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## Effect of seed soaking with nitrophenolate–based biostimulant on germination and growth of chilli var. Kanchanaburi 1

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**Abstract** The results showed that there was significant difference in germination rate. Three ppm of nitrophenolate–based biostimulant increased the germination rate more than the other treatments. However, there was no significant difference among treatments in the leaf number at 14 and 21 days after planting and also the plant height at 14 days after planting. The seeds which were soaked in 7 ppm of nitrophenolate–based biostimulant for 5 hours, resulted in the tallest plant height at 21 days after planting.

**Keywords:** Chilli, Nitrophenolate, Biostimulant, Germination, Growth

### Introduction

Chilli (*Capsicum annuum* L.) is the most important spice crop in Thailand. This plant belongs to the family Solanaceae family. Fresh chilli fruit is well-known to be the quality source of pro-vitamin A, vitamin C and E, carotenoids and phenolic compounds, metabolites with renowned antioxidant property that bring good impacts on human health conditions which seem to able against cancers, preclude from gastric ulcer as well as trigger the immune system (Materska and Perucka, 2005; Sun *et al.*, 2007; Khaba *et al.*, 2020). Thailand produced 251, 665 tonnes of chilli from an area of 21,416 hectare and the productivity is 11.75 t/ha. (ITCC DOAE, 2022). Chilli in Thailand has many varieties and can be differentiated according to different sizes and shapes. Chilli var. Kanjanaburi 1 was the one of famous variety in Thailand. The specific characteristics of this variety was guaranteed by Department of Agriculture in 2016. The important characteristics of var. Kanjanaburi 1 was good smell, extreme spicy, high production, market demanded shape and grew well under sunlight (DOAE, 2016).

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The most critical stage of the plant life cycle is germination (Diel *et al.*, 2019). Seed germination will affect the establishment of plants which related to crop yield and quality (Yuniati *et al.*, 2019). They have been reported that the germination of chili pepper is slow and non-uniform under the favorable and also unfavorable conditions (Yadav *et al.*, 2011; Barchenger and Bosland, 2016). This problem also occurred in Chilli var. Kanjanaburi 1.

In order to overcome these issues, there are several techniques and method including using biostimulant. Nitrophenolate-based biostimulant (atonik) is the aromatic nitro phenolic compound which consists of sodium ortho-nitrophenol, para-nitrophenol and sodium nitro-guaiacol as active ingredients. Atonik stimulates plant activity without causing malformation or toxicity to the plants and accelerates the plasma streaming of the cells by increase in the endogenous auxin level (Djanaguiraman *et al.*, 2005). It has been reported that atonik increased the germination rate of cotton and tomato (Djanaguiraman *et al.*, 2005). Therefore, the objective was to study the effects of seed soaking with nitrophenolate-based biostimulant on germination and seedling growth of chilli var. Kanjanaburi 1.

## **Materials and methods**

The experiment was designed as a Completely Randomized Design (CRD) with 5 treatments and 3 replications. Each replication consisted of 50 chilli seeds. The treatments included: control (non-soaking), soaking in distilled water and three concentrations of nitrophenolate-based biostimulant (3, 5 and 7 ppm). The chilli seeds were soaking in distilled water and nitrophenolate-based biostimulant for 5 hours after that they were planted in cell plug trays with peat moss. Water was daily supplied in the morning. The experiment was conducted from November to December 2023 at Plant Propagation Center No. 1, Chonburi Province, Thailand. The germination rate was recorded at 14 days after planting. The plant height and leaf number were recorded at 14 and 21 days after planting. At the end of experiment, the leaf increasing number and plant increasing height were calculated. Statistical comparisons were performed by analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT).

## **Results**

### ***Germination rate***

The results showed that there was significant difference in percentage of germination rate among treatments. The significantly highest germination rate of

chilli seeds was found in the 3 ppm of nitrophenolate-based biostimulant treatment ( $52.00 \pm 1.90\%$ ). Then the lower germination rates of chilli seeds were followed with 7 ppm of nitrophenolate-based biostimulant, distilled water, 5 ppm of nitrophenolate-based biostimulant which the germination rates were  $41.33 \pm 2.19\%$ ,  $39.33 \pm 1.52\%$  and  $38.67 \pm 2.46\%$ , respectively. The lowest germination rate of chilli seeds was showed in the control treatment ( $26.00 \pm 2.67\%$ ) (Table 1).

**Table 1.** Germination rate of chilli var. Kanchanaburi 1 at 14 days after planting

Treatments	Germination rate (%) <sup>1/</sup>
Non-soaking (Control)	$26.00 \pm 2.67^c$
Soaking in distilled water	$39.33 \pm 1.52^{bc}$
Soaking in 3 ppm of nitrophenolate-based biostimulant	$52.00 \pm 1.90^a$
Soaking in 5 ppm of nitrophenolate-based biostimulant	$38.67 \pm 2.46^{bc}$
Soaking in 7 ppm of nitrophenolate-based biostimulant	$41.33 \pm 2.19^{bc}$

<sup>1/</sup> Means with different letters in each column are significantly different ( $P \leq 0.05$ ) according to DMRT.

**Table 2.** Leaf number of chilli var. Kanchanaburi 1 at 14 and 21 days after planting

Treatments	Leaf number (leaves) <sup>1/</sup>	
	14 days	21 days
Non-soaking (Control)	$2.42 \pm 0.85$	$3.98 \pm 0.66$
Soaking in distilled water	$2.69 \pm 0.62$	$4.36 \pm 0.92$
Soaking in 3 ppm of nitrophenolate-based biostimulant	$2.77 \pm 0.57$	$4.50 \pm 0.84$
Soaking in 5 ppm of nitrophenolate-based biostimulant	$2.72 \pm 0.28$	$4.59 \pm 0.70$
Soaking in 7 ppm of nitrophenolate-based biostimulant	$2.90 \pm 0.41$	$4.95 \pm 0.82$

<sup>1/</sup> Means without letters in each column are not significantly different ( $P \leq 0.05$ ) according to DMRT.

### **Leaf number**

The results of the leaf number of chilli at 14 and 21 days after planting is shown in Table 2. The resulted showed that the chilli leaf numbers at 14 days after planting of non-soaking, distilled water and 3 – 7 ppm of nitrophenolate-based biostimulant treatments were  $2.42 \pm 0.85$ ,  $2.69 \pm 0.62$ ,  $2.77 \pm 0.57$ ,  $2.72 \pm$

0.28 and  $2.90 \pm 0.41$  leaves, respectively. There was no significant difference in leaf number among the treatments at 14 days after planting. Similarly, the chilli leaf numbers at 21 days after planting of non-soaking, distilled water and 3 – 7 ppm of nitrophenolate-based biostimulant treatments were  $3.98 \pm 0.66$ ,  $4.36 \pm 0.92$ ,  $4.50 \pm 0.84$ ,  $4.59 \pm 0.70$  and  $4.95 \pm 0.82$  leaves, respectively. The significant difference in leaf number also was not found among the treatments at 21 days after planting (Table 2).

**Table 3.** Leaf increasing number of chilli var. Kanchanaburi 1 at the end of experiment

Treatments	Leaf increasing number (leaves) <sup>1</sup>
Non-soaking (Control)	$1.56 \pm 0.47$
Soaking in distilled water	$1.67 \pm 0.36$
Soaking in 3 ppm of nitrophenolate-based biostimulant	$1.73 \pm 0.31$
Soaking in 5 ppm of nitrophenolate-based biostimulant	$1.87 \pm 0.44$
Soaking in 7 ppm of nitrophenolate-based biostimulant	$2.05 \pm 0.52$

<sup>1</sup>/ Means without letters in each column are not significantly different ( $P \leq 0.05$ ) according to DMRT.

### ***Leaf increasing number***

There was no significant difference in chilli leaf number among the treatments at 14 and 21 days after planting (Table 2). However, the different increasing trend of leaf number was found at the end of experiment. Namely, the chilli seeds treated with 7 ppm of nitrophenolate-based biostimulant treatment showed the highest of leaf increasing number ( $2.05 \pm 0.52$ ) and followed with 5 ppm of nitrophenolate-based biostimulant ( $1.87 \pm 0.44$ ), 3 ppm of nitrophenolate-based biostimulant ( $1.73 \pm 0.31$ ) and distilled water treatments ( $1.67 \pm 0.36$ ). The lowest of leaf increasing number was found in the control treatment ( $1.56 \pm 0.47$ ) (Table 3).

### ***Plant height***

The plant height of chilli soaked with the non-soaking, distilled water and 3 – 7 ppm of nitrophenolate-based biostimulant treatments were  $3.05 \pm 0.56$ ,  $3.20 \pm 0.61$ ,  $3.25 \pm 0.80$ ,  $3.42 \pm 0.49$  and  $3.81 \pm 0.93$  cm., respectively, at 14 days after planting and there was no significant difference in plant height of chilli at 14 days after planting. However, the plant height of chilli treated with distilled

water and 3 – 7 ppm of nitrophenolate–based biostimulant treatments trended to be higher than that of the control treatment. At 21 days after planting, the significant difference among the treatments was found. The highest plant height was expressed in the chilli treated with 7 ppm of nitrophenolate–based biostimulant treatment which was  $6.13 \pm 0.47$  cm. when compared to the other treatments. The plant height of chilli treated with non-soaking, distilled water, 3 and 5 ppm of nitrophenolate–based biostimulant treatments were  $4.89 \pm 0.46$ ,  $4.91 \pm 0.71$ ,  $5.08 \pm 0.66$  and  $5.19 \pm 0.34$  cm., respectively and they did not significantly differ. However, the plant height of chilli treated with distilled water and 3 – 5 ppm of nitrophenolate–based biostimulant treatments trended to be higher than that of the control treatment at 21 days after planting (Table 4).

**Table 4.** Plant height of chilli var. Kanchanaburi 1 at 14 and 21 days after planting

Treatments	Plant height (cm.)	
	14 days <sup>1</sup>	21 days <sup>2</sup>
Non-soaking (Control)	$3.05 \pm 0.56$	$4.89 \pm 0.46^b$
Soaking in distilled water	$3.20 \pm 0.61$	$4.91 \pm 0.71^b$
Soaking in 3 ppm of nitrophenolate–based biostimulant	$3.25 \pm 0.80$	$5.08 \pm 0.66^b$
Soaking in 5 ppm of nitrophenolate–based biostimulant	$3.42 \pm 0.49$	$5.19 \pm 0.34^b$
Soaking in 7 ppm of nitrophenolate–based biostimulant	$3.81 \pm 0.93$	$6.13 \pm 0.47^a$

<sup>1</sup>/ Means without letters in each column are not significantly different ( $P \leq 0.05$ ) according to DMRT.

<sup>2</sup>/ Means with different letters in each column are significantly different ( $P \leq 0.05$ ) according to DMRT.

**Table 5.** Plant increasing height of chilli var. Kanchanaburi 1 at the end of experiment

Treatments	Plant increasing height (leaves) <sup>1</sup>
Non-soaking (Control)	$1.81 \pm 0.30$
Soaking in distilled water	$1.71 \pm 0.26$
Soaking in 3 ppm of nitrophenolate–based biostimulant	$1.83 \pm 0.46$
Soaking in 5 ppm of nitrophenolate–based biostimulant	$1.77 \pm 0.18$
Soaking in 7 ppm of nitrophenolate–based biostimulant	$2.32 \pm 0.51$

<sup>1</sup>/ Means without letters in each column are not significantly different ( $P \leq 0.05$ ) according to DMRT.

### ***Plant increasing height***

The resulted showed that there was no significant difference in the plant increasing height among all treatments. Namely, the plant increasing height of chilli treated with the non-soaking, distilled water and 3 – 7 ppm of nitrophenolate–based biostimulant treatments were  $1.81 \pm 0.30$ ,  $1.71 \pm 0.26$ ,  $1.83 \pm 0.46$ ,  $1.77 \pm 0.18$  and  $2.32 \pm 0.51$  cm., respectively, at the end of experiment (Table 5).

### **Discussion**

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate. It is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones abscisic acid and gibberellins (Finch-Savage and Leubner-Metzger, 2006). However, this issue has been obviously affected on chilli production around the world for a long time. The seed dormancy caused the delay and low germination which may extremely reduce the quality and yield of chillies. There were a lot of reports on the techniques for enhancing the chilli seed germination including biostimulant (Kaewsorn *et al.*, 2017; Alcalá-Rico *et al.*, 2019; Khaba *et al.*, 2020; Surendar *et al.*, 2020; Yuniati *et al.*, 2019).

In this study, nitrophenolate–based biostimulant (atonik) was used as a biostimulant to enhance the germination rate and growth of chilli var. Kanchanaburi 1. The results showed that 3 ppm of nitrophenolate–based biostimulant treatments significantly increased the germination rate and growth of chilli var. Kanchanaburi 1. This result agreed with Djanaguiraman *et al.* (2005) reported that seed treatment of atonik at 3 ppm in both cotton and tomato was found to be best in recording maximum germination. This may due to the aromatic nitro phenolic compound in atonik such as sodium ortho-nitrophenol, para-nitrophenol and sodium nitro-guaiacol. Phenolic acids are natural compounds of several plants, which can influence cell morphology, physiology and metabolism. It has also been observed that these compounds can effect seed germinations (Djanaguiraman *et al.*, 2005).

Moreover, the result of leaf number and plant height revealed that atonik trend to increase the seedling growth by enhancing the leaf increasing number and plant increasing height of chilli seedlings var. Kanchanaburi 1. Similarly, Al-Musawi (2023) also found that atonik increased in plant height, number of branches, and leaf area of sweet pepper. This may because plants treated with nitrophenolates have greater a higher activity of naturally synthesized auxins, increase cytoplasm streaming (Yamaki *et al.*, 1953; Wilson and Kaczmarek, 1993) and uptake more nutrients from the medium (Stutte and Clark, 1990;

Przybysz *et al.*, 2014). According to all benefits of atonik, the growth of chilli seedling var. Kanchanaburi 1 finally increased when compared with the control treatment.

In conclusion, the nitrophenolate-based biostimulant could enhance the germination and growth of chilli seed var. Kanchanaburi 1. Namely, 3 ppm of nitrophenolate-based biostimulant enhanced the highest germination rate of chilli seed var. Kanchanaburi 1. Seven ppm of nitrophenolate-based biostimulant induced the best growth of chilli seedling var. Kanchanaburi 1.

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