

**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***

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(2017-ES-33-M)



**Division of Environmental Science**

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**Sher-e-Kashmir University of Agricultural Sciences and  
Technology of Kashmir  
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**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***

**Sumaya Manzoor  
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**Thesis**

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**The Faculty of Horticulture  
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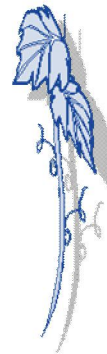


**DEDICATED**

**TO**

*My Beloved Parents*

*Whose affection, love,  
encouragement and prays of day  
and night make me able to get  
such success and honour.*



**Sher-e-Kashmir**  
**University of Agricultural Sciences and Technology of Kashmir**  
**Faculty of Horticulture, Division of Environmental Sciences**

**Certificate – I**

This is to certify that the thesis entitled “**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Environmental Sciences** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Sumaya Manzoor (Regd. No. 2017-ES-33-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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**ABSTRACT**

The present investigation was carried out during 2018-2019 to assess the quality of vermiwash produced from aquatic weeds using two earthworms species *Eisenia fetida* and *Aporrectodea caliginosa*. The vermiwash produced from cowdung and aquatic weeds in the ratio of 3:1 using test species of earthworm were collected and subjected to various assays. There were a total of 5 treatments T<sub>1</sub> (cow dung), T<sub>2</sub> (cow dung + *Eisenia fetida*), T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*), T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*). The vermiwash produced from these treatments was tested for chemical and biological parameters. The results revealed that the treatment combination containing *Eisenia fetida* along with cow dung and aquatic weeds (T<sub>3</sub>) showed significantly high nutrient status. This treatment showed the maximum values for EC (1.44 dS m<sup>-1</sup>), nitrate-nitrogen (23.77 mg/l), total phosphorus (19.33 mg/l), total potassium (552.73 mg/l), calcium (174 mg/l), magnesium (165.50 mg/l), sodium (1221.77 mg/l), copper (0.12mg/l), iron (5.91mg/l), manganese (3.02mg/l) and zinc (1.80mg/l). But pH (7.88), organic carbon (13.31%) and C: N ratio (16.44) was higher in treatment T<sub>1</sub> i.e. Cow dung. The population of total viable bacteria (243.50 x 10<sup>5</sup>), total viable fungi (7.50 x 10<sup>5</sup>), Azotobacter (83.00 x 10<sup>6</sup>), *Azospirillum* (78.00 x 10<sup>6</sup>) and phosphorus solubilising bacteria (68.25 x 10<sup>6</sup>) was highest in T<sub>3</sub>. The vermiwash

was also tested against *Phloeospora maculans* causing leaf spot of mulberry. The maximum inhibition zone (21.25 mm) was found due to vermiwash produced from T<sub>3</sub> i.e. *Eisenia fetida* along with cow dung + aquatic weeds. The study showed that the treatment combination T<sub>3</sub> containing *Eisenia fetida* and cow dung + aquatic weeds in the ratio of 3:1 was found to be the best combination. The earthworm species *Eisenia fetida* was found to have better composting potential as the nutrient status and microbial population was maximum in the vermiwash produced from treatments containing the species.

**Key words:** Vermiwash, aquatic weeds, *Eisenia fetida*, *Aporrectodea caliginosa*, *Phloeospora maculans*.

Signature of Student  
Dated: \_\_\_\_\_

Signature of Major Advisor  
Dated: \_\_\_\_\_

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## Chapter-1

### INTRODUCTION

Extensive usage of inorganic fertilizers and pesticides in agriculture has led to numerous environmental and ecological problems which include greenhouse gas emissions, water pollution, changes in soil properties, declining productivity, deterioration of agricultural production quality, pesticides residues in food commodities, bioaccumulation and biomagnifications of pesticides in food chains, as well as loss of biodiversity and ecosystem services. Owing to wide spectral problems with the use of these chemicals, organic farming is gaining popularity among the scientists and farming community. Vermiwash (a liquid manure), has been integral part of non-chemical based farming system such as organic farming, sustainable farming or eco-friendly farming. It is a liquid extract of organic waste material, which is collected by allowing excess water to saturate the actively vermicomposting substrate in such a way that the water washes the nutrients from the vermicast excreted by the earthworms feeding on the substrate as well as the earthworm's body surface (Gopal *et al.*, 2010). Earthworms are one of the most important components of the soil biota. They are highly resistant to many chemical contaminants including heavy metals and organic pollutants in soil. They have been reported to bio-accumulate heavy metals in their tissues and either bio-degrade or bio-transform them to harmless products with the aid of enzymes (Hemalatha, 2012). They are considered as bioengineers, as they enrich the soil fertility through their cast. They have efficiency to consume all types of organic waste material including wastes from vegetables, fruits, aquatic weeds as well as from industrial origin. Aquatic weeds are those unwanted plants which grow in water and complete at least a part of their life cycle in water (Varshney *et al.*, 2008). In Jammu and Kashmir, the world famous lakes such as Dal lake, Wular lake etc. are infested with aquatic weeds. These weeds cover 2.5-6 per cent water surface of most lakes (Varshney *et al.*, 2008). Since the growth of aquatic

weeds is very fast and they spread at an alarming rate, they can be utilized by converting them into high quality vermicompost and vermiwash.

Vermiwash is produced by different methods in which, it is assumed that microbial activities and nutrients are transformed from vermicompost into its extraction. It contains several enzymes, plant growth hormones like cytokinins, gibberlines, vitamins, beneficial microbes, along with micro and macronutrients (Suthar, 2010) which increases the resistance power of crops against various diseases and enhance the growth and productivity of crops (Suthar *et al.*, 2005; Yadav *et al.*, 2005). The nutrients that are present in vermiwash are water-soluble and immediate requirements from a number of components can be met from a single source. In vermiwash, there is collection of excretory product and mucus secretion of earthworms along with micronutrients from the soil organic molecules. Vermiwash contains high quantities of nitrogen, phosphorus, potash, calcium, magnesium, zinc and is alkaline in nature. Freshly collected vermiwash contains many beneficial microbes that promote the plant growth and prevent infections. It must be diluted before its application on the plants. Vermiwash plays an important role in the plant growth and development; contributes to initiation of rooting, root growth, plant development, promotion of growth rate and improvement in crop production, increasing the soil organic matter and increase in nutrient content which are readily available for the plants, resulting in good crop yield (Sivasubramanian and Ganeshkumar, 2004). Vermiwash can be used both as foliar spray and in the root zone of the plants (Meghvansi *et al.*, 2012). Generally, foliar spray of vermiwash of vermicomposting would offer a method of supplying nutrients to higher plants more rapidly than methods involving soil and root application (Marschner, 1995). Vermiwash protects the environment from various chemical fertilizers. It also has the capacity to encounter worms thereby saving the crops and their productivity. No pathogen can survive in this fluid, thereby protecting the earthworms from the diseases caused by pathogens. It has anti-spawning effects on insects (Zhu *et al.*, 2001). It can be used as a potent

biofertilizer to improve the germination and seedling survival rates in crop plants growing on nutrition depleted soils thus paving the way for sustainable agriculture (Fathima and Sekar, 2014). It could be utilized effectively for sustainable plant production at low input basis (Edwards *et al.*, 2004). It acts as a plant tonic and thus helps in reducing many plant pathogenic fungi. It increases the rate of photosynthesis in crops/plants. It also increases the number of micro-organisms in the soil which help in decomposing soil organic matter (Tripathi *et al.*, 2005). It is one of the potential bio-pesticide that could be used to enhance plant productivity and resistance to some harmful plant pathogens, as well as provide benefits through the disposal of waste matter.

Vermiwash shows positive effect on vegetables (Ismail, 1997; Lalitha *et al.*, 2000) and ornamentals (Shivsubramanian and Ganeshkumar, 2004). The effect of vermiwash spray significantly increased dry chilli yield (George *et al.*, 2007). Karuna *et al.* (1999) have studied the stimulatory effect of vermiwash on crinkle red variety of *Andurium reanum*. Hatti *et al.* (2010) reported that the seedling of *Vigna munga*, *Vigna radiate*, *Sesamum indicum*, showed increase in growth parameters like the root length, shoot length, number of twigs and leaves and total biomass of the plant after spraying the vermiwash of *Perionyx excavates*. Zaller (2006) has reported improved fruit quality of tomato with application of vermiwash and suppression of Phytophthora disease. The treatment of vermiwash has been shown to reduce disease by necrotrophs as well as biotrophs (Al-Dahmani *et al.*, 2003) and significantly decreases in soil borne pathogens and various pests. It was demonstrated that growth of ornamental plant after adding vermiwash showed similar growth pattern as with addition of auxines, gibberellins and cytokinines through the soil (Tomati and Gallii, 1995). It recorded significant growth and productivity in the black gram (Sobha *et al.*, 2003). Vermiwash is a natural growth supplement for tea, coconut and horticultural crops (Weerasinghe *et al.*, 2006). According to Haynes (1986), vermiwash has the property of binding mineral particles like calcium, magnesium

and potassium in the form of colloids of humus and clay, facilitating stable aggregates of soil particles for desired porosity to sustain plant growth. Plants treated with vermiwash are green much more resistant to pests and disease and also more vigorous in growth (Jayashree, 2006). Varghese and Prabha (2014) study suggests that, vermiwash revealed potential application in sustainable development in agriculture biotechnology with respect to its origin, cost effectiveness, availability, reproducibility, reliability as well as biopesticide and ecofriendly soil conditioner.

Mulberry (*Morus alba*) is a woody, deciduous and economically important tree, with moderate tolerance to various environmental stresses (drought, water-logging, salinity, heavy metals etc.). Mulberry leaves are able to reduce wind speed, and leaf surface can adsorb and detain the large particle dust. Generally, one hectare of mulberry can assimilate 27.2 tons of dust a year (Tan *et al.*, 2010). Therefore, mulberry is good green tree species and is cultivated on both sides of the roads. It is a good carbon sink tree. According to the data, 1 ha mulberry trees were able to absorb about  $6.24 \times 10^4$  kg of CO<sub>2</sub> and released  $4.60 \times 10^4$  kg of O<sub>2</sub> each year (Jian *et al.*, 2012). Mulberry leaf has strong absorption ability for atmospheric pollutants and can effectively absorb industrial waste gas, such as sulfide, fluoride, chloride and nitrogen compounds (Liu, 2011; Qin *et al.*, 2010; Tan *et al.*, 2010). It has the potential to remediate heavy metals contaminated soils (Zhou *et al.*, 2015) and is the main food source of silkworm (*Bombyx mori*. L). Mulberry is highly capable of resisting drought and other natural hazards. It is also very effective in resisting wind and consolidating sand and soil. At present mulberry is used as an ecological tree species for water and soil conservation and management of ecological environment. Since mulberry leaf is available throughout the year, it makes the plant prone to various diseases and pests. Various pathogens like fungi, bacteria, viruses and nematode cause diseases in mulberry resulting in 12-25 per cent loss in leaf yield which in turn adversely affects the silkworm growth and finally the silk industry. Of the various mulberry

foliar diseases, leaf spot caused by *Phloeospora maculans* is the major foliar disease in Kashmir valley which are the impediments in the production of quality leaf (Khan *et al.*, 2004). To control this disease a number of management strategies are followed which include physical, chemical and biological methods. Among these synthetic chemicals are commonly used and are more effective but continuous usage of chemicals cause environmental pollution resulting in adverse effects on soil flora, fauna and also human health due to residual toxicity.

So, there is a great need to think of environmentally safe methods for protecting the crop. In the light of information presented above, the present study is proposed with the following objectives;

- To study the nutrient status and microbial population of vermiwash developed from aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa*.
- In vitro study of antifungal activity of vermiwash against *Phloeospora maculans* in mulberry.

## Chapter-2

### REVIEW OF LITERATURE

Zambare *et al.* (2008) evaluated composition of vermiwash by considering biochemical and microbiological approaches for sustainable development of plant growth promoting factor or as a soil conditioning agent on cowpea plant. Vermiwash was found to contain enzyme cocktail of proteases, amylases, urease and phosphatase. Microbiological study of vermiwash revealed that it contains nitrogen-fixing bacteria like *Azotobacter* sp., *Agrobacterium* sp. and some phosphate solubilizing bacteria.

Meenatchi *et al.* (2009) assessed the microbial load of vermicompost and vermiwash prepared by using different food substrates and earthworm species. The results revealed that the best substrate-earthworm combination with respect to beneficial microflora such as bacteria, fungi and actinomycetes population in vermicompost was kitchen waste + *Perionyx excavates* ( $65 \times 10^7$  CFU/g), soybean post harvest waste + *Eudrilus eugeniae* ( $41 \times 10^4$  CFU/g) check (65% soybean post harvest waste + 35% cowdung) + *P. excavates* ( $15 \times 10^4$  CFU/g), respectively. Whereas, vermiwash obtained from Paddy straw + *P. excavatus* recorded maximum bacterial population ( $67 \times 10^7$  CFU/ml) and check + *P. excavatus* recorded maximum fungal population ( $10 \times 10^3$  CFU/ml). Vermiwash obtained from soybean harvest waste + *Eudrilus eugeniae* recorded maximum actinomycetes population ( $14 \times 10^3$  CFU/ml).

Nath *et al.* (2009) studied the potential of an epigeic earthworm *Eisenia fetida* to convert the different combination of variety of wastes into rich nutrient vermicomposts/vermiwash and pre and post chemical analysis of feed mixtures. The experimental results showed significant increase of TKN, TP, TK and TCa and significant decrease in TOC, C: N ratio, EC and pH in final vermicompost of different animal/ agro and kitchen wastes.

Sugapriya and Mahalingam (2010) studied the microbiological and physico-chemical analysis of vermiwash of *Eudrilus eugeniae* and *Eisenia fetida*. Microbiological studies of vermiwash revealed the presence of higher number of bacteria and actinomycetes. The decrease of pH, reduction of organic carbon and increase of electrical conductivity, phosphorous, potassium and sodium in vermiwash were found to be significant due to the earthworm activity.

Gopal *et al.* (2010) conducted an experiment with an aim of producing coconut leaf vermiwash (CLV) from actively vermicomposting coconut leaf litter + cow dung substrate (10: 1 w/w) using *Eudrilus* sp. It significantly increased the seedling vigour index of cowpea and paddy at 1:10 and 1:15 dilutions in laboratory trials. Field trials carried out in red sandy loam soil (Arenic Paleustults) resulted in an increase of 36 per cent fresh biomass weight of cowpea with application of CLV at 1:10 dilution. In maize, increase in cob yield by 5-10 per cent and in okra 22-33 per cent increase in fruit yield were recorded at 1:5 dilutions of CLV. A concomitant increase in populations of general and plant beneficial microorganisms and soil enzyme activities in the rhizosphere of CLV-applied plants were also recorded. Soil organic carbon content increased in the CLV-applied plots in all the crops studied, but the total N, available P and K content in soil varied in different crops.

Hatti *et al.* (2010) studied the effect of vermiwash of *Perionyx excavatus* on the growth of *Vigna mungo*, *Vigna radiata* and *Sesamum indicum*. The root length, shoot length, number of twigs, leaves, flowers, pods, grains and biomass were increased significantly compared to that of control in all the species treated with vermiwash. The analysis of vermiwash showed high level of Ca, K, P, organic carbon, Fe, Mn, Cu and Zn. Therefore, it may be concluded that significant increase in the growth of vermiwash treated plants and their grain yield is due to high level of macro and micronutrients available in the vermiwash.

Sundaravadivelan *et al.* (2011) carried out an experiment to compare the physico-chemical, nutritional and biochemical status of the vermiwash obtained

using the popular composting earthworm species *Eudrilus eugeniae* and *Lampito mauritii* from three different leaf litters namely, mango (*Mangifera indica*), guava (*Psidium guajava*) and sapota (*Achras sapota*). The results showed substantial increase in the nutrient quality of the vermiwash produced with time in all of three cases. However, the vermiwash produced from guava leaf litter showed high electrical conductivity, magnesium, calcium, nitrite, phosphorus, carbohydrate, protein, lipid and amino acid compared with the vermiwash produced from the other two types of leaf litter. Comparison of physico-chemical, nutritional and biochemical parameters of the vermiwash produced from the experiment was commercially produced. It was revealed that experimental control had attained most of the parameters on 60<sup>th</sup> day. Whereas, the vermiwash produced by two composting earthworm species and leaf litters from three different plants attained the values of the parameters of the standard at early days. It revealed that the quality of vermiwash can be achieved as in the standard even within 45 days with the use of these earthworms.

Govindarajan and Prabakaran (2012) studied the antibacterial activity of vermiwash of *Eisenia fetida* against four bacterial species (*Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Escherichia coli*). The antibacterial activity of *E. fetida* directed against gram-positive and gram-negative bacteria was analysed. The disc diffusion assay indicated inhibition of the growth of bacteria. Vermiwash was able to inhibit the growth of *Pseudomonas aeruginosa* at a maximum level of 20mm and less inhibited at a level of 10mm. The present study clearly indicates that the *Eisenia fetida* vermiwash has bioactive compounds to inhibit the growth of bacteria.

Esakkiammal and Lakshmibai (2013) prepared vermicompost and vermiwash from *Eudrilus eugeniae* and tested for antibacterial and antifungal activities. For antibacterial assay five species of bacteria viz. *Escherichia coli*, *Salmonella abony*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and two concentrations of the vermicompost and vermiwash (50 µl

and 100 µl) were used. The disc diffusion assay indicated a dose dependent effect of the vermicompost and vermiwash to inhibit the growth of bacteria. Vermicompost and vermiwash at a dose of 100 µl was able to inhibit the growth of *P. aeruginosa* at a maximum level (15.25± 0.33mm) in vermicompost and *S. abony* (63.5±0.57) in vermiwash. *B. subtilus* (12.22± 0.57mm) in vermicompost and (25.6±0.33) in vermiwash were less inhibited when compared with others.

Anti fungal assay was also carried out using two species of fungi (*Candida albicans*, *Aspergillus flavus*). The fungal species was inhibited by vermicompost and vermiwash in a dose dependent manner. The high dose of vermicompost and vermiwash (100µl) showed highest anti fungal potential. Of the two species tested, the growth of *Candida albicans* was maximum inhibited in vermicompost (13.5± 0.66mm) and in vermiwash (29.0±0.33). The inhibitory potential of the vermicompost and vermiwash was less in the case of *Aspergillus flavus* in vermicompost (11.75± 0.57mm) and in vermiwash (15.33±0.66). The results indicated that the *E. eugeniae* treated vermicompost and vermiwash have bioactive compounds to inhibit the growth of bacteria and fungi.

Manyuchi *et al.* (2013) studied the vermicompost and vermiwash obtained from vermicomposting waste corn pulp blended with cow dung manure over 30 days using *Eisenia fetida*. The pH, temperature, moisture content and electrical conductivity were daily monitored. The feedstock, vermicompost and vermiwash were analysed for nutrient composition. The average temperature and moisture content in the vermi-reactor was 22.5°C and 42.5 per cent respectively. The vermicompost and vermiwash had an almost neutral pH while the electrical conductivity was 21 per cent higher in the vermicompost. The nitrogen and potassium content was 57 per cent and 79.6 per cent respectively richer in the vermicompost compared to the vermiwash. However, the vermiwash was 84 per cent richer in phosphorous as compared to vermicompost. Furthermore, the vermiwash was 89.1 per cent and 97.6 per cent richer in Ca and Mg respectively

and 97.8 per cent richer in Na salts compared to the vermicompost. The vermiwash also indicated a significantly higher amount of micronutrients.

Elumalai *et al.* (2013) studied the effect of vermiwash in comparison with the plant growth regulators on the exo-morphological characters of *Abelmoschus esculentus*. The results showed that vermiwash exhibited growth promoting effects on the exo-morphological characters such as plant height, length and diameter of the internode, number of leaves, leaf surface area, root length, wet and dry weight of the shoot and root of *Abelmoschus esculentus*. Among the various foliar treatments used in the study, 15 per cent vermiwash (V-II) showed growth enhancing effects followed by 10 per cent vermiwash (V-I), Gibberelic acid (100 g/ml) and Naphthalene acetic acid (100 g/ml). Maximum root length and plant biomass was recorded in V-II. These results clearly indicate that vermiwash can be exploited as a potent biofertiliser and foliar spray.

Samadhiya *et al.* (2013) examined the effect of vermiwash on the growth and development of tomato. When vermiwash was sprayed on the tomato plants, it showed a significant growth i.e. length of shoot as well as number of leaves per plant. Vermiwash when mixed with vermicompost increased the shoot length to  $19.61 \pm 0.18$  cm as compared to control ( $17.92 \pm 0.21$  cm). When vermiwash was directly sprayed on the plants the length of shoot was recorded to be  $19.72 \pm 0.30$  cm which was higher as compared to control group ( $17.92 \pm 0.21$  cm). It can be concluded that the growth of tomato plants showed much positive results when grown in vermicompost. However the results were apparent when the plants were treated with vermiwash. Hence, vermiwash proves to be an effective fertilizer which contributes to the growth of plants when sprayed directly as well as mixed with a definite ratio of vermicompost. It was also observed that the plants treated with vermiwash were disease resistant and no any worms like leaf eaters were seen on the leaves and other parts of plants.

Varghese and Prabha (2014) assessed the potency of *Eudrilus eugeniae* with respect to biodegradation of jackfruit (*Artocarpus heterophyllus*) into a liquid

biofertilizer vermiwash. The experiment was conducted to evaluate the physico-chemical characteristics, micronutrients and macronutrients, proteins and carbohydrates present in vermiwash before and after inoculation of earthworm species and also a comparative study was done on the effect of vermiwash on growth parameters such as root and shoot length, number of leaf count in *Capsicum frutescens* after 30<sup>th</sup> day of planting. The results obtained revealed that treatment with vermiwash showed a decrease in total organic carbon and C/N ratio than control. The total macronutrients (N, P, K and Ca) and micronutrients (Fe, Cu, Mg and Zn) showed elevated levels in vermiwash treated when compared with control. Treatment with vermiwash in *C. frutescens* showed increased root and shoot length as well as number of leaves than untreated plant.

Phukan and Savapondit (2014) studied the development of the technology for the preparation of vermiwash from different organic waste (mixed grass, mikania and guatemala grass) with cowdung as feedstock available in and around tea garden. *Eudrilus eugeniae* was used for vermicomposting. On the basis of physical and chemical properties, mikania produced better vermiwash when it was used as feedstock. Microbiological study of vermiwash revealed that it contains nitrogen fixing bacteria like *Azotobacter* sp., *Azospirillum* sp. and phosphate solubilising bacteria. Application of vermiwash in pot and field experiments through foliar application led to remarkable improvement in the growth, nutrient contents of shoot and yield of young and mature tea compared to the untreated plants.

Sivananthi and Paul (2014) studied fungicidal activity of vermicompost water extract on *Rhizoctonia solani*. Clear zone of inhibition was observed on *R. solani* mycelium by vermicompost extract. Increase in concentration increases diameter of zone.

Jaikishan *et al.* (2014) conducted an experiment with the objective of using vermiwash produced from different plant sources against tomato fruit fungus *Alternaria alternata* and growth of tomato. Vermiwash was produced

using bagasse, neem and paddy straw in different combination (T1 to T9) and was used as foliar spray on tomato plants. Vermiwash in combination with neem + earthworm + cattle dung in T3, T6 and T9 were very effective depending on their concentration or percentage to get rid of pathogens and improve yeild and growth parameters of tomato fruit. The treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>6</sub>, T<sub>7</sub>, T<sub>8</sub> and T<sub>9</sub> at 10 per cent dilution of vermiwash were effective against *A. alternata* affecting tomato fruits. At 5 per cent there was no inhibition of *A. alternata*, T<sub>7</sub> had a maximum of 12 colonies and T<sub>1</sub> on the other hand had a maximum of 5 colonies. At 10 per cent dilution growth of *A. alternata* was killed for most treaments except T<sub>3</sub>, T<sub>4</sub> which consisted of two colonies each and T<sub>5</sub> a colony count of 6. At 15 per cent dilution there was growth of *A. alternata* in all treatments except T<sub>8</sub>. Maximum growth was observed on petri plates containing T<sub>1</sub> and T<sub>2</sub> with a count of 8 colonies and a minimum growth of 1 colony present in T<sub>7</sub>. At 20 per cent growth of *A. alternata* was observed in all treaments. The maximum growth was seen in T<sub>1</sub> with 27 colonies and minimum growth in T<sub>5</sub> with one colony. This indicated that at a concentration of 10 per cent vermiwash would inhibit growth of *A. alternata*.

Blancas *et al.* (2014) evaluated the worm-bed leachate as an antifungal agent against pathogenic fungus, *Colletotrichum gloeosporioides*. Worm-bed leachate was stored and characterized while its possible antifungal activity was investigated in vitro. The pH decreased from 9.6 at the onset of the storage to 8.9 after 28 days, while the total N and C nearly halved. The concentration of NH<sub>4</sub><sup>+</sup> tended to decrease with time and the amount of NO<sub>3</sub><sup>-</sup> increased, while the concentration of NO<sub>2</sub><sup>-</sup> remained constant. The EC of the worm-bed leachate did not change significantly over time. Sterilized worm-bed leachate reduced the growth of *C. gloeosporioides*, but unsterilized worm-bed leachate inhibited growth completely (100%).

Jadhav *et al.* (2014) studied the effect of different levels of vermiwash spray on growth and yield of radish cv. Local variety. The experiment was laid out in Randomized Completely Block Design with five treatments and four

replications. The experiment consisted of five treatments involving application of water and vermiwash in the proportion of 1:1, 1:2, 1:3 and 1:4 and control were imposed and the foliar application was made by using knapsack sprayer in the evening hours twice at 15 and 30 days after sowing seeds. The data clearly revealed that the yield obtained with treatment T<sub>4</sub> (100 % RDF as fertigation and vermiwash foliar twice times spray 1:4 at 15 and 30 days after sowing seeds) was significantly higher than all other treatments in growth and yield attributes such as root diameter (10.41 cm), length (23.79 cm), weight (85.21 g), yield/plot (17.04 kg), yield/ha (56.80 t/ha) and marketable yield (49.25 t/ha) of radish cv. Local variety. Thus, it can be concluded that foliar spray of vermiwash (1:4) was effective than control.

Sundararasu and Jeyasankar (2014) evaluated the effect of vermiwash on growth and productivity of *Solanum melongena*. The results revealed that vermiwash spray enhanced the growth parameters (plant height and number of leaves) and yield parameters (number of flowers and fruits/plant). Flowering and fruiting ratio was significantly increased in experimental plots. From the results it could be seen that extracts from earthworms offer a valuable resource which could be effectively exploited for increasing the production of brinjal. The foliar spray of vermiwash can be economically and environmentally suitable for the soil environment. Therefore, it may be concluded that significant increase in the growth of vermiwash treated plants and their yield is due to high level of macro and micronutrients available in the vermiwash.

Pattnaik *et al.* (2015) made an attempt to find out in vitro and in vivo effect of vermiwash on common plant pathogens. A total of eight bacteria were isolated from the freshly prepared vermiwash and were identified by using standard biochemical tests as *Flavobacterium* sp., *Burkholderia* sp., and six different species of *Bacillus*. Fresh and preserved vermiwash were treated against five fungal and bacterial phytopathogens respectively. Out of the eight bacterial isolates, *Bacillus* sp.V08 showed a significant antibiotic activity against bacterial

phytopathogen *Xanthomonas campestris*. *Bacillus sp.V08* and *Burkholderia sp.* were most effective against fungal phytopathogens such as *Fusarium oxysporium*, *F. solani*, *Rhizoctonia solani*, and *Alternaria solani*. The bacterial isolates *Bacillus sp.V08* showed antibiosis against bacterial and fungal phytopathogens was identified by 16S-rRNA gene sequencing and the sequence was submitted to gene bank with accession no. KF543076. Thus, this in-vitro and in-vivo studies indicates that application of vermiwash can control these bacterial and fungal phytopathogens of various plants.

Chattopadhyay (2015) carried out an experiment with an aim at producing the vermiwash from *Eisenia fetida*, under field condition and also in the laboratory under cold and heat stress methods. These washes were used in different ratios for seed germination of mung (*Vigna radiate*). The results showed that the vermiwash produced by cold stress diluted at the ratio of 1:5 showed 100 per cent germination of mung seed followed by 1:5 ratio vermiwash produced naturally that resulted in 95 per cent of seed germination. Undiluted vermiwash produced naturally and by cold stress showed 90 per cent of germination of mung seed than the undiluted vermiwash produced by heat stress. Cold stress produced vermiwash diluted with 1:5 ratio showed a vigour index of 1650 followed by naturally produced 1:5 ratio diluted vermiwash which showed a vigour index of 1092. However, the undiluted naturally produced vermiwash showed the lowest vigour index of 450.

Nath and Singh (2015) analysed vermiwash singly and in combination with different biopesticides for management of the pod borer (*Helicoverpa armigera*) infestation in gram (*Cicer arietinum*). Significant reduction in the number of infested pods was reported after spraying of vermiwash obtained from different combination of animal dung and agro wastes with neem oil (*Azadirachta indica*), custard apple leaves (*Annona squamosa*) and garlic (*Allium sativum*). Vermiwash obtained from animal dung with gram bran with neem oil was also highly effective against the pod borer (*H. armigera*). Spray of vermiwash with

biopesticide increased the productivity of gram crop up to 3 times with respect to control. Present study revealed that the use of vermiwash with plant product is more applicable in organic farming. It is helpful to balance the deficiency of essential nutrients along with certain plant growth hormones, enzymes and vitamins. Vermiwash and its combination with plant products were effective against pod borer which are injurious to the gram plant.

Sahoo *et al.* (2015) analysed the physico-chemical, biochemical and antimicrobial properties of vermicompost and vermi-wash produced from green wastes digestion by the action of night crawler earthworm *Eudrilus eugeniae*. The pH level in the vermicompost was found acidic (pH 5.38±0.64) while in the vermi-wash was found 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 was also detected in the vermi-wash. Mycofloral analysis of vermicompost revealed the presence of 43 fungal species. Most of the isolated fungi showed a proteolytic and amyolytic activity. The antimicrobial property was also exhibited by vermicompost which inhibited the growth of some plant pathogens while the vermi-wash samples did not show any inhibitory effect on the tested plant pathogens.

Das *et al.* (2015) conducted an experiment by employing *Eudrilus eugeniae* to produce vermiwash by utilizing kitchen waste. Vermiwash collected was sprayed on to priorly germinated seeds of *Pisum sativum* and *Cicer arietinum* and planted on plastic pots containing sterilised sand and soil. The growth study was monitored for 28 days. Physiochemical characteristics and biochemical composition of soil amended with vermiwash and those deprived from vermiwash was studied by standard protocols. Soil amended with vermiwash had an alkaline pH, higher electrical conductivity and was rich in organic carbon, sodium,

potassium, iron, copper and zinc. *P. sativum* and *C. arietinum* plantlets grown under vermiwash treatment showed better growth in terms of shoot and root length, internodal length, number of leaves and biomass over control. However, the root length in vermiwash amended plantlets of *C. arietinum* was shorter, suggesting that soil had good amount of nutrients that were easily available.

Jaybhaye and Bhalerao (2015) studied the influence of vermiwash on the seed germination and seedling characters of *Vigna radiata* and *Vigna mungo*. Vermiwash at three different concentrations of 10 per cent, 20 per cent and 30 per cent was used along with Gibberellic acid at 100µg/ml to compare its growth promoting effects and distilled water used as control. The seeds were treated with various test solutions and germination percentage and seedling characters studied. This study revealed that vermiwash at lower concentration was effective in bringing about seed germination and growth of seedling characters. The germination percentage was increasing as concentration of vermiwash increases in *Vigna radiata* and in *Vigna mungo* all seeds showed 100 per cent germination. The seedling growth parameters such as hypocotyls length increases in *Vigna radiata* compare to *Vigna mungo* in 10 per cent, 20 per cent and 30 per cent vermiwash. Radical length was more in *Vigna mungo* compared to *Vigna radiata*.

Ansari *et al.* (2016) tested the efficacy of vermiwash produced from different medicinal plants on fungus affecting cucumber. Vermiwash was prepared from various medicinal plants tulsi (*Ocimum tenuiflorum* L.), neem (*Azadirachta indica* A. Juss), Lemon grass (*Cymbopogon citratus* (DC. ex Nees) Stapf), and paddy straw (*Oryza sativa* L.) and applied, in vitro, to *Alternaria alternata* to investigate their effectiveness as organic alternatives to traditional fungicides. The inhibitive effects on the fungus appeared most effective when *A. indica* and *O. tenuiflorum* were used in the production of the vermiwash. Using the serial dilution method, vermiwash from *O. tenuiflorum* showed the highest per cent of inhibition (93.2 per cent), followed by *C. citratus* (74.7 per cent), *A. indica* (68.7 per cent), *O. sativa*, combination, and combination without worms. Using

the sterile disc diffusion method, all of the samples produced zones of inhibition against *A. alternata*. Vermiwash from *A. indica* produced a zone of inhibition, averaging 15.3 mm, followed by *O. tenuiflorum* 14.0 mm, combination without worms, combination, *C. citratus* and *O. sativa*. Nystatin produced a zone of inhibition of 10mm. The results indicate that vermiwash is not simply an organic alternative to more traditional chemical fungicides, but it may in fact be a better and more effective product in treating certain fungal plant infections, particularly *Alternaria alternata*.

Soni *et al.* (2016) carried out an experiment with an aim at the standardization of aeration and time period to achieve maximum population of beneficial microflora in vermiwash. Significantly higher microbial loads of N<sub>2</sub> fixers, actinomycetes, fungi and phosphate solubilizing bacteria (7.56, 6.78, 6.78 and 7.08 log CFU/ml, respectively) in fresh vermiwash was obtained with 24 hours of aeration at room temperature (20±2°C). Further one month study revealed that the maximum population counts for N<sub>2</sub> fixers (8.70 log CFU/ml) and actinomycetes (7.0 log CFU/ml) were obtained after 5 days incubation, while, fungi (6.59 log CFU/ml), PSB (7.20 log CFU/ml) and total microbes (9.54 log CFU/ml) showed maximum population at 10<sup>th</sup> day. A gradual reduction in microbial counts was observed after 15 to 30 days and aeration showed negligible effect on microbial population. Nutritional profile analysis of 10 day old aerated vermiwash showed that it was a rich source of carbon, nitrogen, iron, manganese and zinc. This reflects vermiwash should be used up to 10 days of preparation with 24 hours of proper aeration to attain maximum microbial population and nutrients.

Maheswari *et al.* (2016) studied the growth promoting effects of vermiwash and panchagavya on *Dolichus lablab*. Vermiwash was obtained from the vermicomposting unit and panchagavya was prepared using cow products such as milk, curd, urine, ghee and dung. Different concentrations of vermiwash and panchagavya (5:2%, 10:3% and 15:4%) were applied to the bean plants once

in every week for 90 days. The exomorphological characters such as shoot length, internode length, diameter of the internode, number of leaves, leaf surface area and chlorophyll content were recorded prior to every treatment in all the groups. Interestingly, 10:3 per cent vermiwash and panchagavya treated lablab beans showed better growth promoting effects than the other plants. The results showed that 10:3 per cent vermiwash and panchagavya could be used as effective foliar spray in the near future.

Prakash *et al.* (2016) examined the influence of various concentrations of vermiwash and humic acid and their combinations on the growth of *Allium cepa*. In this study, the concentration of 3:1.5 per cent (vermiwash: humic acid) was found to be the most effective formulation which was manifested in increase in growth, soft shoots, number of branches, total carbohydrate content and total protein content, followed by a concentration of 3:2 per cent (vermiwash: humic acid). This study was effective in formulating an efficient chemical free fertilizer that can be further used in agriculture on a large scale.

Chithra *et al.* (2016) compared the effect of seaweed fertilizers and vermiwash on seed germination of Cluster bean or Guar (*Cyamopsis tetragonoloba*). Seeds were soaked in various concentrations (0.5 per cent, 1 per cent, 2 per cent, 3 per cent and 5 per cent) of seaweed fertilizers (liquid and paste) and vermiwash separately at different time periods (30, 60 and 120 minutes). After 7 days, the seeds were analyzed for their germination capacity, shoot length and root length. The study infers that seaweed liquid fertilizers with the concentration of 1 per cent showed maximum seed germination (100%) and the optimum shoot and root length was recorded as 6.04 cm and 3.34 cm respectively whereas in seaweed paste fertilizers, 3 per cent showed maximum seed germination (100%) with the shoot and root length of 6.58 cm and 3.46 cm after 120 minutes. Also, the vermiwash with the concentration of 0.5 per cent showed maximum seed germination (100%) and the optimum shoot and root length was recorded as 6.32 cm and 3.52 cm respectively after 30 minutes soaking.

Sayyad (2017) examined the effect of vermiwash on the pest infestation (at 20, 30, 40 and 50 per cent) in tomato. Vermiwash caused significant reduction in pest infestation. The vermiwash concentrations of 20 and 30 per cent were less effective as compared to 40 and 50 per cent solutions against insect pest of the crop.

Prabina *et al.* (2018) conducted an experiment with an aim at selecting a suitable substrate for active decomposition of organic wastes with *Eisenia sp.* and for extraction of vermiwash that could sustain the soil capacities both chemically and biologically. Different substrates namely *Prosopis*, *Albizia*, *Azadirachta*, *Azolla*, *Leucaena*, *Eichornia* and *Banana pseudostem* were used for preparation of vermicompost along with cowdung (10:1w/w). From the active compost, vermiwash was collected @ 40 litres of vermiwash from one complete cycle and different parameters were assessed. Among the different substrates tested, neem leaf vermiwash proved to be a better source of nitrogen fixing, phosphate solubilising, cellulolytic and starch degrading microorganisms. Nitrogen fixing *Azospirillum sp.* registered  $200 \times 10^4$  CFU ml<sup>-1</sup> followed by *Azotobacter sp.*  $61 \times 10^4$  CFU ml<sup>-1</sup>. Phosphorus solubilizing bacteria numbered to  $4 \times 10^2$  CFU ml<sup>-1</sup> and that of fluorescent *Pseudomonads* was  $37 \times 10^4$  CFU ml<sup>-1</sup>. The cellulose and starch degraders outnumbered the other microbes each with a count of  $325 \times 10^4$  CFU ml<sup>-1</sup>. The nutrient composition of the neem leaf vermiwash was 53.30 ppm of nitrogen, 12ppm of phosphorus and 223 ppm of potassium. The micronutrients zinc, iron, copper and manganese were present at 0.06, 1.08, 0.04 and 0.01 ppm levels. The presence of the plant hormones viz. indole acetic acid (1.25ppm) and gibberellic acid (1.65 ppm) added value to the vermiwash.

## Chapter-3

### MATERIALS AND METHODS

The present investigation entitled “**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***” was carried out at the Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar. The detailed description of the materials used and the procedures followed have been described in this chapter.

#### 3.1 Experimental site

The present investigation was carried out in the year 2018-2019 at the Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar.

#### 3.2 Climate

The climate of experimental site was temperate type with moderately hot summers and very cold winters. The valley mostly remains covered with snow during the winter months. The maximum rainfall is received during March to April.

#### 3.3 Experimental details

##### 3.3.1 Number of factors: 02

Test species earthworms

Test treatment

##### 3.3.2 Number of test species earthworm: 02

E<sub>1</sub>: *Eisenia fetida*

E<sub>2</sub>: *Aporrectodea caliginosa*



*Eisenia fetida*



*Aporrectodea caliginosa*

**Plate 1: Test earthworm species**



**Cow dung**



**Partially decomposed aquatic weeds**

**Plate 2: Test substrates**

### 3.3.3 Number of test treatments: 05

### 3.3.4 Details of the treatment

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Code	Details
T <sub>1</sub>	Cow dung (control)
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>
T <sub>3</sub>	Cow dung + Aquatic weeds + <i>Eisenia fetida</i>
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>
T <sub>5</sub>	Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>

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Number of replications : 04

Design of Experiment : Completely Randomized Design (CRD)

### 3.4 Collection of raw materials

Aquatic weeds both free floating comprising of *Salvina* sp. and *Azolla* sp. and submerged comprising of *Potamogeton pectinatus*, *Myriophyllum spicatum* and *Ceratophyllum demersum* were collected from Dal Lake and were bought to experimental site.

### 3.5 Pre-decomposition of raw materials

Submerged weeds were chopped into small pieces and both types of weeds were subjected to pre decomposition for two weeks.

### 3.6 Preparation of vermiwash

The vermiwash unit was set up using plastic buckets with taps fitted near the base. The buckets were placed on a table to facilitate collection of vermiwash. The lowermost part of the bucket was filled with broken bricks and pebbles with a layer of coarse sand above it. On the top of the sand layer, a layer of moist loamy soil was laid. The unit was filled with a mixture of partially decomposed cow dung and aquatic weeds in the ratio of 3:1. The test species of earthworms *Eisenia*



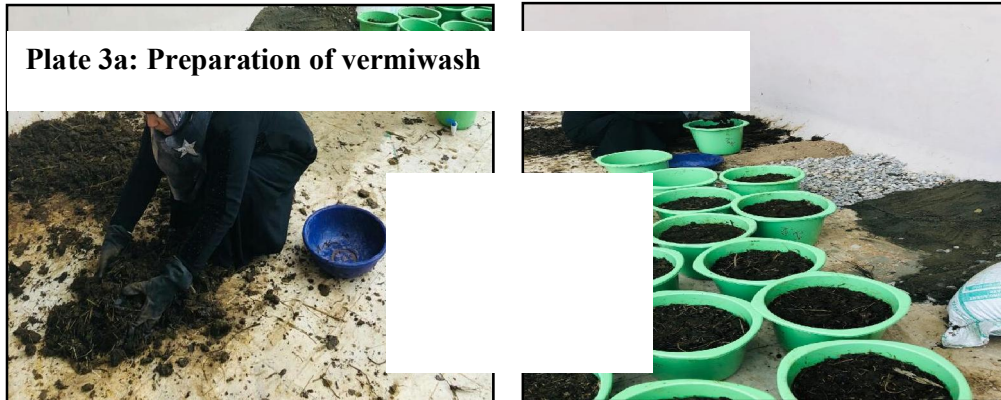
**Filling of gravels into the buckets**



**Filling of soil into the buckets**

**Filling of sand into the buckets**

**Filling of soil into the buckets**



**Plate 3a: Preparation of vermiwash**



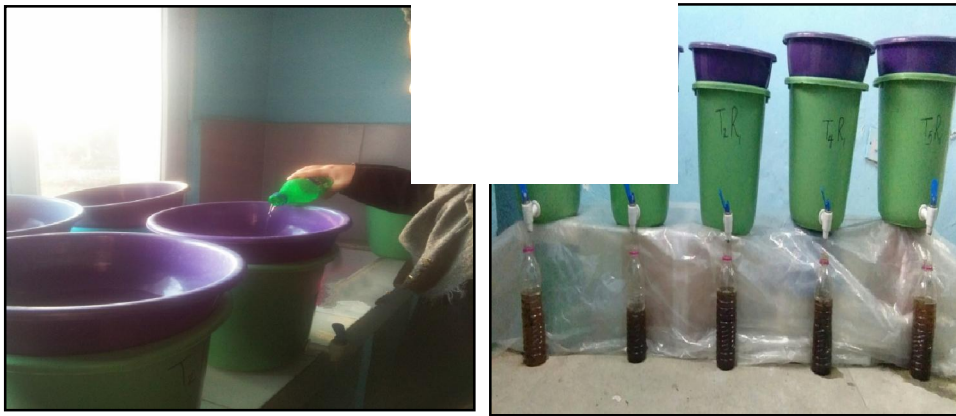
**Mixing of the cow dung and aquatic weeds**

**Filling of the cow dung and weed mixture into the buckets**

**Inoculating earthworms into the buckets**

**Pots filled with substrates**

**Plate 3b: Preparation of vermiwash**



**Filling of water into the collection of Vermivash**



**Final vermivash**

**Plate 3c: Preparation of vermivash**

*fetida* and *Aporrectodea caliginosa* procured from Faculty of Agriculture, SKUAST-K, Wadura were introduced into buckets containing cow dung and weed mixture as mentioned in treatment detail. The unit was kept undisturbed for 10-15 days. Then another pot of 1 liter capacity with a hole at the bottom fitted with a cotton wick was placed over the unit. Water was filled in the pot and allowed to trickle through the wick drop by drop and was refilled regularly. Vermiwash collected daily was stored in sterilized bottles at 4°C and analysed.

### **3.7 Analysis of vermiwash**

#### **3.7.1 Determination of chemical parameters of vermiwash**

##### **3.7.1.1 pH**

The pH of the vermiwash was determined by pH meter as described by APHA (1998). A digital pH meter was used after setting it to zero by using distilled water and standardizing it with standard buffer solutions (pH 4, 7 and 9). The probe of pH meter was kept immersed in vermiwash and pH values were noted from pH meter.

##### **3.7.1.2 Electrical conductivity**

The conductivity of vermiwash was determined with the help of digital conductivity meter as described by APHA (1998). The cell constant was determined by placing N/10 potassium chloride solution in the given conductivity cell. Vermiwash was taken in a clean beaker and conductivity cell was immersed in it and conductivity was measured in  $\text{dSm}^{-1}$ .

##### **3.7.1.3 Organic carbon**

Organic carbon was determined by wet digestion method as outlined by Walkley and Black (1934). One ml of vermiwash was taken in a dry 500 ml conical flask, 10 ml of 1 N potassium dichromate was added and swirled a little. Then 20 ml of sulphuric acid was added, swirled again two or three times and the flask was allowed to stand for 30 minutes and then 200 ml of distilled water, 10

ml of phosphoric acid and 1 ml of diphenylamine indicator were added. The content was then titrated with 0.5 N ferrous ammonium sulphate solution till colour changed from blue violet to green.

#### **3.7.1.4 Nitrate nitrogen**

The nitrate-nitrogen was determined by phenol disulphonic acid method as described by APHA (1998). 25 ml of vermiwash was evaporated to dryness in a 100 ml beaker on a hot water bath. 3 ml of phenol disulphonic acid was added to the residue. After 10 minutes 15 ml of distilled water was added and stirred with glass rod. Contents were cooled and ammonium hydroxide was added slowly till the solution became alkaline as indicated by the development of a stable yellow colour. The final volume was brought to 100 ml with distilled water. Absorbance was measured with spectrophotometer at 420 nm. By comparing the absorbance of vermiwash samples with standard curve prepared by using series of dilution standards of potassium nitrate solution, nitrate-nitrogen of the vermiwash sample was calculated.

#### **3.7.1.5 Total phosphorus**

Total phosphorus was determined by colorimetric method as described by APHA (1998). 25 ml of vermiwash was evaporated to dryness in a 100 ml beaker on a hot water bath. 1ml of perchloric acid was added to the residue and was heated gently till the contents become colourless. When contents were cooled, 10 ml of distilled water and 2 drops of phenolphthalein indicator were added. The content was then titrated against sodium hydroxide solution till light pink colour appeared and volume was made to 25ml with distilled water. Then 10 ml of reagent B was added. The final volume was brought to 50 ml with distilled water and the flask was allowed to stand for 30 minutes till blue colour appeared. Absorbance was measured with spectrophotometer at 660nm.

### **3.7.1.6 Total potassium**

Total potassium was determined by flame photometer method as described by APHA (1998). 50ml of the vermiwash sample was digested using the diacid mixture of nitric acid and sulphuric acid. Then the total potassium was measured using flame photometer.

### **3.7.1.7 Sodium**

Sodium was determined by flame photometer method as described by APHA (1998). 50 ml of vermiwash sample was taken in 100 ml beaker and then fed to flame photometer for the determination of sodium by using respective filter. Sodium was calculated by comparing the reading with the standard curve prepared by using different dilution series of standard sodium chloride in terms of parts per million (ppm).

### **3.7.1.8 Calcium**

Calcium was determined by EDTA titrimetric method (APHA, 1998). 5 ml of vermiwash sample was taken in a conical flask followed by addition of 5 ml of sodium hydroxide and a pinch of murexide indicator. The contents were then titrated against EDTA solution (0.01N) till colour changed from orange red to purple.

### **3.7.1.9 Magnesium**

Magnesium was determined by EDTA titrimetric method (APHA, 1998). 5 ml of vermiwash sample was taken in a conical flask followed by the addition of 1 ml of ammonium chloride-ammonium hydroxide buffer and 3 to 4 drops of Eriochrome Black T indicator. The contents were then titrated against EDTA solution (0.01N) till wine red colour changed to blue.

### **3.7.1.10 Micronutrient cations**

The micronutrients in the vermiwash was extracted using acid digestion given by APHA (1998). 50 ml of vermiwash sample was placed in an evaporating

porcelain dish, acidified to methyl orange with concentrated sulphuric acid and 5ml concentrated nitric acid and 2 ml 30 per cent hydrogen peroxide were added to reduce chromate and evaporated by lacing over a water bath to about 10 ml. Then 125 ml was transferred to a conical flask using 5ml concentrated nitric acid. Further 10 ml concentrated sulphuric acid and a few glass beads were also added. Then the contents were evaporated on a hot plate until white dense fumes characteristic of SO<sub>3</sub> appeared in the flask. Then after cooling to room temperature, 50 ml distilled water was added, and boiled to dissolve the solids if any present and filtered through porcelain filter crucible. The filtrate was transferred to a 100 ml volumetric flask and volume was made up to mark with distilled water.

The digested samples were then subjected to Atomic Absorption Spectrophotometer (AAS) analysis for presence of elements (iron, copper, manganese and zinc) by using appropriate hollow cathode lamp for the element of interest. A mixture of compressed air as oxidant and acetylene as fuel were used.

### **3.7.2 Determination of microbial parameters of vermiwash**

The microbial population of the vermiwash was estimated by the serial dilution technique given by Aneja (2001). One milliliter of vermiwash sample was added to a test tube containing 9 ml of sterilized distilled water. 1 ml of the above solution was pipetted out and transferred to another test tube containing 9 ml of sterilized distilled water. The process was repeated as many times as required to attain the desired dilution (six times for bacteria and five times for fungi). The total viable bacteria, fungi, *Azotobacter*, *Azospirillum* and phosphorus solubilizing bacteria were determined by this technique.

#### **3.7.2.1 Total viable bacteria**

To determine the bacterial population in the vermiwash samples, nutrient agar medium (MacFaddin, 2000) was used. The nutrient agar was autoclaved at 121°C for 15 minutes. It was then allowed to cool but not solidify. The medium

was poured into sterilized petriplates and allowed to solidify. 1 ml of the dilution was poured and spread evenly on these petriplates. Then the petriplates were incubated at 28°C for 1-2 days. The bacterial colonies formed on the medium were counted.

### **3.7.2.2 Total viable fungi**

For the estimation of the fungal population in the vermiwash samples, potato dextrose agar (MacFaddin, 2000) was used. The potato dextrose agar was autoclaved at 121°C for 15 minutes. The agar medium was cooled and poured into sterilized petriplates and solidified. One ml of the desired dilution was spread evenly onto these petriplates and the plates were incubated at 20-25°C for 48-72 hours. The fungal colonies formed on the medium were counted.

### **3.7.2.3 Free living nitrogen fixers**

#### **3.7.2.3.1 *Azotobacter***

The *Azotobacter* population in the vermiwash sample was determined by using Azotobacter agar medium (Subba Rao, 1977) and the agar medium was autoclaved at 121°C for 15 minutes. It was then allowed to cool but not solidify. The medium was poured into sterilized petriplates and allowed to solidify. 1 ml of the dilution was poured and spread evenly on these petriplates. Then the petriplates were incubated at 28°C for 1-2 days. The *Azotobacter* colonies formed on the medium were counted.

#### **3.7.2.3.2 *Azospirillum***

The *Azospirillum* population in the vermiwash sample was determined by using Azospirillum agar medium (Jorgensen *et al.*, 2015) and the agar medium was autoclaved at 121°C for 15 minutes. It was then allowed to cool but not solidify. The medium was poured into sterilized petriplates and allowed to solidify. 1 ml of the dilution was poured and spread evenly on these petriplates. Then the petriplates were incubated at 28°C for 1-2 days. The *Azospirillum* colonies formed on the medium were counted.

#### **3.7.2.4 Phosphorus solubilizing bacteria**

The phosphorus solubilizing bacteria in the vermiwash was determined by using Pikovskayas agar medium (Pikovskaya, 1948) and the agar medium was autoclaved at 121°C for 15 minutes. It was then allowed to cool but not solidify. The medium was poured into sterilized petriplates and allowed to solidify. 1 ml of the dilution was poured and spread evenly on these petriplates. Then the petriplates were incubated at 28°C for 1-2 days. The colonies formed on the medium were counted.

### **3.8 Antimicrobial activity determination**

#### **3.8.1 Collection of samples**

Ten random leaf samples of mulberry exhibiting leaf spot disease were collected from Temperate Sericulture Research Institute, Mirgund. All the symptomatic leaves were brought to the Research Laboratory, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, in perforated polythene bags. The samples were stored under refrigerated conditions at 4°C and 95% relative humidity for further investigations.

#### **3.8.2 Isolation of pathogen**

Isolation of the fungus (*Phloeospora maculans*) from mulberry (*Morus* spp.) leaves causing leaf spot disease was made. The spotted leaves were cut into small bits of 3-5 mm<sup>2</sup> bearing healthy and diseased tissue. The bits were surface sterilized with 0.1% mercuric chloride for 30 seconds and then washed thrice in sterilized distilled water under aseptic conditions of laminar air flow. These bits were dried on blotter paper and five bits were placed at equidistant on potato dextrose agar medium in petri-plates (90mm diameter). After inoculation, the petri-plates were properly labeled and incubated at 25±1°C for 7 days and regular observations were carried out. Pure culture was obtained by fungal tip method and subsequently maintained by repeated transfers.

### **3.8.2 Identification of pathogen**

Detailed morphological characteristics of the fungi (*Phloeospora maculans*) such as colony characters in the form of colour, margin, aerial growth and microscopic observations with respect to nature of mycelium (septation), reproductive structure (conidia), the type and size of spore, etc were observed and recorded.

### **3.8.3 Preparation of fungal inoculums**

The isolates were maintained on plates of potato dextrose agar medium at 4°C until the experiment was performed. Active cultures were prepared by mixing carefully small amount of test organism (*Phloeospora maculans*) in a sterilized petri-plate with autoclaved water under UV laminar air flow and then spreaded.

### **3.8.4 Antimicrobial susceptibility test**

Agar disc diffusion was used to evaluate antimicrobial activity of the vermiwash. The antimicrobial susceptibility of the vermiwash was tested against the fungal pathogen (*Phloeospora maculans*). 20 ml of sterilized potato dextrose agar medium were poured into sterilized petri-plates and plates were allowed to solidify. Then the previously inoculated fungal suspension were swabbed on the agar surfaced petri-plates by streaking it in horizontal and vertical directions to ensure the uniform distribution of the organism. The paper discs of 6 mm diameter were prepared from Whatman filter paper No.1, placed in a petri-dish and sterilized. The sterile paper discs were soaked in vermiwash sample and placed in fungal inoculated agar plates by using sterile forceps. Then the paper discs were adhered to the agar by gently tapping. Before inoculating, the agar plates were allowed to stand for 5 minutes to ensure the diffusion of vermiwash. The plates were then incubated at 25°C for 72 hours in an inverted position. The zone of inhibition which was formed around the discs was measured including diameter of discs with transparent ruler from back of the plates. The experiment was replicated three times.

The experiment was performed under aseptic conditions. The work surface area of laminar air flow was cleaned with alcohol and UV light was left on 15 minutes before starting the work. The experiments were performed wearing gloves and masks to avoid contamination.

### **3.9 Statistical analysis**

Standard statistical procedures were followed for analyzing the data following Gomez and Gomez (1984).

## Chapter-4

### EXPERIMENTAL FINDINGS

The results pertaining to present study entitled “**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***” are presented in this chapter under following headings.

- 4.1 Chemical parameters of the vermiwash
- 4.2 Microbial parameters of the vermiwash
- 4.3 Antifungal property of the vermiwash

#### **4.1 Chemical parameters of the vermiwash**

The findings of chemical parameters of the vermiwash are described as under:-

##### **4.1.1 pH**

The data presented in the table 1 revealed that the pH of vermiwash varied from slightly alkaline to neutral. The maximum pH (7.88) was recorded in T<sub>1</sub> i.e. cow dung (control). The vermiwash obtained from the combination T<sub>4</sub> containing cow dung + *Aporrectodea caliginosa* showed the minimum pH (7.52).

##### **4.1.2 Electrical conductivity**

According to the data presented in table 1, vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* had the highest (1.44 dS m<sup>-1</sup>) electrical conductivity value and the lowest (1.15 dS m<sup>-1</sup>) was recorded in T<sub>1</sub> containing cow dung (control). The electrical conductivity of vermiwash produced from the substrate combination of cow dung and aquatic weeds were significantly higher than that of substrate cow dung. The electrical conductivity of vermiwash produced from *Eisenia fetida* along with cow dung + aquatic weeds was statistically significant than all other interactions.

### 4.1.3 Organic carbon

The data given in the table 1 shows that the organic carbon was higher (13.31%) in T<sub>1</sub> containing cow dung (control) and the vermiwash produced from the combination T<sub>4</sub> containing cow dung + *Aporrectodea caliginosa* showed the lower (8.59%) organic carbon.

**Table 1: Chemical attributes of vermiwash produced from cow dung and aquatic weeds**

Treatments		pH	EC (dS/m)	Organic carbon (%)
T <sub>1</sub>	Cow dung (control)	7.88	1.15	13.31
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>	7.54	1.36	9.49
T <sub>3</sub>	Cow dung + Aquatic weeds + <i>Eisenia fetida</i>	7.61	1.44	10.64
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>	7.52	1.27	8.59
T <sub>5</sub>	Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>	7.57	1.38	9.62
CD (P ≤ 0.05)		0.014	0.002	0.012

### 4.1.4 Nitrate-nitrogen

After examining the data given in table 2 it was revealed that vermiwash obtained from T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) was the best as the maximum value (23.77 mg/l) of nitrate- nitrogen was recorded in this combination followed by T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) which recorded 22.72 mg/l of nitrate- nitrogen, T<sub>2</sub> (cow dung + *Eisenia fetida*) which recorded 20.82 mg/l of nitrate- nitrogen and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) which recorded 19.83 mg/l of nitrate- nitrogen and lastly the lowest value (15.77mg/l) for nitrate-nitrogen was observed in T<sub>1</sub> containing cow dung (control). Nitrate- nitrogen of vermiwash produced from the treatment

combination of *Eisenia fetida* along with cow dung and aquatic weeds was significantly superior to all other treatments.

#### **4.1.5 Total phosphorus**

The data given in the table 2 shows that vermiwash produced from *Eisenia fetida* along with cow dung and aquatic weeds exhibited higher value (19.33 mg/l) for total phosphorus as compared to the control i.e. cow dung which revealed a comparatively lower value of total phosphorus (13.22 mg/l). Total phosphorus in the vermiwash produced from the action of *Eisenia fetida* on cow dung and aquatic weeds was statistically significant than all other treatment combinations. The treatment combination T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.

#### **4.1.6 Total potassium**

According to the data given in the table 2 the maximum concentration of total potassium (552.73 mg/l) was recorded in vermiwash acquired by using the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* followed by the vermiwash obtained from treatment combinations of T<sub>5</sub>, T<sub>2</sub>, and T<sub>4</sub> which produced the total potassium content of 550.62, 549.37 and 547.37 mg/l. The minimum total potassium of 501.52 mg/l was recorded in control i.e. cow dung. Total potassium in the vermiwash produced from treatment combination of *Eisenia fetida* along with cow dung + aquatic weeds was statistically significant than all other treatment combinations.

#### **4.1.7 C: N ratio**

According to the data presented in the table 2 the maximum value (16.44) of C: N ratio was recorded in T<sub>1</sub> containing cow dung (control) and the minimum value (10.67) of C: N ratio was observed in the vermiwash made by using the treatment combination of *Aporrectodea caliginosa* and cow dung.

**Table 2:      Macronutrients status and C: N ratio of vermiwash**

Treatments		Nitrate-nitrogen (mg/l)	Total phosphorus (mg/l)	Total potassium (mg/l)	C: N ratio
T <sub>1</sub>	Cow dung (control)	15.77	13.22	501.52	16.44
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>	20.82	16.64	549.37	11.56
T <sub>3</sub>	Cow dung + Aquatic weeds+ <i>Eisenia fetida</i>	23.77	19.33	552.73	12.01
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>	19.83	14.53	547.37	10.67
T <sub>5</sub>	Cow dung + Aquatic Weeds + <i>Aporrectodea caliginosa</i>	22.72	17.58	550.62	11.71
<b>CD (P ≤ 0.05)</b>		<b>0.951</b>	<b>1.103</b>	<b>1.145</b>	<b>0.156</b>



**Plate 4: Chemical analysis of the vermiwash**

#### 4.1.8 Calcium

Data presented in table 3 revealed that the vermiwash obtained by the use of cow dung + aquatic weeds + *Eisenia fetida* recorded maximum (174 mg/l) calcium content and the minimum (160.49 mg/l) was recorded in T<sub>1</sub> containing cow dung (control). The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) and also T<sub>3</sub> (cow dung + aquatic weeds+ *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.

#### 4.1.9 Magnesium

The data given in table 3 revealed that vermiwash developed by using the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded highest value (165.50 mg/l) for magnesium and the least (151.88mg/l) was recorded in the combination containing only cow dung. The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) and also T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.

#### 4.1.10 Sodium

Data displayed in table 3 indicates that the highest value of sodium (1221.77 mg/l) was recorded in the vermiwash produced due to the application of *Eisenia fetida* on cow dung and aquatic weeds. Lower value for sodium (1017.93 mg/l) was recorded in T<sub>1</sub> containing only cow dung. Sodium in the vermiwash obtained from the treatment combination of *Eisenia fetida* along with cow dung and aquatic weeds was the best and statistically superior to all other treatment combinations.

**Table 3: Macronutrients status of vermiwash produced from cow dung and aquatic weeds**

Treatments		Calcium (mg/l)	Magnesium (mg/l)	Sodium (mg/l)
T <sub>1</sub>	Cow dung (control)	160.49	151.88	1017.93
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>	171.52	160.55	1179.75
T <sub>3</sub>	Cow dung + Aquatic weeds + <i>Eisenia fetida</i>	174.00	165.50	1221.77
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>	168.99	159.00	1171.97
T <sub>5</sub>	Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>	172.74	162.25	1195.99
CD (P ≤ 0.05)		1.621	1.852	7.021

#### 4.1.11 Micronutrient cations

##### 4.1.11.1 Copper

Data displayed in table 4 reveals that higher concentration (0.12 mg/l) of copper was observed in vermiwash acquired from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* and lower value of 0.06 mg/l copper was recorded in T<sub>1</sub> containing cow dung (control). Copper content of vermiwash produced from the treatment combination of *Eisenia fetida* along with cow dung + aquatic weeds was statistically significant than all other treatment combinations.

##### 4.1.11.2 Iron

According to the data presented in the table 4 the treatment combination T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) recorded maximum iron (5.91 mg/l) and T<sub>1</sub> (cowdung) recorded minimum iron (2.17 mg/l). Vermiwash obtained from the treatment combination of *Eisenia fetida* along with cow dung + aquatic weeds was statistically superior to all other treatments.

#### **4.1.11.3 Manganese**

The data displayed in table 4 indicates that vermiwash obtained from *Eisenia fetida* along with cow dung and aquatic weeds recorded higher manganese content of 3.02 mg/l and the lower manganese content of 1.21 mg/l was recorded in control i.e. cow dung. The manganese content of vermiwash derived from the treatment combination of *Eisenia fetida* along with cow dung and aquatic weeds was significantly superior to all other treatments.

#### **4.1.11.4 Zinc**

According to the data given in the table 4 the vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* was best as it recorded 1.80 mg/l zinc and the least (1.20 mg/l) was recorded in T<sub>1</sub> containing only cow dung. The zinc in vermiwash made by using treatment combination of *Eisenia fetida* along with cow dung + aquatic weeds was statistically significant than all the treatment combinations.

### **4.2 Microbial parameters of the vermiwash**

The findings of microbial parameters of the vermiwash are described as under:-

#### **4.2.1 Total viable bacteria**

According to the data presented in the table 5 the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* was the best as the vermiwash obtained from this combination recorded maximum bacterial population ( $243.50 \times 10^5$  CFU/ml). The minimum count ( $192.75 \times 10^5$  CFU/ml) was observed in cow dung (T<sub>1</sub>). The total viable bacterial population of vermiwash produced from the treatment combination of *Eisenia fetida* along with cow dung and aquatic weeds was statistically superior to all other treatments.

**Table 4: Micronutrients status of vermiwash produced from cow dung and aquatic weeds**

Treatments		Copper (mg/l)	Iron (mg/l)	Manganese (mg/l)	Zinc (mg/l)
T <sub>1</sub>	Cow dung (control)	0.06	2.17	1.21	1.20
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>	0.09	3.76	1.67	1.38
T <sub>3</sub>	Cow dung + Aquatic weeds + <i>Eisenia fetida</i>	0.12	5.91	3.02	1.80
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>	0.08	3.22	1.61	1.32
T <sub>5</sub>	Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>	0.10	4.95	2.48	1.43
<b>CD (P ≤ 0.05)</b>		<b>0.004</b>	<b>0.014</b>	<b>0.003</b>	<b>0.002</b>



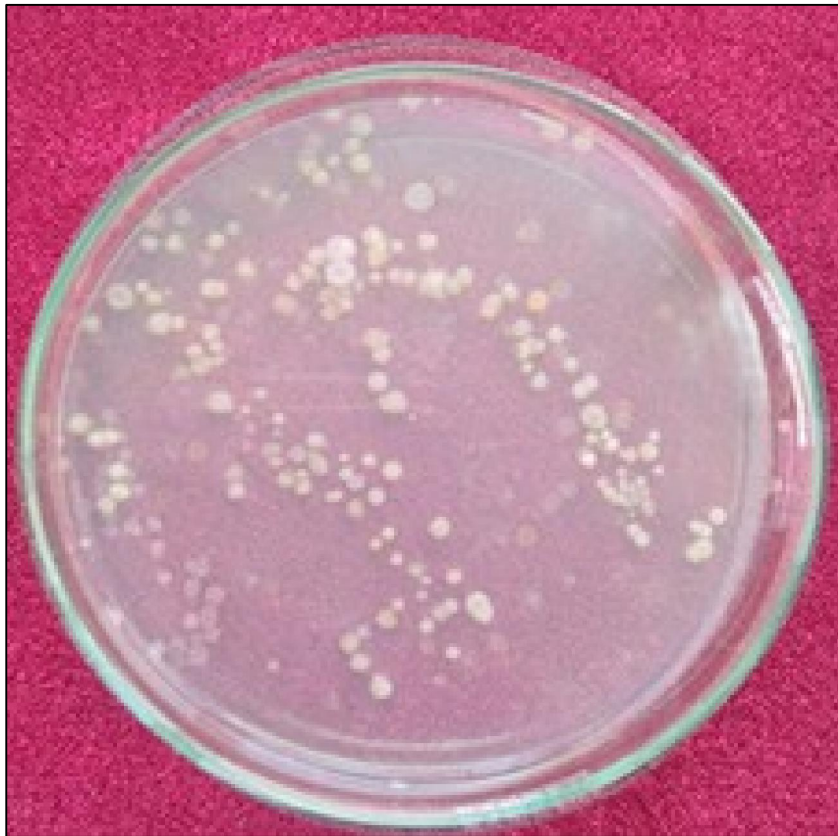
**Plate 5: Microbial analysis of the vermiwash**

#### 4.2.2 Total viable fungi

The data presented in table 5 shows that the vermiwash produced from *Eisenia fetida* along with cow dung + aquatic weeds recorded maximum fungal population ( $7.50 \times 10^5$  CFU/ml) as compared to the control i.e. cow dung which recorded minimum fungal population ( $2.00 \times 10^5$  CFU/ml). The fungal population of vermiwash developed from the treatment combination of *Eisenia fetida* along with cow dung and aquatic weeds was significantly superior than majority of the treatments. The treatment combination T<sub>1</sub> (control) and T<sub>2</sub> (cow dung + *Eisenia fetida*), T<sub>1</sub> (control) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*), T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.

**Table 5: Bacterial and fungal population of vermiwash produced from cow dung and aquatic weeds**

Treatments		Total viable bacteria (10 <sup>5</sup> CFU/ml)	Total viable fungi (10 <sup>5</sup> CFU/ml)
T <sub>1</sub>	Cow dung (control)	192.75	2.00
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>	203.50	3.00
T <sub>3</sub>	Cow dung + Aquatic weeds + <i>Eisenia fetida</i>	243.50	7.50
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>	198.25	2.25
T <sub>5</sub>	Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>	226.75	4.25
CD (P ≤ 0.05)		4.814	1.581



**Plate 6: Bacterial population of vermiwash**

### **4.2.3 Free living nitrogen fixers**

#### **4.2.3.1 *Azotobacter***

Vermiwash produced from *Eisenia fetida* along with cow dung and aquatic weeds recorded  $83.00 \times 10^6$  CFU/ml *Azotobacter* count (Table 6). The minimum population of *Azotobacter* ( $65.50 \times 10^6$  CFU/ml) was recorded in T<sub>1</sub> containing cow dung (control). *Azotobacter* population of vermiwash produced by using the treatment combination of *Eisenia fetida* along with cow dung and aquatic weeds was statistically significant than all other treatments.

#### **4.2.3.2 *Azospirillum***

Data presented in table 6 shows that vermiwash produced by *Eisenia fetida* along with cow dung + aquatic weeds recorded higher *Azospirillum* population ( $78.00 \times 10^6$ CFU/ml). Comparatively lower *Azospirillum* count ( $61.50 \times 10^6$  CFU/ml) was recorded in control i.e. cow dung. The *Azospirillum* population in vermiwash made by using treatment combination of cow dung + aquatic weeds + *Eisenia fetida* was statistically significant than all the treatment combinations. The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.

#### **4.2.3.3 Phosphorus solubilizing bacteria**

According to the data given in table 6 vermiwash produced from cow dung + aquatic weeds + *Eisenia fetida* recorded significantly enhanced level of phosphorus solubilizing bacterial population ( $68.25 \times 10^6$  CFU/ml) and minimum population ( $51.50 \times 10^6$  CFU/ml) was observed in T<sub>1</sub> containing cow dung (control). The treatment combinations T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.



*Azotobacter*



*Azospirillum*



Phosphorus solubilizing bacteria

**Plate 7: Free living nitrogen fixers and phosphorus solubilizing bacteria of vermiwash**

**Table 6: Free living nitrogen fixers and phosphorus solubilizing bacteria of vermiwash**

Treatments		<i>Azotobacter</i> (10 <sup>6</sup> CFU/ml)	<i>Azospirillum</i> (10 <sup>6</sup> CFU/ml)	Phosphorus solubilising bacteria (10 <sup>6</sup> CFU/ml)
T <sub>1</sub>	Cow dung (control)	65.50	61.50	51.50
T <sub>2</sub>	Cow dung + <i>Eisenia fetida</i>	75.00	69.00	63.50
T <sub>3</sub>	Cow dung + Aquatic weeds + <i>Eisenia fetida</i>	83.00	78.00	68.25
T <sub>4</sub>	Cow dung + <i>Aporrectodea caliginosa</i>	69.50	66.00	57.00
T <sub>5</sub>	Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>	79.75	72.50	66.50
CD (P ≤ 0.05)		2.742	3.571	1.945

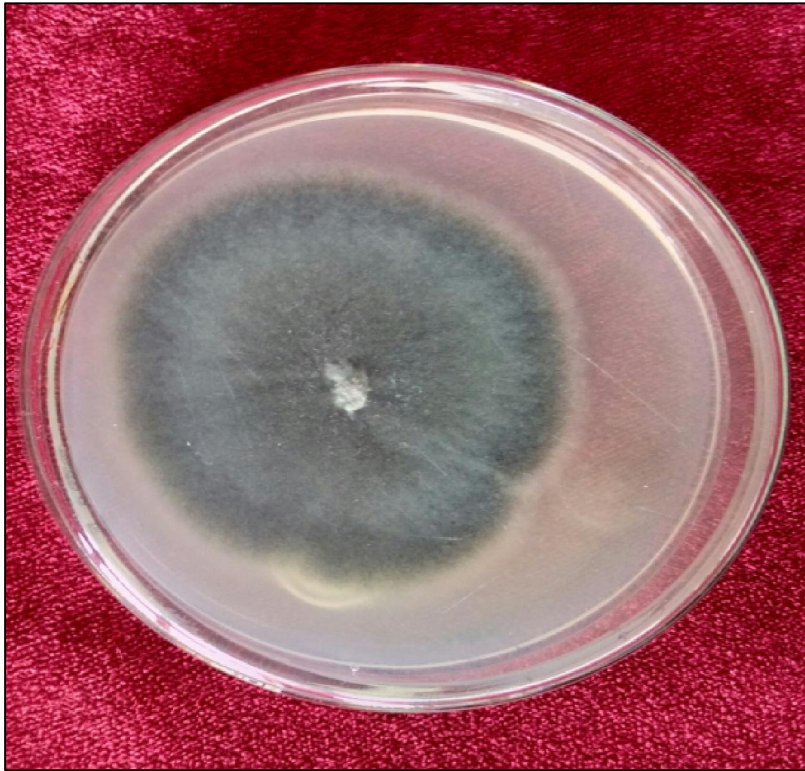
### 4.3 Antifungal property of the vermiwash

#### 4.3.1 *In vitro* antifungal activity against *Phloeospora maculans*

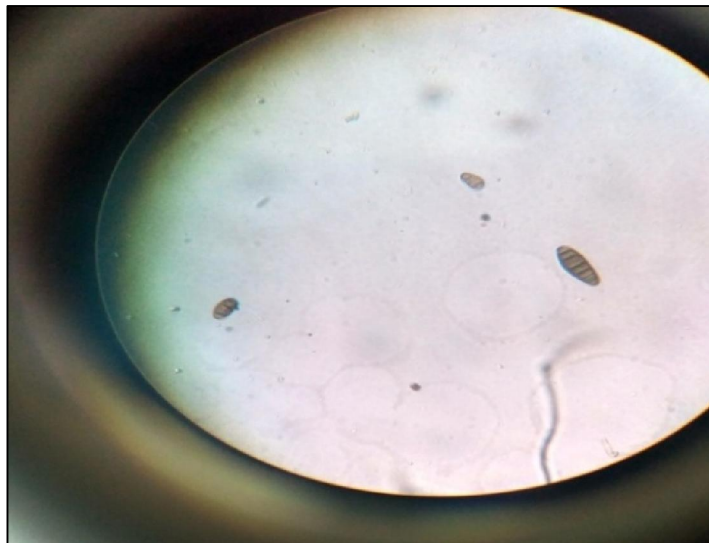
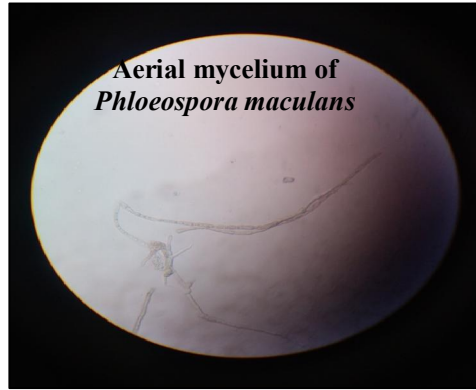
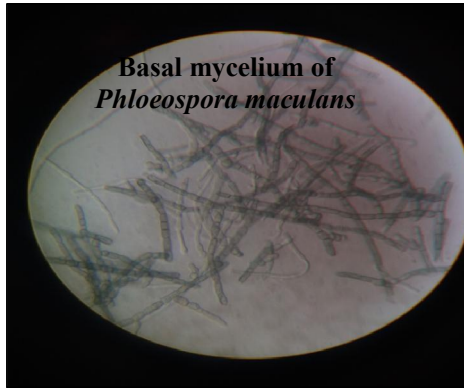
According to the data given in table 7 the maximum inhibition zone (21.25 mm) was observed in vermiwash containing *Eisenia fetida* along with cow dung + aquatic weeds and the minimum (12.25 mm) was observed in T<sub>1</sub> containing cow dung (control). The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*), T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*), T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.



**Plate 8: Leaf spot disease of mulberry (*Morus alba*)**



**Plate 9: Culture of *Phloeospora maculans***



Spores of *Phloeospora maculans*

**Plate 10: Basal mycelium, Aerial mycelium and Spores of *Phloeospora maculans***

**Table 7:** *In vitro* antifungal activity of vermiwash produced from cow dung and aquatic weeds against *Phloeospora maculans*

Treatments	Inhibition zone (mm)
T <sub>1</sub> Cow dung (control)	12.25
T <sub>2</sub> Cow dung + <i>Eisenia fetida</i>	17.50
T <sub>3</sub> Cow dung + Aquatic weeds + <i>Eisenia fetida</i>	21.25
T <sub>4</sub> Cow dung + <i>Aporrectodea caliginosa</i>	15.75
T <sub>5</sub> Cow dung + Aquatic weeds + <i>Aporrectodea caliginosa</i>	19.25
<b>CD (P ≤ 0.05)</b>	<b>3.568</b>



**Control**



**Formation of Inhibition zone**

**Plate 11: *In vitro* antifungal activity of vermiwash against *Phloeospora maculans* by disc diffusion method**

## Chapter-5

### DISCUSSION

The results of the present investigation entitled “**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***” have been discussed in this chapter.

#### 5.1 Chemical parameters of the vermiwash

##### 5.1.1 pH

pH is a measure of  $H^+$  concentration. It is an indicator of the acidity or basicity of a solution. In the present study the pH of vermiwash varied from slightly alkaline to neutral. The maximum pH (7.88) was recorded in treatment containing cow dung (control) whereas the minimum pH (7.52) was exhibited by vermiwash developed from the combination of cow dung + *Aporrectodea caliginosa*. It was observed that pH was reduced during the process of vermicomposting that is in accordance with the results of John Paul *et al.* (2011). This reduction in pH value can be due to the production of  $CO_2$  and organic acids by microorganisms and earthworm. Bioconversion of organic matter to different intermediate materials, intensive mineralization of organic nitrogen to nitrate and nitrite and phosphorous to ortho-phosphates can be considered as other reasons of pH reduction (Gunadi *et al.* 2002). Similar discoveries have been accounted by Sugapriya and Mahalingam (2010) and Sundaravadivelan *et al.* (2011).

##### 5.1.2 Electrical conductivity

Electrical conductivity is the measure of soluble salts in the test samples. The vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded the maximum ( $1.44 \text{ dS m}^{-1}$ ) electrical conductivity and the minimum ( $1.15 \text{ dS m}^{-1}$ ) electrical conductivity was recorded in control i.e. cow dung. The higher value of electrical conductivity may be because of the mineralization of organic matter and release of mineral salts in

available forms such as ammonium, phosphate and potassium. Similar findings were also reported by Bai and Vijayalakshmi (2000), Sugapriya and Mahalingam (2010) and Sundaravadivelan *et al.* (2011)

### **5.1.3 Organic carbon**

Organic carbon constitutes about 58% of organic matter. The stability of soil aggregates, release of essential nutrients for plant growth, maintenance of soil microbial dynamics and biochemical changes in the soil are attributed to the organic carbon content of the soil (Paul and Clark, 1989). The organic carbon was higher (13.31%) in treatment containing cow dung (control) and the lower organic carbon (8.59%) was recorded in vermiwash obtained from cow dung + *Aporrectodea caliginosa*. There was a significant decrease in the organic carbon, it could be due to degradation and mineralization of organic matter. Also organic carbon was lost as carbon dioxide due to the respiration of earthworms and microbes. Similar results were reported by Elvira *et al.* (1998), Garg and Kaushik (2005), Suthar (2007), Sugapriya and Mahalingam (2010), Sundaravadivelan *et al.* (2011) and Varghese and Prabha (2014).

### **5.1.4 Nitrate-nitrogen**

Nitrate-nitrogen is the most oxidized form of nitrogen which can be utilized by the plant roots for growth and development. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) recorded the maximum of 23.77 mg/l nitrate-nitrogen and the minimum (15.77 mg/l) was recorded in treatment containing cow dung (T<sub>1</sub>). The most probable reason for higher value of nitrate-nitrogen in the vermiwash obtained from the T<sub>3</sub> could be due to the decomposition of organic matter, accumulation of excretory products, mucus and other body fluids of earthworms which are rich in nitrogen. This result is supported by Hand *et al.* (1988), Tripathi and Bhardwaj (2004) and Phukan and Savapondit (2014). The other reason may be the transformation of organic bound insoluble nitrogen to soluble form by the action

of nitrogen fixing microorganisms present in the substrates. Moreover, the treatments with *Eisenia fetida* produced higher values for nitrate-nitrogen as compared to *Aporrectodea caliginosa*, this may be because of individual capability of *Eisenia fetida* to convert substrate to nutrient rich vermicast.

#### **5.1.5 Total phosphorus**

Phosphorus is one of the most important plant nutrients, which is required in optimum amount for proper growth of plants. It is a constituent of cell membranes, certain proteins, all nucleic acids and is required for all phosphorylation reactions. The total phosphorus was significantly high (19.33 mg/l) in the vermiwash produced from cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) and relatively low value of total phosphorus (13.22 mg/l) was found in control i.e. cow dung. The treatment combination T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant. The increased total phosphorus content in vermiwash produced from T<sub>3</sub> could be because of the degradation of organic matter and also transformation of organic bound phosphorus to its available state. The results are in consistence with the findings of Lee (1985), Varghese and Prabha (2014). The increase in total phosphorus can also be due to the mineralization and mobilization of phosphorus by bacterial and fecal phosphatase activity of earthworms which is in accordance with the findings of Jeyanthi *et al.* (2010).

#### **5.1.6 Total potassium**

Potassium is essential for protein synthesis, photosynthesis and respiration. It regulates stomatal movement, osmoregulation and helps to maintain anion-cation balance in cells. Vermiwash produced from cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) recorded a higher concentration of total potassium (552.73 mg/l) as compared to the control which recorded a lower value for total potassium (501.52 mg/l). The most probable reason for enhanced level of total potassium in vermiwash produced from T<sub>3</sub> could be organic matter combustion and

mineralization by the activity of earthworms and microbes. Similar discoveries were also reported by Benitez *et al.* (1999), Suthar (2007), Varghese and Prabha (2014). Higher content may also be attributed to the utilization of potassium rich initial substrates.

#### **5.1.7 C:N ratio**

C:N ratio is one of the most widely used indices for maturity of organic wastes. Lower range of C: N ratio ensures better nutritional composition compared to high range. The reduction in C: N ratio to less than 20 indicates an advance degree of organic matter stabilization and reflects a satisfaction degree of organic wastes. In the present study vermiwash produced from all the treatment combinations recorded C: N ratio below 20. The C: N ratio was higher (16.44) in treatment containing cow dung (control) and the lower C: N ratio (10.67) was recorded in vermiwash obtained from cow dung + *Aporrectodea caliginosa*. The reduction of the C: N ratio in the vermiwash relative to that of control could due to combustion of organic carbon during respiration which lowers the carbon content. Simultaneously there was an increase in the concentration of nitrogen due to the mineralization of organic nitrogen. This further enhanced the level of nitrogen in respect to carbon. Similar findings were also reported by Nath *et al.* (2009), Varghese and Prabha (2014) and Hussain *et al.* (2016).

#### **5.1.8 Calcium**

Calcium is the major constituent of cell wall in the form of calcium pectate. It is required for cell division and cell enlargement. It activates certain enzymes and plays an important role in regulating metabolic activities. Calcium was highest (174 mg/l) in vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) and least (160.49 mg/l) in treatment containing only cow dung (T<sub>1</sub>). The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) and also T<sub>3</sub> (cow dung + aquatic weeds+ *Eisenia fetida*) and T<sub>5</sub> (cow

dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant. The most probable reason for high level of calcium in the vermiwash produced from T<sub>3</sub> may be due to the calciferous glands present in the earthworm gut which produces calcium carbonate and increases the availability of calcium. The result is in accordance with findings of Varghese and Prabha (2014). Sivakumar and Subbhuraam (2005) also reported that the enhanced calcium level is due to the gut process associated with calcium metabolism which increases the calcium content in the vermicast.

#### **5.1.9 Magnesium**

Magnesium is a constituent element of chlorophyll. It also acts as an activator of some enzymes especially those concerned with carbohydrate metabolism and those involved in synthesis of RNA and DNA. It is also essential for binding the components of ribosomes. Maximum magnesium (165.50 mg/l) was found in the vermiwash produced from cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) and minimum (151.88 mg/l) was found in the cow dung (T<sub>1</sub>). The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) and also T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant. The most probable reason for high level of magnesium in vermiwash obtained from T<sub>3</sub> could be mineralization of magnesium in substrate by earthworms. Similar discoveries have been accounted by Sitre (2014) and Chattopadhyay (2015).

#### **5.1.10 Sodium**

Sodium is a beneficial element for the growth and development of plant. In higher plants, it is essential and to some extent it can supplement for potassium. The concentration of sodium was highest (1221.77 mg/l) in vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) and minimum (1017.93 mg/l) in control containing cowdung (T<sub>1</sub>). The

increased concentration of sodium in vermiwash produced from T<sub>3</sub> was probably due to the decomposition of organic matter and mineralization of the substrate material by the earthworm. The results were in conformity with the earlier findings of Sugapriya and Mahalingam (2010) and Sahoo *et al.* (2015).

### **5.1.11 Micronutrient cations**

#### **5.1.11.1 Copper**

Copper is a component of several enzymes and plays an important role in plant metabolism. It is also a component of plastocyanin which is involved in electron transport in photosynthesis. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) recorded the maximum (0.12 mg/l) copper content as compared to control (T<sub>1</sub>) which recorded minimum (0.06 mg/l) copper content. The higher level of copper in the vermiwash may be due to the presence of copper containing oxidizing enzymes and also because of mineralization of copper due to earthworm activity. The results are in accordance with Lee (1985), Suthar (2007), Varghese and Prabha (2014) and Prabina *et al.* (2018).

#### **5.1.11.2 Iron**

Iron is an important constituent of proteins like ferredoxin and cytochromes involved in electron transport system in plants. It acts as catalyst in nitrate reductase and is essential for the formation of chlorophyll. The iron content was significantly enhanced in the vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) which recorded 5.91 mg/l iron and the cow dung (control) recorded comparatively lower (2.17 mg/l) iron content. The increased level of iron in the vermiwash might be due to the combustion of organic matter in the compost leading to mineralization of iron. Presence of certain enzymes and co factors in the earthworm gut also led to high concentration of iron. These results are in consistence with Mall *et al.* (2005) and Varghese and Prabha (2014).

### **5.1.12.3 Manganese**

Manganese is an activator for a number of enzymes involved in photosynthesis, respiration and nitrogen metabolism. It plays an important role in the splitting of water to liberate oxygen during photosynthesis and also protects the structure of chloroplast. Significantly enhanced level of manganese (3.02 mg/l) was noted in the vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) as compared to the control which recorded relatively less amount of manganese (1.21 mg/l). This enhancement was probably due to the mineralization and mobilization of manganese by the activity of earthworms and microorganisms. These results are in accordance with Vasanthi and Kumaraswamy (1999), Varghese and Prabha (2014).

### **5.1.11.4 Zinc**

Zinc is essential for the synthesis of indole acetic acid, a plant growth hormone. It also serves as an activator for various enzymes especially carboxylases. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) recorded maximum zinc (1.80 mg/l) and minimum (1.20 mg/l) content was recorded in control containing cowdung. The increasing trend of zinc in vermiwash in relative to that of control might be due to the degradation of organic matter leading to the release of zinc in its soluble form. The results were in consistence with the findings of Varghese and Prabha (2014) and Prabina *et al.* (2018).

## **5.2 Microbial parameters of the vermiwash**

### **5.2.1 Total viable bacteria, total viable fungi, free nitrogen fixers (*Azotobacter* and *Azospirillum*) and phosphorus solubilizing bacteria**

Microorganisms are the primary decomposers of organic matter. Microbial activity in earthworms gut and cast is very essential for the breakdown and release of nutrients in available form. The microorganisms and earthworms act symbiotically to accelerate and enhance the decomposition of organic matter.

Microorganisms especially bacteria are largely responsible for decomposition of organic matter. They participate extensively in mineralization of organic materials into available form which can be utilized by plants (Pingle, 2015). Vermiwash obtained from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* produced the maximum population of bacteria ( $243.50 \times 10^5$  CFU/ml) and minimum population ( $192.75 \times 10^5$  CFU/ml) was produced in treatment containing only cow dung (control).

Fungi are active decomposers of organic matter. They produce enzymes which degrade the organic matter of plant origin. They are efficient in carbon assimilation from decomposing organic residues into fungal hyphae, which serve as source of energy and nutrients for bacteria and other microflora to carry forward the biochemical transformations. The vermiwash produced from cow dung + aquatic weeds + *Eisenia fetida* (T<sub>3</sub>) recorded maximum fungal population ( $7.50 \times 10^5$  CFU/ml) as compared to the cowdung (T<sub>1</sub>) which recorded minimum fungal population ( $2.00 \times 10^5$  CFU/ml). The fungal population of vermiwash developed from T<sub>3</sub> was significantly superior than majority of the treatments. The treatment combination T<sub>1</sub> (control) and T<sub>2</sub> (cow dung + *Eisenia fetida*), T<sub>1</sub> (control) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*), T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.

*Azotobacter* is a non-symbiotic heterotrophic nitrogen fixing bacterium which converts the atmospheric nitrogen into the forms readily taken by the plants. *Azotobacter* also promotes growth and development of crop plants by helping in synthesis of auxins, vitamins, growth substances and antifungal antibiotics. Vermiwash generated from cow dung + aquatic weeds + *Eisenia fetida* recorded maximum *Azotobacter* population ( $83.00 \times 10^6$  CFU/ml). The minimum population of *Azotobacter* ( $65.50 \times 10^6$  CFU/ml) was recorded in treatment containing cow dung (control).

*Azospirillum* is a plant growth promoting soil bacterium which plays an important role in nitrogen fixation. *Azospirillum* is also involved in synthesis of growth promoting substance (indole acetic acid) and also provides disease resistance to plants. Vermiwash developed from cow dung + aquatic weeds + *Eisenia fetida* recorded higher *Azospirillum* population ( $78.00 \times 10^6$  CFU/ml) as compared to control which recorded lower *Azospirillum* count ( $61.50 \times 10^6$  CFU/ml). The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.

Phosphorus solubilizing bacteria includes those which help to convert insoluble phosphate into simple and soluble forms and increase the availability of phosphorus to the plants. Vermiwash prepared by using cow dung + aquatic weeds + *Eisenia fetida* recorded significantly higher population of phosphorus solubilizing bacteria ( $68.25 \times 10^6$  CFU/ml) and minimum population ( $51.50 \times 10^6$  CFU/ml) was observed in control containing cow dung. The treatment combinations T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.

The increased microbial population in the vermiwash might be due to the availability of nutrient rich earthworm casts which supports the enormous growth of microorganisms. The results were in consistence with the findings of Meenatchi *et al.* (2009), Phukan and Savapondit (2014) and Esakkiammal *et al.* (2015). The enhanced bacterial count especially *Azotobacter* and *Azospirillum* was also reported by Prabina *et al.* (2018). The treatment combination of cow dung + aquatic weeds + *Eisenia fetida* was found to have most abundant in microbial population as the substrate used was rich in nutrients.

### 5.3 Antifungal property of the vermiwash

#### 5.3.1 *In vitro* antifungal activity against *Phloeospora maculans*

*Phloeospora maculans* is the causal organism of leaf spot disease of mulberry. The pathogen is soil borne and over seasons in the plant residues. During rainy season, when there is high humidity and temperature around 24°C, the pathogen gets activated and produces conidia in the soil and plant residues. With the splashing action of rains, the conidia get released and deposited on the leaves where they germinate within 24 hours producing one or two germ tubes that enter the leaves through stomata and spread infection on the lower surface of leaves. Vermiwash are reported to control plant pathogens through different mechanisms. The most reported factor influencing the efficacy of vermiwash in inhibiting the development of plant disease is their microbial content. The microorganisms present in the vermiwash may act as pathogen antagonists through their ability to compete for space and nutrients, to destroy pathogens by parasitism, to produce antimicrobial compounds, or to induce systemic resistance in plants (Mehta *et al.*, 2014). In the present study vermiwash obtained from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* produced the maximum inhibition zone (21.25 mm) and the minimum (12.25 mm) was observed in treatment containing cow dung (control). The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*), T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*), T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant. The treatment combination of cow dung + aquatic weeds + *Eisenia fetida* showed maximum inhibition zone as highest population of microorganisms was found in this combination. Similar discoveries have been reported by Esakkiammal and Lakshmibai (2013) and Ansari *et al.* (2016).

## Chapter-6

### SUMMARY AND CONCLUSION

The present investigation entitled “**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***” was carried out during 2018-2019 at Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar. In this study the aquatic weeds were used as substrate for preparation of vermiwash using two species of earthworms *Eisenia fetida* and *Aporrectodea caliginosa*. Various chemical and biological parameters of the final vermiwash were determined. The findings of the study are summarized below:-

#### 6.1 Chemical parameters of vermiwash

- The pH of the vermiwash ranged from 7.52-7.88. The maximum pH (7.88) was recorded in treatment containing cow dung (control) where as the minimum pH (7.52) was exhibited by vermiwash developed from the combination of cow dung + *Aporrectodea caliginosa*.
- Electrical conductivity of the vermiwash varied from 1.15-1.44 dS m<sup>-1</sup>. Highest electrical conductivity (1.44 dS m<sup>-1</sup>) was found in the vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* and lowest value of electrical conductivity (1.15 dS m<sup>-1</sup>) was observed in the control containing cowdung (control).
- Organic carbon of the vermiwash ranged from 8.59-13.31%. Highest value of organic carbon (13.31%) was recorded in treatment containing cow dung (control) and the minimum was recorded in vermiwash produced from the combination of cow dung + *Aporrectodea caliginosa*.
- Nitrate-nitrogen in the vermiwash ranged from 15.77-23.77 mg/l. Vermiwash produced from the treatment combination of *Eisenia fetida* along with cow dung + aquatic weeds showed the maximum nitrate-

nitrogen (23.77 mg/l) and cow dung (control) showed the minimum (15.77 mg/l) nitrate-nitrogen.

- The total phosphorus in the vermiwash varied between 13.22-19.33 mg/l. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded maximum (19.33 mg/l) total phosphorus and minimum (13.22 mg/l) was recorded in control i.e. cow dung. The treatment combination T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.
- Total potassium in the vermiwash ranged from 501.52-552.73 mg/l. Highest concentration of total potassium (552.73 mg/l) was recorded in the vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* and lowest concentration of total potassium (501.52 mg/l) was recorded in treatment containing cow dung (control).
- C:N ratio in the vermiwash ranged from 10.67-16.44. The maximum value (16.44) of C: N ratio was recorded in cow dung (control) and the minimum (10.67) was recorded in the vermiwash of cow dung treated with *Aporrectodea caliginosa*.
- The value of calcium in the vermiwash ranged from 160.49-174 mg/l. Vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded the maximum calcium (174 mg/l) as compared to control which recorded minimum (160.49 mg/l) calcium content. The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) and also T<sub>3</sub> (cow dung + aquatic weeds+ *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.

- In case of magnesium the values varied from 151.88-165.50 mg/l. Maximum magnesium (165.50 mg/l) was recorded in the vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* and minimum magnesium (151.88 mg/l) was recorded in control i.e. cow dung. The treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) and also T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.
- Sodium ranged from 1017.93-1221.77 mg/l. Maximum sodium (1221.77 mg/l) was found in the vermiwash of cow dung + aquatic weeds + *Eisenia fetida* and minimum sodium (1017.93 mg/l) was recorded in the treatment containing only cow dung.
- The concentration of micronutrient cations was found higher in the vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* and the least was found in control i.e. cow dung. Copper, iron, manganese and zinc content in the vermiwash varied from 0.06-0.12 mg/l, 2.17-5.91 mg/l, 1.21-3.02mg/l and 1.20-1.80 mg/l respectively.

## 6.2 Microbial parameters of the vermiwash

- Total viable bacteria ranged from  $192.75 \times 10^5$ - $243.50 \times 10^5$  CFU/ml. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded maximum bacterial population ( $243.50 \times 10^5$  CFU/ml) and minimum population of bacteria ( $192.75 \times 10^5$  CFU/ml) was found in cow dung (control).
- Total viable fungi ranged from  $2.00 \times 10^5$ - $7.50 \times 10^5$  CFU/ml. Vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded maximum fungal ( $7.50 \times 10^5$  CFU/ml) population and minimum population of fungi ( $2.00 \times 10^5$  CFU/ml) was recorded in

treatment containing cow dung (control). The treatment combination T<sub>1</sub> (control) and T<sub>2</sub> (cow dung + *Eisenia fetida*), T<sub>1</sub> (control) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*), T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be statistically non-significant.

- Population of *Azotobacter* ranged from  $65.50 \times 10^6$ - $83.00 \times 10^6$  CFU/ml. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded the maximum *Azotobacter* population ( $83.00 \times 10^6$  CFU/ml) and minimum count ( $65.50 \times 10^6$  CFU/ml) was recorded in cow dung (control).
- Population of *Azospirillum* ranged from  $61.50 \times 10^6$ - $78.00 \times 10^6$  CFU/ml. Vermiwash generated from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded the maximum *Azospirillum* population ( $78.00 \times 10^6$  CFU/ml) and minimum ( $61.50 \times 10^6$  CFU/ml) was recorded in control i.e. cow dung. But the treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) were found to be non-significant.
- Population of phosphorus solubilizing bacteria varied from  $51.50 \times 10^6$ - $68.25 \times 10^6$  CFU/ml. Maximum population of phosphorus solubilizers ( $68.25 \times 10^6$  CFU/ml) were recorded in the vermiwash developed from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* and minimum count ( $51.50 \times 10^6$  CFU/g) was recorded in the cow dung (control). Here also the treatment combinations T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.

### 6.3 *In vitro* antifungal activity of the vermiwash against *Phloeospora maculans*

- The inhibition zone varied from 12.25- 21.25mm. Vermiwash produced from the treatment combination of cow dung + aquatic weeds + *Eisenia fetida* recorded maximum inhibition zone (21.25 mm) and minimum (12.25 mm) was recorded in cow dung (control). But the treatment combinations T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*), T<sub>2</sub> (cow dung + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*), T<sub>3</sub> (cow dung + aquatic weeds + *Eisenia fetida*) and T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) were found to be statistically non-significant.

### CONCLUSION

On the basis of the results obtained the following conclusions can be drawn:-

The treatment combination T<sub>3</sub> (*Eisenia fetida* with cow dung + aquatic weeds in the ratio of 3:1) was the best followed by T<sub>5</sub> (cow dung + aquatic weeds + *Aporrectodea caliginosa*) > T<sub>2</sub> (cow dung + *Eisenia fetida*) > T<sub>4</sub> (cow dung + *Aporrectodea caliginosa*) > T<sub>1</sub> (cow dung) as this combination showed maximum electrical conductivity, nitrate-nitrogen, total phosphorus, total potassium, calcium, magnesium and sodium, micronutrient cations and viable bacteria, fungi, free living nitrogen fixers, total phosphorus solubilizing bacteria and antifungal activity against *Phloeospora maculans*.

Though the nutrient status and microbial population were found highest in the treatment combination T<sub>3</sub>, however all treatment combinations showed statistically significant nutrient status and microbial population than control hence vermiwash can be utilized as an effective liquid manure. Based on results it can be concluded that vermiwash have bio-active compounds to inhibit the growth of *Phloeospora maculans*. Hence vermiwash can be used as a potential bio-control agent against the pathogen.

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**CERTIFICATE**

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Bashir Ahmad Ganaie**, Professor, COD and Head, Environmental Sciences, University of Kashmir during Viva-Voce examination held on **28-02-2020** have been incorporated in the manuscript entitled “**Quality assessment of Vermiwash produced from Aquatic weeds using *Eisenia fetida* and *Aporrectodea caliginosa***” submitted by **Ms. Sumaya Manzoor** (Regd. No. 2017-ES-33-M).

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