



Effect of Vermicompost and Terrestrial Isopod (*Porcellio laevis*) Fertilizers on The Yield and Quality of Lettuce (*Lactuca sativa* var. *capitata* cv. Wismar)

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Abstract—Terrestrial isopods (woodlice) (Isopoda.: Oniscidea) are important members of soil macrofauna in many habitats. Although the role of woodlice in decomposing organic matter and recycling nutrients is well known, there is no available data on the utilization of fertilizer obtained using terrestrial isopods in agricultural production. To evaluate the effects of the addition of vermicompost and the fertilizer obtained using terrestrial isopod species *Porcellio laevis* Latreille, 1804 in different ratios to plant growing media on nutrient content and yield of lettuce, nine different mixtures containing 1, 5, 10, and 20% of each fertilizer [v/v, including agricultural soil (control)] were used. As result, while the total and head weight of the control plant was 69.66 g and 59.53 g respectively, the highest values were obtained from the mixtures containing 20% vermicompost with 154.33 g and 150.66 g, and this followed by isopod fertilizer (20%). The vitamin C and chlorophyll (SPAD) content of plants grown in all mixtures were higher than control and there were no significant differences in respect to phenolic matter and nitrate. The fertilizer produced by using *P. laevis*, which can evaluate many kinds of agricultural and household organic wastes can be confidently used or added to media.

Keywords—Lettuce, *Porcellio laevis*, quality, Terrestrial isopod, vermicompost.

I. INTRODUCTION

Lettuce, a cool-season crop rich in Vit C, A, and minerals, is the major salad vegetable in many countries of the world also in Turkey (Yamaguchi 1983; Liebster 1990; Krug 1991). It has nutritious properties and important for a balanced diet. Existing (conventional) agricultural production systems are threatening living and environmental health. For instance, the excessive use of nitrogen for high yield is a widespread practice, especially in leafy vegetables like lettuce. This situation not only the soil's ability to produce healthy plants affect, but also causes salinization and pollution in soil and groundwater. Therefore, there is a demand for naturally derived plant nutrient elements and soil conditioners for the sustainability of agriculture and healthy vegetable products. In this context, it is increasing the interest in the

use of materials such as vermicompost, especially for the utilization of agricultural wastes and environmentally friendly sustainable production. Vermicompost, processed organic material by earthworm, which is increasingly used in agricultural practices, has high porosity, drainage, water-holding capacity, and microbial activity. It improves absorbability and retention of nutrient due to having a large surface area, and thus contain nutrients in forms that are readily taken up by the plants. There are a number of researches in the literature dealing with the positive influence of vermicompost on growth, productivity and quality of vegetables (Edwards and Burrows 1988; Orozco et al. 1996; Atiyeh et al. 1999; Sharma and Banik 2014).

Terrestrial isopods (Isopoda: Oniscidea), a member of soil macrofauna, are invertebrate species that playing an important role in the decomposition of agriculture and

livestock waste material (Drobne 1997; Odendaal and Reinecke 1999; Hussein et al. 2006; Loureiro et al. 2006). They are identified as an integral part of the decomposition process, which recycles essential nutrients of the soil and maintains its fertility by the fragmentation of organic matter and stimulating and/or ingesting fungi and bacteria. Due to their high physiological adaptation capacity and exhibiting a broad distribution, they have become an important model organism for the monitoring of pollution and to test the hypotheses in global change biology (Kammenga et al. 2000; Zimmer 2002). *Porcellio laevis* is one of the most widely and intensively used woodlice species for this purpose, as it is cosmopolitan and shows plasticity in physiological and life-history traits in response to different geographical climatic condition (Powers and Bliss 1983; Castañeda et al. 2004; Bacigalupe et al. 2007; Lardies and Bozinovic 2008; Folguera et al. 2009; Da Silva Junior et al. 2014).

Nowadays, many ingredients such as vermicompost, perlite, coir, and peat are used to improve the qualifications of growing media in growing seedling and horticultural crops (Tuzel et al. 2020). Furthermore, interest in different resources to be used for this purpose in the production of horticultural crops is increasing due to reasons such as increased environmental awareness, high input cost, and increasing demand for waste recycling. Although many studies have reported the positive impacts of vermicompost on the growth and yield of vegetable crops, to our knowledge, no information is available on the role of woodlice-mediated fertilizer in crop production. The main objective of this study was to evaluate the usability of terrestrial isopod (*Porcellio laevis*) fertilizer added to growing media at different proportions for lettuce production and to compare it with vermicompost.

II. MATERIALS AND METHODS

2.1 Isopod fertilizer and preparing

In this study, agricultural soil, vermicompost, and isopod fertilizer were used as the main media to show the usability potential of woodlice-mediated fertilizer in horticulture through comparative data. The agricultural soil was taken from the vegetable research field of the department of horticulture to prepare growing media had the clay loam texture (35.13% clay, 24.40% silt, 40.47% sand). Cow manure vermicompost, processed by red earthworm (*Eisenia foetida* L.), suitable for vegetable production was provided by a commercial firm. To prepare isopod fertilizer, the plastic pot having dimensions 40x50x40 cm was used. Firstly, three-liter agricultural soil was put into the pot, laid to cover the bottom, soaked with tap water, and compressed to a depth of about 1-2 cm.

Then, two kg dry cow manure (old cow dung), lettuce and carrot waste, wheat straw (each one 0.5 kg) were added. The mixture was stirred thoroughly and again was moistened. Three hundred adult *P. laevis* longer than 8 mm were collected from the field, identified morphologically according to the species-specific keys (Hale, 1927-1929), released into the pot, and the container was covered with fine mesh and left at room temperature for three months (Fig. 1a). During this composting period, the studied settlement was regularly checked at intervals of no more than one week. The contents were moistened and mixed gently to allow the isopods to access the different parts of foods, and new food materials were provided when it is necessary. At this point, in order to prevent the excess food supply from spontaneously decomposing into the fertilizer independently of the isopod effect, food particles that were covered with mold were removed. In all the rearing processes of the isopods, the known data about their environment preferences, moisture requirements, food preferences, daily feed consumption amounts, and other requirements were considered (Warburg 1993; Zimmer 2002; Lardies et al. 2004; Catalán et al. 2008). At the end of the three-month composting period, all the contents were gently sieved (pore size, ca. 3x3 mm) to eliminate insufficiently decomposed food pieces and the isopods. Some chemical properties of the agricultural soil, isopod fertilizer) and vermicompost used in the experiments were determined using (Table 1).

By taking into consideration the previous studies (Edwards and Burrows 1988; Ali et al. 2007; Kiran 2019) concerning vermicompost, the following growing media were tested in the experiments:

- (1) Agricultural soil (collected from the top 30 cm depth)
- (2) Agricultural soil mixed with 1% vermicompost (v/v)
- (3) Agricultural soil mixed with 5% vermicompost (v/v)
- (4) Agricultural soil mixed with 10% vermicompost (v/v)
- (5) Agricultural soil mixed with 20% vermicompost (v/v)
- (6) Agricultural soil mixed with 1% isopod fertilizer (v/v)
- (7) Agricultural soil mixed with 5% isopod fertilizer (v/v)
- (8) Agricultural soil mixed with 10% isopod fertilizer (v/v)
- (9) Agricultural soil mixed with 20% isopod fertilizer (v/v)

2.2 Experiment site, plant materials, and growth conditions

The experiments on the lettuce cultivation were carried out at the unheated greenhouse of the experimental field of the Department of Horticulture, Faculty of Agriculture, Tekirdag Namik Kemal University, Turkey (40°59'33"N, 27°34'43"E, altitude 18 m). Seedlings of commercial lettuce (*Lactuca sativa* L.), iceberg type, 'Wismar' (Vilmorin-Anadolu Vegetable Seeds, Istanbul, Turkey)

were planted into bags (dimension in 22x40 cm) containing soil and vermicompost or isopod fertilizer at different ratios. Bags were put on benches inside the greenhouse. In order to clearly see the effect of different growing media, no additional fertilization was made during the growing period. Regular watering by hand to

keep the soil with adequate water supply was made. Towards the end of the experiment, low tunnels were set up over the plants and covered with shade cloth to protect the plants from high temperatures. (Fig. 1b). Maximum and minimum temperatures and relative humidity were recorded daily during the experiment period (Fig. 2).

Table 1. The basic chemical properties of the agricultural soil, isopod fertilizer (If) and vermicompost (Vc) used in the experiments.

	pH	Salt (%)	Lime (%)	Organic matter (%)	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
Soil	7.74	0.13	4.72	1.73	0.025	32.31	95.37	531.41	56.20	0.43	1.37	0.71	16.07
If	7.02	0.74	1.71	6.52	0.33	480.61	9591.45	7763.27	2528.88	6.89	3.18	42.43	21.59
Vc	6.08	0.22	-	42.80	1.40	2619.71	2945.74	4455.50	-	-	-	-	-

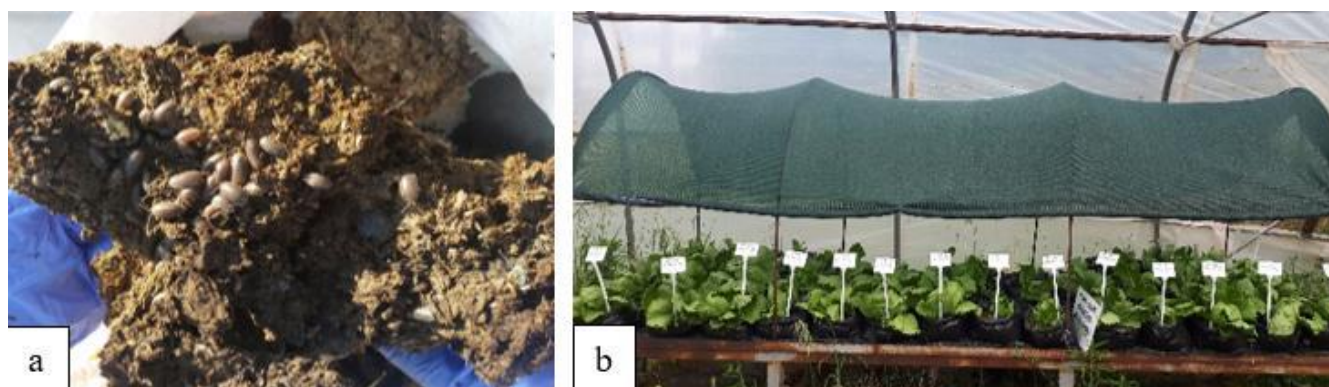


Fig.1. Isopods (*Porcellio laevis*) (a) and a view of plants grown in a greenhouse (b).

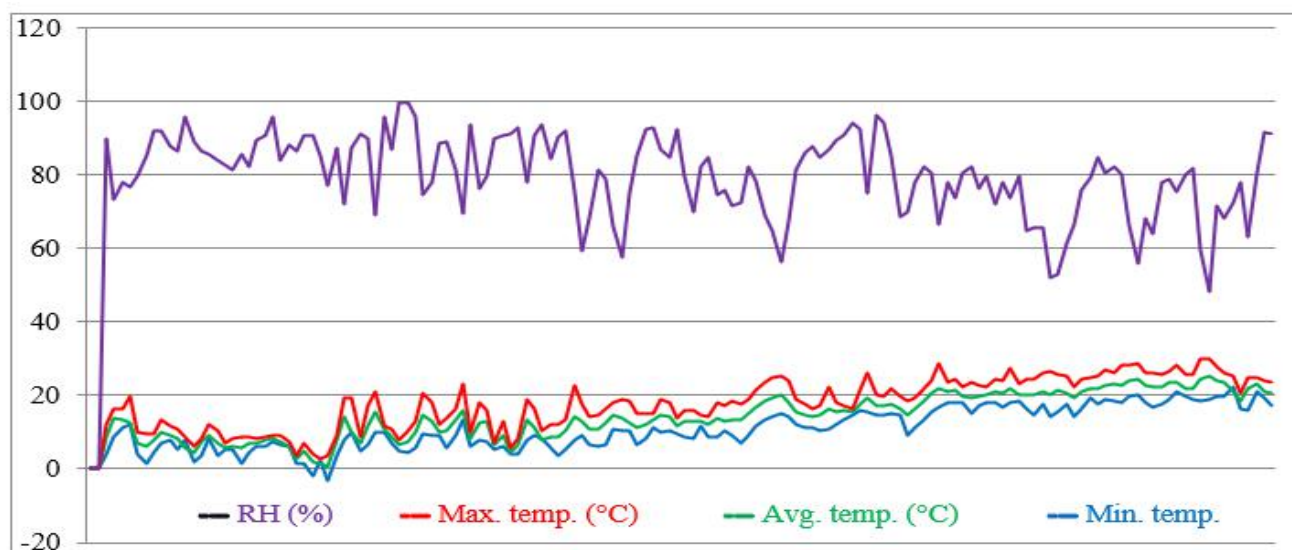


Fig.2: Daily relative humidity (RH, %), minimum, maximum and average temperatures (°C) during the growing period.

2.3 Measurement and analysis of plant

After 60 days from transplanting all plants were harvested by cutting above soil surface using a steel knife, and plant

weight (without roots), head weight, and head circumference were weighed or measured in the samples randomly selected from each plot and application. Vitamin C was determined by using the 2,6, dichlorophenol-indophenol dye titration according to Pearson (1970) and presented as mg/100g fresh weight. Chlorophyll content of leaves was measured nondestructively by Minolta Chlorophyll Meter-SPAD 501 (Ghoname et al. 2017). The content of the total phenolic compounds was determined by Folin-Ciocalteu assay and was expressed as mg gallic acid equivalent/100 g of sample (Leamsomrong et al. 2009). The nitrate content of leaves was determined by salicylic acid method and expressed as ppm (Cataldo et al. 1975). Nitrogen was quantified by micro-Kjeldahl method in dried and ground leaf samples, the determination of mineral content of leaf was used atomic absorption spectrophotometer (Hernandez et al. 2010).

2.4 Statistical analyses

The experiment design was a randomized complete block with three replications, each including nine different growing media. Data were subjected to analysis of variance (ANOVA), followed by the LSD test at $p < 0.05$ (Montgomery 1991).

III. RESULTS AND DISCUSSION

3.1 Head properties

The addition of vermicompost (Vc) or isopod fertilizer (If) to the growing medium led to an increase in plant and head weight, and this increase showed a linear relationship with the amount of them (Table 2). The highest plant and head weights were obtained from 20% Vc with 154.33 g. and 150.66 g. respectively, this was followed by media containing the same proportion of If with 137.77 g. and 135.00 g. The same situation was also seen in the head circumference and plants growing in medium containing Vc and If had larger heads than the soil (control). The addition of Vc or If to the growing medium may have brought the soil pH closer to neutral. Likewise, the fact that they contain a higher rate of nutrients than the soil may have led to this result. Similar to the results obtained from this experiment, as reported by many authors (e.g., Hernandez et al. 2010; Singh et al. 2010; Sharma and Banik 2014), the addition of vermicompost to the medium increases plant growth. Nagavallema et al. (2006) stated that the application of vermicompost can directly modify the physicochemical properties of agricultural soil in a way that promotes plant growth. In our previous study that the vermicompost and isopod fertilizers at the same ratio were added to the lettuce seedling growing medium, there was no difference among applications in terms of seedling emergence and seedling characteristics, probably due to

the short seedling growing time. However, fresh and dry weights of seedlings in If and Vc media were higher than those grown in soil (Arin and Dinçsoy 2020).

3.2 Nutrient composition and mineral content

The vitamin C and chlorophyll contents of plants grown in media containing vermicompost and isopod fertilizers were higher than the soil (control), and this increase was parallel to the amount of them at media (Table 2). Besides all applications have a higher value than control, the highest SPAD value was recorded at 20% vermicompost with 38.56 (a), followed by 20% isopod fertilizer with 35.91 (ab). A similar case existed in the results regarding the vitamin C content of the leaves. The likely reason for the increase in vitamin C, chlorophyll, with the addition of these to the growing medium, could be that these materials contain higher organic matter and plant nutrient elements compared to the soil (Table 1). Other hand, Kiran (2019) stated that vermicompost increases water uptake of roots due to its capacity of holding water and the microorganisms including mycorrhizal fungi. Ayyobi et al. (2014) reported that the increase in chlorophyll content with organic matter applications such as vermicompost or municipal solid waste compost can be attributed to an increase in photosynthesis and CO assimilation which improve mineral uptake by the plant.

There were no significant differences among the content of phenolic matters of plants grown in different media but, the lowest value with 175.33 mg/100 g was determined in control (soil). Also, in terms of nitrate, the differences of growing media were not statistically important (Table 2). It is known that excess nitrate is harmful to human health. During various processes in the human body, nitrates are converted into nitrites, which causes various diseases, such as blue baby syndrome (methemoglobinemia) and cancer. Leafy vegetables, including lettuce, are plants that accumulate high amounts of nitrate (Mensinga et al. 2003; Colla et al. 2018; Bian et al. 2020; Salehzahed et al. 2020). Gorenjak and Cencic (2013) reported that lettuce takes place in the remarkably high group in the classification made according to the nitrate content of vegetables, Krug (1991) that the nitrate content of lettuce varies 380-3520 mg/kg FW as depending on many factors such as the variety, growing season and fertilization. According to EU Commission regulation (No 1258/2011), concerning with maximum nitrate content for the commercialization of fresh vegetable, the nitrate threshold established for lettuce is 3000-5000 mg/kg FW, and the highest daily nitrate intake 222 mg for a 60 kg individual (Colla et al. 2018). In this study, besides the nitrate content of lettuce leaves was not significantly affected by the addition of vermicompost or isopod fertilizers to growing media, the nitrate levels of the plants

were within the aforementioned values or did not exceed the recommended maximum nitrate intake value per day.

Table 2. The effect of different growing media on the plant weight (g), head weight (g), head circumference (cm), vitamin C (mg/100g), chlorophyll (SPAD), total phenol (mg/100g), nitrate content of lettuce (ppm).

	Plant weight (g)	Head weight (g)	Head circum. (cm)	Vitamin C (mg/100g)	Chlorophyll (SPAD)	Total phenol (mg/100g)	Nitrate (ppm)
Soil	69.66 d	59.53 d	28.7 b	9.47 f	24.37 c	175.3	1064.0
1% Vc*	99.67 bcd	96.33 bcd	37.0 ab	11.90 de	28.35 bc	195.6	1230.5
5% Vc	112.00 abc	108.66 abc	41.3 a	13.97 cd	30.95 abc	248.0	1256.0
10% Vc	128.00 ab	125.33 ab	41.0 a	16.43 ab	31.49 abc	184.6	1070.0
20% Vc	154.33 a	150.66 a	42.0 a	18.68 a	38.56 a	218.7	1433.5
1% If**	79.00 cd	76.33 cd	35.0 ab	11.62 ef	28.99 bc	179.3	2164.5
5% If	93.20 bcd	90.33 bcd	34.0 ab	12.16 de	29.12 bc	230.7	1189.5
10% If	120.33 abc	116.66 abc	33.6 ab	15.46 bc	31.29 abc	254.6	1972.5
20% If	137.77 ab	135.00 ab	39.4 a	16.83 ab	35.91 ab	192.0	1745.5

Within columns, values followed by different letters are significantly different ($P < 0.05$)

*Vc: Vermicompost, **If: Isopod fertilizer

The differences in the mineral content of the leaves among growing media were not significant except for zinc. With the increase of isopod fertilizer in the mixture, the zinc content of the leaf increased regularly and the highest zinc content with 11.58 ppm was determined at 20% If (Table 3). As known well, Zinc has a particularly

important role in protein synthesis, membrane stability, and enzyme activation in plants, and it supports and activates the plant immune and resistance system, especially in stressful conditions (Cakmak 2008; Rehman et al. 2019; Farooq et al. 2021).

Table 3. The effect of different growing media on the mineral content of lettuce leaves.

	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Fe (ppm)
Soil	0.93	7187.3	8762.6	11105.9	1717.5	1511.5	41.20	8.53 bc	1.33	12.70	650.8
1% Vc*	0.97	5601.2	8213.4	10581.4	1641.5	1524.8	43.26	7.46 bcd	1.32	12.42	872.7
5% Vc	1.10	7329.2	15254.8	10347.7	1441.6	1422.1	39.58	7.03 bcd	1.15	9.63	445.0
10% Vc	1.08	5741.8	6467.4	9295.6	1502.3	1375.1	34.70	6.08 d	1.10	6.53	481.8
20% Vc	1.05	6348.9	11987.3	9554.7	1310.6	1270.1	33.68	6.60 cd	1.23	9.20	597.7
1% If**	0.87	6735.5	13471.2	10566.8	1581.1	1533.2	37.05	8.16 bcd	1.15	7.30	448.7
5% If	0.88	6649.0	5056.8	10128.8	1609.0	1425.5	40.56	8.63 bc	1.27	13.57	794.3
10% If	0.76	8053.7	10908.5	11017.2	1848.8	1574.3	35.75	9.00 b	0.98	11.05	561.0
20% If	1.14	8046.0	6311.0	10222.4	1677.0	1553.3	39.33	11.58 a	1.38	9.80	608.3

Within columns, values followed by different letters are significantly different ($P < 0.05$)

*Vc: Vermicompost, **If: Isopod fertilizer

3.3 Evaluation of results as deals with health, environment, and agriculture

The use of sustainable organic materials can increase fertility without negative effects on human health and the environment. They maintain quantity and quality of yield and can be less costly than synthetic fertilizers. Vermicompost is widely one of the most used materials for this purpose. However, it should be kept in mind that the content and characteristics of vermicompost vary depending on the raw material, the type of earthworm, the methods of processing, etc. and it has a high cost and can cause salinity when used continuously or at high rates (Atiyeh et al. 2002; Ayyobi et al. 2014; Guitierrez-Miceli et al. 2007). Today is being emphasized the importance of sustainability to protect human life, animals, and plants by cutting pollution. Therefore, within the scope of zero waste, studies are being carried out and the decisions and measures are taking to reach this goal (Marshall and Farahbakhsh 2013; Lim et al. 2015). The European Union, which has focused on environmental and social sustainability issues such as combating climate change, reducing greenhouse gas emissions, and using renewable energy published a new environmental plan called The European Green Deal in November 2019 (EU Commission 2020). All the relevant data indicate that the interest in materials that can be used as plant nutrients and as soil conditioners from waste with natural processes will continue to increase.

This study suggested that woodlice can be a promising alternative that may be utilized in the eco - friendly agricultural practices. Furthermore, our results and observations, and some well-known features of *P. laevis* as follows indicate that this species is a good candidate in this context: i) it can effectively feed on a great variety of fresh or dead plant materials (Warburg 1993) and dry cattle feces, ii) it is able to avoid the foods contaminated with some heavy metal (Odendaal and Reinecke 1999), iii) it ingests more food than some other woodlice species (Dallinger and Wieser 1977; Warburg 1993), iv) the retention time of food in the digestive tract is mostly less than 24 h, depending on some factors such as food quality (Zimmer 2002), v) it is bigger than many other species, and the adults can reach a length of about 13 mm (Amari et al. 2019), vi) during the life span that can be as long as two years (Nair et al. 1976; Amari et al. 2019), each female can breed repeatedly (interparous), the number of broods per year is usually two under field condition (Warburg 1993), this number is most probably higher under constant proper circumstances, and the number of eggs per female in each brood can exceed 100, depending on the size of individual, food quality, and some other

environmental conditions (Amari et al. 2019), vii) it has cosmopolitan distribution over the world and synanthropic feature, is commonly associated with stables, cattle yards, and dung heaps, and can effectively inhabit such indoors (Pierce 1907; Harding 2016), viii) it is predominantly active during the night (nocturnal) or at the shady shelters (Warburg 1993), therefore it does not need direct sunlight, ix) it has obviously higher interest in elevated humidity and lower desiccation resistance compared to most other terrestrial isopods (Warburg 1993; Ghemari et al. 2016), taking advantage of that it is fast (Pierce 1907) and timid species, it is easy to get them to gather at a humidified focus in the rearing container, and x) its integument characteristics are different from most of the other woodlice species (Warburg 1993), in shiny and fine appearance, and possibly the content of the chitin is less than some other species such as *Armadillo officinalis* and *Porcellio dilatatus*. Furthermore, we are also of the opinion that most of these characteristics indicate that *P. laevis* reared for fertilizer production has the potential to be used as animal feed additive.

IV. CONCLUSION

Results show that the tested vermicompost and terrestrial isopod fertilizer in the present study can improve the yield and quality of lettuce, and the isopod fertilizer was as effective as the vermicompost. Considering that vermicompost production is a high cost and technical method, and knowledge for producing are required, isopod fertilizer may be preferred due to it is a cheap, simple, eco - friendly method that does not require highly equipped facility. Moreover, by this way, agricultural and household organic wastes will be evaluated, and useful recycling will be provided through isopod fertilizer production.

DECLARATION OF INTEREST

The authors declare that they have no competing interests.

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