Current Trends in Natural Sciences Vol. 7, Issue 13, pp. 38-46, 2018

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

EFFECT OF DIFFERENT PRIMING TREATMENTS ON SEEDS GERMINATION AND EARLY SEEDLINGS GROWTH OF TOMATO

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Abstract
*Germination is one of the main stages of a plant's life cycle. Since seed hydration by imbibition is a prerequisite for germination, seed priming techniques provide not only water that is absolutely necessary for the initiation of processes related to strict germination, but also additional physiologically effects due to the qualitative and quantitative composition of the solution used. The objectives of this study were to evaluate the effects of some priming treatments (Hidropriming, ASFAC and Diatomaceous earth) on seeds germination and vigour of tomato (*Lycopersicon esculentum *Mill.) seedlings. The effects of treatments have been differentiated in terms of germination process characteristics and / or early seedlings growth, in close interdependence with the concentration of the substance used and especially in relation with the effects of their chemical nature on physiological and biochemical processes. Tomato seeds priming with ASFAC 0.5% assures some benefits on germination efficiency and early seedling vigour. Priming with diatomite may improve seedling growth, in a concentration dependent manner. We appreciate the need to further studies to investigate more varieties, more treatments times and a wider range of concentrations.*

Keywords: ASFAC, diatomite, germination, priming, tomato.

1. INTRODUCTION

Germination is an important stage in the life cycle of plants (Khan et al., 2017), including tomatoes, one of the main vegetables species in Romania, next to cabbage, onions, peppers and eggplants (Popescu, 2017).

Seeds physiological quality is influenced by pre-harvesting conditions, by the harvesting and post-harvesting practices, as well as by the storage conditions (de Mattos and de Carvalho, 2016; Sano et al., 2016; Singkaew et al., 2017). Moreover, studied performed by Rosental et al. (2016) emphasized that there is a relationship between the dry seed metabolic profile and the germination ability of the seeds. This suggests a potential for determining a metabolic signature that can be used for assessing and possibly predicting the germination vigour of seed batches.

Germination, as an initial step of the plant ontogenetic cycle generally has a major role in plant's life (Bewley et al., 2013). Thus, a rapid and uniform germination is essential to a successful overcome the incidence of different stress factors (Jisha şi al., 2013), as well as to obtain a high yield, with a superior quality (Zhang şi al., 2012).

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In this context, seeds priming is a tool by which the seeds are partially hydrated and the physiological processes occurs, without the germination took place, then a re-drying with a view to later manipulation (Bradford, 1986; Khan şi al., 2017) determines the germination parameters improving, such as: imbibition period reduction, a fast and uniform germination, a shorter germination time, a more vigorously seedlings and more tolerant to the stress factors (Badek şi al., 2016; Varier şi al., 2010; Lutts şi al., 2016). As noted in the bibliographic synthesis written by Varier et al. (2010), as well as those of Sivasubramaniam et al. (2011), during the seed priming a series of processes take place, such as: water absorption, changes in protein profile, enzyme activation in relation to seed priming, molecular changes, as in the course of ordinary germination (Nonogaki et al., 2014).

Among the seeds priming techniques described in the literature (see review by Khan et al., 2017) and experimented by different researchers (Al-Amri, 2013; Delian et al., 2015; Gamel et al., 2017; Jyoti et al., 2016; Khan et al., 2016; Theerakulpisut et al., 2016; Patel and Rai, 2018), there can be mentioned: hidropriming, osmopriming, matripriming, nutripriming, halopriming, hormonal-priming, bio-priming, vermi-priming, magneto-priming, fitopriming, chemopriming etc. In particular, the benefits of tomato seeds priming were recently reviewed by Delian et al. (2017), and these are not few.

In this study, we tested the effects of ASFAC (as a bio stimulator product) and diatomaceous earth (diatomite) (as a silicon supplier) on tomato seeds germination indicators and early seedlings growth. To our knowledge, in Romania, it is for the first time when the diatomite effect is studied as a seed priming agent.

2. MATERIALS AND METHODS

A laboratory experiment was carried out in 2017, at the Faculty of Horticulture, USAMV Bucharest. The biological material was represented by tomato seeds Chihlimbar cv..

2.1. „Seeds priming” treatments

Before seeds priming treatments, seeds were treated with sodium hypochlorite (5%) for 10 minutes, then washed three times with distilled water, to totally remove the substance, and dried on filter paper. After that, seeds were immersed in the priming solutions (*Table 1*) for 24 hours. At the end of the priming period seeds were dried back to their original moisture contents, at room temperature (about 23^0C, 45% relative humidity).

Table 1. Seeds priming treatments

Variant	Treatment
Control	Dried seeds – without seeds priming
2.HP	„Hidropriming” with distilled water
3.AP 1 %	„Priming” with ASFAC 1%
4.AP 0.5 %	„Priming” with ASFAC 0.5 %
5.AP 0.25 %	„Priming” with ASFAC 0.25%
6. DEP 1 %	„Priming” with diatomaceous earth 1 %
7. DEP 0.5 %	„Priming” with diatomaceous earth 0.5 %
8. DEP 0.25 %	„Priming” with diatomaceous earth 0.25 %

ASFAC BCO-4 is a bio stimulator with an auxinic action on plants physiological processes, including seeds germination (based on 4-clor-2 potasium amidosulfonil-fenoxiacetat +

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microelements and additives) produced in our country, by Romchim Protect, Bacău (invention of Corneliu Oniscu), with applications in any type of agricultural crop, agriculture, forestry, horticulture, viticulture, floriculture. Diatomaceous earth (DE) was obtained from ICDPP Bucharest, were a research team was concerned with the development of new eco-efficient products with insecticidal-fungicidal effect and veterinary nutritional supplement with antimicrobial antidote action (Lupu et al., 2017). From the biological action point of view of diatomite it has been shown to be effective for insect control, due to its ability to cause rapid tissue dehydration and death due to water loss (Dumitraş şi al., 2015).

2.2. Germination tests and early seedlings growth

The experiment was carried out in laboratory conditions (with naturally day/night photoperiod and a mean temperature around 25^0 C). Two replicates of 50 seeds of control and primed seeds were germinated on filter paper (with 5 ml distilled water) placed in Petri dishes with a diameter of 9 cm. Seed germination tests were performed in accordance with International Seed Testing Association (ISTA, 2015) rules, with some minor modifications. Germinated seeds (in which the radicle had pierced the tegument and reached a length of at least 2 mm) were counted daily for 7 days.

2.2.1. The germination percentage was recorded daily, during the study period of 7 days and different germination indicators have been calculated (*Table 2*).

Table 2. Germination indicators and calculating formulas

Indicators	**Formula**	**References**
Final germination percentage (FGP) (%)	no. of germinated seeds at the end of the specific period/total number seeds used) × 100	ISTA (2015)
Germination speed (GS) (% day $^{-1}$)	G1/T1 + G2/T2 +........+Gn/Tn, where, G1 is the number of germinated seeds and T1 is the day 1	Czabator, 1962
Mean germination time (MGT) (days)	(n1 x d1)+(n2 x d2)+.......+ (nn x dn) /no. of observation days	Ellis and Roberts, 1981
The average number of seeds germinated per day (MDG) (number day $^{-1}$)	final germination percentage / number of observation days for the standard germination test	Sandeep et al., 2016
Maximum germination value (PV- „peak value”)	final germination percentage / the number of days required to reach the maximum germination value	Czabator, 1962
Germination value (GV)	PV×MDG. PV=Peak value of germination MDG= Mean daily germination	Czabator, 1962

2.2.2. Seedlings vigour. *Seedlings growth* (i.e. radicle and plumule length) was evaluated in 7-day-old plants (Delian and Lagunovschi, 2015). For each variant and replication, manually measurements were made (for 10 seedlings) using a ruler, as regard as the radicle length (RL) (cm) and the plumule length (PL) (cm). *Seedlings vigour index (SVI)* = seedling length (cm) x final germination percentage (%) (Pati şi Chowdhury, 2015). *Radicle vigour index (RVI)* = radicle length (cm) × final germination percentage (%) (Patil et al. 2012). *Shoot vigour index (SVI)* = shoot length (cm) × final germination percentage (%) (Patil et al. 2012).

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2.3. Statistical analysis

Statistical analysis of the obtained results was done by two-way analysis of variance (ANOVA). To evaluate pairwise significant differences between treatments, T test was used.
Differences were taken as significant when P value was ≤ 0.05.

3. RESULTS AND DISCUSSIONS

Germination Indicators

Regarding the germination dynamics, the obtained results are shown in *Figure 1*. The seed germination started on the second day after the seeds were placed in the Petri dishes, on the filter paper wetted with distilled water, but not for all the studied variants. Also, the number of germinated seeds was influenced by the application of a certain pre-treatment or lack thereof. Thus, in the case of the control variant (seed without any pre-treatment), germination started only on the third day and the number of germinated seeds was very small.

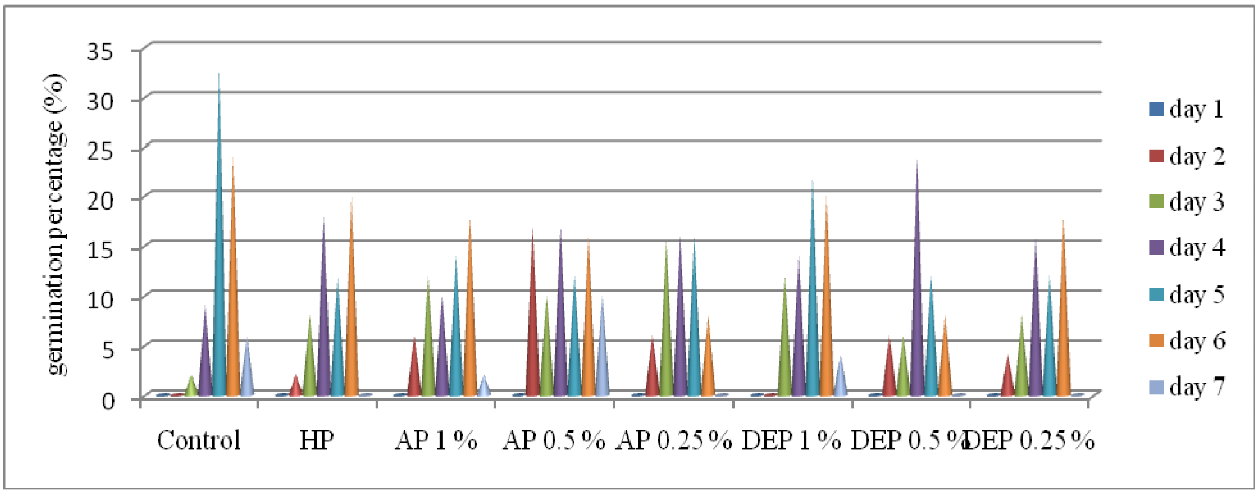

Figure 1. Germination dynamics of tomato seeds (Chihlimbar cv.)

As can be seen in the figure above, in the case of pre-treatment with ASFAC 0.5%, germination was faster and more uniform compared to other variants.
In fact, the dynamic of seed germination is under the coordination of many molecular, physiological and environmental factors (Das et al., 2018).
At the same time, the maximum final germination percentage (FGP) (82%) was recorded for this variant and data are shown in *Figure 2.A.*.
From statistical view point, there were not registered statistically significantly differences as compared with the control variant. Lower values, statistically different compared to the control were recorded for pre-treatments with ASFAC 1% and ASFAC 0.25%. As for pre-treatment with diatomite, there were no statistically significant differences compared to the control.
Increasing the percentage of germination in pre-treatment with ASFAC can be attributed to the growth stimulant effect that this substance exerts, coupled with the enhancement of enzymatic activity.
As noted in the data presented in *Figure 2.B.*, generally, pre-treatments applied did not statistically significantly affected the rate of germination, excepting the seeds priming with ASFAC. It can be noted that in the case of ASFAC 0.5% pre-treatment, the value of this indicator was 26.94 %, i.e. 1.87 times higher than the control. Also, for pre-treatment with 0.25% ASFAC, the germination rate

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was at 16.70%. Our results are in line with those obtained by Trofin and Oniscu (2009). In pre-treatment with diatomite the germination rate showed values close to those recorded at the control and hydro-priming.

Rapid and uniform germination is a basic condition for achieving the biological potential of the species and ensuring quality production with maximum profit. These features may be achieved by pre-treatment of seeds, which can provide a better germination, and uniform emergence, due to the beneficial metabolic processes that occur during imbibition period, in relation to the increase of the metabolic activity and the recovery of some seeds damages (Ebrahimi et al., 2014).

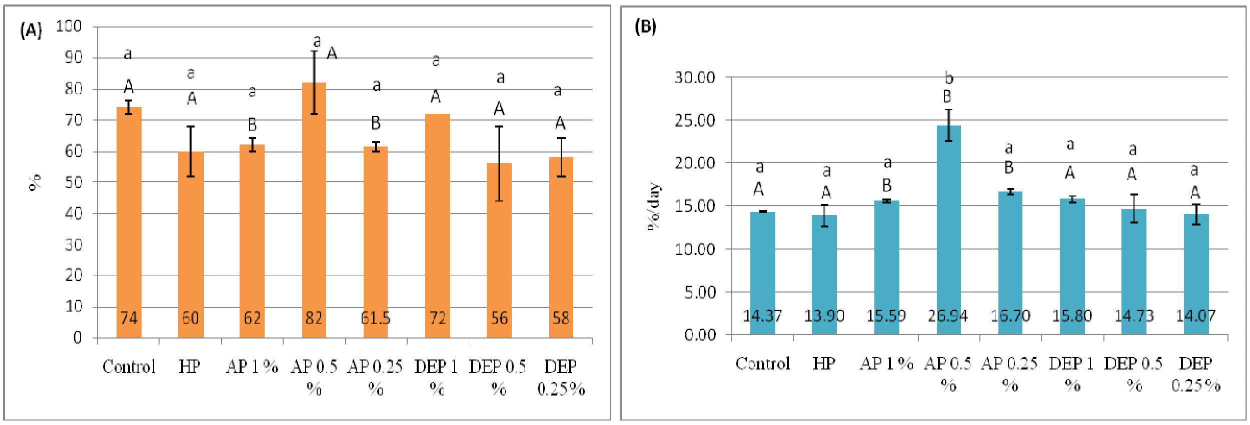

Figure 2. Final germination percentage (A) and germination speed of tomato seeds (Chihlimbar cv.)(mean±standard error)(n=2). Mean (±standard error) designed by the same letters (s) are not significantly different at P≤ 0.05. Capital letters – as compared with the control; Lowercase letters- as compared with hidro-priming.

The average number of seeds germinated per day was maximum in the pre-treatment with ASFAC 0.5% (11.71), but as can be seen from *Figure 3.A.*, the differences between the experimental variants do not appear to be significant as against the control, excepting the treatment with ASFAC 1%. However, the lowest values were recorded in pre-treatment with diatomite, but there were not registered significantly differences as against the control, or hydro-priming variants.

The average number of seeds germinated per day is closely related to the final germination percentage and the number of days corresponding to standard germination. As for the mean germination time (MGT), according to the ANOVA test, there were no significant interactions between the variables. Data presented in *Figure 3.B.* revealed that MGT ranged between 4.07 days (0.25% ASFAC) and 5.31 days (Control). Although there are some differences between the experimental variants, statistically, there are no significant differences, according to the results obtained by the T test (by ANOVA two factor with replication test, P value = 0.36; Fcalc.=1.31 and Fcrit.=3.86).

Maximum germination value ("peak value" - PV) (*Figure 4.A.*) follows the same trend, as the mean daily germination. The maximum PV was recorded for pre-treatment with ASFAC 0.5% (11.72), and the lowest one for pre-treatment with diatomite 0.25% (9.67). The maximum PV is due to the high germination percentage and the shorter number of days required reaching it. Germination value (GV) (PV×MDG) is shown in *figure 4.B.* Note that for pre-treatment with ASFAC 0.5%, the maximum GV (137) was recorded, while the minimum value was calculated for pre-treatment with 0.25% diatomite (80.12).

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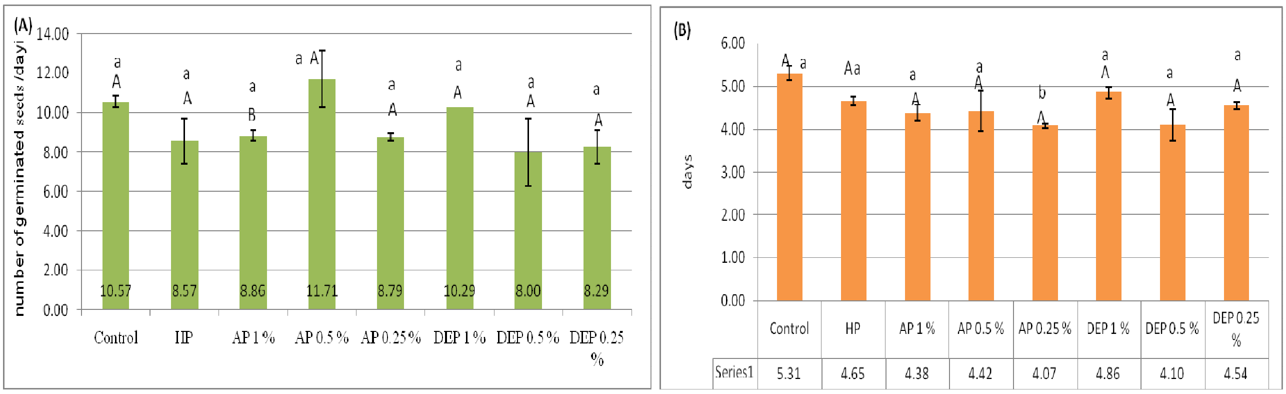

Figure 3. The average number of seeds germination per day (A) and mean germination time of tomato seeds (Chihlimbar cv.)(mean±standard error)(n=2). Mean (±standard error) designed by the same letters (s) are not significantly different at P≤ 0.05. Capital letters – as compared with the control; Lowercase letters- as compared with hidro-priming.

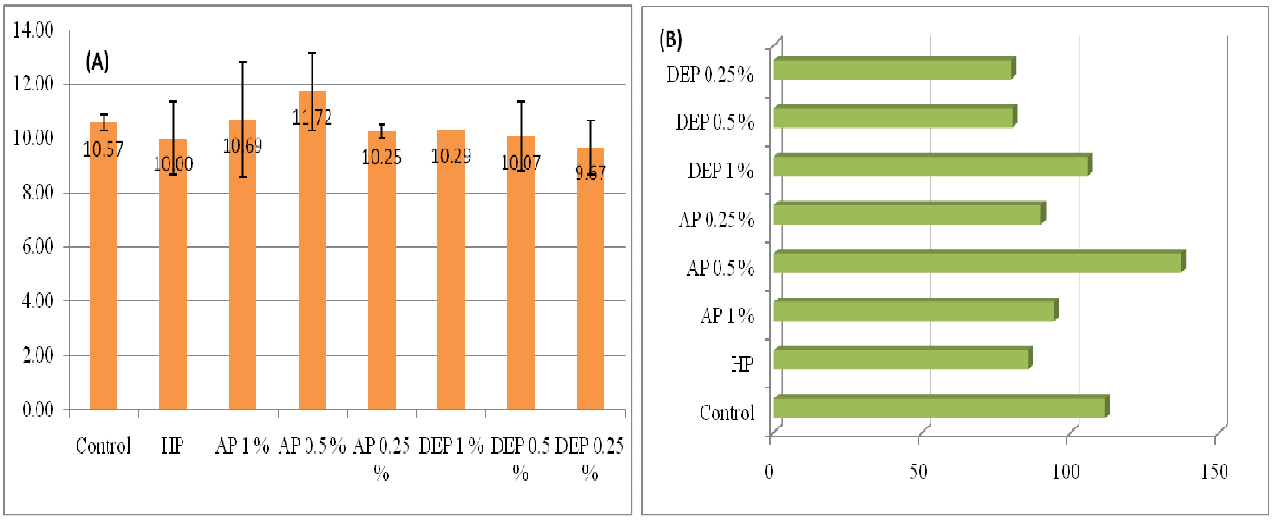

Figure 4. Germination "peak value" (A) and germination value (B) of tomato seeds (Chihlimbar cv.)(mean±standard error)(n=2).

Seedlings vigour

The seedlings vigour parameters are shown in *Table 2*. It is noted that for the treatment with 0.5% diatomite, the maximum value (5.79 cm) was recorded for radicle length (without significantly differences as against the control), while the minimum value was calculated for pre-treatment with 1% diatomite (2.33 cm) (significantly lower value as compared with the control). The higher shoot length was recorded in the case of ASFAC 0.5% and DE 0.5 % priming and there was a significantly differences for the former, as compared with the control. DEP at 1% inhibited the shoot growth (1.67 cm). As regard as the seedling length, as we can see in *Table 2*, the maximum value (8.88 cm) was recorded for the ASFAC 0.5% priming, while the minimum value was calculated for the pre-treatment with 1% diatomite (4.00 cm). It should be noted that diatomite treatments (0.5 % and 0.25 %) have favoured the growth of the seedlings. The maximum value for SVI (617. 44) was noticed for ASFAC 0,5% , while the minimum mean (287.82) was calculated

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for pre-treatment with 1% - diatomite. Also, significantly higher value were registered for shoot vigor index in the case of ASFAC 0.5 %.

Table 2. Tomato seedlings vigor indicators

Treatment		**Seedling length (cm)**	**Radicle length (cm)**	**Shoot length (cm)**	**Seedling vigour index**	**Radicle vigour index**	**Shoot vigour index**
Control		7.93	5.07±0.43 A	2.86±0.03 A	586.91±0.09 A	375.02±0.21 A	211.01±0.99 A
Hidropriming		7.99	5.62±1.05 A	2.38±0.11 A	480.75±0.45 B^{000}	336.45±0.75 B^{00}	143.66±0.58 B^{000}
ASFAC	1 %	7.02	4.15±0.08 A	2.87±020 A	436.12±0.88 B^{000}	257.13±0.17 B^{000}	178.22±0.28 B^{000}
	0.5 %	7.53	4.45±0.38 A	3.09±0.01 B	617.44±0.02 B^{xxx}	368.49±4.00 A	252.98±0.00 B^{xxx}
	0.25 %	6.99	4.32±0.65 A	2.67±0.23 A	429.55±0.03 B^{000}	268.12±2.44 B^{000}	164.89±0.99 B^{000}
DEP	1 %	4.00	2.33±0.34 B	1.67±0.33 B	287.82±0.18 B^{000}	175.50±7.74 B^{000}	122.21±2.33 B^{000}
	0.5 %	8.88	5.79±1.38 A	3.09±0.08 A	496.86±0.14 B^{000}	327.10±3.14 B^{00}	173.00±0.24 B^{00}
	0.25 %	8.70	5.58±0.15 A	3.12±0.05 B	504.40±0.20 B^{000}	327.42±3.78 B^{00}	179.19±1.77 B^{00}

Mean (±standard error) designed by the same letter (s) in the column are not significantly different as compared with the control at $P \leq 0.05$.

Our best results due to ASFAC priming 0.5 % (as abiostimulator) are in line with those obtained by Jyoti et al. (2016), in the case of tomato, by using giberelic acid. The authors pointed out that giberelic acid favoured seeds germination and shoot growth, also seedlings vigour was improved. This effect was explained by its involvement in seed dormancy breakdown, with favourably consequences to germination promoting and determined a shorter germination time. Also, studies performed on three tomato cv. emphasised that seeds pre-soaking with giberelic acid (100 ppm) or β-sitosterol (10^{-5} M) determined a better plants resistance to extreme heat stress (higher or lower temperatures) (Gamel et al., 2017). Moreover, as Al-Amri (2013) noticed, multiple benefits effects can be obtained when seeds are primed with shikimic acid. Thus, tomato growth was improved; higher productivity and yield quality was noticed in the field tomato culture, too.

Concerning the diatomite usefulness, recent studies carried out by Popescu et al. (2016) pointed out the increasing interest for the bio stimulated materials, as seeds coating components. According to Amirkhani et al. (2016) experimentally results, broccoli seeds coating (with soy flour/cellulose fiber/diatomaceous earth) determined a better shoots and roots growth, as compared to uncoated seeds. Even if the germination characteristics were negatively influenced by seeds coating, in relation to gas restriction diffusion, the beneficial effects on plant physiology were registered after 1 month under greenhouse conditions.

As our experiments emphasised, silicon (Si), as a component of diatomite can favour the seedling growth in a concentration dependent manner. In this context, Zhang et al. (2015) experimented on *Glycyrrhiza uralensis* seeds subjected to different levels of salt stress and noticed that low Si concentration (4 mM) enhanced seed germination, seedling emergence, and the seedling vitality index (SVI), while high concentrations of Si (6 mM) inhibited seed germination. From the physiological view point, silicon is qualified as a beneficial element for higher plants (Epstein, 1999). Also, it has been experimentally demonstrated that the application of SiO_2 nanoparticles and prechiling in tall wheatgrass (*Agropyron elongatum* L.) could be a new possible alternative for seed dormancy breakdown (Azimi et al., 2014).

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As Delian et al. (2017) mentioned, in the context of sustainable horticulture, it is necessary to translate successful experimental tests performed in vitro, in greenhouses and/or in field conditions, into an environmentally friendly and economically viable tool to improve tomato plant growth and development.

4. CONCLUSIONS

Tomato seeds priming with ASFAC 0.5 % assured some benefits on germination efficiency (significantly higher germination speed) and early seedlings vigour (significantly higher value of seedlings vigour and shoot vigour index were registered as against the control). Priming with diatomite may improve seedlings growth, in a concentration dependent manner. We appreciate the need to further studies investigating more varieties, more treatments times and a wider range of concentrations, with a view to elucidate the effects on germination performances and seedlings growth, in order to recommend the more appropriately seeds priming treatments.

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