Journal of Medicinal Plants Research Vol. 4(16), pp. 1617-1623, 18 August, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.465
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Growth response of *Artemisia afra* Jacq. to different pH levels in a closed hydroponics system

R. Koehorst, C. P. Laubscher and P. A. Ndakidemi*

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, P. O. Box 652, Cape Town, 8000, South Africa.

Accepted 25 May, 2010

In this study the effect of varying levels of pH on the fresh and dried yield and chlorophyll content of *Artemisia afra* Jacq. was investigated. Five groups of plants received deionised water adjusted to a certain pH. The pH for the treatments were 4.5, 5.5, 6.5 (the control), 7.5, and 8.5. The fresh weight of the plants was highest for the pH treatment of 6.5, while the fresh weight of the 4.5, 5.5 and 7.5 treatments did not differ significantly. The plants grown with a pH of 8.5 were significantly reduced in fresh weight. The total dry weight, shoot dry weight and root dry weight of the plants did not show significant variation between the 5.5, 6.5 and 7.5 treatments, but was significantly lower for the 4.5 and 8.5 treatments. The chlorophyll content of the leaves of the plants showed a marked variation between treatments. The chlorophyll content of the plants grown at pH 6.5 was highest, followed by those grown at the pH of 5.5 and the pH 4.5. The chlorophyll content of the pH 7.5 treatment was the second lowest, while the content of the 8.5 treatment was the lowest. These results indicated that the optimum pH of water supplied to *A. afra* can have a significant effect on the fresh and dried yield of this plant. The study shows that, although *A. afra* can survive at a range of acidic pH's, it does not fare well with regards to chlorophyll content, fresh weight, root dry weight and shoot dry weight in an alkaline or acidic situation.

Key words: Chlorophyll content, traditional medicine, yield, *Artemisia afra*, pH level, acidic stress.

INTRODUCTION

Indigenous cultures have relied on plants to supply their medicinal needs for centuries (Taylor et al., 2001). In South Africa alone it is estimated that about 27 million people depend on traditional medicine for their health care needs (Fennell et al., 2004). One estimate of the economic value of medicinal plants in South Africa is that the trade generates roughly $6 million annually (Keirungi and Fabricius, 2005). By far the majority of these plants are collected from the wild (Van Andel and Havinga, 2008). It has been predicted that around 700,000 tonnes of medicinal plants will be harvested in 2009 (Makunga et al., 2008). In the last 10 years the commercialization of Traditional African Medicines has been rapidly gaining momentum (Van Wyk, 2008). In the past, low population densities helped to limit the demands placed on the natural ecosystems by harvesters (Netshiluvhi, 1999), most of whom are traditional healers (Van Andel and Havinga, 2008). However, rising unemployment levels combined with the entry into a cash economy has led to a breakdown of traditional conservation methods (Netshiluvhi, 1999).

As the effectiveness of medicinal plants is more widely acknowledged and accepted, over harvesting and extinction can result (Strangeland et al., 2008). According to McGeocha et al. (2008), "Over exploitation is a growing problem for many medicinal species in Africa". As an example, in Tanzania alone there are nine plants of medicinal value that are reported to be of conservation concern (Strangeland et al., 2008). Although there is still a drive towards sustainable harvesting, increasing demand coupled with the loss of habitats is quickly leading to the only real solution being the cultivation of important medicinal plants (Fennell et al., 2004).

According to Netshiluvhi (1999), the supply of the most commonly used plants could be ensured only by using a

*Corresponding author. E-mail: ndakidemip@cput.ac.za. Tel: 2721-460-3196. Fax: 2721-460-3193.

"...firm scientific basis for propagation". Although it is agreed that there is a need for the cultivation of medicinal plants, there is a lack of relevant information available as to the specific requirements of these plants (Makunga et al., 2008; Fennell et al., 2004; McGeocha et al., 2008). Little information exists on the effects of cultivation practices on the growth and biological activity of African medicinal plants (Fennell et al., 2004). There is a pertinent need to determine which plants would be suitable for cultivation on a medium to large scale (Van Wyk, 2008). Street et al. (2008) reported that 82% of traditional healers would cultivate the medicinal plants that they use. While there is information regarding specific crops it is generally circumstantial and general in nature, with little scientific justification.

The most widely utilized medicinal plant in southern Africa is undoubtedly *Artemisia afra* Jacq. (Liu et al., 2008; Diederichs, 2006; Van Wyk, 2008). The most common method of use is as either dry or fresh leaves and shoots boiled and then used as a tea. Sometimes the roots are also used (Diederichs, 2006; Liu et al., 2008). It is often also used fresh to pack around painful teeth, or as a decoction that is used against gum infections by holding in the mouth (Diederichs, 2006). *A. afra* Jacq. contains many chemical compounds (Liu et al., 2008; Van Wyk, 2008). The most common components are scopoletin, found in the flower heads, and α–thujone, ß-thujone, artemisyl acetate and artemisia ketone. *A. afra* also contains camphor, santolina alcohol, and borneol, as well as a large number of secondary metabolites (Bohlmann and Zdero, 1972; Liu et al., 2008).

The main traditional uses of *A. afra* are to treat chest problems, such as coughs, asthma, pneumonia, croup, influenza and upper respiratory tract infections. It can be used to treat stomach problems like gastritis, gastric derangement, dyspepsia, poor appetite, indigestion, constipation, flatulence, colic and intestinal worms (Diederichs, 2006; Liu et al., 2008; Gurib-Fakim, 2006). It is also used to treat gout, malaria, fevers, colds, chills, bladder and kidney disorders, diabetes, convulsions, heart inflammation, rheumatism, and sore throats (Diederichs, 2006; Liu et al., 2008; Gurib-Fakim, 2006). It is sometimes used as a purgative (Diederichs, 2006; Liu et al., 2008; Gurib-Fakim, 2006). *A. afra* has shown some antimicrobial and antioxidative activity in *in vitro* tests (Viljoen, 2007).

A. afra can tolerate a wide range of environments, (Diederichs, 2006) but is reported to grow best in a sandy/ loam soil (Grey, 2009). Although there have been studies that investigate the role of pH, nitrogen fertilization and other growth factors on this genus (Ozguven et al., 2008; Liu et al., 2003) there is little research available on *A. afra* in particular, specifically with regards to suitable pH ranges. The pH of the soil is an important factor influencing the choice of crop to grow (Diederichs, 2006; Hartmann et al., 2002; Stern, 2006). Although the pH of the soil can be manipulated via the addition of certain products, such as the application of sulphur to lower the pH or lime to increase the pH (Denisen, 1979), it is often not practical for the small scale subsistence farmer. pH is a critical variable in plant growth (Rengel, 2003). As well as affecting the availability of various elements to the plant (Kunh et al., 1995, Marschner 1995), research has indicated that pH can have a significant influence on the growth and essential oil yield of various plants (Ram et al., 1997). Research by Kuhn et al. (1995) has shown that pH can have an adverse effect on plant growth, particularly on those that are being cultivated in hydroponic cultures. As the pH approaches 5.5 and below calcium, magnesium, zinc and copper are less readily available for plant uptake (Brady and Weil, 2008). Despite the fact that these elements (with the exception of magnesium) do not play a direct part in chlorophyll formation, they do contribute to the action of enzymes and thereby affect the action of certain metabolic processes, which in turn influence plant weight (Stern, 2006). As the pH rises above 7.5 phosphorus, iron, manganese, boron and zinc are reduced in their availability to plants (Brady and Weil, 2008, Kunh et al., 1995; Marschner, 1995). According to Stern (2006) the lack of minerals such as phosphorus and iron can lead to a loss of chlorophyll. A lack of these nutrients, especially iron, could lead to the restricted development of chlorophyll in *A. afra*. Before a plant can be recommended for cultivation it is essential that the pH range that it will be most productive in is known. In this study the aim was to determine the optimum pH for the cultivation of *A. afra*, which could assist future growers with improved commercial success in the cultivation practices of this important medicinal plant species.

MATERIALS AND METHODS

Experimental design

Glasshouse experiment

The experiment was conducted from July to October 2009. It was located in the research greenhouse of the Cape Peninsula University of Technology in the Western Cape of South Africa. The latitude and longitude are S33°55' 58 E18°25' 57. The climate controlled greenhouse had temperatures ranging from 16 - 36°C during the days, and 10 - 18°C at night. The relative humidity of the glasshouse averaged 35%. There is a 40% Alunet shade cloth suspended 2 m above the ground of the glasshouse. The light intensities ranged from 030 lux to 600 lux, as measured by a Toptronic T630 light meter. Irrigation water was supplied from a Hager IP65 Water Filtration Plant de-ioniser, and had an average temperature of 16°C.

Hydroponic experiment

A recirculation soilless medium setup was used to supply the treatments to the plants. 15 cm plastic pots were filled with approximately 220 g of medium grade horticultural perlite. This medium was chosen due to its neutral pH and lack of nutrients. The fluoride content of the perlite was reduced by a series of flushes with deionised water. The pots were lined at the bottom with discs

of shade cloth to prevent any medium leaving through the drainage holes. Each treatment had 20 pots, each containing one plant. Every pot functioned as an experimental unit and was placed randomly in one of the five treatments. The treatments were placed on galvanized steel tables which were divided into five separate compartments (each of which was 40 cm × 100 cm), one for each of the treatments. Each treatment drained into its own plastic 65 L container which was used as a reservoir to hold the water treatment. Each reservoir contained its own 1350 L/h hour Boyu submersible pump. The water was supplied to the pots via spaghetti tubing inserted into the medium. A TopTronic TMT24 analogue timer was used to activate the pumps used to irrigate the plants. The timer was set to provide water for 15 min every 90 min. This resulted in each pot receiving 2L of water every 90 min, ensuring that the medium was wet to carrying capacity and then had time to drain. As the water drained out of the pots it drained back into the reservoirs.

Factors controlled in the experiment

After setup but before planting the system was turned on and allowed to run for 24 h with a 1 ml per 2 L of water concentration of SporeKill (supplied by Hygrotech) (active ingredient Didecyl Dimethyl Ammonium Chloride 120 g/l) to disinfect the medium and system. After the 24 h period the whole system was flushed three times with deionised water and allowed to run for another 24 hour period with deionised water, before being filled the final time with the prepared treatments.

Plant selection and planting process

Two month old *A. afra* Jacq. plants were obtained from Good Hope Nursery. They all originated from one mother stock plant identified as a suitable phenotype for medicinal use by a group of local traditional healers (Grey, 2009). Prior to planting the plants in the hydroponics system they were thoroughly washed in deionised water to remove any foreign matter from their roots.

Treatment preparation

The experiment was laid in a randomised complete block design. There were 5 treatments, each of which was applied to 20 plants. The treatments were pH 4.5, 5.5, 6.5 (the control), 7.5, and 8.5. Hydroponic pH was maintained by the addition of NaOH to raise or HCl to lower the pH. The application of the treatments was via the hydroponic nutrient solution adjusted to the required pH. The plants were fed by adding 2 g/L of the commercially available hydroponic fertilizer CHEMICULT® [Chemicult Products (Pty) ltd, 133 Camps Bay, South Africa, 8040] to the water supply. The first dose of fertilizer was prepared according to the instructions supplied with the fertilizer, and the electrical conductivity (EC) of the water was tested. The EC of the water was determined to be 1600 µs at the recommended dose of fertilizer. After the first dose the EC was maintained at 1600 µs by the addition of Chemicult dissolved in a small amount of deionised water. The pH and EC were monitored every two days using a Martini Instruments PH55 handheld pH meter and a DIST handheld EC meter respectively.

Data collection

The plants were grown in the hydroponic system for 2 months. They were planted on the 24th of July, and were harvested on the 2nd of October, when the plants were still in their active stage of growth. On the day before harvesting the chlorophyll content of the leaves was measured. Average chlorophyll content (SPAD value) of four leaves was taken for each plant, using a Chlorophyll Meter (Konica Minolta SPAD-502, Spectrum Technologies, Plainfield, Illinois). Immediately after harvesting, the fresh plant weights were determined, after which they were sun-dried to constant weight for 4 weeks and the roots and shoots separated and their respective weights determined once again (Diederichs, 2006; Liu et al., 2008).

Statistical analysis

Mean values of data collected of yield components were analyzed statistically using a one way analysis of variance (ANOVA). These computations were performed with the software program Statistica version 8 (Hill and Lewicki, 2006) Fisher's Least Significant Difference (LSD) was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Results of the determination of the effect of pH on the chlorophyll content of the plants are shown in Figure 1. The chlorophyll content was significantly affected ($P \leq 0.001$) by the variations of pH. Results showed that plants grown at a pH 6.5 (control) had significantly higher levels of chlorophyll content, followed by those grown at pH 5.5 and 4.5 respectively. Results also showed that at pH 7.5, the chlorophyll content of the plants was significantly reduced when compared with the control. The pH 8.5 plants showed the lowest levels of chlorophyll and were significantly lower than those of the control and all other treatments. In this study, nutrient availability was not measured. However, it seems that nutrient availability was adversely affected by pH extremes and this was in agreement with the findings of Edmond et al. (1975), Reed (1996); Preece and Read (2005). At the pH of 5.5 and below calcium, magnesium, zinc and copper are less readily available for plant uptake (Brady and Weil, 2008). The reduction of the availability of these minerals is due to the impairment of the net extrusion of H^+, combined with the displacement of the various nutrients' bivalent cations from adsorption sites such as cell walls and membranes by aluminium (Kunh et al., 1995, Marschner, 1995). Although these elements (with the exception of magnesium) do not play a direct part in chlorophyll formation, they do contribute to the action of enzymes, which in turn affects the action of metabolic processes, and thereby the creation of plant weight (Stern, 2006). Magnesium does play a part of chlorophyll synthesis, and this could explain the low chlorophyll content of the plants in the pH 4.5 and 5.5 treatment when compared with the control. At a pH above 7.5 phosphorus, iron, manganese, boron and zinc are reduced in their availability to plants (Brady and Weil, 2008). In an alkaline situation phosphorus becomes unavailable to the plants due to adsorption and precipitation reactions (Bertrand et al., 2003). The precipitation of ferric oxide is the major factor influencing the availability of iron in alkaline soils. With a soil pH that is in the alkaline range, zinc becomes less

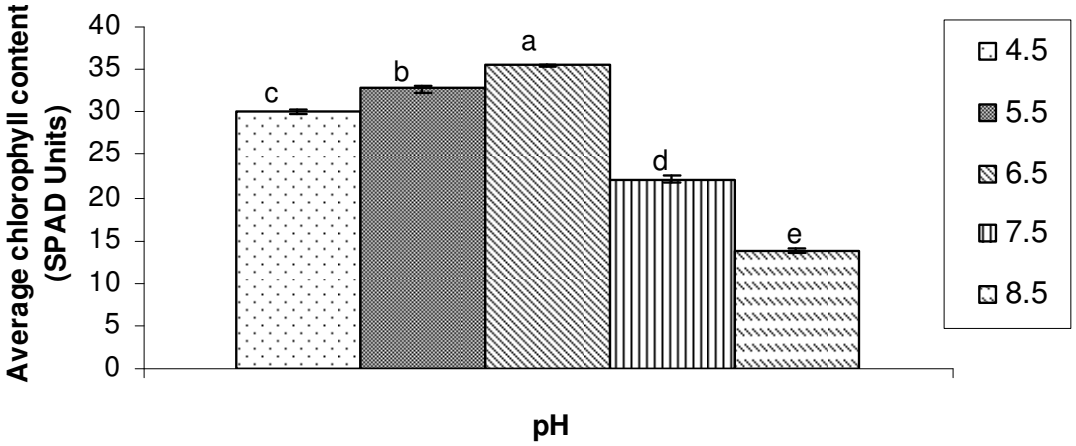

Figure 1. Effects of pH values on chlorophyll content of *Artemisia afra*. Bars presented are means ± SE. Mean values within each bar followed by different letter differ significantly at P ≤ 0.05 according to Fishers least significant difference.

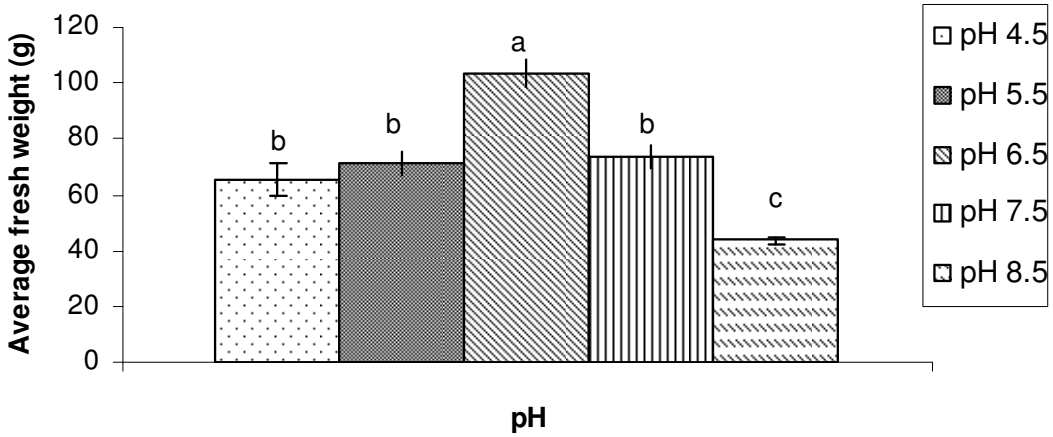

Figure 2. Effects of pH values on average fresh weight of *Artemisia afra*. Bars presented are means±SE. Mean values within each bar followed by different letter differ significantly at P ≤ 0.05 according to fishers least significant difference.

available to the plants due to the adsorption of zinc by soil constituents. Manganese is less available for plants in a soil with an alkaline pH due to the manganese forming into insoluble oxide forms (Wilkinson, 2000). Although not measured, it is proposed that the lack of minerals such as phosphorus and iron can lead to a loss of chlorophyll (Stern, 2006). The deficit of these nutrients, especially iron, could lead to the restricted development of chlorophyll in the pH 7.5 and 8.5 treatments.

The manipulation of the pH significantly ($P \leq 0.001$) affected the average fresh weight of the plants. The highest measurement was obtained in the control treatment of pH 6.5 (Figure 2). The plants that were grown in pH adjusted to 4.5, 5.5 and 7.5, all had fresh weights that were significantly lower than the control. However, they did not vary in a statistically significant way from each other. The plants exposed to the pH 8.5 treatment were significantly lower in fresh weight when compared with the control. They were also significantly lower than the 4.5, 5.5 and 7.5 treatments (Figure 2). The pH 5.5 and 7.5 treatments producing similar fresh weights may be attributed to the fact that at these pH values there is no major impact on nutrient availability (Brady and Weil, 2008; Van Oorschot et al., 1997). As pH approaches 4.5, calcium, magnesium and copper become less available. As Reed (1996) has shown, these are needed in large quantities in the development of the plants. This could explain the fact that the 4.5 treatment differed significantly from the control in fresh weight, which is probably due to the unavailability of magnesium, copper and calcium. Research has shone that as pH is raised above 7.5 minerals such as phosphorus, iron,

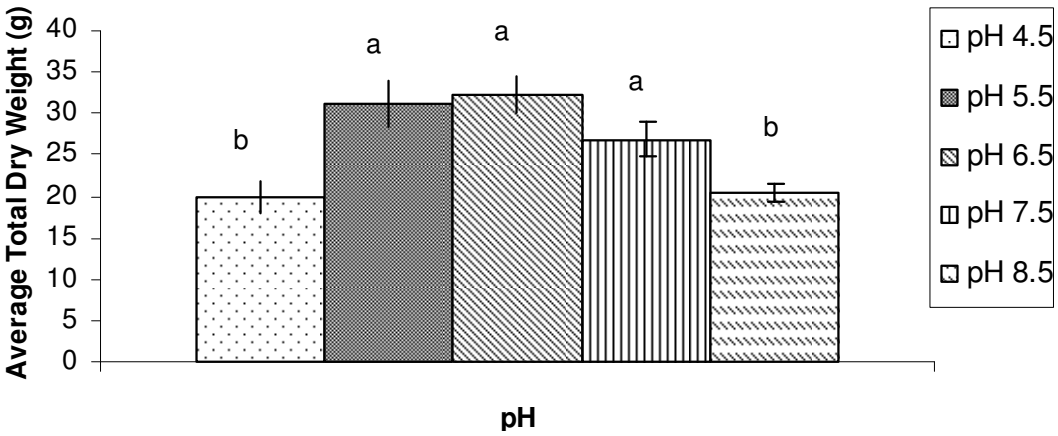

Figure 3. Effects of pH values on average total dry weight of *Artemisia afra*. Bars presented are means±SE. Mean values within each bar followed by different letter differ significantly at P ≤ 0.05 according to Fishers least significant difference.

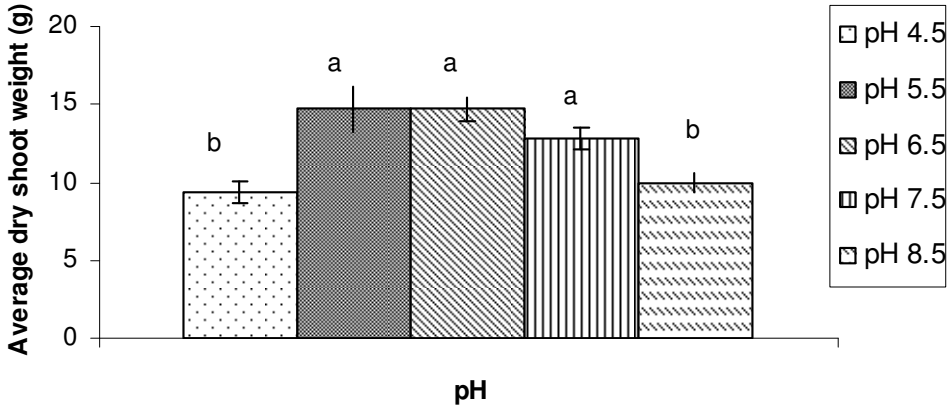

Figure 4. Effects of pH values on average dry shoot weight of *Artemisia afra*. Bars presented are means±SE. Mean values within each bar followed by different letter differ significantly at P ≤ 0.05 according to Fishers least significant difference.

manganese, and boron begin to become unavailable to the plants (Edmond et al., 1975; Reed, 1996; Preece and Read, 2005; Brady and Weil, 2008). These minerals are essential for plant development, and this could contribute to significantly lower fresh weight of the plants grown at a pH of 8.5 as compared to near neutral pH.

The total dry weight was significantly affected ($P \leq 0.001$) by pH treatments (Figure 3). The average total dry weight of the control was not significantly different to the average total dry weight of the plants grown at the pH values of 5.5 and 7.5. The pH 4.5 and 8.5 had an effect upon the total dry weight of the plants, which was significantly lower than that of the control. It is likely that this is also an effect of the lower availability of nutrients at these pH levels. It is interesting to note that while the fresh weight of the plants grown at the control of 6.5 was significantly higher than that of the 4.5, 5.5 and 7.5, the total dry weight of the control, 5.5 and 6.5 treatments was not significantly different.

Shoot dry weight was significantly influenced ($P \leq 0.001$) by different pH treatments. When compared with the control of pH 6.5, the plants at a pH of 5.5 and 7.5 were not significantly different in terms of shoot dry weight (Figure 4). However, the plants grown in the medium adjusted to pH 4.5 and pH 8.5 had significantly lower shoot dry weights than those of the control.

A similar significant ($P \leq 0.001$) trend with pH adjustment was noticed with the dry weight of the roots (Figure 5). The control was not significantly varied from the pH 5.5 and pH 7.5 treatments in terms of root dry weight. However, the pH 4.5 and pH 8.5 treatments produced significantly lower weights of dry roots than the control.

When a comparison between the total dry weights and the chlorophyll content of the different treatments is made it can be seen that there is a relationship between

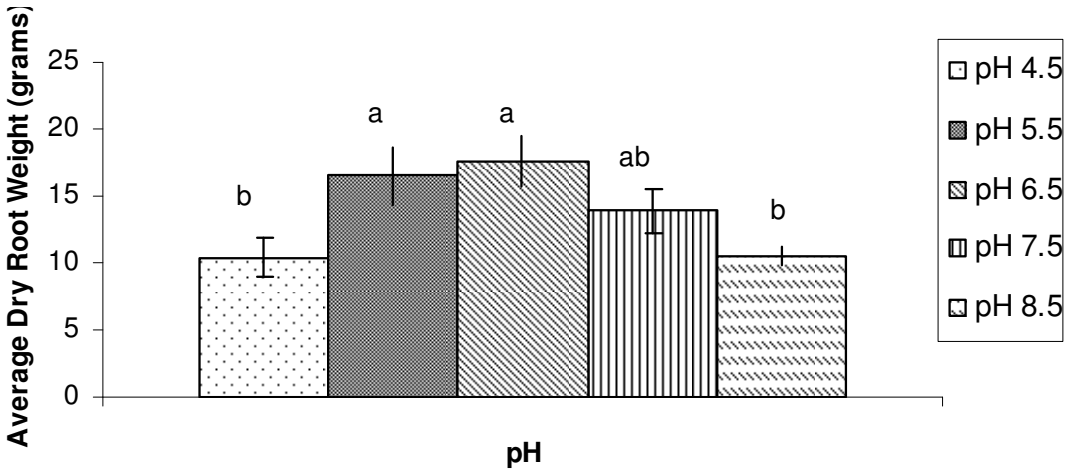

Figure 5. Effects of pH values on dry root weight of *Artemisia afra*. Bars presented are means±SE. Mean values within each bar followed by different letter differ significantly at P ≤ 0.05 according to Fishers least significant difference.

chlorophyll content and dry weights. The lower average dry shoot and root weights and chlorophyll content of the pH 4.5, and 8.5 pH values could be attributed to the lower levels of nutrients such as iron, manganese and boron that are available at these pHs (Edmond et al., 1975; Reed, 1996; Preece and Read, 2005). As the nutrients become unavailable to the plant, various metabolic processes such as chlorophyll synthesis, photosynthesis and respiration are restricted (Stern, 2006). As pH is raised above 7.5, minerals such as iron, manganese and boron become unavailable to the plants (Edmond et al., 1975; Reed, 1996; Preece and Read, 2005). Below a pH of 5.5, nitrogen, phosphorus and many others begin to become unavailable to the plants (Edmond et al., 1975; Reed, 1996; Preece and Read, 2005; Brady and Weil, 2008). The lack of minerals such as phosphorus at a low pH and iron at a high pH can lead to chlorosis and hence a loss of chlorophyll (Stern, 2006). This could contribute to the chlorophyll levels of the plants exposed to the pH 4.5 treatment being significantly lower than that of the pH 5.5 and 6.5 treatments, while the total dry weight is significantly lower than that of the control, but similar to the pH 7.5 and 8.5.

The results clearly indicated that there is a relationship between the pH of supplied irrigation water and the yield and chlorophyll content of *A. afra*. Although there was a significant difference between the fresh weights of all the treatments, with the highest weight being that of the control treatment, the dry yield was not significantly different between the treatments below pH 7.5. In the South African context, information regarding *A. afra*'s response to pH is important knowledge, because many of the small scale cultivators of this medicinal plant cannot afford soil amendment products (Makunga et al., 2008).

In conclusion, this pilot study has demonstrated that pH can play a significant part in the growth and yield of *A. afra*. It has indicated that this plant is tolerant of a wide range of pH levels, but performs best (in terms of fresh yield and chlorophyll content) in a pH range from 5.5 to 7.5. Although the yield of the plant is the primary focus of most small scale growers, to the medicinal industry the most important factor is the yield of useful metabolites (Fennell et al., 2004). Further studies are recommended as to the effect of varying pH levels on the production of secondary metabolites and other chemical components with medicinal values. In-depth studies as to the relationship between mineral requirements of *A. afra* and its production of useful secondary metabolites would yield useful data pertaining to the commercial cultivation of this plant. It would also be relevant to investigate the effect that the combination of factors such as pH and nutrient availability would have on the metabolite content of the plant.

ACKNOWLEDGEMENTS

We would like to thank Bruce James and Fiona Milanese for their technical assistance in the setup of the hydroponics system used in this study and the Cape Peninsula University of Technology for their financial support.

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