Effects of Microwave Irradiation (MWI) on Quality of Vermicasts, and Growth of Kale (*Brassica oleracea* var. *sabellica*) Plants

by

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ABSTRACT

The application of microwave irradiation (MWI) technique has gained considerable attention in agriculture such as its use to enhance soil properties and extraction of active principles in natural products. It was therefore hypothesized that variables such as MWI power output levels, duration of exposure, and moisture content (MC) of vermicasts may different influence the quality and efficacy of vermicasts. Thus, the present study evaluated the main and the interaction effects of MWI power output levels (0-1000 W), exposure time (0-10 min) and vermicasts moisture content (35-85%) on physical and electrochemical properties, and the efficacy on potted kale (*Brassica oleracea* var. *sabellica*) plants growth and tissue chemical content. The quality of vermicasts was significantly ($P<0.05$) affected by MWI power output levels and exposure time at varying MC. It showed that the 85% MC vermicasts treated at 400 W for 2.5 min was the best treatment for vermicasts to promote kale plants growth. Kale plants treated with 35% and 45% MC vermicasts treated at 400 W for 2.5 min had the least growth parameters. Considering that this research was a pot-experiment performed in the greenhouse, there are some limitations to this research. However, it is recommended that further studies should focus on the large-scale field study and how the MWI influence the biological properties of vermicasts.

Keywords: *microwave irradiation, vermicasts, kale, power output level, moisture content, exposure time*

LIST OF ABBREVIATIONS USED

ACI   Anthocyanin Content Index

cm   Centimeter

dBmW   Decibel Milliwatts

EC   Electrical Conductivity

FMV   Fresh, Moist Vermicasts

g   Gram

GHz   Gigahertz

hrs   Hours

Hz   Hertz

kg   Kilogram

MC   Moisture Content

mg   Milligram

MHz 10^6Hz

min   Minutes

MWI   Microwave Irradiation

NUT   Nephelometric Turbidity Unit

ppm Parts-per-million

ppt Parts-per-trillion

S/cm Siemens/Centimeter

SOM Soil Organic Matter

SPAD Soil-Plant Analyses Development

TDS Total Dissolved Solids

V Volt

W Watt

% Percent

°C Degrees Celsius

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Chapter 1 Introduction

1.1 General Introduction

Kale (*Brassica oleracea* var. *sabellica*) is a leafy vegetable that has a large exposed surface area; it is commonly cultivated in central and northern Europe, as well as in North America and globally acknowledged for its numerous health benefits (Neugart et al. 2012). Kale belongs to the *Brassicaceae* family together with broccoli, brussels sprouts and cabbage (Neugart et al. 2012). The leaf tissue of kale is abundant due to the high proportion of structurally different flavonoids, which is higher than other vegetables, such as broccoli and onion (Hertog et al. 1992; Schmidt et al. 2010). The flavonoids have health-promoting effects on humans including protection against chronic diseases due to their various biological activities (Knekt et al. 2002; Schmidt et al. 2010). Kale also contains health beneficial phytochemicals such as vitamin C, phenolic compounds, and glucosinolates (Cartea et al. 2011; Jahangir et al. 2009; Olsen et al. 2012; Podsędek 2007). Thus, kale has gained attention from producers and consumers due to its health-related potential.

Natural growing medium amendments include rock powder, vermicasts, composts, green and animal manure, extracted humates, volcanic minerals and their derivatives (Iheshiulo et al. 2017). A soil mixed with natural growing medium amendments may increase the harvest quality of plants because different natural growing medium amendments have different effects on growth and harvest quality of plants. For instance, the yield and quality of kale were improved following treatment with dry vermicasts as compared to potassium humates and volcanic minerals (Iheshiulo et al. 2017).

In recent years, vermicasts (earthworm excreta) have gained more attention compared to other natural growing medium amendments due to their overall preferable characteristics, convenience and low production input, i.e. earthworms and organic wastes as feedstock (Munroe 2007). Vermicasts are thus ready-to-use natural organic growing medium amendment for growing crops (Munroe 2007). The whole production process and use of vermicasts are pollution-free.

Recent trend shows that consumers prefer safe and organic food. Consumers are concerned about the sources of their foods and how the foods are grown. As a result, the demand for organic food is increasing due to the pressure from consumers and environmental group to cut down the use of synthetic chemical fertilizers and pesticides (Iheshiulo et al. 2017). Consequently, the use of natural growing medium amendments including vermicasts to produce sustainable and organic foods is on the rise.

Microwave irradiation (MWI) has gained considerable attention in domestic, commercial and industrial settings because for its high heating efficiency and smooth operation (Li et al. 2016; Zhu et al. 2005). MWI was previously used to pretreat rice straw (Zhu et al. 2005); compost to produce hydrogen (Song et al. 2012); soil (Kim and Kim 2013) and more recently, vermicasts (Abbey et al. 2017). Although the use of MWI technology in growing media industry is understudied, existing preliminary studies demonstrated that MWI technology might improve the efficiency of organic growing medium amendments. For instance, Kim and Kim (2013) reported that MWI might enhance the humification of soil organic matter (SOM), which may contribute to successful soil and groundwater remediation. It was found that microwave pretreatment of compost suppressed the activity of hydrogen-consuming bacteria, which resulted in a high yield of hydrogen production (Song et al. 2012).

More importantly, Abbey et al. (2017) reported that MWI increased the nutrient density of vermicasts and enhanced yield and quality of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). Variables such as duration of exposure to MWI and moisture content of vermicasts may differentially influence the effect of the MWI when MWI power output level was varied. However, these effects were not reported by Abbey et al. (2017), and there is limited literature information available. The present study evaluated the individual and interaction effects of MWI power output levels, exposure time on the quality and efficacy of vermicasts with different moisture content. Also, the growth response of kale was assessed in greenhouse pot-experiments.

1.2 Thesis Objectives and Organization

The hypothesis of this project was that the quality of vermicasts will be influenced by MWI pretreatment, and kale plants growth and quality will be enhanced by microwaved vermicasts. The overall goal of this project was to evaluate the effects of MWI on the overall quality of vermicasts, and their impacts on kale plants growth. Three detailed experiments were conducted in this project. The specific objective of the first study focused on elucidating the MWI power output level effects on vermicasts quality indices, and response of kale plants growth. The aim was to find the optimal MWI power output level on vermicasts that show the best kale plants growth response to do the second study. The specific objective of the second study was to evaluate the interaction effects of MWI power output levels and exposure times on vermicasts quality indices, and the response of kale plants growth and quality. The aim was to find the optimal combination of MWI power output level and exposure time on vermicasts quality and kale plants growth response, which will be used to explore MWI effects on vermicasts with varying moisture content, and kale plants growth response in the third study. Finally, the third study's aim was to evaluate moisture content effects on quality indices of vermicasts following treatment with the selected optimal combination of MWI power output level and exposure time, and response of kale growth.

The thesis has been organized into six chapters. Chapter 1 is the introduction and divided into the general introduction of the background of this project and thesis objectives and organization. Chapter 2 is the literature review. It provides a literature review related to the definition, processing, properties, applications, and previous research of vermicasts and MWI. Chapter 3 is an original manuscript exploring the MWI power output level effects on vermicasts quality indices, and response of kale growth response and quality. The objectives of this study were addressed. Chapter 4 is also an original manuscript exploring interaction effects of MWI power output level and exposure time on vermicasts quality indices, and response of kale growth and quality. Similarly, Chapter 5 is to explore moisture content effects on quality indices of vermicasts after MWI treatment using the selected optimal combination of MWI power output level and exposure time, and response of kale growth and quality. Chapter 6 is the conclusion and recommendation for future research, followed by an appendix.

Chapter 2 Literature Review

2.1 Vermiculture and Vermicasting

Vermiculture is the raising of earthworms, i.e. controlled-environment rearing of worms in specialty structures. Vermicasting is a non-thermophilic process by which earthworms are used to change organic wastes into a humus-like material termed as vermicasts, a natural or organic amendment (Munroe 2007). The vermicasting process is low-input biotechnology, which does not require mechanical ventilation or mixing. Comparatively, earthworms take about 22-32 days to turn organic wastes into vermicasts depending on micro-environment for the earthworm (Chaoui 2010) as compared to thermophilic composting that takes about 3-4 months and additional curing time (Chaoui 2010). During the vermicasting process, organic waste is ingested, digested and excreted from the earthworm (Pattnaik and Reddy 2010). Vermicasts is the final product of vermicasting obtained through the interaction of both earthworms and various types of microorganisms in their guts. Vermicomposts are the mixture of decomposing vegetable or food wastes, bedding materials, and vermicasts.

Overall, there are two different stages regarding earthworm activity in vermicasting as shown in Fig. 2.1. These are: (1) Gut-associated processes (GAPs) - earthworms process the organic substrate by changing its physical state, and microbial composition; and (2) Cast-associated processes (CAPs) – micro-organisms decompose the processed substrate by the earthworm (Gómez-Brandón et al. 2013; Lores et al. 2006).

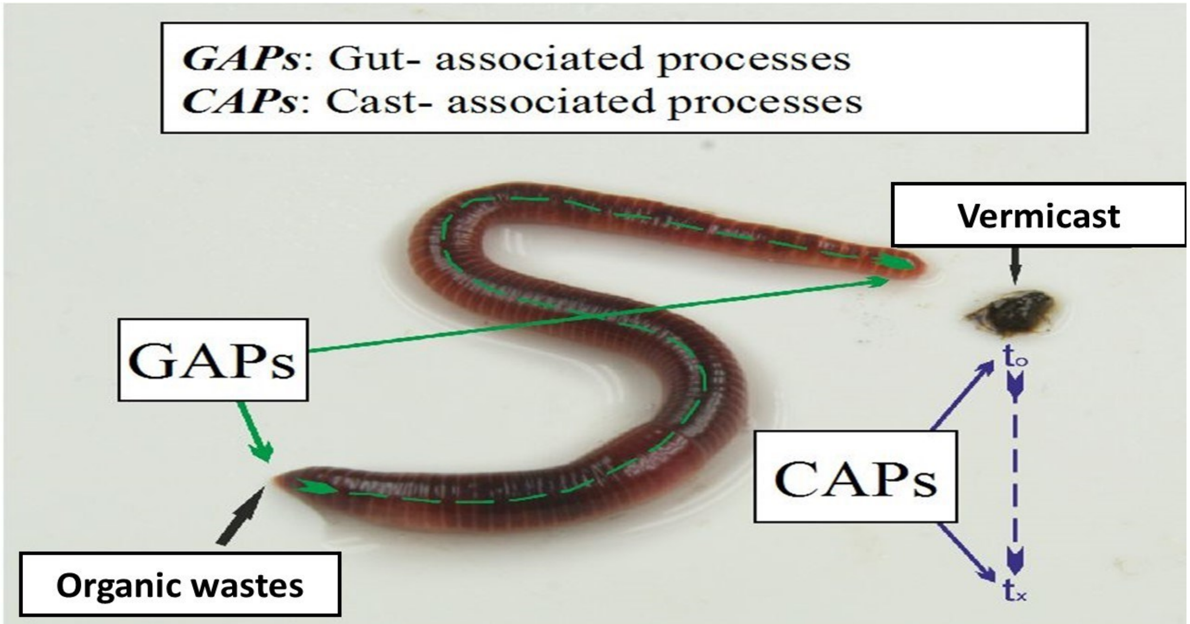

Fig. 2.1. The two process of vermicasting (source: Gómez-Brandón et al. 2013).

2.1.1 Worm biology

There are over 1000 species of earthworms worldwide, but the three most common species of earthworms in Canada are the African night crawlers (*Eudrilus eugeniae*), Red wigglers (*Eisenia foetida*), and Indian blue worms (*Perionyx excavatus*) (Pattnaik and Reddy 2010). The Indian blue worms have the highest rate of growth and productivity compared to the African night crawlers or the Red wigglers (Pattnaik and Reddy 2010). However, the African night crawlers have the most efficient ability of returning bioconversion of urban green waste into nutrient-rich and microbiologically-active vermicasts; the vermicasts produced by the African night crawlers include higher calcium (Ca), phosphorus (P), potassium (K), nitrogen (N) and magnesium (Mg) compared to the other two species (Pattnaik and Reddy 2010).

2.1.2 Vermicasts compared to thermophilic compost and synthetic fertilizer

Vermicasts are nutrient-rich, microbiologically active, and environmentally friendly natural growing medium amendment used in agriculture (Chaoui 2010). Thermophilic compost is a kind of compost by breaking down biological waste with thermophilic bacteria. Synthetic fertilizer usually derived from by-products of the petroleum industry. Vermicasts have high social acceptability and high economic value as a soil conditioner for plant growth compared to thermophilic compost and synthetic fertilizer (Chaoui 2010; Dominguez et al. 1997; Pattnaik and Reddy 2010) because vermicasts contain higher contents and more soluble mineral nutrients (i.e. macro-nutrients and micro-nutrients) than thermophilic composts and synthetic fertilizer. Vermicasts have been shown to improve plants resistance to many diseases, increase yield, and protein content of plants as compared to other synthetic chemical fertilizers (Chaoui 2010). As such, vermicasts provide 30-40% higher yield of crops and reduce crop water requirement by 30-40% over synthetic chemical fertilizers (Sinha et al. 2010).

2.2 Properties of Vermicasts

The physical and chemical properties of vermicasts are dependent on the material digested by the diverse species of earthworms. Thus, vermicasts from similar feedstock origins have similar properties (Tomati et al. 1983). Vermicasts are well known to enhance soil physicochemical and biological properties. Sinha et al. (2010) observed that due to the properties of vermicasts, i.e. physical (structure), chemical (essential plants nutrients), and microbiological (beneficial soil microbes) qualities of soil were remarkably improved when vermicasts were added to the soil.

Vermicasts may have effects on plant growth directly or indirectly (Fig. 2.2). As regards the direct effects on plant growth, vermicompost constitutes a source of plant macro- and micronutrients (Lazcano and Domínguez 2011). These nutrients are present in inorganic forms and are readily available to plants (Lazcano and Domínguez 2011). However, in the indirect mechanisms, vermicasts could inhibit plant disease development (Lazcano and Domínguez 2011). Edwards et al. (2006) observed that the suppressive effect exerted by several types of vermicasts on several plant pathogens such as *Pythium*, *Rhizoctonia*, *Verticillium*, and *Plectosporium*, disappeared after sterilization of the vermicompost, and concluded that disease suppression may be related to the presence of biological suppressive agents in vermicasts.

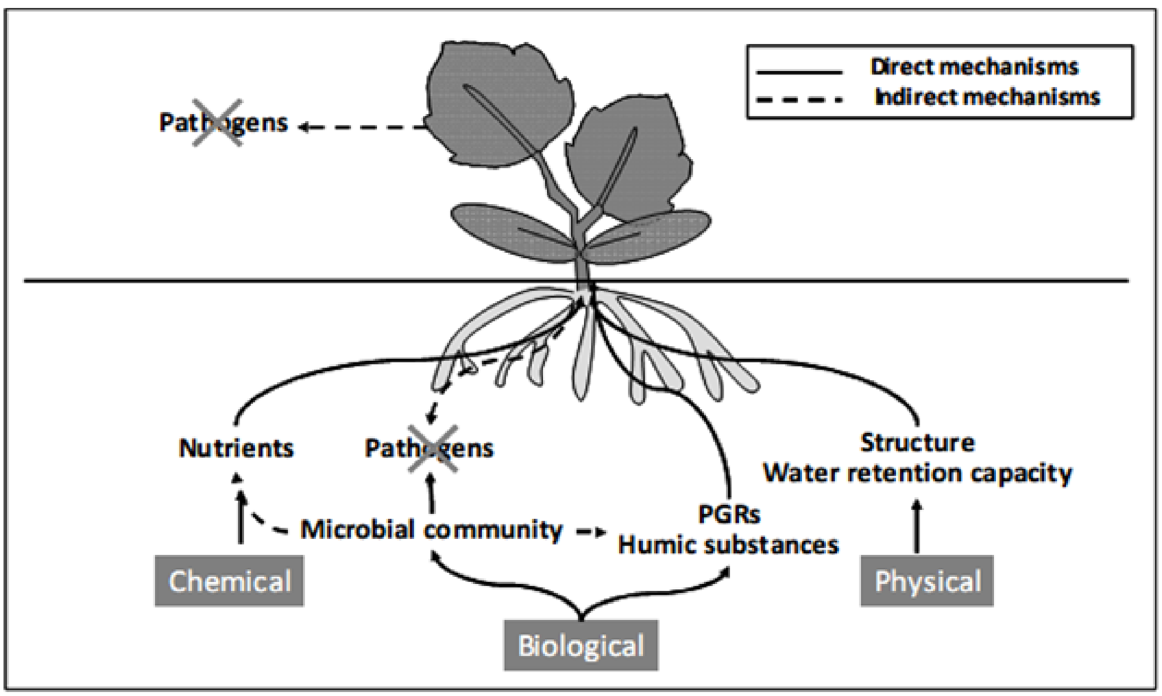

Fig. 2.2. The influence of plants by vermicasts (source: Lazcano and Domínguez 2011).

2.2.1 Physical properties

Vermicasts are nontoxic, and are characterized by finely divided peat-like material with excellent and stable structure (Sinha et al. 2010). Vermicasts have remarkably high porosity for good aeration, drainage and high moisture-holding capacity (Dominguez et al. 1997). Freshly harvested vermicasts granules are usually moist and break easily as compared to granules of synthetic chemical fertilizers that are adapted to the mechanized application (Abbey et al. 2013). The bulk density and moisture content of vermicasts varied in addition to unknown shelf-life, these deter its large-scale production, processing, transportation and use when freshly harvested (Abbey et al. 2013). Hence, dehydration was seen as a convenient way of large-scale vermicasts application.

2.2.2 Chemical properties

Vermicasts contain high amounts of plant-available and long-term reserved nutrients, which include nitrogen (N), available phosphorus (P), exchangeable potassium (K), calcium (Ca), and magnesium (Mg) (Sinha et al. 2009). These nutrients were found in significantly higher amounts than those in thermophilic composts (Sinha et al. 2009). However, the proportion of the essential nutrients was found to be primarily influenced by the different species of earthworms used for vermicomposting (Pattnaik and Reddy 2010). The temperature of vermicasts ranged from 22.3 to 29.8; the pH ranged from 6.3 to 7.4; the electrical conductivity (EC) was from 152.2 to 3354.4 S/cm, and moisture content was from 35 to 65%, which resulted in high microbial activity (Pattnaik and Reddy 2010).

2.2.3 Microbiological properties

There is not much detailed documented research on the study of microbiological properties of vermicasts. Pattnaik and Reddy (2010) reported that the guts of worms are inhabited by some beneficial micro-organisms that could convert insoluble N, available P, exchangeable K, Ca, and Mg into soluble forms for plants uptake. According to Sinha et al. (2010), vermicasts have many useful micro-organisms such as actinomycetes, phosphate solubilizing, nitrogen-fixing bacteria and mycorrhizal fungi that increased biological resistance in plants. These protect the plants against pests and diseases and improve the biological properties and total microbial counts of the growing medium when vermicasts were added to the soil.

2.3 Vermicasts Applications and Benefits

By virtue of their many properties, vermicasts granules retain and slowly release nutrients and other biomolecules such as free amino acids to plants, and they also protect crops from pests and diseases (Munroe 2007; Sinha et al. 2010). Vermiwash is the body fluids produced during vermicomposting and from the production of vermicasts tea, i.e. a fermented solution of vermicomposts produced in water. Vermiwash is known to be highly effective ‘bio-pesticides’ with good control of crop pests and diseases (Munroe 2007). Nath and Singh (2012) reported that the growth and productivity of paddy rice (*Oryza sativa*), maize (*Zea mays*) and millet (*Pennisetum typhoides*) crops were significantly increased by vermiwash treatment. French dwarf bean (*Phaseolus vulgaris*) treated with vermiwash had the tallest plants, most extended pods, the highest number of pods per plant and the highest number of lateral branches (Ayyobi et al. 2014).

2.3.1 Vermicasts efficacy as the soil amendment

The use of vermicasts as natural soil amendments is increasing due to the benefits from its physical, chemical and microbiological properties that protects and enhances soil health and the overall agro-ecosystem in addition to improving food quality (Abbey et al. 2017, 2018; Arancon et al. 2006). Compared to synthetic chemical fertilizers, vermicasts are rich in humus, N, P, K, Ca, Mg and micronutrients, amino acids, beneficial soil microbes that may restore and improve soil fertility and reduce soil degradation (Sinha et al. 2010). Vermicasts also contain beneficial soil microbes, polysaccharides, proteins and other nitrogenous compounds that may boost crop productivity when added to soil or soilless growing medium (Sinha et al. 2010). Arancon et al. (2006) reported that soil microbial biomass was significantly increased with a single application of vermicompost when growing strawberries (*Fragaria ananassa,* var. Chandler) as compared to the application of synthetic chemical fertilizer. Also, Aira et al. (2010) observed that vermicompost was shown to strongly influence rhizosphere microbial communities, which increased the growth of rhizosphere microbial biomass of bacteria and fungi in maize (*Zea mays*). Microbes are essential for exchanging nutrients into their plant available forms and also for facilitating nutrients uptake by plants (Arancon et al. 2006).

Application of vermicasts increased SOM content, i.e. soil carbon content to a more sustainable level above 3 - 5% and improved soil fertility (Munroe 2007). SOM acts as a ‘glue’ that binds soil particles into aggregates, thus improving soil structure, infiltration, porosity, water, and nutrients retention and release capacity of the vermicasts amended soil. As SOM decomposes over time, it results in the development of a more stable carbon compound called humus, which is essential for plant growth (Munroe 2007). The best percentage of vermicasts as the growing medium to grow plants is between 10 to 40% (Arancon et al. 2004).

2.3.2 Effect on plants growth and yield

Vermicasts promote plant growth and increase yield of a wide range of plants species such as peppers (*Capsicum annum* L. var. California) (Arancon et al. 2004); brinjal (*Solanum melongena*) (Gajalakshmi and Abbasi 2004); garlic (*Allium sativum* L.) (Argüello et al. 2006); sweet corn (*Zea mays*) (Lazcano et al. 2011); and kale (*Brassica oleracea* L. var. *acephala* (DC) Alef.) (Iheshiulo et al. 2017). The functions of humus include supporting plants to absorb nutrients from the soil; increased mineralization of nutrients; promoting plant root growth; and improving plant tolerance to environmental stress (Sinha et al. 2010; Tomati et al. 1983). Vermicasts contain actinomycetes, and the actinomycetes mechanism of protecting the plants against pests and diseases is through repelling pests' activities (Arancon et al. 2004; Rodríguez-Navarro et al. 2000). According to Sinha et al. (2010), vermicasts consistently improved seed germination, seedling establishment and development, and increased plant productivity even when plants were already receiving optimal nutrition. Interestingly, vermicasts did not only increased the taste, quality, and shelf life of grapes but also boosted grape yield by two-fold as compared to synthetic chemical fertilizers (Buckerfield and Webster 1998).

2.3.3 Effect on harvest quality

Shankar and Sumathi (2008) reported significantly higher vitamin C content of selected vegetables such as spinach, tomato, turnip, cabbage and fruits of apple and pears were grown with vermicasts. They also reported that the total antioxidants, total carotene, iron (Fe), zinc

(Zn), crude fiber, and lycopene content of tomato plants grown with vermicasts were significantly higher than tomato plants grown in other sources of organic amendments.

The presence of antibiotics and actinomycetes in vermicasts increases biological resistance in plants to protect them against pests and diseases (Sinha et al. 2010). For example, Yardim et al. (2006) reported that the application of vermicasts reduced the damage caused by spotted cucumber beetle (*Diabotrica undecimpunctata*), striped cucumber beetle (*Acalymma vittatum*) in cucumber and larval hornworms (*Manduca quinquemaculata*) in tomatoes in both greenhouse and field experiments. Arancon et al. (2002) reported that vermicasts application to pepper (*Capsicum annuum*), cabbage (*Brassica oleracea*) and tomato (*Lycopersicum esculentum*) inhibited the infection of insect pests. Compared to synthetic chemical fertilizers, vermicasts increased the protein content of wheat grains by 12% (Palanisamy 1996). Strawberry fruit malformations were reduced from 12% to 4% in addition to significant reductions in grey mould and botrytis rot upon application of vermicasts (Bhat and Khambata 1959).

The conclusions from these studies were that the addition of vermicasts as natural growing medium amendment plays an essential role in plant growth and productivity. Vermicasts did not only increase the quantity of the food produced but also improve its nutritional quality. The study from Sinha et al. (2010) showed that vermicasts could produce nutritious and more flavorful food at economical costs because they promoted crop growth better than conventional compost by between 50 and 100%, and synthetic chemical fertilizers by between 30 and 40%.

2.4 Microwave Irradiation (MWI) Technology in Agriculture

Microwave irradiation (MWI) is a non-traditional heating method where microwaves heat objects uniformly and more quickly (Li et al. 2016). MWI is becoming popular and has long been used in many disciplines including food science, plant science, and environmental science (Abbey et al. 2017; Gibson et al. 1988). Many of the previous studies on microwaving demonstrated its potential impact on different substrates including composts and vermicasts. For example, MWI of soil increased the release of nutrients from soil microbial biomass (Hendricks and Pascoe 1988) and improved birch (*Betula pendula*) seedlings establishment and growth (Gibson et al. 1988). Furthermore, Hu and Wen (2008) showed that enzymatic hydrolysis ratio was significantly increased by using microwave pretreatment of switchgrass (*Panicum virgatum*), which was higher than using alkali. Zheng et al. (2015) showed that levoglucosan yield from fast pyrolysis of corncobs at MWI power output level 150 W for 18 min exposure was higher than the no microwave treatment.

2.4.1 Mechanism of operation of MWI

MWI uses an electromagnetic wave, which is between infrared radiation and radio waves in the electromagnetic spectrum (Li et al. 2016). Compared to traditional heating techniques such as hotplate heating, when biomass treated with MWI, heat is generated inside the biomass (Motevali et al. 2014). Hot plate is a simple portable appliance that has a flat heated surface for heating by transfer through the process of convection or radiation or conduction. The mechanism of MWI is the movement of molecules resulting in collision and friction between the molecules to produce thermal energy while conventional heating such as the hot plate (Motevali et al. 2014).

Gabhane et al. (2011) observed that lignocellulosic substrate pretreatment by MWI at 200°C (i.e. 700 W) had a better effect than hot-plate heating and sterilizer. Azuma et al. (1984) reported that furfural production was significantly increased following the application of MWI pretreatment of lignocellulosic wastes. As such, MWI technology has enormous benefits in biomass pretreatments such as high heating rate, thermal efficiency, and well-proportioned heating (Li et al. 2016). Unfortunately, MWI cannot be viewed as an ideal approach because it is still considered as a primary heating method, and it has some problems including the high cost of pretreatment, lack of large equipment while the non-thermal effect of the pretreatment is still understudied (Li et al. 2016). For these reasons, it is necessary to develop a high-efficiency microwave and scale up microwave pretreatment to meet large-scale treatment for different study areas.

2.4.2 Growing medium treatment with MWI

There are several related studies concerning MWI treatment of growing medium for plants. Gibson et al. (1988) reported that the growth of birch was enhanced by microwave treated soil and suggested that soil with MWI treatment could benefit the growth of birch seedlings since many of the harmful bacteria are heat-sensitive. Ferriss (1984) found that soil treated with MWI could improve the growth of both roots and shoots of sorghum x Sudan grass (*Sorghum × drummondii)* hybrids, and only enhanced the root growth of soybean (*Glycine max*) seedlings. In a related study on foods, a higher percentage of mineral nutrients in broccoli were preserved by microwave treatment compared to other cooking methods such as boiling (Schnepf and Driskell 1994). Song and Thornalley (2007) found that the glucosinolates content of broccoli (*Brassica oleracea* var. *italica*) was maintained after microwave cooking but was reduced by boiling due to the glucosinolates leaching into the cooking water. As well, the content of polyphenols and vitamin C contents of broccoli were reduced when boiled. Similar observations were made on growing media substrates. For instance, Song et al. (2012) found that microwave pretreatment of compost may suppress the activity of hydrogen-consuming bacteria and resulted in higher hydrogen production than compost without microwave pretreatment. Abbey et al. (2017) found that vermicasts treated at MWI power output level 400 W MWI for 5 min improved Chinese cabbage (*Brassica rapa* subsp. *Pekinensi*) growth more than vermicasts without MWI treatment. However, there is no report on the interaction effect of substrate (i.e. food, compost nor vermicasts) moisture content, microwave power output level and exposure time.

2.5 Conclusion

It is essential to use an appropriate substrate such as vermicasts to improve the growth and quality of plants in production systems. Vermicasts play essential roles in the growth, development, protection, and nutrition and functional properties of plants through the supply of enough water and nutrients to plants. Also, vermicasts are characterized by good drainage and high water-holding capacity. Furthermore, vermicasts promote oxygen dispersion to the roots and gaseous exchange between the inside and outside of plant roots due to its ability to improve porosity (Edwards and Burrows 1988; Vo and Wang 2014). According to the literature, microwaved vermicasts have potential to replace synthetic chemical fertilizers in promoting plant growth. However, further research into the detail physiological and metabolic mechanisms is necessary in order to improve consumer confidence in the use of this natural amendment (Lazcano and Domínguez 2011). As far as pretreatment with MWI is concerned, a better and comprehensive understanding of the interaction and relationship between microwave and biomass, especially for non-thermal effect is necessary (Li et al. 2016). Other studies showed that vermicasts treated at different MWI power output levels have different effects on plant growth of Chinese cabbage (Abbey et al. 2017) and dahlia (Cai et al. 2018). However, there is no study on microwaved vermicasts effect on kale. Moreover, there are no studies on the interaction effect of MWI power output level, exposure time and moisture content on vermicasts quality indices which are the focus of this thesis. Thus, it will be necessary to study the effects of MWI on the overall quality of vermicasts, and its impact on kale plant growth.

Chapter 3 Effects of Microwave Irradiation Power Output Level on Quality of Vermicasts, and Growth Response of Kale

3.1 Abstract

Vermicasts are rich in organic carbon compounds, enzymes, chemical nutrients and diverse beneficial microorganisms to enhance soil health and plants growth. Microwave irradiation (MWI) has many benefits to the agricultural food industry but under-exploited in farm settings. The present study focused on the effects of different MWI power output levels on vermicasts quality indices and response of kale plants growth. The treatments were MWI power output levels: (0, i.e. control, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 W) at an exposure time of 5 min per MWI treatment. The results indicated that the temperature and water loss from the fresh, moist vermicasts (FMV) increased with an increase in MWI power output level from 0 to 1000 W. Turbidity, pH, total dissolved solids (TDS), electrical conductivity (EC) and mineral nutrients of the vermicasts were significantly ($P<0.05$) affected differently. Overall, turbidity and pH value decreased while total dissolved solids, electric conductivity and mineral nutrients increased with an increase in MWI power output level. The 400 W treatment significantly ($P<0.05$) increased chlorophyll and anthocyanin contents, fresh weight and plant height of the kale plants compared to the treatments with other power output levels. Therefore, the present study demonstrated that the efficacy of vermicasts was enhanced by MWI power output level at 400 W and treatment time of 5 min. Also, the yield and quality of kale plants increased with the addition of vermicasts treated at 400 W.

Keywords: *microwave irradiation, vermicasts, kale, power output level, growing medium*

3.2 Introduction

Kale is a leafy vegetable, which is easy to grow in gardens or containers. It belongs to the *Brassicaceae* family (Neugart et al. 2012). The leaf tissue of kale contains high levels of health-promoting flavonoids (Hertog et al. 1992). However, there is increasing concerns about the potentially harmful chemicals residues in foods, especially leafy vegetables like kale due to an increasing use of synthetic chemical fertilizers and pesticides (Iheshiulo et al. 2017). Consumers prefer safe, organic food and are concerned about the sources of their foods and how they are grown. As a result, the demand for safe organic food is increasing due to pressure from environmental groups (Iheshiulo et al. 2017). Also, synthetic chemical fertilizers have a negative impact on soil quality and ecosystems (Iheshiulo et al. 2017; Suge et al. 2011).

Natural growing medium amendment includes rock powder, vermicasts, composts, green and animal manure, vermicompost, volcanic minerals, and their derivatives. Different natural growing medium amendments have different effects on growth and harvest quality of plants. For instance, the productivity of kale treated with dry vermicasts was higher compared to potassium humates and volcanic minerals (Iheshiulo et al. 2017). Vermicasts are nutrient-rich and microbiologically active, socially acceptable, and easy to produce (Chaoui 2010). Vermicasts also contain plant growth regulating substance such as auxins, gibberellins, and cytokinins that directly influence plant growth (Lazcano and Domínguez 2011). Consequently, vermicasts enhancement of soil health, plant growth, and bioactive compounds are well documented (Chaoui 2010; Lazcano and Domínguez 2011).

MWI technology has gained considerable attention in many areas in agriculture and industry since the advent of the commercial microwave oven in the 1970s (Li et al. 2016). Microwaves are electromagnetic waves of which the frequency is higher than ordinary radio waves but lower than infrared light (Li et al. 2016). The frequency used to display the different properties of various materials is most often 2.45 GHz. Microwave can pass through glass, plastic, and porcelain without being absorbed. However, when biomass or water is heated by MWI, the movement of molecules in the biomass or water results in the collision and friction between the molecules which manifests itself as heat (Galema 1997; Li et al. 2016). MWI may decrease the response time strikingly by making the product formation fast and accompanied by the fast reduction of the response activation energy (Li et al. 2016; Motevali et al. 2014). These advantages offer MWI considerable benefits in biomass pretreatment.

MWI was previously used to study the effect of enzymatic hydrolysis on rice straw (Zhu et al. 2005); the effect of cow dung compost on bio-hydrogen process from corn stalk by dark fermentation (Song et al. 2012); the effect of humification of SOM (Kim and Kim 2013); and more recently, the effect on vermicasts and growth of Chinese cabbage (*Brassica rapa* subsp. p*ekinensi*) (Abbey et al. 2017). Although the use of MWI remains underexplored, existing studies demonstrated that MWI technology could improve the efficiency of organic growing medium amendment. For instance, Kim and Kim (2013) reported that MWI could enhance the humification of SOM, which can contribute to successful soil and groundwater remediation. It was also found that microwave pretreatment of compost resulted in a high yield of hydrogen production (Song et al. 2012). More importantly, MWI was reported to have increased the nutrient density of vermicasts and enhanced yield and quality of Chinese cabbage (Abbey et al. 2017). Therefore, the MWI application of vermicasts may have a positive impact on plant growth. However, the studies on the effects of microwaved vermicasts on plant growth are still sparingly. Notably, there is no research report on the effect of microwaved vermicasts on kale plants.

The present study sought to determine the effects of different MWI power output levels on vermicasts quality indices and response of kale plants growth in pots. The objectives were to determine the effects of different MWI power output levels at a specific exposure time (5 min) on vermicasts quality indices including moisture loss, heat-load, chemical properties, and mineral nutrients and response of kale plant growth including plant height, fresh weight, and anthocyanin content and chlorophyll contents.

3.3 Materials and Methods

3.3.1 Location and materials

The experiment was performed in the greenhouse in the Department of Plant, Food, and Environmental Sciences, Faculty of Agriculture, Dalhousie University (45.37°N, 63.26°W) from July 2017 to October 2017. Organically-certified and film-coated seeds of kale 'Red Russian' (Ontario Seed Company Ltd., Waterloo, ON, Canada); FMV (Growing Green Earthworm Castings Inc., Wedgeport, NS, Canada) and Promix-BX™ potting medium (Premier Horticulture Inc., Quakertown, PA, USA) were purchased from local retailers in Truro, NS. A General Electric Microwave Oven (Model No. JES1295STC01, Mabe Canada Inc., Mississauga, ON, Canada) was used to irradiate the vermicasts granules.

3.3.2 Vermicasts preparation and MWI

One-hundred (100) g of the FMV (initial moisture content was about 64.5%) were weighed, transferred into a microwavable ceramic glass bowl and irradiated for 5 min at 10 different MWI power output levels: 0 (i.e. control, no treatment), 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 W. The control treatments were FMV without MWI and 100 g Promix-BX™ potting medium alone. As described by Hendricks and Pascoe (1988) and adopted by Abbey et al. (2017), the microwave equipment was paused half-way to the exposure time during heating, and the mass of vermicasts was stirred before restarting to minimize disruptive heat buildup in the vermicasts granules. Four-hundred (400) mL of distilled water were added to each 100 g portion of the microwaved vermicasts and vortexed for 10 min using an Isotemp stirring plate (model LT1892X1; Thermo Fisher Scientific Inc., Markham, ON, Canada). The mixtures were allowed to stand for 24 hrs prior to analysis.

3.3.3 Heat-load and water loss analyses

The heat-load of the mass of microwaved vermicasts granules was recorded using a glass thermometer (Fisher Scientific Company Ltd., Ottawa, ON, Canada) immediately after removal from the microwave oven at the end of the MWI treatment. Water loss was determined from the difference between the initial and final mass of vermicasts.

Water loss was calculated as:

[((initial weight-final weight) * 1000)/initial weight], and the unit is mg/g.

3.3.4 Chemical properties and mineral nutrients analyses

The turbidity of the individual solutions was determined using turbidity meter (Oakton Instruments, Vernon Hills, IL, USA). Electrical conductivity (EC), potential hydrogen content (pH), total dissolved solids (TDS) and salinity of the water extract were determined using Oakton multi-parameter PCTesterTM 35 (Oakton Instruments, Vernon Hills, IL, USA). Nitrate (N), potassium (P), sodium (Na), and calcium (Ca) in solutions were determined using LAQUA meters (Spectrum Technologies Inc., Aurora, IL, USA). All the determinations were measured in triplicates.

3.3.5 Plant growth response

Kale seeds were started in a Promix-BX™ potting medium contained in a 72 cell-tray without vermicasts amendment. Seedlings were transplanted into 6-inch diameter plastic pot containing 400 g of Promix-BX™ potting medium two weeks after germination. Each pot received two seedlings. One week later, one-hundred (100) g of microwaved vermicasts per treatment was prepared and kept at about 10°C for 24 hrs before incorporating into the Promix-BX™ potting medium. The kale plants were later thinned to one plant per pot two weeks after transplanting. All the plants were watered when needed. No additional nutrient supplement nor vermicasts were added.

Anthocyanin content of the kale leaves was determined using Anthocyanin Content meter (Opti-Sciences Inc., Hudson, NH, USA). Chlorophyll content of kale leaves was determined using SPAD 502 Chlorophyll meter (Spectrum Technologies Inc., Aurora, IL, USA). Plant height was measured from the tip of the longest leaf to the collar of the stem while the total number of green leaves and fresh weight were recorded at final harvest.

3.3.6 Experimental design and statistical analyses

The experiment treatments, i.e. MWI power output levels (0, i.e. control, no treatment, 100, 200, 300, 400,500, 600, 700, 800, 900, 1000 W) were arranged in a completely randomized design with five replications on a galvanized-steel bench in the greenhouse. The pots were rearranged biweekly on the bench to offset any random occurrence due to variations in the environment. Data collected were analyzed by one-way analyses of variance (ANOVA) using

Minitab version 18.1 (Minitab Inc., State College, PA, USA). Fisher's least significant difference (LSD) method was used to separate treatment means when the ANOVA indicated a significant difference at $P<0.05$. Microsoft Excel was used to plot graphs.

3.4 Results and Discussion

3.4.1 Effects of MWI on vermicasts quality

The heat-load as measured by the temperature of the microwaved FMV changed as the MWI power output levels increased from 0 to 1000 W as shown in Table 3.1. The heat-load of the microwaved FMV increased quickly from 29℃ to 86℃, as the MWI power output level increased from 100 W to 400 W. The principle of operation of MWI is based on activation and collision of water molecules in a microwaved material (Galema 1997; Li et al. 2016; Motevali et al. 2014), which generate intense heat within the structure of the microwaved material. This might be the cause of the dehydration of the vermicasts with MWI treatment. The water loss from the vermicasts continued to increase from 15.2 mg/g at MWI power output level 100 W to 118.9 mg/g at 400 W. Although the heat-load of the vermicasts increased rapidly with MWI power output level from 0 to 400 W, the dehydration of vermicasts increased slightly. The results partially confirmed the observation in the study on Chinese cabbage conducted by Abbey et al. (2017).

Unexpectedly, when the MWI power output level increased from 400 to 1000 W, the heat-load decreased from 86℃ to 68℃ (Table 3.1). Meanwhile, the water loss of vermicasts increased quickly from 118.9 mg/g to 472.9 mg/g. Ferriss (1984) clarified that the heat-load and heat capacity of the vermicasts mass were reliant on moisture content. Thus, an obviously increased dehydration led to less collision and friction between water molecules, and then generate less thermal energy. Meanwhile, increased dehydration absorbed a lot of heat. These reasons may explain the decrease of the heat-load as found in the present study.

Table 3.1. Microwave[a] irradiation (MWI) power output levels for the treatment of fresh, moist vermicasts (FMV) and the corresponding heat-load and rate of water loss recorded immediately after 5 min of exposure.

Treatment – Power output level (W)	Power output[b] (W)	% output energy	Heat-load (°C)	Water loss (mg/g)
0	0	0	25	0
100	100	10	29	15.2
200	200	20	61	30.4
300	300	30	82	43.3
400	400	40	86	118.9
500	500	50	84	188.8
600	600	60	81	271.4
700	700	70	76	354.1
800	800	80	76	400.8
900	900	90	70	422.9
1000	1000	100	68	472.9

[a] Total power output is 1000 W at power output level P1000.

[b] Specifications: power consumption, 120 V, 60 Hz and 1450 W; operation frequency, 2450 MHz, and power output, 1000 W equivalent to 60 dBmW and 300°C.

A downward trend was observed for both turbidity and pH as the MWI power output level increased (Fig. 3.1 and Fig. 3.2). Turbidity gradually increased with MWI power output level from 0 to 200 W, which then continued to decline to the lowest level (75.4 NTU) at MWI power output level 900 W (Fig. 3.1). The value of pH gradually decreased from MWI power output level 0 (pH = 6.41) to 200 (pH = 6.38) W, then followed by a steep rise to a peak at 300 W (pH = 6.47). There was a fluctuation in the decline of pH to the lowest level at 1000 W (Fig. 3.2). It was demonstrated that MWI helped in extracting and removing organic materials (Cid et al. 2001) and contaminants (Kawala and Atamanczuk 1998) from compost. Meanwhile, with the increase in MWI power output levels, organic materials charred, which might be the reason for the decline in turbidity of the extracted samples. The decrease in pH may be related to changes in the distribution of salts, extractives, acidic groups in hemicelluloses and soluble and insoluble organic acids in vermicasts by MWI treatment (Poonia and Tripathi 2017).

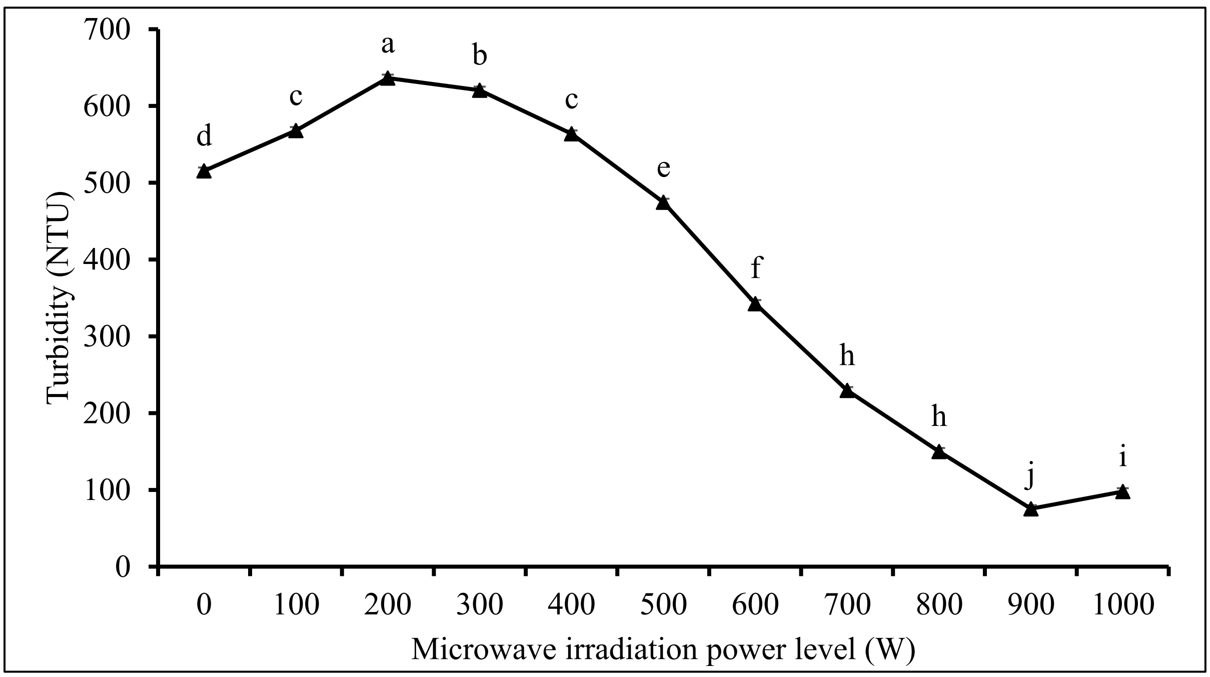

Fig. 3.1. Microwave irradiation (MWI) effect on turbidity of fresh, moist vermicasts (FMV) at different power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

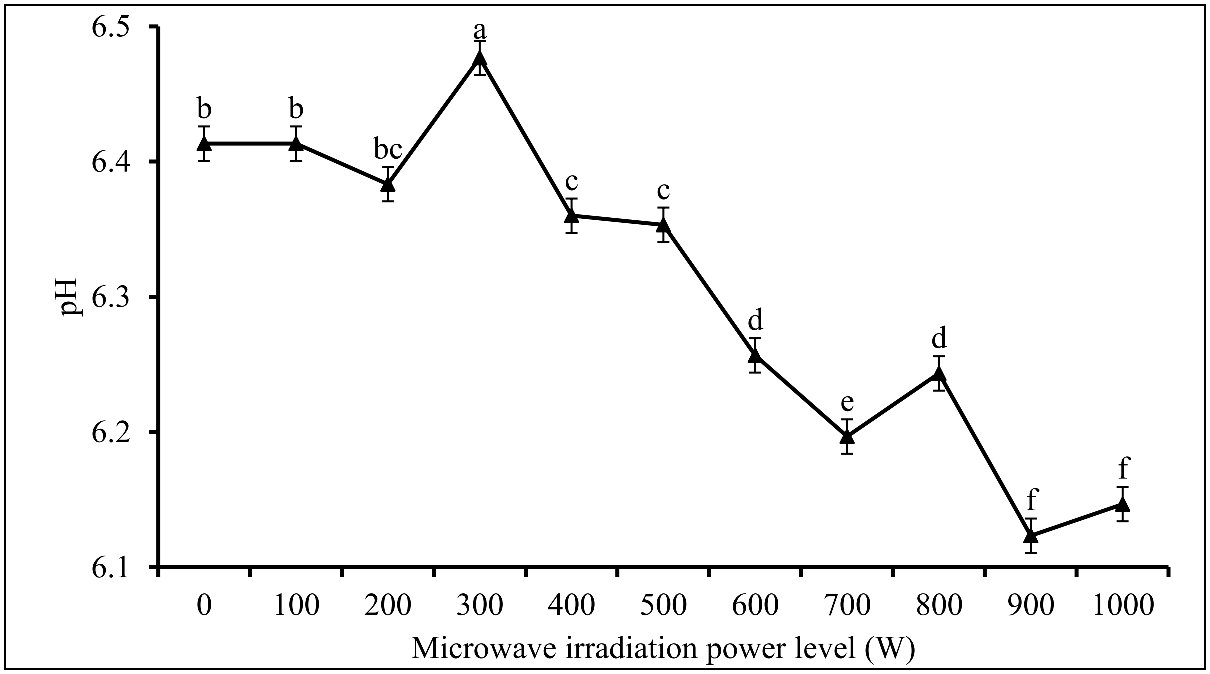

Fig. 3.2. Microwave irradiation (MWI) effect on pH of fresh, moist vermicasts (FMV) at different power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

Comparatively, the TDS and the EC showed a similar trend as the MWI power output level increased (Fig. 3.3 and Fig. 3.4). The values of the TDS and the EC gradually decreased from MWI power output level 0 to 300 W. The crystalline structure, and chemical properties of vermicasts may be changed by high temperature leading to a significant rise in volatilization and reductions in chemical activity and/or solubility (Abbey et al. 2017). The heat-load of the microwaved FMV increased quickly from 100 W (29°C) to 300 W (82°C). These may explain the reductions in TDS and EC values from 100 to 300 W. The main reason for the increased values of TDS and EC from above 300 to 1000 W microwave power output levels can be attributed to the heat-load which probably, increased the solubility of organic and inorganic materials such as proteins, carbohydrates, mineral nutrient and other microbial cellular materials (Abbey et al. 2017; Speir et al. 1986).

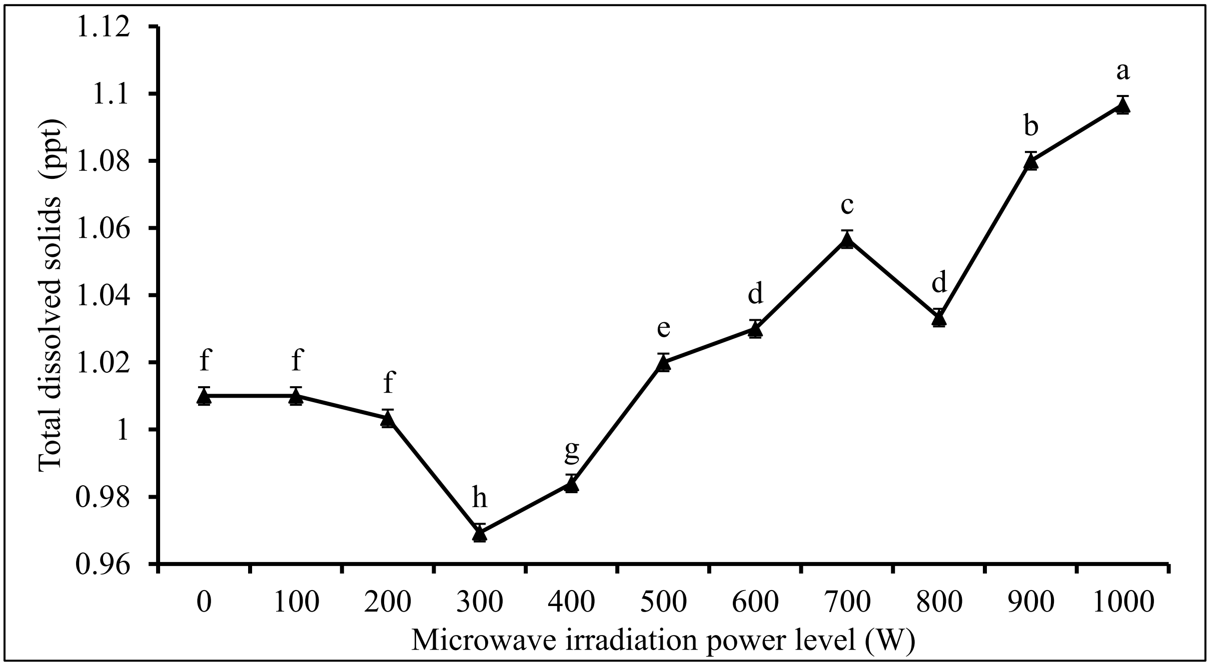

Fig. 3.3. Microwave irradiation (MWI) effect on total dissolved solids (TDS) properties of fresh, moist vermicasts (FMV) at different power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

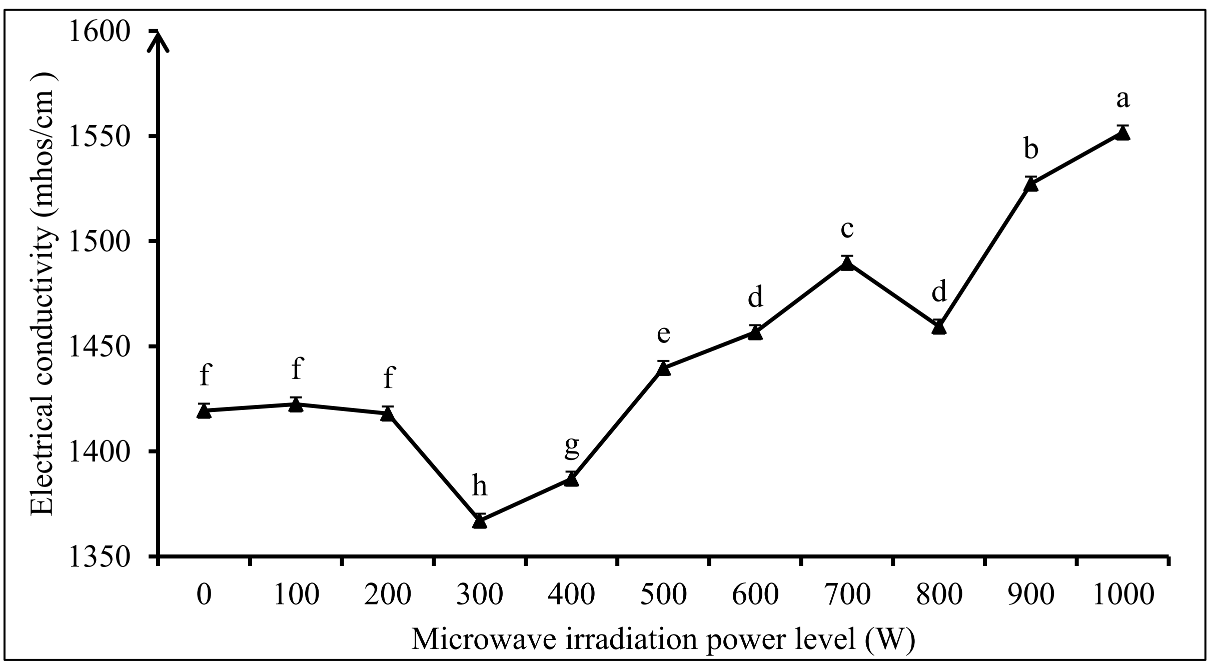

Fig. 3.4. Microwave irradiation (MWI) effect on electrical conductivity (EC) properties of fresh, moist vermicasts (FMV) at different power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

There were similar changes in the contents of N, K, Na and Ca in the vermicasts as MWI power output level increased (Fig. 3.5). Overall, the contents of N, K and Na were all increased as the MWI power output level increased from 0 to 100 W, followed by a sharp decline to the lowest level at 200 W. The content of Ca increased from MWI power output level 0 to 200 W, followed by a sharp decline to the least level at MWI power output level 300 W. As the MWI power output level increased from 300 to 1000 W, the contents of N, K, Na and Ca were all substantially increased.

Vermicasts are rich in humic substances, plant supplements, enzymes and different types of microorganisms (Abbey et al. 2017). Like soils, the physicochemical and microbial properties of the vermicasts may be influenced by MWI as it changed the crystalline structure of the vermicasts granules. The effects of thermal reactions on some essential plant mineral elements in the vermicasts varied according to MWI power output level. With the increase in MWI power output level from 300 to 1000 W, organic substances, for example, amino acids and carbohydrates may have been disrupted and broken down into their simpler, active and soluble forms as suggested by Abbey et al. (2017).

N is one of the essential nutrients for plant vegetative growth and productivity and a core component of many plant structures (Massignam et al. 2009; Ullah et al. 2010). Previous work showed that MWI could cause damage to microbial cells leading to leakage of cell materials including nutrients (Song et al. 2012; Spier et al. 1986). As such, the high MWI power output level may have disrupted microbial cell membranes in the vermicasts that resulted in the increases in some of the nutrients such as N, K, Na and Ca in the microwaved vermicasts.

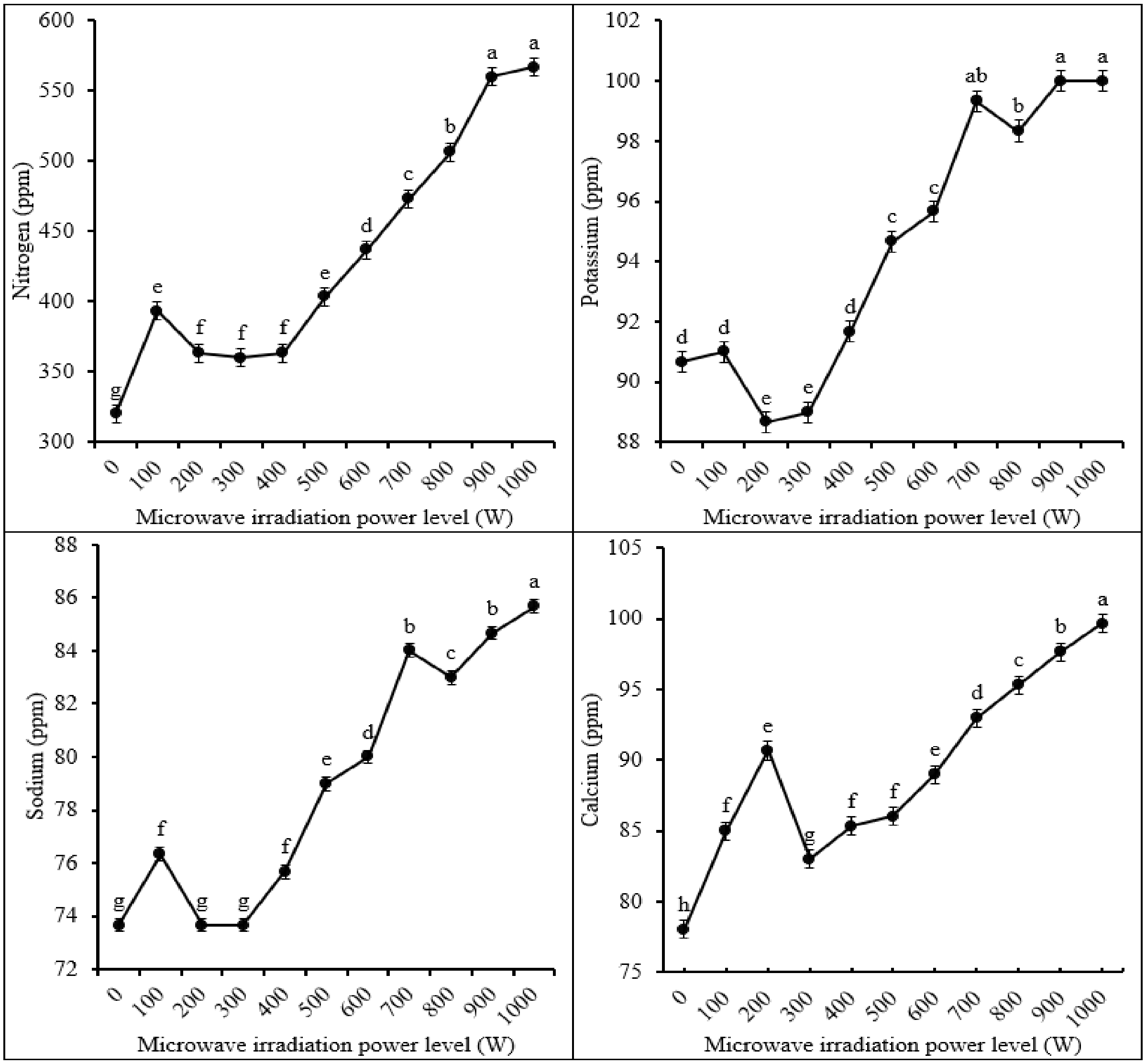

Fig. 3.5. Microwave irradiation (MWI) effect on mineral nutrient contents of fresh, moist vermicasts (FMV) at different power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

3.4.2 MWI effects of vermicasts on kale plants growth response

Overall, growth parameters and yield of kale plants significantly ($P<0.05$) responded positively to the microwaved vermicasts. The chlorophyll content of kale plants treated with microwaved vermicasts were all significantly higher than kale plant grown in Promix-BX™ alone, and the chlorophyll content of kale plants applied with vermicasts treated at 400 W was the highest as compared to the other treatments (Fig. 3.6). The results partially confirmed the results of the study on Chinese cabbage by Abbey et al. (2017) that Chinese cabbage applied with vermicasts treated at MWI power output level 400 W showed the highest chlorophyll content. However, the difference in plant species probably led to the different response observed in different MWI power output levels situations.

Compared to other treatments, the nutrients content of microwaved vermicasts at 400 W was not the highest, but the chlorophyll content of kale plants grown in this vermicasts was the highest. This suggested that the various substances of microwaved vermicasts at MWI power output level 400 W were more balanced and available for plant use as compared to the other treatments.

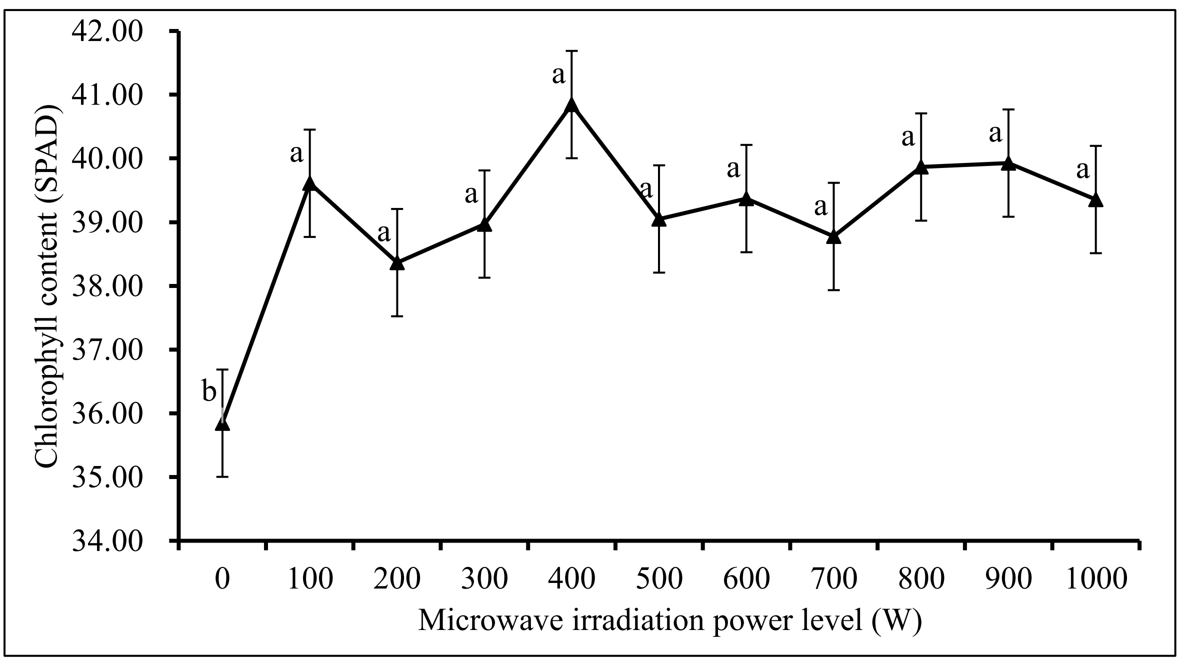

Fig. 3.6. The chlorophyll content of kale leaf as affected by different microwave irradiation (MWI) power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 5 replications.

The anthocyanin content of kale plants was increased as the MWI power level treatment of the vermicasts increased from 0 to 400 W followed by a steep decrease at 600 W, and another increment from 600 W to 700 W (Fig. 3.7). The anthocyanin content of kale plants did not change much from 700 to 1000 W. However, anthocyanin content of the kale plants applied with vermicasts treated at 400 W and 700 W were significantly (P<0.05) higher than the other treatments. Thus, those kale plants applied with vermicasts treated at MWI power output level 400 W had the highest anthocyanin content of 6.90 ACI, and that of the 0 W treatment was the least. The reason may be due to balanced vermicasts nutrients and more nutrients availability at the MWI power output level 400 W as compared to the other treatments.

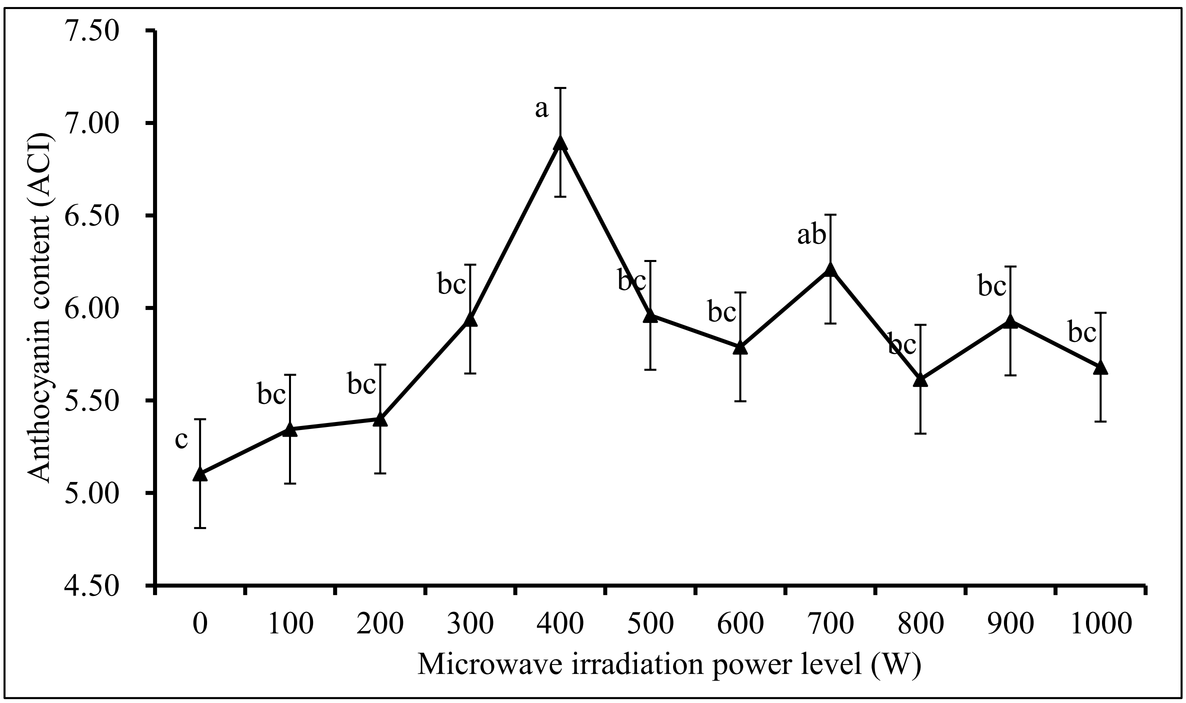

Fig. 3.7. The anthocyanin content of kale leaf as affected by different microwave irradiation (MWI) power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 5 replications.

The fresh weight of kale plants was also increased by the treatment of vermicasts increased from 0 to 400 W, followed a decrease to 800 W and increased at 900 W (Fig. 3.8). The fresh weight of kale plants applied with vermicasts treated at 100, 200, 300, 400, 500 and 900 W were significantly ($P<0.05$) higher than other treatments. However, the fresh weight of kale plants applied with vermicasts treated at 400 W was still the highest. This result was also similar to the results of Abbey et al. (2017) who reported that MWI power output level 400 W produced the highest fresh weight of Chinese cabbage. The increased fresh weight of kale plants applied with vermicasts treated at MWI power output level 400 W might be attributed to increased chlorophyll and anthocyanin contents in the leaf tissues.

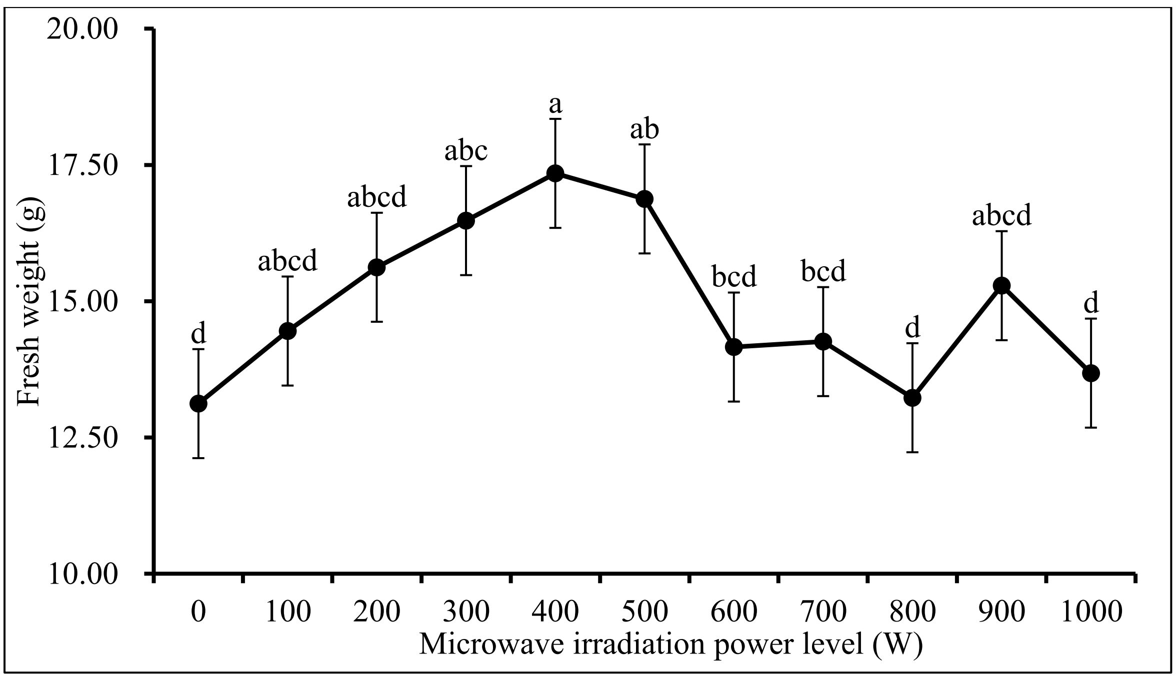

Fig. 3.8. The fresh weight (g) of kale leaf as affected by different microwave irradiation (MWI) power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 5 replications.

The final height of kale plants was still increased by the treatment of vermicasts increased from 0 to 400 W, then decreased to the lowest level at 700 W and increased to 1000 W (Fig. 3.9). Similarly, the plant height of kale plants applied with vermicasts treated at 400 W was the highest. This result was still similar to the results of Abbey et al. (2017) that the MWI power output level 400 W treatments produced the highest height of Chinese cabbage. The increased plant height of kale plants applied with vermicasts treated at MWI power output level 400 W might also be attributed to increased chlorophyll and anthocyanin contents in the leaf tissues.

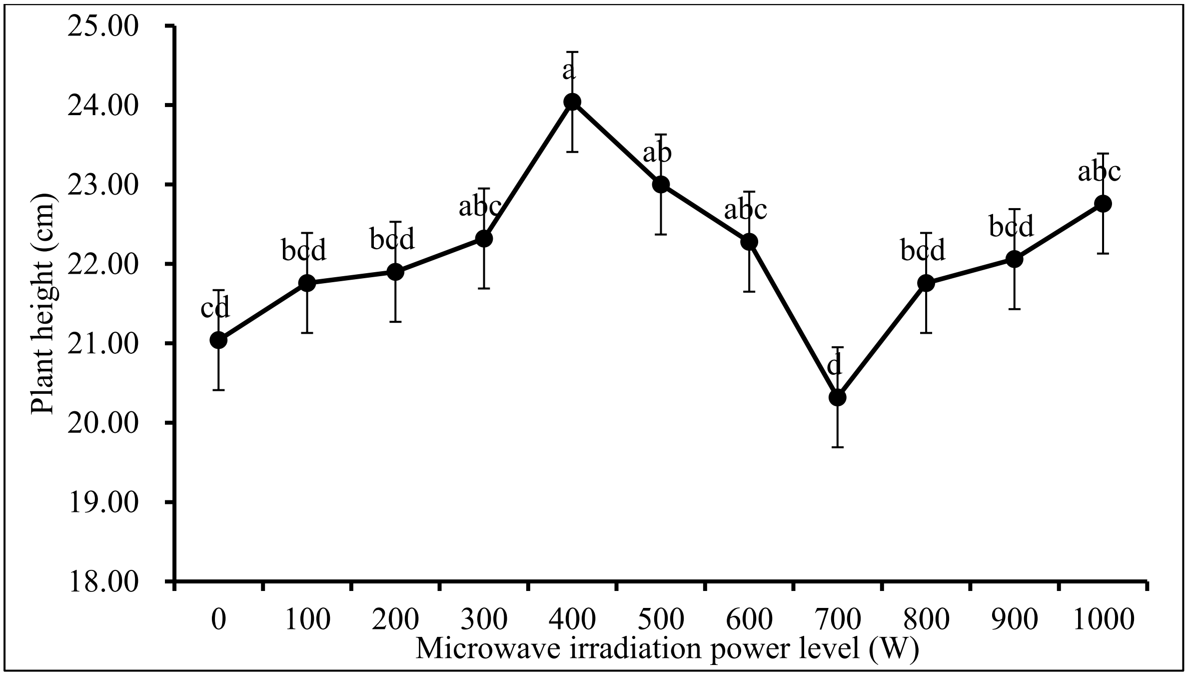

Fig. 3.9. The plant height (cm) of kale leaf affected by different microwave irradiation (MWI) power output levels. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 5 replications.

3.5 Conclusion

Vermicasts treated at MWI power output level 400 W consistently provided the best overall conditions to support kale plant growth as compared to the control group Promix-BX™ amendment and other MWI power output level treatments. Chlorophyll content is significant; it has an essential relationship with photosynthetic capacity, leaf tissue N and plant health status. Anthocyanin is very important for the plants as anthocyanin can increase the content of flavonoid in leaf tissue and promote photosynthesis of leaves. Kale plants grew with vermicasts treated at MWI power output level 400 W had the highest chlorophyll content, anthocyanin content, fresh weight, and plant height. In this research, it seemed microwaved vermicasts have potential to replace synthetic fertilizers in promoting plant growth. Microwaved vermicasts offer a unique opportunity for kale production. Vermicasts treated at MWI power output level 400 W seemed to have provided the best medium nutrient balance leading to improved leaf tissue chlorophyll and anthocyanin contents, and other factors for improved plant growth. This study only worked to find the effects of MWI power output level irradiation on quality of vermicasts, and growth of kale in a stationary time (5 min). Further research will be required in order to study the interaction effects of power output level and exposure time on vermicasts quality indices, and the response of kale growth and quality.

Chapter 4 Power Output Level and Exposure Time of Microwave Irradiation Effects on Quality of Vermicasts, and Growth Response of Kale

4.1 Abstract

The application of microwave irradiation (MWI) technique has gained considerable attention in agriculture due to its unique properties and many benefits in biomass pretreatment. The present study evaluated the MWI power output level and exposure time effects on the potency of vermicasts, and its influence on kale plant growth and nutrient components. Fresh, moist vermicasts (FMV) were irradiated at 0, 2.5, 5, and 10 min at two MWI power output levels: 400 and 1000 W and were tested on the growth of kale (*Brassica oleracea* var. *sabellica*) plants. The quality of vermicasts was significantly ($P<0.05$) influenced by MWI power output level and exposure time. Total dissolved solids, electric conductivity, salinity and nutrient contents of microwaved vermicasts were significantly ($P<0.05$) increased at both power output levels 400 and 1000 W at 10 min. The microwaved vermicasts had higher potency than non-treated vermicasts. Kale plants treated at 400 W for 2.5 min had the highest chlorophyll content, fresh weight, plant height, dry weight, crude protein, crude fiber, fat, ash, iron, and manganese contents. The present study demonstrated that MWI power output level 400 W at 2.5 min exposure time might be the optimal treatments for vermicasts and the growth of kale plants.

Keywords: *microwave irradiation, microwave exposure time, vermicasts, kale, plants growth, plant nutrients, organic amendment*

4.2 Introduction

MWI is electromagnetic radiation at specific frequencies that interact with materials containing water such as soil, composts and vermicasts to produce heat inside the materials (Motevali et al. 2014). MWI is different compared to traditional heating such as a hot plate. The mechanism of MWI is the movement of molecules that results in the collision and friction between molecules, which then produces thermal energy (Motevali et al. 2014). The frequency of MWI is very important. Low frequency (i.e. < 2.45 GHz) does not cause heat because of the less time for water molecules to reorient (Chevalier 2009).

As a non-traditional heating method, MWI technology has gained considerable attention in agriculture, domestic, industrial and medical applications (Remya and Lin 2011). Compared to the conventional heating methods, MWI has some advantages such as higher heating rates, greater control of the heating process. It is also characterized by selective heating, high-energy efficiency and reduced equipment size (Jones et al. 2002). MWI has been used widely in the field of environmental engineering and agriculture in recent years due to its distinctive advantages (Jones et al. 2002; Remya and Lin 2011).

MWI power output level, exposure time and nature of the material (i.e. matrix, mass and volume) are the three main parameters that may impact the outcome from microwave heating (Villar et al. 2007). Many of the previous studies on MWI demonstrated its potential impact on different substrates including food, composts and vermicasts. Hu and Wen (2008) showed that enzymatic hydrolysis ratio was significantly increased by using microwave irradiated alkali on switchgrass (*Panicum virgatum*), which was higher than that not using alkali. MWI has the potential to regulate harmful microorganisms such as fungi and nematodes (Islam and

Weil 1998). Song et al. (2002) reported that cow dung compost treated with MWI produced more bio-hydrogen than non-treated cow dung compost. Pang et al. (2012) investigated the effects of MWI power output level and exposure time on corn (*Zea mays*) stovers. They reported that as MWI power output level and exposure time increased, the release of carbohydrates from the corn stover was enhanced. Mohamadi et al. (2013) assessed two parameters, i.e. the exposure intensity and duration on rose-oil extraction. They concluded that the combination of 650 W and 15 min treatment time produced the optimal extraction. Zheng et al. (2015) showed that levoglucosan yield from fast pyrolysis of corncobs at 150 W microwave power output for 18 min was higher than that with no microwave treatment. Recently, Abbey et al. (2017) found that MWI power output level at 400 W for 5 min exposure time improved vermicasts mineral elements, and the antioxidant activity and the physical properties of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). However, Abbey et al. (2017) did not examine the effect of the exposure time.

The advantages of MWI technology to plants are partially documented (Abbey et al. 2017; Gibson et al. 1988). Iheshiulo et al. (2017) reported that the yield of kale treated with dry vermicasts was increased compared to potassium humates and volcanic minerals. However, there was no research on the interaction effects of various MWI power output level and exposure time on vermicasts quality indices, and the response of plants growth and quality. As such, the impacts of MWI power output level and exposure time interaction on vermicasts quality indices and how the microwaved vermicasts influences plant growth would have to be investigated.

The objectives of the present study were to determine the interaction effects of MWI power output levels and exposure time on vermicasts quality indices (i.e. moisture loss, heat-load, chemical properties), and to assess the growth and yield performance of kale plants.

4.3 Materials and Methods

4.3.1 Location and materials

The study was carried out from November 2017 to February 2017 in the greenhouse in the Department of Plant, Food, and Environmental Sciences (45.37°N, 63.26°W). Organically certified and film-coated seeds of kale 'Red Russian'; FMV and Promix-BX™ potting medium were purchased from local retailers as described in Section 3.3.1 of Chapter 3. The General Electric Microwave Oven was also the same one as described in Section 3.3.1 of Chapter 3.

4.2.2 Vermicasts preparation and MWI

One-hundred (100) g of the FMV (initial moisture content was about 64.5%) were weighed, and transferred into a microwavable ceramic glass bowl and then irradiated at four different exposure times: 0, 2.5, 5, and 10 min at two different MWI power output levels: 400 W and 1000 W. The control treatment was 100 g Promix-BX™ potting medium. The microwave procedure and preparation of water extracts were the same as described in Section 3.3.2 of Chapter 3.

4.3.3 Heat-load, water loss, and chemical properties analyses

The heat-load, water loss and chemical properties include potential hydrogen content (pH), turbidity, electric conductivity (EC), total dissolved solids (TDS) and salinity in solution of microwaved vermicasts granules were recorded using the same materials and procedure as described in Section 3.3.3 and 3.3.4 of Chapter 3. All the determinations were done in triplicates.

4.3.4 Plant growth response, nutrients and proximate analyses

Transplants were produced using the same materials and procedure as described in Section 3.3.5 of Chapter 3. Chlorophyll content of kale leaf was determined using SPAD 502 Chlorophyll meter (Spectrum Technologies Inc., Aurora, Illinois, USA). Plant height was measured from the top of the longest leaf to the collar of the stem with a meter rule, and the total number of green leaves and fresh weight were recorded at final harvest. Kale leaves were sent out to the Central Testing Laboratory Ltd. (Winnipeg, Manitoba, Canada) for essential nutrients and proximate analysis. The method of analysis included Association of Official Analytical Chemists, i.e. 968.08, 990.03, 922.02, 923.03, 935.13A, and 985.01 and American Oil Chemist Society Am 5-04, and Ba6a-05.

4.3.5 Experimental design and statistical analyses

The experiment was a factorial experiment (two factors). The pots of kale plants were relocated biweekly on the bench to offset any random occurrence due to variations in the environment. Data collected were subjected to one-way analysis of variance (ANOVA) using Minitab version 18.1 (Minitab Inc., State College, PA, USA). Fisher's least significant difference (LSD) test was used for mean comparison, and the significant difference was set at $P<0.05$. Microsoft Excel was used to plot graphs.

4.4 Results and Discussion

4.4.1 MWI and exposure time effects on vermicasts quality

Water loss of the microwaved FMV increased with an increase in exposure time from 0 to 10 min at both MWI power output levels 400 W and 1000 W (Table 4.1).

At MWI power output level 400 W, heat-load of the microwaved FMV increased with an increase in exposure time from 0 to 10 min. However, heat-load of the microwaved FMV increased with an increase in exposure time from 0 to 5 min, followed by a decrease from 5 to 10 min (Table 4.1).

The heat-load and heat capacity of the vermicasts mass are reliant on moisture content (Ferriss 1984). The activation and collision of water molecules in a microwaved material is the principle of operation of microwaves (Galema 1997; Li et al. 2016; Motevali et al. 2014). Thus, the reason for the reduction in heat-load of the MWI vermicasts at power output level 1000 W from exposure time 5 min to 10 min may be attributed to the higher water loss leading to less collision of water molecules, and increased dehydration absorbed a lot of heat (Table 4.1).

Table 4.1. Fresh, moist vermicasts (FMV) treated at different microwave irradiation (MWI) power output levels and exposure time, and the corresponding temperature and rate of water loss recorded immediately after irradiation.

Treatment – Power output level (W)	Exposure time (min)	Heat-load (°C)	Water loss (mg/g)
400	0	25.0	0
400	2.5	66.5	20.3
400	5	78.0	117.3
400	10	80.5	263.5
1000	0	25.0	0
1000	2.5	84.0	212.4

Treatment – Power output level (W)	Exposure time (min)	Heat-load (°C)	Water loss (mg/g)
1000	5	88.0	473.2
1000	10	63.5	617.2

Turbidity and pH values of microwaved FMV were highly significant (P<0.001) affected by MWI power output level and exposure time (Table 4.2). Overall, at both MWI power output level 400 and 1000 W, pH increased when the exposure time increased from 0 to 2.5 min but decreased gradually as the exposure time increased to 10 min.

At MWI power output level 400 W, turbidity decreased as the exposure time increased from 0 to 2.5 min, then increased a slightly from exposure time 2.5 to 5 min. Finally, the least turbidity was recorded at exposure time 10 min. At MWI power output level 1000 W, turbidity decreased from exposure time 2.5 to 10 min at both 400 W and 1000 W power output level. The higher MWI power output level may disrupt organic materials in vermicasts, which might be the reasons of decline in turbidity (Abbey et al. 2017).

For the interaction effects, the pH of FMV treated at 400 W for 2.5 and 5 minis were highly significant (P<0.001) than the other treatments (Table 4.2). The pH of vermicasts treated at 400 W for 2.5 min was the highest while vermicasts treated at 400 W for 0 min had the lowest pH value. The turbidity of FMV treated at 400 W for 0 min as the highest and FMV treated at 1000 W for 10 min was the lowest.

Table 4.2. Turbidity and pH of fresh, moist vermicasts (FMV) at different microwave irradiation (MWI) power output level and exposure time.

Treatment	pH	Turbidity (NTU)
Power output level (W)		
400	7.09a	33.54a
1000	6.83b	18.14b

Treatment	pH	Turbidity (NTU)
Exposure time (min)		
0	6.76c	54.87a
2.5	7.08a	13.65c
5	7.01b	28.13b
10	6.97b	6.70d
Power output level * Exposure time		
400 * 0	6.76d	54.87a
400 * 2.5	7.27a	19.06c
400 * 5	7.22a	50.63b
400 * 10	7.09b	9.59d
1000 * 0	6.76d	54.87a
1000 * 2.5	6.94c	8.25de
1000 * 5	6.77d	5.63ef
1000 * 10	6.86c	3.80f
Significance level (P-value)		
Power output level	＜0.001	＜0.001
Exposure time	＜0.001	＜0.001
Power output level * Exposure time	＜0.001	＜0.001

[a] Means with different letters are significantly different at the 0.001 levels.

Overall, EC, TDS, and salinity in the samples of microwaved vermicasts were also highly significantly (P<0.001) affected by MWI power output level and the exposure time (Table 4.3). The EC, TDS, and salinity were higher at 1000 W than vermicasts treated at 400 W. As the exposure time kept increasing, the EC, TDS, and salinity were also increased for both 400 and 1000 W. At 1000 W, the EC, TDS, and salinity increased as exposure time increased from 0 to 5 min, followed by a drop from 5 to 10 min. However, for the interaction effects, FMV treated at 1000 W for 5 min had the significant (P<0.001) highest EC, TDS, and salinity and FMV treated at 400 W for 2.5 min were the lowest.

The possible reason for this observation could be thermal decomposition of materials such as nitrates, proteins, lipids and carbohydrates in the vermicasts (Abbey et al. 2017) as the heat-load (temperature) rose (Table 4.3), which resulted in high TDS, EC and salinity. The reason may when FMV treated at 400 W, heat-load, EC, TDS, and salinity of were all increased with increasing exposure time. Similarly, heat-load, EC, TDS, and salinity of FMV treated at 1000 W were increased from exposure time 2.5 to 5 min, and then decreased from 5 to 10 min.

Table 4.3. Microwave irradiation (MWI) and exposure time effects of fresh, moist vermicasts (FMV) on electrical conductivity (EC), total dissolved solids (TDS), and salinity.

Treatment	Electrical conductivity (S/cm)	Total dissolved solids (ppm)	Salinity (ppm)
Power output level (W)			
P400	957.00b	679.58b	400.00b
P1000	1020.33a	724.58a	426.83a
Exposure time (min)			
0	928.00d	658.67d	387.33d
2.5	976.67c	694.17c	409.00c
5	1018.50b	723.00b	425.83b
10	1031.50a	732.50a	431.50a
Power output level * Exposure time			
400 * 0	928.00e	658.67e	387.33e
400 * 2.5	918.33f	653.00f	384.67e
400 * 5	957.33d	679.67d	399.67d
400 * 10	1024.33c	727.00c	428.33c
1000 * 0	928.00e	658.67e	387.33e
1000 * 2.5	1035.00b	735.33b	433.33b
1000 * 5	1079.67a	766.33a	452.00a
1000 * 10	1038.67b	738.00b	434.67b
Significance level (P-value)			
Power output level	<0.001	<0.001	<0.001
Exposure time	<0.001	<0.001	<0.001
Power output level * Exposure time	<0.001	<0.001	<0.001

[a] Means with different letters are significantly different at the 0.001 levels.

4.4.2 MWI and exposure time effects of vermicasts on kale plants growth response

Growth parameters and yield of kale plants that received treated vermicasts at 400 W were significantly ($P<0.05$) higher than those parameters recorded at 1000 W (Table 4.4). This finding partially confirmed the results from Chapter 3 and Abbey et al. (2017). In these reports, plant height, and fresh weight yield were significantly ($P<0.05$) higher at 400 W among all the MWI power output level treatments. At both 400 and 1000 W, kale plants grown with microwaved vermicasts treated at 400 W or 1000 W for either 2.5 or 5 min had significantly ($P<0.05$) higher plant height, fresh weight and dry weight as compared to the 0 and the 10 min treatments (Table 4.4). Leaf tissue content of chlorophyll was highest at exposure time 2.5 min as compared to the 0, 5, and 10 min exposure time.

Apart from chlorophyll content, there was no significant ($P>0.05$) difference in plant height, total plant fresh weight and total plant dry weight between exposure times 2.5 and 5 min at MWI power output level 400 or 1000 W (Table 4.4). The chlorophyll content was increased as exposure time increased from 0 to 2.5 min and reached the highest at 2.5 min. This was followed by a decrease at exposure time 10 min. The plant height and fresh weight were increased as exposure time increased from 0 to 5 min, then dropped at exposure time 10 min.

Overall, there were no significant ($P>0.05$) interaction effects of MWI power output level and exposure time on growth and quality of kale plants. However, from the results, it showed that kale plants treated by microwaved vermicasts at 400 for 2.5 min had higher chlorophyll content, plant height, fresh weight, and dry weight. Considering economic and environmental protection benefits, it can be suggested that vermicasts under the treatment of MWI power output level 400 W for 2.5 min might be optimal for kale plants growth and yield.

Table 4.4. Microwave irradiation (MWI) and exposure time effects of fresh, moist vermicasts (FMV) on chlorophyll, plant height, fresh weight and dry weight of kale.

Treatment	Chlorophyll content (SPAD value)	Plant height (cm/plant)	Fresh weight (g/plant)	Dry weight (g/plant)
Power output level (W)				
400	43.09a	21.00a	7.28a	0.91a
1000	42.42b	20.46b	6.95a	0.85b
Exposure time (min)				
0	40.08d	19.80b	6.11c	0.76b
2.5	46.09a	21.61a	7.73a	0.97a
5	43.31b	21.63a	7.75a	0.95a
10	41.53c	19.87b	6.86b	0.84b
Power output level * Exposure time				
400 * 0	40.08a	19.80a	6.11a	0.76a
400 * 2.5	46.43a	21.87a	7.90a	0.97a
400 * 5	44.04a	22.12a	7.84a	1.01a
400 * 10	41.78a	20.20a	7.27a	0.91a
1000 * 0	40.08a	19.80a	6.11a	0.76a
1000 * 2.5	45.75a	21.35a	7.56a	0.96a
1000 * 5	42.58a	21.15a	7.67a	0.90a
1000 * 10	41.27a	19.53a	6.46a	0.77a
Significance level (P-value)				
Power output level	0.049	0.041	0.121	0.045
Exposure time	$<$0.001	$<$0.001	$<$0.001	$<$0.001
Power output level * Exposure time	0.473	0.598	0.566	0.312

[a] Means with different letters are significantly different at the 0.001 levels.

Crude protein, crude fiber, fat and ash content of kale at MWI power output level 400 W was higher than at 1000 W. These quality parameters were all increased at exposure time 2.5 min, followed by a decrease at 5 min and slightly increasing at 10 min (Table 4.5). Apart from the ash content, the 0 min followed by the 5 min exposure time recorded the least crude protein, crude fiber and fat contents. Vermicasts treated at exposure time 2.5 min at MWI power output level 400 or 1000 W consistently enhanced kale crude protein, crude fiber, fat and ash contents.

Table 4.5. Microwave irradiation (MWI) and exposure time effects of fresh, moist vermicasts (FMV) on the crude protein, crude fiber, fat and ash content of kale leaves.

Treatment	Crude Protein (%)	Crude Fiber (%)	Fat (%)	Ash (%)
Power output level (W)				
400	8.91	6.56	3.04	12.13
1000	8.86	6.41	2.95	12.00
Exposure time (min)				
0	8.38	6.00	2.85	11.96
2.5	9.57	7.16	3.14	12.41
5	8.63	6.21	2.99	11.81
10	8.96	6.57	3.02	12.07
Power output level * Exposure time				
P400 * 0	8.38	6.00	2.85	11.96
P400 * 2.5	9.47	7.25	3.19	12.6
P400 * 5	8.84	5.8	3.11	11.63
P400 * 10	8.94	7.17	3.02	12.32
P1000 * 0	8.38	6.00	2.85	11.96
P1000 * 2.5	9.66	7.06	3.08	12.22
P1000 * 5	8.41	6.62	2.87	11.98
P1000 * 10	8.95	5.97	3.01	11.82

Kale leaf tissue content of copper, iron, manganese and zinc followed similar trend following MWI power output level at 400 W and 1000 W, with values for the 1000 W treatment being higher than 400 W (Table 4.6). As the exposure time increased, the trends of copper and zinc contents became similar but were decreased gradually from exposure time 0 to 10 min. Iron and manganese showed a remarkable increase as exposure time increased from 0 to 2.5 min, followed by a reduction at 5 min and an increase at 10 min.

Table 4.6. Microwave irradiation (MWI) and exposure time effects of fresh, moist vermicasts (FMV) on the copper, iron, manganese and zinc content of kale leaves.

Treatment	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Power output level (W)				
400	4.41	55.43	94.47	44.30
1000	4.96	145.32	101.73	45.13
Exposure time (min)				
0	4.96	63.92	96.35	46.27
2.5	4.74	204.99	104.51	45.34
5	4.57	58.25	80.15	44.57
10	4.48	74.34	111.38	42.69
Power output level * Exposure time				
P400 * 0	4.96	67.12	96.35	46.27
P400 * 2.5	4.24	43.03	80.70	44.78
P400 * 5	4.13	60.38	79.92	42.93
P400 * 10	4.29	51.20	120.91	43.22
P1000 * 0	4.96	67.12	96.35	46.27
P1000 * 2.5	5.23	366.94	128.32	45.89
P1000 * 5	5.00	56.12	80.37	46.20
P1000 * 10	4.66	97.48	101.38	42.15

Apart from potassium content, the calcium, magnesium, and sodium content were all decreased from 0 to 5 min, then increased from 5 to 10 min under treatment at both MWI power output level 400 and 1000 W (Fig. 4.1). The potassium content was increased as exposure time increased from 0 to 2.5 min and arrived at the summit at 5 min, followed by a decrease to 10 min.

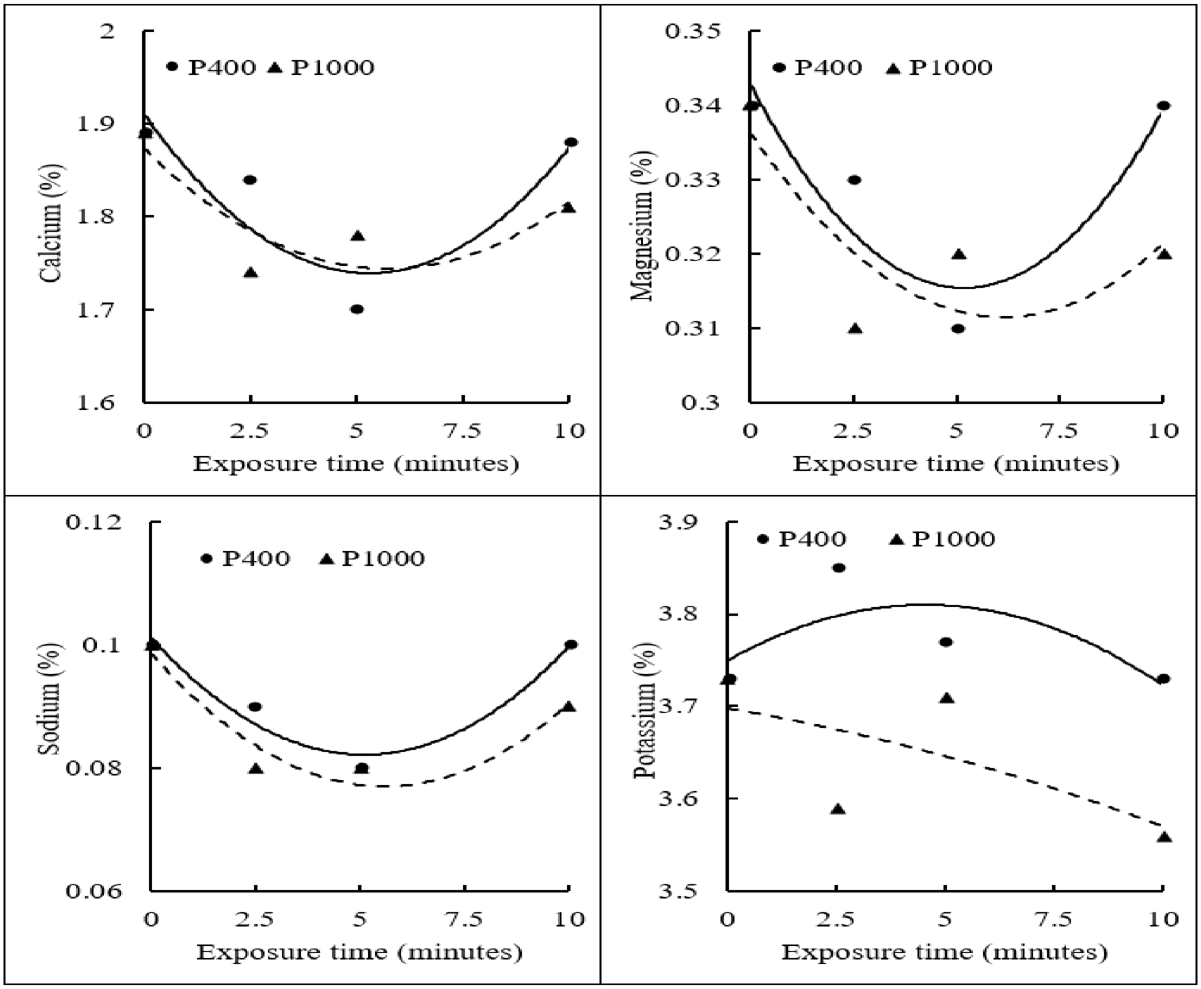

Fig. 4.1. Microwave irradiation (MWI) and exposure time effects of fresh, moist vermicasts (FMV) on calcium, magnesium, sodium and potassium content of kale leaves.

Microwaved vermicasts at either 400 W or 1000 W for 2.5 min seemed to have increased root production compared to the other treatments' combinations (Fig. 4.2). It was observed that kale plants grown with microwaved vermicasts at 400 W for 2.5 min had the most root mass compared to the 1000 W treatment (Fig. 4.2). Similarly, kale under treatment 1000 W at exposure time 2.5 min also produced more root mass compared to the other exposure times.

Fig. 4.2. Microwave irradiation (MWI) power output level and exposure time effects of fresh, moist vermicasts (FMV) on root mass of kale plants.

4.5 Conclusion

Overall, microwaved vermicasts have the potential in promoting plant growth and quality. Microwaved vermicasts have higher potency than non-treated vermicasts. The findings suggested that vermicasts microwaved at 400 W for 2.5 min produced kale plants with higher chlorophyll content, fresh weight, plant height, and dry weight. It also produced kale plants with higher nutrient contents and proximate composition such as potassium, iron, manganese, crude protein, crude fiber, fat, and ash. Hendricks and Pascoe (1988) reported that microwaved soil generated more N and P when 300 g samples were heated at 700 W for 20 min. Similarly, MWI increased the nutrients content of vermicasts, which enhanced the growth and quality of the kale plants. This study confirmed that microwaved vermicasts have the potential to enhance growing medium properties and to promote plant growth and mineral nutrients composition. However, there are some limitations to this experiment, considering that this experiment was a pot-experiment performed in the greenhouse. Some nutrients are released slowly, and plants may contain unconfirmed growth regulatory hormones later in the growing season, that might have influenced plant growth in a long-term. This will require further investigation. Additionally, the moisture content of vermicasts is an essential parameter in MWI treatment research. Therefore, further studies should focus on the effects of moisture content of vermicasts under MWI treatment.

Chapter 5 Microwave Irradiation Effects on Quality of Vermicasts at Different Moisture Content, and Growth Response of Kale

5.1 Abstract

Vermicasts production and utilization in agriculture and environmental sciences are receiving increasing research attention. Moisture content (MC) is essential for vermicasts. Many research studies have confirmed benefits of microwave irradiation (MWI) of vermicasts. The present study evaluated the moisture content effects on quality indices of vermicasts after MWI treatment, and to assess the growth and yield performance of kale plants. Different MC vermicasts (i.e. 35%, 45%, 55%, 65%, 75%, and 85%) were irradiated MWI power output levels 400 for 5 min and were tested on kale (*Brassica oleracea* var. s*abellica*) plants. The quality of vermicasts was significantly affected by MWI power output level 400 W at varying moisture content. Kale plants treated with 85% moisture content microwaved vermicasts have significantly ($P<0.05$) increased chlorophyll content, anthocyanin content, chlorophyll fluorescence indices (Fv/Fo and Fv/Fm), plant height and fresh weight. The results indicated that microwaved vermicasts have the potential to improve the growth response of kale plants. Meanwhile, microwaved vermicasts with higher moisture content enhanced kale plants growth compared with microwaved vermicasts with low moisture content. Further studies should focus on large-scale field study and response of diverse species of plants.

Keywords: *microwave irradiation, vermicasts, kale, moisture content, moisture content*

5.2 Introduction

Vermicasts production and utilization in agriculture and environmental sciences are receiving increasing research attention. Vermicasts as organic soil amendment are becoming the most preferable choice because of the low technological input, low-cost, higher mineral nutrients composition and presence of plant growth factors compared to aerated or anaerobic compost (Chaoui 2010; Dominguez et al. 1997; Pattnaik and Reddy 2010). Iheshiulo et al. (2017) reported that the yield and quality of kale were improved following treatment with dry vermicasts as compared to potassium humates and volcanic minerals.

Moisture content is essential for microbial activities and decomposition of organic matter into compost or worm ingestion to produce vermicasts (Liang et al. 2003). Some investigators have conducted experiments and identify that 50–60% moisture content is suitable for efficient composting (Suler and Finstein 1977; Tiquia et al. 1998). Takahashi et al. (2016) reported that the moisture content of compost strongly affects the bioavailability of phosphorus (P) and P mineralization of poultry manure compost was enhanced at higher moisture contents. Liang et al. (2003) proved that temperature and moisture content are dominant factors impacting aerobic microbial activity in compost. The composition and density of microbes are dramatically influenced by temperature, the activity of the microbial community reduced when the temperature is more than 60°C (Miller 1993).

In recent years, the use of MWI technology is becoming widespread in many agricultural applications because of its high heating efficiency, fastness and ease of operation. Villar et al. (2007) reported that extraction volume, MWI power output level and exposure time are the three main parameters that may impact the outcome of microwave heating. Many research studies have confirmed benefits of MWI of biomass, soil, compost and vermicasts. These studies revealed microwave enhancement potential and positive impact on different substrates including growing media. Some of these previous microwave studies were MWI power output level at specific exposure time on rice straw and its enzymic hydrolysis (Zhu et al. 2005); bio-hydrogen production of cow dung compost (Song et al. 2012); extraction of essential oil from rose flowers (Mohamadi et al. 2013); and nutrient density in vermicasts and growth performance of plant (Abbey et al. 2017). Additionally, more recent works on microwaved vermicasts as reported in Chapters 3 and 4 (not yet published) further confirms microwave enhancement of vermicasts efficacy. However, none of these previous and recent works reported the effects of MWI on vermicasts containing varying amounts of moisture content. Therefore, in the present study, the best MWI power output level of 400 W and exposure time of 2.5 min as recommended in Chapters 3 and 4 were adopted as the MWI conditions for treating vermicasts containing varying amounts of moisture content in this study.

Thus, the objectives of the present work were to evaluate moisture content effects on quality indices of vermicasts after MWI treatment using the selected optimal combination of MWI power output level and exposure time, and to assess the growth and yield performance of kale plants.

5.3 Materials and Methods

5.3.1 Location and materials

The study was carried out from March 2018 to June 2018. The location, equipment and materials used were the same as described in Chapter 3.

5.3.2 Vermicasts preparation and MWI

Fresh, moist vermicast (FMV) (initial moisture content was about 64.5%) were placed in a plastic box and air-dried under room temperature (22°C) and relative humidity (78%) conditions for 3 days to a moisture content of about 28%. Distilled water was added using a sterilized tablespoon to adjust the moisture content of the vermicasts to varying levels as follows: 35%, 45%, 55%, 65%, 75% and 85%. The mixture was mixed thoroughly and allowed to stabilize under room temperature and relative humidity conditions for 24 hrs prior to MWI treatment. One-hundred (100) g samples of the individual treatments were weighed and transferred into a microwavable ceramic glass bowl and irradiated at MWI power output level 400 W for 2.5 min. After MWI and cooling to room temperature, 400 ml of distilled water was added to each 100-g portion of the microwaved vermicasts and vortexed for 10 min using an Isotemp stirring plate (model LT1892X1; Thermo Fisher Scientific Inc., Markham, ON, Canada). The mixture was allowed to stand for 1 hr for the particles to settle. The control treatment was 100 g Promix-BX™ potting medium.

5.3.3 Heat-load, water loss and chemical analyses

The heat-load, water loss and chemical properties of microwaved vermicasts granules were recorded using the same materials and procedure as described in Section 3.3.3 of Chapter 3. All the determinations were done in triplicates.

5.3.4 Chemical properties and mineral nutrients analyses

The measurement of (pH), turbidity, electric conductivity (EC), total dissolved solids (TDS) and salinity in solution were determined using the same materials and procedure as described in Section 3.3.4 of Chapter 3. All the determinations were done in triplicates.

5.3.5 Plant growth response and analyses

Transplants were produced using the same materials and procedure as described in Chapter 3. Anthocyanin content, chlorophyll content, plant height, fresh weight, the total number of kale leaves were determined using the same tools and methods as described in Section 3.3.5 Chapter 3 as well.

5.3.6 Chlorophyll fluorescence activities

A portable OS30p+ Chlorophyll fluorometer (Opti-Science Inc. NH, USA) was used to measure chlorophyll fluorescence indices. The middle portion of the 3rd and 4th youngest leaves were attached with dark adaptation (light exclusion) clip with the 3-mm diameter window closed for 25 min to reach a steady-state between photochemical and non-photochemical quenching (*Maxwell* and *Johnson*, 2000). The measured indices were maximum quantum yield (Fv/Fm) calculated from the ratio of variable fluorescence (Fv = Fm - Fo) to maximum fluorescence (Fm) at the dark-adapted state with the application of saturating flash of light; and potential photosynthetic capacity (Fv/Fo) calculated from the ratio of Fv to minimum fluorescence (Fo) at the steady state immediately after the clipped portion of the leaf was exposed to weak modulated light. The dark adaptation phase was characterized by maximum photochemical efficiency and minimum heat dissipation, which provided a baseline for Fm (Garousi et al. 2016; Maxwell and Johnson 2000).

5.3.7 Experimental design and statistical analyses

The experiment was a one-factor experiment that was arranged in a complete randomized design with five replications on a galvanized-steel bench in the greenhouse. The potted kale plants were relocated biweekly on the bench to offset any random occurrence due to variations in the environment. Data collected were subjected to one-way analyses of variance (ANOVA) using Minitab version 18.1 (Minitab Inc., State College, PA, USA). Fisher's least significant difference (LSD) test was used for mean comparison, and the significant difference was set at $P<0.05$. Microsoft Excel was used to plot graphs.

5.4 Results and Discussion

5.4.1 MC effects on vermicasts quality under MWI treatment

The heat-load (temperature) of microwaved vermicasts increased with increasing moisture content (MC) from 75.2ºC for the 35% MC treatment to 86.8ºC for the 65% MC treatment before decreasing from to 55.3℃ for the 85% MC (Table 5.1). The water loss of microwaved vermicasts increased with increasing MC from 35% to 85% (Table 5.1).

Ferriss (1984) explained heat-load of microwaved soil was dependent on its MC. The results showed that there is a range of moisture content for the vermicasts that the heat-load increased with the increased MC under the same microwave treatment condition. However, the heat-load decreased when the moisture content of vermicasts was higher than the critical threshold level, which could possibly be between 55 – 65% MC.

Table 5.1. Heat-load and water loss characterizing microwaved vermicasts at different moisture content.

Moisture content (%)	Heat-load (℃)	Water loss (mg/g)
35	75.3	32.6
45	80.0	29.4
55	85.0	24.1
65	86.8	22.7
75	64.5	17.9
85	55.3	8.6

Overall, the pH of microwaved vermicasts was increased from 35% to 85% (Fig. 5.1). The turbidity of different moisture content vermicasts was increased from 35% to 75%, followed by a steep decline at 85% (Fig. 5.2).

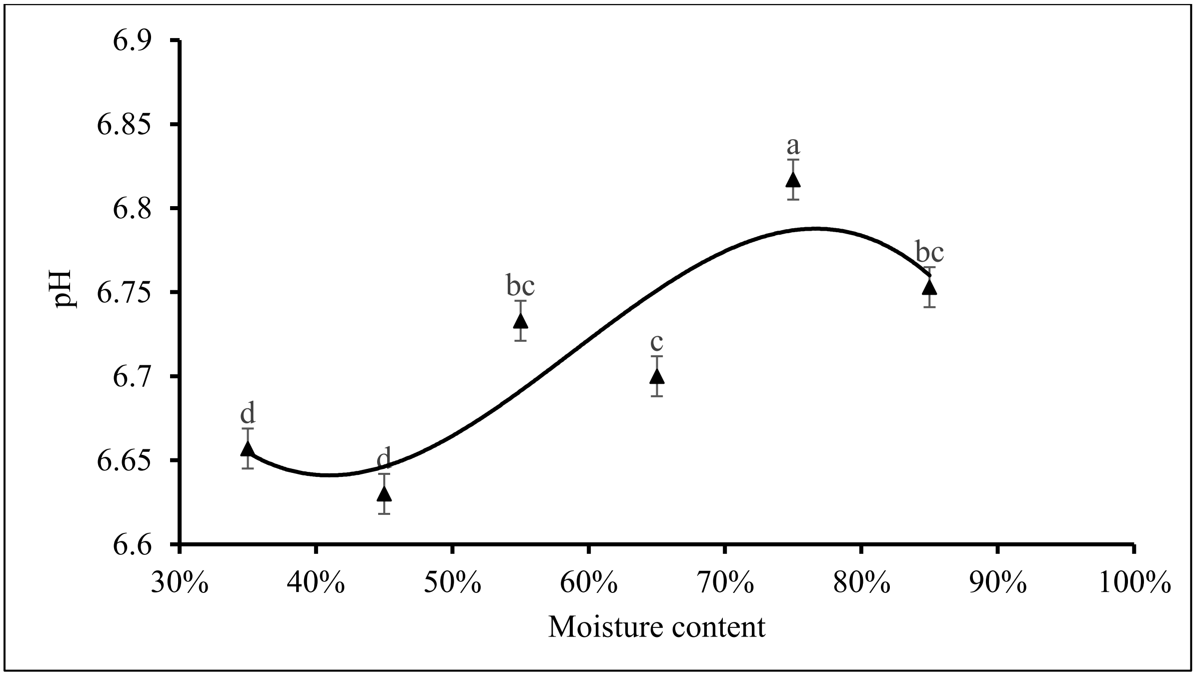

Fig. 5.1. The effect of microwave irradiation (MWI) on the pH of vermicasts at different moisture content. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

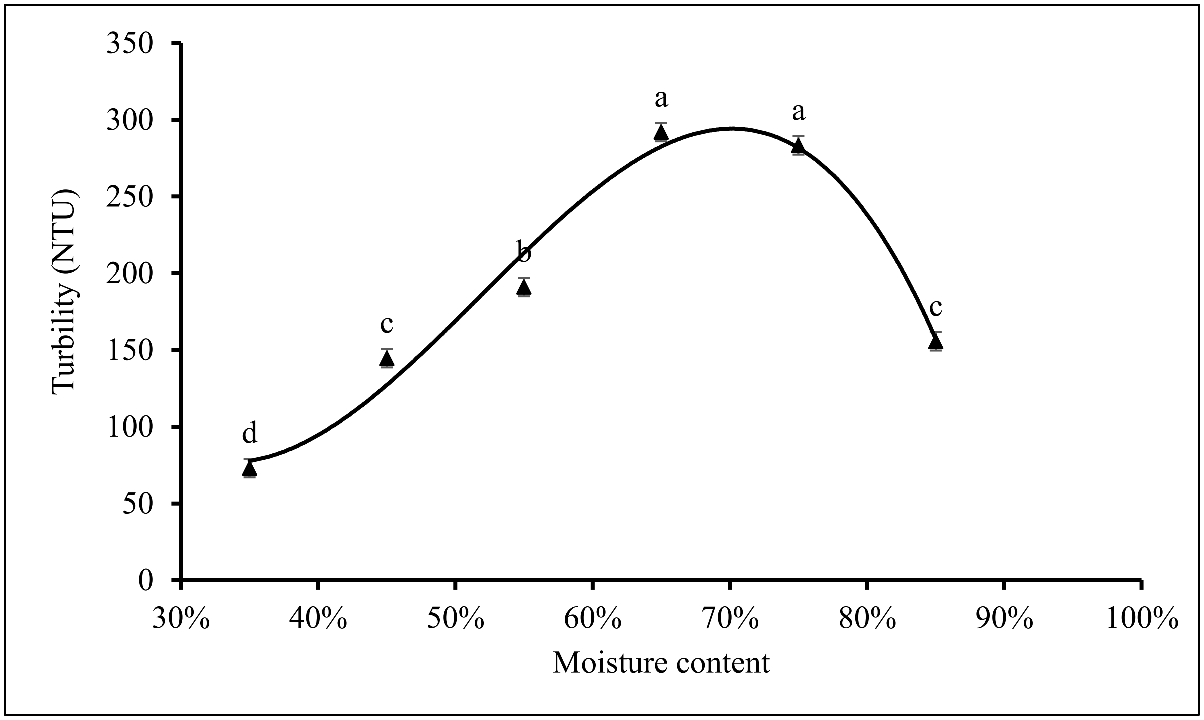

Fig. 5.2. The effect microwave irradiation (MWI) on the turbidity of vermicasts at different moisture content. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

TDS, EC, and salinity of different moisture content vermicasts treated at MWI power output level 400 W for 2.5 min exposure almost showed similar trends as the moisture content increased (Fig. 5.3, Fig. 5.4 and Fig. 5.5). The values of TDS, EC, and salinity gradually increased from the moisture content of vermicasts of 35% to its peak at moisture content of 55%, followed by a sharp decline to the lowest level at moisture content was 85%.

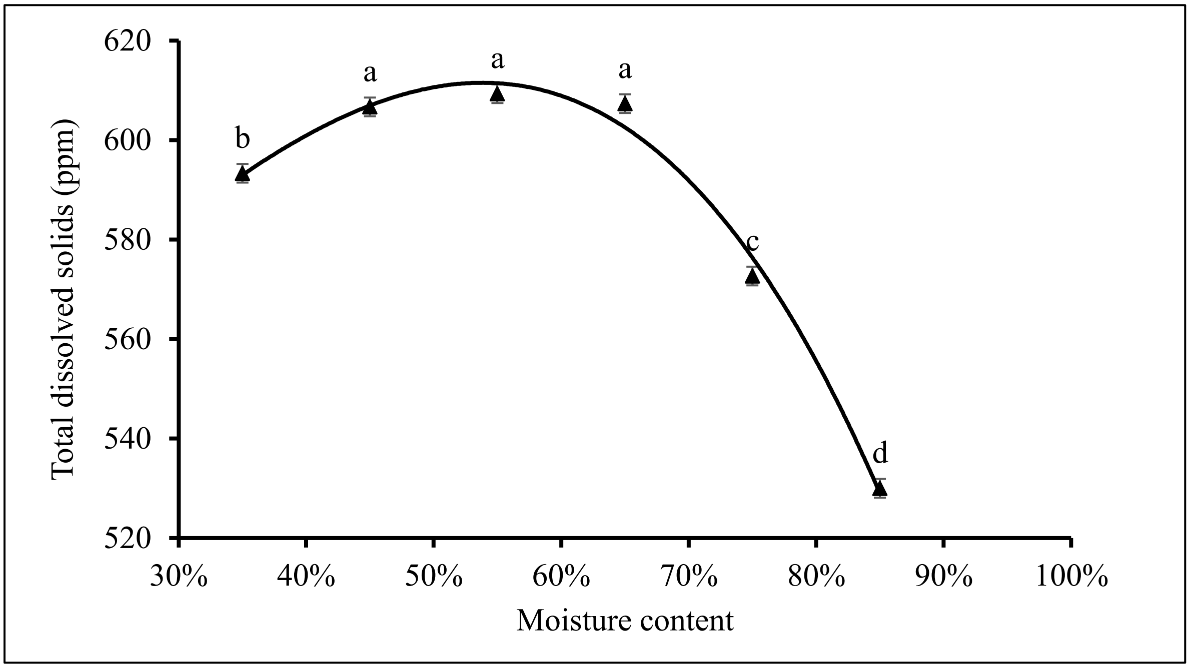

Fig. 5.3. The effect microwave irradiation (MWI) on the total dissolved solids of vermicasts at different moisture content. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

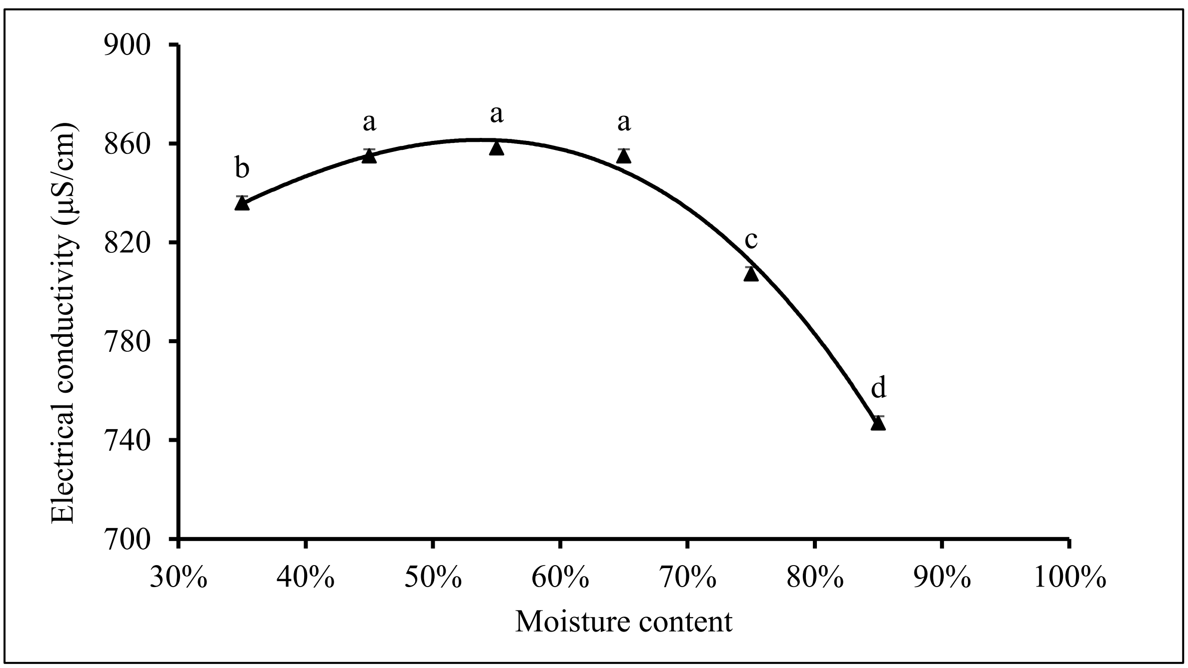

Fig. 5.4. The effect microwave irradiation (MWI) on the electrical conductivity (EC) of vermicasts at different moisture content. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

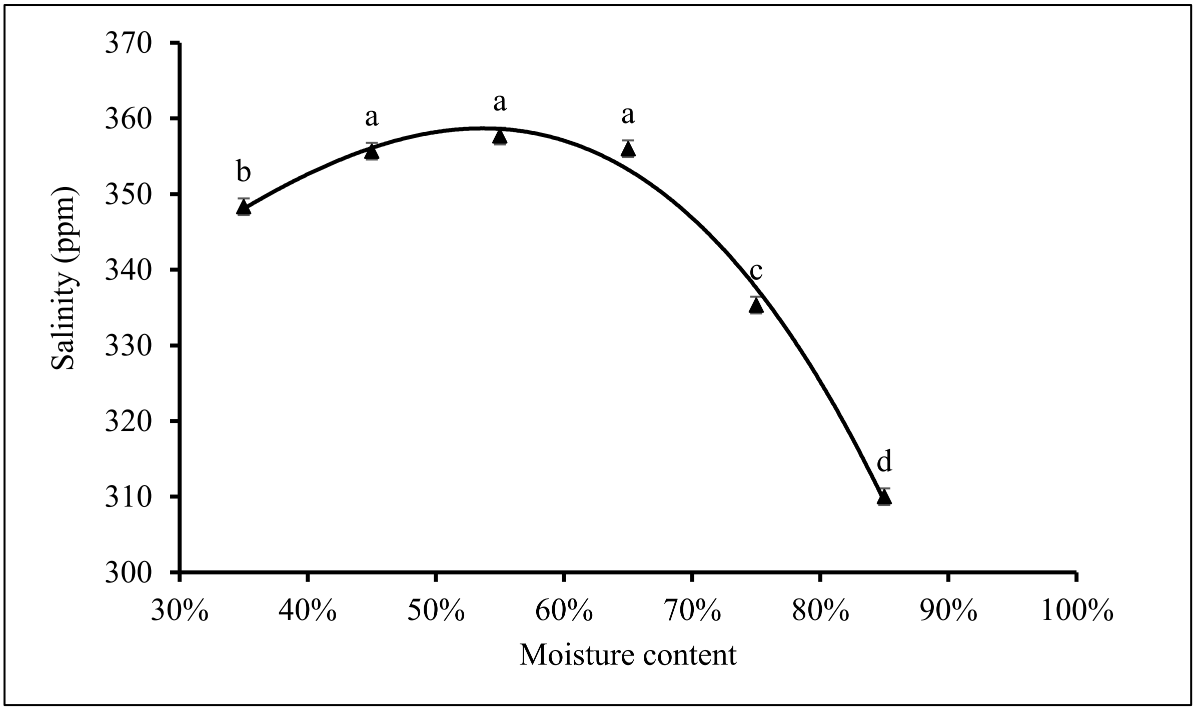

Fig. 5.5. The effect microwave irradiation (MWI) on the salinity of vermicasts at different moisture content. Means with different letters are significantly different at the 0.05 levels. Values represent the mean ± SD of 3 replications.

5.4.2 MC effects of vermicasts on kale plants growth response

Chlorophyll and anthocyanin content of kale plants applied with microwaved vermicasts at 85% MC was significantly ($P<0.05$) higher than the other MC vermicasts treatments (Table 5.2). The plant height and fresh weight of kale plants applied with 85% MC microwaved vermicasts were also significant ($P<0.05$) higher than MC vermicasts treatments (Table 5.2). These findings further confirm that microwaved vermicasts, as an organic amendment, promote plant growth and increase yield.

There were no apparent differences in chlorophyll fluorescence indices (Fv/Fo and Fv/Fm) of kale plants with different MC microwaved vermicasts (Table 5.2). Kale plants applied with 35%, 45%, 65%, 75%, 85% moisture content vermicasts, the Fv/Fo and Fv/Fm were all significant (P<0.05) higher than 55% moisture content vermicasts and Promix-BX™ amendment. Chlorophyll fluorescence is essential to the physiology of a plant (Maxwell and Johnson 2000). However, the kale plants applied with 85% MC microwaved vermicasts showed the highest value of Fv/Fo and Fv/Fm, which means it had the best photosynthetic performance.

Table 5.2. Microwaved vermicasts at different moisture content effect on kale plant growth components.

Moisture content (%) of microwaved vermicast	Chlorophyll content (SPAD value)	Anthocyanin content (ACI)	Fv/Fo	Fv/Fm	Plant height (cm)	Fresh weight (g)
35	34.10d	7.30d	3.90ab	0.80ab	14.40c	5.20c
45	34.40d	7.40cd	3.90ab	0.80ab	14.80c	5.30c
55	36.20c	8.00bc	3.80bc	0.79bc	14.40c	5.80c
65	36.40bc	8.10bc	3.85ab	0.79ab	15.60b	6.00c
75	37.40b	8.50ab	3.90ab	0.79ab	17.30a	7.80b
85	39.90a	8.80a	4.00a	0.80a	17.90a	8.80a
No vermicast	32.50e	5.90e	3.60c	0.78c	13.00d	3.20d
Significance level (P-value)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

[a] Means with different letters are significantly different at the 0.05 levels.

5.5 Conclusion

Effect quality indices of different moisture content vermicasts at 400 MWI power output level with 2.5 min exposure time, and the treated vermicasts effects on kale plants growth were investigated in this study. Results showed that with moisture content rising, the pH, turbidity EC, TDS, the salinity of vermicasts treated at MWI power output level 400 W for 2.5 min exposure were all significantly (P<0.05) influenced. For the kale plants treated with different moisture content microwaved vermicasts, chlorophyll content, anthocyanin content, Fv/Fo, Fv/Fm, plant height and fresh weight of kale plants were all significantly ($P<0.05$) increased with moisture content rising. The kale plants treated with 85% moisture content microwaved vermicasts showed the best growth response in this study.

In this research, it showed that microwaved vermicasts had the potential to improve the growth response of kale plants. Meanwhile, vermicasts with higher moisture content treated at MWI power output level 400 W enhanced the growth of kale plants. However, considering that this experiment was a pot-experiment performed in the greenhouse, the conclusion drawn from this study is subject to further examination. Further studies should focus on the large-scale field study.

Chapter 6 Conclusions

The use of natural growing medium amendment such as vermicasts in agricultural production is on the rise due to the pressure from consumers and environmental groups to cut down on the use of synthetic chemical fertilizers and pesticides. The benefits of using microwaved vermicasts in kale growth were proved in this study. The application of vermicasts in the agricultural system can not only contribute toward management of environmental problems but also optimize to improve the production of plants. The ideal production in agriculture is to produce more foods and minimize the damage to the environment.

In this study, we tried to use the microwave irradiation (MWI) technology to change the quality of vermicasts and improve the growth response of kale by using microwaved vermicasts. Overall, this project was focused on 1) determining the effects of different MWI power output levels at a specific exposure time on vermicasts quality indices and growth response of kale plants; 2) determining the interaction effects of MWI power output levels and exposure time on vermicasts quality indices, and to assess the growth and yield performance of kale plants; 3) evaluating the effects of moisture content on the quality indices of vermicasts after a selected optimal combination of MWI power output level and exposure time, and to assess the growth and yield performance of kale plants.

In Chapter 3, we concluded that MWI was effective at changing the quality of vermicasts. Turbidity, potential hydrogen content (pH), total dissolved solids (TDS), electrical conductivity (EC), nitrogen, potassium, sodium, calcium of vermicasts were significantly ($P<0.05$) influnced by MWI. Meanwhile, microwaved vermicasts was also active at improving the growth response of kale plants. It showed that vermicasts treated at MWI power output level 400 W for 5 min provided the best overall condition to support kale plants growth because kale plants grown with this vermicasts had the highest chlorophyll content, anthocyanin content, fresh weight, and plant height. This study only investigated the effect of MWI power output level irradiation on quality of vermicasts, and growth of kale at a specific exposure time of 5 min. Therefore, further research can be conducted to explore the interaction effect of power output level and exposure time on vermicasts quality indices, and the response of kale growth and quality.

Chapter 4 explored the interaction effects of various MWI power output level and exposure time on vermicasts quality indices, and the response of plants growth and quality. Specifically, this chapter was designed to determine the optimal combination of MWI power output level and exposure time on vermicasts and kale growth and quality. In Chapter 4, the quality of vermicasts, i.e. pH, turbidity, EC, TDS, and salinity were also changed with various MWI power output level and exposure time. The kale plants growth parameters (i.e. chlorophyll content, plant height, fresh weight and dry weight) and nutrient contents of kale leaves (crude protein, crude fiber, fat, ash, copper, iron, manganese, zinc, calcium, magnesium, sodium and potassium) were all changed by different combinations of MWI power output level and exposure time. The findings in this Chapter confirmed that vermicasts microwaved at 400 W for 2.5 min produced kale plants with highly enhanced chlorophyll content, fresh weight, plant height, and dry weight, potassium, iron, manganese, crude protein, crude fiber, fat and ash content. However, there are some limitations to this experiment, considering that this experiment was a pot-experiment performed in the greenhouse. Some nutrients were released slowly, and plants might contain hidden hormones later in the growing season that might have influenced plant growth in a long-term, which were not taken into consideration in this experiment. Moisture content of vermicasts is an essential parameter in MWI treatment research. Therefore, further studies should focus on the effects of moisture content of vermicasts under MWI treatment.

The objective of Chapter 5 was to evaluate moisture content effects on quality indices of vermicasts after MWI treatment using the selected optimal combination of MWI power output level and exposure time, and to assess the growth and yield performance of kale plants. In Chapter 5, it was concluded that with moisture content rising, the pH, turbidity EC, TDS, the and salinity of vermicasts treated at MWI power output level 400 W for 2.5 min exposure were all altered. Chlorophyll content, anthocyanin content, chlorophyll fluorescence indices (Fv/Fo and Fv/Fm), plant height and fresh weight of kale plants were also altered moisture content rising. Generally, the kale plants treated with 85% moisture content microwaved vermicasts showed the best growth response in this study. However, considering that this experiment was a pot-experiment performed in the greenhouse, there are some limitations to this experiment. Therefore, further studies should focus on field study.

Finally, findings from Chapter 3, 4, and 5 suggested that MWI have the potential to improve the quality of vermicasts and promote kale plants growth. It showed that 85% moisture content vermicasts treated at MWI power output level 400 W for 2.5 min was the best treatment for vermicasts to promote kale growth. However, how the MWI influence the quality of vermicasts still requires more clarification. Microbiological properties of vermicasts play an essential role in the quality of vermicasts. Hence, it is essential to have a better understanding of the effects of vermicasts' microbiological properties under the treatment of MWI in future research.

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