Australian Journal of Basic and Applied Sciences, 7(1): 556-561, 2013
ISSN 1991-8178

Effect of Pre-Emergence treatment of *Jatropha curcas* L. Seeds with Some Spices Extracts on Germination, Growth, Physiological and Enzymatic Activity

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Abstract: The experiment was conducted at the National Research Centre, Cairo, Egypt during two successive seasons (2010-2011), to determine the effect of two spices extracts (*Rosmarinus officinalis* and *Thymus capitatus*) in different quantities (1% or 2%) for 3 and 6 days on germination percentage, morphological features (Plant height, plant thickness, number of leaves, leafe area, root length, fresh and dry weights of stem, leaves, roots),pigment content and enzymatic activity on *Jatropha curcas* L. The obtained data showed that the pre-emergence treatment of seeds with Thymus extract at 2% for 3 days resulted in the highest germination percentage as well as the highest amount of peroxidase, polyphenol oxidase and Esterase activities. The treatment with Rosmarinus extract at 1% for six days resulted in best results for plant height, number of leaves, plant thickness, stem, leaves fresh and dry weights as well as pigments content (Chl.a,b and carotenoids). Increasing the Romarinus extract to 2% for 3 or 6 days led to highest fresh and dry weights of roots. Thyme extract at 2% for three days caused the highest value of leaf area. The bad effect of these spices which cause a significant reduction in germination percentage, some morphological features and enzymatic activity may be due to poisonous effect of aromatic compounds that are found in these plants.

Key words: Jatropha, spices extracts, Thymus, Rosmarinus, growth, germination, enzymatic activity.

INTRODUCTION

Jatropha curcas L. (physic nut or purging nut) is a drought-resistant shrub or tree belonging to the family *Euphorbiaceae*. It is widely planted as a hedge to protect fields, as it is not browsed by cattle or other animals. It is well adapted to arid and semi-arid conditions and often used for prevention of soil erosion (Heller, 1996). Various parts of the plant hold potential for use as a source of oil, animal feed or medicinal preparations. Recently, their seeds were investigated mainly as a potential source of oil that was recognized as an adequate substitute motor fuel (Openshaw, 2000).

Many essential oils and extracts obtained from spices and plants have recently gained in interest for both the general population and the scientific community (Perez-Alvarez *et al*., 2000).

Many plants are used for different purposes, for example, in the food, drugs and perfumery sectors. Several researchers have shown interest in biologically active compounds isolated from plants and spices for eliminating pathogenic microorganisms because of the resistance that many microorganisms have built up to antibiotics (Essawi and Srour, 2000).

Culinary spices and herbs contain a wide variety of active phytochemicals (including flavonoids, terpens, polyphenols, crucumins, coumarins) and also contain fibre, proteins, sugars, cations and pigments (Fabio *et al*., 2003).

Rosmarinus officinalis and *Thymus capitatus* are used for flavoring foods, food industry, antimicrobial, antifungal and antoxidant agents due to the presence of some important phenolic components in their essential oils (Amal, 2010).

Alberto *et al*. (2004), Atti-Santos *et al*. (2005) and Szumny *et al*. (2009) demonstrated the chemical composition of essential oil of the *Rosmarinus officinalis* L. which are categorized into three groups phenolic diterpenes possessing abietic acid framework, flavonoids and phenolic acids.Moreover Ozlem *et al*. (2007) mentioned that carnosic acid (CA), carnosol, abietene-type diterpenes, rosmarinic acid (RA) and hydroxycinnamic acid ester are the main antioxidant compounds present in rosemary. These compounds, together with other isoprenoids such as sterols, isoprene, mono-and diterpenes, tocopherols or cartotenoids play a photoprotective role and are considered as bioactive constituents.

Several studies have focused on antimicrobial, antifungal, antioxidant and radical-scavenging properties of essential oil of thyme in order to identify the responsible compounds of these actions (Bhaskara *et al*., 1998 and Safaei-Ghomi *et al*., 2009). Spasmolytic as well as antioxidant activities were reported for the phenolic oil extract of the thymus plant (Miguel *et al*., 2004 and Sacchetti *et al*., 2005). There is some evidence that minor components play a critical part in biological activities, possibly by producing a synergistic effect between other components.

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The aim of this work was to determine the effect of some spices extracts in different quantities on germination percentage, some morphological features, and enzymatic activity of *Jatropha curcas* L.

MATERIALS AND METHODS

The experiment was carried out at the National Research Centre, Cairo, Egypt during two successive seasons, 2010-2011.

Jatropha curcas L. seeds were determined from farm in Luxor, Egypt. The seeds were pre-emergence treated with different concentrations (0%, 1% and 2%) of *Rosmarinus officinalis* and *Thymus capitatus* extracts for different periods (3 and 6 days). The concentrations 1% and 2% were prepared by boiling 1 and or 2g of the plant material in 100 ml distilled water.

The seeds were planted equally in Petri-dishes (15 cm) over a moistened filter paper so that every 10 seeds were spread inside each Petri-dish and pre-emergence treated with 1% and 2% of rosemary and thyme, another set of dishes was prepared where the seeds were soaked in a similar volume of distilled water (0.0%) to serve as control. All dishes were kept at 27°C $\pm$ 2 for three and six days. After that, the seeds were planted in the green house in pots (60 cm in diameter) filled with loamy clay soil (mechanical analysis: Sand 24%, silt 47% and clay 29%) at 15th and 10th March 2010 and 2011, respectively in completely randomized design with three replicates. Each replicate consisted of three plants. Water requirements were regularly fulfilled according to weather conditions. Each pot was fertilized twice with 1.5g nitrogen as ammonium nitrate (33.5% N) and 1g potassium sulfate (48.5% K_2O). These fertilizers were applied at 30 and 60 days from sowing. Phosphorous as calcium superphosphate (15.5% P_2O_5) was mixed with soil before sowing at the rate 5g/pot. Other agricultural practices were performed as normal done. The seed germination percentage was taken after 10 days from sowing. At the end of the experimentation (at 90 and 180 days from sowing), three plant samples were taken from each treatment. Plant height and thickness (cm) , leaves number and areas (cm^2), root length (cm), fresh and dry weights of stem, leaves and roots (g) were recorded. The photosynthetic pigments were also determined in fresh green leaves. Chlorophyll (a and b) as well as carotenoids content were determined as mg/100g fresh weight, according to the procedure achieved by Saric *et al*. (1967).

Peroxidase activity was measured as a biochemical marker according to Reuveni *et al*. (1992). The reaction mixture (3ml) consisted of 0.25% (V/V) guaiacol in 10mM sodium phosphate buffer, pH 6.0, containing 10mM peroxide. 25µl of the crude enzyme extract was added to initiate the reaction which was measured specrotphotometrically at 740nm. Total peroxidase activity was expressed as the increase in the absorbance at 470nm $min^{-1}g^{-1}FW$ (0.01 OD=1EU).

Polyphenoloxidase (PPO) activity was assayed spectrophotometrically as described by Leonard (1971) using DL-3,4-dihydroxphenylalamine (L-DOPA) as a substrate. The reaction mixture containing 1.3 ml of 0.03M potassium phosphate (pH 6.5) buffer and 1.2 ml L-DOPA was heated to 30°C for 2 min and finally 0.5 ml o the enzyme extract was added to the cuvette. Changes in the obsorbance at 475nm were measured for 3 min using a Shimadzu UV-120-01 spectophotometer.

Esterase activity was determined spectrophotometrically at room temperature (25-C) using either 1- or 2-naphthylacetate as substrate (Burlina and Galzigna, 1972). Esterase activity was calculated following the increase in absorbance at 322nm (1-naphthylacetate) or 313nm (for 2-naphthylacetate), due to the formation of 1-naphtol (e322nm = 2.0mM-1 cm^{-1}) or 2-naphtol (e313nm = 1.25mM-1 Cm^{-1}) (Balen *et al*., 2003). The reaction mixture contained 100m MTris/HCL, pH 7.4, and 100mM1-or 2-naphthylacetate, both dissolved in absolute methanol. The esterase activities were directed for spontaneous hydrolysis of 1- and 2- naphthylacetate. The specific enzyme activity for all enzyme was expressed as units per milligram of protein (1U=1mol min^{-1}).

The data were statistically analyzed according to Steel and Torrie (1980) and the treatments means were compared using LSD test.

RESULTS AND DISCUSSION

Germination Percentage:

Results in Table (1) indicated that the germination percentage of *Jatropha curcas* L. seeds was increased by 5.27% in response to pre-emergence treatment with the highest concentration of thymus extract (2%) for 3 days, whereas the treatment of the same concentration (2%) for the same period (3 days) of Rosmarinus reduced the germination percentage of the seeds by 47.37% as compared to control treatment. On the other hand, the germination percentage of both seeds treated with Rosmarinus (1% and 2%) and Thymus (1%) for six days showed increment by 128.59%, 142.91% and 128.59%, respectively as compared to control treatment. In this respect, Amal (2010) on *Pisum sativum* revealed that *Thymus capitatus* (1%) for 3 days increased germination percentage by 2.27%, however Rosmarinus treatment in both treatments (1 or 2%) highly significantly reduced the germination percentage by 18.18% and 15.91%. Both seeds treated with Thymus and Rosmarinus at six days showed highly reduction.

Table 1: Effect of pre-emergence treatment with the different concentrations of *Rosmarinus officinalis* L and *Thymus capitatus* L for different periods of treatment on germination percentage (%) of *Jatropha curcas* L.

Treatments	Concentration	3 days	6 days
Control	0.0	63.33	23.23
Rosmarinus	1%	63.33	53.33
	2%	33.33	56.67
Thymus	1%	63.33	53.33
	2%	66.67	23.33
LSD at 5 %		8.97	8.97

Growth Parameters:

Table (2) showed the results of plant height and thickness, leaves number and area and root length of *Jatrapha curcas* produced from seeds treated with *Rosmarinus* and *Thymus* extracts for three and six days. It is clear that plant height and number of leaves produced from treatment with *Rosmarinus* extract (1%) for six days showed highly significant increase by 15.89% and 14.99%, respectively followed by 2% *Rosmarinus* extract for the same period (six days) as compared to control treatmen.

Table 2: Effect of pre-emergence treatment with the different concentrations of *Rosmarinus officinalis* L. and *Thymus capitatus* L. for different periods of treatment on some growth characters of *Jatropha curcas* L. plants.

Period of treatment	Treatments	Plant height (cm)	Plant thickness	Number of leaves	Leaves area (cm^2)	Root length(cm)
3 days	Control (0.0)	82.00	1.60	39.33	87.33	27.00
	Rosemary 1%	65.33	2.07	28.67	70.41	27.00
	Rosemary 2%	64.67	1.67	24.67	54.62	27.00
	Thyme 1%	77.67	1.87	30.00	68.29	21.33
	Thyme 2%	71.33	1.43	22.33	95.33	19.33
LSD 5%		3.28	0.15	1.83	4.74	0.84
6 days	Control (0.0)	65.00	1.37	26.67	74.03	18.33
	Rosemary 1%	75.33	1.80	30.67	63.12	22.00
	Rosemary 2%	70.00	1.87	28.00	37.05	20.67
	Thyme 1%	54.67	2.17	24.67	59.84	25.67
	Thyme 2%	52.33	1.67	19.33	34.94	28.00
LSD 5%		2.94	0.08	0.84	0.84	2.52

On the other hand, thickness of plants produced from treatment with *Rosmarinus* or *Thymus* extract (1%) for three or six days was increased by 29.38% and 58.39%, respectively. While, thyme extract (2%) caused increment by 9.16% in leaves area for three days and 52.76% in root length for six days.

As for fresh and dry weights of stem, leaves and roots, the data presented in Table (3) indicated that plants produced from treatment with *Rosmarinus* extract at 1% for six days showed highly significant increment in stem fresh and dry weights as well as leaves fresh and dry weights by 85.58%, 33.52%, 23.59% and 17.79%, respectively. The treatment of *Rosmarinus* extract at 2% for three and six days resulted in significant increment in roots fresh and dry weights by 12.20%, 51.57%, 39.78% and 15.00%, respectively.

Table 3: Effect of pre-emergence treatment with the different concentrations of *Rosmarinus officinalis* L. and *Thymus capitatus* L. for different periods of treatment on changing of the weights of *Jatropha curcus* L plant parts.

Period of treatment	Treatments	Stem F.W.	Stem D.W.	Leaves F.W.	Leaves D.W.	Roots F.W.	Roots D.W.
3 days	Control (0.0)	128.82	21.64	123.93	17.55	38.37	7.95
	Rosemary 1%	88.22	17.87	76.95	13.32	34.78	6.92
	Rosemary 2%	115.97	19.02	58.35	10.78	43.05	12.05
	Thyme 1%	73.03	11.65	75.45	11.13	27.15	6.18
	Thyme 2%	86.58	12.63	61.43	8.63	32.62	7.50
LSD 5%		14.66	2.52	10.00	2.76	6.18	2.40
6 days	Control (0.0)	53.12	10.77	71.00	12.14	40.15	10.13
	Rosemary 1%	98.58	14.38	87.75	14.30	33.60	7.20
	Rosemary 2%	93.43	12.47	63.52	11.62	56.12	11.65
	Thyme 1%	57.95	9.92	65.63	11.37	47.83	10.77
	Thyme 2%	45.98	8.31	33.47	5.63	16.98	4.58
$LSD_{5\%}$		8.98	2.31	9.46	4.95	8.03	2.73

It is obvious from data recorded in Table (1) and Table (3) that treatments with *Rosmarinus* or *Thymus* extracts at 1% or 2% for 3 days showed a significant reduction in plant height, number of leaves, stem fresh and dry weights as well as leaves fresh and dry weights.

The data recorded by Amal (2010) on *Pisum sativum* indicated that shoot lengths, root length, fresh and dry weights of the seedling produced from seeds treated with *Thymus* and *Rosmarinus* extracts for three and six days showed highly significant increase with 1% and 2% of thymus extract for three and six days. On the other hand *Rosmarinus* extract showed a high reduction especially at treatment for six days. The fresh and dry weights of

seedlings treated with thymus extract showed a highly significant increase at 1% and 2% for three days and a highly significant reduction at 2% for six days.

The bad effect of these spices when used in different quantities may be due to the poisonous effect of aromatic compound including phenolic compounds and terpene-phenolic derivatives which are poisonous and can cause painful rash (Houlihan *et al*., 1985 and Imad *et al*., 2007).

Pigments Content:

Chlorophylls a and b and caratenoids as affected by *Rosmarinus* and *Thymus* extracts (1 or 2%) for 3 and 6 days are indicated in Table 4. Chlorophylls are known for their potential antioxidant capacity especially towards the lipid preoxidation mechanism (Lanfer-Marquez *et al*., 2005). Carotenoids play an important role in plant reproduction along with phenolics which are responsible for bright colours. This chemical class act as antioxidants, with functions that include protection of membranes against damage by free radicals and retardation of ageing processes (Bulda *et al*., 2008). The results revealed that *Jatropha curcas* produced from seeds treated with *Rosmarinus* extract (1%) for six days are the most rich in these components (Chlorphyll a,b and carotenoids). The treatment of Thymus extract (2%) for six days presented the lowest values.

Table 4: Effect of pre-emergence treatment with different concentrations of *Rosmarinus officinalis* L. and *Thymus capitatus* L. for different periods of treatment on pigments content of *Jatropha curcas* plants (mg/100g F.W).

Period of treatment	Treatments	Chl.a	Chl.b	Carotenoids
3 days	Control (0.0%)	184.52	83.27	185.51
	Rosemary 1%	120.80	46.84	136.63
	Rosemary 2%	133.69	48.72	206.29
	Thyme 1%	116.18	87.61	127.71
	Thyme 2%	158.03	69.49	128.66
$LSD_{5\%}$		27.28	7.94	16.34
6 days	Control (0.0%)	156.16	76.93	130.06
	Rosemary 1%	365.97	154.62	229.08
	Rosemary 2%	111.30	48.59	118.70
	Thyme 1%	109.87	52.74	120.21
	Thyme 2%	55.17	22.45	77.15
$LSD_{5\%}$		34.07	13.26	21.46

Enzymatic Activity:

Data presented in Table (5) revealed that the amount of perioxidase, polyphenol oxidase and esterase activity in *Jatropha curcas* plants was found to be the highest when both *Rosmarinus* and *Thymus* extracts (1% or 2%) for three days were used. This increment was in highest value with *Thymus* (2%) treatment (75%, 60.9% and 141.2%, respectively). However, when the same extracts were used for six days resulted in reduction in amount of enzymatic activity (Perioxidase, Polyphenol oxidase and esterase) except the treatment of *Thymus* at 1%. We can notice that the germination percentage was in highest value in response to treatment with *Thymus* extract at 2% for 3 days (Table 1) and this may attributed to the role of enzymatic activity which was in highest amount with the same treatment.

Table 5: Effect of pre-emergence treatment with different concentrations of *Rosmarinus officinalis* L. and *Thymus capitatus* L. on enzyme activity of *Jatropha curcus* L.

Period of treatment	Treatments	Peroxidase (A470/min/gm F.Wt)	Polyphenol oxidase (A470/in/gmF.Wt)	Esterase (Umg protein)
3 days	Control (0.0)	2.00	0.87	0.8
	Rosemary 1%	3.00	1.04	1.87
	Rosemary 2%	3.00	1.20	1.72
	Thyme 1%	2.00	0.93	0.9
	Thyme 2%	3.50	1.40	1.93
6 days	Control (0.0)	2.50	1.05	1.43
	Rosemary 1%	1.00	0.09	0.3
	Rosemary 2%	1.00	0.08	0.3
	Thyme 1%	2.50	2.47	1.31
	Thyme 2%	1.20	0.05	0.7

Researchers correlated different enzymes with the percentage of oil in seeds of *Jatropha* and found that accessions which have greater laccase enzyme activity showed greater oil percentage (Kumar *et al*., 2006). Selection for peroxidase and polyphenol oxidase enzymes activities will improve the nutritive quality of *Jatropha*. Researchers have demonstrated the role of all these enzymes during the development process in several crops. Agrawal *et al*. (2002) reported that polyphenol oxidase, peroxidase and cellulase had a critical role in the physiological process during the development of soybean. Also different enzyme activities and their role in several crops and trees for enhancement of the development process have been reported by Andrews *et al*., 2000 and Tarkka *et al*., 2001.

Esterase are involved in the metabolic processes of germination and maturation of plants. They are constituvely expressed in seeds during germination to release the reserve materials for the growing embryo (Thomas, 1993).

Most of *Lamiacea* plants contain considerable amounts of Rosmarinic acid, which in addition to its well known free radical scavenger properties and a natural antioxidant which is found as secondary metabolite in, rosemary displays antibacterial and antifungal properties (Dkoh *et al*., 2010).

Several studies have reported that many secondary metabolites are released into the environment, either as exudation from living plant tissues or by decomposition of plant material under certain conditions (Rice, 1984, Putnam, 1988 and Einhelling, 1995). These chemicals like phenols, terpenoids and alkaloids and their derivatives are potential inhibitors of germination and seedling growth (Macias *et al*., 1992; Siddiqui, *et al*., 1999 and Siddiqui & Zaman, 2004), enzymes activity (Borua & Das, 2000 and Cremer & Eichner, 2000).

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