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THE EFFECTS OF SOIL DEPTH ON THE GROWTH OF THE CLOVER FERN AND THE USES OF THE CLOVER FERN ON THE GERMINATION OF FERN SPORES

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ABSTRACT: Many native plants have valuable uses that have yet to be discovered. The clover fern is an aquatic fern which could possibly be used as a planting material. The purpose of this study was to determine the effects of soil depth on the growth of the clover fern (*Marsilea crenata* C. Presl.) and also to explore the uses of the clover fern on the germination of fern spores. The soil depths tested were: 5, 10, 15, 20 and 25 cm in planting pots. Observations were made on 3 replications in an RCBD experiment. The mean comparisons were made using Duncan's test at a significance level of 0.05. It was found that the ferns at a soil depth of 15 cm possessed the longest internode length of 7.43 cm. Ferns at a soil depth of 20 cm possessed the longest stalk length of 16.16 cm and ferns at a soil depth of 25 cm showed the widest leaf diameter at 4.00 cm. The clover ferns at a soil depth of 25 cm demonstrated the highest fresh and dry weights at 971.11 g and 146.11 g respectively ($p<0.05$). Uses of these clover ferns on the germination of fern spores were tested along with peat moss. Both germination materials were tested on their own and in a series of mixes. Germination was observed in two of the mix ratios, but that germination rate was low. This study indicates that clover ferns should be studied further for use as a planting material.

Keywords: Clover fern, Water fern, Planting material, Weeds

1. INTRODUCTION

Weeds are defined as native plant species that grow in cultivating fields. Most weeds have an important impact on crop yields [1]. Some of them grow along and compete with the cultivating crops [2] while some clog irrigation networks [3]. In wetland plantations such as rice fields, there are also weed problems. Clover Fern outbreaks have been reported but they are not importance value [4]. The clover fern (*M. crenata* C. Presl) is one of the causes. This fern is known as an aquatic, semi-aquatic fern or water fern [5,6] and has been classified as a weed. It grows well in shallow water such as in a rice field. It is found mainly in Southeast Asia, within rainforest zones with high humidity [7]. The general characteristics of the clover fern are: a rhizome (stem) consisting of nodes and internodes, growing in soil or a muddy surface, having four leaflets (fronds) on the top of a petiole that grows from the node of the rhizome. It has fibrous roots and sporocarps growing from the nodes [8]. The clover fern is an edible wild plant. Some Thais use this fern as a vegetable, and it can be found in many Thai recipes. It is a part of Thai culture [9,10,11].

The clover fern can also be an ornamental plant. It is unique when it is planted in a pot and placed in a garden or a pond [12]. It can also be grown in an aquarium or a shallow water basin [13]. This fern has some medicinal properties as well. Its extract has been used for estrogen therapies [14]. And Thai folk healers in the south of Thailand have long used it for hepatitis and colic treatments [15].

From an environmental point of view, this fern can be used to test the soil for lead contamination. Growth inhibition of the fern was found in relation to increasing amounts of lead in the soil [16].

The research team for this study has been looking for some native plants that can replace peat moss, which is imported. In a nursery environment, it was noticed that the clover fern grew well in clay, in containers with some water, especially in the rainy season. In the dry season, the ferns became withered and died off. They could be harvested, dried, and then used as a planting material. Some other fern species and epiphytic plants can grow from them when they have a suitable moisture content. Drying and cracking of clover ferns produces a material very similar in physical properties to peat moss. Peat moss is popular for sowing seeds and fern spores as well as transplanting some fern seedlings [17]. The stems and petioles of clover ferns consist of aerenchymal tissues which allow for good gas exchange [8,18]. The objectives of this study were to determine

1) the effects of soil depths on clover fern growth and 2) the effects of clover ferns as a germination medium for bird-nest-fern spores.

2. MATERIAL PREPARATION

2.1 Fern stock preparation

Clover fern tips were collected from ferns naturally growing in a rice field. The tips were then planted in clay in large plastic bowls. After two months, the ferns grew and filled each bowl. These ferns were then used as the fern stock for this experiment. Some small clusters of the ferns were collected from the stock with their roots attached in the clay in which they were grown. Each cluster was allowed a 10 x 10 cm clay block with it (fig.1). Five clusters each were then planted in a container containing some clay. The same procedure was applied to other containers. All containers were 60 cm in diameter and 30 cm in height. After planting, some water was added to the top of the containers (fig.2).

Fig.1 Collected clover fern clusters

Fig.2 The experimental clover ferns in the nursery

2.2 Germinating media preparation

Some dried clover ferns were ground and sieved. The ground product was mixed with some peat moss at three different ratios of clover ferns to peat moss: 1:1, 1:2 and 2:1 (table 3). The mixture was put in moisture chambers (6.5 cm in width, 8.5 cm in length and 3 cm in height) and spread evenly. It was then sprayed with some water prior to use.

3. METHOD

3.1 Planting experiment

Some clay was used as the planting soil for the clover ferns in this experiment. It was put in planting containers to achieve five different depths: 5, 10, 15, 20 and 25 cm. The water height was 30 cm. These depths were expected to demonstrate some effects on the growth of clover ferns. The observations were made on 3 replications in a Randomized Complete Block Design (RCBD). The internode lengths, stalk lengths, leaf diameters, fresh weights and dry weights were collected and analyzed. The mean comparisons were made with the Duncan multiple range test at a significance level of 0.05.

3.2 Germinating experiment

The spores from bird-nest ferns (*Asplenium nidus*) were chosen for this experiment. They were sown onto the moistened media prepared earlier at an amount of 2.5 mg per chamber. The chambers were closed and observed for the presence of prothalli.

4. RESULTS

It was found that the clover ferns planted at a depth of 15 cm possessed the longest internode length at 7.43 cm on average. Those planted at a depth of 20 cm demonstrated the longest stalk length at 16.16 cm on average. Those planted at a depth of 25 cm showed a leaf width of 4.00 cm on average. However, there was no significant difference ($p>0.05$). (Table 1, fig. 3-7)

Table 1 The growth of clover ferns planted in clay at different depths over 13 weeks.

Treatment[1]	Growth of Clover Fern ($\bar{x}$/cm)		
	Internode length	Stalk length	Leaf diameter
5cm	6.97	15.00	3.79
10cm	7.26	13.39	3.94
15cm	7.43	14.84	3.91
20cm	7.24	16.16	3.87
25cm	7.16	14.09	4.00
CV%	6.83	13.38	6.18
F test	ns	ns	ns

[1] depth of clay soil

In term of weights, the clover ferns which were planted at a soil depth of 25 cm were the heaviest by fresh weight (971.11 g on average). They also showed the highest dry weight (146.11 g). These figures were significantly different ($p < 0.05$) and the details can be found in the data below. (Table 2, fig. 8-9)

Table 2 The fresh and dry weights of clover ferns at 13 weeks in clay at different depths.

Treatments[1]	Weight of Clover fern ($\bar{x}$/g)	
	Fresh weight/g	Dry weight/g
5cm	524.99 c	66.11 c
10cm	683.88 b	89.99 bc
15cm	798.88 b	118.88 ab
20cm	793.88 b	123.33 ab
25cm	971.11 a	146.11 a
CV%	11.52	2.60
F test	*	*

[1] depth of clay soil, * = significantly different ($p < 0.05$)

The bird-nest fern spores germinated in all chambers with pure peat moss but no germination was found with pure clover fern. Some spores could germinate in the mixed media but the germination rate was low. It was only observed in the chambers containing a mix ratio of clover fern to peat moss of 1:2 and 2:1 respectively. (Table 3, fig. 10-14)

Table 3 The germination of bird-nest fern spores in dried-ground clover fern, peat moss and their mixtures at two months.

Material ratio	Box number					
	1	2	3	4	5	6
C	-	-	-	-	-	-
P	+	+	+	+	+	+
C: P =1:1	-	-	-	-	-	-
C: P =1:2	-	-	+	-	-	-
C: P =2:1	-	+	-	-	-	-

C= clover fern, P= peat moss,
presence of prothalli: + = found, - = not found

Fig.3 The clover ferns at a depth of 5 cm in clay soil

Fig.4 The clover ferns at a depth of 10 cm in clay soil

Fig.5 The clover ferns at a depth of 15 cm in clay soil

Fig.6 The clover ferns at a depth of 20 cm in clay soil

Fig.7 The clover ferns at a depth of 25 cm in clay soil

Fig.8 The fresh clover ferns

Fig.9 The dried clover ferns

Fig.10 The germination of bird-nest fern spores in ground clover fern

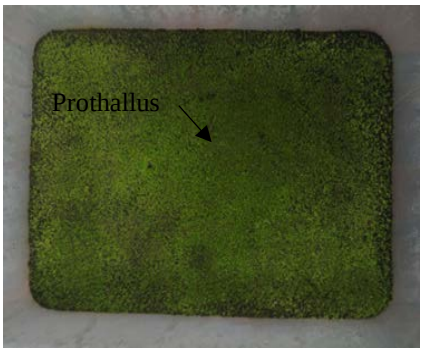

Fig.11 The germination of bird-nest fern spores in peat moss

Fig.12 The germination of bird-nest fern spores in a 1:1 clover fern: peat moss mixture

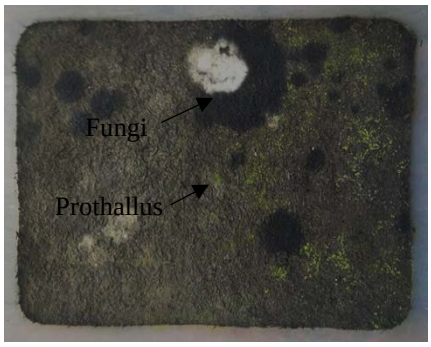

Fig.13 The germination of bird-nest fern spores in a 2:1 clover fern: peat moss mixture

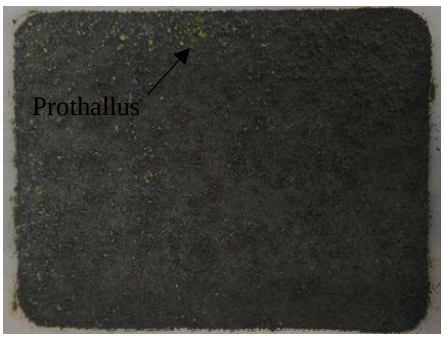

Fig.14 The germination of bird-nest fern spores in a 1:2 clover fern: peat moss mixture

5. CONCLUSIONS

The values of many native plants have not been well studied. This study chose to test the clover fern for its production potential and possible use as a planting material. The study was conducted in a. plant nursery for 13 weeks during the rainy season. On the basis of fresh weight and dry weight, ferns appeared to grow well at a depth of 25 cm in clay soil, and 5 cm in shallow water. They also contained leaves, leaf stalks and roots at the nodes of rhizome. [19].

For use as a germination material, the ground clover fern did not show a positive result with respect to peat moss. Although some bird-nest fern

spores can germinate in a mix ratio of 1:2 and 2:1 clover fern to peat moss, germination was delayed for a few weeks. In contrast, fern spores completed their germination in the first month and the spores develop to be prothallus [20].

The observations made during the experiment noticed that the ground clover fern looked perfectly sound, but it generated some decomposition odor. This may suggest a negative effect in this experiment. It might be caused by some toxins generated during the decomposition process [21] but this will require further study. Clover ferns should be developed for use as agricultural materials because they are a free material from nature. Furthermore, they have also already been shown through research to be commercially viable as edible vegetables, medicine as well as improve quality of irrigation water. for agriculture [22].

6. ACKNOWLEDGMENTS

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