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Response of Artemisia (*Artemisia annua* L.) for Moisture Stress Condition at Different Growth Stages at Koka, Ethiopia

Elias Meskelu*, AyeleDebebe, HenokTesfaye, Mulugeta Mohammed, Seble Bekele

Wondo Genet Agricultural Research Center, P.O.Box 198, Shashemene, Ethiopia

ABSTRACT

The experiment was carried out at Wondo Genet Agricultural Research Center, Koka research station, Ethiopia, Ethiopia 8°26' N latitude, 39°2' E longitude and 1602 m.a.s.l. altitude for three dry seasons (2016/17, 2017/18 and 2018/19) based on an objective to identify Artemisia (*Artemisia annua* L.) growth stages sensitive to soil moisture stress, determine critical time for irrigation application for limited water resources and productivity of water. Fourteen moisture stress at the four growth stages (initial, development, mid-season and late season stages) and one control (irrigated all the four growth stages) were used with three replication in randomized complete block design. The study revealed that moisture stress at different growth stages had a highly significant ($p<0.01$) effect on pooled means of plant height, fresh leaf weight, stem fresh weight, aboveground biomass, essential oil yield and water use efficiency. Higher growth, yield and yield components like plant height, fresh leaf weight, stem fresh weight, aboveground biomass and essential oil yield were associated with the control treatment and for treatments that received higher irrigation water. However, water use efficiency was associated with treatments that received lower irrigation amounts due to moisture stress at different growth stages especially those obtained irrigation on late season stage. The study revealed that despite lower water use efficiency, irrigation of Artemisia during all the growth stages leads to higher yield herbal and essential oil yield. This shows its sensitive to moisture stress at different in all the growth stage especially in the study area and similar semi-arid environment. Therefore, it could be recommended that irrigation of Artemisia during all the growth stages should be practiced in the study area and similar agro-ecology.

Keywords: Artemisia; Irrigation; Growth stages; Moisture stress; Water use efficiency

INTRODUCTION

Large amount of population are affected by malaria in the world especially in Sub-Saharan Africa. Annually 500 million people most from tropical especially Sub-Saharan Africa, affected globally and kills 41% [1].Transmitted by Anopheles mosquitoes, malaria is developing resistance against common antimalarial drugs and causing economic burden to countries, 12 billion US dollar only in Africa, beyond its health problem. On the other hand, *Artemisia annua* has been used to treat symptoms associated with fever and malaria in China since long time. Moreover, it has been used for the elimination of breast cancer cells [1].

Traditional treatment like that of *Artemisia annua* extracts are less affordable and less toxic to health cells. Willcox *et al.*, reported that *Artemisia annua* extracts are three times more effective than artemisinin [2]. Artemisia annua is known for its artemisinin contenet in its essential oil composition which is effective in treating malaria [3]. Therefore, development of anti-malarial drugs for multi-drug resistant malaria from plants like *Artemisia annua* is essential. Artemisinin from Artemisia is affordable, effective against multi-drug resistant malaria, low toxicity and side effect. Its low toxicity resulted they target protozoan cells that are loaded with iron, acquired through the plasmodium feeding on hemoglobin (Ferreira, 2004) [1]. These artemisnin importance leads Artemisia to be considered the best hope against multi-drug resistant malaria which is caused by *Plasmodiumfalciparum*. Therefore, production of medicinal plant like Artemisia has a paramount importance to improve the health and economic of farming community as the demand for products is increasing.

On the other hand, high population growth forces production of additional food and other agricultural commodities from a limited land and water resources. Competition for these limited water resource from different sectors is increasing due to different human activities and further exacerbated by climate change and pollution [4,5]. However, to increase agricultural production, irrigation is vital despite limited water resource. Moreover, irrigation is the

*Correspondence to: Elias Meskelu, Wondo Genet Agricultural Research Center, P.O.Box 198, Shashemene, Ethiopia, Tel: +251916856072; E-mail: emeskelu@yahoo.com

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highest water consuming sector with poor efficiency. Moisture availability via rainfall or irrigation is vital for crop production, which its limited amount affects crop growth, yield and quality. Arid and semi-arid area of Ethiopia like the study areas are characterized under low rainfall with erratic and unreliable rainfall which is challenging food supply in the country [6]. Moreover, some research reports shows that rainfall in the main rainy season, June to September, is declining due to climate change [7]. Moreover, the study area is characterized under highly variable rainfall probability of the threshold limit of 30 mm per decade for crop production [8].

Under such condition with a limited water resource, evaluation of different crops for irrigation strategies that leads to improve water productivity without significant yield reduction is important for irrigated farms. Therefore, this field experiment was initiated with an objective to evaluate Artemisia (*Artemisia annua* L.) under moisture stress at different stages and identify moisture sensitive stage.

Different research findings reveal that different crop have a different response to moisture stress at growth stages. Maize yield highly affected when moisture stress happen at mid-stage especially when combined with stress at development stage [9]. Some crops also showed an improvement on quality despite reduction on yield when deliberate mild moisture stress applied as deficit irrigation practice. Among these, wheat and maize protein content, fiber quality of cotton, sucrose concentration of sugar beet and wine color density was reported by FAO [10]. Moisture stress at whole or parts of the growth stages affect growth and yield of different crop and the sensitivity vary within crops and growth stages as different crops have different mechanism to escape and adapt drought through physiological responses [11]. Different findings revealed that *Artemisia annua* could be produced under lowland humid tropics with capable of producing high leaf biomass and artemisinin yield.

MATERIALS AND METHODS

The study area

A Field experiment was carried out for three consecutive years (2016/17 to 2018/19) during the dry period (October to February) at Wondo Genet Agricultural Research Center, Koka research station, Ethiopia based on an objective to evaluate Artemisia (*Artemisiaannua* L.) under moisture stress at different stages and identify moisture sensitive stage. Geographically the experimental plot was situated at 8°26' N latitude, 39°02' longitude and altitude of 1602 m.a.s.l. Clay is the dominant soil texture in the study area in which the field capacity and permanent wilting point of the soil in the rooting depth found to be 33.5 and 19.0%, respectively. The bulk density of the soil was 1.1 g/cm^3. These leads to a volumetric available water holding capacity per unit meter of the soil profile in the root zone to be 160 mm. The climate of the study area characterized as semi-arid with mean annual rainfall of 831 mm from which only 9.7% fall during the dry season (October to February) and the rest majority of the annual rainfall, falls from March to September (Tables 1 and 2).

Experimental design and procedure

The field experiment was carried out using Randomized Complete Block Design (RCBD) with three replications following the design procedure for RCBD by Gomez and Gomez [12]. The plot size used was 3.00 m×3.00 m with spacing of 1.50 m between plots and 3.00 m between blocks. For this experiment, fourteen moisture stress at different growth stages and one control (full irrigation) were used as treatments. As per the procedure of RCBD each treatments were assigned randomly for each plot in each blocks and replicated three times. Treatments were 1) irrigated all stages as control (no stress), 2) depriving irrigation at initial stage only (I), 3) depriving irrigation at development stage only (D), 4) depriving irrigation at mid-season stage only (M), 5) depriving irrigation at late season stage only (L), 6) depriving irrigation at initial and development stages (ID), 7) depriving irrigation at initial and midseason stages (IM), 8) depriving irrigation at initial and late season stages (IL), 9) depriving irrigation at development and midseason stages (DM), 10) depriving irrigation at development and late-season stages (DL), 11) depriving irrigation at mid-season and late season stages (ML), 12) depriving irrigation at initial, development and midseason stages (IDM), 13) depriving irrigation at initial, development and late season stages (IDL), 14) depriving irrigation at initial, midseason and late season stages (IML) and 15) depriving irrigation at development, midseason and late seasons (DML).

Table 1: Long-term monthly climatic data of the experimental area.

Month	Tmin (°C)	Tmax (°C)	RH (%)	Wind speed (m/s)	Sunshine hours (%)	RF (mm)
January	11.3	27.4	54	4.04	75	13.5
February	12.6	28.3	52	4.08	76	26.1
March	14.4	30	51	4.64	74	51.5
April	15.2	30.3	54	3.8	71	58.5
May	15.1	30.9	53	3.98	68	48.5
June	15.5	30	57	4.91	65	72.7
July	15	26.7	67	4.3	54	212.7
August	15.1	26.3	68	3.15	53	202.4
September	14.9	27.8	66	2.3	57	104.3
October	12.7	28.3	56	3.5	73	21.1
November	11.3	27.4	52	4.09	83	9.9
December	11	26.1	54	4.19	76	9.9

Table 2: Soil physical characteristics of the experimental site.

Soil texture	Bulk density (g/cm^3)	Field capacity (%)	Wilting Point (%)	Available water holding capacity (mm/m)
Clay	1.17	34.5	17.5	170

Artemisia (*Artemisia annua* L.) seedling was prepared in nursery raising for two months prior to plantation time during the rainy season. For seedling preparation, top cutting position was used as per the recommendation for the area for better seedling using stem cutting from one year old Artemisia collected from multiplication plots at Wondo Genet Agricultural Research Center.

After good establishment of seedling at nursery with average height of 25 cm, it was transplanted to the field with population density recommended for the area which was 40 cm between rows and 20 cm between plants in the row. Prior to planting the land preparation was done with plowing, land leveling and layout according to the experiment. During the study period, regular tillage and agricultural operation for Artemisia in the study area were followed beyond the irrigation level as it is applied according to the treatment arrangement. Irrigation water was applied based on to refill the soil to field capacity in the control plot which receive all stage. Accordingly equal amount of water with the same date was applied for treatments to be irrigated in the particular growth stage. Those which miss irrigation in the specific growth stage irrigation water were not applied. All other agronomic practices were similar for all plots as per the recommendation for the area. The calculated gross irrigation depth for each treatment was applied using Parshall flume of size 2-inch. For these soil samples before irrigation was taken to determine the moisture content of the soil using gravimetric method. Next irrigation applied when the moisture depletion level in the control plot attain 60% from the total available water for all season.

Data collection

Yield and yield component data was collected from the randomly selected five central plants from each plots. Border rows and plants were excluded from the sampling to minimize border effect on yield and yield components of Artemisia. The selected five samples were harvested 120 days after transplanting. Harvesting was done manually by cutting the samples at the branching position above the ground level using sickle. Then after the collected samples were punched to separate leaf and steam and weighted separately for each plots. Finally, the samples were submitted to Wondo Genet Agricultural Research Center, Natural Product Laboratory for analysis of essential oil content using Clevenger apparatus and, leaf and steam moisture content using oven dry. Based on the leaf yield and amount of irrigation used for each treatment, water use efficiency was calculated using the following formula.

$$WUE\left(\frac{kg}{m^3}\right) = \frac{Leaf\ yield\ \left(\frac{kg}{ha}\right)}{Irrigation\ water\ applied\ \left(\frac{m^3}{ha}\right)}$$

Data analysis

The data collected were statistically analyzed using Statistical Analysis System (SAS) software version 9.3 using the General Linear Programming Procedure (GLM). Mean comparison was carried out using Least Significant Difference (LSD) at a 5% probability level to compare the differences among the treatments mean.

RESULTS AND DISCUSSIONS

Application of moisture stress at different growth stages of Artemisia significantly affected all recorded yield and yield components including water use efficiency. Significantly highest yield like fresh leaf weight, fresh biomass and essential oil yield of Artemisia were recorded at higher irrigated without moisture stress with any of the growth stages. Moreover, lower yields were recorded when moisture stress happen during the development and mid-stage or when three growth stages are stressed. However, water productivity was improved when moisture stress happen on three growth stages, except when mid-stage is irrigated as the amount of water applied is higher in this case. On the other hand, water use efficiency was minimum when moisture stress happen in few stages including no moisture stress in any of the growth stages as the amount of irrigation water applied is higher.

Plant height

The study revealed moisture stress at different growth stage of Artemisia had a highly significant ($p<0.01$) effect on plant height. The currently study showed that there is a decreasing trend in plant height of Artemisia as the amount of irrigation water decreased due to application at different growth stages. This was especially when the moisture stress happen at development and mid-season continuously. The maximum plant height (125.2 cm) was recorded when Artemisia stressed during at initial and development stages only. However, these maximum plant heights were statistically similar with no stress and when moisture stress occurs only in one of the growth stages except development and mid-season growth stages. On the other hand, the minimum plant height (94.2 cm) was recorded at moisture stress at development, mid-season and late stages. However, this minimum plant height was statistically similar with most of the moisture stress at three growth stages. Exposing Artemisia to moisture stress during development, mid-season and late season growth stages continuously leads to a reduction of 24.8% as compared with the maximum plant height.

Different studies revealed that moisture stresses either full growth stage or at different growth stages leads to a reduction of plant height on different crops including Artemisia [13]. The current finding is in line with the report of Elias *et al.*,who reported that plant height of maize affected by moisture stress at different growth stage and the highest reduction occurs when development and mid-season growth stage is stressed [9]. The reduction in plant height due to moisture stress at different growth stages might be associated with the drought stress which reduces photosynthesis and reduced cell enlargement might be due to low turgor pressure in plants [14-16].

Fresh leaf weight

Moisture stress at different growth stage had a highly significant ($p<0.01$) effect on fresh leaf weight production of Artemisia. The study revealed that there is a decreasing trend in fresh leaf weight of Artemisia as the amount of irrigation water decreased due to application at different growth stages especially when moisture stress happen in three growth stages. The maximum fresh leaf weight (8.3 t/ha) was recorded at no stress treatment (Table 2). The maximum fresh leaf weight recorded at no stress in any of the growth stages was statistically superior to all other treatments. On the other hand, the minimum fresh leaf weight (3.7 t/ha) was recorded when moisture happen in three growth stage of development, mid-season and late season stages (Table 2). However, this minimum fresh leaf weight was statistically similar with that of moisture stress at initial, mid-season and late growth stages as well as moisture stress at mid-season and late season growth stages.

The study revealed that a decreasing trend was observed in fresh leaf weight production when moisture stress at different growth stages extended on Artemisia. Exposing Artemisia to moisture stress during development, mid-season and late season growth stages continuously leads to a reduction of 55.4% as compared with the maximum fresh leaf weight recorded at no stress. Similar findings were also reported by Alhaithloulthat Artemisia fresh leaf weight affected by moisture stress. Soni and Abdin also reported that water stress causes reduction of growth biomass production of arimisia production including fresh leaf production[13].

Fresh biomass

The study showed that moisture stress at different growth stage had a highly significant (p<0.01) effect on fresh biomass production of Artemisia. The increase in moisture stress exposure showed a decreasing trend in fresh biomass production especially when moisture stress occurs in two or three growth stages. The maximum fresh biomass (31.4 t/ha) was recorded at no stress treatment which is statistically superior to all other treatments. On the other hand, the minimum fresh biomass production (15.9 t/ha) was recorded when moisture happen in three growth stage of development, mid-season and late season stages (Table 2). However, this minimum fresh leaf weight was statistically similar with all of the treatments where moisture stress happens at three growth stages and when moisture stress at two stages at initial and mid-season stages and, development and mid-season stages.

The study revealed that fresh biomass production showed a decreasing trend when moisture stress at different growth stages extended on Artemisia plant. Exposing Artemisia to moisture stress during development, mid-season and late season growth stages continuously leads to a reduction of 49.4% as compared with the maximum fresh leaf weight recorded at no stress. The current study is in agreement with former reports of Alhaithloul and Soni and Abdinwho reported that Artemisia fresh biomass production is affected due to moisture stress in all or parts of the growth stages. These might be due to the fact that moisture stress leads to reduce food production through photosynthesis and translocation of food to different parts of the plant. That is because water is the main component of photosynthesis for food production and food transportation done through water solution form in the plant.The reduction in plant biomass production at well irrigated treatments might be associated with low turgor pressure response of crops during drought in their growth stages which reduce cell enlargement [14,15]. Others also reported that soil moisture availability significantly affect most of the agronomic characteristics of Artemisia annua including fresh leaf biomass yield (Table 3).

Essential oil yield

Moisture stress at different growth stages had a highly significant (p<0.01) effect on essential oil yield production of Artemisia. Similar with the production of fresh leaf weight, essential oil yield obtained from hydro distillation of leaf showed a decreasing trend as moisture stress level increased at different growth stages. Maximum essential oil yield (18.6 kg/ha) was recorded at no stress treatment which is statistically superior to all other treatments (Table 2). On the other hand, minimum essential oil yield production (8.9 kg/ha) was recorded when moisture happen in three growth stage of development, mid-season and late season stages (Table 2). However, this minimum fresh leaf weight was statistically similar with all of the treatments where moisture stress happens at three growth stages and when moisture stress at two stages at development and mid-season stages and, mid-season and late season stages. Exposing Artemisia to moisture stress during development, mid-season and late season growth stages continuously leads to a reduction of 52.2% essential oil yield production as compared with the maximum recorded at no stress.

Other researchers also reported abiotic stress like moisture stress affects Artemisia essential oil yield production [16,17]. This might be due to the reduction in leaf production due to moisture stress also affects total essential oil production which is directly associate leaf production. Reports of Elias *et al.*,also revealed that essential oil production of spearmint crop affected due to moisture stress due to deficit irrigation in all growth stages in the study area [9]. Moisture stress affects photosynthesis production

Table 3: Effect of moisture stress at different growth stages on yield, yield component and water use efficiency.

Treatment	PH (cm)**	FLW (t/ha)**	FBM (t/ha)**	EOY (kg/ha)**	WUE (kg/m3)**
T-1	121.3^{ab}	8.3^{a}	31.4^{a}	18.6^{a}	3.6^{def}
T2	120.9^{ab}	7.1^{b}	25.7^{b}	15.6^{b}	3.2^{f}
T-3	112.6^{bcde}	6.5^{b}	23.8^{cbd}	15.4^{b}	3.6^{def}
T-4	107.8^{cdef}	5.7^{c}	20.3^{def}	13.1^{bc}	5.2^{def}
T-5	116.7^{abc}	5.6^{c}	24.1^{bc}	13.7^{bc}	3.2^{f}
T-6	125.2^{a}	7.0^{b}	22.5^{bcd}	14.4^{bc}	4.0^{def}
T-7	104.9^{efg}	4.6^{de}	19.0^{efg}	12.1^{cd}	5.5^{cde}
T-8	114.7^{bcd}	6.5^{b}	22.9^{bcd}	15.4^{b}	4.0^{def}
T-9	101.5^{fgh}	4.6^{de}	16.9^{g}	10.0^{de}	7.5^{bc}
T-10	106.6^{def}	5.6^{c}	20.9^{cde}	12.0^{cd}	3.7^{def}
T-11	104.6^{efg}	4.0^{ef}	16.7^{g}	9.1^{e}	5.8^{cd}
T-12	101.0^{fgh}	4.9^{d}	17.1^{fg}	9.9^{de}	9.7^{b}
T-13	105.1^{efg}	4.8^{d}	18.0^{efg}	9.9^{de}	3.5^{ef}
T-14	96.8^{gh}	3.8^{f}	17.6^{efg}	9.7^{de}	9.6^{b}
T-15	94.2^{h}	3.7^{f}	15.9^{g}	8.9^{e}	23.8^{a}
CV (%)	4.9	7.5	9.9	13.1	21.3
$LSD_{0.05}$	8.9	0.7	3.4	2.7	2.3

Means followed by the same letters in column are not statistically different at 5% level for Least Significant Difference Test. *Significant at p<0.05, and **significant at p<0.01.

and distribution of essential growth components in plants as water is the main component for food production and translocation [14]. Likewise, Artemisia morphological and growth parameter could be affected by drought due to moisture stress at different growth stages. Moreover, Marchese *et al.*,reported that moderate water deficit prior to harvesting the crop may induce artemisinin accumulation in Artemisia plant [18]. Similarly, De Magalhaes*et al.*, reported that artemisinin, the main component of essential oil has a negative correlation with biomass production [19]. However, the higher biomass production at no stress leads to better essential oil yield production in this study due to higher leaf production to compensate the lower essential oil content. Vashisth*et al.*,also observed that artemisinin content was increased in different abiotic stresses like salt, cold and water logging. However, the same author reported that these increments were not observed under the case of drought stress [20].

Water use efficiency

The study revealed that moisture stress at different growth stage had a highly significant ($p<0.01$) effect on essential oil yield production of Artemisia. Generally, the increased in moisture stress exposure showed an increasing trend in efficient utilization of the available water despite some variation due to moisture stress at difference. This is especially seen as Artemisia imposed to moisture stress during late growth stage highly reduced its water use efficiency. The maximum water use efficiency (23.8 kg/m^3) was observed at moisture stress at development, mid-season and late stages which was statistically superior to all other treatments (Table 2). On the other hand, the minimum water use efficiency was recorded at stress only at initial stage and stress only at late stage which both treatments recorded 3.2 kg/m^3. However, this minimum water use efficiency was statistically similar with no stress and all treatments with stress only at one stage. Moreover, it was statistically similar with treatments of moisture at two growth stages (initial and late stages, and development and late stages) and moisture stress at three growth stages of initial, development and late stages.

The study revealed that water use efficiency showed an increasing trend when moisture stress at different growth stages extended on Artemisia plant despite some variation due to stress at different stages. As compared to the maximum water use efficiency observed when Artemisia stressed during development, mid-season and late stage, the control treatment with no stress leads to a reduction of water use efficiency by 83.1%. However, stressing three growth stages of development, mid-season and late stages leads to a reduction of essential oil yield production by 52.2% as compared to the control treatment.

The current study is in agreement with former reports of Elias *et al.*, who reported that water use efficiency of spearmint improved when the level of moisture stress due to deficit irrigation levels increased [9]. This might be due to the use of lower irrigation water which the plant use it efficiently to produce some yield despite lower amount as compared to full irrigation. Tatrai*et al.*, also reported in thyme plant that it showed a morphological drought avoidance mechanism by maintaining the root system development which promoted root absorption capacity which leads to an increase in water use efficiency in stressed plants as water saving strategies [21]. De Magalhaes*et al.*,also reported that artemisinin content and biomass has negatively correlation which higher biomass production per plant leads to lower content and the plants under stress and lower biomass leads to higher content and compensation on total artemisinin yield [19]. This could leads to improve the water use efficiency at lower irrigation treatments as the production of essential oil per irrigation water is higher than the well irrigated once.

CONCLUSION AND RECOMMENDATION

The current study revealed that exposing Artemisia annua to moisture stress at different growth stages leads to a reduction of plant height, fresh biomass, fresh leaf weight, fresh stem weight and essential oil yield. Irrigation of Artemisia during all the growth stages leads to higher essential oil yield better than all the stressed treatments. Contrary to this, irrigating all stage leads to a reduction of water use efficiency due to using higher irrigation water. Higher water use efficiency was associated with irrigation of only during the late stages despite lower essential oil yield and other yield components. The study revealed that despite lower water use efficiency, irrigation of Artemisia during all the growth stages leads to higher yield herbal and essential oil yield. This shows its sensitive to moisture stress at different in all the growth stage especially in the study area and similar semi-arid environment. Therefore, it could be recommended that irrigation of Artemisia during all the growth stages should be practiced in the study area and similar agro-ecology.

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