Response of sow thistle to the level of shading and mycorrhiza

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Abstract. Perennial sow thistle (Sonchus arvensis L.) is one of the Indonesian medicinal plants that contain flavonoid compounds. The research aimed to examine the effects of shading and mycorrhiza application toward the growth, fresh and dry weight of shoot, chlorophyll, nitrogen, phosphorus, potassium, and quercetin content of S. arvensis. The research was conducted in the greenhouse of the Soil Department, Faculty of Agriculture, Gadjah Mada University, Yogyakarta. The experimental design used was a split-plot design, with shading level as the main plot and mycorrhiza application as the subplot. Shading treatment comprised four levels: 0%, 25%, 40%, and 75%, whether subplot comprised two levels: control and with mycorrhizal inoculation. The result showed that shading significantly affected plant growth, fresh and dry weight of shoot, and chlorophyll content. Without shading levels (level 0%) resulted in the highest plant growth, nutrient uptake and yield. The shading level of 0% with AMF application resulted in the highest yield of quercetin. The chlorophyll a, b, and total chlorophyll content was not significantly different at 0%, 25%, and 40% shading levels but decreased significantly at 75% shade levels.

Keywords: Sonchus arvensis L., shading, mycorrhiza, yield, quercetin

1. Introduction

Sow thistle (Sonchus arvensis L.) is a perennial herbaceous plant belonging to the Asteraceae family coming from Eurasia. This plant has a wide adaptation, grows wild in the exposed or slightly protected areas, and the slightly damp soil, like the edge of the ditch, roadside, between the stones, cliffs, and sloping walls with an altitude of 0-1650 m asl., 2000-3000 mm/year rainfall, 75-95% air humidity, and a temperature of 20-30°C [1].

Generally, sow thistle is used as a vegetable and traditional medicine in Indonesia. There have been seven formulas of Scientific Jamu made of a mixture of various preselected crude drugs found in conventional jamu. S. arvensis is one of the local 30 medicinal plants in the research and development program of traditional medicines from raw materials supported by the Indonesian Ministry of Health [2]. S arvensis content flavonoids which are efficacious as lipotropic (kidney stone abolish) and diuretic (urine accelerate) [3]. The plant's ethanol extract compounds are mostly phenolic compounds, and flavonoids such as kaemferol, quercetin, orientin, rutin, hyperoside, catechin, and myricetin act as antioxidants [4].

The need of sow thistle simplicia as a raw material for the Indonesian traditional medicine industry in seven herbal jamu industries in 2007 was 94 tons [5], and tends to increase. The raw material demand, around 90%, was obtained by exploitation or harvesting directly from plants that grow wild, forests, and
yards that a planned and integrated system has not cultivated. This condition might affect the raw material availability and the variation of quality and bioactive content of Sonchus [6].

Each plant has a different light requirement for living in its natural habitat. Sow thistle has a reasonably wide adaptation, often growing in areas with varying light intensity and growth rates. It is necessary to know the optimal environmental conditions for optimum growth and yield of sow thistle. Shading intensity significantly affected plant height, bud number, leaf dry weight, and stem dry weight of sow thistle [7].

Arbuscular mycorrhizal fungi (AMF) is soil fungi that forms the mutualistic symbiosis with plant roots, by developing external mycelium around the infected plant roots, which increased rhizospheric soil volume about 12 to 15 times per cm$^3$ infected root. The principal function of AMF is to increase the soil volume explored for nutrient uptake and enhance the efficiency of nutrient absorption from the soil solution [7]. Application of several types of AMF on $S$. arvensis could increase the shoot's fresh and dry weight and the quercetin content of plant leaf [8].

The research aimed to examine the effects of shading and mycorrhiza application toward the growth, fresh and dry weight of shoot, chlorophyll, nitrogen, phosphorus, potassium, and quercetin content of sow thistle.

2. Methodology
The greenhouse experiment was conducted at Gajah Mada University, Yogyakarta, Indonesia, for 5 months, and harvesting was carried out at 14 weeks after planting (WAP). Soil analysis was conducted at ISMECRI laboratory. Soil growth media had the characteristic: neutral pH (soil pH 7.74), medium C$_{organic}$ (2.07%), medium N$_{total}$ (0.26%), high available P (320 ppm), hight exchangeable Ca, Mg, K, Na (24.26 Ca, 4.66 Mg, 2.62 K and 2.22 Na cmol/kg), and low CEC (16.11 cmol/kg).

Perennial sow thistle seedlings (Manoko accession) were sown for one month and transferred to a polybag (10×15 cm) containing media (soil: manure = 3:1) in the nursery for one month. They were transferred to polybag (5 kg volume), containing soil (sterilized by 40 g/m$^2$ of Basamid soil fumigant) and 1 kg cow dung.

The mixed crude inoculum of AMF ($Glomus$ sp., $Acaulospora$ sp., $Gigaspora$ sp.) was multiplicated using sterilized media of zeolite and soil (1:1). Sorghum infected by AMF, as the host plant, were grown for three months and fertilized with Hyponex nutrient source (low P). The soil was air-dried for two weeks before harvesting time. The inoculum of AMF was obtained from hypha, spores, sorghum roots, and soil that contained spores of AMF.

The trial was arranged in split-plot design, repeated three times. The main plot was shading level, and the subplot was mycorrhiza application. Shading treatment comprised four levels: 0%, 25%, 40%, and 75%, whether subplot comprised two levels: control and 200 spores of AMF inoculated simultaneously with planting time. The number of plants per treatment per repetition was ten plants, and the total population was 240 plants.

The quantitative data of growth parameter (leaves number, length and width of leaf, and diameter of plant canopy) were measured at 10 and 14 WAP. The leaf area (measured by Leaf Area Meter), fresh and dry weight of perennial sow thistle shoot were measured at the first and second harvest (10 and 14 WAP), whereas the percentage of AMF colonization in perennial sow thistle root, nitrogen (N), phosphor (P), and potassium (K) content of leaves, and flavonoid content of leaves, were measured at the second harvest (14 WAP).

Mycorrhizal root colonization was determined by Phyllip and Hayman's (1970) method in [9], using tryphan blue staining solution. N, P, and K nutrients content of leaves were analyzed using the Kjeldahl, spectrophotometer, and AAS method [10]. Furthermore, this macro-nutrient content was calculated for nutrient uptake transported during harvesting. The content of flavonoids (quercetin) in perennial sow thistle leaf was extracted and analyzed with acetone and 25% of HCl, and determined using spectrophotometry.
Microclimate in the greenhouse included light intensity, air humidity and air temperature were measured during the research.

The statistical analysis were carried out by analysis of variance (ANOVA). Significant differences between the means of the treatments were determined by Duncan multiple range tests (DMRT) at α 5% (p<0.05).

## 3. Result and discussions
### 3.1. Microclimate of experiment location
The microclimate in the greenhouse and under the paranet with different levels of shading was presented in Table 1. The higher shading provided, the lower the light intensity and the higher the measured air humidity. The temperature is relatively stable at various levels of shading.

| Shading | Morning | Afternoon |
|---------|---------|-----------|
|         | Light Intensity (lux) | Relative Humidity (%) | Temperature (°C) | Light Intensity (lux) | Relative Humidity (%) | Temperature (°C) |
| P1(0%)  | 12025   | 51.5      | 29.24       | 11290   | 51.5      | 28.55       |
| P2 (25%)| 9165    | 61.5      | 28.99       | 4495    | 57.5      | 28.15       |
| P3 (40%)| 8610    | 67.0      | 28.78       | 5000    | 61.0      | 28.11       |
| P4 (75%)| 1075    | 75.0      | 28.07       | 920     | 76.5      | 28.01       |

### 3.2. Growth of sow thistle

#### Table 2. Effect of shading and mycorrhiza on the growth parameter of sow thistle at 10 WAP.

| Treatment   | Leaves number | Leaf length (cm) | Leaf width (cm) | Leaf area (cm²) | Canopy diameter (cm) |
|-------------|---------------|------------------|-----------------|-----------------|----------------------|
| Shading     |               |                  |                 |                 |                      |
| 0%          | 20.67 a       | 21.39 a          | 5.16 a          | 604.69 a        | 32.66 a              |
| 25%         | 18.90 a       | 21.91 a          | 5.03 a          | 247.33 b        | 31.07 a              |
| 40%         | 19.13 a       | 21.38 a          | 5.03 a          | 223.81 b        | 30.06 a              |
| 75%         | 4.40 b        | 11.86 b          | 4.18 a          | 56.89 c         | 19.79 b              |
| Mycorrhiza  |               |                  |                 |                 |                      |
| control     | 15.70 a       | 20.22 a          | 4.95 a          | 292.73 a        | 29.68 a              |
| Mycorrhiza  | 15.65 a       | 18.06 b          | 4.76 a          | 273.64 a        | 27.12 a              |
| CV          | 5.72          | 8.25             | 11.85           | 12.41           | 11.99                |

Note: The numbers followed by the same letters of each treatment column are not significantly different based on Duncan test of 5 % level

#### Table 3. Effect of shading and mycorrhiza on the growth parameter of sow thistle at 14 WAP.

| Treatment   | Leaves number | Leaf length (cm) | Leaf width (cm) | Leaf area (cm²) | Canopy diameter (cm) |
|-------------|---------------|------------------|-----------------|-----------------|----------------------|
| Shading     |               |                  |                 |                 |                      |
| 0%          | 33.80 a       | 31.18 a          | 7.30 a          | 1267.3 a        | 53.07 a              |
| 25%         | 17.73 b       | 27.26 a          | 5.93 a          | 962.1 ab        | 45.92 b              |
| 40%         | 17.53 b       | 26.87 a          | 6.25 a          | 550.7 bc        | 44.38 b              |

CV
Shading treatment affected the growth and development of the sow thistle. The level of shading significantly affected the number of leaves, leaf length, leaf area, and diameter of the canopy at 10 WAP. In comparison, at 14 WAP, the level of shading significantly affected all the growth parameters of the sonchus plant (Table 2 and 3). The best plant growth was shown by 0% of shading (without shading). Plant growth parameters at the level of 25% and 40% shading treatment had no significant difference. The microclimate conditions in that two-level of shading were almost the same (Table 1). [12] found that the application of shading up to 30% still showed the normal growth parameters of sow thistle (canopy width, number of leaves, and plant height) and was not significantly different from without shading treatment.

The 75% shading level had the lowest number of leaves, leaf length, leaf area, and canopy diameter compared to 0%, 25%, and 40% shading treatments. The 75% shading level had the highest panet density compared to other treatments, so the least amount of sunlight was captured by plant leaves. [13] also found the significant reduction in Shorea sp. plant growth by shading including leaf area growth. Lack of light to the plant causes disruption of the photosynthesis process and plant growth. The auxin as a growth hormone will decrease and cannot maximize its function for plant cells development.

Shading treatments of 0%, 25%, and 40% showed an increase in growth parameters (leaf length, leaf width and canopy diameter) with increasing plant age, while at 75%, shading showed the opposite condition (Table 2 and 3). The growth and development of sow thistle were inhibited. At 14 WAP, most of the plants died, physically stunted. The leaves were few, thin, and pale in color—lack of sunlight due to higher shading level, resulting in less plant photosynthate. The plant will terminate the old leaves to adjust the stress condition to meet the photosynthate requirement of the plant. This is directly related to the intensity, quality, and duration of light radiation received by plants for the plant growth process (Daniel et al. (1992) cit. [13]).

The AMF application was not significantly affected the growth of sow thistle plants. It could be due to the dominant effect of shading more on plant growth and the good soil fertility conditions of the media, which had relatively high macronutrient content.

3.3. Yield parameter of sow thistle
The level of shading treatment significantly affected the fresh and dry weights of shoot and root of sow thistle plants at 10 WAP and 14 WAP (Table 4 and 5). At the same time, the AMF application did not significantly affect the shoot and root weights, although, at 10 WAP shoot fresh weights were significantly higher than control. Without shading (0%) produced the highest fresh and dry weight of sow thistle shoot and root, whether the 75% shading level resulted in the lowest. At 75% shading level, the fresh and dry weight of sow thistle sharply decline. The higher the shading density, the lower the light intensity received by the sow thistle plant. The other study reported that in two-month-old plants, the 20% shading level produced the highest leaf fresh weight (65,88 g/plant) compared to the open area, 10% and 30% shading levels; moreover, the yield decreased at 30% shading level [13]. Sow thistle could grow well in an open area to 10-20% shading level, and at 30% shading level, it started to reduce yield [1]. The lower weight value described the smaller amount of photosynthate produced by the plant. Plants that receive lower light intensity (shaded) up to a certain level of shading had no optimal yield. Plants grow in low light intensity
environments, commonly have smaller root, few in root number and are composed of thin-walled cells due to the inhibition of photosynthate to the roots [14]. The results of photosynthesis process will depend on the plant optimum light intensity requirement. Every plant leaf must produce sufficient energy utilized for photosynthesis and respiration activity.

Table 4. The fresh and dry weight of sow thistle shoot and root (g/plant) at 10 WAP.

| Treatment | Shoot fresh weight | Shoot dry weight | Root fresh weight | Root dry weight |
|-----------|--------------------|------------------|-------------------|-----------------|
| Shading   |                    |                  |                   |                 |
| 0%        | 37.67 a            | 1.98 a           | 11.83 a           | 1.06 a          |
| 25%       | 14.38 a            | 1.67 a           | 10.87 a           | 0.75 ab         |
| 40%       | 16.13 a            | 1.51 a           | 7.22 a            | 0.56 b          |
| 75%       | 2.78 b             | 0.58 b           | 1.97 b            | 0.11c           |
| Mycorrhiza|                    |                  |                   |                 |
| control   | 14.95 b            | 1.54 a           | 8.49 a            | 0.69 a          |
| Mycorrhiza| 20.53 a            | 1.33 a           | 7.45 a            | 0.54 a          |
| CV        | 12.82              | 29.96            | 18.68             | 23.24           |

Note: The numbers followed by the same letters of each treatment column are not significantly different based on Duncan test of 5 % level.

Table 5. The fresh and dry weight of sow thistle shoot and root (g/plant) at 14 WAP.

| Treatment | Shoot fresh weight | Shoot dry weight | Root fresh weight | Root dry weight |
|-----------|--------------------|------------------|-------------------|-----------------|
| Shading   |                    |                  |                   |                 |
| 0%        | 83.38 a            | 10.48 a          | 27.64 a           | 4.86 a          |
| 25%       | 53.00 ab           | 5.98 ab          | 20.19 a           | 2.27 ab         |
| 40%       | 27.57 b            | 3.46 b           | 17.93 a           | 2.29 ab         |
| 75%       | 1.07 c             | 0.50 c           | 1.27 b            | 0.20 b          |
| Mycorrhiza|                    |                  |                   |                 |
| control   | 40.60 a            | 5.10 a           | 15.47 a           | 2.56 a          |
| Mycorrhiza| 41.91 a            | 5.10 a           | 18.04 a           | 2.25 a          |
| CV        | 14.69              | 21.41            | 33.07             | 23.24           |

Note: The numbers followed by the same letters of each treatment column are not significantly different based on Duncan test of 5 % level.

Table 6. Chlorophyll content of sow thistle on shading and mycorrhiza treatments.

| Treatment | Chlorophyll (mg/gfw) |         |         |         |         |
|-----------|----------------------|---------|---------|---------|---------|
|           | a   | b   | Total   | Ratio a/b |
| Shading   |      |     |         |          |
| 0%        | 0.2438 a   | 0.3093 a | 0.5530 a | 0.79     |
| 25%       | 0.2407 a   | 0.2915 a | 0.5320 a | 0.83     |
| 40%       | 0.2285 a   | 0.2832 a | 0.5115 a | 0.81     |
Chlorophyll is the primary substance in chloroplasts, a central component of photosynthesis, a green pigment, in all photosynthetic plant tissues. Chlorophyll functions directly in converting light energy to chemical energy and absorbing and transporting energy to the molecular reaction center. Chlorophyll b functions as an absorber of light energy, which will be forwarded to chlorophyll a [15]. Increasing the shading level up to 40% had no significant effect on reducing the content of chlorophyll a, b, and total chlorophyll (Table 6). However, the 75% shading level significantly reduced the content of chlorophyll a, b, and total chlorophyll of sow thistle leaves. In the open expose area, the leaves of shaded plants have a higher ratio of chlorophyll a:b than leaves that are not shaded. This is a physiological response so that the leaves can absorb more long-wave radiation for photosynthesis [16; 17].

### Table 7. The N, P, K content and uptake of sow thistle at harvested (14 WAP)

| Shading | N  | P  | K  | N (mg/plant) | P (mg/plant) | K (mg/plant) |
|---------|----|----|----|--------------|--------------|--------------|
| 0%      |    |    |    | 316.37       | 25.95        | 388.22       |
| control | 3.17| 0.26| 3.89|              |              |              |
| AMF     | 3.08| 0.22| 3.45| 337.88       | 24.13        | 378.47       |
| 25%     |    |    |    | 128.95       | 13.13        | 268.05       |
| control | 2.16| 0.22| 4.49|              |              |              |
| AMF     | 3.85| 0.24| 4.61| 230.62       | 14.38        | 276.14       |
| 40%     |    |    |    | 112.05       | 7.52         | 144.76       |
| control | 2.98| 0.20| 3.85|              |              |              |
| AMF     | 2.88| 0.20| 3.71| 90.72        | 6.30         | 116.87       |

Analysis of macronutrient content N, P, K, could only be carried out in the treatment of 0%, 25%, and 40% shading, while the 75% shading was not analyzed because the yield of shoot was in short supply (Table 7). Increased shading might reduced shoot dry weight and NPK uptake. At 25% shading level, mycorrhiza application resulted higher N, P, and K nutrients content than other treatments (Table 8). AMF can facilitate the sources of inorganic and organic nutrients in the soil [18]; [19], by supplying nutrients from unavailable to available, through their external mycelium to plants [9]. Therefore, AMF can increase the uptake of various nutrients for plants, such as P [20], N [21], and K [22].

### 3.4. Content of flavonoid (Quercetin)

Increasing the shading level up to 40% did not reduce quercetin content in the Sonchus leaf sharply. However, when it was adjusted for the dry biomass weight of Sonchus, shading treatment reduced quercetin yield by up to 31 to 62%. At the 0% and 25% shading treatment with AMF inoculation, the quercetin content increased by 9 to 14%, from 86.83 mg to 94.34 mg/plant and 52.12 mg to 64.09 mg/plant (Figure 1 and 2). Application of AMF1 and AMF2 on Manoko accession of sow thistle could increase the fresh and dry weight of shoot and the quercetin content and yield of plant leaf [8].
4. Conclusion
Shading level significantly affected plant growth, fresh and dry weight of shoot, and chlorophyll content. Without shading levels (level 0%) resulted in the highest plant growth, nutrient uptake and yield. The shading level of 0% with AMF application resulted in the highest yield of quercetin. The chlorophyll a, b, and total chlorophyll content was not significantly different at 0%, 25%, and 40% shading levels but decreased significantly at 75% shade levels.

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