

RESPONSE OF *ARTOCARPUS HETEROPHYLLUS* LAM. TO SOME NATURAL EXTRACTS AT THE EARLY GROWTH STAGES 2- CHEMICAL CONSTITUENTS OF THE PLANTS

Amira S. Soliman^{*}; A.M.Z. Sarhan^{**}; A. Nabih^{***}; S.A. Gomma^{****} and H.S.A. Elham^{***}

^{*} Institute of African Researches and Studies, Cairo Univ., Egypt.

^{**} Ornamental Hort. Dept., Fac. Agric., Cairo Univ. Egypt.

^{***} Botanical Gardens Res. Dept., Hort. Res. Inst., ARC, Giza, Egypt.

^{****} Ornamental Plants and Landscape Gardening Res. Dept., Hort. Res. Inst., ARC, Giza, Egypt.



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Prof. Dr. E.S. Nofal,
Kafr El-Sheikh Univ.

Prof. Dr. Boshra A. El-Sayed,
Hort. Res. Inst.,
ARC.

ABSTRACT: The experimental trial was consummated under saran house of 65% shading performed at the nursery of Horticulture Research Institute, Giza, Egypt during two successive seasons (2012/2013 and 2013/2014) with the aim of improving plant quality at the early growth stages of *Artocarpus heterophyllus* Lam. by studying the effect of treating plants with some natural botanical extracts (*Moringa oleifera* Lam., *Thymus vulgaris* L. and *Majorana hortensis* Moench) with different levels (0, 5 and 10%) besides, the combinations of moringa + marjoram, moringa + thyme and marjoram + thyme extracts at 2.5%. All of them were applied as a foliar spray commencing from November 20th in both seasons on transplants of *Artocarpus heterophyllus* Lam. 14-15 cm height with 4-5 leaves till June 20th of the following year at 15 days interval. The plants were grown in sand + peatmoss mixture (1:1, v/v). The results emphasized that most plants which received the highest natural extract level (10%) died after about 6 times of spraying. So, the corresponding data of such treatments were excluded. Meanwhile, using either moringa extract at 5% or the combination of marjoram + thyme extracts at 2.5% proved their mastery in improving plant chemical constituents at the terminate of experiment (July, 5th) as that treating plants with moringa extract at 5% followed by that of the combination of marjoram + thyme extracts at 2.5% were the best treatments used for improving all the components which were measured in this respect (chlorophyll a, b and carotenoids, total carbohydrates, N, P, K and protein in leaves).

From the aforementioned results, it could be recommended to use either moringa extract at 5% or the combination of marjoram + thyme extras at 2.5% for improving chemical constituents of *Artocarpus heterophyllus* Lam. at the early growth stages of such plants besides, getting better plants without being forced to use chemical nutrients or synthetic growth regulators that may contaminate the environment with getting a safe clean product.

Key words: *Artocarpus heterophyllus* Lam., natural extracts, *Moringa oleifera*, *Thyme vulgaris*, *Majorana hortensis*., chemical constituents.

INTRODUCTION

Artocarpus heterophyllus Lam. (Jack fruit) is belonged to family Moraceae. It is native to parts of southern and southeast Asia. Jack fruit is the national fruit of Bangladesh. It is also found in East Africa. It

is well suited to tropical lowlands and its fruit is the greatest tree-born fruit reaching as much as 36 kg in weight and up to 90 cm long and 50 cm in diameter. The seeds may be boiled or backed like beans. Seeds from ripe fruits are edible. They have a milky,

sweet taste. The wood of the tree is used for the production of musical instruments. Jack fruit wood is widely used in the manufacture of furniture, doors and windows (Wikipedia, 2011).

In Egypt, Jack fruit (*Artocarpus heterophyllus* Lam.) is considered as an important economic plant, but suffer variable conditions to be maintained in good quality especially at the early growth stages, besides the slow rate of vegetative growth, consequently the delay in fruiting time.

It is well known that the use of natural extracts of certain plants (referred to as biostimulants, botanical activators or botanicals) in improving the growth of agricultural crops is highly recommended as environment friendly and safe approach to get better plants without being forced to use chemical nutrients or synthetic growth regulators that may contaminate the environment.

Moringa oleifera, family Moringaceae is most widely grown. Since leaves of moringa are rich in zeatin, it can be used as natural source of cytokinins (Fuglie, 1999). In addition moringa leaves are also rich in ascorbates, carotenoids, phenols, potassium and calcium which have plant growth promoting capabilities and often applied as exogenous plant growth promoters (Foidle *et al.*, 2001). Antioxidant such as ascorbic acid and glutathione which are found at high concentrations in moringa chloroplasts and other cellular compartments are crucial for plant defense against oxidative stress (Noctor and Foyer, 1998). In view of all these reports, it is hypothesized that priming with leaf extract from moringa, having a number of plant growth promoters, mineral nutrients and vitamins in a naturally balanced compositions which may promote the plant growth.

Thymus vulgaris, L. family Lamiaceae is native of Mediterranean countries, growing abundantly over wide area in France, Spain, Portugal, Italy, Algeria and Morocco (Porte *et al.*, 2000). Moreover, it is also cultivated

in other parts of Europe and North America, North Asia and Ocrania (Prubhi, 1976). Thyme is employed to season and suppress offensive odors, such as trimethylamino odor, in foods (Porte *et al.*, 2000 and Prubhi, 1976). It was found that the main components of the essential oil were thymol and carvacrol and that it had antimicrobial activity against fungi (some aflatoxins produces) virus helminth, Gram positive bacteria and Gram negative bacteria (Nakatani *et al.*, 1989 and Farag *et al.*, 1989).

Majorana hortensis Moench, Family Lamiaceae. Marjoram is indigenous to the Mediterranean area, it is a member of the Origanum genus and similarity of flavor with Oregano (*Origanum vulgare*). It has a strong and sharp spicy odor. Marjoram is a perennial evergreen shrub (treated as annual under cultivation) growing to a height of about 40 cm. It has a square, red brown stem and small and hairy, gray-green leaves. During summer the plant produces tiny, white to pink flowers. The parts used, is the whole plant. The useful compounds are caffeic and rosmarinic acid, carvacrol, flavonoids, linalool, terpenes triterpenoids, sabinen, sabinen hydrate (Health from Nature, 2011)

As for the very limited investigations were performed on the effect of natural extracts of the above mentioned plants (moringa, thyme and marjoram) especially thyme and marjoram extracts on vegetative growth of woody or ornamental plants the following findings may reveal some results were obtained in this regard on other plants. El-Bassiony *et al.* (2005) reported that foliar spray with α -tocopherol (one of the components of thyme extract) on faba bean plants induced increase in growth parameters. Parabhu *et al.* (2010) worked on seared basil (*Ocimum sanctum*) and concluded that spraying the combination of 2% panchakavya + 0.2% humic acid + 2% moringa leaf extract resulted in taller plant height and higher number of leaves. Mvumi *et al.* (2012) on *Lycopersicon esculentum*

var. Rodad stated that applying moringa extract increased growth of the plant. Rana *et al.* (2013) experimented the effect of moringa leaf extract (MLE) on seedling growth of maize (*Zea mays*, L.) where different concentrations of MLE (5, 10, 15 and 20%) were used, and the different treatments were applied at two times (5 and 10 days after sowing). Results indicated that application of 5% MLE at 5 days furnished maximum shoot length.

In this connection, other authors attributed the beneficial effect of moringa extract to its contents of zeatin, ascorbates, phenolic compounds, K and Ca (Makkar *et al.*, 2007).

Lobna *et al.* (2015) on jojoba indicated that the addition of moringa leaves extract positively affected pigments content of the plants.

Therefore, the present experiment was conducted with the aim of determining the most efficient natural extract treatment that can be applied for *Artocarpus heterophyllus* Lam. for producing plants of healthy vegetative growth at the early growth stages besides improving their chemical constituents.

MATERIALS AND METHODS

The present experiment was conducted throughout two successive seasons (2012/2013 and 2013/2014) at the nursery of Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The second season was an exact repetition of the first one. It was intended to study the response of *Artocarpus heterophyllus* Lam. transplant at the early growth stages to different levels of *Moringa oleifera* Lam., *Thymus vulgaris* L., and *Marjoram hortensis* Moench, extracts (0, 5 and 10%) and some combinations (moringa + marjoram, moringa + thyme and marjoram + thyme extracts at 2.5%) on chemical constituents of the plants for producing plants of vigorous growth at the early growth stages.

Plant material:

Seeds of *Artocarpus heterophyllus* Lam. were collected from Zohria garden in both seasons.

The mixture of sand + peatmoss (1:1, v/v) was used in plantation in every season, as a preliminary study was conducted on the effect of some growing media on germination and early growth of newly established plants concerning, sand, peatmoss and the mixture of sand + peatmoss (1:1, v/v), where the later proved its mastery in this regard.

Some physical and chemical properties of the used sand (fine sand granules diameter of 0.20:0.25 mm) and chemical properties of peatmoss are shown in Tables (a) and (b), respectively.

Natural extracts: Different natural extracts of some plants with different levels (*Moringa oleifera* Lam., *Thymus vulgaris*, L. and *Marjoram hortensis* Moench) and some combinations were applied in both seasons, as follows:

- 1- Untreated plants (control)
- 2- Moringa extract at 5%
- 3- Moringa extract at 10%
- 4- Marjoram extract at 5%
- 5- Marjoram extract at 10%
- 6- Thyme extract at 5%
- 7- Thyme extract at 10%
- 8- Moringa + marjoram extracts at 2.5%
- 9- Moringa + thyme extracts at 2.5%
- 10- Marjoram + thyme extracts at 2.5%

Preparation of extracts:

Fresh leaves of moringa (*Moringa oleifera* Lam.), thyme (*Thymus vulgaris*, L.) and marjoram (*Marjoram hortensis*, Moench) were collected directly from mature trees and plants. The sample was cleaned by rinsing bath in distilled water, dried by shaking vigorously with hand. The leaves of every species were air dried in shade place.

Table a. Some physical and chemical analysis of the used sand.

pH	E.C. (ds/m)	S.P.	Cations (meq/l)				Anions (meq/l)			N (ppm)	P (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻							
7.41	1.51	42.30	2.94	1.29	3.89	2.30	3.54	4.73	2.16	232.40	25.05	580	7.48	5.66	2.90	7.18

Table b. Some chemical properties of the used peatmoss.

Organic matter	90-95%	P	0.23%
Ash	8-10%	K	1.77%
Density (Vol. Dry)	80-90 mg/l	Fe	421 ppm
pH value	3.4	Mn	27 ppm
Water relation	60.75%	Zn	41 ppm
Salinity	0.3 g/l	Cu	8.8 ppm
N	1.09 %	Mg	3.3 ppm

After drying, the leaves were ground by an electrical grinder and made powder. The powder was weighed and mixed with distilled water at a ratio of 1:10 (w/v) i.e. 100 g powder + 1000 cm³ distilled water for preparing 10% aqueous extract. The mixture was then shaken for four hours by an electrical stirrer and kept in dark room temperature for 24 h. Thereafter, the solution was heated till just before boiling. Afterwards, it was filtrated through two cheese cloth. The extracts served as the stock solution (10%) for each plant species, where different levels of natural extracts (0, 2.5, 5 and 10%) were prepared, using the crude aqueous extract of 10% of each and distilled water.

Chemical composition of plant extracts:

Moringa oleifera Lam.: The chemical composition of *Moringa oleifera* oil: hemacosan (13.9%), pentacosan (13.3%) and heptacosan (11.4%) are the main components Ultra high performance chromatography. DAD analysis detected the flavonoids quercetin (126 mg/g) and luteolin (6.2 mg/g). The essential oil exhibited a relatively low free radical scarening capacity (Titiana *et al.*, 2013).

Thymus vulgais, L.: Shows polymorphic variations in monoterpene production, the

presence of intraspecific chemotype variation being common in the genus *Thymus*. Each of the six chemotypes geraniol (G), α -terpineol (A), thuyanol-4 (U), linalool- (L), carvacrol (C), and thymol (T), is named after its dominant monoterpene (Thompson *et al.*, 2003).

Marjoram hortensis, Moench: Volatile oil components (%) of marjoram: alfa pinene (1.57%), beta pinene (4.79%), limonene (9.63%), 1.80 cineole (5.22%), Y- terpinen (2.23%), linalool (3.88%), terpinen-4-ol (49.37%), a-terpineol (9.38%), linalyl acetate (2.39%), estragol (1.56%), beta carophyllene (2.21%), eugenol (1.71%), unidentified (6.06%) (Parabhu *et al.*, 2010).

Procedure:

In both seasons seeds of *Artocarpus heterophyllus* Lam. were planted on August 8th in the mixture of sand + peatmoss (1:1, v/v) in 20 cm diameter plastic pots under saran house condition of 65% shading. After two months from planting (October, 8th) where the newly established plants reached about 5-6 cm length, bearing 2-3 leaves, were transplanted into 20 cm diameter of plastic pots (one transplant each) filled with about 2.5 kg of the same mixture used for germination (sand/peatmoss 1:1, v/v) and left to grow under saran house conditions. Thereafter, when the plants reached about 14-15 cm length bearing 4-5 leaves (November 20th), foliar spray of the different natural extract treatments were applied and then at 15 days interval till June 20th of the next year. Besides, untreated plants (control) which received only foliar spray of distilled water. Thus, the plants were treated 15 times with foliar spray throughout the course of the study.

The layout of the experiment was randomized complete block design (RCBD) with three replicates. Every experimental unit contained 4 plants and every treatment represented by 12 plants.

All the plants under various treatments received the usual agricultural practices, such as weeding, watering and fertilization etc. recommended for such plantation whenever required. In this connection, Kristalon (19:19:19) at the rate of 2 g/l was applied at 15 days intervals as a soil drench, during the course of the study.

Chemical constituents of the plant (at the end of the second season on July 15th) were carried out as follows:

- Chlorophyll a, b and carotenoids content (mg/g f.w.) in fresh leaves were determined according to Wettstein (1957).
- Total carbohydrates in dry leaves were determined using colorimetric method described by Smith *et al.* (1956).
- Nitrogen, phosphorus and potassium (%) in dry leaves, nitrogen was determined by microkjeldahle apparatus (Black, 1965), phosphorus was colorimetric by determined in the acid digested using ascorbic acid method (John, 1970), potassium was determined using the flamephotometer (Dewis and Freitas, 1970) and protein was mathematically calculated.

RESULTS AND DISCUSSION

Firstly, plants which received the highest level of the different natural extract treatments (10%) died after about 6 times of spraying. So, the concerning data were excluded in this regard.

Chemical constituents of leaves at the terminate of the experiment (July 15th) in the second season:

Pigments content in leaves:

Data presented in Table (1) indicate that treating plants with moringa extract at 5% proved its superiority in raising chlorophyll a, b and carotenoids content in leaves over the control, followed by those which received the combination of marjoram + thyme extracts at 2.5%. Meanwhile, the third rank for elevating either chlorophyll (a) or carotenoids content in the leaves was belonged to plants which were treated with the combination of moringa + marjoram extracts at 2.5%. However, receiving plants marjoram extract at 5% showed also a favourable effect on raising chlorophyll (b) content in the leaves. In this connection, the promotive effect of some natural extracts on chemical constituents of some plants was also noticed by other workers.

Table 1. Effect of some natural extracts on pigments content in leaves [chlorophyll a, b and carotenoids (mg/g f.w.)] of *Artocarpus heterophyllus* Lam. in the second season (on July 15th 2013/2014).

Treatments	Chlorophyll (a)	Chlorophyll (b)	Carotenoids
Control	0.766	0.255	0.101
Moringa at 5%	1.296	0.497	0.291
Marjoram at 5%	1.010	0.446	0.205
Thyme at 5%	0.924	0.415	0.169
Moringa + marjoram at 2.5%	1.023	0.410	0.245
Moringa + thyme at 2.5%	0.938	0.414	0.216
Marjoram + thyme at 2.5%	1.175	0.453	0.256

El-Bassionny *et al.* (2005) reported that spray with α -tocopherol (one of the components of thyme extract) on faba bean plants induced increase in chlorophyll a, b and carotenoids content. Lobna *et al.* (2015) on jojoba plants indicated that the addition of moringa leaves extract positively affected pigments content of the plants.

Total carbohydrate (%) in the leaves:

Data presented in Table (2) show that the highest records of total carbohydrates % in the leaves were obtained due to supplying plants with moringa extract at 5%, followed by those which received the combination of marjoram + thyme extracts at 2.5%. The third rank was achieved by plants which supplied with either marjoram extract at 5% or the combination of moringa + marjoram extracts at 2.5%. Meanwhile, the lowest records were obtained due to using either thyme extract at 5% or the combination of moringa + thyme extracts at 2.5%.

Table 2. Effect of some natural extracts on total carbohydrates (%) in the leaves of *Artocarpus heterophyllus* Lam. in the second season (on July 15th, 2013/2014).

Treatments	Total carbohydrates (%)
Control	9.95
Moringa at 5%	17.71
Marjoram at 5%	14.53
Thyme at 5%	12.09
Moringa + marjoram at 2.5%	15.83
Moringa + thyme at 2.5%	13.52
Marjoram + thyme at 2.5%	16.42

Minerals and protein contents in leaves:

Minerals content:

N % in the leaves:

It is clear from Table (3) that using moringa extract at 5% proved its mastery in elevating N % in the leaves over control. The second degree for raising the same constituent was achieved by treating plants with either the combination of moringa + marjoram or that of marjoram + thyme extracts at 2.5%. The lowest record was

obtained due to applying thyme extract at 5%. The other treatments gave an intermediate effect in this respect.

P % in the leaves:

Corresponding values of P % in the leaves clear the superiority of supplying plants with either moringa extract at 5% or with the combination of marjoram + thyme extracts at 2.5% in raising such constituent in leaves. However, treating plants with the combination of moringa + marjoram extracts at 2.5% also showed a favourable effect in this regard. The least score, on the other side, was obtained resulting from supplying plants with thyme extract at 5% (Table, 3).

K % in the leaves:

Data presented in Table (3) show that supplying plants with moringa extract at 5% reveal a clear increment on K % in the leaves comparing with that gained from control and other treatments used. The second rank for raising the same constituent was belonged to plants which received the combination of either moringa + thyme extracts or those which received the combination of marjoram + thyme extracts at 2.5%. The lowest record, on the other hand, was a result of treating plants with marjoram extract at 5%. The other treatments gave an intermediate effect in this regard.

Protein content in the leaves:

Data recorded in Table (3) reveal that the effect of the different natural extracts treatments on protein content in the leaves clearly differed according to the different treatments used. In this connection, using moringa extract at 5% proved its mastery in elevating such constituent in leaves. Meanwhile, supplying plants with the combination of marjoram + thyme extracts at 2.5% achieved the second rank for raising the scored values. The third rank for increasing the same constituent was a result of treating plants with the combination of moringa + marjoram extracts at 2.5%. The lowest record, on the other hand, resulted from treating plants with marjoram extract at

Table 3. Effect of some natural extracts on N, P, K and protein (%) in the leaves of *Artocarpus heterophyllus* Lam. in the second season (on July 15th 2013/2014).

Treatments	N (%)	P (%)	K (%)	Proteins
Control	0.74	0.08	0.63	4.60
Moringa at 5%	2.01	0.36	1.39	9.34
Marjoram at 5%	1.21	0.22	0.78	6.71
Thyme at 5%	1.05	0.19	0.84	6.42
Moringa + marjoram at 2.5%	1.50	0.31	0.85	7.24
Moringa + thyme at 2.5%	1.11	0.24	0.88	6.56
Marjoram + thyme at 2.5%	1.38	0.33	0.91	8.69

5%. The other treatments gave an intermediate effects, with means closely near together.

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إستجابة نبات الجاك فروت (الكاكاي) *Artocarpus heterophyllus* Lam لبعض المستخلصات الطبيعية في المراحل المبكرة من النمو - ٢ - المكونات الكيميائية للنباتات

أميرة شوقي سليمان*، عاطف محمد زكريا سرحان**، على نبيه***، صلاح عبد العزيز***، إلهام حسين***

* معهد بحوث الدراسات الأفريقية، جامعة القاهرة، مصر.

** قسم بساتين الزينة، كلية الزراعة، جامعة القاهرة، مصر.

*** قسم بحوث الحدائق النباتية، معهد بحوث البساتين، مركز البحوث الزراعية، مصر.

**** قسم بحوث نباتات الزينة وتنسيق الحدائق، معهد بحوث البساتين، مركز البحوث الزراعية، مصر.

تم إجراء هذه التجربة بمشغل معهد بحوث البساتين، جيزة، مصر خلال موسمين زراعيين متتاليين (٢٠١٢/٢٠١٣ و ٢٠١٣/٢٠١٤) بهدف تحسين جودة نبات الجاك فروت *Artocarpus heterophyllus*, Lam في المراحل المبكرة للنمو وذلك بدراسة تأثير معاملة النباتات ببعض المستخلصات النباتية لبعض النباتات (المورينجا، الزعتر، البردقوش) بتركيزات مختلفة (صفر، ٥، ١٠%) إلى جانب استخدام خليط من مستخلص المورينجا + البردقوش، المورينجا + الزعتر، البردقوش + الزعتر بتركيز ٢,٥% حيث تم معاملة جميع النباتات عن طريق الرش ابتداء من ٢٠ نوفمبر في كلا الموسمين على النباتات الصغيرة لنبات الجاك فروت *Artocarpus heterophyllus*, Lam (١٤-١٥ سم في الإرتفاع، ٤-٥ ورقة/نبات) وحتى ٢٠ يونيو من العام التالي وذلك على فترات كل ١٥ يوم وقد تم نمو النباتات في مخلوط الرمل + البيت موس (١:١ حجما) تحت ظروف الزراعة بالصوبة الساران ذات تظليل قدره ٦٥%. وقد أوضحت النتائج موت

معظم النباتات التي تم معاملتها بالتركيز العالي (١٠%) وذلك بعد ٦ رشات وعلى هذا الإعتبار قد تم إستبعاد البيانات المصاحبة لتلك المعاملات.

أكدت نتائج المحتوى الكيميائي للنباتات فى نهاية التجربة (١٥ يوليو) أن معاملة النباتات بمستخلص نبات المورينجا بتركيز ٥% متبوعا بتلك النباتات التي تم معاملتها بمخلوط البردقوش + الزعتر بتركيز ٢,٥% كانت أفضل المعاملات بالنسبة للمحتوى الكيميائي للنباتات (كلوروفيل أ ، ب ، الكاروتينويدات ، الكربوهيدرات الكلية ، عناصر النيتروجين والفوسفور والبوتاسيوم بالإضافة إلى البروتين فى الأوراق).

من النتائج السابقة يمكن النصح بإستخدام مستخلص نبات المورينجا بتركيز ٥% أو مخلوط مستخلص البردقوش + الزعتر بتركيز ٢,٥% وذلك لتحسين الخصائص المورفولوجية والمحتوى الكيميائي لنبات الجاك فروت *Artocarpus heterophyllus*, Lam. فى المراحل المبكرة لنمو النبات بالإضافة إلى الحصول على نباتات جيدة دون الإضطراب لإستخدام المغذيات الكيميائية ومنظمات النمو المخلقة التي قد تضر بالبيئة بالإضافة إلى الحصول على منتج آمن.