Global Publication House Journal Agricultural Research

GPH-Journal Of Agriculture And Research

Responses of Periwinkle (*Catharanthus roseus*) to soil and foliar applications of Haza (*Haplophyllum tuberculatum*).

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Research Article

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Article Info

Received on: 05-31-2019

Revised on: 06-04-2019

Accepted on: 06-11-2019

Volume 02 || Issue 05 || May 2019

ABSTRACT

This study aimed to investigate the responses of Periwinkle plants to soil and foliar applications of Haza plant in two separate tests under the conditions of the nursery at Shambat, Khartoum North, Sudan. The foliar treatments were for boiled water extracts of hand crushed Haza shoots in concentrations: 0.0, 5, 10, 15 and 20 g/l, while the soil dressing test was for powder of dry shoots of Haza applied in doses of: 0.0, 5, 10, 15 and 20 g per plant. The Periwinkle transplants were planted in 18 inch plastic pots containing River Nile sedimentary soil. The study was arranged in complete randomized design and each treatment was replicated 7 times. Data were collected 4 months after applications. The results showed substantial increments in vegetative and reproductive growth parameters coupled with high alkaloids content from soil dressing with 10 g/plant Haza treatment or the foliar application of the 10 g/l Haza extract. These findings elucidated the bio-stimulating potential of Haza applications for enhanced vegetative and reproductive growth beside alkaloids content of Periwinkle. This stimulating potential may be of value for trials on organic production of other horticultural crops.

KEYWORDS||-

Haza (*Haplophyllum tuberculatum*); Periwinkle (*Catharanthus roseus*); Bio-stimulant; Growth; Alkaloids

INTRODUCTION

The periwinkle (*Catharanthus roseus*) is an important medicinal and ornamental plant from the Apocynaceae family. It is believed to be native to the West Indies but was originally described from Madagascar. It is an important constituent of pharmaceuticals and is of importance in the traditional medicine of many contemporary cultures such as China, India, West Indies, and Japan (WHO, 1999). It has been cultivated as an ornamental throughout tropical and subtropical regions and therefore was naturalized in many regions in Asia, Africa, America, southern Europe and Oceania (Govaerts, 2015). The aerial parts of the plant are used for the extraction of the medicinal alkaloids vincristine and vinblastine. The alkaloids are prescribed in anticancer therapy, usually as part of complex chemotherapy protocols. The dried root is an industrial source of ajmalicine, which increases the blood flow in the brain and peripheral parts of the body. Preparations of ajmalicine are used to treat the psychological and behavioral problems of senility, sensory problems (dizziness, tinnitus), cranial traumas and their neurological complications (Sutarno and Rudjiman, 1999). In Sudan, Periwinkle is among the outdoor flowering herbs, and is of extensive use in public and institutional gardens as the plant proved to be tolerant to high temperatures and minimum human attention. However, it is of no use in Sudan folk medicines. Yet, the outstanding growth performance may suggest commercial production of the plant for export to countries where it can be exploited for pharmaceutical purposes. Nevertheless, research on the agronomy of the plant under Sudan's conditions is very meager.

Haza (*Haplophyllum tuberculatum*), a member of the family Rutaceae, is an indigenous herbaceous plant in Sudan. It is of wide distribution in the country, but of considerable abundance along the Nile's banks. According to Raissi *et al.,* (2016), the plant possesses various pharmacological properties including anti-cancer, anti-bacterial, anti-oxidant, anti-HIV and uterus relaxing properties, which are probably due to the presence of aromatic compounds such as two alkaloids named haplophytin A and B together with essential oils. The constituents of the oil include estragole, myrtenal, spathulenol and a homologous series of medicinally important mono-terpenes and sesqui-terpenes (El Naggar *et al.,* 2014). The plant is of diverse uses in Sudan ethno-medicine, but the main uses are for allergic rhinitis, spasmo-colon, asthma and gynecological disorders (Mohamed *et al.,* 1996).

Recent studies in the last decade pointed to the growth and yield stimulation potential of some Sudanese local flora when applied to horticultural crops as foliar or soil dressings (Idris *et al.*, 2011; Abdelrahman, 2016; Eisa 2016; Hamed, 2016). However, Haza application as priming treatment of mango seeds enhanced the speed and percentage of germination of mango seeds coupled with stimulations in seedling growth attributes (Idris and Modawi, 2016). According to Eisa (2016), Haza applications to (*Aloe vera*) plants resulted in stimulations of growth and gel yield. Therefore, the aim of this study was to investigate the growth and alkaloids responses of Peiwinkle plants to foliar and soil applications with Haza.

Materials and methods:

These studies were conducted in complete randomized design in the nursery of Sudan University of Science and Technology, at Shambat, Khartoum North, Sudan to determine the responses of Periwinkle plants to foliar applications of shoot boiled water extracts and soil dressing with dry shoots of Haza. Periwinkle transplants grown in 25X30 cm plastic pots containing River Nile silt soil were used as experimental materials. A month after establishment, in complete randomized design, these transplants were employed in two separate tests as follows:

1. Test of Haza extracts: The shoot system of Periwinkle transplants was sprayed to run-off with boiled water extracts of: 0.0, 5, 10, 15, and 20 g/l of hand crushed dry Haza shoots.
2. Test of soil dressing with dry Haza shoots powder: The treatments were 0.0, 5, 10, 15 and 20 g/ plant.

Each treatment was replicated 7 times and each plant in a plastic pot was considered a replicate. The Haza applications were repeated at 2 months' interval and irrigation was applied according to need. Final data were collected 4 months after treatments for the plant height, number of branches, number of leaves, length and width of leaves, number of flowers and fruits, seeds weight and shoot fresh and dry weights. The collected data were subjected to analysis of variance, and means were separated at 95% confidence limits according to Duncan's Multiple Range Tests with the aid of MStatC computer program.

Results:

The soil applications

All Haza soil dressings increased plant height significantly over the control, and the 10 and 15 g/plant were the most enhancive. They also increased the number of branches per plant significantly over the control. The highest number of branches per plant resulted from the 5 and 10 g treatments that shared the top rank. It is noteworthy that increase above the 10 g level was deteriorative to this parameter (Figure 1 and 2). Regarding the number of leaves per plant, the 10 and 15g treatments were equally most enhancive for this character while the 20 g treatment was second in rank. All treatments increased the length of leaf significantly over the control and treatments 10, 15 and 20 g per plant shared the top rank. Likewise, leaf width was increased by Haza soil applications and the best result was recorded for the 5, 10 and 15 g treatments that shared the top rank. As for shoot fresh and dry weights, these parameters were enhanced by all Haza treatments significantly over the control without difference between them (Table 1). All Haza soil treatments increased the number of flowers per plant significantly over the control. Although their impact on this character was statistically similar, the 10 and 15 g/plant treatments were of relatively higher values (Figure 3). According to Table (2), these treatments were also the best for the number of fruits per plant, followed in rank by the 5 and 20 g/ plant treatments, which were of significant higher values compared to the control. The seed weight per plant was best enhanced by the 10 g/ plant treatment while the 15 and 20 g treatments ranked second. The 5 g treatment that ranked third was also superior compared to the control. However, all Haza soil applications were enhancive for alkaloids content compared to the control and in particular, the 5 and 10 g/ plant treatments were the most inductive for this parameter (Table 2).

The foliar applications

According to Figure (4), all Haza foliar applications increased plant height significantly over the control, but without significant difference between them. The number of branches per plant was highly enhanced by the 15 g treatment, followed by the 10 g/l while the 5 and 20 g/l treatments were statistically equal to the control (Figure 5). The number of leaves per plant was highly increased by the 10 and 15 g/l treatments that shared the top position, while the 5 g/l treatment was also superior when compared to the control. Besides, at an equal statistical level, all Haza extracts increased the length and width of leaf significantly over the control. According to data on shoot fresh and dry weights, only the 5 and 10 g/l Haza extracts increased these characters significantly over the control, whereas higher doses were deteriorative (Table 3). The 5, 10 and 15 g/l Haza extracts resulted in the highest number of flowers per plant and shared the top rank, while the 20 g/l dose decreased this parameter compared to the control (Figure 6). Except the 20 g/l Haza treatment, all extracts increased the number of fruits per plant significantly over the control. In particular, the 10 g/l treatment resulted in more than triple folds increases in this parameter. All extracts increased number of seeds per fruit significantly over the control at statistical equal level. Besides, the data revealed significant increments in Periwinkle alkaloids content upon spray with any of Haza extracts except the 20 g/l treatment that equaled the control. (Table 4).

Discussion:

The results of this study revealed enhancements in vegetative and reproductive growth and alkaloids content upon treating Periwinkle plants with foliar extracts of Haza, particularly when applied in concentration of 10 g/l as well as soil dressing with 10 g Haza powder/plant. Although growth gains in Periwinkle plants were obtained, we cannot attribute these stimulations to specific constituents of Haza tissues other than hormonal effect. The interpretations of the positive impact of bio-stimulants on growth of diverse plant species had been based on assumptions. Bio-stimulants are not fertilizers in the sense they do not contain nutrients intended to be delivered to the plant (Halpern *et al*., 2015). However, they may facilitate nutrient acquisition, *e.g.* by mobilizing elements in the rhizosphere or by developing new routes of nutrient acquisition, like fixation of atmospheric N by the recruitment of bacterial endosymbionts (Calvo *et al*., 2014). According to Ali *et al*., (2009), the scarce scientific literature on natural growth enhancers shows that the growth-enhancing effect of different products can be grouped into three categories: compounds enhancing nutrients availability or facilitate their uptake, or decreasing damage by pests, and/or interfere with the plant hormone system either directly or indirectly through microbes. Besides, Sato *et al.* (1990) and Wagentrisl (2003) reported that commercial products based on extracts from saponin-containing plant parts, had been shown to enhance the yield, quality and growth characters of tomatoes, cucumbers, and strawberries and owed the effect to improved uptake of nutrients from soil and air, effectiveness against fungal infections, nematodes, and pathogens and growth stimulating properties. Moreover, Foidle *et al.* (2001) reported that a spray made from Moringa leaves extract resulted in increased strawberry production and claimed the possibility of its use as a foliar spray to accelerate growth of young plants. Other studies had also shown that treating soil or spraying plants with saponins-containing plant products based on the bark from the soap bark tree (*Quillaja saponaria*) had a positive effect on the yield and quality of strawberries, tomato, and grapes (Wagentrisl, 2003). The study of Sims *et al*. (2009) on the bio-stimulating property of tea seed extract applied on *Euphorbia obtusifolia*, grown under non-nutrient-limiting conditions, revealed that the application resulted in increase in number of leaves, plant height, number of branches and roots compared to the untreated plants. They claimed that tea seed powder contains substances with hormone-like properties or substances that interfere with endogenous hormones, which can stimulate or affect biomass allocation in plants. Apart from the growth-stimulating effects, plant extracts also displayed growth-reducing effects at increased doses in seeded plants such as corn, mustard, barley, and the soil-treated strawberries (Wagentrisl, 2003). Growth-reducing effects were also reported for early water grass (*Panicum crusgalli* L.), green foxtail (*Setaria viridis* Beauv. L.) and white clover (*Trifolium repens* L.) in response to different concentrations of saponins (Kohata *et al*., 2004). In this study the high levels of Haza were not as effective as the relatively low ones; an indication for need for dose optimization to get the desired stimulations. Nevertheless, our findings reported in this investigation are in conformity with those reported by Eisa (2016), who obtained growth and gel yield enhancements in *Aloe vera* plants, treated with Haza applied foliarly or as soil dressing. Inasmuch as, Idris and Modawi (2016) reported accelerated germination and increase in germination percentage coupled with enhancements in seedling growth attributes upon priming seeds of mango with extracts of Haza plants. Regardless of solid interpretation, the demonstrated potential of Haza applications in this study might be of practical value if traied on other horticultural crops heading towards organic farming.

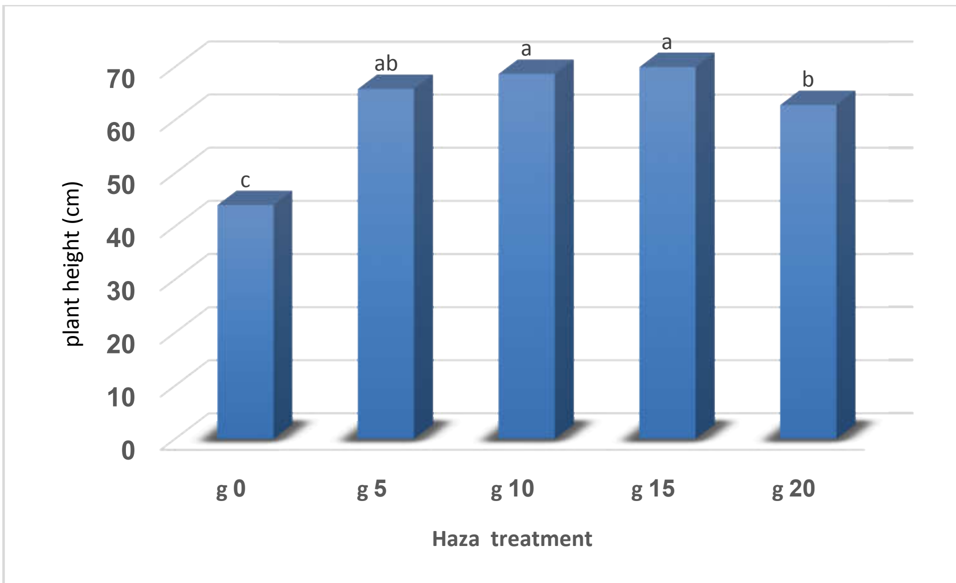

Figure 1. Impact of Haza soil applications on height of Periwinkle plant at Shambat, Sudan in 2018.

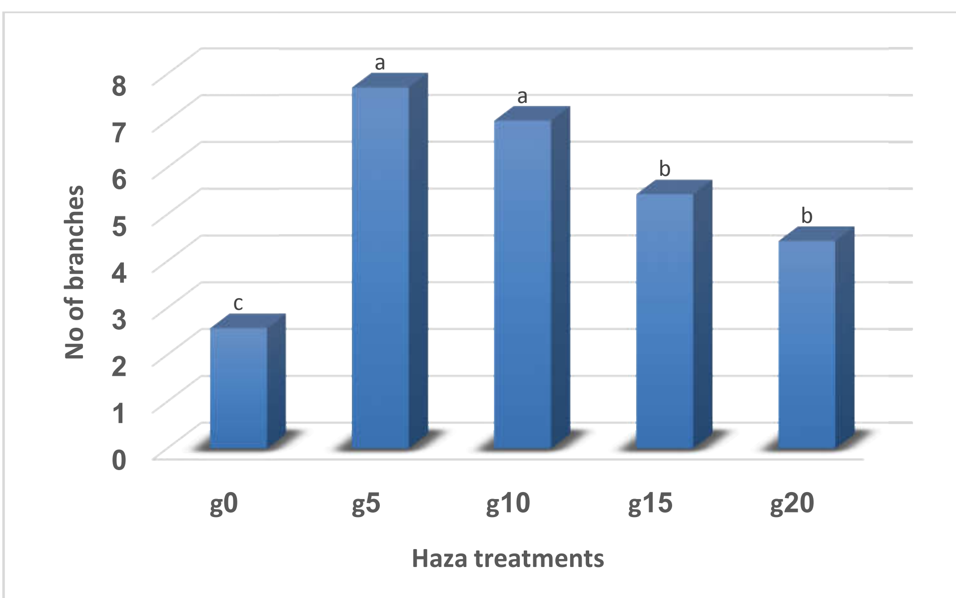

Figure 2. Impact of Haza soil applications on the number of branches per Periwinkle plant at Shambat, Sudan in 2018.

Table 1: Impact of Haza soil dressings on growth attributes of Periwinkle plant at Shambat, Sudan in 2018.

Haza soil treatments (g/plant)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
0.0	59.71c	4.48c	2.11c	18.53b	3.74b
5.0	69.43bc	6.81b	3.70a	30.31a	6.34a
10.0	106.10a	7.71a	3.50a	32.11a	6.86a
15.0	117.60a	8.13a	3.44a	34.93a	7.60a
20.0	84.57b	8.14a	3.06b	29.93a	6.70a
CV (%)	23.06	10.97	10.82	15.26	20.43

*Means within column with the same letter(s) are not significantly different at P≥ 0.05% according to DMRT.

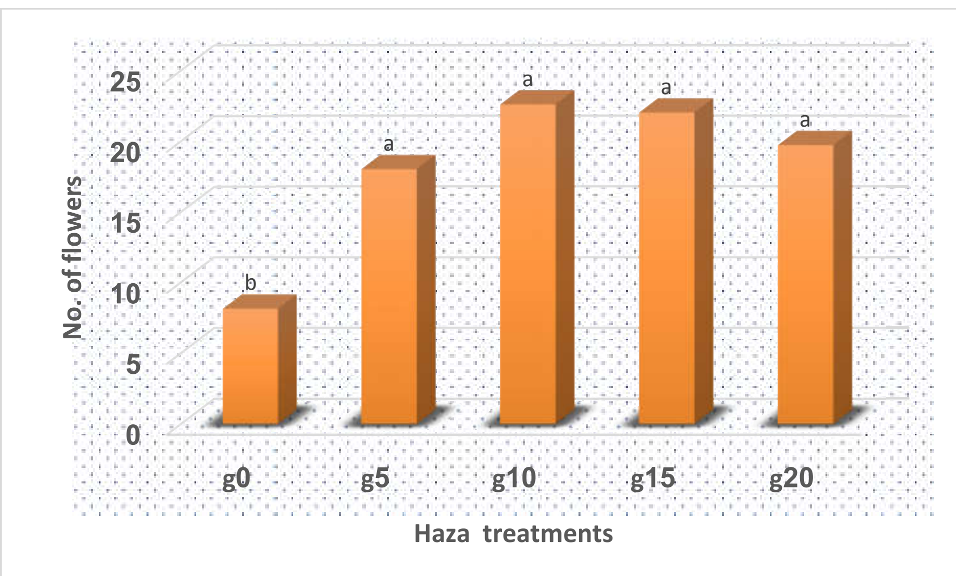

Figure 3. Impact of Haza soil applications on the number of flowers per Periwinkle plant at Shambat, Sudan in 2018

Table 2. Impact of Haza soil dressing on number of fruits, weight of seeds and alkaloids content at Shambat, Sudan in 2018

Haza trt. (g/plant)	No. of fruits per plant	Weight of seeds/ plant (g)	Alkaloids content (%)
0.0	2.86c	0.11d	0.440d
5.0	6.14b	0.20c	0.778a
10.0	8.00a	0.40a	0.778a
15.0	7.57a	0.31b	0.711b
20.0	6.43b	0.31b	0.508c
CV(%)	25.80	25.95	5.24

*Means within column with the same letter(s) are not significantly different at P ≥ 0.05% according to DMRT.

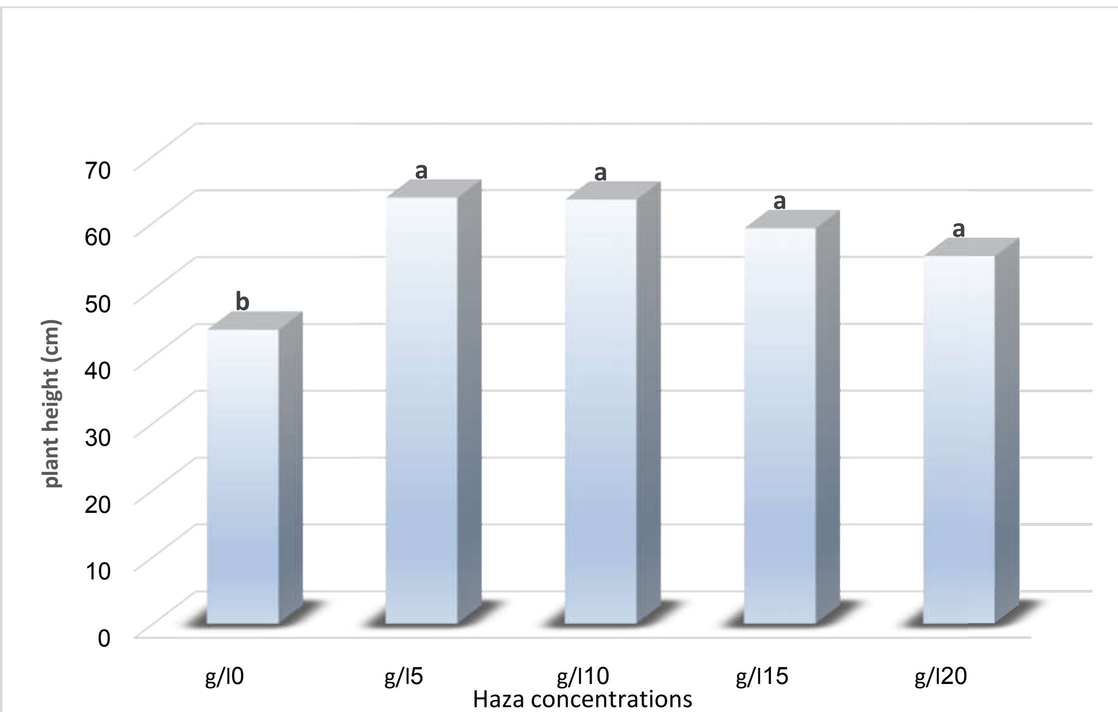

Figure 4. Impact of Haza foliar applications on height of Periwinkle plants at Shambat, Sudan in 2018.

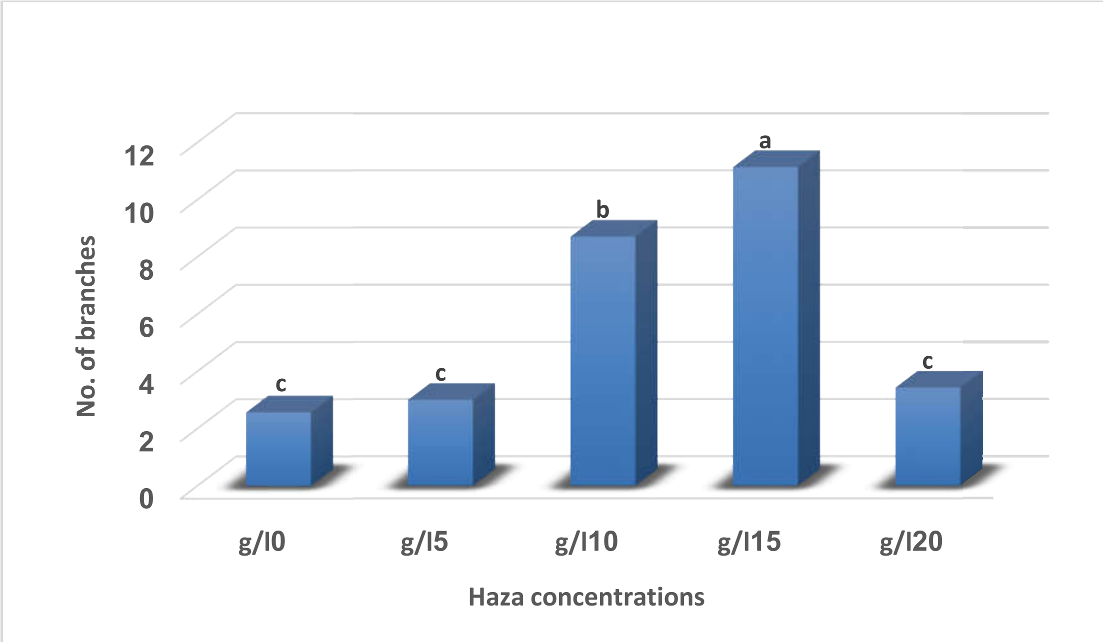

Figure 5. Impact of Haza foliar applications on the number of branches in Periwinkle plant at Shambat, Sudan in 2018.

Table 3. Impact of foliar applications of Haza extracts on vegetative growth attributes of Periwinkle plant at Shambat, Sudan in 2018.

Haza extract conc. (g/plant)	No. of leaves/ plant	Leaf length (cm)	Leaf width (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
0.0	59.71c	4.49b	2.11b	21.94b	5.17b
5.0	84.43b	7.87a	3.07a	30.97a	6.83a
10.0	131.4a	7.26a	3.23a	29.03a	6.14a
15.0	115.3a	7.57a	3.21a	17.41c	4.44cd
20.0	65.71bc	7.03a	3.13a	18.53c	3.74d
CV(%)	22.36%	14.55%	16.38%	12.55	22.89

*Means within column with the same letter(s) are not significantly different at P≥ 0.05% according to DMRT.

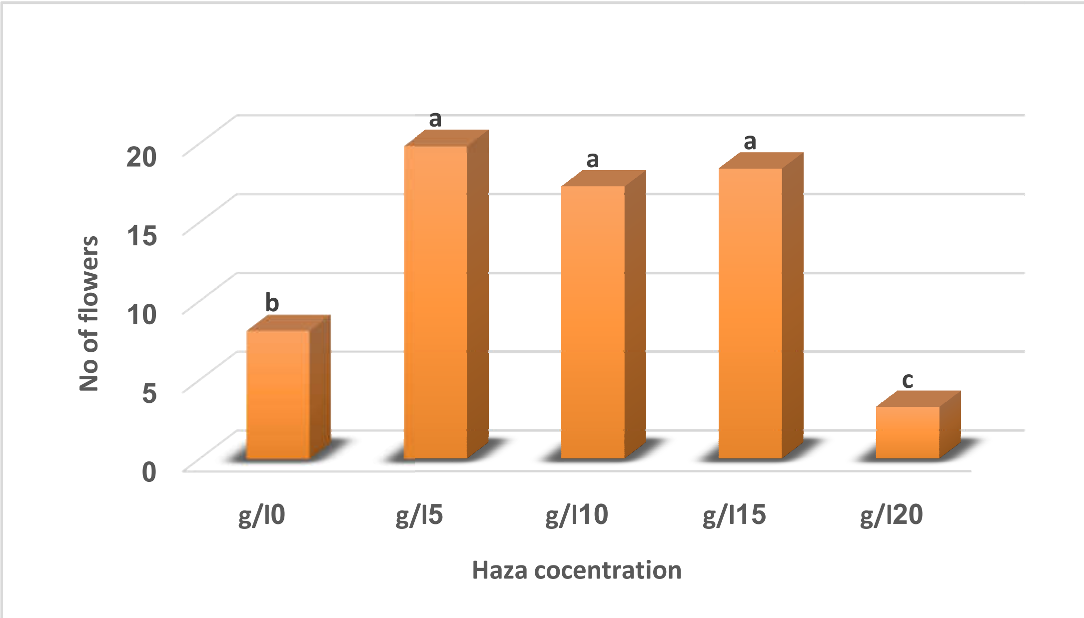

Figure 6. Impact of Haza foliar applications on the number of flowers in Periwinkle plant at Shambat, Sudan in 2018.

Table 4. Impact of Haza extracts on Periwinkle's number of fruits, seed weight and alkaloids content at Shambat, Sudan in 2018.

Haza conc. (g/l)	No of fruits	Seed weight (g)	Alkaloids content (%)
0.0	2.86c	0.11b	0.440b
5.0	6.86ab	0.23a	0.710a
10.0	8.57a	0.24a	0.885a
15.0	5.43ab	0.26a	0.846a
20.0	4.57bc	0.24a	0.406b
CV (%)	17.78%	20.82	4.19

*Means within column with the same letter(s) are not significantly different at P≥0.05% according to DMRT.

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