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Analysis of Fungal Flora, Physicochemical and Antimicrobial Properties of Vermicompost and Vermi-Wash Developed Through Green Waste Digestion by *Eudrilus eugeniae*- A Night Crawler Earthworm

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Abstract

The formulation of vermicompost from green wastes digestion by the action of night crawler earthworm *Eudrilus eugeniae* was carried out in vermicompost tank which also results in the generation of liquid leachate vermin wash or vermi-wash. In the present study, we have analysed the physicochemical, biochemical and antimicrobial properties of vermicompost and vermi-wash. ThepH level in the Vermicompost found to be acidic (pH 5.38±0.64) while in the vermi-wash found to be alkaline (pH 8.41). The C: N ratio was higher in the vermicompost when compared to the vermi-wash. The biochemical analysis of both samples indicated the presence of DPPH free radical scavenging activity, alkaloid and carotenoid. However, flavonoid, lycopene, B-carotene and phenolic content were absent in both the samples. The presence of extracellular enzymes such as amylase, protease, cellulase and xylanase also detected in the vermi-wash. Mycofloral analysis of vermicompost revealed the presence of 43 fungi, most of the isolated fungi showed a proteolytic and amylolytic activity. The antimicrobial property was exhibited by vermicompost which inhibited the growth of some plant pathogens while, the vermi-wash samples did not show any inhibatory effect on the tested plant pathogens.

Keywords: Vermicompost; Vermi-wash; *Eudrilus eugeniae*; Fungi; Extracellular activity; Antagonism

Introduction

Earthworms are found to be responsible for the development of soil fertility that has led to their more utilization for agricultural purposes [1,2]. Presently, the green wastes obtained from garden, local plantation and household droppings are difficult to be manage, it requires high energy in the form of manpower and mechanical management and also considered as time-consuming. These problems has led to the search for an alternative(s) to put live energy into another gainful use. In this context, vermicomposting is an eco-friendly technology for rapid conversion of any organic waste to value manure by using earthworms. Hence, vermicompost or castings is worm manure and considered to be best soil amendment available. Now-a-days, vermicomposting is a popular technique of composting. Earthworms are used to degrade the organic waste, (dairy farm wastes, industrial and garden waste, hatchery waste, leaf litter waste, municipal wastes, sugar mill residues, slaughterhouse waste and vegetable wastes etc.) to produce the final vermicompost product [3-5]. Vermicompost obtained after degradation process is peat like containing factory of soluble nutrients, plant growth regulators as well as a good microbial activity because of high porosity, aeration and water holding capacity [6]. Vermicomposting should regularly be done to build up the soil's physical, chemical and biological properties so that its natural fertility is restored. Moreover, it increases the available nutrient content for plants in the soil (nitrates, phosphates, exchangeable calcium and soluble potassium), growth regulators as well as the benificial microbial populations [7]. It contains 1.2-6.1% more nitrogen, 1.8-2.0% more phosphate and 0.5-0.75% more potassium compared to the farmyard manure. Fungi are the important component in the vermicompost which play a major role in the biodegradation and conversion process of composting. Fungi undertake rapid decomposition of lignocellulosic material including cellulose, hemicellulose, ligninetc resulting in the maturation of compost matter [8]. However, a little is known about fungal communities

associated with vermicomposting [9]. It is also known that differences in the composition of the fungal community are observed in different composting methods and different material sources. So, the resident fungal population needs to be monitored to determine the quality of vermicompost material [10], many reports on fungal population associated with vermicompost are also presented [11,12].

The Vermicompost technology can be also used for generating a liquid leachate called vermin wash or vermi-wash [13]. Vermi-wash is the best alternative to chemical fertilizer and can be use as a foliar spray. Generally, the excretory products of earthworms along with some trace nutrients leached from soil organic molecules. This foliar spray produced from vermicomposting can be used against plant diseases. Presently, vermi-wash is also used as liquid manure. It also possesses plant growth promoting factors [14]. The present investigation was carried out to study the mineral composition, biochemical components, and antagonistic properties of vermicompost and vermi-wash. Mycofloral analysis and extracellular activity from the fungal isolates of vermicompost and enzymatic potential of vermi-wash was also studied.

Materials and Methods

Source of vermicompost and vermi-wash

The vermicompost used in this study was produced by the collection of green wastes which includes leaf litter; garden mowed grasses, garden waste, etc. Primary decomposition of the raw material was carried out inside the pit by spraying of cow dung slurry in layers. The 21-day old pre-decomposed material was dumped into vermin tank and inoculated by night crawler earthworm *Eudrilus eugeniae*. Regular watering was done with sprinkler and left for 45-90 days. Vermicompost was formulated in the tank and vermi-wash sample is obtained from liquid leachate after 90 days.

Physicochemical analysis

The vermicompost and vermi-wash samples were collected. The pH and mineral content such as C, N, P, K, Na, Ca, Mn, Fe, Cu and Zn were analysed in the collected samples.

Biochemical and antioxidant analysis

Alkaloids: The collected samples were extracted in 100 ml of 10 % ethanolic glacial acetic acid. Five millilitre of the extracted solution was taken and pH was adjusted to pH 2-2.5, then Two millilitre of Draggendroff's reagent was added and centrifuged for 10 min. at 5000 r.p.m. after the centrifugation, the supernatant was discarded and the precipitate obtained was washed for 3-4 times using 95% ethanol then 2 ml of 1% sodium sulfide solution was added, the sample then centrifuged 10 min at 5000 rpm. The resulted precipitate was dissolved in 2 ml of 70% concentrated HNO_3 then diluted using 10 ml of distilled water, one milliliter of the diluted sample was mixed with 5 ml of 1 % thiourea solution. The absorbance was measured at 435 nm and amount of alkaloids was dermined from standard curve with Bismuth nitrate pentahydrate stock solution [15].

Beta carotene and lycopene: The concentration of β-carotene and lycopene in extracts was estimated calorimetrically [16,17]. Hundred milligram of dried methanolic extract prepared using soxhlet was shaken vigorously with 10 ml of acetone and hexane mixture (4:6) for one minute continuously and filtered through whatman filter paper. The contents of β-carotene and Lycopene were estimated by following formula:

$$Lycopene = -0.0458 \times A.663nm + 0.372 \times A.505nm - 0.0806 \times A.453nm$$

$$\beta - carotene = 0.216 \times A.663nm - 0.304 \times A.505nm + 0.452 \times A.453nm$$

Carotenoid content: The determination of carotenoids was done according to the method described by Arnon [18]. Ten ml of 80 % acetone was added to One gram of the sample in a mortersetted in an ice bath then the sample grounded and centrifuged at 2500 g for 10 minutes at 4 ^{0}C. The procedure was repeated untile the final residue became colourless. 80 % acetone was added to the Resulted extract up to 10 ml then assayed spectrophotomerically. The obtained extract was measured at 480, 645 and 663 nm and expressed as mg carotenoids per one gram sample by using following formula:

$$Carotenoid\ content = A.480\ nm + \left(0.114 \times A.663\ nm - 0.638 \times A.645\ nm\right)$$

Where A= absorbance

DPPH Free Radical scavenging activity: Radical scavenging activity in the methanolic extract was analysed [19]. One ml of methanolic extract was added in a test tube containing two ml of DPPH solution then incubated for 30 minutes in the dark at room temperature. The Absorbance of the resulting solution was measured spectrophotometrically at 517nm. Ascorbic acid Equivalent Antioxidant Capacity (AEAC) was calculated by putting the value of absorbance in standard ascorbic acid curve and AEAC value was expressed in terms of mg per g.

$$\%\ RSA = \left[\frac{\left(A_{DPPH} - A_S\right)}{A_{DPPH}}\right] \times 100$$

Flavonoids: The flavonoid content in the sample was estimated by using aluminium chloride colorimetric technique and expressed in terms of mg quercetin equivalents per gram [20]. Hundred micro litres of methanolic extracts were diluted with 1.5

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ml of methanol and incubated for 5 minutes at room temperature. Hundred micro litres of $AlCl_3$ was added and again incubated at room temperature for 5 minutes. The reaction mixture was mixed with 0.1 ml of 1 M Potassium acetate and total volume was made up to 5 ml using distilled water. The mixture was incubated for 30 minutes at room temperature then the optical density was measured at 415 nm.

Total phenolic content: The Total phenolic content in the samples were estimated by using the folin phenol method [21]. 1g sample extracted with 10 ml of methanol by grinding in mortar and pestle then centrifuged at 2000 g for 15 minutes. The supernatant obtained was served as methanolic extract. Hundred micro litre of extract was added to 900 micro litre of distilled water followed by the addition of 1 ml of Fc reagent and shaken well. 2 ml of 10 % sodium carbonate solution was added to the reagent mixture. The Phenolic content in different extract were measured at 765 nm and expressed in as Gallic acid equivalent (GAE) in mg per gram.

Mycofloral analysis and their extracellular activity

Serial dilutions of the vermicompost sample were prepared and then a portion of the prepared dilution separated on Sabouraud's Dextrose medium in Petri-dishes to isolate the mycoflora. The fungal isolates obtained were identified through slide culture technique and morphological characteristics on plate such as colony colour, medium colour, growth, margin, form, texture and elevation were recorded. All the isolates were screened for their extracellular enzymatic activity such as amylase, cellulase, lipase and protease; phosphate solubilisation potential and organic acid production at 28ºC [22]. Vermi-wash samples were also detected for extracellular activity.

Amylase activity (Starch hydrolysis): Starch agar medium containing 2% starch was used to determine amylase activity. Each isolate was centrally inoculated on starch agar media and incubated at 28 ºC for 5 days. After incubation period, the result was observed using 1% iodine solution. The appearance of clear zone surrounding the colony indicated a positive amylase production.

Cellulase activity: Cellulolytic activity was determined in Glucose Yeast Extract Peptone agar medium amended with 0.5% Carboxymethyl cellulose. After 5 days of incubation, the plates were stained with 0.2% aqueous Congo red solution followed by destaining with 1M NaCl for about 15 minutes. Transparent zone appearance around the growing colony in red medium shows positive cellulase activity.

IAA test: Indole acetic acid production was estimated in JNF medium amended with 0.1% tryptophan. After incubation, the plates flooded with Salkowski's reagent. The appearance of light pink color shows after adding the reagent indicates a positive result.

L-Asparaginase activity: L-Asparaginase activity was evaluated using 1% L-Asparagine and phenol red containing medium. After 5 days of incubation, plates were flooded with Nessler's reagent and screened on the basis of the appearence of a pink zone around the colony.

Lipolytic activity: Lipase activity was studied in Peptone agar medium amended with 1% Tween 20. A visible precipitate around the growing colony indicates a positive lipase activity.

Organic acid production test: Samples were investigated by using Glucose Yeast Extract Peptone agar medium amended with $CaCO_3$ Bromocresol green is used as indicator. After 5 days of incubation, the presence of clear zone around the colony indicates a positive organic acid production.

Phosphate solubilization activity: Phosphate solubilisation activity of the tested microbial flora isolated from vermicompost and vermi-wash sample wereinvestigated on Pikovskaya's agar plate containing 0.5% Tricalcium phosphate. After the incubation period the appearance of a clear halo zone around the colony indicate a positive phosphate solubilization activity.

Protease activity: Fungal isolates were grown on Gelatin agar medium (2% gelatin). After 5 days of incubation at 28ºC, 2 ml of 15% $HgCl_2$ and 20% HCl solution was used as an indicator.

Xylanase activity: Xylanase activity was evaluated using xylan as substrate in the Glucose Yeast Extract Peptone agar medium. After the incubation period, the surface of the medium flooded with 0.1% Congo red solution then destained using 1M NaCl for 15 minutes. The Appearance of transparent zone around the colony indicates positive activity of xylanase.

Antimicrobial properties

The antagonistic activity assay was performed on Sabouraud dextrose agar medium plates by dual culture method [23]. Five plant pathogen cultures (*Alternaria triticina, Botrytis cinerea, Curvularia lunata, Phoma tropica, Trichoderma viride*) were used for the study. Medium prepared with pH maintained at 4.5±0.2, sterilized at 121 ºC at 15 lbs pressure for 15 minutes. 6mm agar well are made using cork borer at a distance of 2 cm in the plates prepared. At one side, vermi-wash/vermicompost was inoculated with the culture at the other side and incubated for the period of growth at 28 ºC.

Results and Discussion

Data presented in Table 1 showed that the vermicompost produced by using the earthworms on plant wastes was acidic (pH 5.38±0.64), this might be due to that, the micro-organisms began the biochemical degradation on the most available substrates such as sugars and starch [24]. The Presence of (17.73±2.92) % organic C, (0.69±0.24) %N, (0.17±0.071) % P and (0.3±0.115) % K content was determined from mineral analysis. The C: N ratio in the vermicompost was found to be 25:1 some reports revealed that, the C: N ratio in vermicomposted olive cake was 29:1 [12]. Elements such as Na, Ca, Fe, Mn was presented as (0.059±0.015) %, (1.475±0.55) %, (1.23±0.63) % and (0.03±0.03) % respectively. However, Cu and Zn scored (22.575±7.24) and (74.425±19.01)

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mg/Kg respectively. The obtained minerals compositions of vermicompost were within the range other previous studies [12]. On the other hand, vermi-wash samples showed an alkaline pH of 8.41 with electrical conductivity of 4.06 ds/m (Table 2). The increase of pH in vermi-wash might be due to the participation of microbes in the degradation of organic wastes representing aerobic metabolism. Loss of organic matter and release of mineral salts in available forms (such as phosphate, ammonium and potassium) result in an increase of electrical conductivity in the vermi-wash. The mineral analysis of vermi-wash samples showed the presence of 1.03% N, but the amount of Organic C, P, K is found to be 0.03%, 0.04% and 0.02% respectively which is very low. The N content in vermin-wash was more due to the addition of nitrogenous excretory substances by the earthworms as a part of nitrogen cycle where as C content is less as carbon is lost in the form of CO_2 during respiration in earthworms [25]. Hence, the level of C/N ratio was found to be significantly decreased. Na is higher than Ca in vermi-wash samples. The concentration of heavy metals such as Fe, Mn, Cu and Zn in vermi-wash samples is 58.5, 6.0, 0.47 and 0.25 mg/L respectively.

Table 1: Physicochemical analysis of Vermicompost.

Parameters	Vermicompost
pH	5.38±0.64
OC (%)	17.73±2.92
Total N (%)	0.69±0.24
Total P (%)	0.17±0.071
Total K (%)	0.3±0.115
Total Ca (%)	1.475±0.55
Total Na (%)	0.059±0.015
Total Fe (%)	1.23±0.63
Total Mn (%)	0.03±0.03
Cu (mg/kg)	22.575±7.24
Zn (mg/kg)	74.425±19.01

Table 2: Physicochemical analysis of Vermi-wash.

Parameters	Vermi-wash
EC (dS/m)	4.06
pH	8.41
OC (%)	0.03
Total N (%)	1.03
Total P (%)	0.02
Total K (%)	0.04
Total Ca (%)	0.24
Total Na (%)	96.2
Fe (mg/L)	58.5
Mn (mg/L)	6
Cu (mg/L)	0.47
Zn (mg/L)	0.25

The biochemical and antioxidant assay of vermicompost and vermi-wash samples showed the presence of DPPH free radical scavenging activity, Alkaloid and Carotenoids (Table 3). However, flavonoid, lycopene, β-Carotene and phenolics content in vermicompost and vermi-wash were not obtained. The quantity of alkaloids in both the samples is recorded as (1±0.05) mg/g in vermicompost and (0.112±0.08) mg/ml in vermi-wash. Carotenoid content in vermicompost (23.5±21.23) mg/g as compared to vermi-wash (1.68±1.24 mg/ml). DPPH free radical scavenging activity was recorded in vermicompost and vermi-wash samples. In vermicompost, (98.25±0.64) % activity is exhibited whereas in vermi-wash it is (68.8±4.58)%. Extracellular enzymatic content and secondary metabolites in vermi-wash was also measured and presented in (Table 4). Vermi-wash showed positive result for amylase, protease, cellulase and xylanase enzymes only. Amylase and protease activity in vermi-wash was also reported [5]. Most of the cellulase and chitinase enzymes are secreted by the earthworms that occur in their intestinal canal [26].

Table 3: Biochemical analysis of Vermicompost and Vermi-wash.

Parameters	Vermicompost	Vermi-wash
Alkaloid	1±0.05 mg/g	0.112±0.008mg/ml
B-carotene	0	0
Carotenoid	23.5±21.23 mg/g	1.68±1.24 mg/ml
DPPH	98.25±0.64%	68.8±4.58%
Flavonoid	0	0
Lycopene	0	0
Phenolics	0	0

Table 4: Qualitative analysis of vermi-wash for presence of enzymes and secondary metabolites.

Extracellular activity	Test
Amylase	+ve
Cellulase	+ve
IAA	-ve
L- Asparaginase	-ve
Lipase	-ve
Organic acid	-ve
Phosphate solubilisation	-ve
Protease	+ve
Xylanase	+ve

The mycofloral analysis of the vermicompost samples resulted in 43 fungi which are representing different genera such as *Aspergillus, Penicillium, Articulospora, Orbilia, Endophragmia, Oidiodendron* and Sterile mycelium identified through slide culture technique (Table 5). Isolates belong to *Aspergillus* and mycelia sterilia genera were dominant. 42% belonged to *Aspergillus* genera, 32% belonged to sterile mycelium and 14% from *Penicillium* genera. In few studies, 142 fungi were

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isolated from vermicompost by one group [11] and 77 fungi by another [27] which is more than the number of fungi obtained from our study (43 fungi). *Aspergillus* and *Penicillium* genera in vermicompost was also reported [8,11]. Sterile mycelia also prevailed in vermicompost as previously demonstrated [28]. Fungal colony characteristics and growth pattern of all the isolates were noted down. The front and reverse coloration of all the isolates were noted and specific features of some isolates were highlighted. The growth pattern of the fungal isolates varied from least to maximum growth. 2 fungi showed least growth, 14 fungi showed moderate growth whereas good growth was observed in 15 fungi on medium plate. The colony form of the fungi were mostly irregular, few are filamentous and circular. The margin of the fungal colony included undulate, filiform, lobate and entire. The colony texture ranged from very slight cottony to cottony, powdery, velvety and cottony with powdery. The elevation was either flat or raised except few were umbonate. These isolates were also screened for their extracellular enzymatic activity, phosphate solubilising ability and organic acid production capacity on specific culture medium (Table 6). Most of the isolates have proteolytic activity and amylolytic activity. Almost same number of isolates has lipolytic, phosphate solubilisation and organic acid production potential.

Table 5: Mycofloral Analysis of Vermicompost.

Fungi	Front colour	Back colour	Growth	Form	Margin	Texture	Elevation
Articulospora sp 1	White	White	++	Irregular	Filiform	Cottony	Raised
Articulospora sp 2	White with a yellow ring	Off white	++	Irregular	Entire	Slight cottony	Flat
Aspergillus sp. 1	White	White	++++	Irregular	Undulate	Cottony	Raised
Aspergillus sp. 2	Olive green	Pale white	+++	Circular	Entire	Powdery	Flat
Aspergillus sp. 3	Light pink	Off white	++++	Irregular	Filiform	Powdery	Flat
Aspergillus sp. 4	Light pink	Off white	++++	Irregular	Filiform	Powdery	Flat
Aspergillus sp. 5	White cottony with yellowish green spor	Off white	+++	Irregular	Filiform	Cottony and sporulating	Raised
Aspergillus sp. 5	White cottony with yellowish green spor	Off white	+++	Irregular	Filiform	Cottony and sporulating	Raised
Aspergillus sp. 6	White cottony with yellowish green spor	Off white	+++	Irregular	Filiform	Cottony and sporulating	Raised
Aspergillus sp. 7	Light pinkwith white border	Off white	++	Irregular	Undulate	Cottony	Flat
Aspergillus sp. 8	Green	Off white	++++	Irregular	Undulate	Powdery	Flat
Aspergillus sp. 9	Black	Off white	++++	Irregular	Lobate	Powdery	Flat
Aspergillus sp. 10	Creamy	White	+++	Irregular	Undulate	Powdery with slight cottony	Flat
Aspergillus sp. 11	Olive green	Pale yellow	++++	Irregular	Undulate	Powdery	Flat
Aspergillus sp. 12	Off white	Pale yellow	++++	Filamentous	Filiform	Slight powdery	Flat
Aspergillus sp. 13	White cottony with yellowish green spor	Off white	++++	Irregular	Filiform	Cottony and sporulating	Raised
Aspergillus sp. 14	Olive green	Pale yellow	+++	Irregular	Undulate	Cottony with powdery	Flat
Aspergillus sp. 15	Green with white excurate	Pale yellow	++++	Irregular	Undulate	Cottony with powdery	Raised

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Aspergillus sp. 16	Black with pale yellow	White	+++	Irregular	Entire	Cottony with powdery	Raised
Aspergillus sp. 17	Ash with green	Pale brown	++++	Irregular	Undulate	Powdery	Flat
Endophragmia sp.	Greyish to pale pink	Center black *to orange*	+++	Irregular	Undulate	Slight cottony	Flat
Oidiodendron sp.	White	White	++++	Irregular	Entire	Cottony	Raised
Orbilia sp.	Sap green	Back	++	Irregular	Undulate	Velvety	Flat
Penicillium sp. 1	Light pink	Pale yellow	+++	Circular	Entire	Powdery	Flat
Penicillium sp. 2	Light pink	Pale yellow	+++	Circular	Entire	Powdery	Flat
Penicillium sp. 3	Whitish with light olive green	Pale white	+++	Irregular	Undulate	Velvety	Flat
Penicillium sp. 4	Light olive green	Pale yellow	++++	Irregular	Undulate	Slight cottony	Raised
Penicillium sp. 5	Grey	Pale white	++++	Irregular	Undulate	Powdery with slight cottony	Flat
Penicillium sp. 6	Grey	Yellow	++++	Irregular	Undulate	Slight cottony	Flat
Sterile mycelium sp 1	Blackish white	Off white making medium pink	++	Irregular	Undulate	Cottony	Flat
Sterile mycelium sp 2	White	Yellow	++	Irregular	Lobate	Slight cottony	Flat
Sterile mycelium sp 3	White cottony	Center black and black white mix	++++	Filamentous	Filiform	Cottony	Flat
Sterile mycelium sp 4	Pale yellow with white border	Pale yellow making medium yellow	++	Irregular	Undulate	Velvety	Flat
Sterile mycelium sp 5	Ash colour	Pale yellow	++	Irregular	Undulate	Velvety	Raised
Sterile mycelium sp 6	White	White	+++	Irregular	Undulate	Velvety	Flat
Sterile mycelium sp 7	White	Yellow making medium yellow	++	Irregular	Undulate	Velvety	Umbonate
Sterile mycelium sp 8	Olive green	Pale white	++	Irregular	Entire	Velvety	Flat
Sterile mycelium sp 9	Light brown with white border	White	+++	Irregular	Undulate	Powdery	Flat
Sterile mycelium sp 10	White	White	+	Irregular	Undulate	Very slight cottony	Flat
Sterile mycelium sp 11	White	White	++	Irregular	Undulate	Cottony	Umbonate
Sterile mycelium sp 12	Greyish	Black and off white	+	Irregular	Undulate	Slight cottony	Flat
Sterile mycelium sp 13	Pale yellow with white border	Brown to black	+++	Irregular	Lobate	Slight cottony	Flat
Sterile mycelium sp 14	White	White	++	Irregular	Undulate	Leathery	Flat

(+++++)- Maximum growth, (++++)-Good growth, (+++)-Moderate growth, (++)-Less growth, (+)-Least growth based on linear growth of fungi on medium plate, after 5 days of incubation.

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Table 6: Extracellular activity of fungi isolated from Vermicompost.

Fungi	Amylase	Cellulase	Lipase	Organic acid	Phosphate Solubilisation	Protease
Articulospora sp 1	-	-	++	-	-	++++
Articulospora sp 2	+	++	-	-	-	-
Aspergillus sp. 1	-	-	-	-	-	-
Aspergillus sp. 2	-	+++	+	++	++	+++
Aspergillus sp. 3	-	-	-	-	-	-
Aspergillus sp. 4	-	-	-	-	-	+++
Aspergillus sp. 5	+	-	-	-	-	+++
Aspergillus sp. 5	+	-	-	-	-	++++
Aspergillus sp. 6	-	+	-	-	-	-
Aspergillus sp. 7	-	-	-	-	-	+
Aspergillus sp. 8	++	-	-	-	-	++
Aspergillus sp. 9	++	++++	+++	++++	+++	++
Aspergillus sp. 10	+	-	+	-	-	+++
Aspergillus sp. 11	-	-	-	-	-	-
Aspergillus sp. 12	-	+++	+++	+++	+++	-
Aspergillus sp. 13	+	-	-	-	-	-
Aspergillus sp. 14	-	-	-	++	+	-
Aspergillus sp. 15	+++	+++	-	+	-	++
Aspergillus sp. 16	-	+++	+++	+++	+++	++
Aspergillus sp. 17	-	-	-	-	-	++
Endophragmia sp.	-	-	+	-	-	++
Oidiodendron sp.	-	-	-	+++	-	+++
Orbilia sp.	+	-	++	-	-	++++
Penicillium sp. 1	+	++	-	+++	+++	+++
Penicillium sp. 2	++	+++	-	+	++	-
Penicillium sp. 3	++	+++	-	+++	+++	-
Penicillium sp. 4	-	++	-	+++	-	-
Penicillium sp. 5	-	+++	-	+++	-	-
Penicillium sp. 6	-	+++	-	+++	+	-
Sterile mycelium sp 1	+	-	-	++	-	+++
Sterile mycelium sp 2	-	-	-	-	-	-
Sterile mycelium sp 3	-	-	-	-	-	+++
Sterile mycelium sp 4	+++	-	+	-	++	-
Sterile mycelium sp 5	+	-	+++	-	+	-
Sterile mycelium sp 6	-	-	++	-	-	-
Sterile mycelium sp 7	+	-	-	++	++	-
Sterile mycelium sp 8	++	-	++	-	-	+++
Sterile mycelium sp 9	++	-	+++	-	-	++
Sterile mycelium sp 10	-	-	++	-	+	+
Sterile mycelium sp 11	+	-	+	-	-	+++
Sterile mycelium sp 12	-	-	-	-	-	++
Sterile mycelium sp 13	-	-	-	-	+	+++
Sterile mycelium sp 14	-	-	++	-	++	++

(+++++)- Highest activity, (++++)-High activity, (+++)-Good activity, (++)- Medium activity, (+)- Low activity, (-) - No activity.

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Table 7: Antimicrobial activity of Vermicompost and Vermi-wash *in vitro*.

S. No	Plant pathogens	Vermicompost	Vermi-wash
1	*Alternaria triticina*	+	-
2	*Botrytis cinerea*	+	-
3	*Curvularia lunata*	+	-
4	*Phoma tropica*	+	-
5	*Trichoderma viride*	+	-

Vermi-wash along with vermicompost possess plant disease control ability was reported by some investigators. Studies also suggest that fungal species obtained from vermicompost caused inhibition of growth of pathogenic fungi such as *F. oxysporum* and *Rhizoctonia* sp. [29]. In our study, antagonistic effect of both against five plant pathogens (*Alternaria triticina, Trichoderma viride, Curvularia lunata, Phoma tropica and Botrytis cinerea*) was evaluated, the results obtained shows that vermicompost was little effective against all the five pathogens however, vermi-wash depicted negative activity (Table 7).

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