



Review article

Vermiwash: An agent of disease and pest control in soil, a review

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ABSTRACT

Vermiwash is a liquid extract produced from vermicompost in a medium where earthworms are richly populated. It comprises a massive decomposer bacteria count, mucus, vitamins, different bioavailable minerals, hormones, enzymes, different antimicrobial peptides, etc. This paper aimed to assess how these natural products in vermiwash suppressed the pathogen and pests. Thus, we have reviewed the importance of vermiwash/vermicompost in disease control, the mechanism of disease suppression, the components of vermiwash applied in disease suppression, and pest control to use the scientific facts in agriculture to enhance the productivity of the crops. The bioactive macromolecules from the skin secretion of earthworm, coelomic fluid, and mucus directly able to defend pathogenic soil microbes against the worm and thereby freed the environment from the disease. Earthworms establish symbiotic relations with microbes, produce an essential product that supports the growth of plants, and suppress plant's root disease. It is recommended that earthworm should be inoculated in an agricultural field, or prepare and apply its vermiwash/vermicompost as a spray or as additive bio-fertilizer in the soil to enhance the productivities of the crops.

1. Introduction

Vermiwash is a natural product formed by vermicomposting of organic matter from rich population of earthworms (Aghamohammadi et al., 2016; Thakur and Sood, 2019). The composition and quality of vermicompost/vermiwash vary depending on the raw organic matter used for vermicomposting. The composition of vermicompost and vermiwash prepared from the same organic matter are essentially the same. It comprises numerous chemicals viz, hormone, mucous, enzyme, vitamins, proteins, different macro and micronutrients, and a large number of microbes that distinguishes the two products (Das et al., 2014; Tripathi et al., 2005; Nadana et al., 2020). Besides its application as a fertilizer to enhance crop productivity, it can also be applied in disease suppression and pest control due to the presence of essential antimicrobial and anti pest chemicals (Kanchan et al., 2013; Thakur and Sood, 2019; Nadana et al., 2020).

Vermiwash/vermicompost is rich in many different nutrients, vitamins, growth hormones, which acted as disease and pest suppression agents (MacHfudz et al., 2020). As compared to application of solid vermicompost, its liquid form (vermiwash) is more suitable due to its bioavailability to reach quickly to targeted area around the roots of plants

(Sulaiman and Mohamad, 2020). The more it liquefied, the more it became bioavailable and readily absorbed by plants for suppressing the disease. There was a report that the effect of vermiwash and mucus extracted from *Eisenia fetida* on *Fusarium graminearum* greatly inhibited the growth of pathogenic fungus, which significantly influenced both quality, and production of wheat (*Triticum aestivum* L.) (Akinuoye-Adelebu et al., 2019).

Combined application of vermiwash with other plant-based pest controlling method gave a synergetic effect to minimize infestation of pests such as thrips and mites, and produce a large number of healthy pods to enhance productivity (Kanchan et al., 2013). Acarids are parasitic arachnids that severely affect productivity of crops. For instance, in laboratory condition Aghamohammadi et al. (2016) reported leaf of bean (*Phaseolus vulgaris* L.) treated with vermiwash displayed a significant repellent effect against Acarina (*Tetranychus urticae*) as compared with untreated leaves.

Thakur and Sood (2019) reported vermiwash efficiently inhibited the growth of eggs of plant-eating red spider mite (*Tetranychus urticae*), which showed that it could be applied in the agricultural field as an insecticide for controlling red spider mite infestation. The repellent effect of vermiwash attributed to the presence of mucus in it (Nadana et al.,

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2020). In earthworms, coelomic fluid secreted through dorsal pores in the form of mucus as a mechanism of defense when irritated and reported to possess insecticidal, antifungal, and pesticidal bioactive compound (Nadana et al., 2020).

Many studies showed that vermiwash/vermicompost applied as a liquid bio-fertilizer as well as a spray (Shafique et al., 2021). By using growth parameter, effect of vermiwash had been evaluated on different crops, such as *Linum usitatissimum* L. (Makkar et al., 2019), *Pennisetum glaucum* (Naroila and Poonia, 2011), *Trigonella foenum* (Tadayyon et al., 2018), *Solanum melongena* (Sundararasu and Jeyasankar, 2014). Sundararasu and Jeyasankar (2014) reported vermiwash was rich source of micro and macronutrients, enzymes, and growth regulator, which improved the growth and productivity of plants and reduced biotic stress.

The vermiwash is a natural product that its application is compatible with environmental protection. Thus, we have reviewed the importance of vermiwash in disease control, mechanism of disease suppression, components of vermiwash that are applied in disease suppression, and pest control in order to use these scientific facts in agriculture to enhance crop productivity.

2. Methodology

The manuscript was reviewed from data of well reputed (85% Scopus indexed) article using Google Scholar search engine. All the articles were read thoroughly, important concepts of data squeezed out, constructed then rewritten in a meaning full manner. Most of the basic data of the manuscript obtained from Scopus indexed and other non-Scopus articles were used due to lack of published data in Scopus indexed journals on the desired subjects.

2.1. Preparation of vermiwash

Vermiwash is the liquid extracted from vermicompost, which is prepared by feeding earthworms with raw materials like leaf litter or cow dung or other organic materials (Tharmaraj et al., 2011). Vermiwash/vermicomposting could be prepared at larger and smaller scales by batch and continuous mode. Batch mode requires periodic inoculation of verms/worms whereas continuous mode of preparation involves continuous production of the products once the worms are inoculated with continuous supply of raw materials (Munroe, 2007). Tharmaraj et al. (2011), employed *Lampito mauritii*, an anecic indigenous earthworm to India, and two exotic species *Eisenia fetida* and *Eudrilus eugeniae* to produce vermicompost/vermiwash.

The vermipits were prepared with (2 × 2 × 2m) (length x breadth x depth) by Tharmaraj et al. (2011) with the arrangement of layers performed from bottom to top as shown in (Figure 1). The pebbles or coconut shell filled on the bottom layer to absorb excess water from the composting pit that added from the top. The second layer filled with sandy soil to prevent accumulation of extra water in the medium, and the third layer contained organic soil and old compost inoculated with earthworms. Cow dung and leaf litter blended at the ratio of 1:2 and added to the pit in the fourth layer. Finally, on the top layer the coconut fronds used to cover the pit to prevent from direct sunlight and keep the medium moist enough. The contents of the pit turned once in 2 or 3 days to enhance aeration in the course of vermicomposting. After 30 days of the composting process, vermiwash started to collect as demonstrated by Tharmaraj et al. (2011).

Raphael and Velmourougane (2011) performed another method of vermicomposting in shaded brick tanks, each measuring 1 m × 1 m × 0.75m (length×breadth×depth), with a capacity to hold 500 kg of waste and a hole at the bottom. The waste material consisted of composted coffee pulp mixed with the cow dung inoculated with exotic (*Eudrilus eugeniae*) and native (*Perionyx ceylanensis*) earthworms at the rate of one kg of worms per tank. The tanks were covered with gunny bags to shade the contents and prevent desiccation and periodical sprinkling of water performed to maintain proper moisture and maintain the comminution of

organic matter into vermicomposting/vermiwash (Raphael and Velmourougane, 2011).

2.2. Decomposer bacteria as pathogen suppressor

Earthworms are able to clean up various pathogens in soil and accumulate heavy metals in their bodies from it (Usmani and Kumar, 2015). The decomposer bacteria are reported to suppress pathogenic bacteria and fungi due to metabolites produced from them. There was a report that decomposer bacteria such as *pseudomonads* produced metabolite, which could suppress the fungal disease of important plants (Dowling and O'Gara, 1994; Kalantari et al., 2018). The decomposer bacteria reported to win the pathogenic bacteria in competition and in the place where decomposers bacteria are there, the disease-causing bacteria cannot survive. The decomposer bacterias in the gut of earthworms and in drilosphere were able to suppress pathogenic bacteria, which could cause disease to soil animals and plants (Rostami et al., 2014).

Species richness of soil ecosystem implies healthier soil environment in which pathogens cannot endure. Therefore, those plants grow in a soil where disease suppressed by biodiversity possess a tendency to be healthier than those plants growing in less diversified soil (Datta et al., 2016). Drilosphere is the region of soil influenced by burrowing, secretion, and casts of earthworms applied to support the growth of plenty decomposer bacterias (Ojha and Devkota, 2014). Vermiwash comprises decomposer bacteria, fungi, secretion, and mucous of earthworm, which could help in disease suppression and production of metabolites that prevent growth of pathogenic bacteria and different plant pests (Dowling and O'Gara, 1994; Nadana et al., 2020; Sulaiman and Mohamad, 2020).

Vermiwash appeared to have an inherent quality, which acted as liquid organic fertilizer as well as mild biocide, and tended to utilize it a powerful contribution to organic agriculture, soil wellbeing, disease control and sustainable crop production (Das et al., 2014; Tripathi et al., 2005). Vermiwash at 5–10 percent dilution inhibited mycelial growth of pathogenic fungi. It also had capacity to encounter worms, thereby save crops and enhance productivity. As a foliar spray, it initiated flowering and enduring inflorescence. Likewise, it could be utilized as a liquid fertilizer applied to the rhizosphere (Das et al., 2014; Khan et al., 2015). No pathogens could survive in vermicompost, thereby protect earthworms from diseases. It acted as a plant tonic and thus helped in reducing many plant's pathogenic fungi (Das et al., 2014; Tripathi et al., 2005).

Higher protease and acid phosphatase activity in vermicompost treated soils might be responsible for higher nitrogen and phosphorus content in soil extracts (Gosh et al., 2018), and different types of hormones and macronutrients such as cytokines, auxin, different amino acids, and vitamins, enzyme cocktails of proteases, amylases, urease and phosphatase possessed by and contributed to soil wellbeing. Some other secretions and many useful microbes such as saprophytic bacteria, fungi, actinomycetes including nitrogen-fixing bacteria such as *Agrobacterium* spp., *Azotobacter* spp., *Rhizobium* spp., phosphate solubilizers were available in the vermiwash (Das et al., 2014; Tripathi et al., 2005) (Table 1).

Due to the presence of plenty vital bioavailable nutrients, vermiwash is demonstrated to enhance growth of plants and improve nutrients. The growth indices of green vegetable, *Amaranthus viridis* L. using different concentration of vermiwash prepared from cow dung, aquarium water and tap water with formulation of (0, 25, 50, 75, and 100%) (Deepthi et al., 2021). The growth indices of plant viz., root weight, root length, shoot weight, number of primary and secondary roots, area of leaf, number of leaves, and wet weight were significant in application of pure (100%) vermiwash. Nutritional qualities of plants treated by 100% vermiwash indicated that the concentration of protein and carbohydrates 1.96 mg/g and 0.98 mg/g respectively as compared to untreated plants (Deepthi et al., 2021).

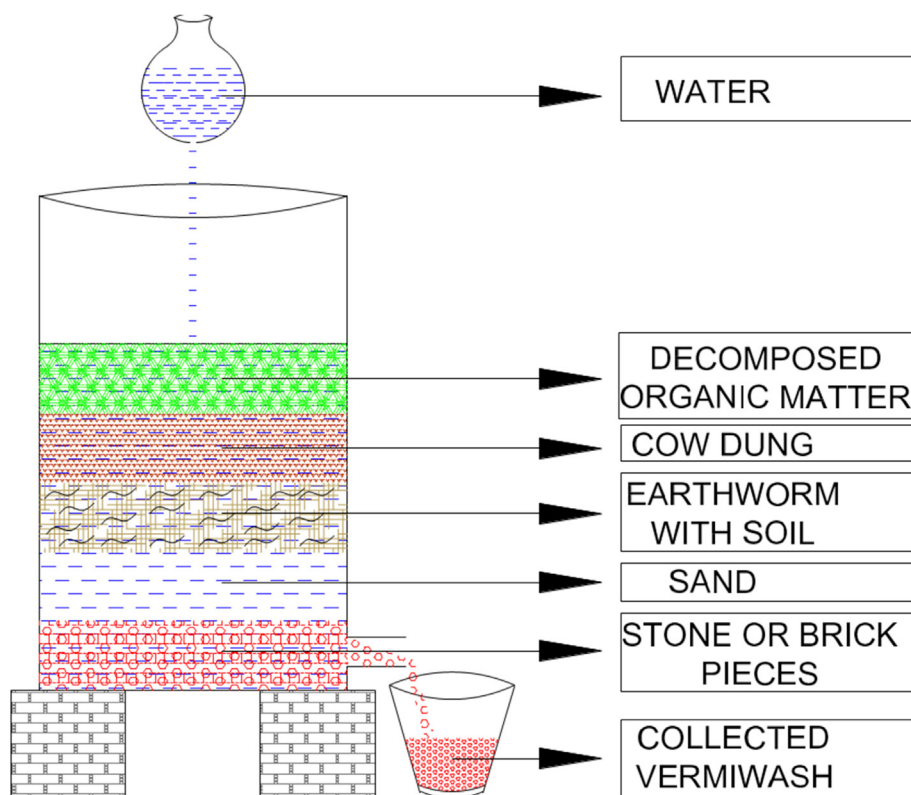


Figure 1. Preparation of vermiwash. It showed a basic means of vermiwash preparation. The bottom layer contains stone or brick pieces in coarse grain. The next layer contains sand, which helps to drain water through it to maintain moderate moisture. In the third layer earthworms inoculated with organic soil, in the fourth layer cow dung, or any organic waste which used as a main raw material. On the top layer the decomposed organic matter such as leaf litter, straw, etc an additional raw material that mainly prevents direct exposure from sunlight. The periodic sprinkling of water should be done to prevent desiccation and facilitate the comminution of organic matter. Finally, pure vermiwash harvested for application.

Table 1. Composition of vermiwash and its benefit.

Contents of vermiwash	Benefit of vermiwash	References
Decomposer bacteria	Suppress pathogen	(Mondal et al., 2015)
Hormones (Cytokines, auxin)	Facilitate plant root and shoot growth	(Das et al., 2014; Tripathi et al., 2005)
Amino acid and mucosal secretion	Suppress pathogen and pest	(Liu et al., 2004; Valembois et al., 1984)
Vitamines	Facilitates growth and development of plant	(Das et al., 2014; Tripathi et al., 2005)
Phosphatase	Stabilize physical, chemical and biological properties of soil as well suppress pathogen and solubilize phosphorus	(Gosh et al., 2018)
Actinomycetes	Suppress pathogen	(Das et al., 2014; Tripathi et al., 2005; Balachandar et al., 2018a,b)
Amylase, cellulase	Stabilize physical, chemical and biological properties of soil as well applied in carbon turnover by degrading organic matter	(Das et al., 2014; Tripathi et al., 2005)
Varieties of micronutrients Ni, Mg, Fe, Ca, K	Facilitate the growth and productivities of plant	(Ndegwa and Thompson, 2000)

2.3. Selected mutualist decomposer bacterial and fungal family with earthworm

Symbiotic bacteria and fungi with earthworms are all decomposers that catalyze ingested larger and complex organic materials either by their enzymes or by initiating secretion of an enzyme of earthworms (Davidson et al., 2013). In this symbiotic relationship, bacteria and fungi get shelter and protection while earthworms benefited through microbial initiated enzymatic secretions for digestion (Davidson et al., 2013). Earthworms also benefitted from the relationship in that the bioactive compounds secreted by microbes such as actinomycetes, which produces potent antibiotics to protect them from pathogens (Balachandar et al., 2018a,b).

Species of decomposer bacteria and fungi, which are known to make mutualistic relation with earthworm and facilitated decomposition in its gut, its cast, drilosphere and burrows (Table 2). The microbial metabolite

contains bioavailable nutrients such as carbon, nitrogen, potassium, phosphorous, and many other micronutrients that resulted from decomposition of organic matter. Mucus, skin secretion, and coelomic fluid create an ideal condition for the growth of these microbes (Tripathi et al., 2005; Nadana et al., 2020). The gut of earthworm serves as a microhabitat for survival of different microbes including actinomycetes.

The castings of endogeic (*Aporrectodea caliginosa* and *Octolasion tyraeum*) and an epigeic *Dendrobaena octaedra* earthworms were observed to harbour higher actinomycete counts than the surrounding soil (Jaya-singhe and Parkinson, 2009). Population of ingested soil microorganisms possibly increased during their passage through the earthworm's intestinal tract (Scheu 1987). Earthworm casts are reported to be much more microbiologically active and richer in microflora than surrounding soil (Daniel and Anderson, 1992).

One of the fascinating natures of actinomycetes would be important to make the vermiwash/vermicomposting to act against a pathogen.

Table 2. Family of decomposer bacteria and fungi making symbiotic relation with earthworm and found in vermicompost/vermiwash.

Family/Genera/spp	Bacteria/fungus	References
<i>Agrobacterium</i> spp	Bacteria	Tripathi et al., (2005)
<i>Azotobacter</i> spp	Bacteria	Tripathi et al., (2005)
<i>Rhizobium</i> spp	Bacteria	Tripathi et al., (2005)
<i>Proteobacteria</i>	Bacteria	Singh et al., (2015)
<i>Firmicutes</i>	Bacteria	Singh et al., (2015)
<i>Actinobacteria</i>	Bacteria	Singh et al., (2015)
<i>Bacillus circulans</i>	Bacteria	Idowu et al., (2008)
<i>Staphylococcus aureus</i>	Bacteria	Idowu et al., (2008)
<i>Clostridium absonum</i>	Bacteria	Idowu et al., (2008)
<i>Penicillium funiculosum</i>	Fungus	Tiwari and Mishra (1993)
<i>Absidia glauca</i>	Fungus	Tiwari and Mishra (1993)
<i>Alternaria alternata</i>	Fungus	Tiwari and Mishra (1993)
Actinomycetes	Bacteria	Singh et al., (2015); Balachandar et al., (2018a),b
Mycorrhizae	Fungus	Singh et al., (2015)

Balachandar et al. (2018a,b) isolated the potent actinomycete from vermiwash that could produce a bioactive compound that acted against the pathogen. In another experiment they tested antimicrobial activities of actinomycetes isolated from vermicast, which were acted against both Gram +ve and Gram -ve pathogenic bacteria viz., *Bacillus subtilis*, *Bacillus circulans*, *Escherichia coli*, *Pseudomonas aeruina* and *Staphylococcus aureus* (Balachandar et al., 2018a,b).

2.4. Mechanism of disease suppression of vermiwash

Both vermicompost and vermiwash are reported to possess ability to suppress disease. According to Mehta et al. (2014), microbial content of vermicompost and vermiwash was the most influencing factor in hindering the development of plant disease. Microorganisms in the vermicompost and vermiwash may act as antagonistic agents of a pathogen by their ability to compete for nutrients and space, destroying parasitizing pathogens by producing an antimicrobial compound and systematically reducing their resistance (Mehta et al., 2014; Naseri, 2019).

Due to the abundance of different micro and macronutrients in vermicompost and vermiwash (Tables 2 and 3), it becomes inconvenient for a pathogen to survive and grow in vermicompost/vermiwash as compared to the decomposers. The latter are able to survive and even grow exponentially in vermicompost-amended soil because they directly consume it as a saprophytic mode of nutrition (McLean et al., 2006). The concentration of both micro and macronutrients improved, the cell count of the decomposer organisms increased by half from 2.4×10^8 CFU g^{-1} to 4.8×10^8 CFU g^{-1} (Mondal et al., 2015) (Table 3).

In heterogenous soil ecosystems caused by earthworms' activities, disease-triggering microbes become suppressed (Datta et al., 2016). Disease causing agents cannot tolerate the influence exerted by the

decomposers in soil where the population of the latter is very high due to their ability to secrete antibiotics and metabolites. Earthworm is considered as keystone species due to its effect to facilitate the survival of massive soil microbes around its habitat (Jiménez and Decaens, 2004).

Kuzyakov and Blagodatskaya (2015) gave the term 'microbial hot-spots' having higher microbial process rates as compared to bulk soil indicating earthworms positively influenced microbial growth. High microbial count in medium where earthworms were cultured could change healthier biochemical properties of soil that suited for the survival and growth of different soil microbes. On the contrary, the physiology of pathogen did not resist greater concentration of microbial metabolites in the vermicompost and vermiwash (Rashtbari et al., 2020).

As earthworm survive in soil where pathogenic microbes subsist, they have evolved immune system enabling them to defend against pathogens (Li et al., 2011). They possess both humoral and cellular defense mechanism to defend themselves from microbial pathogens (Vaillier et al., 1985). Antimicrobial peptides in earthworm's mucus, skin secretions and coelomic fluid display phagocytosis, encapsulation, agglutination, opsonization, clotting and lysis properties, which can identify pathogens and act against them (Homa, 2018). Cellular defense is responsible for phagocytosing the pathogen whereas humoral defense response, agglutination, opsonization, clotting and lysis found in skin secretion, mucus and coelomic fluid (Valembois et al., 1984; Vaillier et al., 1985; Field et al., 2004).

2.5. Antimicrobial peptide from earthworms

Lysenin lysed and killed cells by the sphingomyelin-dependent mechanism in eukaryotic mammals and sphingomyelin-independent mechanism in bacteria as the latter lacked sphingomyelin (Kobayashi et al., 2004). An earthworm protein (38.6 kDa) from coelomic fluid of *Eisenia fetida* was isolated and purified by using different isolation procedures (Massicotte et al., 2004). Eiseniapore (38.6 kDa) also needed a reaction with sphingomyelin to lyse cells by ion formation with glycoproteins and phospholipids binding action and lysing of bacterial cells through the formation of pores (Massicotte et al., 2004). Lysenin and eiseniapore were toxic to mammalian cells because they directly developed a link reaction with sphingomyelin to lyse cells. Lumbricin PG also identified from the skin secretion of *Pheretima guillelmi* acts as a primary defense to protect the earthworm from soil pathogenic bacteria.

The bioactive molecules such as lysenin (33 kD), and 41 kDa), cytolytic eiseniapore (38.6 kDa), and fetidin (40 kDa), hemolysin, and fetidin (45 kDa), lysozyme (39 kDa), Lumbricin I (7.2kDa), Lumbricin-PG, coelomic cytolytic factor (42 kDa), OEP3121, bacteriostats obtained from coelomic fluid of earthworm responsible to defend earthworms from pathogen (Gupta and Yadav, 2016; Vaillier et al., 1985; Liu et al., 2004) (Table 4). These antimicrobial peptides are oozed out through the dorsal pore of earthworm secreted as skin secretion during irritation or unfavorable condition to them and to make their burrow moist enough. The synergetic effect of all these peptides influenced the pathogen and able to suppress the pests in the soil. In addition, these antimicrobial

Table 3. Characteristics of both cow dung and vermicomposting from which vermiwash extracted (Adopted from Mondal et al., 2015).

Chemical and biological characteristics of cow dung		Chemical and biological characteristics of vermicomposting	
Characteristics	Value	Characteristics	Value
Nitrogen in %	0.98 ± 0.07	Nitrogen in %	1.71 ± 0.08
Phosphorus in %	1.01 ± 0.02	Phosphorus in %	1.18 ± 0.07
Potassium in %	0.54 ± 0.03	Potassium in %	0.98 ± 0.02
Zinc in %	0.0056 ± 0.001	Zinc in %	0.0088 ± 0.001
Iron in %	0.077 ± 0.01	Iron in %	0.094 ± 0.01
Manganese in %	0.016 ± 0.005	Manganese in %	0.024 ± 0.008
Copper in %	0.009 ± 0.001	Copper in %	0.012 ± 0.004
Total bacteria count (CFU g^{-1})	2.4×10^8	Total bacteria count (CFU g^{-1})	4.8×10^8

Table 4. Species of earthworms from which different antimicrobial peptide extracted.

Antimicrobial peptide	Species of earthworm	References
OEP3121	<i>Eisenia fetida</i>	Liu et al., (2004)
Lumbricin I	<i>Lumbricus rubellus</i>	Cho et al., (1998)
A Lumbricin I analog named PP 1	<i>Pheretima tschiliensis</i>	Wang et al., (2003)
EFAF (<i>Eisenia fetida andrei</i> factors)	<i>Eisenia fetida andrei</i>	Valembos et al., (1984)
Lysozyme	<i>Eisenia fetida andrei</i>	Cooper et al., (2002)
Eseniapore	<i>Eisenia fetida</i>	Valembos et al., (1984)
Fetidins	<i>Eisenia fetida</i>	Gupta and Yadav (2016); Josková et al., (2009)
Bacteriostatsins	<i>Eisenia fetida</i>	Vaillier et al., (1985)
Lysenin	<i>Eisenia fetida</i>	Shogomori and Kobayashi (2008)
Coelomic cytolytic factor (CCF)	<i>Lumbricus</i> sp.	Šilerová et al., (2006)
Lumbricin-PG	<i>Lumbricus</i> sp.	Cho et al., (1998)

peptides enable earthworms to make the soil healthier (Kobayashi et al., 2004; Kaur et al., 2010; Li et al., 2011).

2.6. Vermiwash and earthworm extract as antifungal agent

Globally, the pathogenic fungus *Fusarium graminearum*, severely affected the roots and reduced the production of and quality of wheat (*Triticum aestivum* L.) by 20%. But, after the application of vermiwash into the farm of wheat, the effect of this harmful fungus was controlled (Akinnuoye-Adelabu et al., 2019). Akinnuoye-Adelabu et al. (2019) applied vermiwash to a wheat plantation and measured the efficiency of the extract from the earthworm. Vermiwash has significantly promoted mycelium growth inhibition for the parameters (shoot length, root, root biomass, and disease index). They reported the effect of vermiwash and mucus extracted from *Eisenia fetida* on *Fusarium graminearum* that greatly inhibited the growth of pathogenic fungus from a wheat farm.

The antagonistic properties of extract of earthworm *Eisenia fetida* not only inhibited growth of fungus *Fusarium graminearum* significantly but also enhanced overall growth of the plant. The mucus of the worm effectively acted as anti-fungus up to 26% (Akinnuoye-Adelabu et al., 2019) because of presence of peptides, which inhibited the growth of pathogenic microbes in the soil (Li et al., 2011; Liu et al., 2004). On the other hand, the vermiwash from fourteen days vermicomposting inhibited the growth of fungal mycelium by 16% (Akinnuoye-Adelabu et al., 2019) indicating that the concentration of fungal growth inhibitor agents in vermiwash was less as compared with its concentration in mucus. Extracts of earthworms, thus, acted as bio-fungicide in suppressing harmful fungus (*Fusarium graminearum*).

In contrast, *Arbuscular mycorrhizal* fungi in combination with vermiwash effectively suppressed diseases of a plant (Khan et al., 2015). Application of vermiwash on plant leaf and inoculation of *Arbuscular mycorrhizal* fungi improved physical and chemical properties of soil enhancing growth of plant by facilitating nutrients uptake. The combined consequence of both vermiwash and AM fungi was far more as compared to their individual effects (Khan et al., 2015). One of conditions that reduced the productivity of a plant was due to physiological stress caused by biotic and abiotic factors. *Arbuscular mycorrhizal* fungi possessed inherent properties of reducing biotic and abiotic stress caused by drought, higher temperature, soil and plant root pathogens by stimulating the physiology of plants, minimizing loss of water, and facilitating availability of vital mineral (Rouphael et al., 2015).

2.7. Application of vermiwash as pesticide

Thakur and Sood (2019) evaluated pesticide activities of different materials with different compositions. One of the materials tested was

vermiwash from vermicompost prepared from cowdung (3kg) and biomass (2kg) decomposed by 200–300 adult earthworms. Vermiwash is reported to possess plant growth-promoting agents as well as killing properties of pests. Vermiwash spray efficiently controlled all types of diseases and pest conditions, and served as an ecofriendly bio-pesticide.

A study was carried out on application of vermiwash, liquid vermicompost, and coelomic fluid extracted from earthworm *Eisenia fetida* to control root-knot nematode (*Meloidogyne javanica*) in vitro and under greenhouse conditions (Rostami et al., 2014). The results showed that the three products (vermicompost, coelomic fluid, and vermiwash) had the highest effect on egg hatching inhibition respectively. This indicated that the vermicompost had the highest concentration of nutrients followed by coelomic fluid and vermiwash.

In-vitro application of coelomic fluid exhibited the highest mortality rate in larvae as it possessed bioactive molecule that directly affected physiological activities of the larva. Generally, it concluded that all earthworm products effectively suppressed the number of juvenile nematodes and gall index in greenhouse. The best recommended combination of earthworm products to suppress the influence of pest was vermicompost with 10% liquid vermicompost and vermicompost with 10% vermiwash (Table 5).

2.8. Role of vermiwash to improve productivity and Rhizobial nodulation of legumes

The vegetative parameters viz., carotenoid, leaf weight, chlorophyll, the height of *Phaseolus vulgaris*. L bean reported by Belmeskine et al. (2020) to increase significantly. Plants treated with pure vermiwash during their growth could be productive (in nutritional values and growth indices). It provided copious amount of bioavailable macro and micronutrients for synthesis of their biomass. Soya bean, a leguminous plant, harnesses nitrogen-fixing bacteria in their nodules and contain healthy protein. In an experiment, Mahendra and Narendra (2012) observed enhancement of higher nodule counts and root biomass in a soya bean field treated with vermiwash/vermicompost, which may prove to be a boon for improving productivity of agricultural crops. As the number of nodules per plant rises, the fixation rate of nitrogen increases resulting in higher biomass, leaf size, pod and seed size. Vermiwash contains higher concentration of available nitrogen improving *Rhizobial* colonization of nodules. Sufficient availability of soluble nitrogen enhances plant growth and increases productivity (Pathma and Sakthivel, 2012).

The sufficient availability of soluble nitrogen enhanced the fast growth of plants, and increased their productivity. Vermiwash contain a higher concentration of available nitrogen Pathma and Sakthivel (2012), improve the *Rhizobial* colonization of nodules, and hence suppress root disease in the soil (Parveen et al., 2019). Water is the most essential raw material for the productivity of plants in the process of photosynthesis. Vermicomposting able to retain water in the soil in a dry area and enhanced the rate of photosynthesis of chickpea (*Cicer arietinum* L.) and was demonstrated the plant to resist drought by using vermicomposting (Hosseinzadeh et al., 2016).

2.9. Role of coelomic fluid to suppress plant disease

Earthworms secrete coelomic fluid through their dorsal pores as a defense mechanism against stress. The coelomic fluid contains immunity components, which effectively controls protozoan parasite monoxystis (Field et al., 2004). The coelomic fluid secreted from earthworm (*Eudrilus eugeniae*) by cold stress tested for different plant growth parameter viz., seed germination, enhanced shoot and root length, and seedlings. It significantly promoted the growth of the plant (*Oryza sativa* L. sub sp. *Indica*) as compared to the plant treated by Gibberellic acid (Nadana et al., 2020). Besides, the biochemical compounds analyzed by spectrometry and liquid chromatography revealed that it possessed pesticidal, antifungal, insecticidal properties, and chemicals that mimicked the role

Table 5. Repellent activity of bio-pesticide products/bio-pesticide formulations against *Tetranychus urticae* adult females at different exposure durations (Adopted from Thakur and Sood, 2019).

Treatment concentration	Repellence (%) over untreated check at indicated exposure duration					Mean
	1h/2h	1h	2h	4h	8h	
10%	16.37 (23.85)	16.67 (23.85)	16.63 (23.85)	23.33 (28.77)	23.33 (28.77)	19.27 (25.82)

of hormones. A derivative of plant hormone, indole-3-acetyl-L-valine was discovered from the coelomic fluid of earthworm. The coelomic fluid of earthworm effectively suppressed the fungus (*Rhizoctonia solani*) and enhanced the growth and production of rice (Nadana et al., 2020).

2.10. Adaptation of earthworm against soil pathogen

Earthworms live in an environment of disintegrating dead natural organic matter surrounded by microbes (Li et al., 2011). Night crawlers (*Lumbricus terrestris*) protect themselves against pathogenic microorganisms in dirt by secreting an assortment of humoral and cellular toxic substances (Massicotte et al., 2004). According to Ghosh (2018), earthworm's defensive mechanisms include cellular, humoral, immune response, and antimicrobial peptides. The cellular immunity comprises eleocytes, chloragocytes, granulocytes, oelomocytes, natural killer (NK) cells and NK-like effectors cells. Humoral immune system encompasses antibacterial molecules, cytokines, cytotoxic proteins, that play a role in encapsulation, phagocytosis, clotting, agglutination, and lysis (Cooper et al., 2006).

The immune response in earthworms achieved by coelomic cells controls the response to different irritation in its environment. In an experiment, Yadav (2016) showed colder stimulus causes higher secretion and concentration of coelomic cells enabling earthworm's response to it. Antimicrobial peptides (AMP) protect earthworms from pathogenic microbes in an unfavourable environment. These AMP are OEP3121, Lumbricin I, Lumbricin I analog named PP 1, EFAF (*Eisenia fetida andrei* factors), Lysozyme, Eсениapore, Fetidins, Bacteriostatins, Lysenin, Coelomic cytolytic factor (CCF), Lumbricin-PG (Ghosh, 2018; Köhlerová et al., 2004).

Humoral method of anti-microbial defense mechanism is a basic vital biological activity in earthworms (skin protection, mucus and coelomic fluid), which secured their survival and protecting them from pathogenic bacteria and other irritants via several ways. Possibly, an evolutionary adaptation enabled them to protect themselves from pathogens (Vaillier et al., 1985), including several bioactive peptide chains of different amino acids (Gupta and Yadav, 2016). The humoral method could be achieved through lysing cells (Prakash and Gunasekaran, 2011; Procházková et al., 2006; Field et al., 2004), by forming pore on the cell membrane of pathogen (Kobayashi et al., 2004; Shogomori and Kobayashi, 2008), and hemolysis (Pan et al., 2003; Vasanthi et al., 2013).

Earthworms have very poor physical defense system instead have evolved varieties of humoral mechanisms to defend against irritants and pathogens. In earthworms, Biruntha et al. (2020) reported that coelomic fluid played a vital role in countering irritants by-synthesis of zinc sulfide nanoparticles using *Abrus precatorius*. As a result, they secreted higher concentration of coelomic fluid enabling them to resist its toxicity. Higher secretion of coelomic fluid observed in response to unfamiliar and hazardous substances in the soil. It has positive impact on wellbeing of environment and bioremediation by its inherent properties to neutralize the toxic substances (Yadav, 2017).

2.11. Experimental application of G-90 and coelomic fluid against microbes

G-90, a tissue extract of earthworm comprising lipopolypeptides, which effectively acted against four species of bacteria and two species of fungi with significant results (Vasanthi et al., 2013). Antimicrobial macromolecules are identified and referred to as antimicrobial

vermipeptide family (AVPF) from coelomic fluid and tissue homogenate of earthworm (Wang et al., 2007). They examined and separated using crude peptide preparation, ultrafiltration purification, ion-exchange chromatography, gel filtration, and HPLC chromatography. They determined the antibacterial activity for Gram-ve, Gram +ve bacteria, and anti-fungal property with significant positive results (Wang et al., 2007).

It investigated that the coelomic fluid extracts from *Eisenia fetida andrei* acted against gram-ve bacteria (Shogomori and Kobayashi, 2008). Coelomocyte concentrations in *Lumbricus terrestris* using as the independent variable was evaluated to determine parasitic load and it was indicated that as invading parasite increased, the coelomocyte concentration also increased (Field et al., 2004). The coelomic fluid has anti-microbial property, which benefited the animal as an agent of immunity (Field et al., 2004). The coelomic fluid of *Eisenia fetida* possesses short peptide coded as OEP3121, which acted against bacterial species of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* (Liu et al., 2004).

In an experiment, antimicrobial activity of vermi-extract of *Perionyx excavatus* was reported against four human pathogenic microorganisms (*Escherichia coli*, *Proteus* spp., *Providencia* spp., *Morganella* spp.), which caused urinary tract infection in children (John and Packialakshmi, 2007). They concluded continuous taking of earthworms as a meal could recover people from urinary tract infections. Earthworms were able to defend against pathogenic bacteria by secreting bioactive macromolecules from their organs mostly when they exposed to soil environment harboring a variety of pathogens. Engelmann et al. (2004) and Bilej et al. (2000) opined earthworm's coelomocytes prevented pathogenic bacteria not only by phagocytosis and intoxicating them but also by lysing extracellularly. In vivo analysis of the antimicrobial activity of tissue homogenate (G-90) extracted from *Eisenia fetida* on non-pathogenic bacteria and facultative pathogenic bacteria demonstrated that the bacteriostatic influence of G-90 was higher for facultative pathogenic bacteria than non-pathogenic bacteria (Bilej et al., 2000).

Higher concentration of tissue extract (G-90) of earthworm showed greater effect to inhibit facultative-pathogenic bacteria than non-pathogenic bacteria (Bilej et al., 2000). The activity of different bioactive molecules of coelomic fluid against diverse bacterial species is identified by the recognition of lysozyme (Fiołka et al., 2012). Invertebrates could identify chemical structure of either bacteria's cell wall by detecting peptidoglycan and phospholipid or cell membrane in higher animals by detecting sphingomyelin (Fiołka et al., 2012).

3. Conclusion

Vermiwash is the by-product of vermicompost applied as fertilizer by directly adding it into soil and as a liquid spray overall part of the plant body to prevent fungal, bacterial pathogen, and pests. Bioactive macromolecules found in the coelomic fluid, mucus, and skin secretion of earthworms are very important to act against pathogenic soil microbe and pests. The metabolites produced as a vermicomposting product applied to promote growth of plants and create an unfavorable condition for soil dwelling pathogens. As earthworm survives in soil, full of pathogenic microorganisms, and evolves cellular and humoral defense mechanisms protecting them from infection. Their cellular defense mechanism fight against pathogen by phagocytosis. They also defend themselves against pathogenic microbes and pests through their humoral defense mechanism which includes bioactive molecules such as lysenin, cytolytic eiseniapore, fetidin, hemolysin, lysozyme, Lumbricin I,

Lumbricin-PG coelomic cytolytic factor, OEP3121, bacteriostats obtained from coelomic fluid, mucus and skin secretion of earthworm. Other metabolites such as hormones, enzymes, vitamins, proteins, different macro and micronutrients, and a massive amount of microbes facilitate a conducive environment for the growth of a plant by reducing stress conditions and create unfavorable condition for pathogenic soil microbes and pests. Furthermore, the exponential multiplication of soil microbes around earthworm in vermicompost/vermiwash competed and defended the disease-causing. Therefore, application of vermiwash/vermicompost as fertilizer adding into soil or spraying on the surface of plants significantly suppress a pathogen and pest.

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Author contribution statement

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The authors declare no conflict of interest.

Additional information

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