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Growth and Essential Oil Yield of Artemisia (*Artemisia annua* L.) as Affected by Growth Stage

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ABSTRACT

A study was conducted to observe the growth trend and essential oil yield of *Artemisia annua* L. under varying growth ages at the Wondo Genet Agricultural Research Center. The experiment consisted of six growth stages (2, 3, 4, 5, 6 and 7 months after transplanting). The treatments were arranged in randomized complete block design with three replications. Data on plant height, branch number/plant, fresh leaf weight/plant, dry leaf weight/plant, fresh leaf/stem ratio, dry leaf/stem ratio essential oil content (EOC) and essential oil yield (EOY) were collected and analyzed. As mean squares from analysis of variance indicated, different growth ages affected plant height, fresh leaf/stem ratio and dry leaf/stem ratio very highly significantly ($P < 0.001$) and affected EOC and EOY highly significantly ($P < 0.01$). Fresh leaf weight/plant and dry leaf weight/plant were affected significantly ($P < 0.05$) by different growth ages. Branch number/plant did not vary significantly ($P > 0.05$) at different age of growth. In this study, the highest values were recorded at 5 months after transplanting (MAT) for plant height (194 cm), fresh leaf weight/plant (382.47 g) and dry leaf weight/plant (98.36 g) and at 2 MAT for fresh leaf/stem ratio (0.69) and dry leaf/stem ratio (0.64). The highest EOC (1.08%) and EOY (21.78 kg) values were obtained at 6 MAT.

Keywords: artemisia, essential oil, Ethiopia, growth ages, Wondo Genet

INTRODUCTION

Artemisia is one of the largest genera of the Asteraceae family (Bertea *et al*. 2005). This genus belongs to a useful group of aromatic and medical plants comprising about 300 species which are distributed throughout the world (Bertea *et al.* 2005). *Artemisia annua* L., called annual or sweet wormwood, is an annual herb native of Asia and has been used for many centuries in the treatment of fever and malaria (Brown *et al*. 2003). *A. annua* is grown for its aromatic and medicinal leaves. Its leaves yield artemisinin, which is proven to be effective medicine for the treatment of malaria (Wilairatana *et al*. 2002). Current research also shows that artemisinin drugs are effective against cancer, *Leishmania* (Sen *et al*. 2007), *Trypanosoma* (Mishina *et al*. 2007), and some viruses (Li *et al*. 2005). In addition, *A. annua* has a high content of flavonoid compounds which are responsible for its high antioxidant activity. There are potential uses of *A. annua* plant extracts for humans and livestock based on the synergistic effects of flavonoids; artemisinin precursors, including antimalarial effects were reported for traditional *A. annua* tea (Blanke *et al*. 2008). *A. annua* is used traditionally in China to treat fevers and hemorrhoids (Hsu 2006). It is also used in the crafting of aromatic wreaths, as a flavoring for spirits such as vermouth, and as a source of essential oils for the perfume industry (Ferreira *et al*. 1997). The essential oil of the plant has been reported to possess antimycotic, antimicrobial and repellent effect on certain beetles (WHO 2006).

Growth and essential oil contents of *A. annua* are affected by numerous factors such as geographical conditions, harvesting time, temperature, fertilizer application, population density and age of the plant (Wright 2002). Ramezani *et al*. (2009) also stated the influence of growth stage of the plant on quantity and quality of essential oil in most essential oil-bearing plants. Some efforts have been done on effect of spacing and harvesting age on yield and yield components at Wondo Genet, Ethiopia. But the result does not clearly indicate the growth trend of the crop at earlier age of development. Therefore, this experiment was conducted to observe the growth and essential oil yield of *A. annua* under different growth ages starting from earlier age of development.

MATERIALS AND METHODS

This experiment was carried out at Wondo Genet Agricultural Research Center experimental site. The Center is geographically located at 07° 03' 19.1" to 07° 04' 00.2" North latitude and from 38° 30' 08.4" to 38° 31' 01.8" East longitude. The site receives mean annual rain fall of 1000 mm with minimum and maximum temperature of 11.47 and 26.51°C, respectively. The soil textural class of the experimental area is sandy loam with pH of 6.4 (National Soil Research Center 2006).

The experiment was conducted based on randomized complete block design with three replications and six growth ages. The considered ages were 2, 3, 4, 5, 6 and 7 months after transplanting (MAT). The experiment includes 3 blocks and each block is contained 6 plots. Each plot size was 7.2 × 7.2 m and 60 × 60 cm spacing was maintained between plants and rows. Distance between blocks and plots were 2 m and 1 m, respectively. Each plot was consisted of 13 rows. All agronomic practices were keeping normal and uniform for all of the treatments. Ten plants were selected at random from each plot for recording individual plant observations. Data on plant height, number of main branches, fresh leaf weight, fresh stem weight and fresh leaf/stem ratio were recorded. A separate composite sample from all 10 plants (100 g of fresh leaves and fresh stem) was dried in a hot oven at 100°C for 24 h to determine dry leaf weight, dry stem weight and dry leaf/stem ratio. Essential oil content was determined from 500 g fresh leaves of composite samples using hydro-distillation in a Clevenger apparatus according to Guenther (1972). All the data were analyzed using

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Table 1 Mean squares of *Artemisia annua* L. as affected by growth stages.

Source of variation	Df	PH	BN	FLWP	DLWP	FLSR	DLSR	EOC	EOY
Replication (Rep)	2	34.43	540.95	70.42	11.15	0.01	0.007	0.033	12.96
Treat	5	161.86***	175.70ns	20186.04*	1077.76*	0.10***	0.14***	0.12**	127.28**
Error	10	115.06	225.64	4287.57	221.24	0.01	0.004	0.02	13.22
CV%		6.7	25.89	24.15	21.71	12.3	21.46	15.39	26.6

*** = Significant at $P < 0.001$; ** = Significant at $P < 0.01$; * = Significant at $P < 0.05$; ns = Non significant at $P < 0.05$, PH = plant height, BN = branch number, FLWP, fresh leaf weight/plant, DLWP = dry leaf weight/plant, FLSR = fresh leaf to stem ration, DLSR = dry leaf to stem ratio, EOC = essential oil content, EOY = essential oil yield

Table 2 Performance of growth and yield traits of *Artemisia annua* L. under different growth stages.

Treatments (months after transplanting)	PH	BN	FLWP	DLWP	FLSR	DLSR	EOC	EOY
2	88.13 d	60.93	231.54 c	39.38 c	0.64 a	0.69 a	0.62 c	6.58 c
3	126.00 c	62.87	363.16 ab	67.39 b	0.46 b	0.41 b	1.03 a	5.30 c
4	156.87 b	63.80	249.31 bc	70.26 b	0.31 c	0.26 c	0.73 bc	14.10 b
5	194.00 a	63.60	382.47 a	98.36 a	0.27 cd	0.19 dc	0.66 c	18.11 ab
6	193.2 a	45.93	218.17 c	72.93 ab	0.20 de	0.16 dc	1.08 a	21.78 a
7	201.0 a	50.93	182.08 c	62.79 bc	0.15 e	0.11 d	0.91 ab	16.13 ab
LSD	19.52	ns	119.12	27.06	0.08	0.12	0.23	6.62
CV	6.7	25.89	24.15	21.71	12.3	21.46	15.39	26.6

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test; ns = Non significant at $P < 0.05$, PH = plant height, BN = branch number, FLWP, fresh leaf weight/plant, DLWP = dry leaf weight/plant, FLSR = fresh leaf to stem ration, DLSR = dry leaf to stem ratio, EOC = essential oil content, EOY = essential oil yield

SAS version 9 computer soft ware and significant mean comparison was done by using least significant difference test at 5% probability level.

RESULTS AND DISCUSSION

Plant height, fresh leaf/stem ratio and dry leaf/stem ratio were significantly ($P < 0.001$) different at different growth stages of the plant. Similarly, growth stage significantly ($P < 0.01$) affected essential oil content and yield, as well as ($P < 0.05$) leaf fresh weight/plant and leaf dry weight/plant. Growth stage did not significantly ($P > 0.05$) affect branch number/plant (**Table 1**).

Performance and essential oil yield of artemisia as affected by different growth stages

Minimum plant height (88.13 cm) was recorded for the measurement taken at 2 MAT and increased with the age of the plant and attained a maximum value (194 cm) at 5 MAT. After 5 MAT, no significant increments in height were observed (**Table 2; Fig. 1A**). Increasing age from 2-3, 3-4 and 4-5 MAT, plant height increased by 43, 24.5 and 23.67%, respectively. Belay (2007) also reported an increase in plant height with increasing age in *A. annua.* Similar results were also reported by Zewdinesh *et al.* (2012) for rosemary (*Rosmarinus officinalis*) and Mastiholi (2008) in *Coleus forskohlii* BRIQ. Branch number/plant was not significantly affected by growth stage but slightly higher values were recorded at 2, 3, 4 and 5 MAT and started to decrease after 5 MAT (**Table 2; Fig. 1B**). With increasing age, auxiliary buds failed to initiate new branches while existent branches died, explaining why branch number decreased with the age of the crop.

Minimum fresh leaf weight/plant and dry leaf weight/plant (231.54 g and 39.38 g, respectively) were recorded at earlier stage of the crop and these parameters increased with increasing age and attained maximum values at 5 MAT (382.47 g and 98.36 g for fresh leaf weight/plant and dry leaf weight/plant, respectively). Both values started to decline after 5 MAT (**Table 2**; **Figs. 1C, 1D**). The increase at 2 to 5 MAT was 65.2 and 149.77% for fresh leaf weight and dry leaf weight/plant, respectively. Higher dry leaf weight/plant of *A. annua* at 5 MAT was also reported by Zewdinesh *et al*. (2011) and by Laughlin (1993). An increment of fresh leaf yield and dry leaf yield with increasing age and a decrease in these values after attaining a maximum value was also reported by Beemnet *et al*. (2011) for peppermint (*Mentha piperiata* L.) and by Zewdinesh *et al.* (2012) for rosemary (*Rosmarinus officinalis*). The decrease in dry leaf weight/plant after 5 MAT was due to leaf senescence observed in the field during the experiment.

For fresh leaf/stem ratio and dry leaf/stem ratio, higher values (0.64 and 0.69, respectively) were at 2 MAT. These values decreased as plant age increased, reaching minimum values (0.15 and 0.11, respectively) at 7 MAT for both parameters (**Table 2; Figs. 1E, 1F**). The decrease in fresh leaf/stem ratio and dry leaf/stem ratio with increasing age was 76.56 and 84.06%, respectively. Similar findings were reported by Beemnet *et al*. (2011) for peppermint and by Solomon and Beemnet (2011) for Japanese mint (*Mentha arvensis*). Similarly, Zewdinesh *et al.* (2012) reported a decreasing trend of dry leaf/stem ratio with increasing age for rosemary. The decrease in leaf/stem ratio with increasing age of the plant indicated that the proportion of leaves is better at earlier age than at a later age. With increasing age, the amount of leaves decrease due to leaf senescence. On the other hand, the stem become thicker and the weight increased that is why leaf/stem ratio decreased with age of the plant.

Essential oil content varied from 0.62 to 1.08%. The highest values were obtained at 6 MAT while lowest values were recorded at 2 MAT (**Table 2; Fig. 1G**). Minimum essential oil yield (6.58 kg/ha) was recorded at 2 MAT. Essential oil yield increased with increasing growth stage of the plant and reached a maximum value (21.78 kg/ha) at 6 MAT (**Table 2; Fig. 1H**). Similar to this finding, lower essential oil content and essential oil yield at a younger age and an increased values of these parameters with increasing age was reported for rosemary (Zewdinesh *et al.* 2012). An increasing trend of essential oil content with increasing age was also reported by Solomon and Beemnet (2011) and Jahangir *et al*. (2008) in spearmint (*Mentha spicata* L.) and by Verma *et al.* (2010) for menthol mint and spearmint.

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REFERENCES

Abayneh E, Demeke T, Ashenafi A (2006) *Soils of Wondo Genet Agricultural Research Center*, National soil Research Center, Addis Ababa, Ethiopia, 67 pp

Beemnet MK, Teixeira da Silva JA, Solomon AM (2011) Agronomic characters, leaf and essential oil yield of peppermint (*Mentha piperiata* L.) as influenced by harvesting age and row spacing. *Medicinal and Aromatic Plant Science and Biotechnology* **5 (1)**, 49-53

Belay Bekele (2007) Assessment of Crop Growth and Artemisinin Content of the Medicinal Plant *Artemisia annua* Anamed in the Gamo Highlands of SW

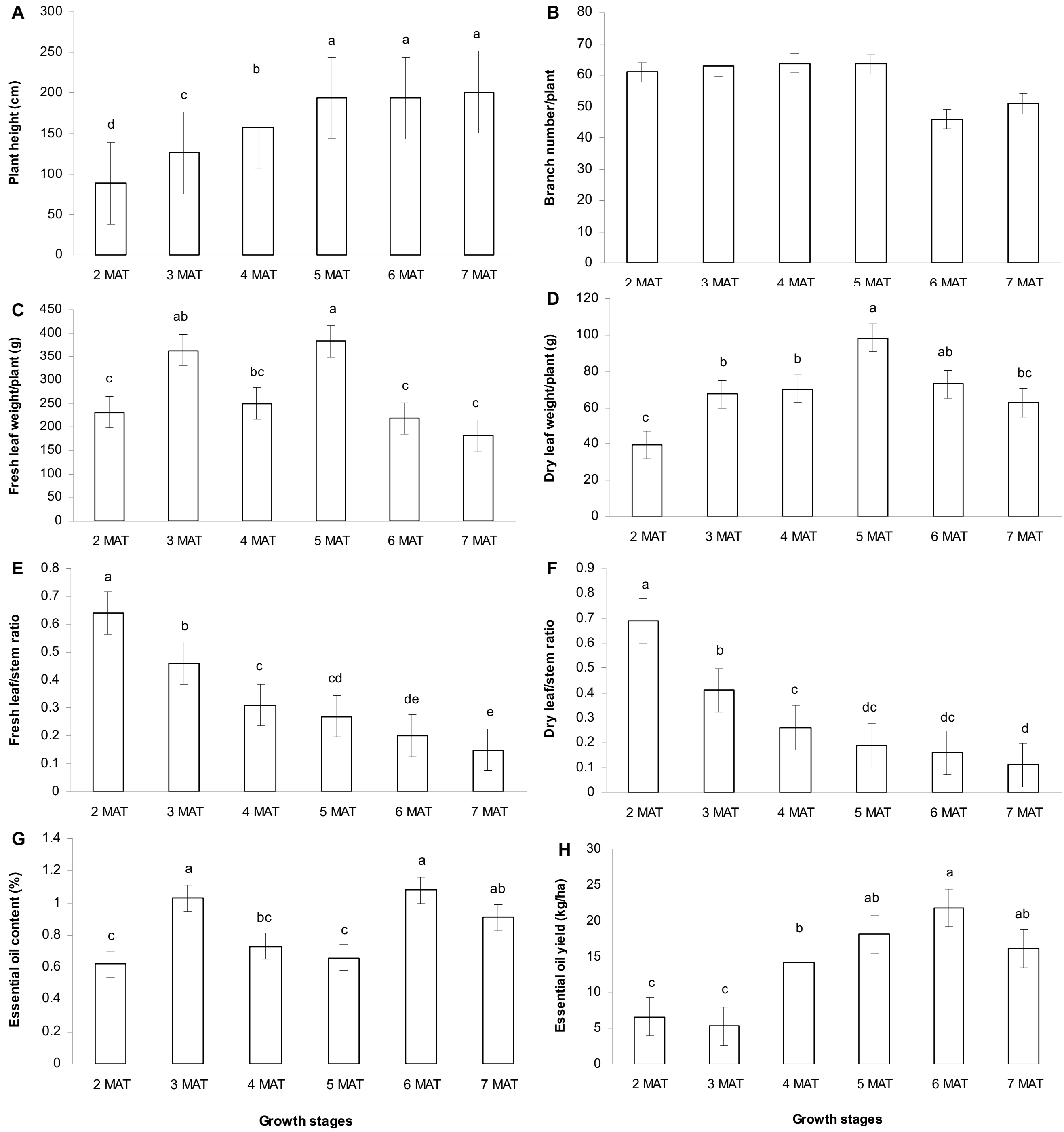

Fig. 1 Effect of growth stage on (**A**) plant height, (**B**) branch number/plant, (**C**) fresh leaf weight/plant, (**D**) dry leaf weight/plant, (**E**) dry leaf /stem ratio, (**F**) fresh leaf /stem ratio, (**G**) essential oil content and (**H**) essential oil yield of *A. annua* L. Values presented are mean ± standard error (SE), n = 6.

Ethiopia, Department of Agronomy, University of Hohenheim, Sttutgart, Germany, 84 pp

Bertea CM, Freije JR, van der Woude H, Verstappen FWA, Perk L, Marquez V, De Kraker JW, Posthumus MA, Jansen BJM, de Groot A, Franssen MCR, Bouwmeester HJ (2005) Identification of intermediates and enzymes involved in the early steps of artemisinin biosynthesis in *Artemisia annua*. *Planta Medica* **71**, 40-47

Blanke CH, Naisabha GB, Balema MB, Mbaruku GM, Heide L, Muller MS (2008) Herbal Artemisiae annuae tea preparation compared to sulfadoxine-pyrimethamine in the treatment of uncomplicated falciparum malaria in adults: A randomized double-blind clinical trial. *Tropical Doctor* **38**, 113-116

Brown GD, Liang Gy, Sy L (2003) Terpenoids from the seeds of *Artemisia annua*. *Phytochemistry* **64**, 303-323

Ferreira JFS, Simon JE, Janick J (1997) *Artemisia annua*: Botany, horticulture, pharmacology. *Horticultural Reviews* **19**, 319-371

Guenther E (1972) *The Essential Oils: History - Origin in Plants Production - Analysis* (Vol I), Robert E. Kriger Publishing Co., Malabar, Florida, 427 pp

Hsu E (2006) The history of *qing hao* in the Chinese *materia medica*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 505-508

Jahangir AA, Nada K, Begum F, Hossain F, Sarker MAM, Moniruzzaman M (2008) Influence of nitrogen-phosphorus fertilization and time of harvest on the growth, yield and oil content of *Mentha spicata* L. *Bangladesh Journal of Scientific and Industrial Research* **43 (1)**, 47-54

Laughlin JC (1993) Effect of agronomic practices on plant yield and antimalarial constituents of *Artemisia annua* L. *Acta Horticulturae* **331**, 53-61

Li S, Chen C, Zhang H, Guo H, Wang H, Wang L, Zhang X, Hua S, Yu J, Xiao P, Li R, Tan X (2005) Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antiviral Research* **67**, 18-23

Mastiholi AB (2008) Effect of spacing, time of harvest and NPK levels on growth, yield and quality of medicinal coleus (*Coleus forskohlii* BRIQ.) under irrigated condition. PhD thesis, Department of Agronomy, University of Agricultural Sciences, Dharwad, 224 pp

Mishina YV, Krishna S, Haynes RK, Meade JC (2007) Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense in vitro* growth. *Antimicrobial Agents and Chemotherapy* **51**, 1852-1854

Ramezani S, Ramezani F, Rasouli F, Ghasemi M, Fotokian M (2009) Diurnal variation of the essential oil of four medicinal plant species in central region of Iran. *Journal of Biological Science* **4 (1)**, 103-106

Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, Saha P, Chatterjee M (2007) Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. *Journal of Medical Microbiology* **56**, 1213-1218

Solomon AM, Beemnet MK (2011) Row spacing and harvesting age affect agronomic characteristics and essential oil yield of Japanese mint (*Mentha arvensis* L.). *Medicinal and Aromatic Plant Science and Biotechnology* **5 (1)**, 74-76

Verma RS, Rahman L, Verma RK, Chauhan A, Yadav AK, Singh A (2010) Essential oil composition of menthol mint (*Mentha arvensis*) and peppermint (*Mentha piperiata*) cultivars at different stages of plant growth from Kumanon region of West Himalaya. *Open Access Journal of Medicinal and Aromatic Plants* **1**, 13-18

WHO (World Health Organization) (2006) Monograph on good agricultural and collection practices (GACP) for *Artemisia annua* L. Geneva, Switzerland, pp 1-58

Wilairatana P, Krudsood S, Treeprasertsuk S, Chalermrut K, Looareesuwan S (2002) The future out look of anti-malarial drugs and recent work on the treatment of malaria. *Medicinal Research* **33**, 416-421

Wright CW (2002) *Artemisia*. *The Journal of the American Botanical Council* **57**, 1-65

Zewdinesh D, Bizuayehu T, Daniel B (2011) Leaf, essential oil and artemisinin yield of artemisia (*Artemisia annua* L.) as influenced by harvesting age and plant population density. *World Journal of Agricultural Science* **7 (4)**, 404-412

Zewdinesh D, Beemnet M, Tsion T (2012) Effects of harvesting age and spacing on leaf yield and essential oil yield of rosemary (*Rosmarinus officinalis* L.). *The African Journal of Plant Science and Biotechnology* **6 (Special Issue 1)**, 9-12