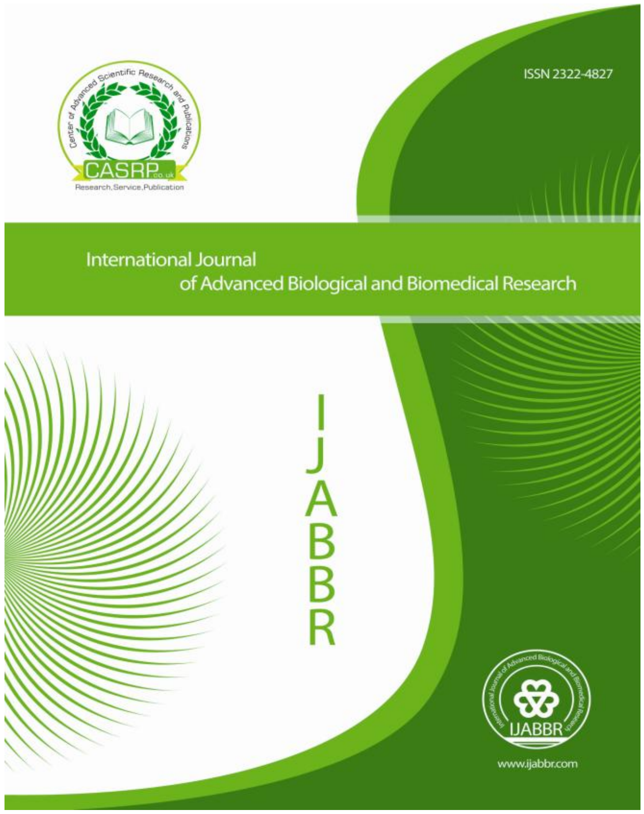
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Effect of plant population density on growth and yield of Artemisia (*Artemisia annua L.*)

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Abstract

A field experiment was conducted at wondo genet Agriculture research center under irrigated condition to determine the effect of intra and inter-row spacing on growth and yield of Artemisia (Artemisia annua L.) during the two successive seasons of 2013 and 2014. Factorial combinations of four intra-rows (40, 60, 80, 100 cm) and four inter-row plant spacing's (60, 80, 100, 120 cm) were laid out in a randomized complete block design with three replications. Interaction effect of the two factors brought about significant variation on above ground biomass, leaf fresh weight, leaf dry weight and Essential oil yield. However, plant height, number of primary branch per plant and essential oil content were not influenced by the interaction effect. In this study, the maximum above ground biomass 72605 kg/ha, leaf fresh weight 9510 kg/ha, leaf dry weight 5392.7 kg/ha and Essential oil yield 23.39 kg/ha were attained due to spacing combination 40cm intra-row and 60cm inter-row spacing's. Therefore, it could be concluded that Artemisia annua could be planted at optimum spacing of 40 x 60 cm in Wondo genet area to attain maximum yield.

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1. Introduction

Artemisia (Artemisia annua L.) is an aromatic and medicinal plant (Singh and Lai, 2004). It is one of the largest and most widely distribute genera of family Astraceae comprised over 450 diverse species. It has originated in China and currently distributed in North America, Europe, Africa, Asia, South America and Australia (Laughlin JC, Heazlewood GN and Beattie BM, 2002). It is grown for its aromatic and medicinal leaves, which yields artemisinin and essential oil. Artemisinin has been proven to be forceful and effective medicine for the treatment of malaria including cerebral malaria and multi drug-resistance Plasmodium falciparum (Wilairatana, 2002). In addition, medicinal herbal tea can be prepared from dried leaf of A. annua for the treatment of malaria without negative side effect (Hirt and Lindsey, 2000).

Malaria is a major health problem in many developing countries, mostly in Africa and Southeast Asia. According to WHO report on malaria (2004), 40% world's population is living with risk of malaria, over 1.5 million death occur per year and the cost of malaria treatment is $1800 million US dollar. The first effective ant malarial drug was quinine, which was isolated from the bark of cinchona. Since then malaria has been treated with quinoline based drugs. However, Plasmodium falciparum developed resistant globally against two of the most common ant malarial drugs: chloroquine and the combination sulphadoxine / pyrimethamine (Ferreira Janick, 2002).

The World Health Organization has thus recommended that all countries experiencing resistance to use combination therapies preferably antimalaria medicines in combination with artemisinin derivatives for Plasmodium falciparum (WHO, 2004). Not only malaria but also some other medical problems such as haemorrhoids, bronchitis, bilharzias and certain form of cancer can be treated with *A.annua* tea and showed good result (Hirt and Lindsey, 2000). Apart from artemisinin, essential oils are another active research interests from A. annua as it could be potentially used in perfumery, cosmetics and aromatherapy (Muzemil, 2008). Artemisinin and essential oil levels in the leaves of A. annua ranges from 0.01 to 1.4 % and 0.04 to 1.9 %, respectively (Delabays et al., 2002). The contents are affected by numerous factors such as geographical conditions harvesting time, temperature, population density, age of harvesting and inorganic fertilizer application (Wright, 2002).

Zaffaroni and Schneiter (1991) noted that three production variables that a producer can manipulate to influence the production of a given crop are plant population, row arrangement and hybrid selection. There are many factors that influence agronomic characteristics, biomass and EO yield of aromatic and medicinal plants. Among these plant spacing deserves special attention (Yasin et al., 2003; Khazaie et al., 2007; Ramamneh, 2009). Singh (2002) also explained that establishment of optimum population per unit area of the field is essential to get maximum yield. Under conditions of sufficient soil moisture and nutrients, higher population is necessary to utilize all the growth factors efficiently. The level of plant population should be such that maximum solar radiation is utilized. The full yield potential of an individual plant is fully exploited when sown at wider spacing. Yield per plant decreases gradually as plant population per unit area increases. However, the yield per unit area is increased due to efficient utilization of growth factors.

As the current economic policy of Ethiopia is market oriented, by giving emphasis to this species the country could have gained a great deal of income from this new Aromatic and Medicinal crop. However, most of its appropriate agronomic practices and requirements of the crop were not yet well studied and determined. Hence, realizing the importance of developing appropriate cultural practices such as plant spacing for optimum production of this crop, this study was conducted. Therefore, the objective of the experiment was to determine the effect of intra and inter-row spacing on growth and yield of Artemisia (*Artemisia annua* L.).

2. Materials and Methods

The field experiment was carried out under irrigated condition for two consecutive years (2013 and 2014 cropping season) at Wondo Genet Agricultural Research center experimental site, south Ethiopia. The experimental site was geographically located at 07° 19.1' North latitude, 38° 30’ East longitude and an altitude of 1780m.a.s.l. It was received mean annual rain fall of 1128 mm with minimum and maximum temperature of 11 and 26°c, respectively. The texture of the top soil (0-25cm) was sandy clay loam with PH 8.84(1:2.5soil water suspension. Factorial combinations of 4 intra-row plant spacing's (40, 60, 80, 100 cm) and 4 inter-row spacing's (60, 80, 100, 120 cm) were laid out in a randomized complete block design (RCBD) with three replications. Plot size for each treatment was 4.80m x 4m.

Seedlings of Artemisia were raised in the nursery for three months and transplanted to actual field on well tilth land for planting. The number of plants per row and the number of rows per plot were determined by intra and inter-row spacing, respectively. All required manage mental practices were done as and when required. Five plants were selected randomly from each plot by excluding the borders to collect yield and yield contributing characters such as plant height(cm), Number of primary branch/plant, above ground biomass(gm), Leaf fresh weight, stem fresh weight(gm), leaf dry weight(gm), essential oil yield(gm), and essential oil content(w/w, wet/dry based%) of the plant. After recording leaf fresh weight at harvest, Essential oil yields analysis was done using gas chromatography-mass spectrophotometer or modified Clevenger collector apparatus.

The collected data were statistically analyzed using SAS computer soft ware version 9.0 English and the significance difference between any two treatments means were tested by least significant difference (LSD) at 5% probability level. It must be noted that data for each trait measured for the two years were pooled and analyzed to determine the year effect.

3. Results and discussion

3.1. Plant height

During first harvesting season, plant height was not significantly influenced by intra-row, inter-row and their interaction. However, second harvesting season and pooled mean analysis result revealed that plant height was significantly ($p>0.05$) affected by intra-row spacing but not inter-row and their interaction. In this study, highest plant height was recorded in the lowest intra and inter-row spacing. Relatively lower plant height was observed in wider intra- and inter-row spacing. The result of this study in line with previous finding of (Zewdenesh, 2011) who reported plant height has direct association with population density. At higher density, there was greater intra and inter plant competition for available light. As a result, plants responded by diverting their resource to vertical growth to capture the available light.

3.2. Number of branch per plant

In 2012-2013 harvesting season, number of branches per plant was significantly ($p>0.05$) influenced by intra-row spacing. The highest and lowest number of branches per plant 188.33 and 127.08 were recorded at 100 cm and 40cm of intra-row spacing, respectively. On the other hand, number of branches per plant was not significantly ($p>0.05$) influenced by inter-row and their interaction. During 2013-2014 harvesting season and pooled mean analysis result demonstrated that, number of branches per plant were significantly ($p>0.05$) influenced by intra- and inter-row spacing. The highest number of branches per plant 206.04 and 191.21 were recorded in pooled mean at 100 cm and 100cm of intra- and inter-row spacing, respectively. Conversely, number of branches per plant was not significantly ($p>0.05$) influenced by their interaction. This indicate that, the variation in intra-row spacing means may be attributed to the effect of the different levels of that factor and was not significantly affected the different levels of inter-row spacing.

Generally, as the intra and inter-row spacing gets narrower, the number of branches per plant decreased significantly. The result observed in the current investigation supports earlier findings where greater numbers of branches were recorded due to wider spacing's and lesser plant density (Abebe, 2007). Similarly, (Simon, 1990) reported that higher branches number per plants were observed at lower density, while the lowest branches number per plant at the highest planting density of *A. annua*. The reduction in branches number per plants with deceasing intra and inter-row spacing may be due to greater inter-plant competition for incident light, soil nutrition, soil moisture and mutual shading of each other at high plant density than at low plant density. There was also failure of auxiliary buds to initiate branches or death of branches at higher density due to competition for assimilates between vertical and lateral growth.

Table 1
Main effect of intra and inter-row spacing on plant height, number of primary branch and Essential oil content.

Treatments	Plant height			NBPP			EOC		
	2013	2014	Pooled	2013	2014	Pooled	2013	2014	Pooled
Intra-row spacing cm									
40	144.67	175.35[a]	160[a]	127.08[b]	179.5[b]	153.25[b]	0.13[a]	0.29	0.216
60	137.43	162.1[ab]	149.76[ab]	125.08[b]	200.1[b]	162.58[b]	0.16[a]	0.25	0.207
80	139.15	154.25[b]	146.69[ab]	160.08[ab]	229.33[a]	194.71[a]	0.14[a]	0.27	0.207
100	137.44	138.72[b]	138.08[b]	188.33[a]	223.75[a]	206.04[a]	0.14[a]	0.27	0.206
LSD	12.88	28.82	15.43	38.03	22.93	22.24	0.028	0.04	0.026
Significance level	ns	*	*	*	**	***	ns	ns	ns
Inter-row spacing cm									
60	140.22	169.43	154.83	135.90[a]	194[b]	164.96[b]	0.15[a]	0.28	0.217
80	141.52	166.07	153.79	151[a]	202.08[b]	176.54[ab]	0.15[a]	0.28	0.213
100	142.77	147.9	145.33	153.33[a]	229.08[a]	191.21[a]	0.13[a]	0.27	0.204
120	134.16	147	140.58	160.15[a]	207.5[ab]	183.88[ab]	0.14[a]	0.26	0.202
LSD	12.88	28.82	15.42	38.03	22.93	22.24	0.028	0.04	0.026
Significance level	ns	ns	ns	ns	*	*	ns	ns	ns
Intra*Inter-row	ns	ns	ns	ns	Ns	ns	ns	ns	Ns
CV%	11.06	21.93	17.98	30.38	13.21	21.51	23.66	20.56	22.13

Essential oil content, in this particular study no significant variation were eminent in different levels of intra, inter-row spacing and their interaction. In agreement with the present study, non significant influence of population density on Essential oil content was reported for peppermint (Beemnet et al., 2011). As well similar Essential oil content over different population density was also reported for Artemisia (Zewdinesh, 2010).

3.3. Above ground biomass and Leaf fresh weight

In both harvesting seasons and pooled mean analysis result showed that, Above ground biomass and Leaf fresh weight were highly significantly ($p>0.01$) influenced by intra-row, inter-row spacing and their interaction. These indicate that the effects of different levels of intra-row spacing were affected by different levels of inter-row spacing. The maximum above ground biomass 72605 gm and leaf fresh weight 9510 gm were observed at 40*60cm and the minimum above ground biomass 15726.17 gm and leaf fresh weight 3162.39 gm were observed at 100*120 intra and inter-row spacing. The increasing trend of above ground biomass and leaf fresh weight per hectare observed in this study is in agreement with the result of Rao (2002) on rose scented geranium, Zewdinesh (2010) on A. annua. Similarly, the current investigation supports the previous research findings conducted by (Abebe Terefe, 2007) on Vernonia galamensis. Highest herbage resulted by the highest population (narrow spacing) and the lowest ones resulted by the lowest population (wider spacing). This is probably due to the occurrence of higher number of branch and leaves contributed from greater number of plants per unit area; resulted in higher use of light in that experiment.

3.4. Leaf dry weight and essential oil yield

In both harvesting seasons and pooled mean analysis result showed that, Leaf dry weight and Essential oil yield were highly significantly ($p>0.01$) affected by intra, inter-row spacing and their interaction. The maximum Leaf dry weight 5392.7 gm and Essential oil yield 23.39gm were observed at 40*60cm and the minimum Leaf dry weight 1758.55 gm and Essential oil yield 5.59 gm were observed at 100*120 intra and inter-row spacing. Higher dry leaf yield per ha at higher planting density compared to the lower density on *A. annua* was also reported by

Diemer and Griffee, 2005 and Charles et al., 1990. The present finding is in agreement with the result of (Zewdinesh *et al*., 2011) who found higher essential oil yield at the highest plant density in *A. annua.* Similar findings were also reported by Yasin et al., 2003 and Saeed et al., 1996 on *Mott Elephantgrass*. An increase in biological yield with increasing plant population density was also reported by Nekonam and Razmjoo, 2007 and Najafi and Moghadam, 2002 on *Plantago ovata*. According to the study made by Board et al., (1990), narrow-row spacing at normal and high densities had significantly higher yield than wide rows. Likewise, in corn mint, highest biomass and maximum essential oil yield were produced due to the narrow spacing's (Rao, 2002). The increasing in essential oil yield at higher densities may be due to the contribution of higher above ground biomass, fresh leaf yield and dry leaf yield at higher densities. This is supported by the very highly significant and positive association of essential oil yield with above ground biomass (r= 0.72***), leaf fresh weight (r= 0.71***) and leaf dry weight (r= 0.90***).

Table 2

Pooled mean comparison of Above ground biomass and Leaf fresh weight as affected by the interaction effects of intra and inter-row spacing.

Intra-row Spacing (cm)	Above ground biomass				Leaf fresh weight			
	Inter-row spacing (cm)				Inter-row spacing (cm)			
	60	80	100	120	60	80	100	120
40	72605^{a}	52105.2^{b}	39706.67^{c}	33132.5^{c}	9510^{a}	7677.09^{b}	4359.17^{c}	4469.38^{c}
60	30725.0 ^{c}	29902.08^{cd}	24205^{cd}	22582.5 ^{de}	4142.5^{c}	4854.17^{b}	3793.89^{bc}	3640.83^{bc}
80	29001.25^{cd}	23585.42^{d}	24985^{cd}	26804.06^{cd}	4142.52^{c}	3691.85^{b}	3701.25^{bc}	4969.53^{bc}
100	28292.19^{cd}	26097.22^{cd}	21751.74^{d}	15726.17^{e}	5042.72^{ab}	3487.42^{b}	3518.06^{bc}	3162.39^{bc}
LSD	***				**			
CV	22.07				26.08			

Table 3

Pooled mean comparison of Leaf dry weight and Essential oil yield as affected by the interaction effects of intra and inter-row spacing.

Intra-row Spacing (cm)	Leaf dry weight				Essential oil yield			
	Inter-row spacing (cm)				Inter-row spacing (cm)			
	60	80	100	120	60	80	100	120
40	5392.7^{a}	4317.55^{a}	2512.74^{ab}	2522.23^{ab}	23.39^{a}	15.98^{ab}	8.98^{ac}	9.48^{ac}
60	2252.23^{ab}	2803.69^{ab}	2143.33^{b}	2120.35^{b}	6.66^{ab}	10.09^{b}	7.78^{bc}	7.28^{bc}
80	2377.69^{ab}	2023.47^{b}	2109.76^{b}	2817.^{91ab}	8.67^{ab}	6.66^{b}	7.51^{bc}	9.73^{bc}
100	2816.33^{ab}	2022.57^{ab}	2006.69^{b}	1758.55^{b}	10.22^{ac}	7.57^{b}	7.29^{bc}	5.59^{bc}
LSD	***				**			
CV	22.42				32.51			

Table 4

Association among growth, yield and yield related characters of A. annua tested under varying intra and inter-row spacing's .

	PH	NB	AGB	LFW	SFW	LDW	EOY	EOC
PH	1							
NB	0.25*	1						
AGB	0.44***	-0.09ns	1					
LFW	0.47**	-0.08ns	0.90***	1				
SFW	0.43***	-0.09ns	0.99***	0.87***	1			
LDW	0.58***	0.32**	0.56***	0.57***	0.55***	1		
EOY	0.48***	0.17ns	0.72***	0.71***	0.7***	0.90***	1	
EOC	0.17ns	0.42***	0.12ns	0.02ns	0.13ns	0.65***	0.64***	1

***= Significant at p < 0.001; **= p < 0.01; *= p < 0.05 probability level; and ns= non significant at 0.05 probability level; PH= Plant height, NB= Number of branch per plant, AGB= Above ground biomass, LFW= Leaf fresh weight, SFW= Stem fresh weight, LDW= Leaf dry weight, EOY= Essential oil yield and ECO= Essential oil content.

4. Conclusion

The analysis of variance revealed that, plant height, number of primary branch per plant, above ground biomass, leaf fresh weight, leaf dry weight and Essential oil yield were significantly ($p>0.05$) influenced by the main effect. While interaction effect of the two factors brought about significant variation on above ground biomass, leaf fresh weight, leaf dry weight and Essential oil yield. However, plant height, number of primary branch per plant and essential oil content were not influenced by the interaction effect. In this study, the maximum above ground biomass 72605 kg/ha, leaf fresh weight 9510 kg/ha, leaf dry weight 5392.7 kg/ha and Essential oil yield 23.39 kg/ha were attained due to spacing combination 40cm intra-row and 60cm inter-row spacing's. Therefore, it could be concluded that *Artemisia annua* could be planted at optimum spacing of 40 x 60 cm in Wondo genet area to attain maximum yield.

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