Response of *Artemisia annua* L. to shade and manure fertilizer application in lowland altitude

H H Permana¹, Y Widyastuti², Samanhudi³ and A Yunus⁴  
¹Student of Study Program of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret Surakarta, Indonesia  
²Research Center for Medicinal Plant and Traditional Drug, Tawangmangu, Indonesia  
³Lecturer of Study Program of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret Surakarta, Indonesia  
Email : yunus.uns7@yahoo.com

**Abstract.** Artemisia is a plant producing artemisinin substance which is the main compound in the treatment of malaria. Artemisia comes from China, usually grows wild in native habitats in the plains with an altitude of 1,000-1,500 meters above the sea level. Artemisia development efforts in Indonesia hampered by limited land with the required altitude due to their competition with vegetable crops. Based on this reason, this research is conducted to observe the growth of artemisia planted in lowland with the help of shade and manure. This study aims to determine the level of shade and best manure on the growth of Artemisia. Research conducted at the Laboratory of the Faculty of Agriculture UNS Jumantono using nested design with two factors, shade as main factor and manure fertilizer as sub factor. The data analysis used F-test with confidence level of 5%, if significant, then continued with DMRT (Duncan Multiple Range Test). The results showed the treatment of shade gave no difference in growth within 50% shade, 75% shade as well as without shade treatment. Goat manure fertilizer gave the highest result and able to increase plant height, number of branches, flower weight and root volume.

1. **Introduction**  
*A. annua* L. is a medicinal plant that has been used since a long time ago in China as an antimalarial medicine. Artemisia containing terpenoids complex compounds, called artemisinin which known to be able to eradicate Plasmodium falciparum as a major cause of malaria and treat breast cancer[1]. Artemisinin is a compound that is effective for the types of malaria that resistant to quinine and chloroquine and cerebral malaria caused by Plasmodium falciparum. In Artemisia, artemisinin is synthesized in the roots and accumulated in the leaves. The artemisinin content in flower is quite high and can be compared with artemisinin contained in the leaves [2]. Artemisinin production is influenced by climate, soil conditions, plant age and genetic variations [3].

Treatment for malaria usually use quinine that has been used for a long time by traditional society. Along with the development of malaria, resistency of malaria occurring to various existing drugs. Resistency happens to drugs such as chloroquine and sulfadoxine-pyrimethamine (SP) so that medical efforts are made with treatment of Artemisinin based Combination Therapy [4]. Artemisia development as the only producer of artemisinin have a major problem, that is the existing genotype...
provided relatively low artemisinin content between 0.01% - 0.5% with the time of planting about 6 months plus the time of extraction to produce finished materials between 2-5 months [5]. Other problems are in the tropic area, this plant grows in the highlands at an altitude of over 1,000 meters, while in Indonesia the area with that altitude usually used for the cultivation of vegetables and fruit, so that there is competition of land with vegetable crops in the cultivation of these crops.

2. Methods

2.1. Location and Time Research
The study was conducted in the village of Sukosari, District Jumantono, Karanganyar, Surakarta from July to November 2015.

2.2. Tools and Materials
The tools used are 30cm x 20cm size planting pot (36 units), paranet 50%, 75% paranet, bamboo poles, hoes, tape measure, ruler, measuring cups and analytical scale. The material used in the research are the seeds of *A. annua* L., Jumantono Alfisol soil (growing media), goat manure fertilizer, chicken manure fertilizer and cow manure fertilizer.

2.3. Research Design and Data Analysis
This research used a nested design with manure fertilizer nested in shade. The main factor was the level of shade and sub factors that nested on the main factors was manure fertilizer. The shade consisted of 3 levels: without shade (N0), 50% shade (N1) and a shade 75% (N2). The manure fertilizer consisted of 4 levels which were without fertilizers (P0), goat manure (P1), chicken manure (P2) and cow manure (P3) with a dose of 200 gr on each pot. In total 12 combinations of treatments were obtained. Every combination treatment was repeated three times, then the total number of samples obtained was 36 units. The variables observed in this study were plant height, branch number, date of flowering, flower weight, root volume and root length. The data were tested using the F test with significance level of 5%. If significant then continued by Duncan Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Plant height
Treatment of shade does not provide a remarkable difference on plant height, while the treatment of manure that nested in the shade effect provided significant difference on plant height. Based on the data table (Table 1), it can be seen that the combination of goat manure and without shade gave the highest plant height with an average of 178.67 cm and increase in plant height by 22% when compared to treatment without fertilizer. Goat manure fertilizer also gave an average of the highest plants with significant results at the level of shade 50% and 75%. At the level of 50% shade highest yield was 177.33 cm with an increase in plant height by 35% of the treatment without fertilizer and at the level of 75% shade, the plant height was 181.33 cm and a high increase of 34% compared to without fertilizer treatment. Manure fertilizer on the cultivation of *A. annua* L. aims to fulfill essential nutrient needed. With sufficient soil nutrients then productivity of biomass will increase. Goat manure containing 1.19% N, 0.92% P and 1.58% K₂O. From the various elements, nutrient elements nitrogen (N) is the most influential element on plant growth, especially vegetative growth [6]. Manure has a nitrogen content that serves to form amino acids that are the result of protein degradation and function to form growth hormone [7]. Cell division that affects the growth of plant height depends on the supply of carbohydrates resulting from photosynthesis process involving chlorophyll and nitrogen elements play a role in chlorophyll formation [8].
3.2. **Number of Branch**

The shade treatment does not give significant difference to the number of branches, whereas the treatment of manure fertilizer nested in the shade gave a very significant difference to the number of branches, presented in table 1. Based on the table it can be seen that in the treatment without shade, the largest number of branches produced in the treatment of chicken manure fertilizer with branch number of 53.67 and able to increase the number of branches as much as 26.7% compared to without fertilizer. Goat manure fertilizer produces the highest number of branches in the shade treatment of 50% with the number of branches as much as 55.67 and increasing the number of branches by 37.5%. Goat manure fertilizer also give the largest number of branch in the shade 75% with the number of branches 52 and 33% increase in the number of branches than the treatment without fertilizer. Increased vegetative growth includes plant height, number of leaves and number of branches is strongly influenced by the presence of nitrogen (N), Phosphor (P) and Potassium (K) [9]. Increased macro nutrients, especially nitrogen, will provide an increase in vegetative growth. Nitrogen serves as a builder of chlorophyll, proteins, fats and enzymes in cells that stimulate the growth of stems, leaves and branches. Chicken manure produces the largest number of branches on each shade level. Chicken manure has the most nitrogen content than other types of manure [10]. At 50% shade level and 75% goat manure also gives a significant effect with the highest value both on the variable height of the plant and also the variable number of branches. This is because goat fertilizer has more nutrient variant and optimal amount compared to chicken or cow manure [11], so it will give the best effect on the number of branches.

3.3 **Days of Flowering**

Treatment of shade does not give a significant difference to the days of flowering, as well as treatment of manure fertilizer that is nested in the shade does not provide a real difference to the life of flowering. From the results obtained, the analysis variance calculation showed that none of the treatments give significant result on days of flowering. However, when seen in Table 1, the average age of flowering in the shade of 75% was slightly higher than the other shade level even though no significant difference in the analysis of variance calculation. At the days of flowering table shows the average days of flowering ranges on day 70 until day 95. These results are consistent with the results of Firdaus [12] which showed that A. annua L. grown in the lowlands will flowering at the age of 2-3 months. A. annua L. is a plant that in its natural habitat, these plants will flowering at day 190 to day 200 [5]. Artemisia itself is a short-day plant that will became flowering when the sun shines shorter than the critical exposure period. With their natural habitat located in a sub-tropical climate that has long photoperiodism > 13 hours/day, this duration of radiation can cause the time the emergence of flower in A. annua L. will be longer, so that harvesting will also be longer. Therefore, when the planting is done in areas with radiation are short (<13 hours/day) this plant will quickly form a flower [13].
3.4. Flower Weight
Flowers on *A. annua* L. have components such as antioxidants, anti-microbial, anti-fungal and artemisinin content. These chemical components are affected by harvest age, fertilizer and soil pH, geographical location, plant clones and extraction methods [14]. According to [15] the concentration of artemisinin reaches its peak when flowering plants bloom entirely. The shade treatment does not give any significant difference to the weight of the flower, while the treatment of manure fertilizer nested in shade gave a very significant difference to flowering age, presented in table 2. Based on the table it can be seen that at the highest shade level the highest observation of flower weight is the treatment of goat manure with the weight of 14.18 gram and increase the average weight of flower by 35% compared to the treatment without fertilizer. At 50% shade, the highest yield weight on goat manure treatment with the weight of 9.06 gram and 50% increase compared to the treatment without fertilizer. Goat manure also has the highest yield at 75% shade level with weight 10.40 grams and raising the flower weight by 62% compared to the treatment without fertilizer. Treatment of manure fertilizer gives a very significant effect for the flower weight. This shows that plants need nutrients to form generative organs (flowers). It is also supported by Ahmad et al. [16] which states that the application of nitrogen (N) and phosphor (P) elements contained in the fertilizer gives positive results on flower weight and flower blooming. Nitrogen element is the most needed element in plant growth. In general, nitrogen absorbed by plants is used to form chlorophyll that makes the plants green and amino acids that play a role in cell division and plant development. Phosphor is very important when the plant enters the generative phase. The presence of a phosphorus element is used in the transfer of energy for all plant metabolism, so in addition to stimulate the plant growth, phosphorus is useful to spur the formation of flowers and fruit maturation, so the percentage of flower formation and fruit ripening will increase [17].

3.5. Root Volume
Root is a part of Artemisia for synthesized artemisinin. [18] revealed in artemisia’s root hair are contained artemisinin could reach up to 0.4%. Shade treatment does not give a real difference to the volume of roots, while the treatment of manure fertilizer that is nested within the shades provides very real difference to the volume of roots, presented in (Table 2). Based on the table it can be seen that the manure fertilizer can increase root volume significantly, especially goat manure. Goat manure fertilizer got the highest volume results at every level of shade. At without shade level, goat manure fertilizer application is able to produce root volume amounted to 34.67 ml and improve root volume by an average of 67% than treatment without fertilizer. In the shade level of 50%, the goat manure fertilizer treatment had the highest root volume results amounted to 30.67 ml and an average increase root volume by 43% compared to the treatment without fertilizer. In the shade level of 75%, the goat manure fertilizer

| Table 1. Table of average plant height, number of branches and day of blooming of *A. annua* L. on treatment of shade and manure fertilizer |
|-------------|-------------|-------------|-------------|
| Shade (N)   | Manure Fertilizer (P) | Plant Height (cm) | Number of Branch | Day of flowering (days) |
| Without Shade (N0) | Without Fertilizer (P0) | 145.00±1.70⁹ | 42.33±6.40⁹ | 79.33±5.80⁹ |
|              | Goat (P1) | 178.67±7.80abc | 50.67±4.00abc | 85.33±16.60⁹ |
|              | Chicken (P2) | 169.67±15.50abc | 53.67±8.50abc | 73.67±2.00⁹ |
|              | Cow (P3) | 160.00±5.20ab | 43.00±6.60ab | 86.00±14.00⁹ |
| Shade 50% (N1) | Without Fertilizer (P0) | 131.67±24.08ab | 40.00±12.50ab | 90.00±12.10⁹ |
|              | Goat (P1) | 177.33±6.60⁹ | 55.67±3.20bc | 82.00±14.00⁹ |
|              | Chicken (P2) | 157.00±6.50abc | 45.67±6.70abc | 86.67±16.10⁹ |
|              | Cow (P3) | 128.33±27.20ab | 42.00±5.20ab | 73.00±17.00⁹ |
| Shade 75% (N2) | Without Fertilizer (P0) | 135.57±25.10ab | 39.00±2.60ab | 90.00±15.60⁹ |
|              | Goat (P1) | 181.33±9.60⁹ | 52.00±5.50bc | 85.33±12.90⁹ |
|              | Chicken (P2) | 164.67±19.60abc | 49.00±4.90abc | 94.00±19.70⁹ |
|              | Cow (P3) | 112.00±10.40⁹ | 35.00±2.00⁹ | 95.33±18.60⁹ |

Description: The numbers followed by different letters in each shade levels and variable show different significant values (P<0.05) on duncan multiple range test.
treatment had the highest root volume amounted to 37.33 ml and an average increase root volume by 85% compared to treatment without fertilizer. Manure contains nutrients and organic matter which is good for the soil. The organic material is this which is the source of nitrogen, phosphorus and sulfur for plant growth [19]. The addition of nitrogen does not only affect the plant biomass but also on root growth [20]. Nutrients are not the only factor that determines the root volume. Volume roots other than influenced by the level of root distribution and the availability of nutrients, are also affected by the water content in the soil. Organic materials other than as a primary source of nutrients establishment also serves as a binder of water in the soil. Manure fertilizer input into the soil, will improving soil organic matter content so that the water stored in the soil also increased. The availability of sufficient water in the soil will affect the optimal nutrient absorption by the roots. The roots will respond when the plants are in condition of water shortage by reducing transpiration rate conserve water [21]. The longer transpiration occurs without water supply from the soil will lead to reduce sized of plant roots. Position of roots spread out and supported by sufficient soil nutrient availability will increase the volume of roots.

3.6. Root Length

Treatment of shade does not give a real difference to the length of the roots, as well as treatment of manure that is nested within the shades provides very real difference to the length of the roots. Based on table 2, the treatment of manure fertilizer can be seen that the highest yield observations of root length is on chicken manure fertilizer. Chicken manure fertilizer give root length results highest at all levels of shade treatment although based on the statistical calculation influence of manure that is nested in the shade does not provide a real different on the length of the root as well as the treatment level of shade, so that the variable length of the root is not followed by DMRT, Excellence manure is able to improve soil structure and restore the soil ecosystem that increases the activity of microorganisms in the soil. According to [22], the increased of microorganisms activity in the soil will lead to increased availability of nutrient elements from the activity of bacterial binding nitrogen [23] and also from the organic material was decomposed to available nutrient elements to plants by the activity soil microorganisms, this affects root development which then allows increased absorption of nutrient elements. In line with the [24] which States, land that had a lot of activity of the organism has a porous soil that much that can facilitate the growth of the roots penetrate the ground through soil pores that can absorb water and nutrient elements are dissolved.

Table 2. Table of average flower weight, root volume and root length of A. annua L. on treatment of shade and manure fertilizer.

| Treatment | Manure Fertilizer (P) | Flower weight (gram) | Root volume (ml) | Root length (cm) |
|-----------|-----------------------|----------------------|-----------------|------------------|
| Without Shade (N0) | | | | |
| Goat (P1) | Without Fertilizer (P0) | 10.45±5.1abc | 20.00±9.1a | 22.80±0.8a |
| Chicken (P2) | | 14.18±1.8abc | 34.67±4.1c | 25.43±5.1a |
| Cow (P3) | | 11.09±2.6abc | 29.33±5.1abc | 26.40±1.0a |
| Shade 50% (N1) | | 8.18±0.8abc | 21.67±6.6abc | 24.03±0.9a |
| Goat (P1) | Without Fertilizer (P0) | 6.03±1.2abc | 21.00±6.2abc | 21.20±3.4a |
| Chicken (P2) | | 9.06±0.6abc | 30.67±4.0abc | 21.03±3.3a |
| Cow (P3) | | 8.43±0.3abc | 29.67±4.7abc | 26.00±3.1a |
| Shade 75% (N2) | | 5.72±1.6abc | 20.33±1.5abc | 23.40±4.7a |
| Goat (P1) | Without Fertilizer (P0) | 6.42±1.8abc | 20.33±3.2abc | 21.60±4.6a |
| Chicken (P2) | | 10.40±0.6abc | 37.33±5.5abc | 25.50±1.6a |
| Cow (P3) | | 10.03±2.2abc | 31.67±4.6abc | 26.63±2.1a |
| | | 7.32±0.5abc | 22.67±2.5abc | 20.10±4.9a |

Description: The numbers followed by different letters in each shade levels and variable show different significant values on duncan multiple range test.
4. Conclusions
The conclusion of the study are the various shade as main factor showed that there were no difference in growth within 50% shade, 75% shade as well as without shade treatment. Treatment of goat manure fertilizer as a sub factor nested in 3 levels of shades overall gave the highest results in the variable plant height, number of branches, flower weight and root volume.

Acknowledgement
This research was supported by the Ministry of Research Technology and Higher Education and Dr. Ir. Yuli Widyastuti as field from Research Center for Medicinal Plant and Traditional Drug, Tawangmangu, Indonesia. Artemisia annua seeds in this research were obtained from B2P2TOOT, Tawangmangu, Karanganyar.

References
[1] Ferreira JFS 2004 Artemisia annua L.: The Hope Against Malaria and Cancer. Medicinal and Aromatic Plants: Production, Business & Applications Proceedings of the Jan 15-17/2004 meeting. Mountain State University, Beckley, WV.
[2] Kardinan A 2008 Artemisia (Artemisia annua) Tanaman Anti Malaria Warta Penelitian dan Pengembangan Tanaman Industri Vol. 14 No. 2. Badan Penelitian dan Penelitian dan Pengembangan Pertanian.
[3] Klayman DL 1985 Qinghaosu (Artemisinin): An Antimalarial Drug From China. Science Vol 228.
[4] Bjorkman A, Bhattarai A 2005 Public Health Impact of Drug Resistant Plasmodium falciparum Malaria. Acta Tropica 94 (2005) 163-169.
[5] WHO (World Health Organization) 2006 WHO Monograph On Good Agricultural and Collection Practices (GACP) For Artemisia annua L.
[6] Hikmah 2008 Pemberian Beberapa Bahan Organik Pada Budidaya Tumpang Sari Tanaman Brokoli (Brassica oleracea) dan Petai (Brassica pekinensis) Serta Pengaruhnya Terhadap Pertumbuhan dan Serapan Cu dan Zn. Skripsi. Institut Pertanian Bogor. Bogor.
[7] Dewi WW 2016 Respon Dosis Pupuk Kandang Kambing Terhadap Pertumbuhan Dan Hasil Tanaman Mentimun (Cucumis Sativus L.) Varietas Hibrida. Journal Viabel Pertanian 10(2) 11-29.
[8] Dapoiny L, Fleury A, Robin P 1997 Relation between relative growth rate and nitrogen content during growth of lettuce (Lactuca sativa L.) effect of radiation and temperature Agronomie 17(1):13-41.
[9] Hertos M 2015 Pengaruh Pemberian Pupuk Kandang Kotoran Ayam Dan Pupuk NPK Mutiara Yaramila Terhadap Pertumbuhan Dan Hasil Tanaman Terung (Solanum melongena L.) Pada Tanah Berpasir. Anterior Jurnal, Volume 14 Nomor 2, Juni 2015, Hal 147-153.
[10] Lingga P 1991 Jenis dan Kandungan Hara pada Beberapa Kotoran Ternak. Pusat Pelatihan Pertanian dan Pedesaan Swadaya (P4S) ANTANAN Bogor.
[11] Nursharti DF 2009 Pengaruh Pemberian Pupuk Organik Terhadap Pertumbuhan dan Hasil Tanaman Sawi Caisim (Brassica Juncea L.) Jurnal Agronobis, Vol. 1, No. 1, Maret 2009.
[12] Firdaus R, Muhamad S, Endang GL 2009 Evaluasi Daya Hasil Artemisia (Artemisia annua L.) Hasil Mutasi Di Dataran Tinggi, Dataran Sedang dan Dataran Rendah Makalah Seminar Departemen Agronomi Dan Hortikultura Fakultas Pertanian Institut Pertanian Bogor.
[13] Gusmaini dan Hera N 2007 Potensi Pengembangan Budidaya Artemisia annua L. di Indonesia. Jurnal Perspektif Vol. 6 No. 2 Desember 2007 Hal 57 – 67.
[14] Bilia AR, Francesca S, Cristiano S, Maria CB and Rosa D 2014 Essential Oil Of Artemisia annua L.: An Extraordinary Component with Numerous Antimicrobial Properties. Evidence-Based Complementary and Alternative Medicine Volume 2014.
[15] Ferreira JFS, JC Laughlin, N Delabays and PM Magalhaes 2005 Cultivation and Genetics Of Artemisia annua L. for Increased Production of the Antimalarial Artemisinin. Plant Genetic Resources 3(2): 206 – 229.

[16] Ahmad I, Asif M, Amjad A, Ahmad S 2010 Fertilization Enhances Growth, Yield and Xanthophyll Contents of Marigold. Research Article Turk J Agric For 35 (2011) 641 - 648.

[17] Rina D 2015 Manfaat Unsur N, P dan K Bagi Tanaman. Balai Litbang Pertanian Kalimantan Timur. Kementrian Pertanian Republik Indonesia.

[18] Patra N, Srivastava AK and Sharma S 2013 Study Of Various Factors for Enhancement Of Artemisinin in Artemisia annua Hairy Roots. International Journal Of Chemical Engineering and Applications, Vol. 4, No. 3, June 2013.

[19] Mujiyati dan Supriyadi 2009 Pengaruh Pupuk Kandang dan NPK terhadap Populasi Bakteri Azotobacter dan Azospirillum dalam Tanah pada Budidaya Cabai (Capsicum annum).

[20] Wang ZR, Rui YK, Shen JB and Zhang FS 2008 Effects Of Fertilizer On Root Growth in Zea mays L. Seedlings. Spanish Journal Of Agricultural Research 2008 6(4), 677 - 682.

[21] Nio SA dan Torey P 2013 Karakter Morfologi Akar Sebagai Indikator Kekurangan Air Pada Tanaman. Jurnal Bioslogos Vol. 3 No. 1. Februari 2013.

[22] Dyan MSP 2006 Pengaruh Jenis Media terhadap Pertumbuhan Begonia imperialis dan Begonia ‘Bethlehem Star’. Biodiversitas Volume 7, Nomor 2, Hal: 168-170.

[23] Coyne MS and Mikkelsen R 2015 Soil Microorganism Contribute to Plant Nutrition and Root Health. Better Crops Journal Vol. 99. No.1.

[24] Kroon HD, Hendriks M, Van Ruijven J, Ravenek J, Padilla FM, Jongejans E, Visser EJW and Mommer L 2012 Root Responses to Nutrients and Soil Biota: Drivers of Species Coexistence and Ecosystem Productivity. Journal of Ecology Vol 100 : 6-15.