

Bitki Koruma Bülteni / Plant Protection Bulletin

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Original article

Determination of suppression efficiency of vermicompost extracts on some aerial fungal plant pathogens

Vermikompost ekstraktlarının bazı havai fungal bitki patojenleri baskılama etkinliğinin belirlenmesi

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ARTICLE INFO

Article history:

DOI: 10.16955/bitkorb.1238852

Received : 18-01-2023

Accepted : 09-03-2023

Keywords:

vermicompost extract, horse, cattle, plant, fungal pathogen

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ABSTRACT

The use of vermicompost in agriculture has become increasingly common, especially in organic agriculture. In this research, the suppression efficiency of horse and cattle vermicompost extracts against some aerial fungal plant pathogens (*Monilinia laxa*, *Cytospora leucostoma*, *Botryosphaeria obtusa*, *Phomopsis viticola*, *Alternaria mali*, and *Botrytis cinerea*) that damage fruit trees and are common in fruit production areas, was studied for the first time in our country. 25%, 50%, 75%, and 100% concentrations of horse and cattle extracts were tested against fungal plant isolates. The results were evaluated by two-way ANOVA testing the suppression rates of vermicompost extracts in petri plates. While the best results were found in *M. laxa* (90.6%), *P. viticola* (80.2%), *A. mali* (65.1%) at 100% concentration for horse and cattle vermicomposts respectively, the suppression of *B. obtusa* (44.7%), *C. leucostoma* (38.1%), and *B. cinerea* (35.5%) was found to be lower.

INTRODUCTION

While the population of the world has been increasing, the use of limited agricultural resources has become important. Studies conducted in recent years show that vermicompost not only provides nutrients to the soil and improves soil's physical properties, but also suppresses some plant diseases. Therefore, alternative management methods to control diseases and pests that cause damage in terms of quantity and quality in agricultural products are gaining importance. Synthetic chemicals are widely preferred for the control of diseases and pests of cultivated plants as they are practical, easily applicable, and low-cost compared to other control methods. However, chemicals used for this purpose have

brought problems that threaten the environment and human health. In particular, the widespread and uncontrolled use of synthetic chemical plant protection products causes pollution of soil, surface, and underground waters, as well as carcinogens, and teratogens (gene disruption), etc. has become a threat to human and environmental health. Since the use of pesticides causes irreversible disasters, some active substances have been banned or restricted. For similar reasons, organic farming has come to the fore from sustainable farming methods. In sustainable agriculture, cultural and biological control is preferred rather than chemical.

In the process of vermiculture (worm cultivation/worm cultivation), it is generally defined as obtaining vermicompost by using all kinds of organic residues or wastes in a mixture as suitable food for worms. The vermicompost obtained in this process is called vermicompost. Vermicompost, also called "black gold" (Kangmin 2005, Patangray 2014), is obtained in its simplest form by digesting organic residues or wastes by worms and turning them into fertilizer. Vermicomposts are one of the most popular organic fertilizers recently due to their slow release and the physical, chemical, and biological improvements provided by the soil (Yağmur and Eşiyok 2016).

The most important feature that distinguishes vermicompost from other organic fertilizers is its richness in microbial diversity and biomass. The high amount and diversity of microorganisms ensure that vermicompost products are rich in enzymes and hormone-like chemicals produced by microorganisms. In addition, the high aggregation stability of vermicompost ensures that microbiological factors and plant nutrients can be used by the plant for a long time. This feature gives vermicompost a "slow-release fertilizer" feature (Erşahin 2013).

These microorganisms found in vermicompost increase the rate of decomposition of the surrounding organic matter in the soil and facilitate the digestion of the organic matter by the worms. It has been reported that these vermicomposts contain biologically active substances such as plant growth regulators, which are used in plant production as organic fertilizer, soil conditioner, and plant disease and pest control (Edwards and Bohlen 1996).

An average of 30 thousand tons of vermicompost is produced annually in our country. Vermicompost is also effective in suppressing plant diseases and pests. Studies in this area continue to gain momentum around the world and in our country as well (Anonymous 2020). Researches based on the suppression of bacterial and fungal pathogens, which is a problem in plant production, with vermicompost extracts were also conducted around the world. In this area, it has been revealed that especially *B. cinerea* and *M. laxa* (Scheuerell and Mahaffee 2002), *A. alternata* (Din 2018), *P. viticola* (Edwards et al. 2006) are suppressed by vermicompost extracts. In our study related to the suppression of aerial plant fungal pathogens with vermicompost extracts, it was also observed that the growth of *C. leucostoma* and *B. obtusa* were inhibited in vitro.

Scientific studies on vermicompost are not yet at the desired level in our country. In particular, sufficient studies have not been carried out on the possibilities of using vermicomposts in terms of plant health. It is necessary to research the

methods of control that can be alternative to pesticide usage against plant diseases and pests, to increase the studies on this subject and to establish the necessary data and information infrastructure. For these reasons, this study, it is aimed to study the alternative control possibilities of horse and cattle vermicomposts in phytosanitary conditions under laboratory conditions. We preferred horse and cattle vermicomposts because horse and cattle vermicomposts, especially cattle manure, are easily and abundantly available and have some advantages over other fertilizers in composting.

MATERIALS AND METHODS

Horse and cattle manure and vegetable wastes were used in the production of vermicompost; isolates of fungal plant pathogens *M. laxa*, *P. viticola*, *A. mali*, *C. leucostoma*, *B. obtusa* and *B. cinerea* constitute the main materials used in the study.

M. laxa, *P. viticola*, *A. mali*, *C. leucostoma*, *B. obtusa*, and *B. cinerea* isolates constituted the main material of the study. Horse and cattle manure and vegetable wastes for the production of vermicompost were put to use as the other's main materials.

Obtaining Vermicomposts

Horse and cattle vermicompost was obtained from Çankırı Karatekin University, Faculty of Forestry in solid form, and horse and cattle manure with a carbon/nitrogen ratio of 25:1 was used. Humidity was around 70%. Fruit and vegetable wastes were given weekly to the worms in pieces not exceeding 1 cm and 3 times the mass of the worm. Vermicompost was obtained by separating from worms after 3 months and these vermicomposts were used in this study.

Preparation of liquid vermicompost extracts

Vermicompost (worm castings) was mixed with 1:2 (vol; volume) distilled water and the mixture was left at room temperature for 24 hours. The extract was freshly prepared for each application. Vermicompost water mixture was filtered with filter paper (whatman No: 2) and this filtrate was used as "stock filtrate".

Determination of the efficacy of vermicompost extracts against fungal isolates

The isolates obtained from the field and kept on slanted agar, together with the isolates that were previously tested for pathogenicity and stored at -20 OC in slanted agar, were included in the trial. The experiment was set up in a randomized plot design with 10 replications. The effects of the extracts on the mycelial growth of the fungal isolate in petri dishes were tested at different doses. The stock filtrate

was applied at 0% (control), 25%, 50%, 75%, and 100%. 20 µl of the vermicompost filtrate was taken and spread on PDA (potato dextrose agar) medium using a sterile glass baguette. Mycelial discs of 3 mm in diameter were obtained using a cork-borer from the fungal pathogen isolate cultures included in the experiment and incubated for 7-10 days. These mycelial discs were placed in the center of the petri dishes treated with vermicompost filtrate. Petri dishes were kept for the development of the pathogen in the incubator at the appropriate temperature (22±2 OC). As a result of the incubation, when the colonies in the control petri dishes approached the edge of the petri dish, the growth of the colonies in all the petri dishes was measured and recorded as a radius. Colony development in the petri dishes was proportional by measuring the radius of the colonies in the control petri dishes (Levins et al. 2017). Then, the biological percentage efficiencies of the treatments were calculated by the Abbott formula (Abbot 1925).

RESULTS AND DISCUSSION

In this research, the suppression efficiency of vermicompost extracts against the aerial fungal plant pathogens *M. laxa*, *P. viticola*, *A. mali*, *C. leucostoma*, *B. obtusa*, and *B. cinerea* in vitro was investigated.

Differences were found in the effects of 25%, 50%, 75%, and 100% doses of horse and cattle vermicompost according to the diseases. The relationships between pathogen-dose (P<0.01), fertilizer-dose (P<0.01), and pathogen-fertilizer (P<0.01) were found to be significant. Disease, dose, and fertilizer interaction were not significant.

In the study, the effects of different doses of vermicompost on aerial plant fungal pathogens were examined. The relationship between dose and pathogen is given in Figure 1 showing the suppression of diseases in different doses of vermicompost extracts.

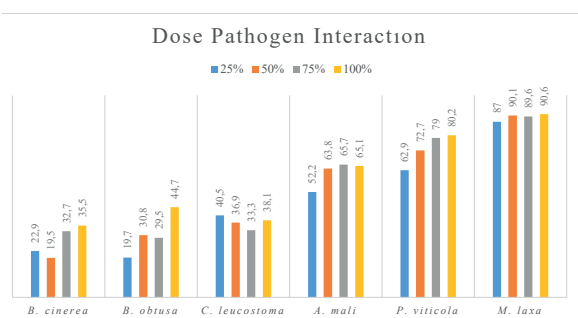


Figure 1. Effect of different doses of vermicompost extracts on aerial fungal plant pathogens.

As it is indicated in Figure 1, 100% doses of vermicomposts were found to be effective in general. It was determined that *M. laxa*, which is especially found in stone fruits and known

as “blossom blight”, was suppressed at the highest rate with 90.6%. While the suppression levels were found to be 80.2% for *P. viticola* and 65.1% for *A. mali*, the suppression rates for *B. obtusa*, *C. leucostoma*, and *B. cinerea* were 44.7%, 38.1%, and 35.5% respectively.

In terms of the pathogen suppression effect of vermicompost types, horse vermicompost suppressed *M. laxa* at a higher rate than cattle vermicompost with 90.9% (Figure 2). Based on the ranking of horse vermicompost according to the suppression rates for *P. viticola*, *A. mali*, *B. obtusa*, *C. leucostoma*, and *B. cinerea* were 69.8%, 56.9%, 28.6%, 24.1%, and 15.6% respectively.

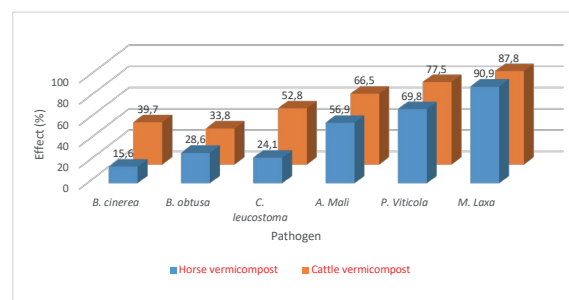


Figure 2. Effect of different doses of vermicompost extracts on aerial fungal plant pathogens.

Cattle vermicompost inhibited the development of the pathogen by suppressing *M. laxa* at the highest level with a rate of 87.8%. The suppression rates for *P. viticola*, *A. mali*, *C. leucostoma*, *B. obtusa*, *B. cinerea* were 77.5%, 66.5%, 52.4%, 33.8%, and 39.7% respectively.

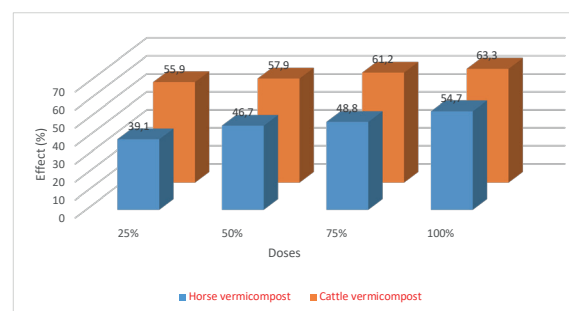


Figure 3. Effect of different doses of vermicompost extracts on aerial fungal plant pathogens.

When the average efficiency of horse and cattle vermicompost extracts at different doses was evaluated, cattle vermicompost doses gave more effective results than horse vermicompost doses. The dose efficiency of cattle vermicompost was 63.3% at 100% dose, 61.2% at 75% dose, 57.9% at 50% dose, and 55.9% at 25% dose. Horse vermicompost was found to be 54.7% at 100% dose, 48.8% at 75% dose, 46.7% at 50% dose, and 39.1% at 25% dose (Figure 3).

Earthworms, which positively affect plant production, increase soil structure and fertility, as well as improve the balance of the soil with nutrition and gallery opening activities. They increase the water-holding capacity and soil porosity in the soil, as well as support plant root growth. Besides, they are effective in reducing the rate of root diseases significantly. Plants grown on vermicompost applied to soils are more resistant to diseases and pests. The antibacterial and antifungal effect of vermicompost on plants is due to the coelomic fluid that worms secrete out of their bodies for various reasons. Enzymes and proteins such as agglutinin, fetidin, lumbricidin, and chitinase are found in the structure of the coelomic fluid that mixes with the environment where they live for months. Therefore, vermicompost is effective against some fungi, bacteria, and pests that contain chitin in their structure, weakening the negative effects of many diseases and pests that contain chitin in their structure (Mısırlıoğlu 2011, Tutar 2013)

In this study, vermicomposts suppressed *M. laxa* at the highest rate, which is one of the aerial fungal pathogens of plants. Vermicomposts had an effect of 90.6% against the pathogen. Scheuerell and Mahaffee (2002) showed that compost suppressed *M. laxa*, which is the causative agent of monilia disease in cherries. The study has similar results with the vermicompost suppression of *M. laxa*. The suppression rates of horse and cattle vermicompost extract were found as 90.9% and 87.8%. Although horse vermicompost appeared to be more successful, both types of vermicompost showed a high level of success in suppressing plant pathogens.

Vermicomposts suppressed the *P. viticola*, grapevine downy mildew, at a high level of 80.2%. Edwards et al. (2006) stated that *P. viticola* was suppressed with vermicompost applications in the field. This result confirms the conclusion obtained with the study. Cattle vermicompost and horse vermicompost were effective in suppressing *P. viticola* at 77.5% and 69.8%.

Vermicompost extract prevented the growth of *A. mali* at 65.1%, which causes leaf spot in apples. Bharadwaj et al. (2014) examined the vermicomposts according to their fungus growth inhibitory (fungistatic) status. It was determined that it had a fungistatic effect against the pathogen as a result of 10-day vermicompost applications. It has been observed that the application of vermicompost to the soil at a rate of 25% prevented the germination of the conidia by 51% to 78%. As a result of this study, the inhibition of the development of *A. mali* with vermicompost extract is similar. In another study, comparing the spray of traditional compost and compost teas on tomato plants, it was found that compost extracts suppressed *A. alternata* more than

conventional composts (Din et al. 2018). These results are in agreement with the results we obtained with vermicompost extracts for the suppression of *A. mali*, which is the pathotype of *A. alternata*. Cattle and horse vermicompost extracts prevented *A. mali* at 66.5% and 56.9%.

B. cinerea is a polyphagous fungal pathogen that causes damping off in plants. It was observed that a 100% dose of vermicompost extracts provided 35.5% suppression against the pathogen. Similarly, Arancon et al. (2007) reported that *B. cinerea* was suppressed at 50% with vermicompost applications. Soylu et al. (2020) reported that bacterial microbiomes obtained from vermicomposts suppressed the growth of *B. cinerea* between 3.44% and 57.18% in vitro. Koné et al. (2010) also revealed that the compost extract obtained from cattle manure suppressed *B. cinerea* at 95% compared to the others. In this study, the suppression effects of horse and cattle vermicomposts on *B. cinerea* were 15.6% and 39.7%, respectively.

The first research on the suppression of the *C. leucostoma*, known as *Cytospora* canker, with vermicompost products under in vitro conditions was performed within the scope of this study. The results showed that while horse vermicompost suppressed *C. leucostoma* at 24.1%, cattle vermicompost was found to be effective at 52.4%.

B. obtusa, which causes fruit rots, leaf spots, stem and branch cankers, gummosis, and dieback of pome and stone fruit trees (Kurbetli and Demirci 2014) was carried out in vitro for the first time within the scope of this study. The results have demonstrated that the horse and cattle vermicompost extract suppressed *B. obtusa* by 28.6% and 33.8% respectively.

The cattle vermicompost extracts we tested gave better results than horse vermicompost extracts in suppressing aerial plant fungal pathogens and in the effectiveness of doses. Our results are consistent with Shanmugasundaram et al. (2013).

In our study; horse and cattle vermicompost extracts were demonstrated to have the potential to suppress aerial fungal plant pathogens. The results show that the vermicompost extracts have the potential to be used in the biological control of the aerial fungal plant pathogens *M. laxa*, *P. viticola*, and *A. mali*. In addition, vermicompost extracts are promising against *C. leucostoma* and *B. obtusa* fungal agents, which were first tried out in this study. Different materials and disease agents are required to perform studies on different scales (laboratory, greenhouse, field) for the results to be used in practice. In this context, the results gained from the study have the potential to form the fundamental and guide for future researches.

ACKNOWLEDGEMENTS

I would like to offer my special thanks to Prof. Şebnem KUŞVURAN, Prof. Sabit ERŞAHİN, Associate Prof. Yurdagül ŞİMŞEK and Dr. Arda ÖZEN.

ÖZET

Tarımda vermicompost kullanımı, özellikle organik tarımda giderek yaygınlaşmaktadır. Bu çalışmada, ülkemizde ilk kez meyve ağaçlarına zarar veren ve meyve üretim alanlarında yaygın olan bazı havai fungal bitki patojenlerine (*Monilinia laxa*, *Cytospora leucostoma*, *Botryosphaeria obtusa*, *Phomopsis viticola*, *Alternaria mali* ve *Botrytis cinerea*) karşı at ve sığır vermicompost ekstraktlarının baskılama etkinliği araştırılmıştır. At ve sığır vermicompostlarının %25, %50, %75 ve %100 konsantrasyonları, fungal bitki izolatlarına karşı test edilmiştir. Hazırlanan vermicompost ekstraktlarının baskılama oranları petri kaplarında denenerek, sonuçlar iki yönlü ANOVA ile istatistiksel olarak değerlendirilmiştir. At ve sığır vermicompostları için en iyi sonuçlar sırasıyla %100 konsantrasyonda *M. laxa* (%90.6), *P. viticola* (%80.2) ve *A. mali*'de (%65.1) bulunurken, *B. obtusa* (%44.7), *C. leucostoma* (%38.1) ve *B. cinerea*'nın (%35.5) baskılanması daha düşük bulunmuştur.

Anahtar kelimeler: vermicompost ekstraktı, at, sığır, bitki, fungal patojen

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Cite this article: Uzunok, S. & Kuşvuran, Ş. (2023). Determination of suppression efficiency of vermicompost extracts on some aerial fungal plant pathogens. *Plant Protection Bulletin*, 63-1. DOI: 10.16955/bitkorb.1238852

Atf için: Uzunok, S. & Kuşvuran, Ş. (2023). Vermikompost ekstraktlarının bazı havai fungal bitki patojenleri baskılama etkinliğinin belirlenmesi. *Bitki Koruma Bülteni*, 63-1. DOI: 10.16955/bitkorb.1238852