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Optimum Castor Meal Application in the Cultivation of Pak Choi (*Brassica chinensis* L.) with Toxicity Survey for Earthworms (*Eisenia andrei*)

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Abstract: In Taiwan, castor meal (CM) is often used by farmers as an organic fertilizer for the supplement of plant nutrition. It can rapidly increase nitrogen availability for crops. However, the excessive application of CM will affect the ecosystem. This study was conducted to evaluate the optimum concentration of CM that can be used as a fertilizer for balancing crop production and soil ecosystem health (by considering earthworms). Pak choi was selected for the experiment. A randomized block design with three replications was used, with treatments consisting of five concentrations of CM (namely 25, 50, 100, 150, and 200 kg/0.1 ha) and fermented livestock compost 800 kg/0.1 ha, represented as CM_{25}, CM_{50}, CM_{100}, CM_{150}, CM_{200}, and LC_{800}, respectively. The results revealed that soil properties, leaf nutrient concentration, and plant traits, namely plant diameter, plant height, and fresh and dry root and shoot matter, improved with the increasing concentrations of CM, and CM_{100}–CM_{200}, and led to the highest production of pak choi which was equivalent to that with LC_{800}. However, the weight of earthworms decreased as CM concentrations increased. The weight of earthworms was similar between CM_{25}, CM_{50}, CM_{100}, and LC_{800}. In conclusion, given the characteristics of high nitrogen as a fertilizer, the optimal dose of CM was 100–150 kg/0.1 ha for obtaining a balance between crop production and ecosystem safety.

Keywords: castor meal; biological production; ecosystem; earthworm; toxicity

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1. Introduction

Organic residues are widely used as fertilizers because they provide nutrition to plants, improve the physical and chemical properties of soil [1], and promote microbial activity in soil [2–4].

One of the crucial byproducts of organic fertilizer use is the potentially available N [5], which is the most essential nutrient for plant growth [6]. In the soil, N is mainly present in organic form; however, plants can only absorb inorganic N, such as nitrate or ammonia [7,8]. Organic matter (OM) must be mineralized with a suitable carbon to nitrogen (C/N) ratio. When organic residues are added to soil, N and P content temporarily decline through immobilization by microorganisms [9]. Thereafter, N is gradually released from the residue [10]. The C/N ratio is a crucial factor affecting the decomposition rate of organic material [11]. For using any matter as compost, its C/N ratio should ideally be in the range of 25–30 [12]. Because the C/N ratio of plant meal is <20, it can be considered a rapidly available organic fertilizer [13].

Castor (*Ricinus communis* L.) is widely cultivated in India, China, and Brazil [14]. Castor oil contains abundant ricinoleic fatty acid, which is useful for industrial application; thus, it differs from other oils [15,16]. Castor meal (CM) is produced from the residue left after the extraction of oil from castor seeds. CM is toxic to animals because it contains a toxic protein ricin; however, it is not toxic to plants when used as an organic fertilizer, which is the most common use of this byproduct [17]. CM is often imported and sold as fertilizer to farmers in Taiwan because its N content is approximately 7–8% [18], which is higher than that of many organic fertilizers [19]. However, the high ricinine content in CM was found to cause toxicity in plants and the ecosystem if used in high doses. Following CM application to soil, a temporary toxic effect is occasionally observed for some days because of rapid mineralization, leading to the release of excessive N in the soil; however, the toxicity eases after 1–2 weeks [17,20]. Some studies have revealed that doses >4% (*v:v*) reduce seedling emergence, impair plant growth, and even cause plant death [21,22]. Moreover, CM contains high concentrations of ricinine, which poisons earthworms in soil if excessively applied [23]. Hence, use of an appropriate CM dose can promote remarkable plant growth.

This study examined the optimum CM dose for improving the growth of pak choi while minimizing its harmful effects on the ecosystem (e.g., earthworms).

2. Materials and Methods

2.1. Experimental Design and Treatments

The experiments were conducted using pots in replicates of four at the Department of Plant Industry, National Pingtung University, Pingtung, Taiwan, between September and December 2020 by using a completely randomized design. Each experimental pot (diameter 31.5 cm and depth 22 cm) was composed of five pak choi plants growing in a blend of sandy loam soil with various organic fertilizers mixed according to the experimental design. The temperatures of the daytime and nighttime were 25 and 18 °C, respectively. The soil temperature and soil moisture were 20–25 °C and 55–60% for the growth of earthworms. In each pot, 55 ± 5 g (10 individuals) of earthworms was introduced. The earthworms were obtained from Tai-Ping No. 2 (*Eisenia andrei*). Five concentrations of organic fertilizers were employed with 800 kg/0.1 ha application of livestock compost (LC), namely CM 25, 50, 100, 150, and 200 kg/0.1 ha (CM_{25}, CM_{50}, CM_{100}, CM_{150}, and CM_{200}, respectively). The amounts of organic fertilizers to be used in pots were calculated on the basis of pot area and weight of the topsoil. The CM contained OM (90.0%), N (5%), phosphorus oxide (P_2O_5; 2.1%), and potassium oxide (K_2O; 1.5%). The LC contained 61% OM, 2.5% N, 2.3% P_2O_5, and 2.0% K_2O.

2.2. Soil and Plant Analysis

A composite soil sample (0−30 cm) was collected before cultivation. The physiochemical characteristics of the studied soil and plant samples were assessed. Soil texture was determined using the hydrometer method according to Beretta et al. (2014). Soil OM was measured using the Walkley and Black dichromate oxidation method [24]. Soil salinity was measured through the salt bridge method by using an electrical conductance (EC) meter [25], and the soil pH was determined using a pH meter [26]. A mixture of ammonium fluoride (1 N) and hydrochloric acid (0.5 N) was used for P extraction from soil samples, and ammonium acetate (1 M adjusted at pH 7.0) was used for K extraction. P was measured through the molybdenum blue method by using a spectrophotometer (Hitachi U-2000, Japan) at 650 mm [27]. K was measured using a flame photometer (Corning 410, England) [28]. The available nutrients were measured after soil samples were digested according to the procedure by El-Ramady et al. (2014) [29]. The metals in soil and plant extracts were determined using inductively coupled plasma optical emission spectrometry (Jobin Yvon, Ultima 2000). A composite soil sample (0−30 cm) was collected from each experimental unit for studying available nutrients and physiochemical characteristics. The ground leaf samples were digested using concentrated sulfuric acid. Subsequently, the digested extracts were analyzed for N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn with standard methods used in soil analysis.

2.3. Determination of Pak Choi Traits

Ten plant samples from each experimental unit were sampled randomly to determine their traits. Plant samples were washed with tap water followed by distilled water and then converted to a mixture by using a blender. The total soluble solid values were measured using a digital refractometer according to the method by Ali et al. (2018) [30]. The plant height, leaf number, leaf width, root length, wet weight, and dry weight were estimated. The leaf area was calculated using Image J software.

2.4. Estimation of Earthworm Weight

After the plant traits were investigated, the soil in the pots was poured out, and earthworms were carefully collected and weighed. The weight of earthworms in each pot was recorded after removing the soil on their surface.

2.5. Statistical Analysis

The obtained results were statistically analyzed with one-way analysis of variance and Duncan test (at 0.05% probability), for which SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) was employed.

3. Results

The soil properties before the experiment were as follows: pH = 5.14, EC = 53.5 μS/cm, OM = 3.5%, P_2O_5 = 23.0 kg/ha, and K_2O = 43.6 kg/ha (Table 1). After treatments with organic fertilizers, the pH of all soils increased. Among the different treatments, LC application led to the highest increment in soil pH. However, the CM application exhibited the opposite trend, with the application of 200 kg/0.1 ha showing the lowest pH increment. Conversely, because LC leads to higher salinity and slower nutrient release compared with CM [31], the increase in pH was the highest with LC_{800}. Soil EC increased with CM concentration. The OM (organic matter) of soil was highest with the LC_{800} (4.4%), and it increased following CM application. Among the treatments of CM, it was the highest with CM_{150} and CM_{200} (4.3%). The P contents of soil were similar across all the CM_{200} and LC_{800} treatments, and they were significantly higher than CM_{25}. The K contents of soil increased following CM treatment, and the highest level was observed with LC_{800}. The K contents in the treatments of LC800 were significantly higher than CM. N concentration in plants increased with CM concentrations (Table 2). N in plants with CM_{200} treatment was significantly higher than that with CM_{25} treatment. However, N with CM_{25} treatment was higher than that with LC800 treatment by 8.3%. Both P and K concentrations in plants were highest with CM_{200} than other treatments. The plant height and leaf number were not significantly different between all treatments. The leaf area, leaf width, fresh weight and sugar degree of pak choi were all highest with the LC_{800}. However, the leaf area, leaf width, fresh weight, dry weight, and sugar degree of pak choi increased progressively with the increased application of CM. Although the traits of pak choi were optimal with the LC_{800} treatment, they were not significantly different from those with the CM_{200} treatment (Table 3). Comparison of pak choi traits with various soil samples is presented in Figure 1.

Table 1. The soil properties before and after treatments of different rate of organic fertilizer.

Treatments	pH	[3] EC (μS/cm)	[4] OM (%)	P_2O_5 (kg/ha)	K_2O (kg/ha)
Before experiment	5.14 b [5]	53.5 ab	3.5 c	23.0 a	43.6 bc
[1] CM_{25}	5.61 a	35.3 bc	3.9 bc	18.5 b	27.1 c
CM_{50}	5.54 a	26.8 c	3.8 bc	23.4 a	27.3 c
CM_{100}	5.53 a	33.1 bc	4.0 bc	20.2 ab	29.4 c
CM_{150}	5.45 a	42.3 b	4.3 a	21.8 ab	29.9 c
CM_{200}	5.43 a	79.7 a	4.3 a	23.0 a	35.1 bc
[2] LC_{800}	5.65 a	42.9 b	4.4 a	23.4 a	71.8 a

[1] CM_{25}, CM_{50}, CM_{100}, CM_{150}, CM_{200}: The application rate of castor meal 25, 50, 100, 150 and 200 kg/0.1 ha. [2] LC_{800}: the compost in accordance with national standard 800 kg/0.1 ha. [3] EC: Electrical conductance. [4] OM: Organic matter. [5] The same letter in the same column of means no significant difference with 0.05 level according to Duncan's multiple range test.

Table 2. The nutrient concentrations in the leaves of Pak Choi after the application of organic fertilizers.

Treatments	N (%)	P (%)	K (%)
[1] CM_{25}	3.00 bc [3]	0.239 c	3.40 c
CM_{50}	3.01 bc	0.264 bc	3.67 bc
CM_{100}	3.17 bc	0.280 bc	3.69 bc
CM_{150}	4.02 a	0.281 bc	3.98 a
CM_{200}	4.13 a	0.334 a	4.01 a
[2] LC_{800}	2.75 c	0.299 a	4.39 a

[1] CM_{25}, CM_{50}, CM_{100}, CM_{150} and CM_{200} represented the rate of castor meal 25, 50, 100, 150 and 200 kg/0.1 ha. [2] LC800 represented the application of livestock compost 800 kg/0.1 ha. [3] The same letter in the same column of means no significant difference with 0.05 level according to Duncan's multiple range test.

Table 3. The traits of Pak Choi by the treatments of organic fertilizers.

Fertilizers Rate (kg)	Plant Height (cm)	Leaves Number (No.)	Leaf Area (cm^2)	Leaf Width (cm)	Fresh Weight (g)	Dry Weight (g)	Root Length (cm)	Sugar Degree (°Bx)
[1] CM_{25}	24.2 a [3]	12 a	82.6 c	5.3 c	18.8 c	1.30 c	12.5 c	1.4 c
CM_{50}	22.2 a	12 a	90.2 c	6.6 b	20.3 b	1.52 b	12.6 c	1.9 b
CM_{100}	25.3 a	12 a	105.4 b	6.6 b	23.7 ab	1.76 ab	15.9 b	2.5 a
CM_{150}	25.1 a	11 a	120.9 b	7.5 ab	24.1 a	1.85 a	16.7 b	2.5 a
CM_{200}	20.4 a	10 a	169.6 a	7.8 a	27.1 a	1.98 a	18.0 a	2.8 a
[2] LC_{800}	22.7 a	10 a	231.4 a	8.3 a	28.2 a	1.85 a	21.4 a	3.0 a

[1] CM_{25}, CM_{50}, CM_{100}, CM_{150} and CM_{200} represented the rate of castor meal 25, 50, 100, 150 and 200 kg/0.1 ha. [2] LC800 represented the application of livestock compost 800 kg/0.1 ha. [3] The same letter in the same column of means no significant difference with 0.05 level according to Duncan's multiple range test.

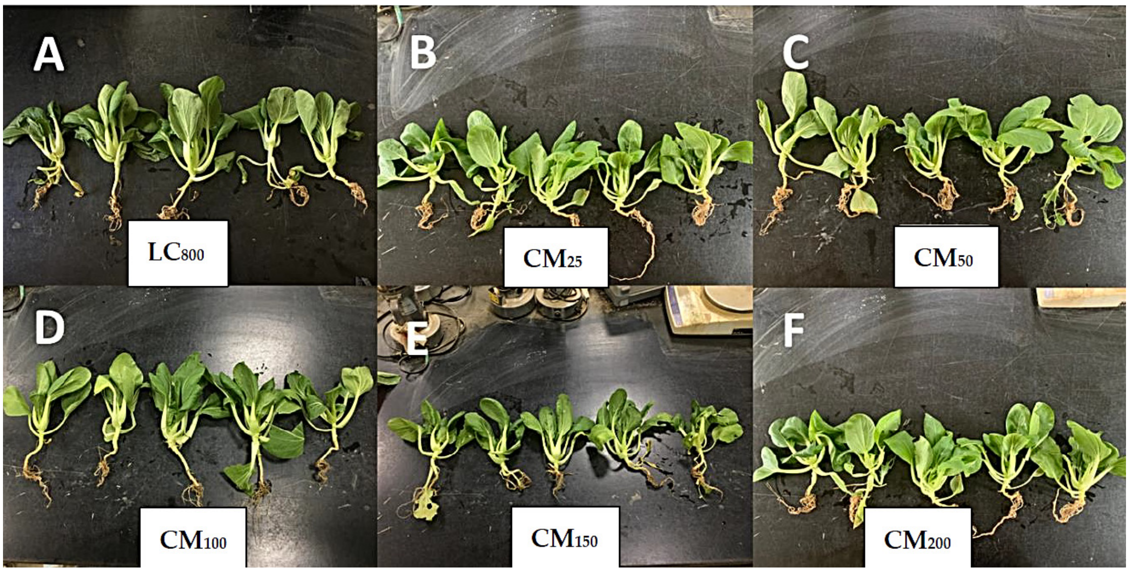

Figure 1. The growth of Pak Choi (*Brassica chinensis* L.) by the treatments of organic fertilizers. (**A**) LC_{800}, (**B**) CM_{25}, (**C**) CM_{50}, (**D**) CM_{100}, (**E**) CM_{150}, (**F**) CM_{200}. CM_{25}, CM_{50}, CM_{100}, CM_{150} and CM_{200} represent the rate of castor meal 25, 50, 100, 150 and 200 kg/0.1 ha. LC800 represented the application of livestock compost 800 kg/0.1 ha.

The investigation of earthworms in soil samples revealed that the weight of earthworms in soils decreased with all treatments compared with that before the treatment. No significant difference was observed between treatment with 25, 50, and 100 kg/ha CM; however, treatment with 150 and 200 kg/0.1 ha CM revealed significant difference compared with the aforementioned three treatments (Figure 2).

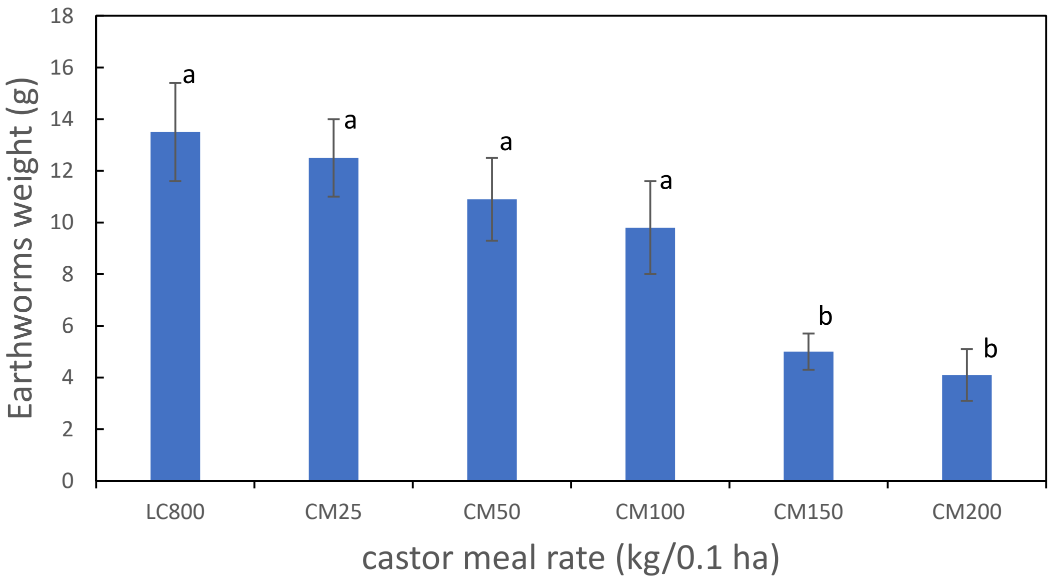

Figure 2. The earthworms variation after the harvesting of Pak Choi (*Brassica chinensis* L.) in soil by the treatments of organic fertilizers. (CM_{25}, CM_{50}, CM_{100}, CM_{150} and CM_{200} represented the rate of castor meal 25, 50, 100, 150 and 200 kg/0.1 ha. LC800 represented the application of livestock compost 800 kg/0.1 ha). The same letters (a, b) in the graphic represent no significant difference with 0.05 level according to Duncan's multiple range test.

4. Discussion

Appropriate application of organic fertilizers may improve soil physicochemical properties, consequently increasing root growth and the uptake of essential nutrients [32]. Organic fertilizer application can improve soil moisture in diverse terrains [33]. In the current study, the pH and EC of soil increased after the application of organic fertilizers because OM has higher cation exchangeable capacity and can adsorb hydrogen ions in soil solution; however, the rapid release of large amounts of minerals causes temporary soil acidification [34]. Conversely, because LC leads to high salinity and slow nutrient release [31], the pH increment was the highest with LC_{800} treatment (near CM_{200}). Moreover, leaf area, leaf width, leaf weight, root length, and sugar degree of pak choi increased with the application dose of CM. The highest concentration of CM (200 kg/0.1 ha) significantly enhanced the traits of pak choi in comparison with the lowest CM concentration (25 kg/0.1 ha). Although LC_{800} application exhibited the greatest improvement in all plant traits, the main traits, including plant height, plant weight, dry weight, and sugar degree, were not significantly different from those with CM_{100}, CM_{150}, CM_{200}, and LC_{800} application (Figure 1). Abe, Songmuang, and Harada (1995) [35] studied the response of rice plants to various CM concentrations and reported that 200 kg/0.1 ha CM improved the growth and quality of rice in comparison with the nonamended soil. Gupta, Antil, and Narwal (2004) [36] indicated that CM of approximately 100–150 kg/0.1 ha should be applied at least three weeks before sowing of wheat and the field must be kept moist for the degradation of toxicants. Approximately 100 kg/0.1 ha castor cake increases the production of marketable fruits (1.78 kg per plant) compared with controls [37]. The current study clearly demonstrated that CM concentrations of 100, 150, and 200 kg/0.1 ha significantly increased the fresh weight, dry weight, root length, and sugar degree by 31%, 34%, 30%, and 50%, respectively, in comparison with 25 kg/0.1 ha. Moreira et al. (2014) [38] assessed the effect of CM concentration on pak choi plants grown in a greenhouse, and they found that 100–150 kg/0.1 ha CM increased the diameter of lettuce head and leaf numbers by 5.5 and 19.6%, respectively. Hilioti et al. (2017) reported that CM as an organic fertilizer can enhance soil fertility and increase the nutrient uptake in crops [39]. The results of the present field trials illustrated that fortifying the soil with CM_{200} increased soil EC, OM, P, and K by 56%, 9%, 20%, and 23%, respectively, compared with CM_{25}; furthermore, this CM concentration significantly enhanced the uptake of N, P, and K by 27%, 28%, and

15%, respectively, compared with CM_{25}. Increasing the OM in soil improves the nutrient availability and enhances the physiochemical properties of the soil [40,41]. In the current study, fortification of the soil with CM (CM_{200}) increased the soil EC and OM by 32.9% and 19.4%, respectively, compared with before the experiment, and by 56% and 10%, respectively, compared with CM_{25} (Table 3 and Figure 1). The leaf nutrient concentration increased with CM concentration (Table 2). CM_{200} increased the N, P, and K concentration by approximately 27%, 28%, and 29%, respectively. These findings are similar to those of relevant studies indicating higher N, P, and K uptake by wheat and solanaceous plants after CM application [36,42].

CM contains ricin residue, and, hence, CM application to the soil introduces ricin in the soil. Ricin toxin was confirmed to be harmful to animal cells [43]. Stillmark observed that ricin causes agglutination of red blood cells and promotes protein precipitation in serum. The molecule is formed by two chains (A and B) bonded by a single disulfide bond [44]. Chain A is a ribosome-inactivating enzyme of 32 kDa, whereas chain B is a galactose/N-acetylgalactosamine-binding lectin of 34 kDa [45]. Both chains are required for the high toxicity of ricin and inhibition of protein assimilation in cells. In the current result, the traits of pak choi were better or similar with the application of CM_{100} to CM_{200} kg/0.1 ha. However, CM application of <150 kg/0.1 ha was most effective at ensuring the survival of earthworms. Therefore, the optimal dose of CM for obtaining a balance between crop production and the reduction of toxicity to earthworms based on primary evaluation was 100–150 kg/0.1 ha.

5. Conclusions

Not only crop productivity but also ecosystem safety should be considered. Castor meal (CM) is a fertilizer of high nitrogen and is often applied by farmers for the supplement of plant nutrition. However, the effect of CM on the ecosystem (e.g., survival of the earthworm) has rarely been considered. This study primarily evaluated the optimum concentration of CM required to balance crop productivity with ecosystem safety. The optimal dose of CM was determined to be 100–150 kg/0.1 ha for obtaining a balance between crop production and the reduction of toxicity to earthworms.

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