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The Effect of Organic Priming with Marigold Herbal Tea on Seeds Quality in Aji Pepper (*Capsicum baccatum* var. p*endulum* Willd.)

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Abstract

The influence of priming (KNO_3, *Patula* and *Erecta*) on the germination percentage, mean germination time, germination index, seedling emergence, mean emergence time, seedling weight and emergence index investigated in which harvested during five different developmental stages (D1, D2, D3, D4 and D5) of *C. baccatum* var. *pendulum* pepper seeds. The seeds were harvested at various stages of development based on fruit skin colour. The results revealed that physiologically mature seeds (after D3 stage) showed higher seed quality and performance. The greatest advantage of priming was observed at seed stages D2 and D3. Particularly, *Patula* treatment at the D2 stage resulted in 10% higher germination, 4-day faster mean germination time, 15% higher seedling emergence rates, 52 mg heavier seedling weights, and higher emergence index with respect to untreated seeds. Consequently, *Patula* priming, an inexpensive, organic, eco-friendly and simple technique, has been proposed to improve seed quality in Aji pepper. In addition, priming had minimal effect on lower average emergence time, higher seedling weight and emergence index for seeds in the D4 and D5 stages.

Key words: Development stages, organic priming, pepper seedling, mass maturity, priming agents

Çan Biberinde(*Capsicum baccatum* var. *pendulum* Willd.) Tohum Kalitesi Üzerine Kadife Çiçeği Bitki Çayı ile Yapılan Organik Primingin Etkisi

Özet

Çan biberinde (*C. baccatum* var. *pendulum*) farklı tohum gelişim aşamalarındaki (D1, D2, D3, D4 ve D5) tohumların kalitesini organik priming (KNO_3, *Patula* and *Erecta*) tekniği ile arttırılması hedeflenen çalışmada çimlenme oranı, ortalama çimlenme süresi, çimlenme indeksi, çıkış oranı, ortalama çıkış süresi, fide yaş ağırlığı ve çıkış indeksi belirlenmiştir. Gelişim safhaları meyve dış rengine göre sınıflandırılmıştır. Sonuçlar fizyolojik olgun (D3'den sonra) tohumların tohum kalitesi ve performansının daha yüksek olduğunu göstermektedir. Primingin en önemli avantajı D2 ve D3 gelişim dönemlerindeki tohumlarda tespit edilmiştir. Özellikle *Patula* uygulaması kontrole göre D2 gelişim dönemindeki tohumlarda çimlenme oranını %10 arttırmış, ortalama çimlenme süresini 4 gün kısaltmış, çıkış oranını %15 arttırmış, yaş fide ağırlığını 52 mg artırmış ve çıkış indekisinde de artışa sağlamıştır. Bu nedenle, *Patula* priming ucuz, organik, çevre dostu ve basit uygulanabilir bir yöntem olarak çan biberinde tohum kalitesini artırmada etkili bir teknik olarak bulunmuştur.

Key words: Tohum gelişimi, organik priming, biber fidesi, kütle olgunluğu, priming ajanı

Introduction

The Capsicum genus includes 30 species with different plant habitats, flowers, and fruits. Five *Capsicum* species (*Capsicum annuum* L.*, C. baccatum* L., *C. chinense* Jacq.*, C. frutescens* L.*, C. pubescens* Ruiz & Pav.)

have been cultivated worldwide. *Capsicum baccatum* var. *pendulum* (Willd.), commonly referred to as "Aji or Peruvian hot pepper", is one of the five cultivated species in this genus (Jarret, 2007). Optimal pepper seed germination and seedling emergence occur at 25-28 °C. Under sub-optimal temperatures, low-level seed germination is a common problem for pepper seedling farmers during late winter and early spring worldwide. *C. baccatum* is important for genetic material of low temperature emergence (Gerson and Honma, 1978). The mother plant is an important component for seed quality development in fleshy fruit species, such as Aji. Aji (*Capsicum baccatum*) seed development has been rarely studied compared with cultivars of *Capsicum annuum*.

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). To promote seed priming, the use of organic materials, such as herbal tea, and salts, such as KNO_3, is efficient for germinating pepper seeds at low temperatures (Sachs et al., 1980; Mavi et al., 2013). Furthermore, the stage of seed maturation is a significant aspect of germination performance at lower temperatures and responses to priming treatment (Olouch and Welbaum, 1996). Improved germination performance has been observed in well-grown seeds under stress temperatures compared with seeds harvested during the early and late stages; however, priming had greater effects at early harvest stages (e.g., premature seeds). Consequently, priming is a beneficial technique for increasing germination and the uniformity of heterogeneously well-grown seed lots in melon (Olouch and Welbaum, 1996). Organic seeds and seedlings should be used in organic farming, and priming is also important for organic production. Primed onion and carrot seeds showed greater seedling emergence than non-primed seeds when cultivated on organic soils (Groot et al., 2004). In this study, the effect of salt (KNO_3) and organic (*Patula, Erecta*) priming on the germination, seedling emergence and seedling growth of Aji pepper seeds was examined using seed lots harvested at five different fruit maturity stages.

Material and Methods

Seed material

Plants of the Aji pepper cultivar 'Çan' (*Capsicum baccatum* var. *pendulum*) were grown between March and October in Hatay in 2011. The methods for cultivating these plants were similar to those of George (1985) for the entire developmental stage. Sixty fruits were harvested at five different development stages (D1 green, D2 mature green, D3 breaker, D4 mature and D5 over mature). The fruit skin colour at each development stages was measured using a Minolta CR200. The skin colour was expressed as *L, a, b, C* and hue angle (*H*). The seeds were manually collected from the fruit and dehydrated over mesh trays under shadow for 2 days at 25 °C and 45-50% RH.

The seed moisture contents were determined using the high-temperature oven technique (130 °C, 1 h) for fresh seeds. After drying, the seeds (6.8 and 7.8 % moisture content) were stored at 5 °C in plastic containers until priming was conducted. The seed weight (g) was determined after weighing on a precision balance (0.0001 g) using 8 × 100 seeds. The initial viability of the lots was determined using a standard germination test.

Seed treatment with herbal tea and osmopriming

Marigold flowers of the *Tagetes patula* and *T. erecta* species were growed and gathered in our experimental field. The petals of these flowers were extracted and dehydrated for 10 days under shadow at room temperature. The distinct colours of the flowers of *T. erecta* and *T. patula* are orange and yellow and dark red, respectively. The

flower petals were dried and stored in glass jars in the laboratory prior to treatment. A total of 4 g of the dried petals were brewed in 1000 ml of boiling, distilled water, followed by cooling, and this herbal tea was used as an organic priming solution (Mavi, 2014). The names of species of Erecta and Patula were used to classify the organic priming applications. To compare salt priming to organic priming, 3% KNO3 was used in the present study.

As described in Mavi (2014), the harvested seeds from each group were primed over dampened filter paper using 18 ml of priming solution at 25 °C for 24 h in the dark in 15-cm Petri dishes. During priming, the Petri dishes were covered with plastic film to avoid water loss. The seeds subjected to priming treatments were subsequently washed with tap water and subjected to surface dried.

Germination and seedling emergence tests

Following priming, the germination test was conducted for 3 replications of 50 seeds over 20 cm×20 cm germination paper for 14 days at 25 °C. Germination was determined when the radicle reached 0.2 cm. Based on Maguire (1962) and Ellis et al. (1980), the germination index and mean germination time (MGT) were calculated using daily counts.

To examine emergence, 3 replications of 50 seeds were used for each treatment and each development stage. The seeds were sown at a 1 cm depth in peat moss packed into plastic boxes (30 cm length, 19 cm width and 5 cm depth). The emergence tests were cultivated inside the growing cabinet at 23 ± 2 °C for 24 days. Emergence was considered when the hypocotyl hook showed on the compost surface, and emerged seedlings were recorded daily. The emergence index and mean emergence time (MET) were calculated according to Maguire (1962) and Ellis et al. (1980), respectively. After a sufficient number of emerged seedlings reached stability, destructive harvesting was performed on 21 random and normally grown seedlings of each treatment to determine the seedling performance. Fresh and dry (under 80 °C for 24 h) seedlings were recorded as mg/plant.

Statistical analysis

SPSS was used for data analysis. Statistical significance for primed and control seeds were evaluated using Percentages were arcsine transformed prior to analysis and were present the untransformed data in the tables and figures.Duncan's multiple tests, with $p \leq 0.05$ at each developmental stage.

Results and Discussion

Hue angle (*H*) and *L* decreased while *a*, reflecting a colour change from green to red, increased in the same stages, but Croma (*C*) and *b* were not affected change. The original colours of the fruit skins are shown in Table 1. The seed moisture content declined from 59 % at D1 to 37 % at the D5 development stage (Figure 1). After drying, the seed moisture contents were changed from 7.8 % to 6.8 % (Table 2). The seed weight progressively increased with maturity, and reached mass maturity at D3 with 5.6 g (Figure 1).

Seed moisture content was changed between 65% and 51% depending on the priming treatment and developmental stages. Total germination for unprimed seeds was 2 % at the D1 stage. Thereafter, this rate increased from 46 % to 94 % between stages D2 and D4 in unprimed seeds. Priming treatments weren't changed total germination. Priming with *Patula* increased the germination index and decreased the mean germination time at all development stages, except D1. Priming treatments resulted in a significant ($p \leq 0.05$) increase in the germination index and a decrease in the mean germination time at all development stages. The germination index reflects the strength of a seed to germinate as an exceptional indicator of seed vigour, demonstrating that higher the germination index value the greater the vigour of the seed. The germination index was higher in

the treated seeds compared with unprimed seeds (Table 2).

Table 1. Fruit skin colour in different developmental stages described by means of *L*, *a*, *b*, chroma (*C*) and hue angle (*H*)

Çizelge 1.Farklı gelişim safhalarındaki meyvelerin L, a, b, kroma(C) ve hue açı(H) renk değeri ortalamaları

Development stages	*L*	*a*	*b*	*C*	*H*	Original
D1	65.6	-17.3	49.8	52.8	109.1	
D2	68.5	-9.3	55.9	56.8	99.4	
D3	65.6	9.4	59.9	61.2	81.1	
D4	52.3	36.5	44.1	57.3	50.2	
D5	47.8	40.5	37.0	55.0	42.3	

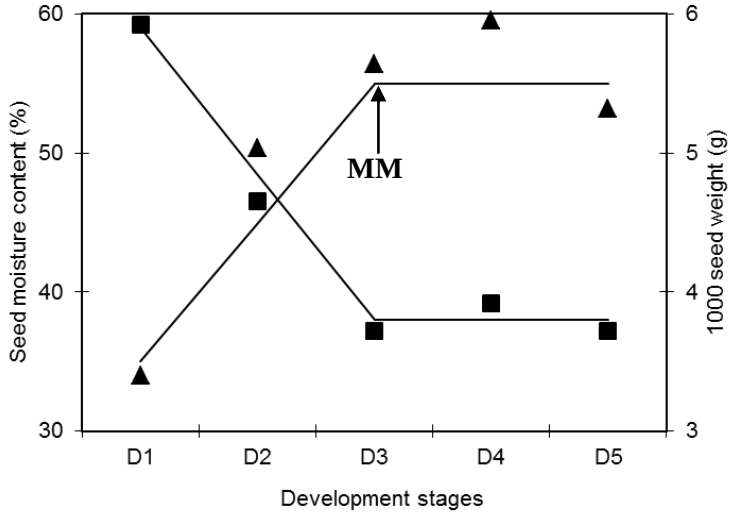

Figure 1. Seed moisture contents (■) and 1000 seed weight (▲) in different development stages of seeds. An arrow shows that mass maturity.

Şekil 1. Tohum gelişim safhalarına bağlı olarak tohum nem içeriği (■) ve 1000 tohum ağırlığı (▲) değişimleri, MM kütle olgunluğunu göstermektedir.

Emergence and MET are shown in Table 3. All priming methods increased emergence percentages at the D2 stage. While unprimed seeds emerged 45%, *Patula*-primed seeds emerged 60 % at this stage. The maximum effect was determined using *Patula* (91 %) priming at the D3 stage. Because of increasing seed quality, the effect of priming was decreased at stages D4 and D5. MET was also decreased in primed seeds at stages D2 and D3, except for *Patula* priming at the D2 and D3 stages (Table 3).

Table2. Seed moisture content (smc), germination (%), mean germination time (MGT) and germination index in different developmental stages of primed and unprimed seed lots

Çizelge 2. Farklı gelişim safhalarındaki prime edilmiş tohumların tohum nemi (%) çimlenme oranı (%), ortalama çimlenme süreleri (gün) ve çimlenme indeksleri

Stages	Treatment	SMC (%)	Germination (%)	MGT (days)	Germination Index
D1	Control	7.8	2	13.00	0.11
	KNO_3	59.0	1	3.00	0.33
	Patula	64.0	3	6.00	0.26
	Erecta	65.0	5	7.00	0.29
D2	Control	7.2	46 a	9.91 a	1.39 b
	KNO_3	56.0	23 b	7.97 b	0.85 b
	Patula	57.0	54 a	5.70 c	2.61 a
	Erecta	55.0	56 a	5.88 c	2.66 a
D3	Control	7.1	84 a	7.96 a	3.21 b
	KNO_3	52.0	62 b	5.50 b	3.10 b
	Patula	52.0	90 a	4.62 c	5.40 a
	Erecta	52.0	86 a	4.62 c	4.94 a
D4	Control	6.8	94 a	7.08 a	4.16 b
	KNO_3	54.0	90 a	4.24 b	5.67 a
	Patula	53.0	96 a	4.20 b	6.43 a
	Erecta	51.0	93 a	4.03 b	6.45 a
D5	Control	7.0	88 a	5.65 a	4.54 b
	KNO_3	53.0	92 a	3.95 b	5.98 a
	Patula	53.0	94 a	4.37 b	6.15 a
	Erecta	51.0	94 a	4.24 b	6.31 a

Table 3. Seedling emergence (%) and mean emergence time (MET) in different development stages in primed and unprimed seed lots

Çizelge 3. Çıkış oranları ve ortalama çıkış süreleri

Stages	Treat.	Emer. (%)	MET (days)
D2	Control	45 b	13.36 a
	KNO_3	52 ab	11.99 b
	Patula	60 a	14.18 a
	Erecta	52 ab	11.72 b
D3	Control	75 bc	13.32 a
	KNO_3	69 c	11.44 b
	Patula	91 a	13.02 a
	Erecta	83 ab	11.59 b
D4	Control	92 ab	11.25 b
	KNO_3	93 ab	12.34 a
	Patula	89 b	11.24 b
	Erecta	100 a	10.87 b
D5	Control	91 a	11.38 a
	KNO_3	91 a	9.20 c
	Patula	93 a	11.37 a
	Erecta	84 b	10.80 b

The seedling fresh weight and emergence index are shown in Figures 2 and 3. Unprimed and primed seeds showed significant differences at all stages ($p < 0.05$). The emergence index increased as an effect of the treatments, particularly, the seedling fresh weight of KNO_3-primed seeds was better than unprimed group at all development stages. Also *Patula* and *Erecta* priming were higher than unprimed seeds in D2 and D3.

Mass maturity was observed at the D3 stage, and the seeds showed the maximum seed weight at this stage (Figure 1). Nascimento et al. (2011) observed similar results, showing that physiological maturity occurred at 60-70 days after anthesis, when the fruits appeared light red in *C. baccatum* var. *pendulum* cv. Mari.

Seed development and seed priming have been extensively examined in pepper cultivars of *C. annuum* (Sanchez et al., 1993; Demir and Ellis, 1994; Vidigal et al., 2011), however few studies have been conducted in cultivars of *C. baccatum* var. *pendulum*. It has been determined with this present study that D4 and D5 stages are the best stages in term of seed quality in *C. baccatum.* Pagamas and Nawata (2008), reported that elevated temperatures are a critical factor in seed development and quality during this stage of chili pepper (*C. annuum* cv. Shishito).

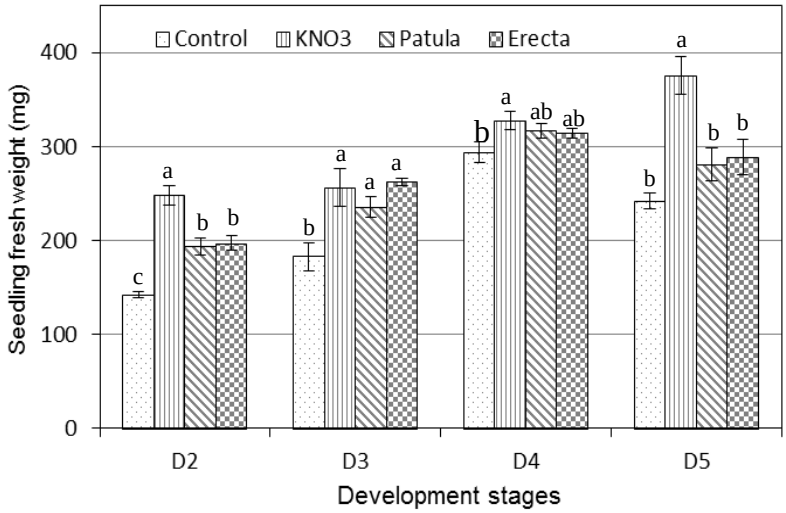

Figure 2. Mean seedling fresh weight (mg) of primed and unprimed seeds ($P > 0.05$). Different letter in same development stage is statically significant.

Şekil 2. Farklı gelişim safhalarındaki prime edilmiş ve edilmemiş tohumların fide yaş ağırlıkları (mg). Aynı gelişim safhasındaki farklı harfler istatistiksel olarak önemlidir.

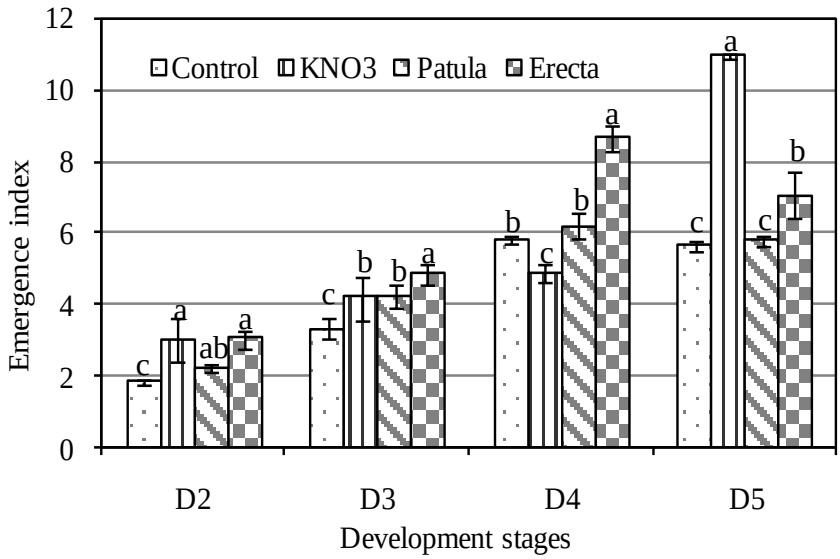

Figure 3. Emergence index of primed and unprimed seeds ($P > 0.05$). Different letter in same development stage is statically significant.

Şekil 3. Prime edilmiş ve edilmemiş tohumların çıkış indeks değerlerindeki değişimler. Aynı gelişim safhasındaki farklı harfler istatistiksel olarak önemlidir.

In general, seaweed (Demir et al., 2006; Liu et al., 2011; Matysiak et al., 2011) is often utilised as an organic seed priming material. Moreover, different plant materials, such as *Moringa oleifera* (Basra et al., 2011), *Melia azedarach*, eucalyptus (Mehta et al., 2010), propolis, maize grain extracts (Semida and Rady, 2014) and grapefruit juice (Szopinska, 2011), have been used to enhance seed germination and emergence in recent years. However, the literature review did not reveal any studies on marigolds, extremely viable organic flowers, as priming materials in *C. baccatum*. These flowers can be used as an easily gathered substitute priming material, with a simple application method (Mavi et al., 2013; Mavi, 2013; Mavi, 2014).

The flowers of *T. patula* and *T. erecta* contain sugar, Gallic acid, lutein, vitamins, amino acids and terpenoids (Vasudevan et al., 1997). Semida and Rady (2014) reported that terpenoids are considered as the precursors of many phytohormones (particularly, gibberellins), which are necessary for growing plants. Thus, these compounds were demonstrated as highly effective priming agents in this study, and this effectiveness has been recently confirmed for different species (Mavi et al., 2013; Mavi, 2013; Mavi, 2014).

Early uniformed emergence and germination were observed in D4 and D5 stage seeds compared with D3, D2 and D1 stage seeds. *Patula* and *Erecta* priming presented the best effects on MGT and the germination index at the D2 and D3 stages. The results of the present study support the findings of Mavi et al. (2013), Vidigal et al (2011) and Nascimento et al. (2011), who reported the early and increased germination of primed pepper seeds. Similar effects were observed with the *Patula*-primed seeds of different species, such as ornamental plants (Mavi, 2013) and eggplant (Mavi, 2014). Similarly, Demir and Mavi (2003) indicated that treatment extended seed quality in immature eggplant seeds compared with mature seeds, consistent with the results of the present study.

Priming reduced MET and increased seedling emergence and fresh weight in development stages of *C. baccatum* var. *pendulum*. The greatest benefit of priming was observed for seeds harvested at the D2 and D3 developmental stages. Seeds harvested later (D4 and D5) showed higher emergence, emergence index and seedling fresh weights than less mature (D2 and D3) seeds. Priming showed smaller effects on more mature seeds. The improved effect of priming with KNO_3, PEG and seaweed extract on the emergence of different *Capsicum* species explains previous findings concerning the germination of pepper seeds at low temperatures under laboratory conditions (Sachs et al., 1980; Demir et al., 2006; Demir and Mavi, 2008].

This positive effect of priming was clearly reflected in subsequent seedling fresh weights, consistent with the observations of Mavi et al. (2013) in pepper seeds. A vigorous seed can produce a better seedling fresh weight at different development stages, particularly stages D2 and D3, compared with non-vigorous seeds.

The emergence index was higher with *Erecta* priming compared with control seeds, except at stage D5, consistent with the results of Mavi et al. (2013) in *C. annuum* cv. Sena and Mavi (2014) in eggplant.

Conclusions

Consequently, to determine the correct time of seed harvest to obtain the highest germination efficiency and establish emergence conditions, *C. baccatum* pepper seeds should be harvested at the D4 and D5 stages. In addition, when the seeds are harvested at earlier stages, *Patula*, *Erecta* and KNO_3 priming can be applied to increase seed quality. *Patula*, *Erecta* and KNO_3 priming effectively increased germination, emergence and the seedling fresh weight at different maturity levels of *C. baccatum* pepper species. Particularly, *Patula* and *Erecta* are organic priming agents that can be used in organic systems. 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, and in the repair of undeveloped seeds. From the finding of the present study, it has been also suggest that investigation of *Patula*, *Erecta* herbal tea to develop fungicidal seed treatments should be further investigated. However, further comprehensive studies on the effects of chemical and organic priming agents will be beneficial for other vegetables seeds.

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