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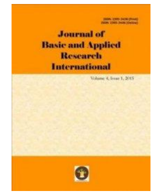

COMBINED EFFECT OF VERMICOMPOST AND EARTHWORM *Pontoscolex corethrurus* INOCULATIONON THE YIELD AND QUALITY OF BROCCOLI (*Brassica oleracea* L.) USING ORGANIC GROWING MEDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author NA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MM and IM managed the analyses of the study. Author IM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Combined effects of three kinds of vermicompost and *Pontoscolex corethrurus* inoculation on plant yield and quality of broccoli were investigated in pot cultures organically. The experiment used a factorial complete randomized design which consisted of two factors. The first factor is the kind of vermicompost consisted of three levels: V1 = the mixture of spent mushrooms waste (SMW), cow dung (CD) and vegetable wastes (VW), V2=SMW, CD and leaf litter (LL), V3 = SMW, CD, VW and LL. The second factor is the population of *P. corethrurus* consisted of five levels: 0, 25, 50, 75, and 100 indiv.m^{-2} and one control (inorganic treatment). Vermicompost was made by earthworm *Lumbricus rubellus* activity. The results of this study showed that the application of various vermicompost had significantly ($p < 0.05$) higher total biomass and marketable weight of broccoli than the control treatment. The highest total biomass (1.40-1.48 kg plant^{-1}) was found on the vermicompost V1 and V2 with population 75 indiv.m^{2} and the vermicompost V3 with population 25-50 indiv.m^{-2}. But the highest marketable weight (378.26-412.10 g plant^{-1}) was found on the vermicompost V1 with population 50 indiv.m^{2}, V2 with population 75 indiv.m^{-2}and V3 without inoculation earthworm. Vermicompost application significantly increased the quality of broccoli compared to the control, with the increase in the contents of sugar and vitamin C by 53% and 35%, respectively. Vermicompost application significantly ($p<0.05$) also increased the shelf life of broccoli by average storage loss of 16,4% for the treatment of 7 days of storage at room temperature and 6,47% for the treatment of 14 days of storage at cold temperature compared to the control treatment by 59,53% and 19,79%, respectively. Overall. the treatments using vermicompost V1 and V3 gave the highest quality. Thus, this study suggested for the broccoli cultivation in organic potting media should use the vermicompost.

Keywords: Vermicompost; *P. corethrurus*; broccoli; vitamin C; sugar content; shelf life.

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1. INTRODUCTION

Currently, the world community as well as people in developing countries, have been increasingly aware of the importance of healthy food products produced by farming systems that are free of agro-chemicals. Healthy food is hygienic food, which does not contain diseases or substances that can harm our health. Healthy food products will be generated by the system of healthy agricultural cultivation such as organic farming. Organic farming is a form of agriculture which avoids or largely excludes the use of synthetic fertilizers and pesticides, plant growth regulator, and livestock feed additives. According to the National Organic Standards Board (NOSB) of the United States Department of Agriculture (USDA), organic agriculture is "an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity [1].

The quality attributes of vegetables, not only are influenced by the genotype, but also the nutritional status of the soil plays a significant role [2]. Continuous use of inorganic fertilizers as source of nutrient in imbalanced proportion is also a problem, causing inefficiency, damage to the environment and in certain situations, harms the plants themselves and also to human being who consumes them. Some studies have suggested that organic manures gave better quality of vegetables when compared to inorganic sources of nutrients [3]. Many investigators studied the combined application of organic manures and amendments can enhance the yield, quality and post-harvest attributes of vegetables crops. Among organic amendments, vermicomposts can have potential applications in horticulture. Because vermicomposting is a cheap process, which can process large amounts of organic wastes. Various wastes can be used as a medium for the cultivation of earthworms epigeic such as *L. rubellus*. The different types of waste used will produce different quality vermicompost. During the vermicomposting process, the entire organic matter is decomposed, and its physical and chemical properties are changed with the degradable organic C being oxidized and stabilized [4].

Many studies report that vermicompost is an excellent soil conditioner and can increase is the growth and yield of vegetables such as tomatoes [5], peppers [6], and Chinese cabbage [7], garlic [8] and strawberry [9]. However, the effect of vermicompost on the growth and yield highly variable. The variability may depend on the cultivation system into the which it is incorporated, as well as on the physical, chemical and biological characteristics of vermicompost, which vary widely depending on the original feedstock, the earthworm species used, the production process, and the age of vermicompost [10,11,12]. In order to accelerate the effect of application of vermicompost on the growth of plants, especially plants short-lived needs to be combined with inoculation of endogeic earthworms which plays an important role in the process of decomposition and mineralization of organic materials in addition to the role and function others are not owned by epigeic worm in vermicomposting. Nurhidayati and Basit [13] reported that the presence of worms endogeic *P. corethrurus* can increase the rate of N mineralization of organic materials of low quality and increase of nutrient availability. Because of their effects on these properties, earthworms often affect plant growth [14,15].

This study used broccoli as an indicator plant. Brassica vegetables such as broccoli is popular and is among the most consumed vegetables in the world. Brassicas are known to possess antioxidant activity [16,17]. The consumption of broccoli has been associated with lower risk of chronic diseases problems like cardiovascular diseases, cancer, hypertension and diabetes. It is due to high nutrients content such as phytochemical compounds and their properties that can protect our bodies [18,19,20]. This study aims to describe the combined effects of three kinds of vermicompost and *P. corethrurus* inoculation on plant yield and quality of broccoli grown in pots organically.

2. MATERIALS AND METHODS

2.1 Study Site and Soil Characteristics

This study was a pot experiment conducted in a polyhouse at Tawangargo village, Karangploso district, Malang regency, East Java, Indonesia with latitude 07°56 S, longitude 112°36 E, altitude 1060 m above sea level and Inceptisol soil type. in March until August 2015 and the average temperature of 17°-22°C. Soil samples were air dried and sieved to pass through a 2 mm sieve. The soil was well drained with the following characteristics; pH (H_20) 5.3, 2.03% organic C by Walkley and Black method; 0.47% total Kjeldahl N; 131.17 mg/kg P (Bray II), 3.26 me/100 g K, cation exchange capacity 42.48 me/100 g soil, and 28% sand, 56% silt and 16% clay.

2.2 Preparation of Vermicompost

Vermicompost used in the study was composed of mushrooms culture waste, vegetable residues, leaf litter, and cow manure. The making process of vermicompost is done in a container with the spent mushrooms waste as bedding in the bottom of vermicomposting container. Bedding is functioning to control temperature and humidity vermicomposting, additional food for earthworms and provide a suitable environment for the proliferation of earthworms. In the process of vermicomposting was needed bedding in the bottom of container thickness of 5 cm, and in the upper layer is as thick as 10 cm feed worm. Earthworms feed used there are 3 kinds: (V1) mixture of cow dung and vegetables waste, (V2) mixture of cow dung and leaf litter, and (V3) mixture of cow dung, vegetables waste, and leaf litter. The amount of feed needed to depend on the abilities of earthworm decomposing the organic material. According to Ndegwa and Thompson [21], feed requirements of earthworm by 0.75 kg/kg of earthworms/day with worms to process vermicomposting density is 1.6 kg/m^2. Earthworms *L. rubellus* (0.32 kg of earthworms for 6.72 kg of organic matter per container with a surface area of 0.2 m^2) were added and incubated for 28 days. The moisture of vermicompost was adjusted to 80%. Then, the vermicompost was dismantled and composted by adding egg shells flour and fish bone meal 5 g /100 g of material. The composting process lasts for 14 days. Every 2 days, the compost was opened and stirred manually to maintain the temperature.

2.3 Experiment Design

Three kinds of vermicompost have been created, added into the plastic pots as the first factor. Every pot was filled with the mixture of soil and cow manure with the ratio of 4:1. The vermicompost was applied by 200 g per 10 kg media. The second factor is the population of earthworm *P. corethrurus* which consisted of five levels: 0, 25, 50, 75, and 100 $indiv.m^{-2}$. There were fifteen treatments combinations. The earthworms were inoculated into each pot corresponding surface area of the pot. One plant was grown in each pots at three days after inoculation earthworm. Each treatments was replicated three times with three samples for each replication and one control treatment (inorganic treatment). The content of the inorganic media just consisted of 100% of soil and added the fertilizer N:P:K (15:15:15) with application dose 500 kg ha^{-1}. Total pot used in this experiment was 144 pots. The pots were placed with block randomized design in the polyhouse. Yield, vitamin C contents, sugar content and shelf life test were determined 70 days after cultivation.

2.4 Chemical Analysis of the Vermicompost

Total nitrogen (N) was measured with the Kjeldahl method and hydrolyzable N by the alkali distillation method. Available potassium (K) was extracted with 1.0-M NH4OAc (pH=7.0) and then determined by Flame photometer [22]. The available P was extracted with Bray II then the P content was determined by the colorimetric method. The pH values of the mixtures were determined with a soil: water ratio of 1:5 (w:v) [23], after shaking for 2 h and filtration through Whatman No.1 Filter Paper. Lignin, cellulose, and ash content by Goering and Van Soest method and polyphenols content by Folin-Denis method Anderson and Ingram [24]. The results of analysis were presented in Table 1.

2.5 Determination of Plant Yield Quantity and Quality

The quantity of broccoli was determined by floret diameter (cm), weight of total biomass and marketable weight which was measured at age of 70 days after planting. The marketable yield was weight of floret as high as 10 cm. Vitamin C content was determined by iodometric titration method. Plant samples crushed with mortar. 30 g slurry was taken and put in a 100 ml volumetric flask. Distilled water is added until the volume reaches 100 ml, then filtered with filter paper. 20 ml filtrate was taken and placed in a 125 ml erlenmeyer flask then added 2 ml of 1% starch solution. The next stage is a standard titration with 0.01 N iodine solution that is made from KI and iodine until the solution blue. Sudarmadji [25] states in 1 ml iodine used equivalent to 0.88 mg of vitamin C, so the calculation of the content of vitamin C can be done by multiplying the volume of iodine solution used in the process of titration with 0.88 mg. The content of sugar is determined with a hand held refractometer (% Brix). Broccoli crop yields for each treatment were taken a sample of 1 g, and then pulverized using a mortar to remove fluid. The liquid is then used for readings with a refractometer.

2.6 Statistical Analysis

The collected data were statistically analyzed using analysis of variance (F-Test) at level ($P \leq 0.05$) and differences of each treatment were adjudged by Tukey test ($P \leq 0.05$) using Minitab Version 14.12. Dunnet test at 5% level was used to compare all treatments with control. For statistical analysis of data (charts), Microsoft Excel was employed.

3. RESULTS AND DISCUSSION

3.1 Effect of Various Vermicompost Materials and Earthworm *P. corethrurus* Population on the Plant Yield

The addition of vermicompost and inoculation of earthworm *P. corethrurus* significantly ($P< 0,05$) increased the floret diameter, total biomass, and marketable weight of broccoli when compared to the inorganic treatment (control) (Table 2). Application of organic fertilizers can improve the physical, chemical and biological soil so that the growth and yield increases. Swarup [26] reported that the increase in soil organic carbon content increases the nutrient availability in the soil. Hence, SOC management is one of the foremost challenges concerning resource management for agricultural systems in the tropics [27]. Organic fertilizer contains complete essential nutrients that are beneficial to plant.

Some treatments using vermicompost and earthworm inoculation showed results that were not significantly different at Tukey-test 5%. However, the highest total biomass (1.40-1.48 kg plant^{-1}) was found on the vermicompost V1 and V2 with population 75 indiv.m^2 and the vermicompost V3 with population 25-50 indiv.m^{-2}. While, the highest marketable weight (378.26-412.10 g plant^{-1}) was found on the vermicompost V1 with population 50 indiv.m^2, V2 with population 75 indiv.m^{-2}and V3 without inoculation earthworm.

Marashi and Scullion [28] reported that inoculation of earthworm into the soil can improve soil properties such as soil aggregate formation water holding capacity, the activity of microflora by mixing plant litter and soil minerals [29]. Earthworms are also an important part of soil functioning by influencing decomposition processes, like fragmentation of organic litter [30] and stimulation of microbial activity [31] and by bioturbation, increasing soil porosity and water infiltration [32,33]. Nurhidayati and Basit [13] reported that the presence of endogeic earthworms *P. corethrurus* can increase the N mineralization rate of low quality organic matter and increase nutrient availability.

Table 1. The chemical composition of three kinds of vermicompost on dry weight basis

No.	Chemical properties	Vermicompost 1	Vermicompost 2	Vermicompost 3
1	C-organic (%)	17.07	15.44	16.48
2	Polyphenol (%)	0.49	0.47	0.45
3	Celulose (%)	37.21	35.34	34.00
4	Lignin (%)	17.74	17.55	18.16
5	Total N (%)	1.33	1.19	1.31
6	C:N ratio	12.69	12.97	12.62
7	P (%)	0.77	0.89	0.92
8	K (%)	0.59	0.46	0.42
9	Ph	6.63	6.97	6.93
10.	Ash (%)	2.72	0.46	0.42

Table 2. Floret diameter, total biomass and marketable yield weight of broccoli plants harvested after 70 days and treated with different vermicompost material (V) and earthworm *P. corethrurus* population (P)

Treatments	Floret diameter (cm)	Total biomass weight (kg)	Marketable yield weight (g)
Control	10.46	1.15	193.67
V1P0	12.12$^{*\ ab}$	1.25$^{*\ ab}$	311.88$^{*\ ab}$
V1P1	12.99$^{*\ abc}$	1.28$^{*\ abc}$	351.14$^{*\ abcd}$
V1P2	13,86$^{*\ c}$	1.35$^{*\ bcd}$	392.52$^{*\ def}$
V1P3	12.71$^{*\ abc}$	1.41$^{*\ de}$	331.11$^{*\ abc}$
V1P4	13.04$^{*\ abc}$	1.31$^{*\ abcd}$	299.60^{*a}
V2P0	13.11$^{*\ abc}$	1.20$^{*\ a}$	363.94$^{*\ bcdef}$
V2P1	12.46$^{*\ abc}$	1.26$^{*\ ab}$	378.26$^{*\ cdef}$
V2P2	12.23$^{*\ ab}$	1.31$^{*\ abcd}$	357.11$^{*\ bcdef}$
V2P3	12.11$^{*\ ab}$	1.40$^{*\ cde}$	337.50$^{*\ abcd}$
V2P4	11.72$^{*\ a}$	1.30$^{*\ abcd}$	325.98$^{*\ abc}$
V3P0	13.46$^{*\ bc}$	1.28$^{*\ abc}$	412.10$^{*\ f}$
V3P1	12.52$^{*\ abc}$	1.42$^{*\ de}$	353.97$^{*\ bcdef}$
V3P2	12.51$^{*\ abc}$	1.48$^{*\ e}$	371.53$^{*\ cdef}$
V3P3	13.05$^{*\ abc}$	1.39$^{*\ cde}$	391.32$^{*\ def}$
V3P4	13.49$^{*\ bc}$	1.30$^{*\ abcd}$	397.48$^{*\ ef}$
HSD 5%	1.53	0.12	59.23
Dunnet 5%	0.79	0.07	30.7

Means followed by different letters in the same column are statistically significant different at Tukey- test, P=0.05;
** = significant; ns = non-significant at Dunnet test, P=0,05*

Table 3. Vitamin C and sugar contents of broccoli plants harvested after 70 days and treated with different vermicompost material (V) and earthworm *P. corethrurus* population (P)

Treatments	Sugar content (% Brix)	Vitamin C content (mg/100 g)
Control	6.67	54.56
V1P0	9.33$^{*\ ab}$	80.61$^{*\ ef}$
V1P1	10.17$^{*\ bcde}$	82.51$^{*\ fg}$
V1P2	10.67$^{*\ cdef}$	83.66$^{*\ g}$
V1P3	10.67$^{*\ cdef}$	84.86$^{*\ g}$
V1P4	10.83$^{*\ def}$	87.73$^{*\ h}$
V2P0	9.00$^{*\ a}$	70.30$^{*\ d}$
V2P1	9.67$^{*\ abc}$	86.66$^{*\ h}$
V2P2	9.83$^{*\ abcd}$	79.27$^{*\ ef}$
V2P3	11.67$^{*\ f}$	70.52$^{*\ d}$
V2P4	9.67$^{*\ abc}$	61.97$^{*\ ab}$
V3P0	11.00$^{*\ ef}$	59.18$^{*\ a}$
V3P1	10.17$^{*\ bcde}$	68.33$^{*\ d}$
V3P2	10.00$^{*\ abcde}$	65.23$^{*\ c}$
V3P3	10.00^{abcde}	63.63$^{*\ bc}$
V3P4	10.00$^{*\ abcde}$	62.15$^{*\ b}$
HSD 5%	1.02	2.87
Dunnet 5%	0.53	1.49

*Means followed by different letters in the same column are statistically significant different at Tukey-test, P=0.05; * = significant; ns = non-significant at Dunnet test, P=0,05*

3.2 Effect of Various Vermicompost Materials and Earthworm *P. corethrurus* Population on the Plant Yield Quality

The kind of vermicompost materials and earthworm *P. corethrurus* population had significantly effect on the quality of the marketable weight of broccoli. The vitamin C and sucrose content were higher in response to vermicompost and earthworm *P.corethrurus* population than in response inorganic fertilization (control) (Table 3 above). The vermicompost V1 combined with inoculation of earthworm *P. corethrurus* by 100 indiv m^{-2} and the vermicompost V2 combined with inoculation of earthworm *P. corethrurus* by 25 indiv m^{-2} had the highest contents of Vitamin C with increases of 61% and 59% respectively, compared to the inorganic treatment (Table 3). Wang et al. [14] reported that the vitamin C of leaves of Chinese cabbage cultivated in plastic pots filled the vermicompost: Soil mixtures with ratios the 4:7 had 5.8-fold higher than that of full soil treatment.

Lisiewska and Kmiecik [34] reported that increasing the amount of nitrogen fertilizer from 80 to 120 kg ha^{-1} decreased the vitamin C content by 7% in cauliflower. It means organic treatment can increase the yield quality of plant. Lee and Kadeer [35] also reported that nitrogen fertilizers at high rates tend to decrease the vitamin C content in many fruits and vegetables. Wang et al. [7] reported that the applications of vermicompost significantly increased the contents of vitamin C, phenols, and flavonoids. Toor et al. [36] also observed that the vitamin C content of tomatoes was decreased by high NO^{3-} levels, but it was increased in plants grown with chicken manure and grass-clover treatments. Asami et al. [37] found that organic fertilizer increased the levels of vitamin C in marion berry, strawberry, and corn. Phenolic compounds are a large group of plant secondary metabolites with different biological activities; for example, flavonoids showed antioxidant activity [38].

The vermicompost made from V1 combined with inoculation of earthworm *P.corethrurus* by 50-100 indiv m^{-2} and the vermicompost made from V2 combined with inoculation of earthworm *P. corethrurus* by 75 indiv m^{-2} had the highest sugar content. While the vermicompost made from V3 had the highest sugar content without earthworm inoculation. The increases of sugar content were 61%, 75% and 52% respectively, compared to the inorganic treatment (Table 3).

It has been reported that applying N, P, K, and organic fertilizers can increase sugar content of plants [22,39]. The timing and mode of mineral application, chemical form of the minerals applied, and tomato genotype affect the response to varying mineral concentrations on fruit total soluble solid such sucrose content [40,41,42,43]. Rembialkowska [44] concluded that the quality of food products was influenced by the quality of the environment (abiotic factors) and the levels of pest and pathogen damage (biotic factors) to which plants are subjected. A high nutritive quality of crops can be obtained if the plants were grown in an unpolluted environment. These contaminants include heavy metals, pesticide residues, nitrogen compounds, plant growth stimulators etc. Climate and weather are also important factors, such as soil type and pH, soil cultivation, fertilization and conditions of crop storage after harvest. Biotic factors can have also a significant impact on crop quality. The main biotic factors are cultivar choice, bacterial and fungal contamination (disease) and pest damage.

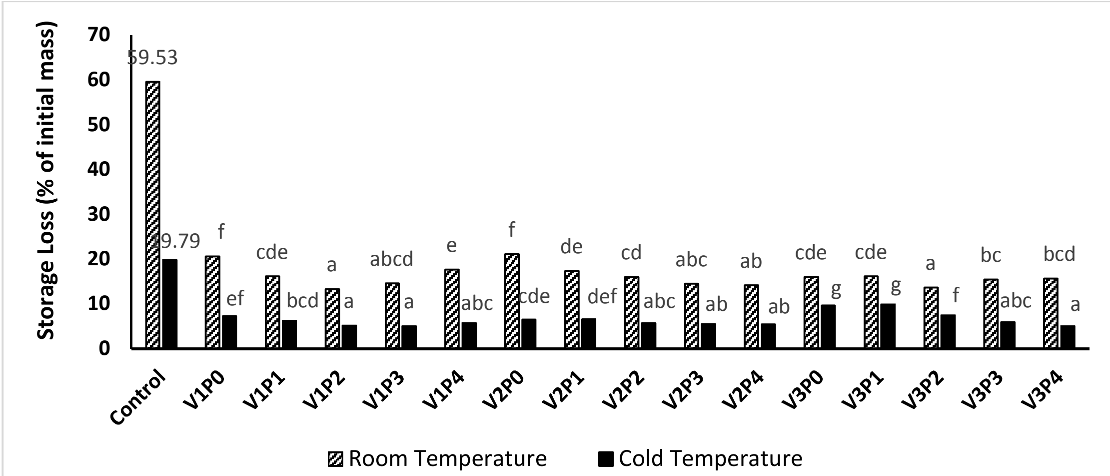

Fig. 1. Storage losses of broccoli from various vermicompost material and earthworm *P. corethrurus* population and control (in organic treatment)

3.3 Effect of Various Vermicompost Material and Earthworm *P. corethrurus* Population on Storage Loss (% of Initial Mass) of Broccoli

The kind of vermicompost material and earthworm *P. corethrurus* population had significant effect on the storage loss (% of initial mass) of broccoli. Fig. 1 above showed that the treatment using the vermicompost had a smaller storage loss of marketable weight than inorganic treatment (control) either cold temperature or room temperature storage. Storage at room temperature gives a higher storage loss of marketable weight. It is caused by storage at higher temperatures result in the loss of dissolved solids in the water substances such as vitamin C [45]. There were some treatments showed the smallest storage loss at room and cold temperature namely the vermicompost V1 combined with population of *P. corethrurus* by 50 indiv.m^{-2}, the vermicompost V2 combined with population of *P. corethrurus* by 100 indiv.m^{-2} and the vermicompost V3 combined with population of *P. corethrurus* by 50 indiv.m^{-2} and 100 indiv.m^{-2}. Among these treatments gave the smallest storage loss was the vermicompost V1 and V3 combined with population of *P. corethrurus* by 50 indiv.m^{-2} at room temperature storage.

Rembialkowska [44] reported that most of the available data indicate that the decay process is slower in organic crops, which therefore show better storage quality after the winter period. The better storage quality of organic crops was probably associated with a higher content of dry matter, sugars and other bioactive compounds in their flesh, resulting in less extensive decay and decomposition. The storage quality of vegetables such as broccoli greatly depends on storage conditions. The important parameters of micro-environment in the storage conditions are gas composition (oxygen, carbondioxide, inert gases, ethylene, etc), the relative humidity (% RH), pressure or mechanical stresses, light and temperature. Intrinsic factor of the food itself such as moisture and pH also affect the storage quality of a product. It may be possible to manipulate these factors to increase the storage quality of a food [35].

4. CONCLUSIONS

Application of vermicompost significantly increased yield of broccoli under organic growing media compared to the plant grown in inorganic media with increase in the marketable yield weight per plant by 46%. Inoculation of earthworms *P. corethrurus* gave positive effect on the application of vermicompost. They accelerated the mineralization of organic matter in the soil and constructed good soil structure branching burrows. Vermicompost application significantly increased the quality of broccoli compared with the control with increase in the contents of sugar and vitamin C by 53% and 35%, respectively. The treatments using vermicompost of the mixture of spent mushrooms waste, cow dung and vegetable wastes with population of *P. corethrurus* by 100 indiv.m^{-2} gave the highest quality. Vermicompost application significantly ($p<0.05$) also increased shelf life of broccoli by average storage loss of 16,4% for the treatment of 7 days of storage at room temperature and 6,47% for the treatment of 14 days of storage at cold temperature compared with the control treatment by 59.5% and 19.8%, respectively. The treatments using vermicompost made from the mixture of cow dung and leaf litter and the mixture of cow dung, vegetables waste and leaf litter with population of *P. corethrurus* by 50 indiv.m^{-2} gave the best shelf life.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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