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Induction of milk thistle (*Silybum marianum* L. Gaertn) growth and phytochemicals production by natural stimulants

Khalil M. Saad-Allah[a,*], Mohammed I. Fetouh[b], Mahmoud A. Elhaak[a]

[a] Botany Department, Faculty of Science, Tanta University, Tanta 31527, Egypt
[b] Horticulture Department, Faculty of Agriculture, Tanta University, Tanta, Egypt

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ABSTRACT

Two field experiments were carried out through 2013/2014 and 2014/2015 seasons in Faculty of Agriculture experimental farm, Tanta University, to investigate the influence of chicken manure (CM) and yeast extract (YE) on *Silybum marianum* (milk thistle) growth, yield and phytochemical constituents. CM was applied to soil with rates of 0.0, 23.81, 35.71 and 47.62 m^3/hectare (ha) and YE was applied after 15 and 75 days of seed sowing, with rates of 0, 25, 50 and 100 g/L as foliar spray. The design of the experiment was as split plot with CM as the main plot. Results indicated that the applied bio-stimulants improved the vegetative traits and seed yield, but without obvious effect on chlorophyll content, though both treatments had resulted in a significant enhancement in PSII photochemical efficiency. Also, both bio-stimulants possessed positive impact on the antioxidant status of seed extracts and silymarin production, as well as the phytochemical constituents. However, these treatments caused a pronounced fluctuation in amino acids profile.

1. Introduction

Milk thistle (*Silybum marianum* L. Gaertn), a member of Asteraceae, is a wild annual winter herb, originated in Mediterranean and abundantly found in the Nile Delta and near water streams (El-Mallah et al., 2003). The fruits of milk thistle are of achene type having shiny dark brown–black color. Two varieties of this species are widely distributed in Egypt; variety *purple* with purple corollas and variety *albiflorum* with white corollas (Boulos, 2002). The two varieties are growing naturally along canal banks, roadsides, and neglected areas. The plant is classified according to its ecological abundance as a common species in the canal bank habitats of the Nile in Egypt (Mashaly et al., 2010).

Milk thistle contains isomeric mixtures of flavonolignans, including Taxofillin (TX), silychristin (SC), silydianin (SD), silybin (SN) and isosilybin(IS) altogether known as silymarin (SM) (Kurkin et al., 2001). These compounds are found throughout the plant, but are most concentrated in the seeds (Belitz and Sams, 2007). *Silybum* seed extracts found to prevent liver problems, as well as treat acute liver poisoning or chronic disease (hepatitis and icterus). Investigations also have been made into the use of milk thistle for treating various types of cancer (Davis-Searles et al., 2005), regulating cholesterol level, promoting nervous system health, and regulating blood sugar in diabetes II type patients (Belitz and Sams, 2007). The hepato-protective effect of these extracts are due to the antioxidant proprieties of flavonoids, which inhibit the synthesis of phosphatidylcholine, and stimulate synthesis of hepatic RNA proteins (Hadaruga and Hadaruga, 2009).

Nowadays, extensive cultivation of *Silybum* as a source for natural silymarin become an urgent demand. Production of high-quality milk thistle achenes depends on conditions of cultivation that directly influence the quality of final product (Spitzová and Starý, 1985). In the last three decades, safe agriculture has been the main targets in the world. Excessive use of inorganic fertilizers proved to potentially cause dangerous effects on plant tissues, as well as the consumers (Ismail et al., 2014). Thus, organic agriculture was endorsed to guarantee safety crops for human and environment. Organic fertilizers also contribute to the physical improvement of the soil structure, maintenance and restoration of humidity, decreasing of water and wind erosion and increasing of the beneficial organisms in the soil (Prakash et al., 2007).

Chicken manure (CM), is an efficient organic fertilizer with relatively high contents of macronutrients as nitrogen, phosphorus and potassium, in addition in organic matter which improves the physical properties of the soil (Ayeni, 2011). Nevertheless, bio-fertilizers are microbial preparations containing living cells

* Corresponding author.
E-mail address: khalilmahfouz@yahoo.com (K.M. Saad-Allah).

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Table 1a
Chemical analysis of yeast extract after Mahmoued (2001).

Amino acid mg.100 g^{-1} dry weight				Vitamins mg.100 g^{-1} dry weight			
Arginine	1.99	Tryptophan	0.45	Vit.B1	2.23	Nicotinic acid	39.88
Histidine	2.63	Valine	2.19	Vit.B2	1.33	Panthothenic acid	19.56
Isoleucine	2.31	Glutamic acid	2.00	Vit.B6	1.25	Pamino benzoic acid	9.23
leucine	3.09	Serine	1.59	Vit B12	0.15	Folic acid	4.36
Lysine	2.95	Aspartic acid	1.33	Thimain	2.71	Pyridoxine	2.90
Methionine	0.72	Cystine	0.23	Riboflavin	4.96		
Phenyl alanine	2.01	Proline	1.53	Insitol	0.26		
Threonine	2.09	Tyrosine	1.49	Biotin	0.09		

of different microorganisms or their active extractions as in the case of yeast. Recently, yeast has been used as a kind of bio-fertilizers in foliar application on the shoots of vegetable crops. Yeast extract effects are ascribed to its high contents of nutrient elements and growth regulator compounds like auxins, gibberellins and cytokinins (Barnett et al., 1990). These natural compounds stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and chlorophyll (Ahmed et al., 2011). In light of the preceding, a field study was designed for two successive seasons to investigate the impact of individual or combined effects of soil amendment with chicken manure and yeast extract foliar application as bio-stimulants on growth, yield, the quality and quantity of flavonolignans, as well as amino acids profile of milk thistle.

2. Material and methods

Two field experiments were carried out in the experimental farm of the Faculty of Agriculture, Tanta University, Tanta, Egypt, during the two successive seasons of 2013/2014 and 2014/2015 in order to study the possibility of enhance the bio-production of silymarin using chicken manure and yeast extract.

2.1. Experimental design and layout

The experiment was designed as split plot. Manure treatments were arranged in four main plots. The main plots area were 36 m^2, each main plot was then splitted to four treatments of bio-yeast extract treatments as subplots with area of 9 m^2 (3 × 3), while bio-yeast extract treatments were considered as sub-plots. The experiment contained 16 treatments.

2.2. Chicken manure application

Before seed sowing, land was divided into four plots each plot was amended with completely-composted chicken manure, to minimize nitrogen losses, with four levels at 0, 23.81, 35.71 and 47.62 m^3/ha. Manure was added during soil preparation one day before seed sowing.

2.3. Yeast extract treatment

Bread yeast extract was prepared after Mahmoued (2001) by using a technique allowed cells to be grown and multiplied efficiently during conducive aerobic and nutritional conditions that allowed producing *denovo* beneficial bio-constituents. Yeast extract was prepared in four concentrations of 0, 25, 50 and 100 g/L and used as foliar application. Seedlings were sprayed with the yeast extract twice, the first was after the formation of first two true leaves (two weeks of seed sowing) with the rate of 1100 L/ha and the second was two-folds of this quantity just before the flowering stage (after 75 days of seed sowing). Foliar spray on plants was made using hand operated compressed air sprayer at the rate of 5 Liter/plot.

Table 1b
chemical composition of chicken manure.

Total N (%)	Total P (%)	Total K (%)
3.13 ± 0.15	1.15 ± 0.19	2.38 ± 0.11

Chemical analysis of yeast extract, quoted from Mahmoued (2001), and chicken manure are presented in Tables 1a and 1b.

2.4. Seed sowing

Silybum marianum variety albiflora (white head flowers) seeds were collected from plants naturally growing in the middle part of the Nile Delta. Seeds were sown on 1st December for both seasons in clayey loam soil. One week after sowing, all the dead seedlings were replanted with fresh seeds. After full germination, seedlings were thinned to four plants per square meter with total 36 seedlings per subplot and 70 cm row distance with a total of 144 seedlings per main plot, keeping uniformed seedlings free from any kind of damage. Plants were irrigated once weekly with 80% field capacity. Elimination of weeds was done manually by hand whenever needed.

2.5. Growth evaluation

One month after seeds sowing, ten plant samples were taken to estimate seedlings growth criteria i.e. total biomass. Samples were dried in forced-air oven at 60 °C till constant weight. Shoot and root dry weights were recorded, and relative dry matter (RDM) was calculated as: RDM = (dry weight/fresh weight) × 100. At the end of experiment (May), growth evaluation of treated plants were assessed by recording plant height, branches no/plant, flowers heads no/plant, air dry seed yield (g/plant)and plant total dry weight (g/plant).

2.6. Chlorophyll content and chlorophyll fluorescence

Total chlorophyll content of plant leaves was measured by non-destructive method using chlorophyll meter (SPAD-502, Minolta Co., Japan) which provides a SPAD value (Zamani et al., 2011). The quantum efficiency of open photosystem II centers (Fv/Fm), was measured according to method described by (Jowkar et al., 2012) at the seedling stage with Opti-Sciences OS-5P pulse amplitude fluorimeter (Opti-Sciences INC., Hudson, NH, USA). Dark-adapted leaves were placed in the leaf clip for measurement of Fv/Fm. Minimal fluorescence (F_o) was measured under a weak pulse of modulating light over a 0.8 s period and maximal fluorescence (Fm) was obtained after a saturating pulse of 0.7 s at 8000 μmol m^{-2} s^{-1}. Fv is the difference between F_o and Fm. Measurements were conducted at seedling stage (30 days-old) on 10 different leaves (replications) in each treatment.