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Effects of Vermicompost and Hog Manure on Growth, Yield and Antioxidant Activity of *Agrimonia pilosa* Ledeb as a Medicine Plant

Quang-Ung Le[1], Horng-Liang Lay[2], Ming-Chang Wu[3], Tien-Dung Nguyen[4] and Thi Thu-Dung Cao[5]

[1]Department of Tropical Agriculture and international Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[2]Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[3]Department of Food Science, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
[4]Institute of Regional Research and Development, No 70, Tran Hung Dao street, Hoan Kiem District, Ha Noi
[5]Institule of Agriculture and Resources, Vinh University, Vinh city

(Corresponding author: Quang-Ung Le)
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ABSTRACT: The effects of different vermicompost and hog manure rates on growth, yield, total flavonoid and phenolic content, antioxidant activity of *Agrimonia pilosa* Ledeb (AL) were investigated. Nine treatments were applied, combining vermicompost (VC) with hog manure (HM) at rates: 0 + 0 (control treatment, T1), 0 + 11.25 (T2), 0 +16.875 (T3), 12.5 + 0 (T4), 12.5 +11.25 (T5), 12.5 + 16.875 (T6), 25 + 0 (T7), 25 +11.25 (T8), 25 +16.875 ton ha^{-1} (T9). The results demonstrated that pH between 6.9 and 7.4, EC from 0.5-0.6 mS cm^{-1} are optimal for herbal growth. Additionally, P_2O_5, K_2O_5 and Mn had coherent coefficient with herbal quality. A mixture of the VC and HM made with the rate of 12.5 + 16.875 ton ha^{-1} produced best parameters and is therefore recommended that these fertilizers served as potential source of nutrient for the AL. Further detailed investigations of them for other herbs are in progress.

Keywords Agrimonia, Herbal quality, Growth, Vermicompost, Yield

INTRODUCTION

Agrimonia pilosa Ledeb is a traditional medicinal plant belongs to Rosaceae in Asian countries and has been reported to possess various medicinal importances (Zhu *et al.* 2009). It has been used traditionally for treatment of abdominal pain, sore throat, headaches, bloody discharge, parasitic infections and eczema in Korea and other Asian countries since centuries (Kato *et al.* 2010). Pharmacologically, the AL has been reported to possess anti-tumor (Koshiura *et al.* 1985), anti-viral (Shin *et al.* 2010), anti-oxidant (Zhu *et al.* 2009), anti-microbial (Yamaki *et al.* 1989), and anti-hyperglycemic activity (Jung *et al.* 2010).

Vermicompost (VC) and hog manure (HM) are environmental friendly materials. VC has many favorable physicochemical characteristics, making it suitable for mixture in substrates, including high porosity and good aeration, drainage, and water-holding capacity (Edwards and Burrows 1988). Vermicompost, in contrast to conventional compost, is the product of an accelerated bio-oxidation of organic matter by the use of high densities of earthworm populations without passing a thermophilic stage (Subler *et al.,* 1998). The earthworm-processed organic wastes are finely divided peat-like materials with high porosity, aeration, drainage, and water-holding capacity (Ewards and Burrows 1988). Compared to conventional compost, which passes a thermophilic stage, VC usually has a much finer structure and larger surface area, providing strong absorbability and retention of nutrients (Shi-wei and Fu-zhen 1991). They contain nutrients in a soluble form that is readily taken up by plants, such as nitrates, soluble potassium, exchangeable phosphorus, and magnesium, and calcium (Grappelli 1987) and may contain biologically active substances such as plant growth regulators. Moreover, vermicompost could release nutrients slowly and steadily into the system and enables the plants to absorb these nutrients over time (Sharma 2003). Based on all these characteristic, earthworm-processed organic waste would have great commercial potential in the herbal industry as container media for growing bedding and herb plants. There is strong scientific proof that vermicomposts have an considerable effect on the growth and productivity of plants (Edwards 1998).

In this study, we have cultured *Agrimonia pilosa* Ledeb in net house condition and examined the effects of VC on growth, yield, total phenolic content, antioxidant activity in this plant. The main objectives of this study are to determine the best organic fertilizer mixture for this herb.

MATERIALS AND METHODS

A. Experimental Design

A field pot experiment was carried out from spring to winter 2016 in the Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung, Taiwan. Before plant culture, the soil and substrates was mixed and analyzed for determination of texture and macro and micro element components. Nine medium mixtures were created by mixing VC with HM at rates: 0 + 0 (control treatment, T1), 0 + 11.25 (T2), 0 +16.875 (T3), 12.5 + 0 (T4), 12.5 +11.25 (T5), 12.5 + 16.875 (T6), 25 + 0 (T7), 25 +11.25 (T8), 25 +16.875 ton ha^{-1} (T9), respectively. A randomized complete block design with nine above treatments and three replications per treatment were used. Each replicate was prepared as one block with 27 pots. One plant 25 days old and uniform seedling of AL having 2-3 leaves were transplanted to per pot. The plants were irrigated based on their water demand with distilled water during the growing period; the irrigation scheduling and water quantity were equal to all treatment. No additional fertilization was provided.

B. Chemical and Physical Characterization of the Substrates

The pH and electrolyte conductivity (EC) of the other media were determined using a doubly distilled water suspension of each medium in the ratio of 1:10 (w/v) (Inbar *et. al* 1993) that has been agitated mechanically for 2 hours, before the measurements were made using a pH meter (UltraBasic-UB10; Denver Instrument, NY) and EC meter (SC-2300 conductivity meter; Suntex Instrument Co. Ltd., New Taipei city, Taiwan). Organic matter (OM) was determined by Walkley Black method. The nutrient elements including P, K, Ca, Mg, Mn, Cu and Zn were analyzed by inductively coupled plasma atomic emission spectroscopy after element extraction with H_2O and 0.1 N HCl acid.

C. Growth and Yield parameters

The height (cm), number of leaves and shoots of the plants were recorded at 10 days intervals after transplanting (from 10 to 120 DAT). Stem and canopy diameter were recorded at 30 days intervals after transplanting (30, 60, 90 and 120). The chlorophyll of leaf was determined by Wintermans and DeMots (1965) method. The plants were harvested at yellow leaves and separated into shoots and roots. The roots were cleaned carefully to remove adhering soil particles. Root volume (cm^3) was measured by submerging roots in a measuring cup with water. The leaves, shoots and roots were air dried for several days oven dried at 65 ^{0}C for 72 hours and dry weights were recorded.

D. Determine total phenolic and flavonoid content

Extract and fraction of extract. The fresh parts of these herbs were washed by distilled water and dried in oven at 44 ^{0}C for 48 hours. The dry materials were crushed into 50-mesh sieve powder. Extracts were obtained as follows: 5 grams of herb was separately extracted twice with methanol 70% (v/v) in circulator bath for 2 hours at 80 ^{0}C and then was filtered. The filtrates were combined and concentrated in a vacuum evaporator at 45 ^{0}C. The dehydrated fractionation were weighted to calculate yield, then dissolved in dimethyl sulforide (DMSO) to a regular concentration and the activity was measured.

Total phenonlic content. The total phenolic content was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The reaction mixture was composed of 0.1 mL of extract, 7.9 mL of distilled water, 0.5 mL of the Folin-Ciocalteu's reagent, and 1.5 mL of 20% sodium carbonate. The absorbance was measured at 765 nm in a UV-3000 Spectrophotometer (the same equipment was used in all the assays). The total phenolic content were calculated using a calibration curve for gallic acid (0-10 microgram/mL) with $y = 0.0149x + 0.0261$; $R^2 = 0.967$, curve standardized in the lab for the calculation of gallic acid equivalent per gram of extracts. All samples were analyzed thrice and results averaged.

Total flavonoid content. The total flavonoid content was determined according to Meda et al. (2005) using quercetin as a standard.Total flavonoids content was determined by using the aluminium chloride colorimetric method. A mixture of 0.5 mL sample, 100 µL 10% aluminum chloride, 100 µL 1 M potassium acetate and 2.3 mL distilled water were incubated at room temperature for 30 minutes. The absorbance was measured at 415 nm. Quercetin (0.2-1 mg/mL) as a standard was used to make the calibration curve with $y = 0.003x + 0.00$; $R^2 = 0.999$. The estimation of total flavonoid in the extracts was carried out in triplicate and the results were averaged.

E. Antioxidant activity

Assay for the scavenging of stable free radical 1.1-diphenyl-2-picrylhydrazyl (DPPH) was carried out as reported earlier with some modification (Blois, 1958). Briefly, 100 µL sample in different solvents was mixed with 1.9 mL of 0.1 mM DPPH in ethanol. The concentration of the tested samples in the mixture was 0, 5, 10, 20, 40, 80, 160, 320 µg/mL, respectively. The reaction mixture was shaken well and incubated in dark at 37 ^{0}C for 30 min and the absorbance was measured at 517 nm. The blanks contained all the reaction reagents except the extract of positive control substances. BHT was used as positive control with IC_{50} 10.08 µg/mL. The percentage scavenging values were calculated from the absorbance of the blank (A_o) and of the sample (A_s) using the following equation: DPPH radicals scavenging activity (%) = $(1-A_s/A_o)$ x 100. Where A_s was the absorbance of the sample and A_o was the absorbance of the blank (without sample).

F. Data Analyses

Data were subjected to a one-way analysis of variance (ANOVA) to test for least significant differences (LSD), and all analyses were performed using the SAS statistical package (SAS Institute 1990).

RESULTS AND DISCUSSION

A. Chemical and Physical Characterization of Media

The pH and EC affect to rate of organic matter, the nutrient uptake and root activity. There is a significant increase for EC of substrates with an increase in the VC and HM mixture proportion at $P < 0.05$ (Table 1) but there is a slight decrease for pH. The greatest value for pH, EC was recorded at T1, T9 (pH 8.33, EC 0.61, respectively) and the lowest value was for T9, T1 (pH 6.97, EC 0.03, respectively) ($P < 0.05$). Sonneveld and Voogt 2009 showed that the optimum pH for availability of essential elements in soilless media is about 6.8. The optimal EC of substrates for plant growth is between 0.07 and 3.5 dS m^{-1} (Warncke 1986). The present study indicate that pH between 6.9 and 7.4, EC from 0.5-0.6 mS cm^{-1} are good for growth, yield and herbal quality of the A L. The OM significantly increased with increasing HM rate. T6 gave the greatest value for OM (3.96 percent) whereas the low OM was obtained with T1, T4 and T7 without HM (1.58-1.82 percent). There were significant differences in concentrations of available macro-and micro-nutrients measured by extraction by 0.1 N hydrochloric (HCl) acid between various media were presented in Table 1. All of macro – and micro nutrients were significant increased when increasing HM rate in media especially in mixture of HM and VC.

B. Morphological parameters of Agrimonia pilosa Ledeb

Vermicompost and hog manure significantly affected plant growth parameters. Table 2a and 2b show some significant difference in the growth of AL under various treatments.

Table 1: Chemical and physical characteristics of different vermicompost rate mixture with hog manure.

Treatment	pH (1:10)	EC (mS cm^{-1})	OM (%)	P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})	CaO (mg kg^{-1})	MgO (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
T1	8.33a	0.03i	1.58h	3.7h	36.29i	587.19g	103.24h	13.61h	0.22f	1.52g
T2	7.88b	0.228g	2.68e	171.82f	107.69h	2570.16d	684.96e	37.63d	2.37d	43.84d
T3	7.67c	0.30e	3.83b	327.24e	137.01g	2565.99d	1188.4b	38.09d	2.59c	45.19c
T4	7.44d	0.217h	1.82h	15.87g	320.36e	592.09g	124.70g	20.22g	0.09g	4.17f
T5	7.28e	0.37d	2.19f	359.41d	288.31f	1896.02e	594.75f	32.96e	1.41e	27.33e
T6	7.21e	0.50c	3.96a	476.99b	470.32b	2947.46b	850.75c	54.20b	2.62b	47.02b
T7	7.3e	0.28f	1.80g	21.107g	330.72d	661.96f	129.40g	21.25f	0.08g	1.75g
T8	7.05f	0.59b	2.90d	429.2c	492.1a	2831.15c	818.07d	47.25c	2.58c	46.57b
T9	6.97f	0.61a	3.49c	619.4a	420.14c	5595.78a	1399.9a	61.62a	4.93a	91.30a
LSD 0.05	0.12	0.005	0.03	12.02	5.61	27.854	8.36	0.47	0.03	0.50

Notes. EC, (electrolyte conductivity; OM, organic matter; LSD, least significant differences. Means followed by the same letters do not significantly differ ($P < 0.05$).

The understanding values for chlorophyll a-b, leaf number, branch number, fresh and dry leaf weight were obtained in T9 with 9.37-3.63 g kg^{-1}, 24.5 leaf, 5.6 branches, 55.62 and 14.08 g $plant^{-1}$, respectively ($P < 0.05$). T6 gave the second place which also had many high values for chlorophyll a, leaf number, branch number, fresh-dry leaf weight and fresh-dry stem weight. This data indicates that at manure mixture including the same amount of hog manure and an increase in vermicompost concentration, some growth indexes correspondingly increased. The lowest values in most of target were in T1 ($P < 0.05$).

These results are in good agreement with the findings of several researchers which revealed that organic manure increasing the vegetative growth and biomass production effectively (Dinesh *et al.* 2010). Plant growth parameters including shoot length, leaf number, shoot and root dry weights, fruit number and fresh fruit weight were better in VC treated pots than the NPK fertilizer treated pots (Kashem *et al.* 2015). The application of VC and HM had no significant effect on the plant height (Table 2a). This could be explained by coherent relationship with themselves genetic characteristic. Ha Duy Truong *et al.* 2015 reported that addition of VC had no significant effect on the seedling diameter, plant height, and leaf number. Stem diameter increased significantly with mixture including similar VC concentration and an increase of hog manure in medium, which illustrate obviously in T4 –T5 and T8-T9 with 8.34-8.51 and 7.84-8.16 cm plant^{-1}. This shows VC was added increasingly hog manure positively influencing on stem growth with horizontal size and suggests that hog manure support suitable physicochemical properties for stem development. The differential response of plants to diverse doses of the VC may be due to release of variable amount of available nutrients and growth promoting substances (Tomati *et al.* 1990).

Table 2a: Effects of different fertilizer rates on the morphological growth of *Agrimonia Pilosa* Ledeb after 120 days.

Treatment	Chlorophyll (g kg^{-1})		Plant height (cm)	Leaf number (leaf)	Branch number	Stem Diameter (cm)	Canopy diameter (cm)
	a	b					
T1	7.65d	1.71i	91.17a	20.5b	1.33d	6.75b	33.5b
T2	9.41a	3.07d	88.5a	23.17ab	3.33c	7.86ab	45.75a
T3	9.2b	2.8g	87.67a	23.33ab	3.17c	8.16ab	41.16a
T4	9.23b	2.87f	84.5a	23.5ab	3.67c	8.34a	41.83a
T5	9.35a	3.33b	91.17a	23.17ab	4.17bc	8.51a	42.75a
T6	9.33a	3.16c	89.5a	24.00ab	5.33ab	7.83ab	43.00a
T7	8.68c	2.17h	80.33a	21.17ab	3.00c	7.96ab	44.42a
T8	9.33a	2.98e	90.67a	21.00ab	3.80c	7.84ab	42.33a
T9	9.37a	3.63a	87.67a	24.5a	5.6a	8.16ab	45.25a
LSD	0.095	0.064	11.11	3.7	1.32	1.48	6.76

Table 2b: Effects of different fertilizer rates on the morphological growth of *Agrimonia Pilosa* Ledeb after 120 days.

Treatment	Root weight (g plant^{-1})		Leaf weight (g plant^{-1})		Stem weight (g plant^{-1})		Root volume (cm^3 plant^{-1})
	Fresh	Dry	Fresh	Dry	Fresh	Dry	
T1	23.39e	3.92g	20.32g	4.70g	16.7h	4.21f	14.00f
T2	38.57b	8.68c	37.70f	8.95f	26.79d	6.49c	27.88e
T3	28.03d	6.44f	48.79cd	11.62cd	28.17b	6.75bc	29.88e
T4	40.35b	7.87d	43.42e	10.35e	23.3e	5.60e	34.89b
T5	40.83b	8.03d	48.88c	12.2bc	23.49e	6.12d	37.21a
T6	34.00c	7.79d	51.48b	12.7b	38.86a	10.27a	34.77bc
T7	48.45a	9.24b	47.59d	11.35d	21.67f	5.42e	37.90a
T8	51.21a	11.76a	49.16c	11.76cd	18.31g	4.53f	32.63cd
T9	41.38b	7.05e	55.62a	14.08a	27.19c	6.95b	32.59d
LSD	3.86	0.47	0.62	1.25	0.37	0.37	2.15

***Notes*:** LSD, least significant difference. Means followed by the same letters do not significantly differ ($P < 0.05$).

The enhancements in plant growth could be due partially to great increase in soil microbial biomass after VC and HM applications, leading to production of hormones in the fertilizer acting as plant – growth regulators independent of nutrient supply. It could be concluded that increasing organic matter to AL herbs induced more growth parameters. This may be due to the ability of organic manure to provide for the growth plants with micro and macronutrients demanding for their growing.

Moreover, increased manure leads to reduction of soil compressibility, because organic matter acts as a cushion and it prevents the transfer of stress to the lower ground, which created suitable conditions for plant growing (Mirshekari and Forouzandeh 2015). There is much proof that the activity of earthworms accelerates organic matter mineralization, decomposition of polysaccharides, increase the humus in the soil, and oppositely reduce the availability of toxic heavy elements to plants (Dominguez *et al.* 1997).

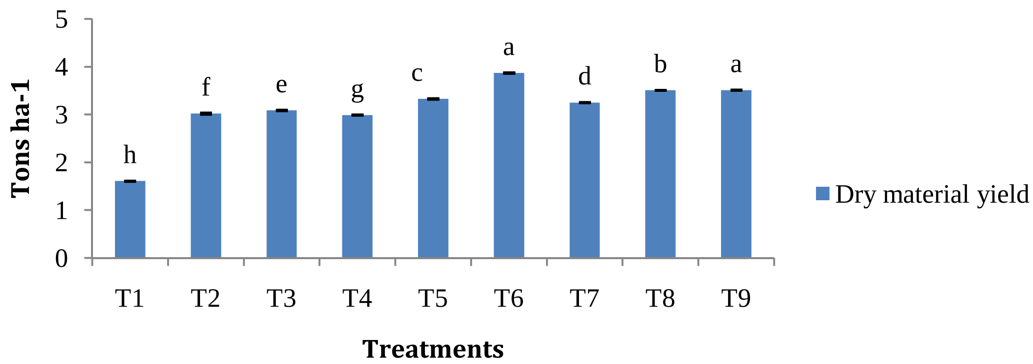

Fig. 1. Dry material yield per ha after 120 days planting of *Agrimonia pilosa* Ledeb.

The Figure 1 provides information about average dry yield per ha after 120 days planting. There are some significant differences ($P < 0.05$) among treatments in average dry material yield per ha. The maximum dry yield per ha 3.868[a] tons was observed in 12.5 VC tons + 16.875 HM tons/ha (T6) fertilizer rate followed by 3.511[b] and 3.507[b] tons ha^{-1} in T9 and T8, respectively. Treatment T1 gave the lowest values for dry yield per ha. This demonstrates obviously that organic fertilizer application positively affect to dry yield. Moreover, the data show that yield slowly increase from 46.47 to 58.47% compared to the control when VC and HM rate were be added more. It could be concluded that the increment in plant dry weight may be attributed to the increase in number of branches/plant shown obviously in Table 3a. It was reported that earthworms and their vermicompost may induce excellent plant growth and enhance crop production. Glasshouse studies made at CSIRO Australia found that the earthworms (*Aporrectodea trapezoids*) increased growth of wheat crops (*Triticum aestivum*) by 39%, grain yield by 35% (Baker et al. 1997). With fruit crops, study found that worm waste (vermicompost) boosted grape yield by two-fold as compared to chemical fertilizers (Buckerfield and Webster, 1998). With vegetable crops, studies on the production of important vegetable crops like eggplant (*Solanum melangona*), tomato (*Lycopersium esculentus*), and okra (*Abelmoschus esculentus*) have yielded very good results (Sinha *et al.* 2009). The sodicity of the soil was also reduced and nitrogen (N) contents increased significantly. The VC increased dry weight in french marigold, pepper, tomato and cornflower (Bachman and Metzger, 2008). In this respect, it is possible that the favorable influence of organic manure on dry yield may be due to their ability to enhance the physiological, biochemical, and biological properties of the soil.

C. Effects of fertilizer on Total Phenolic and flavonoid content and antioxidant capacity of Agrimonia pilosa Ledeb

Phenolics and flavonoids are widely found products derived from plant sources, and they have been shown to possess significant anti-oxidant properties. The high amount of phenols and flavonoids in extracts may explain their high anti-oxidant activities (Ebrahimzadeh *et al.* 2010). The yield of studied herb medicine extracts, content of total phenolics in Table 3 is shown. The total phenol and flavonoid content were increased with using organic fertilizer. This is clear when increasing HM level and it could be explained that HM manure contains macro-and micro-nutrients more than that of VC. The highest these was observed with using 12.5 +16.875 ton ha^{-1} (8.16 mg GAE/g dw and 35.18 mg QE/g dw) followed by T9 and T8 at the second and third place while it is understanding that organic level of these two treatment is higher than that of T6. Moreover, when it is compared with control treatment without using organic fertilizers (5.89 mg GAE/g dw and 27.44 mg/g dw), this results emphasized that the suitable mixture of organic fertilizer caused increased in herbal quality in AL. It is considerable that among all treatments total flavonoid contents were higher than the phenol contents in each treatment.

Table 3: Effects of different fertilizer rates on total phenolic content and antioxidant activity of *Agrimonia pilosa* Ledeb.

Treatment	Total phenol content [1] (mg GAE/g DW)	Total flavonoid content[2] (mg QE/g DW)	IC_{50}[3] of DPPH radical scavenging (µg/mL)
T1	5.89g	27.44e	75.33a
T2	6.18f	30.33c	46.27b
T3	6.23ef	28.34b	38.40cd
T4	6.00fg	29.21d	42.87b
T5	6.83d	33.14b	35.73de
T6	8.16a	35.18a	23.15f
T7	6.48e	29.26cd	39.34c
T8	7.16c	32.84b	34.74e
T9	7.54b	32.61b	23.91f
LSD	0.278	1.12	3.44

Notes: [1]Data expressed as mg of gallic acid equivalents per dry weight (DW)

[2]Data expressed as mg of quercetin equivalents per dry weight (DW)

[3]IC_{50}: Data expressed as µg per milliliter. Lower IC_{50} values indicated the higher radical scavenging activity. Means with different letters were significantly different at the level of P <0.05.

LSD, least significant difference.

Basically, a higher DPPH radical-scavenging activity is related to a lower IC_{50} value. This data indicated T6 and T9 had better antioxidant potential (IC_{50} 23.15 and 23.91, respectively). Lei and Young 2014 reported that the scavenging activity of the AL methanol extract reached to 55.6% at the concentration of 50 µg/mL, which shows antioxidant capacity of two these treatments being higher. All of treatment applied organic fertilizer have IC_{50} being higher than that of BHT (IC_{50} 10.08 µg/mL). This herb strong antioxidant activity and can be used as a good source of natural antioxidants for medicinal purposes. The highest IC_{50} value was recorded in the control treatment without using organic fertilizer. Results emphasized that organic application in herbal production produced better pharmaceutical quality. This can be explained by continuous mineralization of organic manure, which helps them accumulates enough nutrient quality leading to exchange inside quality.

Table 4: Pearson correlation between macro- and micro nutrients and herbal quality of *Agrimonia pilosa* Ledeb.

Traits	P_2O_5 mg kg^{-1}	K_2O mg kg^{-1}	CaO mg kg^{-1}	MgO mg kg^{-1}	Mn mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}
Total phenolic content	0.83^{**}	0.80^{**}	0.67^{*}	0.57^{ns}	0.84^{**}	0.64^{ns}	0.64^{ns}
Total flavonoid content	0.78^{*}	0.77^{*}	0.58^{ns}	0.47^{ns}	0.77^{*}	0.55^{ns}	0.56^{ns}
Antioxidant activity	0.76^{**}	0.80^{**}	0.65^{ns}	0.64^{ns}	0.80^{**}	0.63^{ns}	0.65^{ns}

Notes: Ns-No significant; *- significant at 5% probability; **- significant at 1% probability

Results in table 4 showed that P_2O_5, K_2O_5 and Mn had coefficient with total phenolic and flavonoid content and antioxidant activity; CaO had coefficient correlation with total phenolic content. It can be concluded that total phenolic and flavonoid content on herbal extracts is not limited to these minerals. These results suggest that it can combine application of organic fertilizers with adding more P_2O_5, K_2O_5, CaO, Mn for this herb to increase better herbal quality.

CONCLUSION

In conclusion, this study provides scientific proofs for benefits of organic fertilizer application in herbal productions. It may be concluded that VC and HM could be seen as great potential component of growing media. Our results reveal that pH between 6.9 and 7.4, EC from 0.5-0.6 mS cm^{-1} are good for growth, yield and pharmaceutical quality of AL, which is an important characteristics in AL production. In addition, application of VC and hog manure (12.5 +16.875 ton ha^{-1}) mixture produced the highest parameters of this herb and is therefore recommended.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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