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TRACKING THE PERCENTAGE OF SEED GERMINATION WHEN THE BASIS FOR THE SPROUTS IS PURE COMPOST AND COMPOST WITH ADDITIVES

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Abstract

The present study aimed to monitor the germination percentage of radishes and spinach in compost mass in pure form and with additives. Six variants and one control (V0) were prepared: V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - Min. + microbial fertilizer for each of the studied crops - a total of 14 samples. An initial reading of the microbial community was carried out after the introduction of the additives and immediately before planting the seeds and then at day 5, day 10 and day 15. The temperature, humidity and pH of the samples were measured. The two considered crops give different germination percentages of the different variants. Spinach seeds generally had difficulty germinating and produced fewer sprouts than radishes. The best results for germination and survival of the sprouts were obtained for the spinach in the control and in the variant with mineral fertilizer and the variant with basil. For the radishes, the best germination was shown by the control and by the variants with microbial fertilizer, the variant with microbial and mineral fertilizer.

Key words*: microorganisms, compost, germination.*

INTRODUCTION

Seed germination and seedling preparation are key moments in agriculture. Seed germination is an important process that is influenced not only by external factors, but also by the physicochemical properties of the seed covering tissues (Martin et al., 2010). In their study, Baskin & Baskint et al. (2014) point out that the main factors influencing germination are temperature, water content, the presence of good aeration, light, etc. Some studies have focused on the limiting effect of some environmental factors on seed germination. Thus, in their study, Ghosh et al. (2014) indicated high salinity as detrimental to the development and germination of radish. Temperature is another important factor that can stimulate or inhibit seed germination (Abdel et al., 2016). In a number of studies, temperature and humidity have been identified as the main limiting factors, given their direct involvement in the activation and inactivation of various enzymes responsible for initiating the germination process (Bradford, 2002; Khaeim et al., 2022). In practice, a number of methods for pre-treatment of seeds are applied, with the aim of their easier and more sustainable germination (Kanjevac et al., 2022). It has also been found that the application of bio-priming with certain strains of bacteria increases the germination of radishes (Kaymak et al., 2009). Unfortunately, sometimes germination is unsuccessful due to the use of an unsuitable substrate, often contaminated with certain pathogens that can cause damping off of the seeds. There are a number of articles that emphasize the need for a sterile environment for germination precisely to avoid the unwanted weakening, and sometimes death, of already germinated seeds as a result of pathogenic species (Davoudpour et al., 2020). However, the procedures are often crop-specific and may result in damage to the treated seeds themselves (Sauer & Burroughs, 1986). In general, sterile media ensure the purity of germinating seeds, including the possible presence of pathogens. However, the provision of sterile media is associated with certain economic costs. The use of compost mixes is a potential solution. The positive changes that occur when compost is applied have been established in a number of studies (Hassan et al., 2023). However, there are certain aspects in which compost fertilization, for example, leads to a lower number of inflorescences in lavender compared to plants fertilized with mineral fertilizer (Manescu &

Dudau). Compost has a wide range of applications. However, pathogens are sometimes found in the finished compost, despite the high temperature levels during the thermophilic phase (Gurtler et al., 2018). Given the above, it is advisable to use a compost mix without the presence of animal manure, which minimizes the risk of potential contamination with pathogens.

Another problem mentioned in the literature is the structure and density of the compost, which hinders germination due to the resistance exerted by the roots. It is important to note that similar problems can occur when using poor quality compost and one that has not reached maturity. Quality and mature compost has a soil-like structure of the aggregates. The possibility of using compost as a germination base is related to the final characteristics of the compost. Thus, in their study (Yang et al., 2021), point out the potential toxicity of compost with a predominant share of cow manure, towards germination. Such toxicity is almost completely absent in mature compost, the starting substrates of which are only plant residues. However, the potential moment for the presence of certain pathogens in compost produced on the territory of agricultural farms remains.

There are previous studies of ours that address the possibility of compost decontamination by adding certain plants or plant aqueous extracts, such as lavender, thyme, etc. (Grigorova-Pesheva et al., 2024). Similarly, in their study, Barthod et al., 2018, indicate the possibility of using specific additives that can suppress the development of pathogenic species and improve the quality of the finished compost. Given that compost is an easily generated product from waste plant residues, its use as a basis for seed germination is a prerequisite for reducing costs for any farm. For the present study, radishes and spinach were chosen considering that the first crop is fast-germinating, and the second is indicative of the condition and characteristics of the soil.

The main goal of this study was to track the germination rate of seeds using pure compost and compost with additives as seed base.

MATERIALS AND METHODS

For the purpose of this study, spinach and radish seeds were used. Compost material was placed in sterile plastic containers to serve as a base for seed germination. The parameters of the ready-made compost used for seed germination were previously determined as follows: C:N ratio was 14:1, the acidity of the medium was neutral to slightly alkaline - 7.2, the content of total nitrogen and organic carbon was 1.76% and 4.64%, respectively, the sieving of the compost showed that it had a suitable structure и. The initial microbiological characteristics of the starting compost were determined, including the total microbial number and the amount of individual microbiological groups. Then the compost was distributed among the experimental vessels and the additives were introduced. For the purpose of the experiment, six variants and one control were prepared for each of the tested cultures. The variants are as follows: V0 - control, V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - Min. + microbial fertilizer. A total of 14 containers were prepared, six variants for spinach and six identical variants for radishes + two controls. The temperature and humidity were measured daily in order to maintain optimal moisture levels necessary for the germination process. The acidity of the medium was recorded. In the first six variants, ten high-quality radish seeds were placed, and in the second, ten high-quality spinach seeds. For each of the variants, the total microbial number and the quantity of individual microbiological groups were recorded on the 5th, 10th and 15th day in separate identical containers in order not to damage the seeds. To determine the amount of non-spore-forming bacteria, nutrient agar was used, and the cultivation was carried out at 24°C for 2 days. To determine the bacilli, the samples were pre-pasteurized, with subsequent inoculation on nutrient agar and cultivation under the same conditions. To determine the fungi and actinomycetes, Čapek dox agar and Starch ammonia agar were used, respectively. Cultivation was for 7 days at the corresponding temperatures - 35°C for actinomycetes and 30°C for fungi. The results were averaged based on five-fold measurements. The standard deviation was statistically distributed. The number of germinated seeds for each variant was determined, as well as their condition.

RESULTS AND DISCUSSIONS

The results of microbiological analyses are presented in Table 1. In all variants, including the control, we have an increase in microbial abundance. Compared to the control, the increased amount of microorganisms is due to the introduction of water, for the purpose of comparability with the other results, in which optimal humidity is maintained.

Table 1. Main microbiological parameters (CFU*10^6/g compost)

	Variants	TMN	Non-spore-forming bacteria	Spore-forming bacteria	Actinomycetes	Mucoromycetes	Temperature (°C)	pH	Relative humidity (%)
Initial phase		3.93	2.15	0.60	0.27	0.30	21.2	6.56	30.80
5th day	C	6.05±0.83	3.18±0.89	1.01±0.11	0.81±0.30	0.05±0.06	21.5	6.47	31.7
	V1	6.47±0.83	3.28±0.89	1.11±0.11	0.87±0.30	0.10±0.06	21.0	6.53	30.8
	V2	6.65±0.83	3.19±0.89	1.29±0.11	0.77±0.30	0.10±0.06	21.1	6.48	32.6
	V3	6.69±0.83	3.19±0.89	1.09±0.11	1.18±0.30	0.13±0.06	21.0	6.31	31.6
	V4	6.13±0.83	3.18±0.89	0.91±0.11	1.01±0.30	0.13±0.06	21.1	6.39	31.7
	V5	5.54±0.83	1.44±0.89	1.09±0.11	1.68±0.30	0.24±0.06	21.5	6.57	30.4
	V6	4.37±0.83	1.38±0.89	1.01±0.11	0.87±0.30	0.10±0.06	22.0	6.52	31.0
	V7	4.99±0.83	1.66±0.89	1.09±0.11	1.06±0.30	0.08±0.06	22.0	6.47	29.3
10h day	C	5.91±0.53	2.52±0.43	1.08±0.14	1.18±0.25	0.07±0.02	22.5	6.54	48.25
	V1	6.45±0.53	3.29±0.43	1.11±0.14	0.87±0.25	0.07±0.02	22.0	6.50	48.74
	V2	7.14±0.53	3.33±0.43	1.43±0.14	0.84±0.25	0.12±0.02	22.4	6.54	49.20
	V3	6.05±0.53	3.28±0.43	1.06±0.14	0.59±0.25	0.07±0.02	22.0	6.48	48.02
	V4	6.40±0.53	3.24±0.43	0.92±0.14	1.21±0.25	0.10±0.02	22.0	6.30	48.75
	V5	5.63±0.53	2.18±0.43	1.09±0.14	1.18±0.25	0.08±0.02	23.0	6.42	49.55
	V6	6.12±0.53	3.02±0.43	1.06±0.14	0.87±0.25	0.10±0.02	22.5	6.50	52.01
	V7	7.07±0.53	3.31±0.43	1.14±0.14	1.34±0.25	0.13±0.02	22.5	6.47	50.17
15h day	C	6.00±0.59	2.55±0.43	1.09±0.14	1.18±0.23	0.08±0.03	22.0	6.54	50.25
	V1	6.84±0.59	3.38±0.43	1.18±0.14	1.01±0.23	0.10±0.03	22.7	6.49	51.74
	V2	7.39±0.59	3.36±0.43	1.46±0.14	0.97±0.23	0.13±0.03	22.5	6.48	50.24
	V3	6.72±0.59	3.36±0.43	1.16±0.14	0.94±0.23	0.10±0.03	22.6	6.51	50.35
	V4	6.03±0.59	3.29±0.43	0.97±0.14	0.67±0.23	0.12±0.03	22.4	6.30	51.00
	V5	5.95±0.59	2.27±0.43	1.16±0.14	1.24±0.23	0.12±0.03	22.5	6.42	49.89
	V6	6.50±0.59	3.16±0.43	1.13±0.14	0.97±0.23	0.12±0.03	22.5	7.20	51.11
	V7	7.41±0.59	3.39±0.43	1.21±0.14	1.43±0.23	0.17±0.03	22.8	6.52	50.36

Optimal humidity is the only controlled parameter, in order to ensure optimal conditions for seed germination. The microbial number data show interesting dynamics (Figure 1). On the fifth day of the experiment, all of the studied variants had a higher microbial number compared to the initial phase. Variant V2 with thyme and V3 with basil stood out with the greatest increase in microbial abundance. The microbial number of the variant with introduced mineral fertilizer increased the least. Similar data indicate certain difficulties for the autochthonous microflora when introducing mineral fertilizer into the compost. This is probably due to the moment of adaptation and the specificity of its absorption by the microbial community.

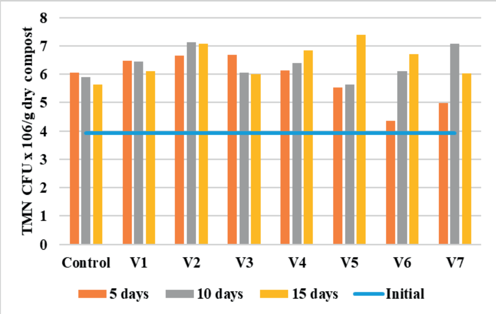

Figure 1. Total microbial number of the tested variants

The variant with only a microbial supplement, as well as the variant with a mix of mineral and microbial supplements, also provide a smaller increase in the total number of microorganisms compared to the variants with plant-based aqueous extracts.

Seed analysis showed initiation of germination in radish seeds, while no development was observed in spinach seeds by day 5. This is an expected result, given that spinach seeds require a longer germination phase.

On the tenth day, the amount of microorganisms in the control decreases. For the variants with plant extracts, we have different dynamics. For Variant 1 and Variant 3, we observe a very slight decrease in microbial abundance, while for Variants 2, 4 and 5, an increase in microbial abundance is observed. The variant with the microbial supplement also shows a slight increase. The data for Variant 6 are interesting, which on the fifth day had the lowest recorded microbial number. On the tenth day, a visible increase in microbial abundance is observed. This indicates that the microbial biota has adapted and has begun to absorb and transform the supplement introduced into the environment. Variant 7 with a mix of microbial and mineral supplement shows the greatest increase in the total microbial number. Variant 2 and Variant 7 stand out with the highest microbial abundance on the tenth day.

On the 10th day after sowing, a higher number of well-developed radish sprouts were observed in the Control (100% germination), Variant 5 (100% germination) and Variant 7 (100% germination). In spinach, sprouts began to develop in the Control (60% germination), Variant 3 with basil (40% germination) and Variant 6 with mineral fertilizer (40% germination). In general, radishes germinate more easily than spinach, regardless of the different speed. In radishes, nice, healthy and fresh sprouts are observed.

On day 15, we again have specific dynamics in the change of microbial communities in the different variants. In the control, the microbial abundance continues to decrease. In addition to the control, a decrease in total microbial number was also recorded in Variant 1, with lavender extract. The variant with a mix of mineral and microbial fertilizer also shows reduced levels of microbial abundance. With a higher total microbial number compared to day ten, V3, V4, V5 and V6 were recorded. Variant with microbial supplement showing the greatest increase.

On the fifteenth day, the trend in the development of sprouts is maintained. Spinach is experiencing visible difficulties. Some of the sprouts that have appeared show signs of damping off. There is a very low percentage of germination overall. Again, the control gives better results than the variants with additives (60% germination). This shows that the use of compost containing the studied additives is not appropriate for use as a basis for the germination of spinach sprouts. We believe that the pure compost used is also not suitable, since even in it the germination is not high enough. In addition, in all variants in which there are germinated seeds, including in the control, some of the sprouts show signs of damping off.

In radishes, there is a better germination rate, with only the variant with mineral fertilizer showing certain signs of damping off. This is also the variant with the lowest germination rate (40%). The variant with lavender (50%) also had a relatively low germination rate. On day 15, the variants with the highest germination rate for radishes were the Control (100% germination, with well-formed and fresh sprouts), the Variant with microbial supplement (100% germination, with well-formed and fresh sprouts), the Variant with mineral and microbial fertilizer (100% germination, with well-formed and fresh sprouts). Variant 3 with basil (90%) and Variant 2 with thyme (80%) showed very good germination. The generated data show that, unlike spinach, compost in its pure form, as well as with additions of mineral and microbial fertilizer, as well as with only a microbial additive, are a good basis for radish germination.

The present study did not find a direct correlation between total microbial number and germination percentage.
For a more detailed analysis, the change in the percentage of individual microbiological groups in the experimental variants was monitored (Figure 2). In the initial phase, the group of non-spore-forming bacteria is dominant. The data show that on day 5, in all variants including the control, the percentage of micromycetes decreases dramatically. This is probably due to the reduced aeration in the containers. Micromycetes are highly sensitive to reduced aeration, in addition, the increased humidity, in order to germinate the seeds, probably also had an impact on this microbial group. Of interest are the data from Variants 5, 6 and 7. In them, after the introduction of the additive, we have a decrease in the percentage of the group of non-spore-forming bacteria at the expense of the development of actinomycetes and bacilli.

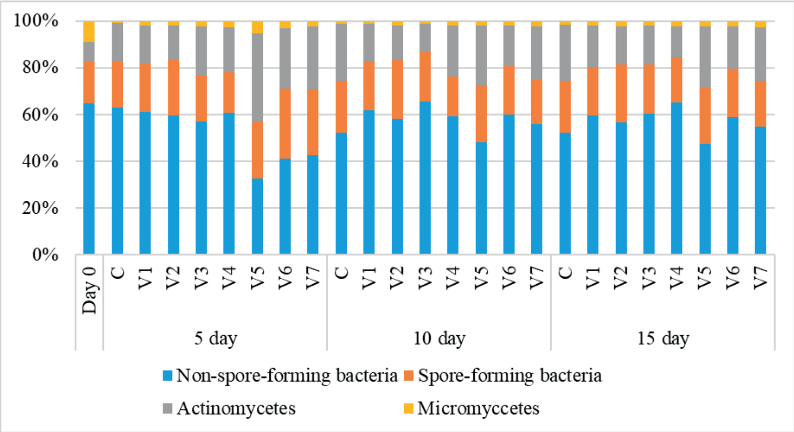

Figure 2. Percentage participation of individual microbial groups

This is most strongly expressed in Variant 5 with microbial supplement. Such a sharp change in the percentage of the main microbiological groups is associated with the introduction of microflora that is allochthonous to the compost mass, which temporarily enters into competitive relations with allochthonous microorganisms. Given that bacteria make up a major percentage of the microbial community, they experience this influence the most. Probably because of this, actinomycetes have temporarily increased their percentage. On the tenth and 15th day, a uniformity of the microbial community is observed, regardless of the studied variant. Again, the group of non-sporeforming bacteria dominates, followed by the group of bacilli and actinomycetes. Micromycetes occupy a minimal share in the microbial community. After performing an analysis for a potential relationship between the distribution of microbial groups and the degree of germination, no such relationship was found.

CONCLUSIONS

The present study aimed to track the percentage of seed germination when the basis for the sprouts is pure compost and compost with additives. The present study did not find a direct correlation between the total microbial number and the percentage of individual microbial groups and the germination percentage. During the experiment, the amount of total microflora in the control decreased over time.
The results for the experimental variants are heterogeneous. Thus, on the fifth day, the variants with imported plant water extracts showed a higher total microbial number compared to the control, while the variants with mineral and microbial additives showed lower values compared to the control. On the fifth day, we found signs of germination only in the variants with radishes.
On the tenth day, only Variants 1 and Variant 3 showed a minimal decrease in their microbial abundance, while an increase was recorded in

the others. On the 10th day after sowing, a higher number of well-developed sprouts were present in the Control (100% germination) and Variant 5 (100% germination) and Variant 7 (100% germination) for radishes.

In spinach, sprouts began to develop in the Control (60% germination), Variant 3 with basil (40% germination) and Variant 6 with mineral fertilizer (40% germination). In general, radishes germinate more easily than spinach, regardless of the different speed. Nice, healthy and fresh sprouts are observed in radishes.

On the 15th day, microbial abundance continues to decrease in the control and Variants 1, 3 and 7.

On the fifteenth day, the data show the appearance of damping off in a large part of the spinach sprouts. Again, the control gives better results than the variants with additives (60% germination). This shows that the use of compost containing the studied additives is not appropriate for use as a basis for the germination of spinach sprouts.

In radishes, there is a better germination rate, with only the variant with mineral fertilizer showing certain signs of damping off. This is also the variant with the lowest germination rate (40%). On day 15, the variants with the highest germination rate for radishes are the Control, the Variant with microbial additive and the Variant with mineral and microbial fertilizer.

The generated data show that, unlike spinach, compost in its pure form, as well as with additives of mineral and microbial fertilizer, as well as with only microbial additive, are a good basis for the germination of radishes.

In conclusion, we believe that compost in its pure form, as well as with imported mineral and microbial additive can be used for the germination of radishes, but not spinach. Overall, further studies are needed on a wider range of crops to enrich the knowledge on the possibility of using compost and compost with additives as a germination medium.

The present study can be used as a basis for future similar studies, as well as a basis for various developments related to the influence of different additives on the microbial community of finished compost.

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