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Review

Vermicompost and Its Derivatives against Phytopathogenic Fungi in the Soil: A Review

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Abstract: Synthetic chemicals, such as fertilizers and pesticides, are abundantly used in agriculture to enhance soil fertility and prevent the occurrence of diseases, respectively. Many studies have reported a negative influence of these chemicals on the soil environment. Natural sources from earthworms and their products, as a result of vermicomposting, may be considered better alternatives. The aim of this review was to reveal the source of antifungal efficiency of vermicompost and its derivatives, such as vermiwash, coelomic fluid, skin secretion of earthworms, and metabolites from decomposer bacteria in vermicompost, in order to highlight their application in agriculture. The synergistic activity of bioactive compounds present in coelomic fluid, mucus, skin secretion, and metabolites from associated bacteria (decomposer) assisted crop plants for effective action against various soil pathogenic fungi, such as *Rhizoctonia solani*, *Alternaria solani*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, and *F. graminearum*. Thus, these bioactive metabolites can be recommended to suppress plant fungal diseases. Vermicompost and its derivatives should be considered for use in agricultural fields to control harmful soil fungi and increase crop productivity.

Keywords: coelomic fluid; earthworm; fungal pathogen; vermiwash; vermicompost

1. Introduction

Synthetic chemicals, such as pesticides and fertilizers, are frequently used in agriculture despite their negative impact on the environment [1]. However, organic products can be a better choice for pest and disease management in agriculture [2]. The use of natural products and resources in agriculture can help to prevent soil damage, nutrient loss, and environmental degradation incurred due to the excessive application of toxic pesticides and chemical fertilizers [3,4]. Promising natural products for application in agriculture for soil fertility and reducing the biotic stress of plants caused by soil pathogens can be found in vermicompost and its derivatives due to the bioactive compounds and metabolites from earthworms and decomposer bacteria [5–7].

Vermicompost, a natural product obtained after decomposition of organic matter by the activity of earthworms, contribute to valuable bioavailable nutrients and use full microbes to increase soil fertility. The application of vermicompost in agriculture has resulted in remarkable improvements in crop yield as well as in crop health and nutritive qualities; it increases the soil mineral content, which enhances the survival of valuable microbes [5,6]. Furthermore, vermicompost possesses antifungal and insecticidal properties by virtue of the coelomic fluid (CF) of earthworms and other bioactive compounds, making it equally effective in controlling pests and suppressing diseases [7]. CF is released through the dorsal pores in the form of mucus, which acts as a defense mechanism due to its antimicrobial properties [2]. Bioactive compounds are synthesized by various chains of amino acids to make them distinct to fight against specific pathogen, and were developed through an evolutionary process enabling earthworms to defend against soil pathogens [7–9].

Vermiwash is an important vermicompost derivative that becomes easily bioavailable to the roots of plants. It is a solution collected after draining vermicompost rich in earthworms. It contains plenty of CF and other bioactive compounds, such as enzymes, hormones, vitamins, mucus, proteins, micro- and macronutrients, and decomposer microbes, establishing a symbiotic relationship with earthworms [2,7,10,11]. The decomposer microbes in vermicompost/vermiwash release important metabolites to prevent plant diseases [3,7,12].

Every year, agriculture faces severe crop loss due to plant diseases. Approximate annual worldwide production tonnage lost in the 21st century has been caused by animal pests (18%), bacterial diseases (16%), and weeds (34%), resulting in an average annual loss in crop production of 68% [13]. The Great Irish famine in 1845-49 was the result of failure of potato crops due to late blight disease caused by *Phytophthora infestans* [14]. Similarly, various fungal pathogens are destroying major crops all over the world; for example, *Fusarium* spp. cause root rot in wheat [15], while infection by *Colletotrichum falcatum* results in red rot in sugarcane [16]. An approximately 80% reduction in rice yield has been recorded due to fungal pathogens [17]. As a postharvest fungal disease, anthracnose causes the spoilage of vegetables, and fruits [18,19].

Considering these fungal infections, which cause great crop loss, both vermicompost and vermiwash can be utilized as antifungal agents to minimize the harmful effects of these pathogens. The objective of this review is to summarize the source of antifungal efficiency of vermicompost and its derivatives, such as vermiwash, coelomic fluid, skin secretion of earthworms, and metabolite secretions from decomposer bacteria of vermicompost, so that farmers can use the products in agricultural fields to control harmful soil fungi and enhance crop productivity.

2. Protective Mechanisms of Earthworm against Diseases

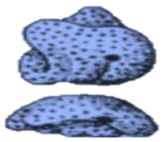
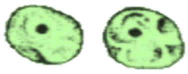
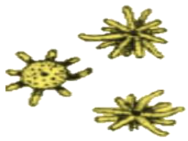
Earthworms live in a complex soil ecosystem where many decomposer bacteria are present in the drilosphere (soil containing earthworm secretions, burrow and cast), and these bacterial species help them to break down organic materials derived from plants and animals [20]. In addition, these decomposer bacteria establish symbiotic relationships with the worms in the drilosphere and in their gut to protect them from pathogenic microbes [7]. Earthworms are also able to protect themselves from pathogenic microbes by virtue of the bioactive compounds found in their CF, mucus and other cutaneous secretions [21]. Earthworm defense mechanisms include both humoral and cellular immune responses [22]. Chloragocytes, eleocytes, coelomocytes, granulocytes, natural killer (NK) cells and natural killer-like (NK-like) cells make up the cellular immune system of earthworms [23], while antimicrobial peptides, cytokines and proteins, which aid in phagocytosis, clotting, encapsulation, lysis, and agglutination, comprise the humoral immune system [7,24].

Coelomocytes are responsible for controlling the immune response of earthworms to numerous irritants in the soil ecosystem [24]. Essential bioactive compounds that are extracted from skin secretions, CF, and mucus of earthworms act as a humoral immune system by lysing cells of pathogenic microbes [22,25,26]. These compounds are important for earthworm survival and protection against pathogens and other stimuli in their surroundings.

3. Coelomic Fluid: Secretion and Biology

Earthworms belong to the phylum Annelida. Their body cavity is a "true coelom", located between the gastrointestinal tract and the epidermis lined by the coelomic epithelium. Intersegmental septa separate the coelom into tiny compartments, which are filled with CF [27]. The earthworm's body contains a significant amount of liquid, which accounts for approximately 85% of its total weight, with CF being a major component [28]. CF is a yellowish-colored alkaline biofluid composed of a watery matrix, plasma, specific proteins, enzymes, salts, and at least four coelomocytes, namely, amoebocytes, mucocytes, circular cells, and chloragogen cells [27,29] (Table 1).

Table 1. Different types of earthworm coelomocytes (adopted from Patil and Biradar, 2017) [27].

Type of Coelomocytes	Shape	Descriptions	Function
Amoebocytes/granulocytes/phagocytes		Large in size and spherical in shape	Helps in removing harmful microbes.
Mucocytes		Elongated and its narrow end bears nucleus	Secretes mucus to keep the skin hydrated for respiratory and other physiological purposes.
Circular cells		Nucleated, circular in shape	Functions of these cells are not known.
Chloragogen cells or yellow cells		Vary in shape	Acts as trophocytes, participates in circulation of nutrients to different cells and organs of the body.

In addition to coelomocyte cells, CF contains many enzymes, such as proteases, metalloenzymes, lysozyme, fibrinolytic enzymes, antimicrobial proteins, and polysaccharides [30]. They have agglutinating, proteolytic, hemolytic, mitogenic, anti-pyritic, tumoratic, and antibacterial capabilities, among other biological activities [31]. In general, innate immunity based on coelomocytes and other components of CF (variety of bioactive components) helps to combat pathogenic microbes [32–35]. Foreign material is recognized by lectin glycoproteins present on the cell surface, resulting in agglutination and lysis [36].

CF is transported between adjacent segments via sphincter-lined channels within each septum. Each compartment of the coelomic cavity has paired nephridia and a single dorsal pore through which CF is ejected when the worm is irritated or while they are moving to moisten and lubricate their burrow, subsequently making CF a component of vermicompost/vermiwash [7,37,38]. CF is important in maintaining homoeostasis and locomotion, acting as a humoral immune factor and promoting communication between the inner and outer environments of earthworms [34].

4. Antifungal Activities of Coelomic Fluid and Skin Secretion of Earthworms

Individual components of CF have also been shown to inhibit fungal growth in vitro [39]. Lumbricin-PG is a bioactive compound derived from the skin secretion of the earthworm *Pheretima guillelmi*. It contains 59 amino acid residues with antibacterial and antifungal properties [20]. In vermicompost/vermiwash, these bioactive substances have a crucial role in suppressing fungal diseases of plants [40]. Another CF component, lysenin, with a size of 33 kDa, is a pore-forming protein recovered from *Eisenia fetida*, which has been shown to play a defensive role against bacterial and fungal infections [41]. Rajesh et al. (2019) [39] reported a reduction in fungal growth on petri dishes when treated with CF.

In another experiment performed by Rajesh et al. (2019) [39], CF from *Eudrilus eugeniae* was shown to effectively inhibit the growth of four different pathogenic fungal species (Table 2). Therefore, in the control group where no coelomic fluid was applied, the fungal growth was higher than that in the experiment where the presence of coelomic fluid greatly reduced the growth of fungal pathogens.

Table 2. The effect of CF of earthworms on growth rate of fungal hyphae (adopted from Rajesh et al., 2019) [39].

Fungal Species	MTCC Number	Growth in Control	Growth with Coelomocytes
Verticillium dahliae	9998	+ + +	+
Aspergillus flavus	873	+ + +	+
Rhizoctonia solani	4634	+ + +	+
Fusarium oxysporum	284	+ + +	+

MTCC: Microbial Type Culture Collection Gene Bank located in Chandigarh, India; greatly dense mycelial growth (+ + +); restricted hyphae growth (+).

Similarly, coelomocytes of the earthworms *Dendrobaena veneta* and *Eisenia fetida* inhibited the growth of the plant-parasitic fungus *Fusarium oxysporum* after 48 or 72 h of treatment [42]. Sethulakshmi et al. (2018) [40] reported the antifungal efficacy of CF from *Eudrilus eugeniae* against *Aspergillus niger* and *Candida albicans,* with the highest inhibitory areas of 16 mm and 18 mm, respectively. Delayed germination of the fungal spores may also account for the antifungal efficacy of CF [39].

The CF of different species of earthworm displays different anti-fungal activities [43]. Researchers have evaluated the effects of coelomic fluid of three species of earthworms (*Allolobophora chlorotica*, *Dendrobaena veneta*, and *Eisenia andrei*) against six species of phytopathogenic fungi, including *Fusarium culmorum*, *Berkeleyomyces basicola*, *Rhizoctonia solani*, *Globisporangium irregulare*, *Sclerotinia sclerotiorum*, and *Macrophomina phaseolina*. The inhibitory effect of the coelomic fluid of *Eisenia andrei* against *Rhizoctonia solani* was higher than that of other earthworm species [43].

In 2020, Nadana et al. [2] performed an experiment on detached leaves of rice plants treated with CF and agar blocks containing *Rhizoctonia solani.* In the experiment, CF-treated leaves showed no signs of necrosis even after inoculation with *R. solani*, while the necrosis spots appeared on untreated leaves. At seven days of post inoculation, the disease index ranged between 25% and 50% in rice leaves treated with CF; in contrast, it ranged between 75% and 100% in control leaves not treated with CF. This experiment suggested that CF can prevent rice plants against fungal pathogens. Other research also confirmed that coelomic fluid from different species of earthworms effectively reduced growth of fungal pathogens (Table 3).

Table 3. Antifungal activity shown by coelomic fluid from different species of earthworms.

Earthworm's Species	Fungal Species	Results	References
Eudrilus eugeniae	*Rhizoctonia solani*	Reduced disease index	[2]
Eisenia fetida	*Fusarium oxysporum*	Reduction of fungal growth	[42]
E. eugeniae	*Aspergillus niger*	Inhibited fungal growth	[40]
E. eugeniae	*Rhizoctonia solani*	Inhibited fungal growth	[39]
Lumbricus rubellus	*Fusarium graminearum*	Reduced its germination	[44]

5. Vermicompost: Antifungal Efficiency

Fungal diseases are usually associated with severe morbidity and mortality in plants. The extensive use of antifungal chemicals has led to the development of drug-resistant pathogens and adverse effects on environmental health [45,46]. Vermicomposting was found to have a disease-suppressive function, making it a better alternative to chemical fungicides. The composition of earthworm secretions (for instance, Lumbricin-PG from skin secretions of earthworms possess antifungal activities) associated with vermicompost are able to protect plants against fungal pathogens [20,47]. One of the antifungal effects of vermicompost is attributed to decomposer bacteria producing a symbiotic association with earthworms. You et al. (2019) [47] demonstrated that ergosterol peroxide is a bioactive metabolite derived from powdered bamboo vermicompost that considerably suppresses *R. solani* mycelium growth. This bioactive compound is secreted by vermicomposting bacteria using bamboo as a resource.

6. Vermiwash as Antifungal Agent

Fusarium graminearum, a well-known pathogenic fungus, has a significant impact on plant roots, reducing the quality and yield of wheat production by 20% [48]. In crops affected by fungal infection, the application of vermiwash (a liquid extract of vermicompost rich in earthworm mucus and bioactive compounds) has been shown to minimise the pathogenic effect, reducing mycelial growth [7]. Naidu et al. (2012) [49] observed that vermiwash could prevent the growth of a powdery mildew on watermelons. It was also used to control onion black mould disease caused by *Aspergillus niger* [50].

The extract of *Eisenia fetida* has been demonstrated to have a substantial antagonistic effect on *F. graminearum* development [48]. When earthworm mucus is applied to agricultural land, it inhibits the growth of disease-causing microbes in the soil and possesses antifungal activity to reduce fungal pathogens by 26% [48]. On the other hand, vermiwash showed only a 16% reduction in mycelium growth, indicating that the efficacy of vermiwash was much lower than that of the applied mucus [20,48,51]. Khan et al. (2015) discovered that spraying vermiwash inoculated with arbuscular mycorrhizal (AM) fungi enhanced the physicochemical parameters of soil, assisted plant growth and development by increasing nutrient availability and boosted disease resistance. AM fungus and vermiwash provided greater synergistic advantages in controlling fungal disease than any of them alone [52].

Antifungal chemicals produced by bacteria during vermicomposting may be responsible for vermiwash's biocontrol properties. *Bacillus* phylotypes found in vermicompost are responsible for the fungicidal properties of vermiwash [53]. Strains of bacteria, such as *Pseudomonas fluorescens* Pf1 combined with *Bacillus subtilis* EPCO 16, play vital roles in suppressing fungal pathogens in vermicomposting. In addition, the fungal species *Trichoderma asperellum* TTH1 was also isolated from vermicompost and found to be more effective as a fungal biocontrol agent against sugar beet root rot than difenoconazole treatment [54]. *Bacillus* species are known to establish mutualistic relationships with earthworms and produce bioactive compounds against fungal pathogens in vermicompost [53]. The combined application of *Bacillus amyloliquefaciens* and dipotassium phosphate (DPP) was used as a decoction to suppress the mycelial growth of *Alternaria solani* [55].

7. Tissue Homogenate (G-90) of Earthworms: Antifungal Activity

Earthworm tissue homogenate, commonly known as G-90 or earthworm paste, is made by mincing the entire body of the earthworm [56]. The tissue homogenate comprises CF, skin secretion, and mucus, all of which contain potent bioactive chemicals that protect earthworms from fungal pathogens. Tissue homogenate from the earthworm *Eudrilus eugeniae* has been proven to be effective against a variety of fungal pathogens [57] (Table 4). Different species of fungal pathogens react differently against the same concentrations of earthworm paste [57]. For instance, there was a reported maximum zone of inhibition of *Aspergillus flavus* growth (15.00 ± 0.57 mm) at 100 μL earthworm paste, suggesting its higher inhibition efficacy at higher concentrations.

Table 4. Antifungal activity of crude earthworm paste through the Disc assay method (adopted from Vasanthi et al. 2013) [57].

Fungal Species	Zone of Inhibition of Fungal Growth under Different Concentrations (Mean ± SD) of Earthworm's Paste	
	50 µL	100 µL
Aspergillus niger (NCIM-501)	11.00 ± 0.57	13.33 ± 0.33
A. flavus (Local isolate)	10.33 ± 0.33	15.00 ± 0.57
Penicillium notatum (Local isolate)	10.66 ± 0.33	14.33 ± 0.33

A comparative study on the antifungal efficiency of the earthworm's paste and fluconazole through inhibition zones demonstrated equivalent or higher efficacy of the earthworm paste at 150 µL and 200 µL concentrations (Table 5). Among four species involved in the experiment (*Aspergillus niger, Penicillium citrinum, A. nidulans* and *Cladosporium herbarum*), to assess antifungal efficacy of earthworm paste in different phytopathogenic species, *P. citrinum* showed a higher zone of inhibition against the paste, but *A. niger* possessed higher resistance with a lower zone of inhibition [58].

Table 5. Efficiency of earthworm paste against fungal pathogens (adopted from Senthil and Sivakami, 2018) [58].

Fungal Species	Concentration of Earthworm Paste and Zone of Inhibition in mm (Mean ± SE)					
	25 µL	50 µL	100 µL	150 µL	200 µL	Fucanazole 20 µL
A. niger	1.2 ± 0.22	4.6 ± 0.72	8.6 ± 0.64	12.8 ± 0.64	11.6 ± 0.92	12.4 ± 0.52
P. citrinum	1.4 ± 0.40	4.8 ± 0.60	9.8 ± 0.72	12.2 ± 0.78	14.8 ± 0.92	16.4 ± 0.49
A. nidulans	2.2 ± 0.34	5.8 ± 0.94	9.6 ± 0.98	13.0 ± 0.72	13.8 ± 0.92	14.6 ± 0.29
C. herbarium	2.4 ± 0.36	5.8 ± 0.82	9.8 ± 0.72	12.0 ± 0.64	13.4 ± 0.92	13.8 ± 0.20

The efficiency of the coelomic fluid and earthworm paste against the fungal pathogens indicated in Tables 2–5 is due to the presence of bioactive compounds that evolutionarily developed in earthworms to protect them against soil pathogens [7].

8. Decomposer Bacteria of Vermicompost/Vermiwash as Antifungal Agents

Compared to chemical fungicides, the application of vermicompost in agricultural land is encouraging to control fungal plant pathogens. *Bacillus subtilis* had the largest range of antifungal activity among the bacteria found in vermicompost, which produce compounds that can help a plant fight off fungal infections. The volatile organic compounds secreted by *B. subtilis*, such as 3-methyl-3-hexanol, were extracted from cow dung vermicomposting and shown to act against the fungal pathogen *Botrytis cinereal* [59].

Additionally, high levels of chitinolytic bacteria, *Actinobacteria*, have been reported in vermicompost. Chitinolytic bacteria can catalyze fungal cell walls at the early germination stage, ultimately hindering the growth of fungal pathogens [60]. Furthermore, various Rhizobacteria species isolated from vermicompost have been shown to strongly inhibit fungal growth on beans by 50–60% [61].

The efficiency of vermicompost/vermiwash to suppress disease is also attributed to metabolites secreted from decomposer bacteria that establish a symbiotic relationship with earthworms in their gut, cast, and burrows [7]. Bioactive substances produced by Pseudomonas as metabolites have either broad-spectrum action against fungal pathogens (Phenazines C-Acelyphloroglucinols) or are species-specific [62].

Vermicompost and associated bacteria from the genera *Pseudomonas*, *Burkholderia*, *Bacillus* and *Streptomyces* are able to produce essential metabolites to suppress pathogenic fungi of plants [63]. The source of these chemicals has been reported from mutualistic bacteria involved in vermicomposting rather than from the raw materials used in the process [47] (Table 6).

Table 6. Metabolites secreted by *Pseudomonas* acting against fungal pathogens of plants.

Fungal Disease	Metabolites	Pathogen	References
Black root-rot of tobacco	Hydrogen cyanide 2,4-2, 4 Diacetylphloroglucinol	*Thielaviopsis basicola*	[62]
Take-all of wheat	Phenazines C-Acelyphloroglucinols	*Gaeumannomyces graminis* Var. *tritici*	[64]
Pre-emergent damping-off of cotton; Sugar beet	Oomycin Pyoluteorin 2, 4 Diacetylphloroglucinol	*Pythium* spp.	[65,66]
Tan spot of wheat	Pyrrolnitrin	*Pyrenophora triticirepentis*	[62]

9. Future Perspective

The efficiency of vermicompost and its derivatives to prevent the development of phytopathogenic fungi in agriculture is due to the synergetic effects of bioactive compounds in the coelomic fluid, mucus, skin secretion of earthworms and metabolites from decomposer bacteria involved in vermicomposting. In the future, these metabolites should be industrially produced in large amounts by culturing these bacteria in vermicomposting so that the valuable products can be commercially available in the market for farmers.

10. Conclusions

The application of vermicomposting to agricultural land increases productivity by a multifaceted impact on soil health and crops, facilitating nutrient enrichment and preventing pathogen development. Vermicompost and its derivatives, such as vermiwash, along with associated decomposer bacteria, act against fungal pathogens. The antifungal efficacy of vermicompost may be associated with bioactive compounds present in the CF, mucus, skin secretion of earthworms and metabolites secreted by decomposer bacteria. The CF of earthworms has an inherent ability to defend worms against diseases. It inhibits the growth of a variety of fungal pathogens, such as *Rhizoctonia solani*, *Alternaria solani*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, and *F. graminearum*. The metabolites from vermicomposting bacteria, CF, mucus, and skin secretion synergistically combat phytopathogenic fungi. As an organic product, vermicompost and its derivatives are environmentally friendly. Thus, these products should be used to boost agricultural productivity by nutrient enrichment and reduction of plant fungal diseases. This review provides new insights into the application of vermicompost and its derivatives in agricultural fields against plant fungal infections.

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