ACTA Acta Sci. Pol., Hortorum Cultus 13(4) 2014, 3-12

USE OF EXTRACT FROM DRY MARIGOLD (*Tagetes* spp.) FLOWERS TO PRIME EGGPLANT (*Solanum melongena* L.) SEEDS

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Abstract. Although chemical priming agents have been successfully used to improve seed germination, emergence and seedling quality in different plant species, still they are not yet useful for organic cultivation. Marigold is a multipurpose plant and rich source of lutein and gallic acid, to be explored as an organic priming agent. The purpose of this study was to identify an organic priming agent for organic growing and to develop appropriate methods by using two different species of marigold (*Tagetes patula* L. and *T. erecta* L.). Herbal tea obtained from the dried flower of marigold was used as a priming agent for eggplant (*Solanum melongena* L) cv. Pala seeds. Hydropriming and control seeds were compared with organic priming (*T. patula* and *T. erecta*) for germination percentage, mean germination time, germination rate, two different emergence tests, seedling height, seedling fresh and seedling dry weights. Data obtained from the application of organic priming (*T. patula* and *T. erecta*) showed a difference in both the germination (82 and 80%), and the emergence (73 and 76%) from Controls and Hydropriming. Seeds emerged in, which unprimed in 52 and 39% respectively, when primed with *T. patula*, emerged in 73 and 56% respectively. The mean germination time and the mean emergence time were shortened compared with Controls and Hydropriming. MET decreased in 1 cm and 2.5 cm depth sowing from 14.0 and 16.1 days than 10.3 and 11.9 days in Emergence-1 and 10.4 and 10.2 days in Emergence 2, respectively. Seedling quality characteristics were examined in the seedling height, the seedling fresh and dry weights of *T. patula* and *T. erecta* applications were also statistically significant. This is the first report which results showed that marigold species can be used as a potential organic priming agent.

Key words: flower seed priming, seed germination, emergence, aubergine, marigold

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INTRODUCTION

Today the environmental issues and concerns are growing rapidly as humans advance their technology and pursue industrial development across the globe. Organic farming has been put forward as a positive response to these issues and concern by encouraging the organic material use at all stages of farming. Organically produced seeds and seedlings should be used in organic farming, i.e. seeds should not be treated with synthetic materials like pesticides, pelleting and priming agents [Groot et al. 2004]. Priming is a common method in which seeds are prepared by commercial applications to promote uniform germination, more even seedling emergence and enhancing germination rate [Brocklehurst and Dearman 1983]. It is a procedure that partially hydrates seeds so that germination processes begin, but radicle protrusion does not occur. Although in priming applications, have commonly been used various chemical compounds and synthetic hormones such as KNO_3 and PEG [Mavi et al. 2010a], $Ca(NO_3)_2$ [Sivritepe and Şentürk 2011], $CuSO_4$ [Patade et al. 2011], salicylic acid [Baninasab and Baghbanha 2013] and GA3 [Mavi et al. 2006] have commonly been used; alternatives for these chemicals are needed to be used in organic farming. The use of organic materials for increasing seed germination rate and uniform seedling emergence has gained importance in the recent years. Different organic materials, such as, seaweed, humic and fulvic acid [Matysiak et al. 2011], Moringa oleifera leaf extract [Basra et al. 2011], and grapefruit juice [Szopinska 2011] were investigated as possible priming agents. Based on these studies, Advantage and Probio commercial priming agents have been developed for sugar beets [Halmer et al. 2004]. Marigold, in addition to being an important ornamental plant, can be used for multiple purposes. The number of studies on virtually and subject in biology has increased over the past 3 decades. While initial studies were pertaining to chemical and biochemical composition of the plant and plant chemistry and biology in general, more recently interest has increased in the bioactivity of various plant extracts and their isolates. Other applications, such as a natural colorant for food from the flower pigments, have also emerged. This species is very important as a source of lutein pigment which showed to delay the effects of aging. In addition, essential oils obtained from marigold have been used as an insecticide, fungicide, bactericide and nematocide and have got positive results for pest control [Vasudevan et al. 1997]. Xu et al. [2011] have isolated 22 different phytochemical substances from the flowers of Tagetes erecta. Gallic acid, one of these substances, is an antioxidant agent that helps to protect cells against oxidative damage. Although the marigold species has a multipurpose, to the author knowledge, there are no published studies on marigold as an organic priming agent for seeds. In addition, the effect of organic priming techniques on the germination of naturally aged seeds is unknown. Hence, the present study was undertaken to test the possibility of using herbal tea obtained from dry flowers of two different species of marigold as an organic priming material and also to determine the effects of this organic priming agent on germination and emergence in naturally aged seeds of eggplant (*Solanum melongena* L.).

MATERIALS AND METHODS

Seed material. Eggplant seeds cv. Pala, which are commonly grown in Turkey, were used in the studies. Initial germination of naturally aged seeds was determined as 59% after conducting the standard germination test 3 times (50 × 3 seeds) [ISTA 2008]. Seed moisture content was identified as 5.7% by the high-temperature oven method (130°C, 1 h) [ISTA 2008].

Seed treatments with herbal tea and hydropriming. Two different species (*T. patula* L. and *T. erecta* L.) of marigold flowers were collected and the extracted petals of the flowers were dried for 10 days in the shade at room temperature. After the flower petals were dried, they were stored in glass jars in the laboratory until the treatment was conducted. The dried petals (4 g) were brewed in boiling, distilled water (1L) and after cooling, this herbal tea was used as an organic priming material.

The used organic priming applications are called in this paper shortly Erecta and Patula. The seeds were primed on top of filter paper moistened with 30 ml of the herbal tea, and kept at 25°C for 72 h, in the dark, in 15 cm Petri dishes. They were covered with plastic film, during the priming treatment, to ensure that there was no loss of moisture. Once the treatment was completed, the seeds were rinsed under tap water. Hydropriming was added in the study for comparison with the organic priming applications. Temperature, duration, and all other conditions were the same for both organic and hydropriming. All treated seeds were used in germination and emergence tests after surface drying. The unprimed dry seeds were kept as control.

Standard germination and seedling emergence tests. After the treatments, standard germination test was conducted by keeping 3 × 50 seeds in germination paper (20 × 20 cm) for 14 days at 25°C and this procedure was repeated twice. Germination was considered to have occurred when the radicle was 2 mm long. Germination rate [Maguire 1962] and mean germination time (MGT) [Ellis and Robert 1980] was calculated by daily counting. In addition, germination percentages were determined on the tenth day. At the end of the germination period, root length (mm) and seedling fresh weights (mg) were determined.

Emergence tests were conducted after surface drying and set up with 3 replications of 50 seeds in each treatment and run. To determine the effect of priming treatment on emergence at two different depths, seeds were sown at 1 cm and 2.5 cm depth in peat moss (pH 5.5–6.5, EC 40 mS/m, Potgrond P, Klasman, Germany) stuffed plastic boxes (30 × 19 × 5 cm). Seedlings were grown in the growing cabinet at 23 ± 2ºC for 24 days. The appearance of the hypocotyl hook on the peat moss surface was used as an emergence criterion and emerged seedlings were recorded daily. To find out the effect of the treatments on uniformity, emergence rate [Maguire 1962] and mean emergence time (MET) [Ellis and Robert 1980] were calculated. To determine seedling growth rate, following the stabilization of the number of emerged seedlings, destructive harvests were taken on randomly selected and normally developed 21 seedlings of each treatments. Such harvested seedlings height, fresh and subsequently dry weights by drying at 80ºC for 24 h were recorded. Fresh and dry weights were expressed as mg/plant.

Statistical analysis. SPSS was used for data analysis. The analysis of variance was done in all comparisons of germination and emergence percentage, MGT and MET, germination and emergence rate, seedling fresh and dry weight within the treatments. Percentages were arcsine transformed prior to analysis and were present the untransformed data in the tables and figures. Statistical significance for comparisons of germination and emergence were set at $p \leq 0.05$ with Duncan's range test.

RESULTS

Seed germination. Germination percentages in aged seeds were significantly ($p < 0.05$) different between those treated with organic priming (Patula and Erecta herbal tea) and the Control seeds in each germination test. The highest germination percentage (81%) was achieved by Patula priming and the lowest germination percentage (59%) was achieved by untreated seeds. Similar to Patula, Erecta treatment was more effective than both the Controls and Hydropriming in the germination tests (fig. 1).

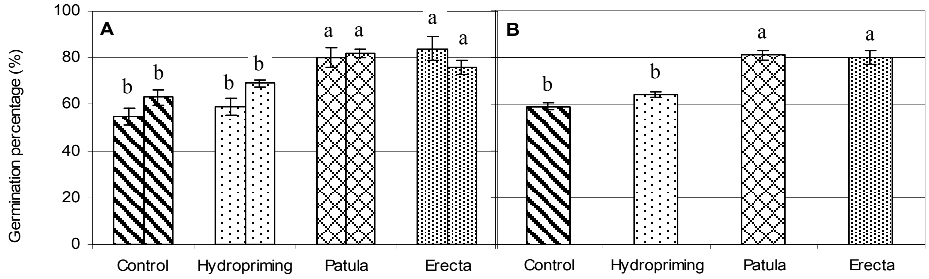

Fig. 1. Effect of priming of eggplant seeds with marigold dry flower extract on their germination (%), A: Repeated germination tests after priming, B: Mean of germination tests. Bars show standard errors, the different letters show statistical significance on treatments

Seed germination was slow because of the use of aged seeds. Germination began after 7 days in Controls, 4 days in Patula and Erecta priming, respectively. Therefore, germination percentages on the 10th day were used for comparison of treated and untreated seeds. Patula primed seeds reached 66% after 10 days, while unprimed seeds had 36% germination. Erecta primed and hydroprimed seeds were between Patula priming and untreated seeds. Parallel to germination percentages, MGT decreased from 11.0 in untreated to 7.9 days in Patula priming (tab. 1).

Germination rate was significantly different between the treatments ($p \leq 0.05$). It was calculated 2.85 in untreated seeds, while it was calculated the highest level 5.52 in Patula primed seeds. The germination rate of Erecta priming and hydropriming varied to 4.90 and 4.08, respectively (tab. 1).

Patula and Erecta priming treatments significantly improved radicle length and seedling fresh weight of naturally aged eggplant seeds compared to non-primed and hydroprimed seeds. The highest radicle length (18.7 and 17.7 mm) and seedling fresh weight (31.8 and 32.1 mg) were obtained from seeds primed in Patula and Erecta (tab. 1).

Table 1. Effect of priming of eggplant seeds with marigold dry flower extract on their germination (10^{th} day, %), mean germination time (MGT) (days), germination rate, root length (mm), and seedling fresh weight (mg)

Treatment	Repeat	Germination percentage (10^{th} day, %)	MGT (day)	Germination rate	Root length (mm)	Seedling fresh weight (mg)
Control	1	29 c	11.0 a	2.56 c	11.4 b	20.5 c
	2	43 b	11.1 a	3.13 c	11.6 b	20.1 c
	mean	36 C	11.0 A	2.85 C	11.5 B	20.3 B
Hydropriming	1	46 b	8.5 b	3.72 b	7.9 b	15.8 d
	2	61 a	8.4 c	4.44 ab	9.7 b	26.0 b
	mean	54 B	8.5 B	4.08 B	8.8 C	20.9 B
T. patula	1	67 a	7.7 c	5.71 a	17.5 a	29.9 b
	2	65 a	8.1 c	5.33 a	20.0 a	33.8 a
	mean	66A	7.9 C	5.52 A	18.7 A	31.8 A
T. erecta	1	73 a	7.5 c	5.94 a	15.9 a	35.2 a
	2	45 b	9.5 b	3.86 bc	19.4 a	29.1 b
	mean	59 AB	8.5 B	4.90 A	17.7 A	32.1 A

Seedling emergence. When compared to those untreated and hydropriming in two different sowing depths, Patula priming had significantly ($p < 0.05$) higher emergence percentage. Treated eggplant seeds with Patula priming had 73 and 56% seedling emergence in 1-cm and 2.5 cm deep sowing, respectively. Corresponding percentages were 52 and 39% in the untreated seeds, respectively. The difference between the Patula priming and untreated seeds at final count was 21 and 17% in 1 cm and 2.5 cm deep sowing, respectively (fig. 2).

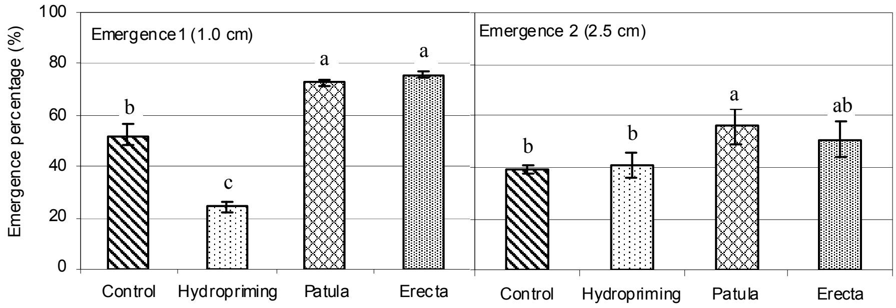

Fig. 2. Effect of priming of eggplant seeds with marigold dry flower extract on their emergences (%) at different depth, A: Sowing at 1 cm depth, B: Sowing at 2.5 cm depth. Bars show standard errors, the different letters show statistical significance on treatments

Table 2. Effect of priming of eggplant seeds with marigold dry flower extract on their mean emergence time (MET) (days), emergence rate, seedling length (cm), seedling fresh weight (mg) and seedling dry weight (mg)

	Treatments	MET (day)	Emergence rate	Seedling length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Emergence 1 (1 cm)	control	14.03 a	1.94 b	2.66 b	390 a	41 b
	hydropriming	11.54 b	1.07 c	2.76 b	461 a	56 ab
	T. patula	10.26 c	3.67 a	3.73 a	517 a	66 a
	T. erecta	10.43 c	3.84 a	3.62 a	533 a	69 a
Emergence 2 (2.5 cm)	control	16.13 a	1.24 b	2.05 c	417 b	41 c
	hydropriming	11.22 b	1.94 ab	2.52 b	563 b	65 b
	T. patula	11.86 b	2.54 a	3.25 a	933 a	96 a
	T. erecta	10.21 b	2.51 a	3.07 a	835 a	90 a

MET, emergence rate, seedling length, seedling fresh and seedling dry weight are depicted in Table 2. Mean emergence time, emergence rate, seedling length, seedling fresh and seedling dry weight were recorded in Patula primed seeds as 10.26 days, 3.67, 3.73 cm, 517 mg, and 66 mg in Emergence-1, 11.86 days, 2.54, 3.25 cm, 933 mg, and 96 mg in Emergence-2, respectively. Patula primed seeds emerged 4 days earlier and caused more uniform seedling than the untreated seeds in both the sowings. The emergence rate increased with effect of treatments, particularly, seedling length, seedling fresh weight and seedling dry weight of Patula priming were better than untreated group in both sowing the conditions (tab. 2).

DISCUSSION

Natural aging in the tested seed lot is likely to be responsible for disrupting the uniformity of germination within the lot. The seed lot was used to test the effectiveness of treatments. The results clearly showed that Patula and Erecta priming resulted in improved germination and emergence in naturally aged eggplant seeds.

During the used priming treatments radicle protrusion did not occur for the effectives of technique. For this reason, treatment durations and temperatures are very important. Varying temperature (10–30ºC) and duration periods ranging from 1 to 15 days were used in previous studies. For example, Trigo and Trigo [1999] reported that 20ºC and 48 h combination was the most appropriate in priming treatment of eggplant seeds, while the present study used 25ºC and 72 h in naturally aged eggplant seeds.

Seaweed [Sivritepe 2000, Yıldırım and Güvenç 2005, Demir et al. 2006, Liu et al. 2011, Matysiak et al. 2011] has been the most widely used organic seed priming material so far. Further, different plant materials as Moringa oleifera [Basra et al. 2011, Thirusenduraselvi and Jerlin 2007], Melia azedarach, eucalyptus [Mehta et al. 2010], grapefruit juice [Szopinska 2011] and blue-green alga [Liu et al. 2011] as Nostoc commune were used to enhance seed germination and emergence in the recent years. However, the literature review did not reveal any study on marigold flowers as the organic priming material. Its flowers found to be important in organic priming as an alternative agent which can be obtained easily and applied with a simple technique.

An extended period of the seedling in the seed bed, as is the case when deep sowing, may increase the risk of infection by 'damping-off' pathogens such as *Fusarium* and *Pythium*. The enhanced and more rapid seedling emergence provided by priming treatments reduce the time that seedlings are covered in the seed bed, thereby alleviating the possibility of pathogenic attack. Fast growing and healthy seedlings may be even more important for the organic farmer than for the conventional one. Moreover, fast growing seedlings can improve competition with weeds for nutrients and light [Groot et al. 2004]. Similar effects were also achieved in this experiment, what suggests that priming with *T. patula* and *T. erecta* improved vigorous seedling in the eggplant seeds.

To determine the activity of Patula and Erecta priming, they were tested in naturally aged seeds. The results of the study indicated that priming with Patula and Erecta resulted in 21% germination and 22% emergence in 1-cm sowing which were higher than the germination and emergence of non-primed seeds. Also, the Patula and Erecta priming resulted in an increase in the quality of seedlings. Similar effects were found that smoke priming of melon [Mavi et al. 2010b] and eggplant [Demir et al. 2009] seeds. However, priming with Kinetin and PEG [Demir et al. 1994] did not produce a positive effect on germination of naturally aged seeds of eggplant.

Seed aging decreases seed quality by reducing resistance to stress conditions, germination and emergence percentages. In the previous studies, priming treatments were effect on a repair mechanism artificially aged seeds [Demir and Mavi 2004, Demir et al. 2009, Mavi et al. 2010b, Kibinza et al. 2011]. Thus, aged seeds could benefit more than unaged seeds from priming treatments. Comparable effects have also been identified in this study used naturally aged eggplant seeds. Treated by the application of certain techniques create a repair effects in a previous phase of DNA synthesis for the repair of damages of aging in seeds. Several different physiological mechanisms may be involved in the repair process effected by priming treatments, and may include enhanced protein and DNA synthesis [Côme 1983], RNA synthesis [Coolbear and Grierson 1979] and changed enzymes activity in L-isoaspartyl methyltransferase and catalase [Kester et al. 1997, Kibinza et al. 2011]. Although enzymes activity, such as catalase and peroxidase, were not examined in the recent study, future research should study the enzyme activity.

CONCLUSIONS

Poor seed germination, emergence and poor seedling quality are a common phenomenon under sub-optimal conditions which is a great concern of growers who grow seedlings in late winter, early spring at deep sowing. This negative effect is more pronounced in aged seeds. In conclusion, seed germination and seedling emergence of aged eggplant seeds can be improved by *T. patula* and *T. erecta* priming in 30 ml solution for 3 days. The emergence and MET of primed seeds was also promoted at different sowing depth (1 and 2.5 cm). The results of this study suggests that organic priming with dry flowers of marigold is a simple, easy, inexpensive and eco-friendly approach and has a significant effect in both improving the emergence of stress conditions, and in the repair of aging seeds. Moreover, the seed germination and vigour could be improved by pre-sowing seed priming treatments with non-toxic and eco-friendly organic sources like *T. patula* and *T. erecta*.

ACKNOWLEDGEMENTS

The author acknowledge with thanks Dr. Hasan F. Mavi and Ms. Shannon R. Mavi for their assistance in English language, grammar and writing. I express my gratitude to the Scientific Research Project Directorate of the University of Mustafa Kemal for financial support (1001 M 0103).

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UŻYCIE EKSTRAKTU Z SUSZONYCH KWIATÓW AKSAMITKI (*Tagetes* spp.) DO STYMULOWANIA ROZWOJU NASION BAKŁAŻANA (*Solanum melongena* L.)

Streszczenie. Chociaż chemiczne czynniki wzrostu są skutecznie stosowane do polepszenia kiełkowania nasion, wschodów i jakości sadzonek u różnych gatunków roślin, to nie są one jeszcze używane w uprawie organicznej. Aksamitka jest rośliną o wielu zastosowaniach: jest jednocześnie źródłem luteiny i kwasu galusowego i jako taką warto badać ją jako organiczny czynnik wzrostu. Celem niniejszego badania było zidentyfikowanie organicznego czynnika wzrostu do uprawy organicznej oraz opracowanie właściwych metod poprzez zastosowanie dwóch różnych gatunków aksamitki (*Tagetes patula* L. i *T. erecta* L.). Herbatka ziołowa przygotowana z suszonych kwiatów aksamitki została użyta jako czynnik wzrostu nasion bakłażana (*Solanum melongena* L) odmiany Pala. Porównano hydrokondycjonowanie oraz nasiona kontrolne z pobudzaniem organicznym (*T. patula* L. i *T. erecta* L.). pod względem procentu kiełkowania, średniego czasu kiełkowania, wskaźnika kiełkowania, dwóch testów wschodów, wysokości sadzonek oraz świeżej i suchej masy sadzonek. Dane uzyskane po zastosowaniu organicznego pobudzania wzrostu (*T. patula* L. i *T. erecta* L.) wykazały różnicę zarówno w kiełkowaniu (82 i 80%), jak i wschodach (73 i 76%) pochodzących z hydrokondycjonowania i kontroli. Nasiona wzeszły, odpowiednio, w 52 i 39%, natomiast gdy były pobudzane za pomocą *T. Patula*, wzeszły, odpowiednio, w 73 i 56%. Średni czas kiełkowania oraz średni czas wschodów były krótsze w porównaniu z kontrolą i hydrokondycjonowaniem. MET zmniejszył się na głębokości siewu 1 cm i 2,5 cm odpowiednio z 14 i 16,1 dni do 10,3 i 11,9 dni dla wschodów oraz z 10,4 do 10,2 dni. Zbadano jakość sadzonek i ich wysokość, świeża i sucha masa *T. patula* L. i *T. erecta* L. były także statystycznie istotne. Niniejsze badanie jest pierwszym, którego wyniki wykazały, że gatunki aksamitki moga być stosowane jako potencjalny czynnik organiczny pobudzający wzrost.

Słowa kluczowe: pobudzanie nasion kwiatów, kiełkowanie nasion, wschody, bakłażan, aksamitka

Accepted for print: 3.03.2014