Effect of Combi VPlantex micronutrient fertilizer on the growth, yield and silymarin content in milk thistle seeds (Silybum marianum (L.) Gaertn.) grown in Phu Tho

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Abstract. The study aims to evaluate the impact of supplementing with Combi VPlantex micronutrients on the growth, yield and silymarin content in milk thistle seeds. The experiment consisted of four treatments, arranged in a completely randomized design. The study monitored growth indicators, yield components, yield and silymarin content in milk thistle seeds. The results have shown that the Combi VPlantex micronutrient had a positive impact on the growth, development and yield of the milk thistle grown in Phu Tho. Combi Vplantex fertilizer sprayed at a concentration of 15% yielded outstanding results with an individual seed yield of 12.8 g/plant, an actual seed yield of 393.8 kg/ha and a silymarin content of 2.39%. However, spraying Combi VPlantex at a concentration of 12.5% also yielded results equivalent to those at a concentration of 15% with 95% reliability, indicating that this could be the optimal concentration for practical production of milk thistle to ensure high economic efficiency while still achieving good yield and quality.

Keywords: Combi Vplantex micronutrients, milk thistle, yield

1 Introduction

Micronutrients play a crucial role in maintaining the overall health and vitality of crops. They activate, support enzymes and promote important metabolic reactions occurring in the plant [1]. The presence of micronutrients ensures the efficient functioning of processes such as photosynthesis, respiration and nitrogen metabolism [2]. During the reproductive stage, micronutrients profoundly affect both the quantity and quality of the seeds produced [3]. Applying micronutrients will also increase the absorption of macronutrients in tissues and organs, helping to improve fruit yield and quality [4]. At the same time, micronutrients also play a central role in helping plants increase their resistance to adverse conditions [5, 6, 7].

Although the demand for micronutrients is quite minimal, their deficiency or imbalance can severely affect the growth, development and productivity of crops. Continuous cultivation, especially without proper supplementation, has reduced micronutrients in the soil. To address the deficiency of these nutrients, many techniques have been employed, such as soil fertilization, foliar fertilization and organic fertilization [8, 9, 10, 11]. Among them, foliar fertilization is considered the most effective method, because it helps the absorption and transportation process to quickly reach other parts, improving the health and meeting the micronutrient requirements of the crops [12].

Milk thistle (*Silybum marianum* (L.) Gaertn.) belonging to the Asteraceae family, is a medicinal plant cultivated for its seeds because of their high content of the active compound silymarin. The milk thistle develops a strong root system, can be grown in various types of soil and easily adapts to different cultivation conditions. Currently, milk thistle is widely cultivated around the world for silymarin extraction, with seed yields ranging from 550 - 1680 kg/ha and silymarin content in the seeds reaching 13.3 - 35.4 kg/ha [13, 14, 15].

In Vietnam, milk thistle has been researched and experimentally cultivated in Phu Tho since 2019. However, the seed yield has only reached 325 - 420 kg/ha, which is still much lower than the global average [16, 17, 18, 19]. For enhanced yield, in addition to factors such as variety and cultivation techniques, the milk thistle requires adequate nutrient supply for its growth. Although the impact of macronutrient factors has been studied before, the effect of micronutrients on the yield and quality of milk thistle has not yet been explored.

Combi VPlantex fertilizer is a preparation containing the elements Mg, Cu, Fe, Mo, B, Mn, and Zn, combined with a high EDTA chelate ratio, which helps stabilize and increase the absorption of essential micronutrients for plants. Organic matter (OM 25%, C/N = 12) helps improve soil structure, increase microbial activity, and provide sustainable nutrition.

Currently, the micronutrient fertilizer Combi VPlantex has been applied in practical production for some agricultural crops such as fruit trees and medicinal plants. However, there have been no thorough investigations evaluating the impact of this type of fertilizer on the growth, yield, and silymarin content of milk thistle. This creates a gap in the scientific and practical basis for micronutrient supplementation for milk thistle plants.

This study was conducted to determine the optimal dosage and method of using Combi VPlantex, contributing to improved productivity, quality of medicinal herbs, increased economic efficiency, and the competitive value of the product.

2 Materials and methods

2.1 Materials

The milk thistle seeds were provided by the Institute for Applied Research and Development, Hung Vuong University. The planting season was from August 2024 to May 2025.

Fertilizers used: Urea (46% N), Lam Thao superphosphate (17% P₂O₅), potassium chloride (60% K₂O), Combi VPlantex micronutrient fertilizer produced by Chelate Asia Agricultural Consulting Joint Stock Company (Vietnam) with the following composition: Mg (1.4%), Cu (0.096%), Fe (6.7%), Mo (0.058%), B (0.8%), Mn (1.9%), Zn (0.38%), EDTA chelate (65.4%), organic matter (25%), C/N ratio (12), moisture (27%).

2.2 Methods

Experimental design method

The experiment was arranged in a completely randomized block design, with three replications. The area of one experimental plot was 30 m². The total experimental area was 360 m² (excluding the border and protective strip). Data were collected from 15 plants per plot, with samples taken from 5 diagonal points. The experimental results are the average values from the replications.

The dosage and concentration of Combi VPlantex fertilizer used in the experiment followed the procedure. Combi VPlantex fertilizer was mixed with clean water according to the concentrations of the experimental formulas and sprayed three times. The first spray was applied

when the plants entered the asterisk stage, the second spray was applied 20 days later, and the third spray was applied before the plants flowered. The amount of solution applied to irrigate 450 liters per hectare.

Table 1. Experimental formula

Formula	Treatment method	
Formula 1 (F1 – control)	Spray with clean water	
Formula 2 (F2)	Spray Combi VPlantex fertilizer at a concentration of 10%	
Formula 3 (F3)	Spray Combi VPlantex fertilizer at a concentration of 12.5%	
Formula 4 (F4)	Spray Combi VPlantex fertilizer at a concentration of 15%	

Experimental background

The seeds were soaked in warm water at approximately 54°C for 6 hours, then drained and wrapped in a damp cloth for two days until the seeds cracked and white sprouts appeared, after which were planted in the fields. The planting density was 40,000 plants per hectare, spacing 50 cm × 50 cm.

Fertilization included 4 tons of organic microbial fertilizer + 150 kg of nitrogen (325 kg urea) + 120 kg of P_2O_5 (705 kg superphosphate) + 120 kg of K_2O (200 kg potassium chloride) per hectare.

For basal fertilization, 100% of organic microbial fertilizer and 100% of P_2O_5 were applied, with 25% of nitrogen.

For top-dressing, the first application was 50% nitrogen at the asterisk stage. The second application was 25% nitrogen and 50% potassium at the flowering stage. The third application was 50% potassium at the seed development stage.

Monitoring indicators

At the time of flowering, various growth and yield indicators of the plants were measured. Plant height (cm) was measured from the ground level to the tip of the highest growing branch. Canopy width (cm) was measured at the widest point of the canopy.

The number of lateral bud/plant, the number of flower/plant, flower diameter (cm), number of seeds/flower and the weight of firm seeds/flower were determined as follows: The milk thistle seeds collected from each flower were dried under conditions of approximately 50°C to a moisture content of 10% according to the Vietnamese Pharmacopoeia V (2017) [20]. The total seed weight (g) and the weight of 1,000 seeds (g) were then measured.

Individual seed Seed yield (g) weight/flower (g) × number of flower/plant. Theoretical seed yield (kg/ha) = (individual seed yield (g) × number of plants/unit area)/103. The actual seed yield was determined at a standard moisture content of 10% according to the Vietnamese Pharmacopoeia V [20] and a seed purity of 100% (kg/ha) by weighing the total dry weight of seeds from the experimental plots (kg). The silymarin content in the seeds was determined by high-performance liquid chromatography according to the Vietnamese Pharmacopoeia V [20] at the Center for Drug, Cosmetic, and Food Testing of Vinh Phuc Province.

Data processing methods

The data were analyzed using analysis of variance (ANOVA) with IRRISTAT 5.0 software. The least significant difference (LSD) between treatments for the monitored indicators was calculated at a 95% confidence level (p < 0.05).

3 Results and discussions

3.1 The effect of Combi VPlantex micronutrient fertilizer on the growth ability of milk thistle grown in Phu Tho

Evaluating the impact of Combi VPlantex micronutrient fertilizer on several growth indicators of milk thistle plants, the results are presented in Table 2.

The data in Table 2 show that, compared to the control (F1), the use of Combi VPlantex micronutrient fertilizer has significantly increased plant height at a 95% confidence level. The height of the milk thistle plants gradually increased with the concentration of micronutrient spray from formula F1 (135.8 cm) to formula F4 (156.7 cm). As compared to the control, the effect was more noticeable when the concentration was raised from 12.5% (F3) to 15% (F4). Micronutrients such as Mg and Fe play a crucial role in accelerating the processes of photosynthesis, protein synthesis, and enzyme synthesis, thereby fostering the growth of stems and leaves [21, 22]. Additionally, micronutrients also help enhance the absorption of macronutrients, thereby stimulating plant height development [4].

The canopy width of the plants also showed an increasing trend from F1 (48.4 cm) to

F4 (62.4 cm). The differences between the treatments were statistically significant at a 95% confidence level. This indicates that Combi VPlantex micronutrient fertilizer has a positive effect on the lateral growth and canopy area of the plants. The mechanism of this effect may be attributed to the role of micronutrients such as Zn and B in stimulating cell division and the formation of lateral meristem tissues, helping the plants to develop wider and more balanced canopies [23, 24].

The number of lateral branches also significantly increased with the use of micronutrient fertilizer, from 6.8 branches (F1) to 7.8 branches (F4), with a statistically significant difference (LSD $_{0.5}$ = 0.70 branches). Micronutrients play an important role in the process of bud differentiation and side branch development, particularly elements like B, Zn, and Cu [24, 25, 26]. Therefore, the increase in the number of lateral branches clearly reflects the positive physiological effect of micronutrients on the milk thistle.

The number of flowers in F4 also showed the best results (7.5 flowers), surpassing F1 (6.6 flowers). F2 and F3 showed intermediate values. However, the differences between the formulas were still statistically significant.

Table 2. The effect of Combi VPlantex micronutrient concentration on some growth parameters of milk thistle

Formula	Plant height (cm)	Canopy width (cm)	Number of lateral branches)	Number of flowers (flowers)
F1 (control)	$135.8^{a} \pm 6.90$	$48.4^{a} \pm 3.22$	$6.8^{a} \pm 0.84$	$6.6^{a} \pm 0.71$
F2	$145.3^{ab} \pm 5.49$	$55.2^{b} \pm 3.29$	$7.2^{ab} \pm 0.84$	$6.8^{a} \pm 0.84$
F3	$154.6^{\text{b}} \pm 7.90$	$58.7^{b} \pm 2.58$	$7.6^{b} \pm 0.65$	$7.2^{ab} \pm 0.84$
F4	$156.7^{\text{b}} \pm 7.97$	$62.4^{\circ} \pm 3.65$	$7.8^{b} \pm 0.69$	$7.5^{\rm b} \pm 0.84$
LSD0.5	14.5	5.6	0.70	0.65
CV%	4.9	5.0	4.8	4.7

^{*} Different letters (a, b, c) in the same column indicate significant differences at p < 0.05.

3.2 The effect of Combi VPlantex micronutrient fertilizer on the yield components of milk thistle

The results of evaluating the effect of Combi VPlantex micronutrient fertilizer concentration on yield components are shown in Table 3.

The data in Table 3 show that Combi VPlantex micronutrient fertilizer has a significant effect (p < 0.05) on the yield components of milk thistle, such as flower diameter, number of seed/flower, number of firm seed/flower, 1000 seed weight and firm seed weight/flower.

The flower diameter is closely correlated with the number of seeds per flower. The data in Table 3 show that the flower diameter increased progressively with the concentration used from 4.2 cm (F1 - control) to 5.4 cm (F4), where formula F3 and formula F4 are not statistically different but are significantly higher than formula F1 and formula F2 at a 95% confidence level.

The number of seeds/flower and the number of firm seed/flower also tend to increase with higher concentrations of micronutrient fertilizer for the plants. Formula F4 achieved the highest number of seed with 78.2 seeds/flower and 64.2 firm seeds/flower, while formula F1 only reached 68.4 seeds/flower and 43.2 firm seeds/flower. This can be explained by the fact

that when spraying the Combi VPlantex micronutrient fertilizer, which has a relatively high content of Zn and B these micronutrients play a crucial role in facilitating the germination processes of pollen grains and the ability to set fruit.

Seed weight is mainly determined by seed size (length, width and thickness). The weight of 1000 seed across the experimental treatments showed no significant difference at a 95% confidence level. The weight of 1000 seed ranged from 20.4 g (F1) to 21.8 g (F4), indicating that micronutrients not only increase the number of seeds but also enhance seed weight. This can be explained by the fact that 1000-grain weight is a highly heritable trait, so when the variety remains unchanged, characteristics such as grain length, width, and thickness are usually stable, and the use of micronutrient fertilizers did not have a strong enough impact to alter the 1000-grain weight.

Seed yield is determined by the number of firm seed/flower. This indicator increased significantly from 0.88 g (F1) to 1.40 g (F4), reflecting the combined impact of the components of the Combi VPlantex micronutrient fertilizer on the plant's yield potential.

Formula	Flower diameter (cm)	Number of seeds/flower (seeds)	Number of firm seeds/flower (seeds)	Weight of 1000 seeds (g)	Weight of firm seeds/flower (g)
F1 (control)	$4.2^{a} \pm 0.20$	68.4°± 4.35	43.2° ± 3.12	20.4a ± 1.26	$0.88^{a} \pm 0.11$
F2	$4.7^{b} \pm 0.37$	$72.8^{a} \pm 4.93$	$53.4^{b} \pm 3.29$	$21.2^{a} \pm 1.50$	$1.13^{b} \pm 0.14$
F3	$5.2^{\circ} \pm 0.40$	$75.3^{ab} \pm 4.64$	$62.8^{\circ} \pm 3.16$	$21.6^{a} \pm 1.19$	$1.36^{\circ} \pm 0.16$
F4	$5.4^{\circ} \pm 0.38$	$78.2^{b} \pm 4.83$	64.2° ± 3.92	$21.8^{a} \pm 0.92$	$1.40^{\circ} \pm 0.18$
LSD0.5	0.36	8.19	5.34	2.19	0.12
CV%	3.7	5.6	4.8	5.2	4.9

^{*} Different letters (a, b, c) in the same column indicate significant differences at p < 0.05.

3.3 effect of Combi **VPlantex** The micronutrient fertilizer on the yield of milk thistle

The results of evaluating the effect of Combi Vplantex micronutrient fertilizer on the yield of milk thistle plants are shown in Table 4. The data in Table 4 show a significant effect of Combi VPlantex micronutrient fertilizer on the seed yield of milk thistle. The individual seed yield progressively increased across the experimental treatments with a significant effect. Formula F1 (control) had the lowest seed yield of 9.2 g/plant, while formula F4 reached the highest yield of 12.8 g/plant.

The theoretical seed yield also showed a linear increase with the concentration of micronutrient fertilizer used. The trend in the yield components (number of firm seeds, firm seed weight, and 1000 seed weight) as shown in Table 3 is in harmony with this increase.

The actual yield is an important indicator reflecting the actual production efficiency. Compared to the control (F1), formula F4 had a higher actual yield of 69.6 kg/ha. Formula F2 did not show a significant difference compared to formula F1. However, formula F3 and formula F4 did not show a statistically significant difference $(LSD_{0.5} = 36.85 \text{ kg/ha}).$

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Formula	Individual seed yield (g/plant)	Theoretical seed yield (kg/ha)	Actual seed yield (kg/ha)
F1 (control)	9.2ª ± 0.71	368.4° ± 18.79	324.2ª ± 16.45
F2	$10.5^{b} \pm 0.78$	419.8 ^b ± 21.62	327.4° ± 16.87
F3	11.7° ± 0.79	468.4° ± 23.55	370.1 ^b ± 19.36
F4	12.8°± 0.69	511.4 ^d ± 19.03	393.8b ± 15.57
LSD _{0.5}	1.19	-	36.85
CV%	5.4	-	5.2

Table 4. The effect of Combi VPlantex micronutrient fertilizer on the seed yield of milk thistle

3.4 effect of Combi VPlantex micronutrients on the silymarin content in milk thistle seeds

The evaluation of the effect of Combi VPlantex micronutrient fertilizer on the silymarin content in milk thistle seeds is presented in Figure 1. The results show that the use of micronutrient fertilizer has a significant effect on accumulation of silymarin in the seeds. Formula F1 had the lowest silymarin content in the seeds at 2.15%. The treatments with additional spraying of Combi VPlantex showed higher silymarin content, ranging from 2.23% to 2.39%. This can be explained by the presence of Fe micronutrient in Combi VPlantex, as Fe not only plays a role in the photosynthesis process but also acts as a catalyst for synthesizing phenolic compounds flavonoids [27]. This result is also consistent with research on the effect of micronutrient (Fe, Zn, B and Mn) supplementation on the antioxidant activity of milk thistle [28].

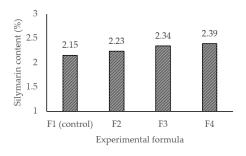


Fig. 1. Silymarin content in milk thistle seeds

^{*} Different letters (a, b, c, d) in the same column indicate significant differences at p < 0.05.

4 Conclusion

Combi VPlantex micronutrient fertilizer has a positive effect on the growth, development and seed yield of milk thistle cultivated in Phu Tho. The addition of micronutrients significantly improves growth indicators, yield components, seed yield and silymarin content in the seeds. The Combi VPlantex fertilizer applied at a 15% concentration gave outstanding results, with individual seed yield reaching 12.8 g/plant, actual seed yield reaching 393.8 kg/ha and silymarin content in the seeds reaching 2.39%. However, VPlantex spraying Combi at concentration also produced results similar to those at the 15% concentration with a 95% confidence level, suggesting that this may be the optimal concentration for practical milk thistle production to ensure high economic efficiency while still achieving good yield and quality.

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