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Effects of *Azolla* Compost Versus Peat and Cocopeat on the Growth and Nutrition of Chrysanthemum (*Chrysanthemum morifolium*) in Pot Culture

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Abstract

This experiment was performed to evaluate the application of *Azolla* compost in growth environments containing peat + perlite in comparison with cocopeat + perlite on the growth and nutrition of chrysanthemums in a completely randomized design with five levels of *Azolla* (0, 25, 50, 75 and 100 %) in three replication. It was found that the nutrients of *Azolla* compost substrates were richer than peat and cocopeat. The results showed that use of *Azolla* as compost increases the elements of phosphorus and potassium in the growing medium, but only the potassium increases in the leaves. The highest amount of leaf nitrogen and potassium was at 50 % compost. 25 to 57 percent compost was not significantly different from peat or cocopeat in terms of final plant hieght, flowering branch length, number of leaves and petal carotenoids. With increasing compost, physical properties of the substrates were within the recommended range, and bulk density (0.31, 0.33, 0.30 and 0.30 g cm^{-3}, respectively) increased compared to peat and cocopeat (0.18 and 0.16 g cm^{-3}). One hundered percent compost had the highest chlorophyll a, chlorophyll b and total chlorophyll. Substitution of 25-100 % compost increased pH and EC of growth media. The results showed that the use of 25-75 % compost could be a good alternative to peat, but was not significantly different from cocopeat. Due to the relatively high price and import of peat and cocopeat, and the negative impact of high amounts of compost on increasing growth medium EC, the use of 50 % *Azolla* compost is economically preferred.

Keywords: *Azolla*, Cocopeat, Compost, Peat, Perlite.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium*) from the family Asteraceae, which has originated from Asia, most probably from China and Japan, is one of the most important cut flowers of the world with over 200 species. This primary plant species are traded as both pot flower and cut flowers in the global markets so that it has the second rank after roses in economic and production aspects (Teixeira and Silva, 2003). The cultivation of plants in soilless substrates is expanding owing to its numerous advantages such as the control of plant nutrition, the reduction of diseases and pests, and higher crop quantity and quality than cultivation in soil. The characteristics of the materials used in a substrate affect plant growth and crop production (Mohammadi Torkashvand *et al.,* 1981).

A study on the effect of different rates of peanut shell compost on the yield and physical characteristics of marigold and violets reported that the use of 25% peanut shell compost performed better for more growth factors of the violets while the marigolds' growth factors responded better to 75% peanut shell compost. They also found that as more peat was replaced with peanut shell compost, in addition to saving on peat consumption, the growth of the violets, marigolds, and dracaena was improved (Mahboub Khomami *et al.,* 2019).

Mahboub Khomami (2015) reported that the collection and commercialization use of *Azolla* not only helps to solve environmental problems, but also has economic benefits. Mohammadi Torkashvand *et al.* (1981) addressed the effects of peanut shell compost as a growth medium for the growth of *Dracaena marginata* L. The control treatment was peat + perlite substrate at 2:1 ratio and they replaced peat with peanut shell compost at a rate of 15, 30, 45, 60, or 100% (v/v). Cristiano *et al.* (2018) concluded that probably due to the physical composition of sewage sludge compost, its replacement in doses of 30 and 60% with peat has the weakest results in terms of plant diameter, branches, leaves and flowers fresh and dry masses. Baran *et al.* (2001) stated that 50% grape waste compost can be substituted for peat in the *Hypoestes* growth medium. The results revealed that the peanut shell compost was more influential on the growth traits, including plant height, leaf number, and shoot dry weight, acting through reducing C/N ratio, creating an appropriate pH, and supplying more nutrients. The treatments containing 15% or 45% peanut shell compost exhibited better growth than the control, and the lowest growth was recorded by the 100% peanut shell compost as well as the control.

Due to the import of peat and cocopeat and its high cost in Iran, the use of suitable alternative substrates is necessary. The aim of this study was to investigate the effect of substrates containing *Azolla* compost on chrysanthemum growth.

MATERIALS AND METHODS

Azolla is collected of the wetlands of Guilan province, and dumped in a cubic meter (2 ×2×2) wooden box (Fig. 1). Every 3 to 5 days temperature of mass controlled, materials aerated and moisture regulated and compost was prepared within 4 months. During 4 months of preparation, the compost temperature was recorded and the prepared composts were used as a growth medium. Some physicochemical characteristics of *Azolla* (nitrogen, phosphorus, potassium, organic carbon, C/N ratio, EC, and pH) were measured before and after composting (Table 1).

The present study was carried out to study the effects of five substrate treatments on the rooted chrysanthemum cuttings in three replications in a completely randomized design at the greenhouse of the Ornamental Plants and Flower Research Station (37° 11' 44" and 50° 01' 03"), Lahijan, Iran.

Table1. Some physicochemical characteristics of *Azolla*, composted *Azolla* and peat used in the experiment.

Property	*Azolla*	Composted *Azolla*
Total nitrogen (%)	2.80	2.41
Total phosphorus (mg/kg)	2360	4300
Total potassium (mg/kg)	5100	8700
Calcium (mg/kg)	5600	6200
Magnesium (mg/kg)	2400	2800
Organic carbon (%)	74.25	22.0
C/N ratio	13.90	9.12
pH (1:5)	5.46	7.40
EC (dS/m)	4.30	5.47

Fig. 1. Wooden box with a volume of 8 m^3 (2 m×2 m× 2 m) for compost production.

After preparing the media (Table 2), *Chrysanthemum morifolium* was transferred to pots. For this aim, first after preparing the beds, the roots of *Chrysanthemum morifolium* were removed from the transplant pot and completely washed. Then for each pot with a new bed, one plant was cultivated and then transferred to the greenhouse to pass the plant growth period. Any 10 days 200 cm^3 solutions consist of OMEX 18-18-18 were used for any pot and irrigation was applied as needed. The chrysanthemum grew at average night temperature of 18 ± 2, a daily temperature of 27 ± 2 °C and a relative humidity of 65-75% (Simon *et al.*, 1976) and medium light, between 75 to 150-foot candles.

Table 2. The growth medium compounds.

Treatment	Combination
75% cocopeat	75% cocopeat + 25% perlite
75% peat	75% peat + 25% perlite
25% *Azolla* compost	25% *Azolla* compost + 50% peat + 25% perlite
50% *Azolla* compost	50% *Azolla* compost + 25% peat + 25% perlite
75 % *Azolla* compost	75% *Azolla* compost + 0% peat + 25% perlite
100 % *Azolla* composts	100% *Azolla* compost

Chlorophyll a and b contents were determined by Fouche *et al.*'s (2010.) method, and total chlorophyll content was estimated by Lichtenthaler and Buschmann's (2001) formula.

Carotenoid was found out by the procedure described in Lichtenthaler and Buschmann (2001) using the following equations:

$$C_a(\mu g/mL) = 12.25A_{663} - 2.79A_{646}$$

$$C_b(\mu g/mL) = 21.5A_{646} - 5.10A_{663}$$

$$C_{(X+C)}(\mu/mL) = \frac{1000A_{470} - 1.82c_s - 85.02c_b}{198}$$

Leaf N content was measured by the method of titration after distillation using the Kjeldahl method (Goos, 1995). Leaf phosphorus content was determined by Murphy and Riley's method (Murphy and Riley, 1962). To measure leaf potassium content, a flame photometer (model Jenway) was used, and leaf calcium and magnesium contents were determined by an atomic absorption device (Houba *et al*., 1989). The total organic carbon was measured according to the method proposed by Nelson and Sommers (1982). EC and pH were measured on an extract of 1:5 ratio of compost to water by weight using the method proposed by Verdonck and Gabriels (1992).

The physical properties of the growing media were measured by using the Fonteno *et al.* (1981) method, and water holding capacity, total porosity, bulk density and air-filled porosity was calculated from table 3 equations.

Table 3. Equations for calculating the physical properties of the growth medium.

Equation	Components of Equation
Bd = (Wdsp-Wp /Vp)	Wdsp = dry weight of substrates and the container
Pd = (Wdsp-Wp/ Vp- Vwd -(Wwsp-Wdsp))	Wp= dry weight of the container
AFP = (Vwd×100/VP)	Vp= Volume of container
CC= ((Wwsp-Wp) ×100/VP)	Vwd = Volume of water drained
TP=AFP+CC	Wwsp= substrates and container fresh weight

Abbreviations: Bd; Bulk density, Pd;particl density, AFP; Air-fill, CC; Container capacity, TP;Total porosity.

Data were analyzed in the SAS software package (SAS Institute Inc. 2001) at the $P < 0.05$ level, and means were compared with the LSD test. The graphs were drawn in MS-Excel.

RESULTS

Physico-chemical properties of the substrates

Table 4 presents the physicochemical characteristics of the substrates used in the research to grow the chrysanthemums. The substrate '75% peat + 25% perlite' had the highest N content and '75% cocopeat + 25% perlite' had the lowest one. N content was increased with the substation of *Azolla* versus '75% cocopeat + 25% perlite'. Mahboub Khomami *et al.* (2021a) reported that the use of cow manure and sawdust vermicompost instead of peat in the substrate of *Aglaonema* increased N content.

Table 4.The physicochemical characteristics of the substrates.

Substrate	N (%)	P (mg/kg)	K (mg/kg)	C/N	OC (%)	EC (1:5) (ds/m)	pH (1:5)
75% cocopeat + 25% perlite	1.02	7.31	317.1	11.85	12.09	0.90	5.93
75% peat + 25% perlite	2.65	51.6	64.7	11.92	31.59	0.49	5.67
25% *Azolla* compost + 50% peat + 25% perlite	2.43	73.8	249.2	11.88	28.86	0.47	6.12
50% *Azolla* compost + 25% peat + 25% perlite	1.21	96.3	356.0	11.92	14.43	0.52	644
75% *Azolla* compost + 0% peat + 25% perlite	1.57	113.0	569.6	11.90	18.72	0.93	6.74
100% *Azolla* compost	1.77	128.6	754.0	11.92	21.06	1.45	6.91

The highest P content (128.6 mg/kg) was related to '100% *Azolla* compost and the lowest P content (7.31 mg/kg) was observed in '75% cocopeat + 25% perlite'. The results of Carstensen *et al.* (2018) experiment showed that ATP synthesis ceases when the orthophosphate concentration in the chloroplast stroma decreases due to phosphorus deficiency.

The highest and lowest K contents (754.0 and 64.76 mg/kg) were related to '100% *Azolla* compost and '75% peat + 25% perlite', respectively. The K content was increased with the increase in substitution of peat with *Azolla*, which is consistent with the results of Grigatti *et al.* (2007).

The substrate '100% *Azolla* compost' exhibited the highest amount of Na content (679.75 mg/kg) whereas '75% peat + 25% perlite' had the lowest amount (139.88 mg/kg). Higher Na content in a substrate reduces osmotic potential and the osmotic adjustment mechanism in plant cells inhibits desiccation and disruption in the uptake and mobilization of nutritional ions, e.g. K and Ca. High concentrations of Na^+ can be directly poisonous to membranes and enzymatic systems (Chen *et al.*, 2003).

C/N ratio was lower than the maximum allowed range (C/N = 30) for the growth of ornamental plants in all substrates. The highest was 11.92 observed in '75% peat + 25% perlite' and '50% *Azolla* compost + 25% peat + 25% perlite' and the lowest was 11.85 observed in '75% cocopeat + 25% perlite'. C/N ratio was in the almost same range in all the studied substrates. Zucconi *et al.* (1981) report that composts whose C/N ratio is lower than 20 are ideal for plant production, but, C/N ratios of >30 may be toxic to plants and kill them.

The substrate '75% peat + 25% perlite' had the highest amount of organic matter (31.59%), whereas the lowest (12.09%) was observed in '75% cocopeat + 25% perlite'.

The substrate '100% *Azolla* compost' had the highest pH (6.91) and the substrate '75% peat + 25% perlite' had the lowest one (5.67). The substrates had pH's in the range of 5-7, which is optimal for the cultivation of ornamental plants. According to Abad *et al.* (2001), the best pH for optimal growth is 5.3-6.5. The highest EC (1.45 dS/m) was for '100% *Azolla* compost' and the lowest (0.49 dS/m) for '75% peat + 25% perlite'. Some desirable factors including flower size and appearance are major criteria to determine the salinity tolerance of ornamental plants. All studied substrates had an EC value in the range of 1-3 dS/m, which is suitable for the growth of ornamental plants (Mahboub Khomami *et al.*, 2019).

Table 5.The physical characteristics of the substrates.

Substrate	**Bulk density (g/cm^3)**	**Water retention capacity (%)**	**Aeration porosity (%)**	**Total porosity (%)**
75% cocopeat + 25% perlite	0.16	58.87	24.56	83.43
75% peat + 25% perlite	0.18	53.11	18.30	71.41
25% *Azolla* compost + 50% peat + 25% perlite	0.31	63.69	22.30	85.99
50% *Azolla* compost + 25% peat + 25% perlite	0.33	63.87	24.44	88.32
75% *Azolla* compost + 0% peat + 25% perlite	0.30	57.58	27.70	85.28
100% *Azolla* compost	0.30	55.34	26.29	81.63

The physical properties of the substrates studied (Table 5) here revealed that '50% *Azolla* compost + 25% peat + 25% perlite' had the highest bulk density of 0.33 g/cm^3 and '75% cocopeat + 25% perlite' had the lowest one of 0.16 g/cm^3. The bulk density of all the studied substrates was within the range defined for ornamental plants. Abad *et al.* (2001) determined the optimal bulk density for substrates to be <0.40 g/cm^3. According to Atiyeh *et al.* (2001), a sharp decline in bulk density increases aeration and decreases available water.

The substrate '50% *Azolla* compost + 25% peat + 25% perlite' had the highest water retention capacity of 63.87%. Based on Nappi and Barberis (1993), ideal substrates have a water retention capacity in the range of 55-85%. Our substrates' water capacity was within this range. However, '75% peat + 25% perlite' had the lowest capacity of 53.11%. This can be attributed to its higher porosity and greater air content, which reduces the amount of water it can hold.

The highest and lowest aeration porosities of 27.70% and 18.30% were related to the substrates '75% *Azolla* compost + 0% peat + 25% perlite' and '75% peat + 25% perlite', respectively. According to Abad *et al.* (2001), the ideal air space in substrates for ornamental plants is 20-30%. Chen *et al.* (1988) reported that physical properties are the most important factor dictating a plant's efficiency in a certain pot substrate so that the lower air content of the peat substrate resulted in the poor growth of *Ficus benjamina* 'Starlight'. They, also, reported that the plant exhibited its best growth in the substrates containing 50% peat + 50% compost (including manure or grape waste compost) versus the control substrate (20% vermiculite + 80% peat). The superiority of the compost-containing substrates was ascribed to their higher nutrient content and higher microbial activity in the root rhizosphere, resulting in the better growth of the plant.

The highest total porosity (88.32%) was observed in '75% *Azolla* compost + 0% peat + 25% perlite' and the lowest (71.41%) in '75% peat + 25% perlite'. Fonteno *et al.* (1981) found that an ideal substrate should have a porosity of >85%. In general, depending on the particle size and real specific mass of peat-based substrates, their porosity is in the range of 85-95%.

Table 6. Means comparison of the growth factors.

Substrate	Final plant height (cm)	Flowering branch length (cm)	Leaf number	Petal carotenoid (µg/mL)	Chl. a	Chl. b	Total Chl.
					(mg/g F.W.)		
75% cocopeat + 25% perlite	48.33 b	33.13 ab	82.00 bc	6881c	27.32 bd	43.91bd	71.24 bd
75% peat + 25% perlite	57.05 a	36.84 ab	115.44 a	8667 ac	24.06 d	38.79 d	62.86 d
25% *Azolla* compost + 50% peat + 25% perlite	54.22 ab	36.30 a	95.78 ab	7349 ac	24.84 d	40.00 d	64.84 d
50% *Azolla* compost + 25% peat + 25% perlite	52. 33 ab	35.27 ab	97.78 ac	9062 a	25.89 cd	41.78 cd	67.67 bc
75% *Azolla* compost + 0% peat + 25% perlite	52.05 ab	35.47 ab	91.11 ac	8734 ab	25.54 cd	41.18 cd	66.73 cd
100% *Azolla* compost	46.11 b	29.67 b	84.22 bc	7457 ac	30.46 ab	49.32 ab	79.79 ac

Means with similar letter(s) in each column show the lack of a significant difference at the $P < 0.05$ level based on the Duncan's test.

Plant height

Based on the results in table 6, the highest plant height (57.05 cm) was obtained from '75% peat + 25% perlite'. This substrate showed significant differences only with '75% cocopeat + 25% perlite' and '100% *Azolla* compost' in which the plant height reached 48.33 and 46.11 cm, respectively. This implies that *Azolla* provided a similar plant height than the peat-containing substrate while reducing peat consumption by 75%. According to the results in the table of different crops' yield, the plants' growth and development response depend on several factors such as substrate properties and the plant's moisture requirement in the growing season (Katsoulas *et al.*, 2006).

Flowering branch length

Based on the means comparison for the flowering branch length (Table 6), the trait was the highest (36.30 cm) in '25% *Azolla* compost + 50% peat + 25% perlite', which was significantly different only from the plants grown in '100% *Azolla* compost', in which the plants grew the shortest flowering branch length (29.64 cm).

Leaf number

The comparison of the means for leaf number (Table 6) showed that the treatment of '75% peat + 25% perlite' was related to the highest number of leaves (115.44 leaves), differing significantly from '75% coco peat + 25% perlite' (82.00 leaves) and '100% *Azolla* compost' (84.22 leaves).

Carotenoid

The comparison of the means (Table 6) revealed that the plants grown in '50% *Azolla* compost + 25% peat + 25% perlite' had the highest carotenoid content of 9062 µg/ml and those grown in '75% cocopeat + 25% perlite' had the lowest one of 6881 µg/ml. These two treatments were significantly different. Carotenoids can absorb energy and may lead to the formation of free oxygen from elicited chlorophyll molecules. They may also scavenge free oxygens formed during photosynthesis (Bergquist, 2006). Carotenoids, because they support photosynthetic and non-photosynthetic pigments, have the ability to absorb excess energy at short wavelengths and convert single oxygen to triple oxygen, which acts as an antioxidant due to the removal of oxygen radicals produced (Inze and Montago, 2000). It seems that the treatment of '*Azolla* + peat + perlite', which had a positive impact on nutrient uptake by plants and consequently on increasing their photosynthesizing parts, has a positive effect on the carotenoid content of chrysanthemums because carotenoids are synthesized by all photosynthesizing parts and many non-photosynthesizing parts.

Chlorophyll a

The comparison of the means (Table 6) showed that the plants grown in '100% *Azolla* compost' had the highest content of chlorophyll a (30.47 mg/g F.W.), showing a significant difference with '75% peat + 25% perlite' that was related to the lowest (24.06 mg/g F.W.), but there was not a significant difference between '75% peat + 25% perlite' and '75% cocopeat + 25% perlite'.

Chlorophyll b

It is observed in table 6 that the highest chlorophyll b content (49.32 mg/g F.W.) was obtained from '100% *Azolla* compost'. This treatment differed from the other substrates significantly. The lowest chlorophyll b content (38.79 mg/g F.W.) was related to '75% peat + 25% perlite', but it did not differ from the other treatments significantly.

Total chlorophyll

According to table 6, the highest and lowest total chlorophyll contents were produced in the plants grown in '100% *Azolla* compost' and '75% peat + 25% perlite' (79.79 and 62.86 mg/g F.W.), respectively.

Overall, chlorophyll content is an excellent measure extensively used to assess the physiological condition of plants (Jiang and Huang, 2001). Jiang and Huang (2001) also state that chlorophyll content in plants is a vital factor in determining their photosynthesis capacity. In advanced agronomy, leaf greenness is mainly considered a measure of N nutritional status, which is related to chlorophyll content (Kirkby and Zude, 2001). Allison *et al.* (1997) showed the leaf nitrogen content positively affects photosynthesis. Meinzer and Zhu (1998) observed that with the increase in leaf nitrogen content photosynthesis increases linearly in sugarcane. So, the higher chlorophyll content in the *Azolla* compost substrate in the present study can be related to the higher N content of this substrate.

Leaf nutrients

The comparison of the means showed that the effects of compost level were not significant on N, P, and Mg contents. The results in table 7 present that the highest leaf K content (5.65%) were related to the treatment of '75% cocopeat + 25% perlite' and the lowest (3.37%) to the treatment of '75% peat + 25% perlite'. The other treatments did not differ significantly. Ryan *et al.* (2005) reported that tea waste was a good K source and provided the plants with the essential nutrients for their growth. Mahboub Khomami *et al.* (2021) also observed an increase in P and K contents with the increased substitution with organic composts.

The highest leaf Ca content was 1.63% observed in the plants grown in '100% *Azolla* compost' and the lowest was 0.80% of the plants grown in '75% coco peat + 25% perlite' (Table 7). Mahboub Khomami *et al.* (2019) showed that as the volumic proportion of peanut shell compost was increased at levels lower than 100% substitution of peat, Ca uptake was increased versus the control. Despite the richness of *Azolla* compost and its positive effect on increasing nutrients in the growth medium, the uptake of elements in plant leaves was not significantly different from peat and cocopeat. Therefore, it seems that the significant effect of compost application on plant growth can be due to some compounds such as organic acids and growth accelerators that are provided to the plant through compost.

Table 7. Means comparison of the effects of treatments on leaf nutrients.

Substrate	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
75% cocopeat + 25% perlite	3.87 a	0.45 a	5.65 ab	0.80 b	3.11a
75% peat + 25% perlite	3.91 a	0.52 a	3.73 c	1.24 ab	4.00a
25% *Azolla* compost + 50% peat + 25% perlite	4.17 a	0.46 a	3.88 c	0.97 b	4.41a
50% *Azolla* compost + 25% peat + 25% perlite	4.63 a	0.54 a	3.92 c	1.30 ab	4.65a
75% *Azolla* compost + 0% peat + 25% perlite	4.43 a	0.48 a	4.07 bc	1.51 ab	5.21a
100% *Azolla* compost	3.98 a	0.41 a	4.07 bc	1.63 ab	3.67

Similar letter(s) in each column signifies an insignificant difference at the $P < 0.05$ level based on Duncan's test.

DISCUSSION

The results show that substrates containing *Azolla* compost are richer than those containing peat and cocopeat. However, the results of leaf analysis showed significant differences only in leaf K content. Although, the difference between the amounts of leaf nitrogen is not significant, its trend is increasing and can increase photosynthesis and plant growth. As Meinzer and Zhu (1998) observed that as the leaf nitrogen content increased, photosynthesis increased linearly in sugarcane. The physical properties of the *Azolla*-containing substrates were within thSTIX-Regulare recommended standard range. The results as to the effect of *Azolla* compost application on growth factors showed that the application of 25-75% *Azolla* can be a good substitute for peat and it does not differ from the cocopeat-containing substrate too. It seems that the treatment of '*Azolla* + peat + perlite', which had a positive impact on nutrient uptake by plants and consequently on increasing their photosynthesizing parts, has a positive effect on the carotenoid content of chrysanthemums because carotenoids are synthesized by all photosynthesizing parts and many non-photosynthesizing parts. These results are consistent with the results of Inze and Montago (2000).

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