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Full Length Research Paper

Propagation, growth and yield of *Boweia volubilis* L. bulbs as affected by growth regulators, manure and inorganic fertilization

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***Boweia volubilis* L. is an endangered indigenous bulb that is highly valued for its medicinal properties by local communities. A method has been developed for rapid multiplication of *B. volubilis* L. from bulbs under nursery conditions. Bulbs of two sizes were cut longitudinally into four quarters, treated with solutions of varying concentrations of NAA, BAP and kinetin, germinated in river sand and transplanted into the field. The mean survival rate of the larger bulb quarters (98.1%), their rooting percentage (91.8%), the number of roots (3.8 roots/bulb quarter) and root dry weight (0.11 g/plant) were higher than those of smaller ones at 1 to 2 mg L^{-1} NAA. The percentage of new bulbs formed (98%), number of bulblets (3.6 bulbs/bulb quarter) and bulblet fresh weight (12.78 g/bulb quarter) were highest in larger bulbs treated with 2.0 mg L^{-1} BAP. The combination of manure and inorganic fertilizer gave the highest fresh weight (66.99 g/bulb) and diameter (66.7 mm) at 8 months of growth. These findings suggest that larger bulbs should be selected for propagation because they had superior rooting ability and a high survival rate. It is recommended that BAP at a concentration of 2 mg L^{-1} be used for propagation of this species. Although inorganic fertilization combined with manure gave a better yield, further work is needed to establish their effect on production of phytochemicals that are responsible for its medicinal uses.**

Key words: *Boweia volubilis*, bulb, propagation, 1-naphthalene acetic acid (NAA), N6-benzylaminopurine (BAP), fertilizer, manure.

INTRODUCTION

Boweia volubilis is a member of Hyacinthaceae. It is characterised by bulbs with thick basal leaves and branched racemes. The inflorescence is comprised of actinomorphic, bisexual flowers which range from white, blue and violet. It occurs among rocks from Eastern Cape to East Africa (Pooley, 1998). It is used to treat dropsy, barrenness in women and headaches, sore eyes, skin complaints and as love charm (Hutchings et al., 1996; Pooley, 1998).

In South Africa, several members of the Hyacinthaceae are harvested without permits from wild populations, processed and then sold as traditional medicine. This practice reduces density, distribution and genetic diversity of wild populations. The species *B. volubilis* is scarce, heavily traded with a high price in South Africa (Dold and Cocks, 2002). It was suggested that *ex situ* conservation through cultivation could alleviate pressure on natural resources, whilst meeting the demand for these plants (McCartan and van Staden, 1999).

The plant species *B. volubilis* is naturally propagated through seeds and from offshoots that develop from bulbs (Brown et al., 2003). However, the bulb is removed during harvesting which threatens the opportunity for perennial rejuvenation. Over-exploitation of the plant for medicinal purposes has caused a decline in wild populations resulting in poor seed set and germination (Broadhurst et al., 2008). One of the most important measures against over-exploitation of plant natural resources is to improve propagation and encourage cultivation especially at village level (Lewu et al., 2006).

Table 1. Characteristics of manure and soil at the University of Zululand's research station.

Nutrient	Soil sample	Manure
P (gm L^{-1})	4.5	193
K (gm L^{-1})	52	803
Ca (gm L^{-1})	221	321
Mg (gm L^{-1})	56	201
Exchange acidity (cmol L^{-1})	0.26	0.41
Total cations (cmol L^{-1})	1.94	5.72
Acid sat (%)	11	7
pH (KCL)	4.71	6.34
Zn (gm L^{-1})	4.2	8.3
Mn (gm L^{-1})	4.8	57
Cu (gm L^{-1})	0.4	0.7
MIR clay (%)	8	<5
MIR org C (%)	0.5	2.5
MIR N (%)	0.06	0.16

Plant tissue culture has become a powerful tool to develop micropropagation methods for endangered medicinal plants (Ascough and van Staden, 2010). Central to this technique is the induction of rooting and shoot growth by means of growth regulators. Research has shown that some growth regulators are more effective than others in the induction of roots or shoots. For example 1-naphthalene acetic acid (NAA) at a concentration of 1 mg L^{-1} produced more roots per shoot than IAA or IBA in shoot cultures of *Gladiolus* (Dantu and Bhojwani, 1995). An MS medium containing 2 mg/L BAP and 0.5 mg L^{-1} NAA was effective in the proliferation of bulblets from twin scales of *Ornithogalum oluphyllum* Hand.-Mazz (Ozel et al., 2008). In the species *Leptadenia reticulate* Retz., N6-benzylaminopurine (BAP) produced a larger number of shoots than kinetin at 2 mg L^{-1} concentration (Shekhawat et al., 2006). In the species *Scilla siberica*, the highest number of bulblets from bulb explants was produced using 1 mg L^{-1} BAP (Chaudhuri and Sen, 2002). The growth regulator BAP was more effective than kinetin in terms of corm formation in Gladiolus (Dantu and Bhojwani, 1995). However, kinetin was more efficient in protocorm multiplication at 2 mg L^{-1} concentration in *Cymbridium giganteum* Wall. Ex Lindl. (Hossain et al., 2010). The tissue culture technique requires specialised equipment and expertise, thus it is not affordable to the poor rural communities. On the other hand, vegetative propagation is cheap and can be done under nursery conditions that are affordable to poor communities.

There is a need for cultivation of medicinal plants in Southern Africa due to their scarcity and demand (Van Jaarsveld, 2006). The use of manure and inorganic fertilizer either alone or in combination have been shown by some workers to cause an increase in crop yield (Sharma and Subehia, 2003; Hati et al., 2008; Zobolo et al., 2008). The objectives of this study were: (a) To propagate *B. volubilis* from bulbs; (b) To grow seedlings emanating from propagation in the field for yield determination; (c) To expose plant traders to environmental education through workshops on propagation and growth of *B. volubilis*.

MATERIALS AND METHODS

Plant material

Plant material was obtained from plant traders at Nongoma Muthi market, situated in Northern KwaZulu-Natal, South Africa. For propagation purposes, bulbs of *B. volubilis* were cut longitudinally into four quarters and sterilized with 1% sodium hypochlorite containing 5 drops of Tween-20 for 20 min. The samples were divided into two sizes namely smaller bulbs (20 to 29 mm in diameter) and bigger bulbs (30 to 39 mm in diameter) and were dipped in solutions of varying concentrations of NAA and kinetin respectively. The procedure was repeated once a week for four weeks. The control plants were dipped in distilled water. The samples were germinated in 20 L plastic pots filled with a mixture of river sand and vermiculite (50:50) and placed in the nursery fitted with plastic roof to eliminate rainfall. The pots were placed in a random design at a spacing of 20 × 20 cm. Each treatment consisted of 20 bulb pieces and was repeated thrice. Humidity conditions within the propagators were enhanced by spraying the bulb pieces with water at 07.30 and 17.30 h each day using a hand-held sprayer. The relative humidity ranged from 80 to 90%. The shade cloth provided maximum light intensity of 400 µmol m^{-2} s^{-1}. Air temperature of the nursery ranged from 25 to 38°C depending on the light intensity. Bulbs were assessed weekly for the presence of roots (≥ 2 mm in length), new bulb formation and old bulb piece mortality. The pot experiments were terminated between 60 and 90 days after treatment.

When seedlings were 10 mm in diameter, they were transplanted into the field at Kwadlangezwa (28°51'S, 31°50'E) where the climate is sub-tropical. The nutrient status of compost and soil for Kwadlangezwa research station is presented in Table 1. The study area consisted of 16 m^2 plots with 4 m long furrows. Each plot had 40 seedlings of *B. volubilis* that were planted in furrows at 30 × 6 cm spacing on 16 August 2008. Plant protection measures and hoeing were conducted as necessary during the growing season. Water was provided to the furrows through an irrigation hose once a week. Compost and fertilizer treatments [2:3:2 (22) + Zn] were applied at 20 and 5 g per hole, respectively before transplanting. The field experiments were terminated at the end of the growing season, which lasted for 8 months.

Growth regulators

Solutions of 1-naphthalene acetic acid (NAA), N6-benzylaminopurine (BAP) and kinetin were prepared from chemicals purchased from Sigma (St. Louis, MO, USA) by dissolving the hormone in a mixture of absolute ethanol and methanol in a 1:1 ratio. The concentrations used were 0.5; 1.0 and 2.0 mg L^{-1}.

Statistical analysis

Analysis of variance (ANOVA) was carried out using randomized complete block design. Duncan's multiple range tests and least significant difference (LSD) test at p≤0.05 was used to test the

Table 2. Root growth and survival of bulb quarters made from small and large *B. volubils* L. plants treated with NAA at 60 days after planting.

Growth parameter	Size	NAA concentration (mg L^{-1})			
		0	0.5	1.0	2.0
Survival of bulb quarters (%)	Small	43.80 ± 0.592^{a*}	47.10 ± 1.260^{b*}	62.50 ± 0.897^{c*}	60.90 ± 1.656^{c*}
	Large	89.70 ± 1.342^{a**}	92.70 ± 0.955^{b**}	97.60 ± 0.653^{c**}	98.10 ± 0.546^{c**}
Root formation from bulb quarters (%)	Small	52.00 ± 0.471^{a*}	54.90 ± 0.525^{b*}	55.70 ± 0.558^{b*}	53.30 ± 0.517^{a*}
	Large	84.70 ± 0.731^{a**}	89.40 ± 0.669^{b**}	91.60 ± 1.011^{c**}	91.80 ± 0.512^{c**}
Number of roots per bulb quarter	Small	0.40 ± 0.245^{a*}	0.60 ± 0.245^{a*}	1.00 ± 0.447^{ab*}	1.60 ± 0.245^{b*}
	Large	0.80 ± 0.200^{a*}	1.00 ± 0110^{ab**}	3.60 ± 0.245^{c**}	3.80 ± 0.200^{c**}
Root dry weight per bulb quarter	Small	0.02 ± 0.001^{a*}	0.02 ± 0.001^{a*}	0.04 ± 0.005^{ab*}	0.05 ± 0.005^{b*}
(gram plant^{-1})	Large	0.03 ± 0.006^{a**}	0.03 ± 0.005a**	0.08 ± 0.011^{b**}	0.11 ± 0.010^{c**}

Means followed by different letters within a row differ significantly (p≤ 0.05). n=10. Means followed by a different number of asterisks within a column differ significantly (p≤ 0.05).

differences between the means of individual treatments.

RESULTS

Survival and root growth in NAA treated bulb quarters of *B. volubilis*

Survival of bulb quarters

The percentage survival of large bulb quarters made from *B. volubilis* L. was significantly higher (p≤0.05) than that of small ones at all NAA concentrations. The highest mean survival of both small (62.5%) and large (98.1%) bulb quarters was recorded at NAA concentrations of 1 or 2 mg L^{-1} (Table 2).

Root formation from bulb quarters

Rooting percentage by large bulb quarters was significantly higher than that from small ones. The highest rooting percentage by both small (55.7%) and large (91.8%) bulb quarters was recorded at NAA concentration of 1 or 2 mg L^{-1} (Table 2).

Root numbers

The highest root number from large (3.8 roots per bulb quarter) and small (1.6 roots per bulb quarter) was achieved in 2.0 mg L^{-1}. Large bulb quarters produced significantly more (p≤0.05) roots than the small ones at all NAA concentrations (Table 2).

Root dry weight

The highest root dry weight was observed in large bulb quarters that were treated with NAA concentration of 1 or 2 mg L^{-1}. The root dry weight of small (0.05 g/plant) bulb quarters was significantly lower that that of large (0.11 g/plant) ones at all NAA concentrations (Table 2).

Effect of kinetin and BAP on growth of bulblets formed by *B. volubilis* bulb quarters

Percentage bulblet formation

The highest percentage of bulblets produced by both small and large bulb quarters treated with kinetin was 65.6 and 92.1%, respectively. The small and large bulb quarters treated with BAP produced the highest percentage of 75 and 98.8%, respectively. Both kinetin and BAP treated bulb quarters produced the highest percentage of bulblets at concentrations of 1 or 2 mg L^{-1} (Figure 1). The percentage of bulblets produced in kinetin treatment was significantly lower (p≤0.05) than in BAP treated bulbs (Figure 1). Large bulb quarters also produced a significantly higher percentage of bulblets than the small bulb quarters in both kinetin and BAP treatments at concentrations of 1 or 2 mg L^{-1} (Figure 1).

The number of bulblets per bulb quarter

There were no significant differences (p≤0.05) in the number of bulblets formed by small bulb quarters treated with either kinetin or BAP. The highest number of bulblets

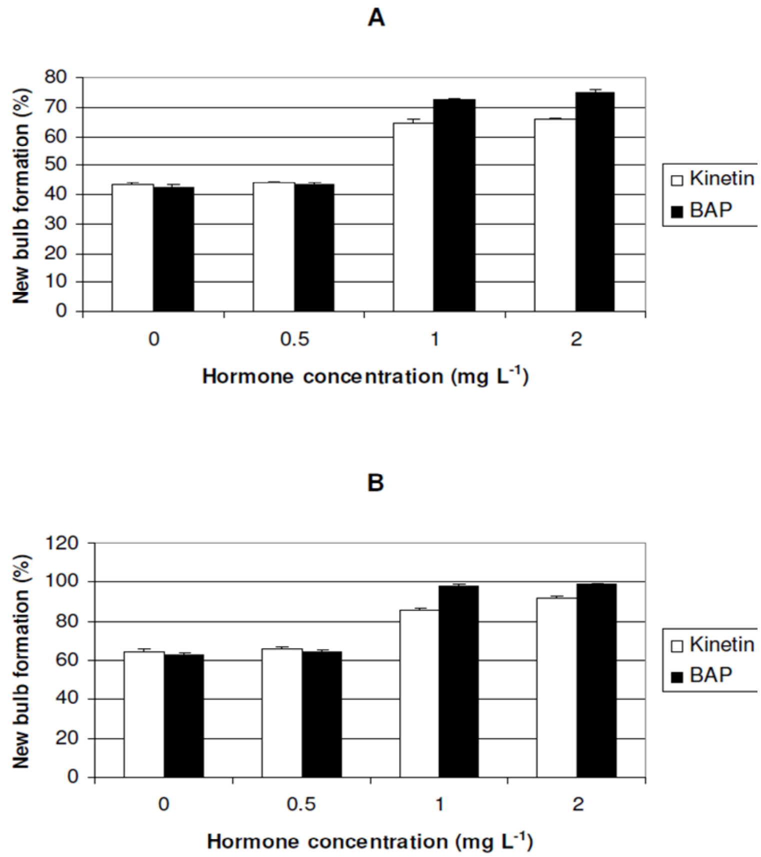

Figure 1. Effect of kinetin and benzylaminopurine (BAP) on the percentage of new bulbs formed from small (A) and large (B) bulb quarters made from *B. volubilis* L. at 60 days after treatment.

produced by large bulb quarters treated with 1 or 2 mg L^{-1} of kinetin and BAP were 1.9 and 3.6 bulblets per bulb quarter, respectively. The treatment of large bulb quarters with BAP resulted in a significantly higher ($p \leq 0.05$) number of bulblets than in kinetin treatment at 1 or 2 mg L^{-1} concentrations (Figure 2).

Fresh weight of bulblets

The highest fresh weight of bulblets in both kinetin (8.85 g/plant) and BAP (12.9 g/ plant) treatments were recorded at concentrations of 1 or 2 mg L^{-1}. The fresh weight from BAP treatments was significantly higher ($p \leq 0.05$) than the one from kinetin treatment at 1 or 2 mg L^{-1} in both small and large bulb quarters (Figure 3).

Effect of manure and fertilizer treatment on growth

Fresh weight of mature bulbs

The highest fresh weights in the 4th (29.12 g/plant) and 8th (66.99 g/plant) harvest dates were achieved in the treatment where manure was combined with inorganic fertilizer. The fresh weight of the control plants was significantly lower ($p \leq 0.05$) than that of manure and fertilizer treatments at both 4th and 8th month harvest dates. A combination of manure plus fertilizer recorded a significantly higher ($p \leq 0.05$) yield than that of manure and fertilizer applied alone (Table 3).

Bulb diameter

The largest bulb diameters at the 4th (40mm) and 8th (66.7 mm) harvest dates were recorded in the treatment where manure was combined with fertilizer. The control plants had significantly smaller ($p \leq 0.05$) bulb diameter than manure and fertilizer treatments at both harvest dates (Table 3).

DISCUSSION

Root growth

The use of a rooting hormone (NAA) in the present study

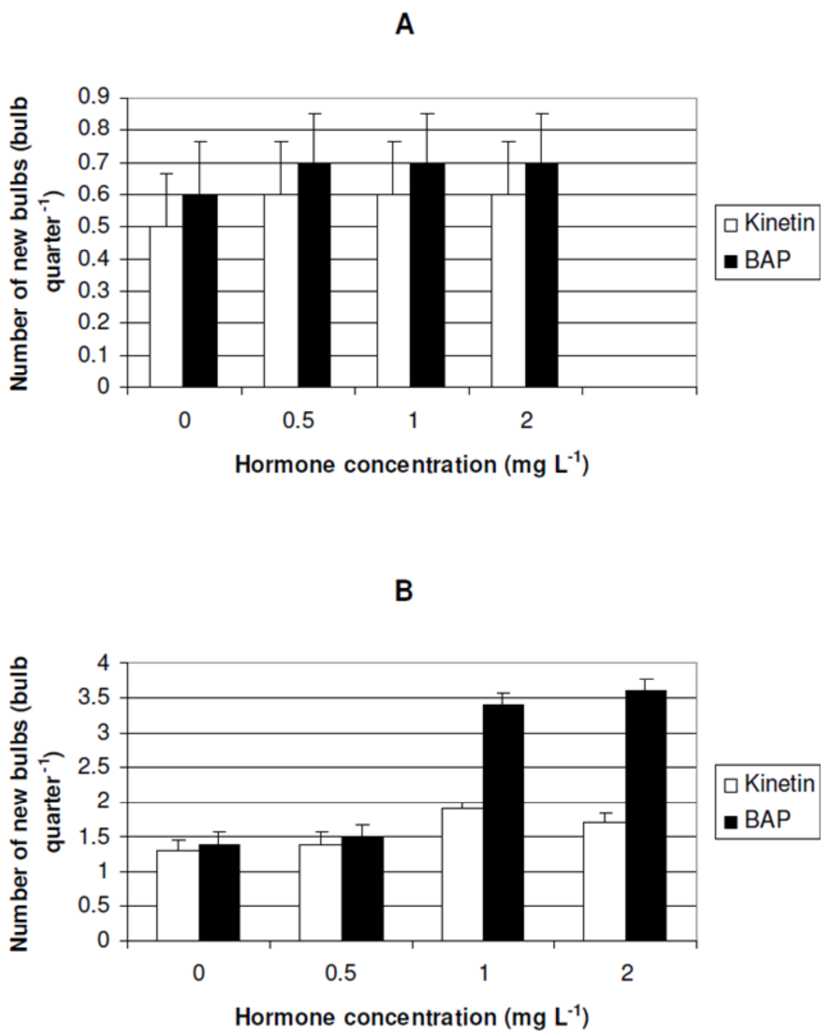

Figure 2. Effect of kinetin and benzyladenine (BAP) on the number of new bulbs formed from small (A) and large (B) bulb quarters made from *B. volubilis* L. at 60 days after treatment.

was intended to enhance growth and survival of *B. volubilis* bulb quarters. The highest percentage of rooted bulb quarters (91.8%) and highest root number (3.8 roots per bulb quarter) were achieved in 2.0 mg L^{-1} NAA (Table 2). The highest mean survival rate (98.1%) of bulb quarters was recorded in large bulb quarters (diameter 30 to 39 mm) at NAA concentrations of 1 or 2 mg L^{-1} (Table 2). The results of the present study are in agreement with the reports from other workers where optimum root initiation without bulblet formation was achieved in media supplemented with 1 or 2 mg L^{-1} NAA (Afolayan and Adebola, 2004; Bekheet, 2006).

The role of kinetin and BAP on bulblet induction

The highest percentage of bulblets produced by bulb quarters treated with either kinetin or BAP at a concentration of 1 or 2 mg L^{-1} were 92.1 and 98.6%, respectively (Figure 1). The percentage of bulblets produced in kinetin treatment was significantly lower ($p \leq 0.05$) than in BAP treated bulbs. The results of the present study agree with the findings of other workers in species such as *Scilla natalensis* (McCartan and van Staden, 1998) and *Ansellia Africana* (Zobolo, 2010) where an increase in kinetin concentration from 1 to 2 mg l^{-1} caused an increase in the number of new shoots. The highest number of bulblets produced by large bulb quarters treated with 1 or 2 mg L^{-1} of kinetin and BAP were 1.9 and 3.6 bulblets per bulb quarter, respectively. The bulb quarters treated with BAP produced a significantly higher ($p \leq 0.05$) number of bulblets than in kinetin treatment at 1.0 or 2.0 mg L^{-1} concentrations (Figure 2). These findings agree with those of other workers who used BAP and found it to be the most effective in induction and proliferation of adventitious

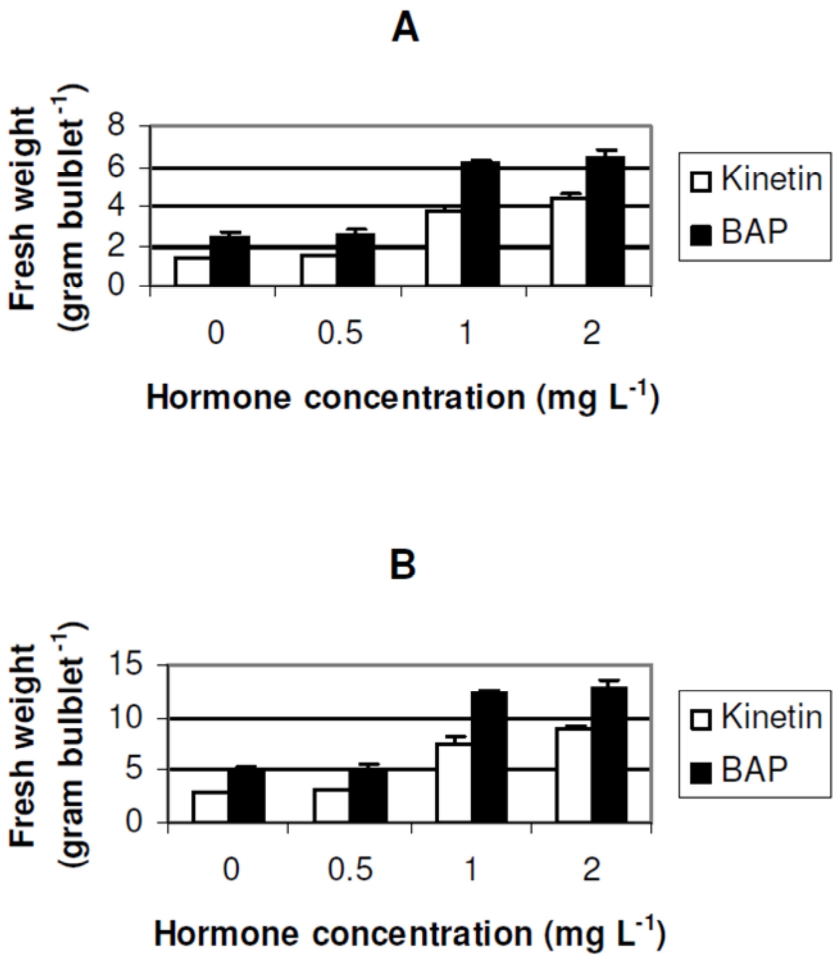

Figure 3. Effect of kinetin and benzyladenine (BAP) on the percentage of new bulbs formed from small (A) and large (B) bulb quarters made from *B. volubilis* L. at 60 days after treatment.

shoots in *Eclipta alba* (Ray and Bhattacharya, 2008; *Allium sativum* (Bekheet, 2006); *Fritillaria imperialis* (Mohammadi-Deheheshmeh et al., 2007).

The highest fresh weight of bulblets from BAP (12.79 g/bulb) treatment was significantly higher ($p \leq 0.05$) than the one from kinetin (8.85 g/bulb) treatment at a concentration of 2 mg L^{-1} (Figure 3). These findings agree with the reports on *Prunus dulcis* (Channuntapipat, 2003) and *Gladiolus* (Prasad and Gupta, 2006), where a concentration of 2 mg L^{-1} BAP (N^6-benzylaminopurine) gave a greater increase in fresh weight than the controls.

Effect of manure and fertilizer treatment on growth

The application of manure has already been established as a recommended fertilizer for improving productivity of vegetable such as *Vigna unguiculata* (Abdel-Moez and Gad, 2002); *Capsicum annum* (Abd El and Faten, 2009), *Thymus vulgaris* (Hendawy et al., 2010), several medicinal and aromatic plants, such as peppermint (O'Brien and Barker, 1996), and *Tagetes erecta* (Khalil et al., 2002). Increase in manure levels from 3.5 to 7.5 t/feddan caused a significant increase in all growth and chemical constituents of three mint species (Khalil and EL-Sherneny, 2003) as well as the yield of *Dracocephalum moldavica* (Hussein et al., 2006). In the present study, manure treatment gave a significantly higher ($p \leq 0.05$) yield than the controls in terms of fresh weight and bulb diameter (Table 3). These results agree with the report on onion where a combination of farmyard manure (23.8 m^3 farmyard manure/ha + 47.6 m^3 chicken manure) gave significantly higher fresh weight/plant (138.4 g) and diameter (8.96 cm) compared to control (Yassen and Khalid, 2009).

The addition of phosphorus as super-phosphate (Shaheen et al., 2007), potassium nitrate (KNO_3) and calcium nitrate [$Ca(NO_3)_2$] plus potassium chloride (KCL) (Ghoname et al., 2007) increased growth parameters (diameter and fresh weight) of onion (*Allium cepa*) bulbs.

Table 3. Effect of manure and fertilizer treatment on growth of *B. volubilis* L. seedlings.

Growth parameter	Duration (months)	Control	Organic manure	Inorganic fertilizer	Manure+ fertilizer
Bulb fresh weight (g/plant)	4	7.77 ± 0.839a*	22.31 ± 1.380b*	25.56 ± 0.749b*	29.12 ± 1.046c*
	8	26.37 ± 0.742a**	38.92 ± 1.912b**	54.43 ± 4.059c**	66.99 ± 4.479d**
Bulb diameter (mm)	4	23.90 ± 1.079a*	35.70 ± 0.989b*	34.70 ± 0.989b*	40.50 ± 1.087c*
	8	36.50 ± 0.778a**	44.50 ± 0.687b**	55.10 ± 1.986c**	66.70 ± 2.459d**

Means followed by different letters within a row differ significantly ($p \leq 0.05$). n = 10. Means followed by a different number of asterisks within a column differ significantly ($p \leq 0.05$).

In the present study, the fresh weight and bulb diameter of plants treated with inorganic fertilizer was significantly higher ($p \leq 0.05$) than both manure and control treatments (Table 3). The results of this study showed a significantly higher yield from inorganic fertilizer when compared to manure treatment. These findings are in accordance with the report on onion where bulb diameter increased significantly when chemical fertilizers were applied compared to controls. Organic manure gave a significantly lower bulb diameter and bulb fresh weight than chemical fertilizers (Lee, 2010).

A combination of manure and an inorganic fertilizer out yielded both organic manure and inorganic fertilizer applied alone in terms of bulb diameter and fresh weight (Table 3). These results agree with those of other workers where the yield of onion and garlic obtained with manure plus inorganic fertilizer was higher than that of manure or inorganic fertilizer alone (Aisha et al., 2007; Nasreen et al., 2009).

Conclusion

This study showed a successful propagation of *B. volubilis* from bulbs using growth regulators in the nursery. Bulb quarters with a larger diameter had a higher root dry weight, root numbers, percentage of rooting and survival than the small ones at all NAA concentrations, suggesting that larger bulbs should be prioritized during propagation. Because their availability is not guaranteed as they are the ones preferred by traditional healers, the alternative is to grow the small ones for one season, and then propagate them during the second season. Bulb quarters treated with 1 or 2 mg L^{-1} of BAP produced more bulblets with a higher fresh weight than kinetin treated ones, suggesting that BAP was a better growth regulator for this species. A high bulb yield that was obtained from manure treatment compared to control plants suggests that the poor communities can improve their yield without using expensive inorganic fertilizers. A combination of manure plus fertilizer recorded a higher yield than that of manure and fertilizer applied alone. Further work is needed to establish the effect of manure and fertilizer treatment on phyto-chemicals that are produced by this medicinal plant. A workshop had not been held with plant traders at the time of submission of the manuscript, but negotiations were in progress between the University of Zululand, community leaders and government officials in the department of environmental affairs and Tourism.

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