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RESEARCH ARTICLE

Application of *Hydrangea macrophylla* flower extract on growth and yield of *Vigna unguiculata* L.

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ABSTRACT

Flower extract of Hydrangea is used as foliar spray as it has ability to induce gibberellins activity. This study investigated the effect of foliar application of Hydrangea macrophylla flower extract (HmFE) on the growth and yield of Cowpea (Vigna Unguiculata L.) at the Eastern University, Sri Lanka. The experimental design was Completely Randomized Design (CRD). Five treatments were defined with five replications viz. T1: Distilled water (Control), T2: 10, T3: 20, T4: 50 and T5: 100% HmFE. All the other management practices were followed uniformly. Growth and yield parameters were measured and analysed statistically using ANOVA test and means were compared with DMRT. Results proved that, application of 20% HmFE significantly increased the plant height (9.6%), chlorophyll content (23.02%), number of leaves (43.66%), leaf area (65%), root length (32.06%), fresh weight of stems (25.8%), fresh weight of leaves (16.5%), fresh weight of roots (32.5%), number of nodules (74.35%), number of effective nodules (90%), weight of the nodules (67.92%), number of seeds per pod (36.36%), weight of seeds per pod (30.14%), number of flowers per plant (71.4%), number of pods per plant (66.66%), 100 seeds weight (22.8%) and the total yield (75.28%) compared to the control plants. Therefore, this experiment concluds that application of 20% HmFE increases the growth and yield on Vigna unguiculata L.

Keywords: *Hydrangea macrophylla flower extract (HmFE), leaf area, nodules, Vigna unguiculata L., yield*

INTRODUCTION

Sri Lanka is a developing country and its economy mainly depends on Agriculture sector. Over 25% of Sri Lankans are employed in the agriculture sector. Sri Lanka has a rich agricultural history dating back more than 2500 years. In ancient time, our agricultural practices have depended on indigenous methods and knowledge of traditional farmers. However, the results of the green revolution in 1960 and the fast growing population have created tremendous pressure on food production in the country and modern agricultural practices have been introduced largely using agrochemicals and synthetic fertilizers. After the introduction of the green revolution, traditional based agriculture was shifted to the technology based agriculture. Uncontrolled population growth in developing countries accelerated the imbalance between human needs and sustainable land use (Anitha *et al.,* 2014). The greatest challenge of today's agriculture is to feed the growing population and restore natural resources.

At present, foliar fertilisation is the current trend of sustainable crop production. Mostly, developed countries are using this system and it is mostly popular among those developed countries. Conversely, sustainable agriculture reduces the synthetic fertilizer and pesticide usage and also contributes to conserve ecosystem. Synthetic fertilizer and pesticides are also dangerous not only for soil but also for aerial environment. Most synthetic fertilizers and pesticides seep down below the root zone in to the ground water and pollute ground water causing to result health issues, i.e. Methemoglobinemia (Naeem *et al.,* 2006).

Global food production needs to be doubled by 2050 to meet the feed requirement of the growing population (Anon, 2009). Farmers are using chemical fertilizers extensively to meet the increasing demand for food. Most of the farmers prefer to get a quick return and hence use inorganic fertilizers and pesticides. The excessive usage of agrochemicals and synthetic fertilizers lead to numerous soil, water and other environmental problems. Agriculture today is not only a leading driver of environmental degradation but also a major force driving the Earth system beyond the 'safe operating space' for humanity (Rockstrom *et al.*, 2009). Nitrate and phosphate concentrations are found to be higher than the permissible limits of World Health Organization standards due to leaching and surface run-off of chemical fertilizers from agricultural lands (Divya and Balagali, 2012).

Hydrangea macrophylla is an ornamental flowering plant which is native to Japan. It is terminated by a massive globular corymbose head of flowers which remain a long period in an ornamental condition. Hydrangeas are widely used as dried flowers in ornamental horticulture industry. *Hydrangea macrophylla* blooms can be blue, red, pink, light purple, or dark purple and is propagated by cuttings. The flower color is affected by soil pH. Flower extract of Hydrangea flowers is used as foliar spray. It has ability to induce gibberellins activity. Studies on modern pharmacology demonstrated that it has functions such as anti-allergic, anti-hyper cholesterolemic, anti-diabetic, antifungal, and plant growth inducing ability (Feng *et al.*, 1997).

Cowpea is the most economically important indigenous legume crop (Langyntuo *et al.*, 2003). It is valued as both human food and livestock feed. Green cowpea seeds and dry mature seeds are boiled and consumed. It is drought tolerant, can be grown on relatively poor soils and fixes nitrogen improving soil fertility. It is mainly cultivated in dry zone areas of Sri Lanka. Cowpea is cultivated in more than an extent of 6807 ha, producing nearly 8576 metric tons in 2018 (Anon, 2018a). However, amount of production is not sufficient to cater the demand. Therefore, it is essential to increase the yield per unit area. Literature reveals that foliar application of *Hydrangea macrophylla* flower extract has the ability to induce growth and yield of crops. Therefore, this study was carried out with the objective to evaluate the effect of *Hydrangea macrophylla* flower extract on growth and yield of *Vigna unguiculata* L.

METHODOLOGY

A pot experiment was conducted in the crop farm of Eastern University, Sri Lanka during the '*Maha*' season from January to April 2019. The pot experiment was arranged out in a Completely Randomised Design (CRD) with five treatments and five replications. 25 pots per treatment were used in this experiment and each pot contained two plants. This experiment was conducted using poly bags. The height of the poly bag was 35 cm and diameter was 20 cm. Eight small holes were made at the bottom of each poly bag to facilitate the drainage of water. The poly bags were filled with top soil and compost in the ratio of 1:1 and a distance of 5 cm was left unfilled from the top of the soil to facilitate irrigation. Cowpea variety '*Waruni*' was selected for the experiment. *Hydrangea* flowers were picked and washed with tap water immediately to remove the foreign particles. Then, it was kept in polythene bag with water spray and immediately transported to the Crop Science Laboratory, Eastern University, Sri Lanka and washed thoroughly using distilled water. Thereafter, flowers were spread on blotting paper to remove excess water and placed on shade to dry. Flowers were shade dried for three to four days. Then, the raw materials were made in to coarse powder using laboratory blender and sieved using 500 MIC (micro meter) aperture sieve. After that, powdered *Hydrangea macrophylla* flower sample was used for the preparation of liquid flower extract. The prepared flower sample was added with distilled water at the ratio of 1:20 (w/v) and autoclaved at 121˚C, 15Ibs/sq inch for 15 minutes. Liquid flower extract was filtered through double layered cloth and allowed to cool at 4 ˚C in refrigerator. After that, the filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was collected and considered as 100% HmFE, stock solution.

The experiment comprised of following treatments. T1: Distilled water (Control), T2: 10% HmFE, T3: 20% HmFE, T4: 50% HmFE and T5: 100% HmFE. The first application was carried out at two weeks after planting. Frequency of application was once a week. Foliar applications were done at early morning for better absorption of nutrients. All the agronomic practices were carried out in accordance with the recommendation made by the Department of Agriculture, Sri Lanka (Anon. 2018b). The parameters *viz.* plant height, chlorophyll content (SPAD meter, Konica Minolta SPAD-502Plus), leaf area (Leaf area meter, LiCOR Inc.), total number of root nodules and weight of the nodules, biomass of leaves, stems and roots, number of seeds per pod, average weight of seeds per pod, 100 seeds weight and total yield.

Data were statistically analysed with ANOVA using SAS 9.1 and means were compared using Duncan's Multiple Range Test (DMRT) at 5% significant level.

RESULTS AND DISCUSSION

Plant height

The results showed that application of *Hydrangea macrophylla* flower extract had significant (P<0.05) increase in the plant height compared to the control plants. The maximum average plant height was recorded in T3 (20%) HmFE followed by T2, T1, T4 while the minimum average plant height was recorded in T5 (Table 1)**.** This might be due to the presence of macro and micro nutrients as well as growth promoting substances (Auxins and Cytokinins) in HmFE. Cytokinins are active at very low concentrations and regulate a number of plant functions including cell division (Koda and Okazawa, 1983). Khosa *et al.* (2011) reported that presence of macro nutrients in foliar sprays increased plant height. Plant height increase also might be due to the potassium (Dkhil *et al*., 2011). Kazemi (2013) also reported that application of Zn and Fe containing foliar application showed a significant effect on vegetative growth. Presence of high amount of potassium combines with naturally occurring plants gibberellins. This could affect to the significant increase of plant height of *Vigna unguiculata* (Priyanka *et al*., 2016; Shukla, 2016).

Table 1: Effect of foliar application of HmFE on plant height (cm) per plant of *Vigna unguiculata.* Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/s indicate significant differences at 5% level of significance by DMRT.

Treatment	Plant height (cm)
T1(Control)	37.10 ± 1.2^{b}
T2 (10%)	36.68 ± 0.7^{b}
T3 (20%)	40.68 ± 1.7^{a}
T4 (50%)	33.64 ± 0.6^{c}
T5 (100%)	33.80 ± 0.1^{c}

Chlorophyll content of leaves per plant (SPAD)

The results showed that application of *Hydrangea macrophylla* flower extract had a significant (P<0.05) increase in the chlorophyll content of leaves compared to control plants. The maximum average chlorophyll content was recorded in T3 (20%) HmFE followed by T2, T1, T4 while the minimum average plant chlorophyll content was recorded in T5 (Table 2). It clearly indicated that application of 20% HmFE had significantly (P<0.05) increased average chlorophyll content compared to control plant. Khosa *et al*. (2011) reported that micro and macro nutrient found in HmLE enhances the chlorophyll content. Phosphate is an important structural signaling molecule with an essential role in photosynthesis, energy conservation, and carbon metabolism. Its deficiency leads to a reduction of growth and an increase of pathogen susceptibility (Lopez-Bucio *et al.,* 2005).

Priyanka *et al*. (2016) reported that gibberellins combined with potassium containing foliar application highly affects to increase chlorophyll content.

Table 2: Effect of foliar application of HmFE on chlorophyll content per plant of *Vigna unguiculata*.Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/s indicate significant differences at 5% level of significance by DMRT.

Treatment	Chlorophyll content (SPAD)
T1 (Control)	39.44±0.7[b]
T2 (10%)	42.92±1.2[b]
T3 (20%)	48.52±1.3[a]
T4 (50%)	38.82±1.2[b]
T5 (100%)	38.82±0.3[b]

Leaf area

Data showed that application of *Hydrangea macrophylla* flower extract had a significant increase (P<0.05) in leaf area when compared to control plants (T1). The maximum leaf area (1131.44 cm^2) was recorded in T3 followed by T2, T1. The minimum leaf area was recorded in T4 and T5 (Table 3).

The present experiment showed that foliar application of HmFE with 20% concentration increased the average leaf area by 39.40%, compared with T1 (control). It might be due to the presence of Zn and Fe in HmFE. It was clearly indicated that presence of micro and macronutrient in foliar extracts, increases the leaf area per plant (Khosa *et al*., 2011). Zn and Fe also might affect on increasing leaf area in *Vigna unguiculata* L. It was reported that Zn and Fe involved in increasing leaf area of plants (Dkhil *et al.,* 2011). Furthermore, general application in Fe had increased the tissue formation with better plant growth which increased leaf area (Rawashdeh and Sala, 2015).

Table 3: Effect of foliar application of HmFE on Leaf area per plant of *Vigna unguiculata.* Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/s indicate significant differences at 5% level of significance by DMRT.

Treatment	Leaf area (cm^2)
T1 (Control)	685.65±0.87[b]
T2 (10%)	722.32±0.24[b]
T3 (20%)	1131.44±0.94[a]
T4 (50%)	569.35±1.08[b]
T5 (100%)	562.39±0.77[b]

Total number of root nodules and weight of nodules

Application of HmFE significantly (P<0.05) increased the total number of root nodules and nodules weight when compared with the control (T1). The maximum number of root nodules and weight of nodules were recorded in T3 (20%) followed by T2 (10%), T1 (control), T4 (50%) and T5 (100%) (Table 4). This result proved that 20% flower extract gave the best performance. Fe is responsible for nitrogen fixation process (Havlin *et al.*, 2014). Further, Milev, (2014) reported that phosphorus and potassium in foliar fertilizers have significant effect of the fixation of atmospheric nitrogen. Zahoor *et al.* (2013) had reported application of Fe significantly increases the weight of the nodules. Cytokinins play a major role in nodule organogenesis. They induce cortical cell division and activate many nodulation related proteins which are involved in early nodulation process (Ferguson and Mathesius, 2003; Mathesius *et al.*, 2000; Murray *et al.,* 2007).

Table 4: Effect of foliar application of HmFE on total number of root nodules and weight of nodules per plant of *Vigna unguiculata***.** Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/s indicate significant differences at 5% level of significance by DMRT.

Treatment	Total number of root nodules	Weight of nodules (g)
T1 (Control)	39±1.14^{b}	0.53±0.96^{bc}
T2 (10%)	44±0.84^{b}	0.62±0.43^{b}
T3 (20%)	68±1.03^{a}	0.89±0.93^{a}
T4 (50%)	33±0.91^{b}	0.50±0.96^{bc}
T5 (100%)	33±1.02^{b}	0.4±1.41^{c}

Dry biomass of leaves, stems and roots

Application of HmFE significantly (P<0.05) influenced the dry weight of the leaves, stems and roots of cowpea. The maximum dry weight of leaves, stems and roots were recorded in T3 (20%) followed by T2, T1 and the minimum in T4 (50%) and T5 (100%) (Table 5). Sutharsan *et al.* (2014) reported that an increase in leaf area leads to an increased dry matter accumulation of crops. HmFE contained several types of micro and macronutrients. This might affect to increase the dry weight of shoots. It was in agreement with Kazemi (2013) who reported that foliar application with micro elements such as Zn and Fe significantly influences dry weight of plants. Further, Asad and Rafique (2002) reported that application of micronutrients increased wheat dry matter content. Thus, there has been an ample supply of N to increase the dry matter content of *Vigna unguiculata*. These findings are in conformity with Dixit and Elamathi (2007). Phosphorus fertilizer enhanced root development of cowpea and also increased the dry matter at the harvest (Ali *et al.*, 2010). Root growth could be influenced by gibberellins activity inducing effect of HmFE. These results are in conformity with the report of Asen *et al.* (1960) and Bottini *et al.* (2004). Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/letters indicate significant differences at 5% level of significance by DMRT.

Table 5: Effect of foliar application of HmFE on dry biomass of leaves, stems and roots per plant of *Vigna unguiculata.*

Treatment	Biomass of leaves (g)	Biomass of stems (g)	Biomass of roots (g)
T1 (Control)	1.84±1.34[b]	2.65±0.94[b]	1.86±0.77[b]
T2 (10%)	1.47±1.65[b]	2.91±0.70[b]	2.10±0.73[b]
T3 (20%)	2.85±1.05[a]	3.90±1.67[a]	3.53±1.56[a]
T4 (50%)	1.18±1.32[b]	1.83±0.64[bc]	1.75±0.61[b]
T5 (100%)	1.16±0.15[b]	1.27±0.57[c]	1.49±1.00[b]

Number of seeds per pod, weight of seeds per pod and 100 seeds weight

Foliar application of HmFE significantly ($P<0.05$) influenced on the number of seeds per pod, Weight of seeds per pod and 100 seeds weight The maximum values were recorded in T3 (20%) HmFE while the minimum values were recorded in T5 (100%) (Table 6). It was reported that, application of micro nutrients such as boron and zinc in foliar spray increased the number of seeds per pod in cowpea. Further, Rawashd *et al.* (2015) report that presence of Iron, Manganese (Mn), Zinc (Zn) and Copper (Cu) in foliar sprays significantly increases 1000 grain weight. Zn, Cu and Mn increased weight of seeds (Dkhil *et al.*, 2011). Further, Ali *et al.* (2012) reported that foliar spray with Fe at different growth stages enhanced 1000 grains weight. Furthermore, Grotz and Guerinot (2006) report a significant increase in 100 grains weight with foliar application of micro nutrients.

Table 6: Effect of foliar application of HmFE on number of seeds per pod, average fresh weight of seeds per pod and 100 seeds weight per plant o*f Vigna unguiculata.* Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/s indicate significant differences at 5% level of significance by DMRT.

Treatment	Number of seeds per pod	Average fresh weight of seeds per pod (g)	100 seeds weight (g)
T1(Control)	11±0.93[b]	1.36±0.88[b]	7.63±0.37[b]
T2 (10%)	12±0.91[b]	1.43±0.83[b]	7.84±0.62[b]
T3 (20%)	15±1.03[a]	1.77±1.00[a]	9.37±1.35[a]
T4 (50%)	10±0.98[b]	1.20±1.16[b]	7.21±1.45[b]
T5 (100%)	10±1.11[b]	1.17±1.05[b]	6.94±0.65[b]

Total yield per plant

The maximum average total yield was recorded in T3 followed by T1 and the minimum in T5 (100%) (Table 7). It might be due to the presence of macro and micronutrients in HmFE. Gibberellin, one of the most probable compounds found in HmFE, is active at very low concentrations and regulates a number of plant functions including cell division, activation of nodulation-related proteins etc. during early nodulation process. Phosphorus promotes root development, Mg promotes root growth and K stimulates flowering. These effects ultimately enhance growth and yield of plant by initiating a robust plant growth.

Table 7: Effects of foliar application of HmFE on total yield per plant per plant of *Vigna unguiculata.* Value represents mean±standard error of five replicates. Mean values in a column having the dissimilar letter/s indicate significant differences at 5% level of significance by DMRT.

Treatment	Total yield per plant (g)
T1 (Control)	3.52 ± 1.05^{b}
T2 (10%)	3.27 ± 1.60^{b}
T3 (20%)	6.17 ± 0.97^{a}
T4 (50%)	2.50 ± 0.39^{b}
T5 (100%)	2.06 ± 0.43^{b}

CONCLUSIONS

Foliar application of 20% *Hydrangea macrophylla* flower extract significantly increased the growth and yield of *Vigna unguiculata* L.. In this investigation, application of low concentration (20%) of *Hydrangea macrophylla* flower extract increased the plant height (9.6%), chlorophyll content (23.02%), number of leaves (43.66%), leaf area (65%), root length (32.06%), fresh weight of stems (25.80%), fresh weight of leaves (16.5%), fresh weight of roots (32.5%), number of nodules (74.35%), number of effective nodules (90%), weight of the nodules (67.92%), number of seeds per pod (36.36%), weight of seeds per pod (30.14%), number of flowers per plant (71.4%), number of pods per plant (66.66%), 100 seeds weight (22.8%) and the total yield (75.28%), compared to the control plant. Among five treatments T3 *Hydrangea* flower extract shows the best results compared with the other treatments. Therefore, this experiment concluded that application of 20% HmFE increased the growth and yield on *Vigna unguiculata* L.

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