carbon-to-nitrogen ratio, electrical conductivity, total organic carbon, total Kjeldahl nitrogen, pH levels, and germination bioassays using seeds like cress (Majlessi et al., 2012).

It's important to note that evaluating compost stability based on CO_2 evolution and maturity based on seed germination are two distinct parameters of compost quality. While low CO_2 evolution may suggest a non-phytotoxic compost, it's not always a reliable indicator. Even though low respiration rates were observed at the end of the process, does not mean that the final product is completely free of phytotoxins. (Domínguez &and Gómez-Brandón, 2013). See Table 3 below about the parameters of the compost maturity.

Table 3: Compost maturity parameters (Paradelo Núñez, Teresa Barral and Paradelo, 2011)

C:N Ratio	
Group A (stability)	Group B (maturity)
Respirometric test	Ammonia/ Ammonium
Oxygen consumption ratio	Ratio ammonia:nitrate
Carbon dioxide production ratio	Biological trials
Dewar self heating test	• Seedlin vigour and emergence • Germination and ratio • Biotrial with earthworms
Solvita co_2	Solvita NH_3
Carbon available for organisms	Volatile organic acids

Biological tests provide a comprehensive and realistic way to evaluate the compatibility of composted materials with plants, taking into account the combined effects of various phytotoxic factors present in the compost (Paradelo Núñez, Teresa Barral and Paradelo, 2011).

(Paradelo Núñez, Teresa Barral and Paradelo, 2011) recommend examining at least three groups of properties: a measurement of the degree of humification, a measurement of the microbial activity or of the water-soluble organic matter, and a plant germination or growth test.

Various plant species are utilized to assess the phytotoxicity of compost. Among these, Garden cress *(Lepidium sativum L.)* is the most commonly used due to its ease of management and rapid germination and growth. Other plant species that have been used include tomato *(Lycopersicum esculentum),* carrot *(Daucus carota L.),* cucumber *(Cucumis sativus L.),* cabbages *(Brassica oleracea L. var. italica*, *Brassica rapa L. var. pekinensis, Brassica parachinensis B.),* radish *(Raphanus sativus L.),* lettuce *(Lactuca sativa L.),* and beans *(Phaseolus vulgaris L.).* Additionally, cereals like barley *(Hordeum vulgare L.),* Italian rye grass *(Lolium multiflorum Lam.),* rice *(Oryza sativa L.),* wheat *(Triticum vulgare L.),* rye *(Secale cereale L.),* soya *(Glycine max. L.),* or corn *(Zea mays L.),* along with plants like sunflowers *(Helianthum annuus L.),* petunia *(Petunia x hybrida),* and amaranth *(Amaranthus tricolor L.)* have been used. It is recommended to use at least one monocotyledonous and one dicotyledonous species in these tests (Paradelo Núñez, Teresa Barral and Paradelo, 2011).

Seed germination and root elongation, plant growth (fresh or dry weight), and root and shoot growth of seeds are the parameters determined. It is necessary to agree on standardized methods for determining phytotoxicity of compost; a researcher reported that a Germination index value of more than 80% indicates phytotoxic-free and mature compost. Unfortunately, the relatively low germination value may be attributed to the release of toxic concentrations of ammonia and low molecular weight short-chain volatile fatty acids, primarily acetic acid (Ravikannan, Manjula and Meenambal, 2013).

2.4. Changes in Properties Over Time

The physical and chemical properties of vermicompost change significantly over a period of three months during the vermicomposting process. The fluctuations in the levels of ammonia-nitrogen, nitrate, and dissolved organic nitrogen indicate the microbial activities and nutrient transformations within the vermicompost. (Domínguez &and Gómez-Brandón, 2013)

Respirometric tests can be used to evaluate the extent of decomposition of organic matter that is readily biodegradable during vermicomposting. The decline in microbial activity levels and changes in nutrient content that occur over time indicate that the vermicompost product is becoming more stable and mature. (Domínguez &and Gómez-Brandón, 2013; Gómez-Brandón et al., 2008) Consequently, using three-month-old vermicompost can alter physical and chemical properties, enhance nutrient availability, and changes in maturity and phytotoxicity levels. Understanding these transformations is crucial for optimizing the vermicomposting process and improving the quality of produced vermicompost for agricultural and environmental applications.

Vermicompost is a type of compost that is rich in nutrients and diverse microbial composition. And it has been proven to enhance seed germination, seedling growth, and overall plant performance as we discussed before.

This organic substrate has been found to have a positive effect on the growth and physiological parameters of plants, as well as the effectiveness of mycorrhizal fungi, which in turn promotes nutrient uptake, dry matter accumulation, and seedling growth in various crops (Atakli et al., 2022). Given these benefits, researchers are currently focused on determine the optimal age of vermicompost at which maximum benefits for seedling growth can be obtained (Ose, Andersone-Ozola and Ievinsh, 2021) therefore, determining the age-related variation plays a crucial role in determining the efficacy of vermicompost as an organic substrate.

The physical and chemical property also improves with age (Shaikh et al., 2021). Studies have shown that fresh vermicomposting can exhibit certain characteristics that are necessary to plant growth, like improved bacterial community and humification indices. The nutrient composition of the substance shows that the availability of phosphorus and potassium was also good. However, during storage, there was a reduction in nutrients like phosphorus which was not significant, but for potassium, it was significant this is due to the assimilation of nutrients by bacterial and fungal grazing macroinvertebrates in the casting, as stated by Kumar et al. in 2020.

According to Kumar et al., (2020) the quality of vermicompost produced is affected by its storage duration. Changes in the physico-chemical properties of the vermicompost can directly impact its effectiveness in enhancing soil health and promoting plant growth.

As storage duration increases, the moisture content decreases due to desiccation, affecting the microbial characteristics and nutrient dynamics of the vermicompost. While bulk density and particle density do not significantly change as storage days increase, low bulk density and particle density are favorable for plant growth, as high bulk density can hinder root penetration and affect water and air movement in the soil. The water-holding capacity in the initial weeks is good due to the high degree of microspores in fresh conditions, but this capacity decreases as microbial secretion, such as polysaccharides, occurs. Electric conductivity shows a significant result over time during storage. pH also plays a role in nutrient availability, abundance of microorganisms, and plant growth, and it was near neutral during storage. As storage time increased, the nitrogen content reduced, likely due to intense nitrification and ammonia volatilization. The reduction in total organic carbon content is due to the dryness of the vermicompost, which affects the growth of microorganisms according to Kumar et al., (2020) Fresh vermicompost, generated from the initial stages of vermicomposting may possesses higher levels of bioactive compounds, such as phytohormones and microbial metabolites, which can enhance seed germination, promote early emergence, and support seedling growth (Cai et al., 2018). On the other hand, aged vermicompost, which has undergone further stabilization processes, may exhibit increased nutrient availability, stability, and maturity, which are essential factors for plant growth (Pattnaik and Reddy, 2010)

Researcher studies have indicated that fresh vermicompost may have rapid nutrient availability and microbial activity, making it a suitable option for applications where immediate plant growth stimulation is desired (Cai et al., 2018) In contrast, aged vermicompost may provide a more stable and mature substrate, which can support long-term plant growth and development (Pattnaik and Reddy, 2010) The nutrient status of vermicompost, including nitrogen, phosphorus, potassium, calcium, and magnesium, can vary with the composting process, with changes in organic carbon and nutrient ratios observed as composting progresses (Pattnaik and Reddy, 2010).

Furthermore, the pH, lignin, cellulose, electrical conductivity, and nutrient contents of vermicompost can differ between fresh and aged vermicompost, influencing their effectiveness as organic substrates (Cai et al., 2018). The age-related variations in vermicompost composition can impact plant nutrient uptake, growth, and overall productivity. While fresh vermicompost may excel in providing immediate benefits for seedling growth, aged vermicompost's stability and nutrient richness make it valuable for sustained plant development over time.

In conclusion, the choice between fresh and aged vermicompost depends on specific agricultural needs, with fresh vermicompost offering rapid benefits and aged vermicompost providing long-term soil enrichment and plant support.

2.5. Advantage and Drawbacks of Vermicompost

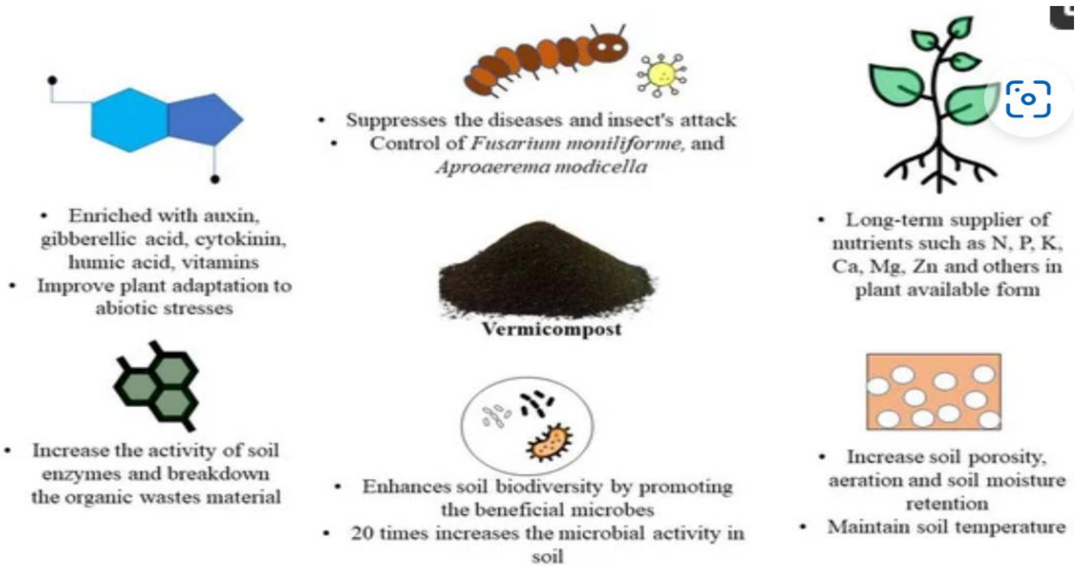

Figure 1- Vermicompost role to encourage plant growth (Rehman et al., 2023)

Vermicompost is a type of fertilizer that provides many benefits to soil and plant growth. The benefits of Vermicompost are vast, including stimulating root and shoot growth, enhancing seed germination, leaf area, branching, yield, nutritional value, and influencing flowering, biomass, photosynthesis, and respiration (Vuković et al., 2021) Recently, Vermicompost has been touted as a crucial alternative in the battle against plant diseases, pests, and pathogens. (Vuković et al., 2021; Prajapati et al., 2023)

Vermicompost can be a protein source for pig feed, fishing bait, or animal feed (Prajapati et al., 2023; Stapathy et al., 2020). When used as a soil amendment during transplanting, vermicompost provides plants with the necessary nutrients, enabling them to establish quickly (Prajapati et al., 2023). The high nutrient and growth-promoting content of the cast, plus its favourable soil microflora, have a significant impact on overall plant growth

(Prajapati et al., 2023) According to (Prajapati et al., 2023) earthworms enhance phosphorus availability in soil, reduce soil compaction, and improve infiltration rate.

The drawbacks of vermicomposting are that it can produce an unpleasant odour if not properly executed. Adding carbon sources like paper to neutralize the smell. The process usually takes around 2-3 months to complete. It's important to maintain the bin's temperature and cover it securely to prevent unpleasant odours and avoid attracting rodents and flies. When done improperly, vermicomposting can emit a foul odour. (Ranga.nr, 2020) The process of vermicomposting is lengthier and takes a minimum of 6 months to transform organic materials into vermicompost. For the production of compost through vermicomposting, one needs to put in high maintenance efforts. (Gupta et al., 2022) Additionally, a higher quantity of application dose is required compared to fertilizers. (Gupta et al., 2022) Compared to other composting methods, vermicomposting demands greater attention and needs to be done regularly.(Gupta et al., 2022).

2.6. Vermicompost technology and its application

The process of vermicomposting begins with the preparation of organic materials such as kitchen scraps, yard waste, and cow manure. These materials are gathered in a specific ratio of approximately 25 pounds of organic matter to worms (25:1) (Sim and Wu, 2010). Once the organic matter is collected, it is placed in a vermicomposting bin or pile. Home composting typically uses the bin method, while the pile method is preferred for large-scale production (Rostami, 2011). After selecting the system, the typical species, such as *Eisenia fetida* or *Eisenia andrei*, should be chosen(Aira, Monroy and Domínguez, 2007). It is important to regularly feed the worms with organic waste and monitor and maintain optimal conditions of moisture and aeration in the vermicompost pile (Rostami, 2011; Sim and Wu, 2010). This is where the decomposition process begins, as earthworms feed on the organic matter and break it down into simpler compounds. The worms digest the material and excrete nutrient-rich castings, forming the vermicompost. The vermicomposting process should continue for 2 to 6 months, allowing the organic material to transform into nutrient-rich vermicompost. (Aira et al., 2007; Sim and Wu, 2010) This process is called maturation, and once the vermicompost has old, it can be harvested from the vermicomposting system. Vermicompost is a valuable organic fertilizer and soil amendment. (Aira, Monroy and Domínguez, 2007)

Every kilogram of worms present produces 0.3 kilograms of vermicompost each day (Sim and Wu, 2010). In a commercial setting, large-scale vermicomposting systems are employed to optimize the process for mass production. These systems utilize controlled environments and specialized equipment for mixing, turning, and harvesting.(Aira, Monroy and Domínguez, 2007)(Sim and Wu, 2010) Vermicompost is crucial in these settings to handle large volumes of organic waste and produce vermicompost efficiently. By employing these systems, commercial operations can increase the efficiency of the vermicomposting process and the productivity of the resulting vermicompost.(Sim AND Wu, 2010)

The location of the site should have a cool and shady environment with adequate moisture. It's important to ensure that the biomass is thoroughly dried before use (Ranga.nr, 2020).

Keeping the temperature below 35ºC, monitor moisture levels between 40-50%. (Stapathy et al., 2020) Maintenance is needed for drainage to prevent water stagnation. (Stapathy et al., 2020). Avoid using chemical fertilizers or insecticides to keep vermicompost healthy. (Stapathy et al., 2020) additionally, prevent ants, frogs, rats, and birds from accessing vermicompost. (Stapathy et al., 2020).

Application of this method employed to transform various forms of biodegradable materials into a valuable soil amendment. This technique proves particularly beneficial in enhancing soil fertility, especially in saline and acidic soils, while also aiding in the reclamation of wasteland, degraded, and infertile soil.

2.7. Influence of vermicompost ratios on seedling growth

In a study by Rodríguez Adriana Hernández et al. (2017), peat moss, vermicompost, and semi-compost were used as substrate mixtures for lettuce and tomato seedlings. These mixtures contained varying levels of nitrogen (N), with the highest level being 2.83% in semi-compost. The study found that the substrate mixtures provided sufficient N to the seedlings during the third stage of germination when they began to rely on external nutrients for growth. The study also found that using vermicompost can be helpful for growing plants, but the amount of plant growth promoters can vary depending on the type of worm that produced it and the material used to make it. In another study conducted by (Ali et al., 2007), replicated growth trials were undertaken using pure wormcasts, while in a study by (Ievinsh, 2011), vermicompost was mixed with sphagnum peat moss at different ratios. In Ali's study, the treatment used included 50/50 and 20/80 (VC/FS) vermicompost and fresh semi compost. In Rodríguez Adriana Hernández's study, five different treatments were applied based on the proportion of vermicompost (V), semi compost (S), and peat moss (PM), with the most suitable proportion for lettuce seedlings being 2V:1PM.

In a 2021 study conducted by Vuković et al., the impact of cow manure vermicompost at varying levels (25%, 50%, and 75%) on the growth and development of thyme was investigated. The study found that only a 25% vermicompost substitution had a positive effect on seedling emergence, while other substitutions did not show any significant benefit. At 50% vermicompost substitution, the maximum length, fresh and dry weight, and photosynthetic efficiency were observed. However, Ievinsh's study in 2011 found that using vermicompost at levels of 10-100% had adverse effects that resulted in shorter seedlings, fewer leaves and decreased germination. This may be due to induced stress by the high-soluble salt concentration or phenolic compounds from vermicompost.

2.8 Organic Seedlings of Lettuce

Improving transplant quality and reducing production time are potential benefits that vermicompost could offer to the vegetable transplant production market. The transplant media should exhibit qualities such as good drainage, moisture retention and small particle size. When evaluating transplant quality, standard criteria include elements such as consistency, robustness, flexibility, timely maturation in the field, and overall well-being. If transplants exhibit accelerated growth rates in a controlled growth environment, while maintaining the same level of quality, it may be possible to shorten the production timeline for vegetable transplants but it's crucial to monitor quality, as rapid growth could compromise transplant quality. (Paul AND Metzger, 2005)

The maturation period for head lettuce after transplanting typically ranges from 60 to 70 days, while most leaf types are ready between 30 to 45 days post-transplanting. Lettuce is prone to attacks from aphids, armyworms, imported cabbage worms, and loopers. Young seedlings are susceptible to a serious disease called damping-off, while mildews and sclerotinia can cause harm to more mature plants (Sanders, 2001)

Lettuce seedlings cultivated in vermicompost-based substrates exhibited improved overall quality. Shoot development was more robust, and seedlings displayed vigorous root systems.(Urechescu, Peticilă and Drăghici, 2023). Application of humates from vermicompost reduced the lettuce production cycle by 21 days, allowing early harvesting without compromising quality and also treatment increased the number of leaves per plant, resulting in improved yields (Hernandez et al., 2015). Lettuce seedlings grown with vermicompost required lower nutrient solution intake. This water and nutrient demand reduction suggests that vermicompost contributes to resource-efficient seedling production (Urechescu, Peticilă and Drăghici, 2023)

The research has reported that using pure vermicompost decreased chlorophyll content in lettuce plants, with no adverse effects on plant growth. This implies that the influence of vermicompost on lettuce seedlings may not be consistent across different growth parameters. Therefore, determining the optimal ratio of vermicompost substitution for lettuce seedlings is crucial, depending on specific growth parameters targeted, such as seed germination and shoot height (Ievinsh, 2011). While working with lettuce, researchers found that vermicompost improves seedling size by 20% due to increased pH, electrical conductivity, nutrient levels, bulk density, and total porosity of the substrate (Arın and Dinçsoy, 2020). Many studies highlight the importance of vermicompost in promoting seedling growth. The production of radish seedlings was also an excellent result under vermicompost usage as a substrate (Jaeggi et al., 2019) Soilless organic production faces hurdles in economics and regulations, but ongoing efforts are needed to overcome these and make it more feasible.

3 MATERIAL AND METHODS

The experiment was conducted in the C block of the Department of Agroecology and Ecological Farming of the Hungarian University of Agriculture and Life Science from 20 June 2023 to 15 April 2024.

3.1. Preparation of vermicompost

The vermicompost utilized in the experiment was prepared in two distinct forms: old and fresh vermicompost. The old vermicompost was six months old, whereas the fresh vermicompost was only one month old. The old vermicompost was started on 20/06/2023 and harvested on 16/10/2023 whereas the fresh compost was started on 16/10/2023 and harvested on 29/01/2024.

Figure 2: Kitchen waste measured on scale and added to wormbin

The vermicomposting process was carried out using specialized worm composting plastic bins, and the worms used in the process were identified as *Eisenia fetida / Eisenia Andrei* complex. The worms were fed with kitchen waste every week, including vegetable scraps, eggshells, and tea bags. In addition, fallen leaves collected from the compost pits were added to the bin to maintain appropriate moisture levels. Occasionally, the vermicompost was sprinkled with water. The worms were usually fed weekly once and no particular limit to feed was maintained, but usually, it was measured at the time of feed. The bin was usually covered with a net cloth and the lid was opened if it was found to be too moist to avoid bad odour and to aerate the compost to maintain the moisture content. Dry feed was also given to balance it.

Following the completion of the process, the vermicompost was harvested, and the worms were carefully transferred to the fresh vermicompost bin through handpicking. The same feedstocks were utilized to prepare the fresh compost. However, in larger farming or industrial settings, the process may be more homogenized.

The old vermicompost was stored in a closed bin in a dark and cool place for six months after harvesting and sieving. We noted that for further studies, measuring the feedstock in volume would be more helpful.

Figure 3: Germination test of different vermi-substrate with different ratio

The utilization of six months of old and fresh vermicompost in the experiment served to differentiate the impact of aging on the composition of the vermicompost on seedlings. The methodical process of preparing the vermicompost, utilizing specialized composting bins and carefully feeding the worms, ensured that the final product was of high quality and suitable for research purposes.

3.2. Germination test (Lepidium test)

In this test vermicompost were mixed with peatmoss for organic seedling growth. Certified organic seed of *Lepidium sativum* was selected for phytotoxicity evaluation.

Germination test was carried out using six different treatments with four replications, each consisting of different ratio mixes of vermicompost and peat moss (0%, 5%, 10%, 15%, 20%, 50%).

The experiment utilized 500 ml of substrate, divided into four 125 ml glass petri dishes, resulting in four replicants for each treatment. In each petri dish, 25 seeds were sown, and 30 ml of water was added at the beginning of the experiment. See Table 4 for treatment description.

Table 4: The six treatments for germination test recipes are described below

Treatment	Vermicompost content ml	Peat content ml	Treatment code
1st. treatment:	0 ml	500ml	VC_0
2nd treatment:	25 ml	475ml	VC_5
3rd treatment:	50 ml	450ml	VC_10
4th treatment:	75 ml	425ml	VC_15
5th treatment:	100 ml	400ml	VC_20
6th treatment:	250 ml	250ml	VC_50

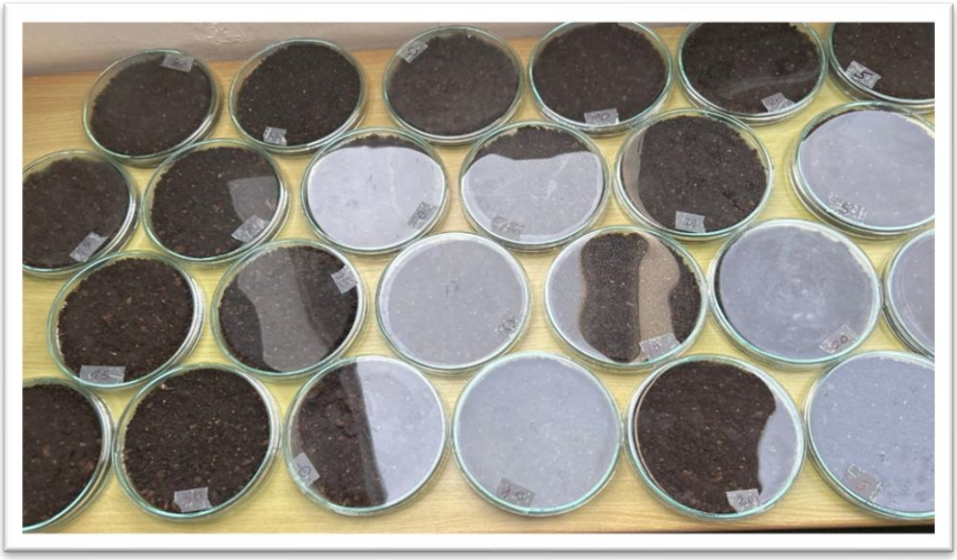

Figure 4: Seeds sown in the Petri dishes containing vermicompost

The seeds were sowed: To old vermicompost on 16/01/ 2024 and measured on 22/01/2024, to fresh vermicompost on 5/02/2024 followed by measurement on 12/02/2024.

This seeds are allowed to incubate in the room temperature at university lab for 6 days. On the 6th day the germinated seeds were counted in all repetition. Shoot length and root length of 50 seedlings in each treatment was measured by a ruler, and data was given to an excel chart.

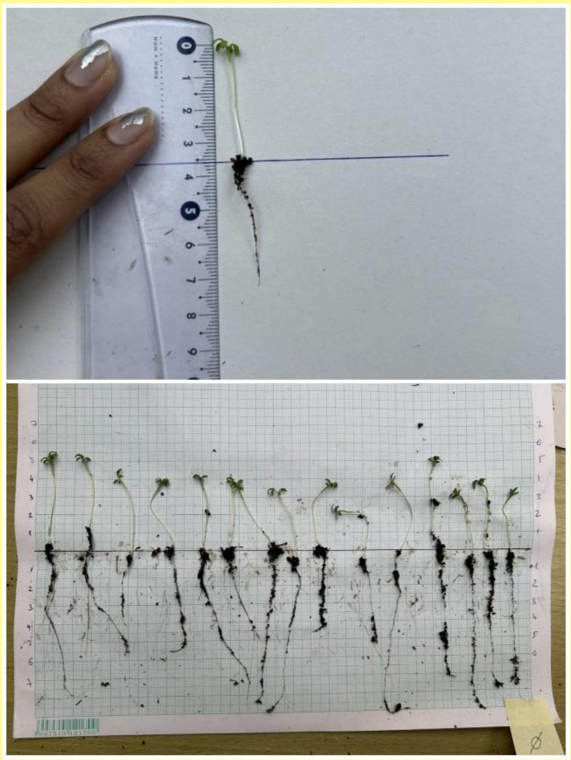

Figure 5 Measuring the shoot length and root length of the seedlings through ruler and graph paper

3.3. Physical and chemical properties of the substrates

The Soil Science Department laboratory of the Institute of Environmental Sciences conducted a comprehensive physical and chemical analysis of fresh and old vermicompost. The analysis comprised measuring various parameters including pH levels, total water-soluble salt, nitrite-N, nitrate-N, ammonia-N, AL-soluble potassium (K2O), AL-soluble phosphorus (P2O5), humus content, calcium carbonate (CaCO3), total organic matter, total nitrogen (Total-N), sulphate content, and sulphide test. The analysis employed various procedures to examine these properties, such as pH meter for pH levels, conversion of total electrical conductivity to total dissolved solids (TDS) or total salts for total water-soluble salt, and colorimetric methods like Griess reaction for nitrite, cadmium reduction for nitrate, and Nesslerization or indophenol blue methods for ammonia for nitrite-N, nitrate-N, and ammonia-N. The humus content analysis method was employed to determine the humus content, while the total carbonate content was determined by hydrochloric acid dripping or by using a Scheibler calcimeter. Extraction with ammonium acetate followed by analysis using atomic absorption spectrophotometry (AAS) for potassium and colorimetric methods for phosphorus were used for AL-soluble potassium and AL-soluble phosphorus, respectively. The Kjeldahl digestion method was used for Total-N, and turbidimetric or gravimetric methods were employed for Sulphate. The methylene blue test was employed for the qualitative detection of sulfide ions in the Sulphide test. These tests were performed to ascertain the suitability of vermicompost as a soil amendment and to determine its level of maturation and readiness for application to seedlings.

3.4. Lettuce seedling test

On 13th of February the lettuce seeds were sown to fresh and old vermicompost substrate. On February 28th, the seedlings were transplanted into different substrate mixtures to observe the final output of seedlings. Each sample treatment consisted of 10 plants. To ensure adequate nutrient supply for the plants, we used a commercially available certified organic growing media, Klasmann KKS Proline (Bio) Potgrond, as the control instead of using only peat. The recipe for both fresh and old vermicompost was the same and was measured as stated in Table 5. The containers used were 125ml in size, and each mixture contained 10 plants.

Figure 6: Measuring and preparing the substrate for transplanting the seedling

Table 5: The measured ingredients for the substrate preparation at different ratio

Vermicompost V/V%	Vermicompost/10 plants	Peat /10 plants	Pot-ground / 10 plants	Treatment code
control	0	0	2000	C
5%	100ml	1900ml	0	VC_5
10%	200ml	1800ml	0	VC_10
15%	300ml	1700ml+	0	VC_15
20%	400ml	1600ml	0	VC_20
50%	1000ml	1000ml	0	VC_50

Figure 7: Lettuce seedling transplantation to fresh and old vermicompost.

3.4.1. Weeds along seedling

We observed the presence of weeds specially tomato weeds with lettuce seedling. It is because tomato waste was also available in the kitchen waste which was fed to worms. The tomato seeds not eaten by the worms are grown with lettuce as weed. Hence these weeds are removed from the lettuce seedlings. We noted that we should be careful with the feeding material of worms to avoid unnecessary weeds in the substrate.

Figure 8:Weeding in lettuce seedling

3.5. Chlorophyll content analysis

Chlorophyll is a vital green pigment found in plants that helps with the absorption of radiation, resulting in energy for photosynthesis. Chlorophyll content is typically measured in micromoles per square meter or mass per area of leaf surface, with the Apogee MC-100 chlorophyll concentration meter being a reliable instrument for this purpose. This innovative tool allows for quick, non-destructive measurements of chlorophyll in multiple leaves, or single leaves by replicating measurement making it an ideal alternative to traditional sampling techniques. In the experiment according to the naming of the lettuce seedling of two different substrates of different ratio the selected leaf is considered to measuring the chlorophyll content in different position with three replications on the same leaf of the seedling hence data was collected.

Figure 9 : Measuring chlorophyll content at different position of leaf using Apogee MC-100 meter for different ratio substrates of vermicompost.

3.6. Lettuce root and shoot measurement

After 66 days (2 months, 7 days) of transplanted on April 8th, the lettuce plant were harvested. The roots were washed, and the shoot and roots were separated and measured using a digital scale. To ensure accuracy, each pair of roots and shoots were measured separately. The wet weight of each pair was recorded and transferred to an envelope labeled accordingly. The envelopes containing the pairs were then placed in a Memmert ULE 600 hot air oven drying machine to remove water content. Finally, each pair of shoots and roots were measured separately for dry weight.

The dry matter content, determined by calculating the dry weight, is expressed as a percentage (%) to signify the amount of solid material in a sample after moisture has been removed. This uniform method of measurement allows for easy comparison among various samples and studies, which is why our experiment also employs this percentage-based system.

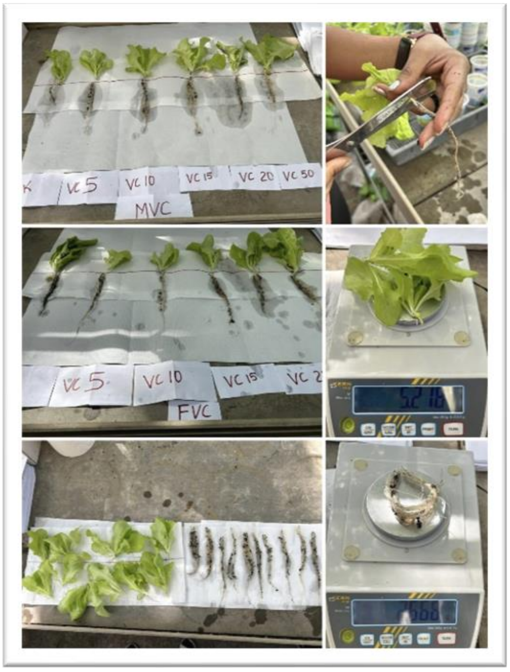

Figure 10: Lettuce seedling shoot and root measured through digital scale

3.7. Statistical analysis

All counted and measured data were collected in excel tables. Statistical analysis were performed by using the statistical software SPSS for windows, (Version .29) (IBM Corporation). We ran an analysis of variance (ANOVA) test. Where 2 factors were used to analyze, age and substrate.
We checked the homogeneity and normality of the data and where the homogeneity was significant, we used Games-Howell test, when and homogeneity was not significant we used the Tukey test.

4.RESULT

4.1 Lepidium test

4.1.1. Germination ability

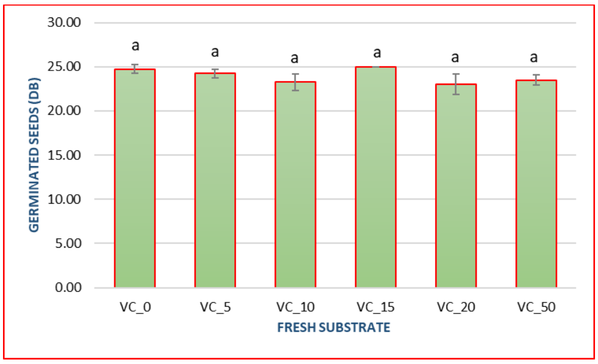

Figure 11 and 12: Germinated seed count (mean±sd) of different ratio of old and fresh vermicompost substrate.

The graph results indicate that varying the ratio of old and fresh vermicompost does not significantly affect the number of germinated seeds in different vermicompost substrate ratios.

4.1.2. *Lepidium sativum* shoot analysis

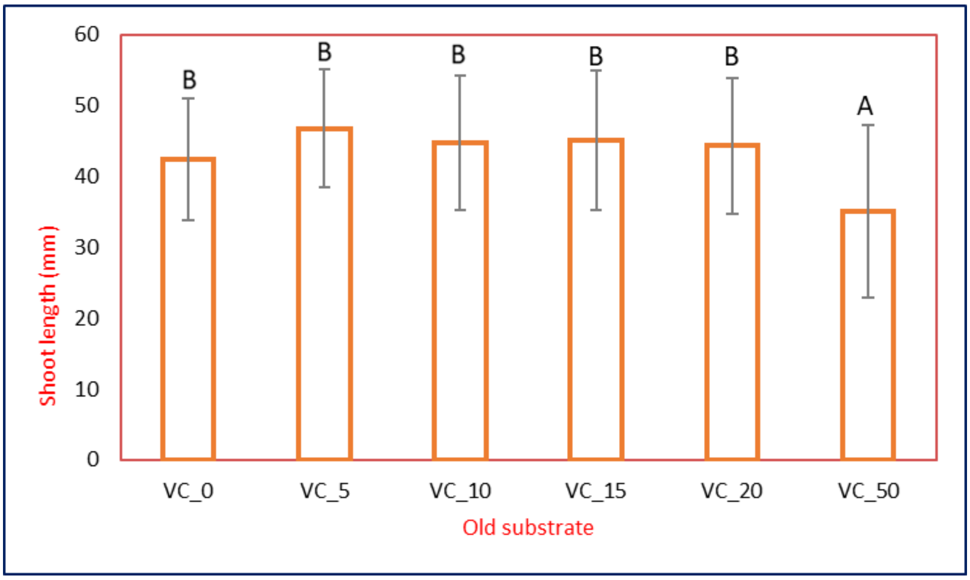

Figure 13: Shoot length of Lepidium sativum (mean±sd) of different ratio of old vermicompost substrate.

Differing letters mean significant difference between samples within old substrates of different ratio ($p<0,05$).

In old substrates, the pattern of shoot length response to different ratio of vermicompost concentration is somewhat consistent across a border range of concentration. Where VC_50 showed a significant difference from VC_0, VC_5, VC_10, VC_15 and VC_20. Whereas VC_0, VC_5, VC_10, VC_15 and VC_20 showed almost uniform response along the concentration

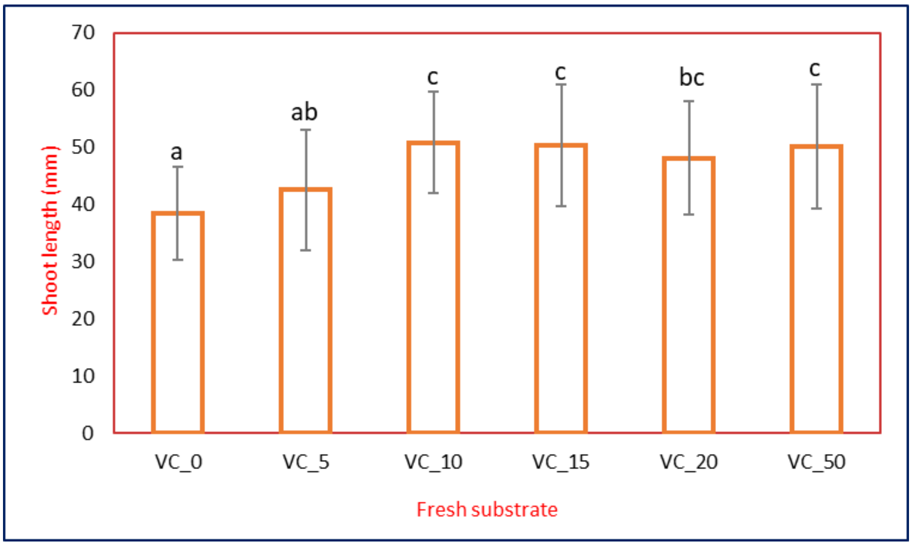

Figure 14: Shoot length of *Lepidium sativum* (mean±sd) of different ratio of fresh vermicompost substrate.

Differing letters mean significant difference between samples within a fresh substrates of different ratio (p<0,05).

In fresh substrate the shoot length of VC_0 show significant difference from VC_10, VC_15,VC_20, VC_50 and VC_5 also show significant difference from VC_10, VC_15, VC_50 whereas VC_0 shows not much difference from VC_5 AND VC_5 doesn't show much difference from VC_20 AND VC_20 shows similar difference among VC_10, VC_15, VC_50.

4.1.3.*Lepidium sativum* root analysis

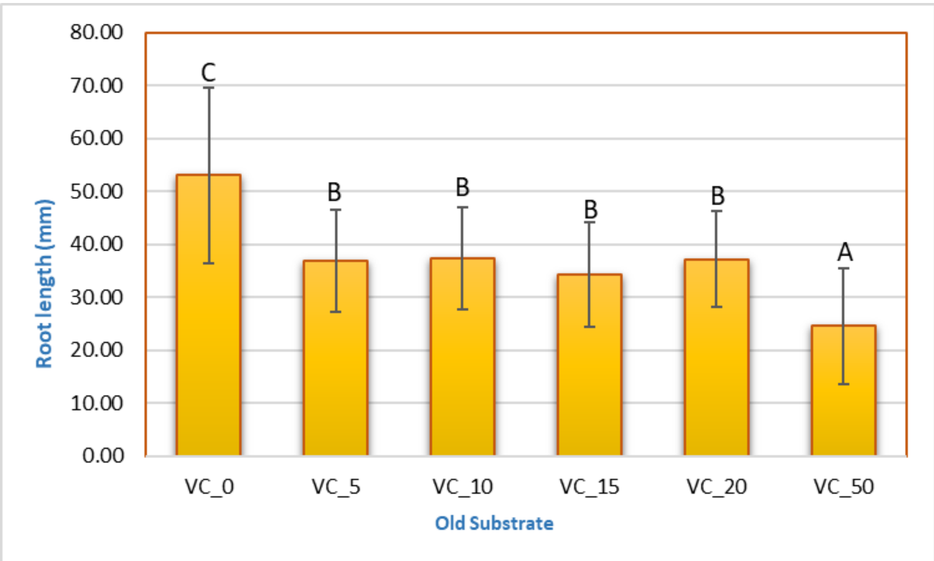

Figure 15:Root length of *Lepidium sativum* (mean±sd) of different ratio of old vermicompost substrate.

Differing letters mean significant difference between samples within a old substrates of different ratio (p<0,05).

Based on the result represented on the graph in old substrate (Fig.15), the pattern of root length response to different ratio of vermicompost concentration is VC_0 shows significant difference from VC_5, VC_10, VC_15, VC_20, VC_50. Whereas VC_50 also show significant differences in root length from VC_0, VC_5, VC_10, VC_15, VC_20. But VC_5, VC_10, VC_15, VC_20 are quite similar in the difference.

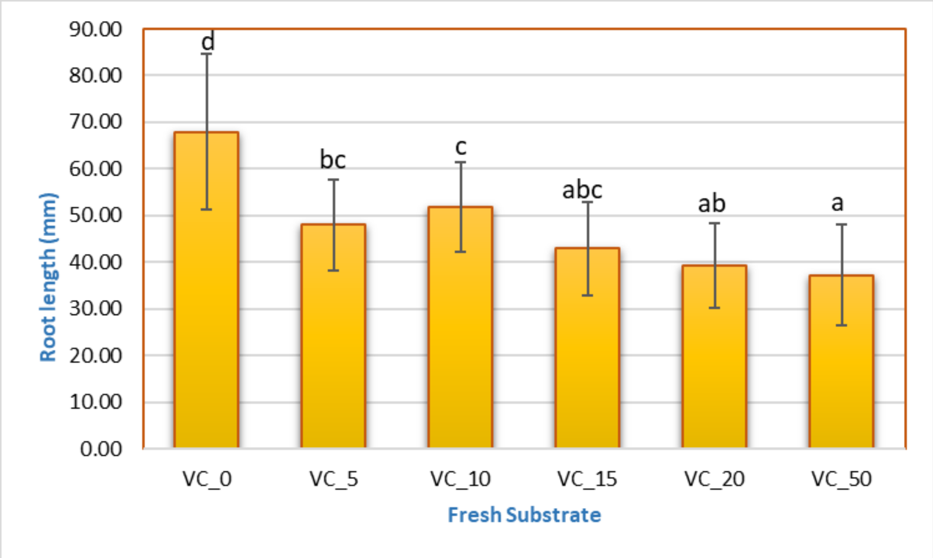

Figure 16:Root length of *Lepidium sativum* (mean±sd) of different ratio of fresh vermicompost substrate.

Differing letters mean significant difference between samples within a fresh substrates of different ratio ($p<0,05$).

In fresh substrates, VC_0 shows significant difference in root length from VC_5, VC_10, VC_15, VC_20, VC_50. VC_10 show significant difference from VC_0, VC_20, VC_50 whereas VC_50 shows similar differences with VC_15 VC_20. VC_5, VC_15, VC_20 show almost similar differences in the root length of different ratios of vermicompost substrate.

4.2. Substrate sample analysis

Table 3 mentioned below of substrate sample analysis shows that as vermicompost matures, its pH level increases in both H2O and KCl solution, indicating an increase in alkalinity. The total water-soluble salts and levels of potassium and phosphorus also increase, suggesting an accumulation of minerals and essential nutrients. The percentage of organic matter and humus content is higher in old, indicating an increase in decomposition and humus formation over time. Calcium carbonate and total-N levels show variations, and sulphate levels and sulphide test results differ between fresh and old vermicompost.

Table 3: Properties of fresh and old vermicompost

Properties/Ingredients	**Fresh vermicompost**	**Old vermicompost**
pH (H_2O)	7,67	8,00
pH (KCl)	7,41	7,59
Total water-soluble salt (mg/kg)(%)	4015 (0,4015%)	13300 (1,33%)
Nitrite-N (mg/kg)	0,2	0,35
Nitrate-N (mg/kg)	189	239
Ammonia-N (mg/kg)	5,5	3,0
AL-soluble potassium K_2O (mg/kg)	13762	29676
AL-soluble phosphorus P_2O_5 (mg/kg)	2108	2223
Humus (%)	14,05	15,88
$CaCO_3$%	3,25	3,99
Total organic matter (%)	40,66	52,11
Total-N (mg/kg)	4190	6598
Sulphate (mg/kg)	600	875
sulphide test	category I.	category 0.

4.3. Lettuce seedling test

4.3.1. Photosynthetic activity

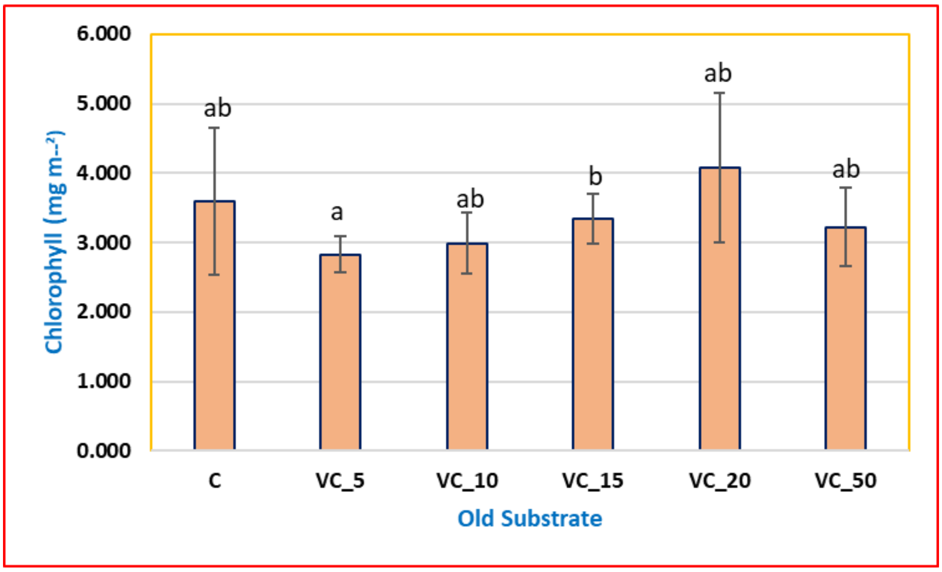

Figure 17: Chlorophyll content of lettuce seedling (mean±sd) grown in different ratio of old vermicompost substrate. Differing letters mean significant difference between seedling within a old substrates of different ratio (p<0,05).

VC_5 shows significant difference with VC_15, but C, VC_10, VC_15, VC_20, VC 50 doesn't show much significant difference in photosynthetic activity of seedling

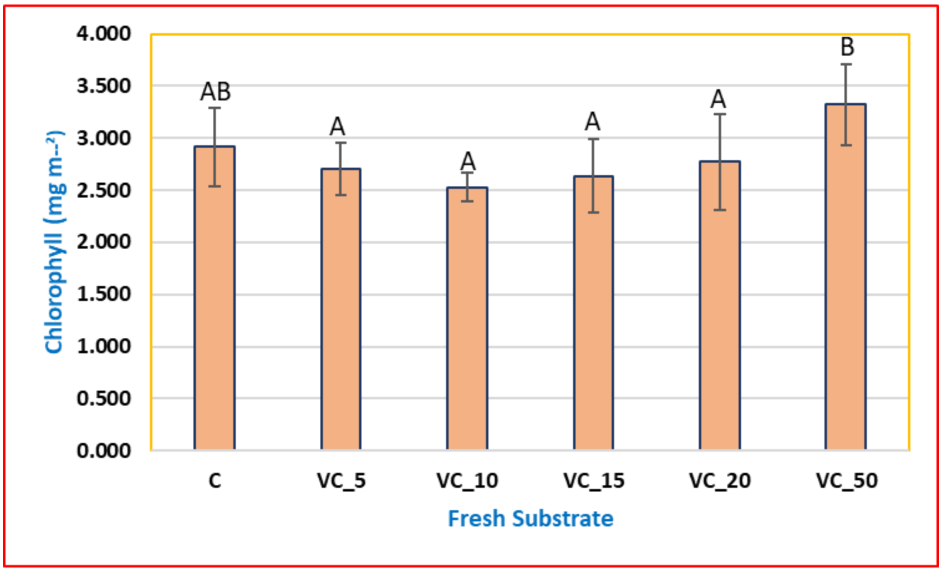

Figure 18: Chlorophyll content of lettuce seedling (mean±sd) grown in different ratio of fresh vermicompost substrate. Differing letters mean significant difference between seedling within a fresh substratefresh substrate of different ratio (p<0,05).

VC_50 shows significant difference with VC_5, VC_10, VC_15, VC_20 whereas VC_50 shows not much difference with C in photosynthetic activity of lettuce seedling.

4.3.2. Lettuce seedling fresh shoot weight

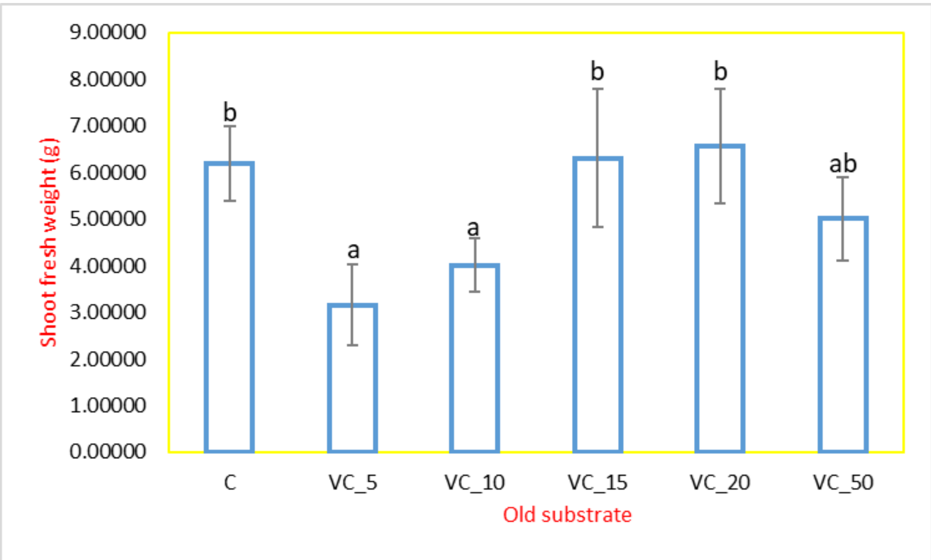

Figure 19: Shoot fresh weight of lettuce seedlings (mean±sd) of different ratio of old vermicompost substrate.

Differing letters mean significant difference between samples within a old substrates of different ratio (p<0,05).

C (6.19 g)shows significant difference from VC_5 (3.15g), VC_10 (4.01g). Whereas VC_5 and VC_10 show significant difference from C, VC_15 (6.31g), VC_20 (6.56g) but VC_50 (5.00g) doesn't show much significant difference from C, VC_5, VC_10, VC_15 and VC_20 in shoot weight.

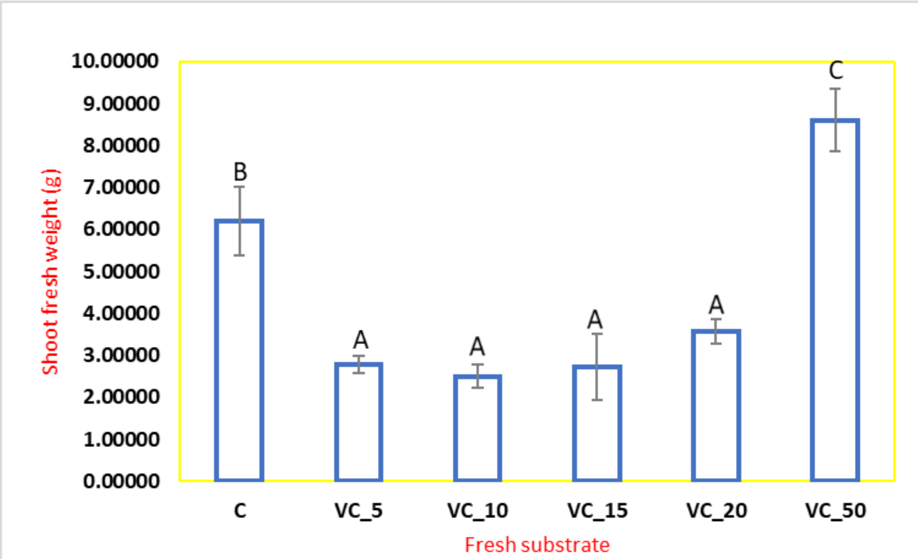

Figure 20: Shoot fresh weight of lettuce seedlings (mean±sd) of different ratio of fresh vermicompost substrate.

Differing letters mean significant difference between samples within a fresh substrate of different ratio (p<0,05).

C (6.19g) shows significant difference with VC_5 (2.77g), VC_10 (2.50g), VC_15 (2.72g), VC_20 (3.57g) and VC_50 (8.59g). Similarly, the significant difference is seen from VC_50 with all other substrates. But there is no significant difference within VC_5, VC_10, VC_15, VC_20 in its shoot fresh weight.

4.3.3. Lettuce seedling fresh root weight

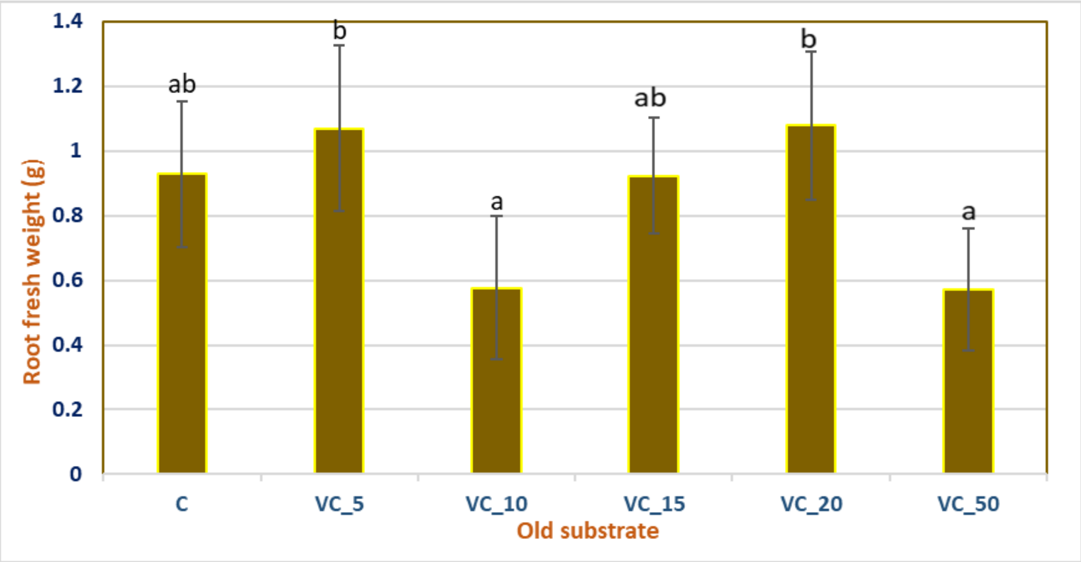

Figure 21: Root fresh weight of lettuce seedlings (mean±sd) of different ratio of old vermicompost substrate.

Differing letters mean significant difference between samples within a old substrates of different ratio (p<0,05)

VC_5 (1.06g) and VC_20 (1.07g) show significant difference between VC_10 (0.57g), and VC_50 (0.57g), but VC_15(0.92g) and C(0.92) show no significant difference from others in root weight.

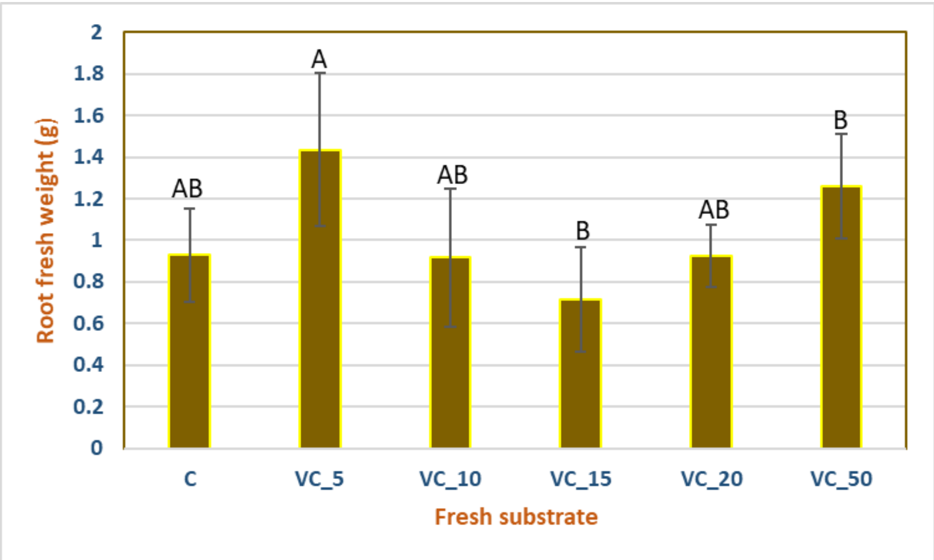

Figure 22: Root fresh weight of lettuce seedlings (mean±sd) of different ratio of fresh vermicompost substrate.

Differing letters mean significant difference between samples within a fresh substrate of different ratio (p<0,05)

VC_5 (1.43g), VC_15 (0.71g) and VC_50 (1.25g) show significant differences in root weight, whereas C (0.92g), VC_10 (0.91g), and VC_20 (0.92g) don't show much significant differences in root weight.

4.3.4. Plant dry matter content of shoot and root

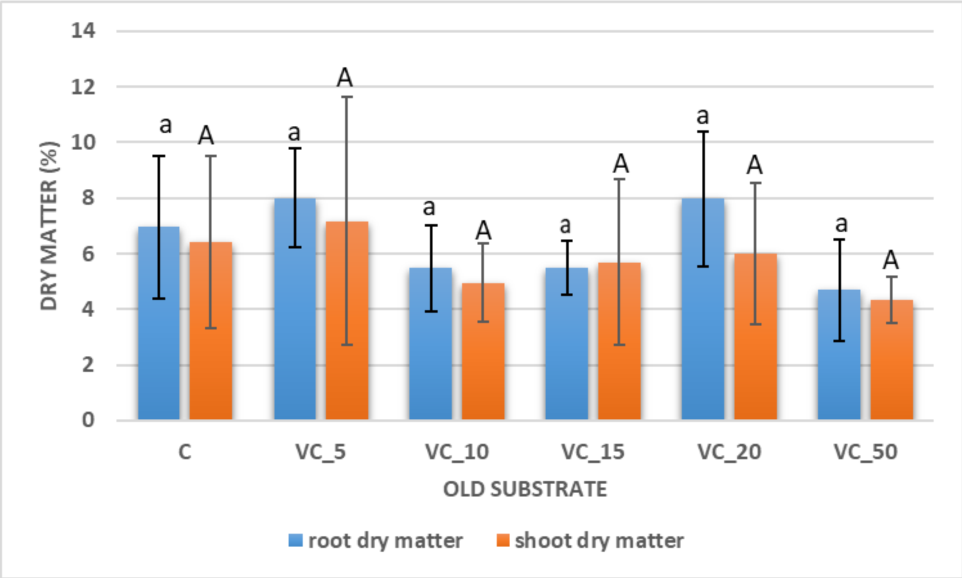

Figure 23: Dry matter content of lettuce seedlings (mean±sd) of shoot and root in old vermicompost substrate

The same letter represents that there is no significant difference observed in dry matter content of root and shoot despite of various ratio of vermicompost treatment in old vermicompost substrate.

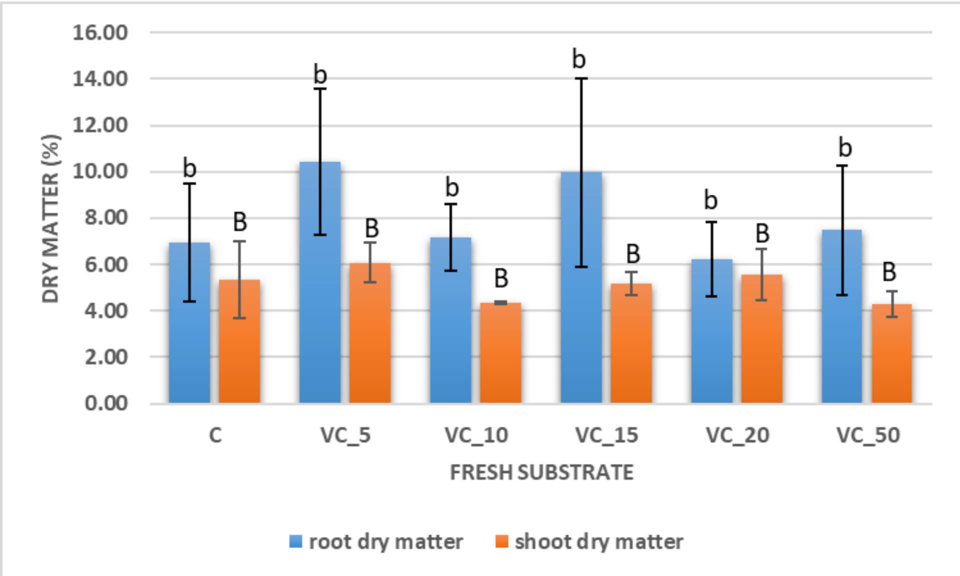

Figure 24: Dry matter content of lettuce seedlings (mean±sd) of shoot and root in fresh vermicompost substrate

The same letter represents that there is no significant difference observed in dry matter content of root and shoot despite of various ratio of vermicompost treatment in fresh vermicompost substrate.

5.DISCUSSION

Eminently I want to highlight that the objective of the experiment was not to conduct a direct comparative analysis of vermicompost based on their age (i.e., Old And Fresh) as several factors were varied throughout the experiment, including the composition of feedstock materials and the known fluctuation in climatic conditions during the vermicomposting process. As it was an experimental setup utilizing kitchen waste, the ingredients varied from week to week, reflecting real-world scenarios where the availability and composition of organic waste may vary. Hence it is the reason why this experiment aims for not direct comparative analysis based on the age of vermicompost.

Effectiveness of germination test

The germination tests with *Lepidium sativum* as it is a sensitive fast-growing plant to assess the maturity and phytotoxicity of the two different substrates (fresh and old vermicompost). As the compost was prepared with different feedstock and climate it is important to test it separately with these two different substrates.

After analyzing the data, it was found that the germination rates of seeds in fresh and old vermicompost substrates did not vary significantly, despite slight fluctuations in different ratios of treatments was observed.

In the old substrates, every treatment was giving equal germination ratio except Vermicompost ratio 50%(VC_50). In the fresh substrates, a little fluctuation is seen Vermicompost ratio 15%(VC_15) resulted in a higher germination ratio than control and Vermicompost ratio 20%(VC_20) was the lowest among all.

This consistency in germination ratio can be attributed to several factors, including the favourable conditions provided by vermicompost, like moisture content, which is crucial for seed germination as it initiates metabolic processes, activates enzyme activity, break down stored food reserves,and softens the seed coat allowing embryo to emerge. Perhaps the nutrients from vermicompost are not really required to sustain early growth as endosperm itself serves as primary source of food storage, able to persist until the seeds attain mature stage called albuminous seeds. Moisture is a critical factor in the germination process. In addition other factors such as temperature, light, and oxygen levels also play crucial roles in the germination process. Also this Optimal conditions for germination vary among plant species.

Hence the germination test with *Lepidium sativum* provided valuable insight into the suitability of different ratios of treatments, were not phytotoxic and they are mature enough and capable for lettuce seedling production. Notably, the maturity of vermicompost is not directly linked with the germination of seeds but there may be changes according to the species. While these germination results may not directly correlate with lettuce seedling growth. Our findings suggest that despite variations in germination rates, the overall performance of lettuce seedlings was comparable across different vermicompost ratios. This highlights the robustness of vermicompost as a substrate for organic lettuce seedling production.

Impact of vermicompost age on lettuce seedling growth

Our analysis revealed notable effects of vermicompost storage time on the growth of organic lettuce seedlings, specifically in terms of shoot and root development. Even though the older vermicompost substrate demonstrates higher levels of shoot and root development in seedlings thereby proving its maturity and stability and suitability for organic seedling over fresh substrate. Similarly, fresh substrate is also exhibited positive result on the seed and seedling condition with some fluctuations, however, the experiment noted that ratio should be highly considered while using vermicompost as a substrate, regardless of whether it is fresh or old substrates. Therefore, we can confidently state that vermicompost can replace peat, regardless of its storage time or fresh usage.

Age-related maturity and phytotoxicity: effects on seedling growth

The results of the soil sample analysis indicate that the maturity of vermicompost plays a critical role in determining its suitability for use in organic seedling production. Old vermicompost exhibits higher maturity levels compared to fresh substrate, as evidenced by increased humic content, total organic matter, and nitrogen levels. This higher maturity is often associated with improved soil structure, nutrient availability, and microbial activity, all of which contribute to enhanced plant growth and development.

However, the analysis also reveals that old vermicompost poses certain challenges related to phytotoxicity. The presence of elevated levels of total water-soluble salts (TSS) in old substrate indicates a higher salinity range, which can lead to osmotic stress in plants, ultimately affecting seedling growth and quality. It is, therefore, important to monitor and manage salt levels in old vermicompost to mitigate phytotoxicity risks.

Interestingly, the analysis indicates lower levels of ammonia in old vermicompost, despite the higher nitrogen content. This discrepancy suggests complex interactions within the vermicompost environment, potentially influenced by microbial activity, nitrogen transformation pathways, and other chemical and physical factors. Resulted in observed fluctuation in seedling shoot and root growth, which can be attribute to varying maturity and phytotoxicity levels of the vermicompost substrates.

Careful consideration of vermicompost ratios with age is the key to balance maturity and phytotoxicity of substrates for effective organic seedling production.

Substitution of peat with vermicompost

The analysis of *Lepidium sativum* using vermicompost revealed interesting findings regarding shoot and root length. Compared to peat-based control In fresh and old vermicompost, except the Vermicompost ratio 50% (VC_50) ratio in old vermicompost, shoot height was higher than the control (peat). However, in the root control, the length was greater than the different ratios of vermicompost utilization as the ratio increased, except for

Vermicompost ratio 10% (VC_10) in both fresh and old vermicompost, where an increased root length was observed.

These results suggest that vermicompost positively influences shoot height compared to peat, indicating its potential to enhance plant growth. The higher shoot height in vermicompost-treated plants may be attributed to the nutrient-rich nature of vermicompost, which can promote above-ground growth. Conversely, the varying effects on root length with different vermicompost ratios indicate a complex interaction between nutrient availability, root development, and the age of the vermicompost.

Factors influencing shoot and root length in vermicompost treated seedlings

The resulted trends in shoot and root length can be attributed to various factors related to maturity and stability of vermicompost. The Shoot length increased with increasing concentration of fresh vermicompost, while old vermicompost treatments showed no significant variation. In fact, an increase in concentration ratio resulted in a decrease in shoot length in old vermicompost treatments. Interestingly, root length decreases as vermicompost concentration increases in old treatments, while fresh treatments show more diverse responses. However, both old and fresh treatments demonstrate a decreasing trend in root length with increasing vermicompost concentration.

The decrease in shoot height with an increase in old vermicompost ratio, can be related to several factor of maturity and stability of vermicompost. as the old compost undergone a longer decomposition process, may show higher content of stable organic components resistant to microbial degradation, the stability shows high amount of humification and lower C/N ratio than the fresh vermicompost (Nogales *et al.*, 2020) and there may be decline of enzymatic activity during the termination phase of vermicomposting (Srivastava *et al.*, 2020). furthermore the presence of nitrifying bacteria can lead to oxidation of NH^{4+} to NO^{2-} and NO^{3-}, which may affect nutrient availability for plants. The higher content of nitrate-N in old vermicompost could potentially influence plant growth, as nitrate is a readily available form of nitrogen for plant uptake (Atiyeh *et al.*, 2000) Hence inverse relationship observed between concentration ratio and shoot length within the old vermicompost treatments could be a result of the complex interactions between nutrient availability, microbial activity, and the stability of organic components in the vermicompost.

The root height decrease with an increased old vermicompost ratio because the maturity and stability alters the root architecture(Atiyeh *et al.*, 2000) as the humic substance increase they enhance the root growth positively, as it regulates plasmalemma (plasma membrane) and the tonoplast in plant cells is the cell membrane system.but it may also vary to different plant species.which is needed for nutrient transportation, storage, maintaining the cell structure.(Zandonadi, Canellas and Façanha, 2006) also according to (Robinson, 1994) higher nutrient availability leads to thicker roots and shorter height, while lower nutrient availability leads to longer roots. This could be the reason for decreased root height in old vermicompost.

Analysis of vermicompost ratio in lettuce organic seedling production

The findings suggest that the use of vermicompost as a soil amendment can have varying effects on the growth and development of lettuce seedlings. While there were observable variations in chlorophyll levels across different substrates when various ratios of vermicompost were employed, no significant differences were observed. Conflicting reports on the effect of vermicompost on chlorophyll content were also noted in the literature. The results indicate that the observed variations in chlorophyll content among different vermicompost treatments may be attributed to factors such as nutrient composition, particularly nitrogen, and humic substances.

Analysis of fresh shoot weights revealed noteworthy variations among different vermicompost ratios, particularly evident in old substrate treatments. Vermicompost ratio 15%(VC_15) and Vermicompost ratio 20%(VC_20) treatments exhibited higher mean shoot fresh weights, suggesting favorable conditions for enhanced shoot growth. In fresh substrates, a consistent increase in fresh shoot weight was observed with the increment of vermicompost ratio, particularly notable in Vermicompost ratio 50%(VC_50), while Vermicompost ratio 5% and 10% (VC_5 AND VC_10) showed relatively lower means, hinting at potential limitations in nutrient availability or other factors affecting shoot development.

Similarly, fresh root weights exhibited variations among different vermicompost ratios, with Vermicompost ratio 5% and 20% (VC_5 and VC_20) treatments displaying higher mean root fresh weights compared to other treatments. Vermicompost ratio 50%(VC_50) treatment exhibited the highest mean root fresh weight, suggesting its effectiveness in promoting root growth under these conditions. Conversely, Vermicompost ratio 10% and 15% (VC_10 AND VC_15) treatments showed relatively lower mean root fresh weights, indicating potential limitations in nutrient availability or other factors affecting root development.

Overall, the results suggest that the use of vermicompost as a soil amendment can have positive effects on the growth and development of lettuce seedlings, with specific vermicompost ratios. However, further studies are needed to determine the optimal vermicompost ratio for lettuce seedling growth and to identify the underlying mechanisms driving the observed effects.

Recommendation for optimal vermicompost ratios in seedling production

We have analyzed various factors, including the age of the substrate, nutrient composition, and seed growth requirements, to determine the best ratio of vermicompost for growing lettuce seedlings. Our study shows that Vermicompost ratio 50%(VC_50) is the optimal ratio for fresh vermicompost, which is even better than Control(C). This ratio consistently demonstrated the highest values for fresh shoot weight, fresh root weight, and dry root weight. For old vermicompost, the optimal ratio appears to be Vermicompost ratio 20%(VC_20), exhibiting the highest values for chlorophyll content, fresh shoot weight, and dry root weight for lettuce seedlings.

Our findings suggest, a balanced ratio that incorporates both fresh and old vermicompost substrates may offer significant benefits for organic lettuce seedling growth and production. However, it is important to consider specific site conditions and production goals when determining the ideal ratio. Our study provides valuable insights into the optimal vermicompost ratio for lettuce seedling production. By following these recommendations, growers can improve their organic lettuce seedling growth and production.

6.SUMMARY

My thesis research focuses on sustainable agriculture and its importance in waste management. I am investigating the potential of vermicomposting as a solution to replace peat moss in seedling production. Specifically, I am testing the effectiveness of different ratios of vermicompost to substitute peat moss for organic lettuce seedling production. My aim is to determine if peat moss can be substituted by vermicompost and, if so, what is the optimal ratio for this substitution.

To conduct my research, I have built categories around two different substrates of vermicompost (fresh and old), two different species of plants (*Lepidium sativum(Garden cress)* and *Lactuca sativa(Lettuce)*), and six different types of treatments for each substrate type with different ratio of vermicompost like Control(C), Vermicompost ratio of 5% (VC_5), Vermicompost ratio of 10%(VC_10), Vermicompost ratio of 15%(VC_15), Vermicompost ratio of 20%(VC_20), Vermicompost ratio of 50% (VC_50).

The methods and material of this work started from June 2023, data collected from January to April 2024Data was collected from vermicompost sample, germination test, shoot and root height, chlorophyll content, and weight of fresh and dry shoot and root, as well as dry matter content of shoot and root, but no direct comparison was made between fresh and old vermicompost. We use two types of control in the experiment for germination test of *Lepidium sativum and Lactuca sativa* we used 100% peat with 0% nutrients, but for lettuce seedling to ensure adequate nutrients supply we used a commercially available certified organic growing media “Klasmann KKS proline (Bio) potground”, instead of using peat alone.

Results have been interpreted accordingly the germination tests of Lepidium sativum didnt show significantly different result. Where germination test is describing that the different ratio of different age (fresh and old vermicompost) are showing substrate suitability for use in organic lettuce seedling production. The data of shoot and root of Lepidium sativum shows that the different ratios of different age fresh and old vermicompost affects seedling growth. Fresh vermicompost with higher ratios favors lettuce seedling production. On the other hand, old vermicompost with higher ratios affects seedling growth. However, it's important to note that while germination rates may not directly correlate with lettuce seedling growth, they serve as an initial indicator of substrate suitability.

The age of the vermicompost did not significantly influence the growth of organic lettuce seedlings. However, the ratio of the vermicompost, in conjunction with its age, showed a significant impact on seedling growth. The observed variations in growth can be attributed to factors such as nutrient release and availability, as well as the stability provided by the substrate for shoot and root development. Overall, this highlights the complexity of the vermicompost ratio and its role in supporting optimal seedling growth.

There were discernible changes in the maturity and phytotoxicity levels of vermicompost corresponding to its age, as indicated by soil sample analysis. The old vermicompost shows higher level of humic content, total organic matter and total nitrogen level it exhibited challenges related to phytotoxicity with elevated level of (TSS)

Total water soluble salt by affecting slightly negative causing fluctuation in growth and quality of organic lettuce seedling.

There was a variation in height, weight of shoot and root in a specific ratio, which highlights the influence of variation is due to the ratio of the vermicompost usage. these variation results in unhealthy root system, not a good growth of the root system along with undesirable shoot yield. so the usage of recommended ratio for both substrates are drawn and presented based on factors such as chlorophyll content, fresh shoot and root weights, and dry shoot and root weight. were these findings underscore the significance of selecting appropriate vermicompost ratios to maximize lettuce seedling growth and quality.

It was found through analysis that a Vermicompost ratio of 50% (VC_50) for fresh vermicompost and a Vermicompost ratio of 20% (VC_20) for old vermicompost were the best ratios for producing lettuce seedlings. These ratios showed excellent performance in promoting growth of both shoots and roots, indicating their suitability for organic seedling production. It's worth noting that the weight of the shoot's fresh produce is important, as it is the edible part. By following these recommendations, growers can enhance the growth and quality of organic lettuce seedlings, contributing to sustainable and eco-friendly seedling production practices.

In conclusion, vermicompost emerges as a promising alternative to peat for organic seedling production, with both fresh and old substrates showing positive results. While old vermicompost demonstrates superior root and shoot development, caution is warranted when adjusting concentration levels. Optimal ratios, such as Vermicompost ratio 50%(VC_50) for fresh and Vermicompost ratio 20%(VC_20) for old vermicompost, exhibit potential for lettuce seedling production. as my experiment doesn't differentiate the two different substrates.

Future research should explore the nuances between this fresh and old vermicompost to optimize seedling growth practices. Furthermore, precise experiments are needed to determine every factor of vermicompost for substituting peat moss, leading to an eco-friendly approach of waste reduction through vermicomposting, as highlighted by Prajapathi's (Prajapati *et al.*, 2023) findings.

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9.ATTACHMENT

DECLARATION

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Title of the document:	Optimal vermicompost ratio for Lettuce seedlings growth
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SUPERVISOR’S DECLARATION

As primary supervisor of the author of this thesis, I hereby declare that review of the thesis was done thoroughly; student was informed and guided on the method of citing literature sources in the dissertation, attention was drawn on the importance of using literature data in accordance with the relevant legal and ethical rules.

Confidential data are presented in the thesis: yes <u>no</u> *

Approval of thesis for oral defense on Final Examination: <u>approved</u> not approved *

Date: Budapest, 2024 April month 19th day

Madaras Krisztina
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Krisztina Madaras (Internal supervisor)

Dr. Kardos Levente
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Dr. Levente Kardos (External supervisor)

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Dr. Anna Divéky-Ertsey (External supervisor)