Assessment of Nursery Methods and Manures for Cultivation of Chirayita (*Swertia chirayita* Buch-Ham.) in Lauri Gewog, Bhutan

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ABSTRACT

Chirayita (Swertia chirayita) is a medicinal plant and is native to the temperate Himalayas. It is widely used in traditional and modern medicines with an increased global demand, leading to unsustainable harvesting, causing the decline, and critically endangering the species. Hence, we hypothesized that chirayita could be domesticated and tested this hypothesis at Lauri Gewog in southeastern Bhutan. Four nursery methods and four organic manure treatments were tested using Completely Randomized Design with three replicates. The average seedling density across the four treatments at mid altitude was 2356 seedlings/m^2, and that of high altitude was 1202 seedlings/m^2. The seedling density at mid altitude was 96% higher compared to that of high altitude; however, there were significant differences between the treatments only in high altitude ($P = 0.003$) and not in mid altitude. The methods were non-significant at mid altitude, mainly due to the large variability of data. Interestingly, the manure treatments were not significant compared to the control implying that the chirayita could be non-responsive to the application of manures in the study conditions. Fresh weight of chirayita in the control plot was 14, 19 and 29% higher than 'compost', 'FYM' and 'compost+FYM (1:1)' treatment plots. Further, this research provides an alternative low-cost nursery technique for adoption by chirayita farmers while also providing information for policymakers and environmental conservationists in developing strategies for conservation of a critically endangered species like chirayita.

Keywords: *Swertia chirayita; Nursery methods; Manure application; Seedling density; Conservation; Domestication*

1. Introduction

Chirayita *(Swertia chirayita* Buch-Ham.) is a medicinal plant indigenous to the temperate region of the Himalayas (Bhargava, Rao, Bhargava, & Shukla, 2009; Naveen, Suresh, Chhavi, & Ritu, 2017; Scartezzini & Speroni, 2000). It is found at altitudes ranging from 1200 to 3000 meters above sea level (masl) in Bhutan, India and Nepal (Aleem & Kabir, 2018; Balaraju, Agastian, & Ignacimuthu, 2009; Bhargava et al., 2009; Khanal, Shakya, Nepal, & Pant, 2014; Shukla, Dhakal,

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Uniyal, Paul, & Sahoo, 2017). It is popularly known as 'chirayita'. The use of this species is reported in many traditional medicine systems viz. Ayurveda, Bhutanese *gSo-ba Rig-pa*, Unani, Siddha, Tibetan, Yunami, British and American pharmacopoeias (DoF, 2008; Joshi & Dhawan, 2005; Negi, Singh, & Rawat, 2011; Pradhan & Badola, 2015a; Sabita, Lal, Kumar, & Amrita, 2019; Saha & Das, 2010; Scartezzini & Speroni, 2000). It is used to treat a wide range of ailments (Brahmachari et al., 2004; Kumar & Staden, 2016; Pradhan & Badola, 2015a). It is valued for its active compounds such as amarogentin, amaroswerin, gentianine, swertinin, swerchirin, mangiferin, lignan, triterpenoid, decussatin and isobellidifolin (Bhargava et al., 2009; Brahmachari et al., 2004; Joshi, 2008; Joshi & Dhawan, 2005; Scartezzini & Speroni, 2000; Tabassum, Mahmood, Hanif, Hina, & Uzair, 2012). It is reported to possess numerous medicinal properties such as antipyretic (Bhargava et al., 2009), anthelmintic (Iqbal, Lateef, Khan, Jabbar, & Akhtar, 2006), anti-inflammatory, anti-diabetic (Karan, Vasisht, & Handa, 1999), antibiotic (Roy et al., 2015) and anti-carcinogenic (Saha & Das, 2010).

The traditional medicine systems use the whole plant (Bhargava et al., 2009; Tabassum et al., 2012), wherein the entire plant, including its roots is uprooted before seed setting, depriving the species of its natural regeneration potential (Aleem & Kabir, 2018). Further, the natural regeneration of chirayita is also affected due to poor seed germination and low viability (Chakraborty, Mukherjee, & Baskey, 2016; Chaudhuri, Pal, & Jha, 2007, 2008, 2009). Due to over-exploitation, unsustainable harvesting practices and habitat loss, the International Union for Conservation of Nature (IUCN) listed the chirayita as one of the critically endangered plant species (Joshi & Dhawan, 2005; Kumar & Staden, 2016). This was why India banned the trade of wild *Swertia chirayita* since 2004 (Cunningham, Brinckmann, Schippmann, & Pyakurel, 2018). On the contrary, the regional and international demand for chirayita is on the rise every year. According to Chaudhuri et al. (2007), the annual demand for chirayita in the Indian subcontinent alone is 400 tons, with an annual growth rate of 10 %. Similarly, Cunningham et al. (2018) estimated an annual export volume of 489.70 to 698.90 tons from Bhutan, India and Nepal of which Bhutan's share constituted about 19 %. The export trend of chirayita from Bhutan (2009 to 2019) (Figure) demonstrates the importance of chirayita for Bhutanese farmers. The majority of the market demand is still met from the wild collection, and a limited share comes from cultivated sources as it is generally not cultivated due to the lack of standard cultivation package of practices (Badola & Pradhan, 2011). However, the past evidence shows that attempts were made to encourage and promote the cultivation of the chirayita in Bhutan (DoFPS, 2012). In recent years, there is a rising interest of pharmaceutical companies in cultivated chirayita for authenticity and sustainability reasons (Badola & Pradhan, 2011). Further, there is a growing concern among the consumers of pharmaceutical products on whether a product has sustainable source. This impetus from market forces provides massive scope for domesticating medicinal plants like chirayita for commercial cultivation. However, there is a lack of understanding of how this species can be cultivated and this study attempts to investigate its domestication potentials under Bhutanese conditions.

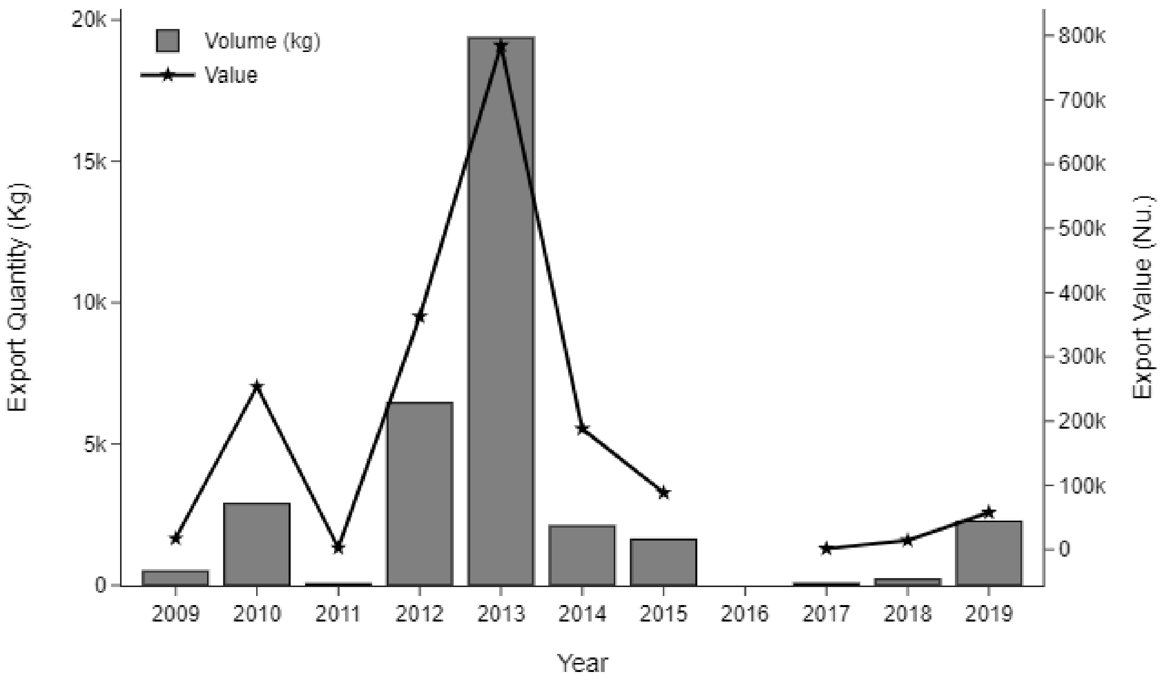

Figure 1. Chirayita export volume (kg) and value (Nu.) from Bhutan (2009-2019), Source – Bhutan Trade Statistics (2009 – 2019).

The people of Lauri Gewog (block) under Samdrup Jongkhar district in Bhutan depend on Non-Wood Forest Products (NWFPs) for cash income, employment, and livelihood. Their dependency and livelihood are at stake due to the depletion of wild chirayita in the forest coupled with the decrease in the market price for wild chirayita in recent years (DoF, 2008). This is a serious concern to the farmers, local leaders and conservationists in Bhutan. The farmers attribute the decline of chirayita plants to the government policy on banning the 'slash and burn' farming system. They believe that the 'slash and burn system' would stimulate the natural regeneration of chirayita. This is further compounded by the indiscriminate and unsustainable harvesting of chirayita from the wild. The resource assessment carried out by the Department of Forests further supports the depletion of wild chirayita resources (DoF, 2008).

Hence, there is an urgent need to domesticate and cultivate chirayita on-farm for a sustainable supply chain and its conservation (Badola & Pradhan, 2011; Jäger & Staden, 2000; Lubbe & Verpoorte, 2011; Phondani et al., 2016; Shukla et al., 2017; Wiersum, Dold, Husselman, & Cocks, 2007). While there were studies that recommended different propagation techniques, including in-vitro propagation (Balaraju et al., 2009; Chaudhuri et al., 2007, 2008, 2009; Pradhan & Badola, 2008, 2011, 2012), such technologies are too expensive and remained inaccessible to the farmers. There is a need to generate low-cost cultivation techniques, including nursery methods and cultivation practices easily accessible and adoptable by farmers. Therefore, the present study attempted to domesticate *Swertia chirayita* with the following specific objectives: (i) to evaluate low-cost nursery methods and (ii) to evaluate yield and yield parameters with different manure treatments.

2. Materials and Methods

2.1. Study Site

The study was conducted in two villages of Tshothang and Dungmanma in Lauri Gewog under Samdrup Jongkhar district in the southeastern part of Bhutan between 2015 to 2017 (Figure). The gewog shares its border with the Arunachal Pradesh state of India in the East, Merak Gewog of Trashigang Dzongkhag in the north and Serthig Gewog in the south. The gewog has a total area of 273.4 sq. km. It lies between the longitude 91˚47’26 E’’ to 92˚ 02’28 E’’, and latitude between 27˚ 04’94 N’’ to 27˚ 14’37 N’’ with altitudes ranging from 1800 - 2600 masl. Maize, paddy, upland paddy, millets, buckwheat, and some vegetables are cultivated as the primary food crops (MoAF, 2020). The people earn cash income mainly from the sales of NWFPs particularly, *Rubia cordifolia*, *Paris polyphylla*, *Litsea cubeba* and *Swertia chirayita*.

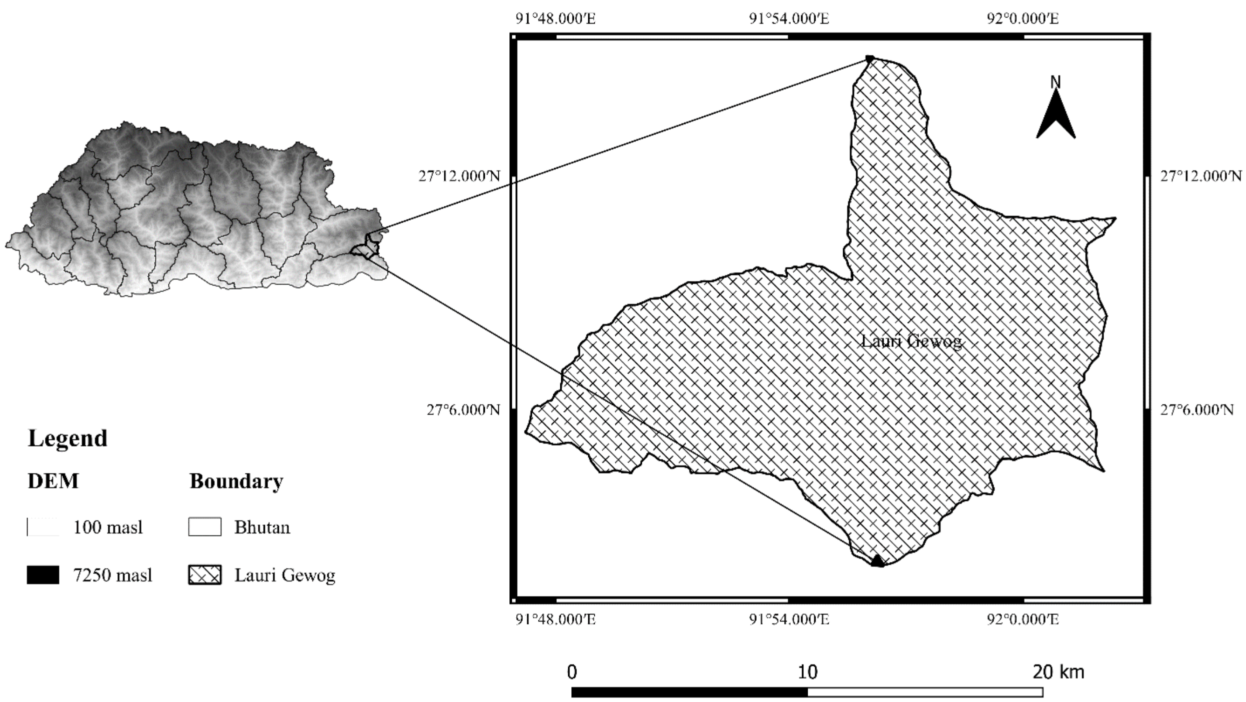

Figure 2. The study site of Lauri Gewog under Samdrup Jongkhar district.

2.2. Experiment Design

All the experiments under the present study were carried out in open field conditions following the recommendations by Badola and Pradhan (2011) for chirayita cultivation in Sikkim. The method also has reference to the findings of Pradhan and Badola (2015b) where chirayita was found to do well in the open field condition in its natural habitats. Both the nursery (detailed in section 3.2) and cultivation (with different organic manures and combinations) experiments were assessed using Complete Randomized Design (CRD) with three replications as the field was fairly homogeneous. The farmer method was used as the control for the nursery experiment. For the cultivation experiment, four types of manure treatments; (i) farmyard manure (FYM) @ 10 t/acre, (ii) compost (based on crop residues and cattle manure) @ 10 t/acre, (iii) Compost and FYM (1:1) @ 10 t/acre and (iv) control without any organic manures were applied.

Seeds collected from the healthy wild population in November 2014 were cleaned, processed (without any seed treatment), and sown at the rate of 6 g per treatment plot. Care was taken to separate external materials, including weed seeds. The seed sowing was done in March - April 2015 (Mukherjee, 2013; Shukla et al., 2017). Manual weeding and routine irrigation were provided to growing seedlings as and when required.

The transplanting of chirayita seedlings in the main field was done five months after sowing when the seedlings attained a 3-5 leaf stage and when the seedlings were strong enough to withstand transplanting shock. The chirayita seedlings (32 numbers) were transplanted on raised beds (1m x 2 m), maintaining row to row and plant to plant distance of 15 cm. The entire trial plots were fenced to ensure protection for the growing plants.

2.3. Nursery Methods

There were four nursery raising methods assessed in the study. The control used was the farmer method. The following section is devoted to providing details of each method for repeatability/reproducibility.

Burning method

This method mimics the slash and burn practice usually practiced by farmers who believe that chirayita grows better in burnt areas. The soil was dug or ploughed three times to prepare raised beds of 1m x 2m dimensions. Stones and large clods were removed and soil was leveled. The beds were then covered with dry plant mass and burnt to ashes. Thick ashes were removed after letting the soil cool overnight. Six grams of chirayita were then broadcast using a perforated plastic bottle (seed broadcaster).

Dolokha method

This method of nursery is practiced by villagers of Dolokha village in Nepal. It was introduced by the researchers after a short study visit to Nepal in 2015. However, the method is not documented. The soil was dug or ploughed five times. Stones and large clods were removed, and the soil was sieved three times using a fine sieve. Then the soil was raised to make 1m x 2m beds. Six grams of chirayita seeds were then broadcast using a perforated plastic bottle (seed broadcaster) and covered with a thin layer of pine leaves.

Farmer method

This method is used by the farmers of Lauri Gewog in raising nurseries of small seeded vegetables. Raised beds of 1m x 2m beds were prepared after digging and ploughing the soil three times. Stones and large clods were removed and soil was leveled. Six grams of chirayita seeds per bed was then broadcast with hand.

Research method

This method is commonly used by the Medicinal and Aromatic Plants Program (MAP), National Centre for Organic Agriculture (NCOA), Yusipang for domestication research. However, it is important to note that this method is nowhere documented. The soil is dug or ploughed three times. The stones and large clods were removed, and the soil sieved two times using a fine sieve. Then the soil was raised to make 1m x 2m beds in dimension and leveled. Six grams of chirayita seeds were then sown in lines at 5 mm depth, maintaining row to row distance of 30 cm and covered with a thin layer of fine soil.

2.4. Parameters studied

Chirayita seedling parameters

The data for seedling density was collected from Tshothang (high altitude) and Dungmanma (mid altitude) villages. The seedling density per unit area was obtained by randomly throwing one square foot quadrat in the treatment plots and counting the number of seedlings within the quadrat. The process was replicated three times. The values obtained were later converted into seedling density per square meter, and an average value was calculated to represent the plot.

The chirayita seedling parameters assessed were (1) plant height, (2) leaf length, and (3) leaf width. The data were collected in August 2015 from the 10 randomly selected seedlings before transplanting (at 3-5 leaf stage). The plant height was measured from the soil surface to the tip of the tallest leaf, while leaf length and width were measured from well-developed leaves.

Yield and yield parameters of chirayita

Due to extended crop gestation period to three years in the high altitude (Tshothang) experimental site and due to unavoidable logistic challenges, chirayita harvest data was recorded only from mid altitude (Dungmanma village) in October 2016. Chirayita was harvested after flowering but before the seed setting when the stem starts turning yellowish. The yield parameters assessed include (1) fresh weight, (2) plant height, (3) root length, and (4) number of shoots per plant. The plant height was measured before the harvest, while the root length and plant biomass measurement (fresh weights) were undertaken after the harvesting operations. The number of shoots per plant were recorded during harvest. Enough care was taken to exclude extraneous materials such as weeds and soil.

2.5. Data processing and analysis

The data collected were processed and analyzed using the general-purpose programming language Python version 3.8.4 (Van Rossum, 2007, June). Various python libraries were used viz. Pandas (McKinney, 2011) for data cleaning and processing, matplotlib (Hunter, 2007), Seaborn (Waskom et al., 2014) and Plotly (Sievert, 2020) for data visualization, and Numpy (Walt, Colbert, & Varoquaux, 2011), Scipy (Virtanen et al., 2020) and Statsmodels (Seabold & Perktold, 2010, June) for statistical analysis.

The means of variables under study were compared using one-way analysis of variance (ANOVA) at a *P*-value (alpha) of <0.05 for statistical significance. Tukey's *HSD* post hoc test was used for multiple comparisons of means (*P*-value = 0.05). Descriptive statistics were used to compute the mean and standard deviation of the variables assessed. All the variable means obtained from Tshothang and Dungmanma villages are presented as high altitude (2015 masl) and mid altitude (1655 masl), respectively.

3. Results and Discussion

3.1. Chirayita seedling parameters

Chirayita seedling density

The means of seedling density per square meter obtained from both the study sites are presented in Table 9. The ANOVA showed that the seedling density per meter square amongst the four nursery methods differed significantly only for high altitude and not for mid altitude. The average seedling density across the four treatments at mid altitude was 2356 seedlings/m^2, and that at high altitude was 1202 seedlings/m^2. The seedling density at mid altitude was 96% higher compared to that of high altitude. However, there were no significant differences between the treatments at mid altitude (Table 9).

The farmer method at the high altitude obtained significantly higher seedling density per square meter than the other methods except for the Dolokha method. While the seedling density in the Dolokha method significantly differed from burning method, it did not differ with the research method in high altitude. Further, the seedling density in research method and burning method did not differ significantly in high altitude. Although not significant statistically, the burning method in the mid altitude obtained higher seedling density in comparison to other methods.

Table 9. Mean seedling density at two study sites.

Nursery methods	High altitude (Number per m^2)	Mid altitude (Number per m^2)
Burning method	481^{c}	3362^{ns}
Dolokha method	1385^{ab}	1622^{ns}
Farmer method	1191^{a}	2020^{ns}
Research method	951^{bc}	2418^{ns}
P-value	0.003	0.25

Different lower-case letters in the superscript indicate statistically significant differences following the Tukey's HSD post hoc analysis at P<0.05; ns = not significant.

Plant height, leaf length and leaf width of chirayita seedlings

The mean of the seedling parameters segregated into high altitude and mid altitude are presented in Table 10. The study found that the seedling parameters amongst the four nursery methods differed significantly for mid altitude (**Error! Reference source not found.**Table 10).

All the seedling parameters in the Dolokha method were significantly different from that in the other nursery methods in the mid altitude, while other pairwise comparisons did not show any significant differences (Table 10). On the contrary, no significant difference in the seedling parameters was observed amongst the nursery methods at high altitude (Table 10). However, the burning method had the tallest seedlings compared to other nursery methods.

Table 10. Mean seedling parameters in different nursery methods at two study sites.

Nursery method	High altitude (2015 masl)			Mid altitude (1655 masl)		
	Height $(cm)^{ns}$	Leaf length $(cm)^{ns}$	Leaf width $(cm)^{ns}$	Height (cm)	Leaf length (cm)	Leaf width (cm)
Burning method	0.88 (0.25)	1.39 (0.36)	0.60 (0.08)	$0.99\ (0.04)^{b}$	$1.16\ (0.21)^{b}$	$0.65\ (0.15)^{b}$
Dolokha method	0.75 (0.17)	0.96 (0.19)	0.49 (0.06)	$2.05\ (0.47)^{a}$	$2.76\ (0.77)^{a}$	$1.18\ (0.08)^{a}$
Farmers method	0.84 (0.02)	1.22 (0.10)	0.53 (0.01)	$1.11\ (0.25)^{b}$	$1.35\ (0.43)^{b}$	$0.74\ (0.09)^{b}$
Research method	0.76 (0.22)	0.93 (0.16)	0.48 (0.08)	$0.92\ (0.13)^{b}$	$1.02\ (0.17)^{b}$	$0.57\ (0.10)^{b}$
P-value	0.817	0.145	0.32	0.004	0.007	0.003

Different letters in superscripts indicate statistically significant differences following Tukey's HSD post hoc analysis at P<0.05; ns = not significant; values in parentheses indicate standard deviations of the means.

It was found that across both sites, on average, all four nursery methods performed better in mid altitude compared to the high-altitude. The poor performance of nursery in the high altitude could have been due to low temperature affecting the seedling growth and development, resulting in shorter height, leaf length, and width. The better performance in the mid altitude could be due to the fulfillment of optimum temperature required for proper growth and development of chirayita seedling. A similar finding was reported in feasibility research of two *Lesquerella* species in

Arizona (Dierig, Adam, Mackey, Dahlquist, & Coffelt, 2006). The significant performance of the Dolokha method at the mid altitude could be possibly due to the combined effect of higher moisture retention by applied mulches and conducive conditions. Further, the performance of different nursery methods did not follow the same trend at the two altitudes, which could be attributed to variation in the micro-climatic conditions.

The findings from the current nursery trial provide an alternative and cheaper propagation method against the current backdrop of expansive and non-adoptable propagation techniques such as tissue culture and in-vivo propagation. It also indicates that the farmers of Tshothang (high altitude) can adopt either 'Dolokha' or 'burning' method, while the farmers of Dungmanma (mid altitude) are recommended to adopt either 'Dolokha or 'farmer' method for raising chirayita seedlings. The current study could not capture the effect of the 'slash and burn system' on the germination and regeneration of chirayita as opined by the farmers. However, the performance of the burning method in both sites suggests the positive effect of the system on chirayita germination and regeneration. It may be noted that the results presented in this article are based on 'one crop season' data, and thus, more similar research in the future is required to substantiate our findings.

3.2. Yield and yield parameters of cultivated chirayita

The current study showed that the yield (Figure) and yield parameters (Table 11) among the treatments were statistically insignificant. However, the average yield obtained under the current study is comparable to the findings of a study conducted in Sikkim (Badola & Pradhan, 2011). It is interesting to note that the manure treatments were not significant compared to the control in terms of all the parameters assessed, implying that chirayita may be non-responsive to the application of manures under the study conditions. Also, the fresh weight of chirayita in the control plot was 14, 19 and 29% higher than 'compost', 'FYM', and 'compost+FYM (1:1)' treatments showing the possible non-responsive nature on the yield. This could be interpreted in three ways. Firstly, possibly due to the fulfillment required soil fertility (soil could have been fertile and no additional fertility enhancement needed) for the crop. Secondly, it could be because the native plants or landrace are rather negatively affected by excess fertilizers since they are adapted to natural conditions. Thirdly, the possible non-responsive nature of chirayita to the local conditions of Lauri Gewog.

Further, the results hint that the application of manures could retard agronomic productivity. Similar findings were reported by Samarakoon (1997), where the combined application of N, P and K treatment reduced the yield of *Rauvolfia serpentina*. It is an important finding that this study has uncovered, and so there is a need to conduct a series of studies to reconfirm it. It is recommended that soil nutrients status in the experimental sites be investigated along with the agronomic parameters so as to rule out any pre-existing high soil fertility status. Non-responsiveness to manures by chirayita observed can be validated once the suspected pre-existing high soil fertility status is ruled out.

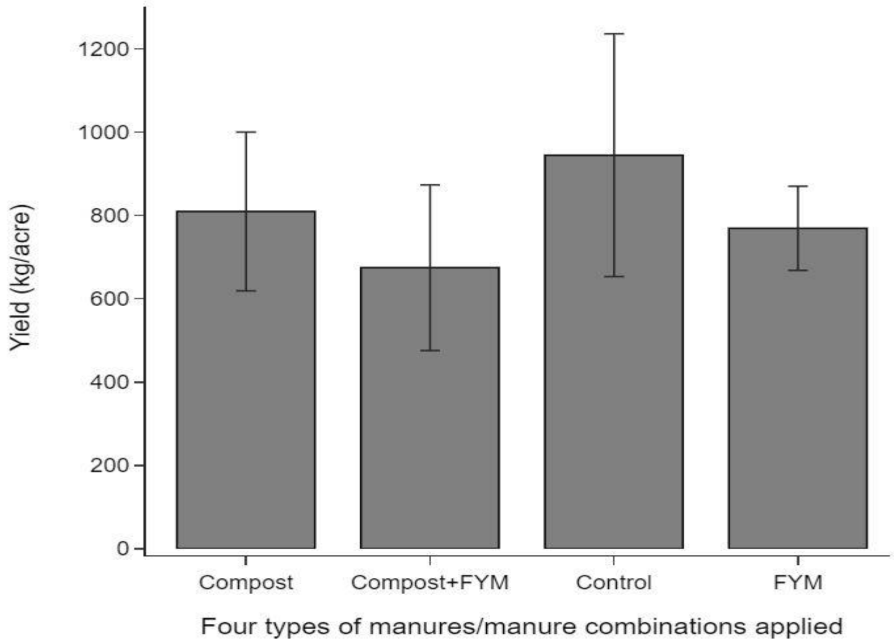

Figure 3. Fresh weight yield including roots (kg/acre) of chirayita from different manure treatments. Vertical bars indicate SE of means. There are no significant differences between treatments.

The tallest plants were observed in the treatment plot applied with ‘FYM’, while the longest root length and number of shoots per plant were recorded in the treatment plots that received ‘compost + FYM (1:1)’. Although not significantly different, the ‘control’ plot produced taller plants compared to the ‘compost’ and ‘compost + FYM (1:1)’ treated plots. Similarly, root length was longer in the control plots compared to that in the ‘compost’ and ‘FYM’ treated plots.

Table 11. Mean yield parameters of plant height, root length, shoots per plant in the four manure treatments. The treatments are not significantly different for the three attributes.

Treatment	Plant height (cm)	Root length (cm)	Shoots / plant (numbers)
Compost	122.06	11.22	6.06
Compost+FYM (1:1)	112.58	12.67	6.92
Control	117.08	12.44	5.33
FYM	130.00	11.72	3.33
P-value	0.78	0.92	0.59

4. Conclusion

The findings corroborate that chirayita can be cultivated like any other crop. In the current study, the seedling density was significantly different for high altitude and not for mid altitude growing conditions. The nursery methods for seedling density did not differ significantly at mid altitude because of the large variability of data. Interestingly, the manure treatments were not significant compared to that in the control plots implying that the chirayita may be non-responsive to the application of manures in conditions similar to the study sites. The fresh weight of chirayita in the control plot was 14, 19 and 29% higher than 'compost', 'FYM' and 'compost+FYM (1:1)' treatments. Further studies are required to validate whether or not chirayita as a cultivated crop is non-responsive to manure applications.

Additionally, this research provides an alternative low-cost nursery technique for adoption by the chirayita farmers while also providing missing information for policymakers and environmental conservationists in developing strategies for conservation of chirayita - a critically endangered and vulnerable plant species.

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