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Response of Artemisia (*Artemisia annua L.*) to Nitrogen and Phosphorus Fertilizers in Wondo Genet and Koka, Ethiopia

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Field experiment was conducted at Wondo Genet Agricultural Research Center and Koka substation in 2013/2014 and 2014/2015 cropping season in order to identify optimum nitrogen and phosphorus fertilizer rate for some selected agronomic characteristics, essential oil yield and oil content of artemisia. Four nitrogen levels (0, 46, 92 and 138 kg N ha^{-1}) and four phosphorus levels (0, 10, 20 and 30 kg P ha^{-1}) would be laid out in factorial RCBD with three replications. At koka condition, results revealed that plant height, leaf dry weight and essential oil content were significantly affected by separate effect of nitrogen and phosphorus fertilizer. The interaction of 46 kg N ha^{-1} and 10 kg P ha $^{-1}$ gave significantly higher above ground biomass, leaf fresh weight and stem fresh weight. Similarly, the maximum essential oil yield was obtained at the interaction of 92 kg N ha^{-1} and control of P though statistically at par with 46 kg N ha^{-1} and 10 kg P ha^{-1}. At Wondo Genet, all the parameters didn't show any significant variation by the application of N and P and its interaction except leaf and stem fresh weight which is at par with the soil analysis result of the center. Thus at Wondo Genet production of artemisia without nitrogen and phosphorus fertilizer is economically visible. For Koka area and other similar soil type and agro ecologies of Ethiopia, N and P fertilizers at the rate of 46 kg N ha^{-1} and 10 kg P ha^{-1} could be recommended.

Key words: Artemisia annua, nitrogen and phosphorus fertilizer, oil content and oil yield

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INTRODUCTION

Nowadays, the promotion of human health is a priority of crop producers. *Artemisia annua (*Asteraceae*)*, commonly known as "sweet or annual wormwood," is an annual medicinal and aromatic herb that possesses a wide range of health benefits and has therefore been widely used as herbal medicine (Obolskiy *et al*., 2011). It has antimalarial effect due to the presence of artemisinin which is one of the most important advances in malaria control in modern times. Medicinal herbal tea can also be prepared from dried leaf of *Artemisia annua* for the treatment of malaria without negative side effect (Hirt and Lindsey, 2000).

Plant nutrition is one of the most important factors that increase plant production. Plant uptake and tissue concentration of elements are mainly dictated by the combined influences of both genetic and environmental factors such as soil composition, use of fertilizers, plant's maturity at harvest and the storage conditions (Sanchez-Castillo *et al*., 1998). Thus, one of the most important needs in agricultural planning is to achieve high yield and good quality plant nutrition evaluation of various systems.

Nitrogen (N) is the most recognized element in plant for its presence in the structure of the protein molecule. Accordingly, N plays an important role in synthesis of the plant constituents through the action of different enzymes (Saber F.H and Khalid A.K., 2011). N limiting conditions increase volatile oil production in annual herbal plants. N fertilization has been reported to reduce essential oil content in creeping juniper (*Juniperus horizontalis*) (Robert and Francis, 1986), although it has been reported to increase total essential oil yield in thyme (*Thymus vulgaris* L.) (Baranauskienne *et al*., 2003).

Phosphorus also plays an important role in various metabolic processes. It activates coenzymes for amino acid production used in protein synthesis. P also aids in root development, flower initiation, seed and fruit development. Hornok (1980) indicated that NP fertilization not only effective on the quantity of vegetative and generation mass, but on the oil content of dill (*Anethum graveolens* L.). According to Emongor (1990), high P rates (more than 7.47 kg P ha^{-1}) decreased chamomile essential oil yield. From all these results, it can be inferred that it is essential to apply N and P fertilizer to medicinal plants supposed to be grown in soils which are deficient in these nutrients.

In this regard, researches show that excessive use of chemical fertilizers decreases crop yield due to soil acidification, loss of biological activity in the soil, changes in soil physical properties and lack of micronutrients in agricultural land (Adediran *et al*., 2004). To reduce these risks, resources and inputs must be used in an optimum level to the current needs of the plant. This implies that there is a need to test and establish optimum N and P rates for adequate production of artemisia. Thus, this research was conducted in Wondo Genet and Koka area to determine optimum N and P rates for improving yield and yield component of artemisia.

MATERIAL AND METHODS

The field experiment was carried out under irrigated condition for two consecutive cropping seasons (2013/2014 and 2014/2015) at Wondo Genet Agricultural Research center experimental site and Koka experimental substation. Wondo Genet 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 (0-20cm) was clay with slightly acidic (pH 5.91, 1:2.5 soil water suspensions). Koka was geographically located at 08°26.1' North latitude, 39° 01' East longitude with an altitude of 1617m.a.s.l. The texture of the top soil (0-20cm) was loam with slightly alkaline (pH 8.01).

An auger was used to take soil samples at random from different spots to make a composite sample per test field at depths of 0-20 cm before fertilizer application. A sizeable quantity of composite soil samples were air-dried and sieved through a 2 mm mesh and subjected to physical and chemical analysis. The soil was analyzed for pH, organic content, total nitrogen and available phosphorus following standard laboratory procedures (Sahlemedhin Sersu and Taye Bekele, 2000). Seedlings of Artemisia were raised in the nursery for three months and transplanted to actual field on well tilth land for planting. Factorial combinations of four nitrogen level (0, 46, 92, 138 kg ha^{-1}) and four phosphorus level (0, 10, 20, 30 kg ha^{-1}) were laid out in a randomized complete block design (RCBD) with three replications. Urea and TSP were used as source of nitrogen and phosphorous fertilizer, respectively. Full dose of phosphorous was applied as basal dressing during planting. Nitrogen containing urea fertilizer was applied in split form (1/3 during planting and remaining 2/3 two month after transplanting). Plot size for each treatment was 4.80m x 4m to which artemisia was planted with intra and inter row spacing of 60 and 60 cm apart respectively. All agronomic practices including weeding and harvesting was done as per the recommendation for the crop.

Five plants were selected randomly from each plot by excluding borders to collect yield and yield contributing characters such as plant height(cm), above ground biomass(g), Leaf fresh weight(g), stem fresh weight(g), leaf dry weight(g), essential oil yield(g), and essential oil content(w/w, wet based%) of the plant. Essential oil yield 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 (SAS, 2000). For those parameters in which their ANOVA results found to be significant, further means separations were done using least significant difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION

Physicochemical Properties of the experimental soil before planting

Selected physicochemical properties of the composite surface soil (0-20 cm) collected before planting showed that the textural class of the soil was clay with slightly acidic (pH 5.91) at Wondo Genet and loam with slightly alkaline (pH 8.01) at Koka (Table 1) indicating that these properties are favorable for Artemisia production. *Artemisia annua* grows in most soil types having deep topsoil and good drainage properties with the pH of the soil between 4.5 and 8.5 (Ferreira *et al*, 1995).

The organic carbon content of the experimental soil for Koka (1.4%) and Wondo Genet (2.8%) are low in accordance with Landon (1991), who classified the organic carbon content of soil <4%, 4-10%, and >10% as low, medium and high respectively. The same author classified total nitrogen content <0.1, 0.1-0.15, 0.15-0.25 and >0.25 as very low, low, medium, and high respectively. Similarly, Tekalign (1991) also classified total nitrogen content of <0.05, 0.05-0.12, 0.12-0.25, and >0.25 as very low, low, medium, and high respectively. The total nitrogen content (0.08%) and 0.24% for koka and Wondo Genet respectively were low and medium (Table 1) in accordance with the ratings of both authors, indicating that nitrogen was a limiting factor for crop growth at Koka probably due to continuous cultivation and lack of incorporation of organic materials and therefore application of nitrogen fertilizer tended to reduce this limiting factor of growth. However, medium N content was observed at Wondo Genet experimental site that is why N and P fertilizer experiments didn't bring significant variation among treatments.

Available P of the two experimental sites were 18 and 44 mg kg^{-1} (Tale 1) could be considered as high accordance with Landon (1991), who classified available P of the soil <5, 5-15 and > 15 as low, medium and high respectively. This indicated that P is not a limiting nutrient for optimum crop growth and yield in the experimental sites. In general, the properties of the experimental soil and the weather conditions at the two sites were conducive for growth of artemisia.

Effects of N and P on Plant height

For both testing sites, plant height didn't show any significant (p>0.05) variation by the application of nitrogen fertilizer and its interaction with phosphorus (Table 2). However, at Koka condition, phosphorus application showed significant variation (p≤0.01) though it was inconsistent at Wondo Genet. The tallest mean plant height (133.97cm) of artemisia was found at 30 kg P ha^{-1} though statistically at par with the control treatment (Table 2). The statistical similarity between the control treatment and 30 kg P ha $^{-1}$ could be due to higher initial P level in the experimental site.

Effect of N and P on Leaf and Stem fresh weight

At Wondo Genet, leaf and stem fresh weight was significantly influenced by different rates of nitrogen fertilizer whereas the different level of phosphorus fertilizer didn't bring any significant influence on the leaf fresh weight of artemisia (Table 2). The maximum leaf fresh weight was found at 46 kg N ha^{-1} compared with the control treatment though statistically at par with the other phosphorus levels. Similarly, the highest stem fresh weight was obtained at 92 kg N ha^{-1}eventhogh statistically similar with 46 kg N ha^{-1}. The non significant effect of Phosphorus might be due higher initial available P in the experimental site (Table 1).

At koka condition, both Leaf and stem fresh weight were significantly (P≤0.01) influenced by the interaction of nitrogen with phosphorus fertilizer (Table 3). The highest leaf and stem fresh weight was found at the interaction of 46 kg N ha^{-1} with 10 kg P ha^{-1} though it showed statistical similarity with control N interact with 30 kg P ha^{-1} for stem fresh weight (Table 3 &4). This evidenced that the right rate of nitrogen and phosphorus fertilizer enhance Artemisia efficiency in utilization of resources by reducing inter and intra specific competition. Prasad *et al*., (2008), in line with this study reported that foliar application of calcium chloride increased herbage yield in rose-scented geranium compared with the control. Sajad *et al*, (2014), in agreement with the above study, also stated that foliar application of 5g Ca (NO3)2 significantly increased fresh matter yield by an average of 68.3 and 42.7% in first and second harvests, respectively compared with the control treatment. Regarding to obtained results, it was clear that nitrogen and phosphorus are considered as one of the most essential elements for growth and development of Artemisia. Many studies proved that N and P play a major role in many physiological and biochemical processes such as cell division and elongation; enzyme activation; stabilization of the native conformation of enzymes and possibly turgor, stomata movement, metabolism of carbohydrates and protein compounds (Espinosa *et al*., 1999; Khalid, 2001; Marschner, 1995; Fawzy *et al*., 2007).

Effect of N and P on above ground biomass and Leaf dry weight

At Wondo Genet location, above ground biomass was significantly influenced by different rates of nitrogen fertilizer whereas the different level of phosphorus fertilizer didn't show any significant influence (Table 5). The maximum leaf fresh weight was found at 46 kg N ha^{-1} compared with the control treatment though statistically at par with the other phosphorus levels. In contrast to above ground biomass, N application and its interaction with phosphorus didn't give any significant effect on leaf dry weight (Table 5). On the other hand, leaf dry weight was significantly (P≤0.05) affected by different level of Phosphorus fertilizer although this was against the initial soil available P in the testing site.

At Koka, above ground biomass was significantly

Table 1. Selected soil physical and chemical properties of Wondo Genet and Koka experimental site before planting

Location	Particle Size Analysis (%)				pH 1:2.5	OC %	Total N (%)	Avail P (mg kg^{-1})
	Sand	Silt	Clay	Class				
Koka	42	32	26	Loam	8.01	1.45	0.08	18
W/Genet	10	40	50	Clay	5.95	2.78	0.24	44

Table 2. Means for plant height, Leaf and stem fresh weight of *Artemisia annua* as affected by nitrogen, phosphorus fertilizer and their interaction at Koka and Wondo Genet during 2013/2014 to 2014/2015 cropping season

Treatments	PH (cm)		LFW (ton ha^{-1})		SFW (ton ha^{-1})	
	Koka	**W/Genet**	**Koka**	**W/Genet**	**Koka**	**W/Genet**
			N levels			
0 kg	123.9	130.25	4.65b	5.32^{b}	22.01	19.54^{b}
46 kg	128.4	133.08	5.23ab	6.09^{a}	22.66	22.60^{ab}
92 kg	127.4	133.50	5.36a	6.03^{ab}	21.53	23.58^{a}
138 kg	128.1	126.29	4.77ab	5.96^{ab}	21.55	22.35^{ab}
LSD	ns	ns	0.61	0.72	ns	3.10
			P levels			
0kg	127.8^{ab}	127.19	4.86	5.78	21.22b	20.48
10 kg	126.2^{ab}	130.88	5.22	6.09	22.13ab	23.09
20 kg	123.4^{b}	130.92	4.94	5.81	21.28b	22.12
30 kg	130.6^{a}	134.13	4.99	5.72	23.12a	22.37
LSD	6.19	ns	ns	ns	1.69	ns
N*P	ns	ns	**	ns	**	ns
CV	8.45	9.67	21.3	21.22	13.3	24.43

***, **,* significant at 0.001, 0.01 and 0.05 probability level respectively, ns= non significant

Means within column followed by the same letter (s) are not statistically different from each other PH= Plant height, LFW= Leaf fresh weight, SFW= Stem fresh weight

Table 3: Mean comparison of above ground biomass and leaf fresh weight as affected by the interaction effects of nitrogen and phosphorus fertilizer rates

Nitrogen Level	Above ground biomass (ton/ha)				Leaf fresh weight (ton/ha)			
	Phosphorus				**Phosphorus**			
	0	10	20	30	0	10	20	30
0	28.38^{bc}	22.47^{e}	25.07^{cd}	30.72^{ab}	4.82^{c}	3.90^{d}	4.42^{cd}	5.46^{b}
46	26.88^{c}	31.39^{a}	25.86^{cd}	27.65^{bc}	5.25^{bc}	6.20^{a}	4.64^{c}	4.84^{c}
92	26.26^{cd}	29.95^{ab}	26.44^{cd}	24.90^{d}	5.64^{ab}	6.04^{ab}	5.06^{bc}	4.69^{c}
138	22.96^{de}	25.59^{cd}	27.52^{bc}	29.20^{b}	3.72^{d}	4.74^{c}	5.64^{ab}	4.99^{bc}
LSD	1.95 12.5				0.61 21.3			

($P \leq 0.001$) affected by the interaction of nitrogen with phosphorus fertilizer (Table 3). Maximum above ground biomass was obtained at the interaction of 46 kg N ha^{-1} with 10 kg P ha^{-1}. In agreement with this finding, Singh & Ganesha Rao, 2009; Singh, 2008; Jeliazkova *et al*., 1999 reported that KNO3 fertilizer increased fresh matter yield by an average of 60.5% compared with the control. The improving effect of nitrogen and phosphorus on above ground biomass could be due to efficiency of the plant for the utilization of nitrogen and phosphorus. On the other

Table 4: Mean comparison of stem fresh weight and essential oil yield as affected by the interaction effects of nitrogen and phosphorus fertilizer rates

Nitrogen Level	Stem fresh weight (ton/ha)				Essential oil yield (kg/ha)			
	Phosphorus				Phosphorus			
	0	10	20	30	0	10	20	30
0	23.56^{b}	18.57^{d}	20.65^{c}	25.26^{a}	13.5^{ab}	9.3^{cd}	9.8^{cd}	12.2^{b}
46	21.43^{c}	25.19^{ab}	21.22^{c}	22.81^{bc}	13.6^{ab}	13.9^{ab}	10.1^{cd}	9.4^{cd}
92	20.62^{c}	23.91^{abc}	21.38^{c}	20.21^{cd}	14.6^{a}	12.3^{b}	12.2^{b}	11.8^{bc}
138	19.25^{cd}	20.85^{c}	21.89^{bc}	24.20^{ab}	8.02^{d}	9.0^{d}	10.6^{c}	12.85^{b}
LSD	1.69				1.59			
	13.3				24			

Table 5. Means for above ground biomass, leaf dry weight, EOY and EOC of *Artemisia annua* as affected by nitrogen and phosphorus fertilizer at Koka and Wondo Genet during 2013/2014 and 2014/2015 cropping season

Treatments	AGB (ton ha^{-1})		LDW (ton ha^{-1})		EOY (kg ha^{-1})		EOC (W/W, wet based)	
	Koka	W/Genet	Koka	W/Genet	Koka	W/Genet	Koka	W/Genet
				N levels				
0 kg	26.66	24.85^{b}	1.86	1.58	11.2^{ab}	8.54	0.25^{a}	0.16
46 kg	27.94	28.78^{a}	2.11	1.83	11.8^{a}	9.69	0.22^{ab}	0.16
92 kg	26.89	29.58^{a}	2.16	1.74	12.7^{a}	9.52	0.24^{a}	0.16
138 kg	26.32	28.31^{a}	2.08	1.80	10.1^{b}	9.60	0.21^{b}	0.15
LSD	ns	3.31	ns	ns	1.58	ns	0.03	ns
				P levels				
0kg	26.12^{b}	26.24	1.96	1.62b	12.4^{a}	9.56	0.25^{a}	0.17
10 kg	27.35^{ab}	29.24	2.10	1.93a	11.1^{ab}	9.31	0.25^{b}	0.16
20 kg	26.22^{ab}	27.94	2.00	1.75ab	10.67^{b}	9.46	0.22^{b}	0.16
30 kg	28.12^{a}	28.10	2.16	1.64b	11.58^{ab}	9.04	0.24^{ab}	0.16
LSD	1.95	ns	ns	0.27	1.58	ns	0.03	ns
N*P	***	ns	ns	ns	**	ns	ns	ns
CV	12.5	20.61	26.8	27.28	24	31.1	20.9	23.82

***, **,* significant at 0.001, 0.01 and 0.05 probability level respectively, ns= non significant
Means within column followed by the same letter (s) are not statistically different from each other
AGB= above ground biomass, Leaf dry weight, EOY= essential oil yield, EOC= essential oil content

hand, the role of N and P in an increasing the yield of artemisia would be attributed to its function in plants which include synthesis of the plant constituents through the action of different enzymes activity and protein synthesis (Jones *et al.,* 1991), energy metabolism and enzyme activation on exchange rate and nitrogen activity as well as enhanced carbohydrate movement from the shoot to storage organs (Mengel & Kirkby, 1980).

Effect of N and P on Essential Oil yield and Essential oil content

At Wondo Genet, both EOY and EOC didn't significantly ($p>0.05$) affected by the application of nitrogen and phosphorus fertilizer and their interaction (Table 5). In contrast to Wondo Genet, essential oil yield at koka was significantly ($P\leq0.01$) affected by the interaction of nitrogen and phosphorus fertilizer rate (Table 5). On the same way, essential oil content was significantly affected by the two main effects. The maximum essential oil content of artemisia was obtained at the control treatment compared with fertilized plots. Essential oil content decreased as the rate of nitrogen and phosphorus fertilizer increased. In line with this research, N fertilization has been reported to reduce essential oil content in creeping juniper *(Juniperus horizontalis*) (Robert, 1986), although it has been reported to increase total essential oil yield in thyme (*Thymus vulgaris* L.) (Baranauskienne *et al*., 2003). Furthermore, different researchers reported that further increase of $CaCO_3$ application decreased essential oil content of *S. hortensis* L. (Mumivand *et al.*, 2011), *Ch. boreale* M. (Lee and Yang, 2005) and *Ch. coronarium* L. (Supanjani *et al.*, 2005).

The maximum essential oil yield (14.6 kg ha^{-1}) was recorded at 92 kg N ha^{-1} with control of phosphorus though it was statistically at par with 46 kg N ha^{-1} and 10 kg p ha^{-1}(Table 5). Rao, (1989), in agreement with this study, reported that application of 100 kg N and 26 kg P per hectare produced the highest biomass and essential oil yields of davana (*Artemisia pallens* Wall.). Similarly, Sharafzadeh, (2011) found that NP treatments produced the highest growth and essential oil of garden thyme (*Thymus vulgaris* L.) compared with the control treatment. Generally, an increase in oil yield of Artemisia was more evident when p level was lower and N level was higher till 92 kg N ha^{-1}, after which any further increase decreased essential oil yield. It seems that Artemisia probably benefited from the N and P applied to the plant. This finding inveterate previous findings of Khalid, 2012 who reported that NP elements positively affect oil yield, growth and chemical constituent's of some medicinal apiaceae (anise, coriander and sweet fennel) plants; Malik *et al.*, 2009, 2012 also stated Artemisia annua responds well to chemical, organic and bio fertilizers in terms of oil quality. These results are in good accordance with the findings of Singh *et al.* (2007) on rosemary (*Rosmarinus officinalis* L.).
laboratory analyses.

CONCLUSION

Nitrogen and phosphorus fertilizers play vital role in maximization of agronomic trait and economic yield of artemisia. The result of the study at koka revealed that essential oil content of *Artemisia annua* was higher in the unfertilized plots of N and P while fertilized plots increased above ground biomass, leaf fresh weight, stem fresh weight and essential oil yield. Therefore, it could be concluded that nitrogen and phosphorus fertilizers at the rate of 46 kg N ha^{-1} with 10 kg P ha^{-1} are ideal to maintain maximum economic yield of *Artemisia annua L.* production at koka area and other similar soil types and agro ecologies of Ethiopia. Further demonstration of this result around the study area through the involvement of stakeholders is recommended. Additionally, in the area further research on artemisia in an integrated approach with organic fertilizers is highly advised. For Wondo Genet, all the parameters didn't show any significant variation by the application of N and P and its interaction except leaf and stem fresh weight which is at par with the soil analysis result of the center. Thus at Wondo Genet artemisia production without nitrogen and phosphorus fertilizer is recommended. Besides to these, in the center further fertilizer investigation should not be conducted unless there was N and P gradient created before the start of the actual field experiment.

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