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Germination and Post Emergence Growth of Lime (*Citrus aurantifolia* Swingle) as Affected by Seed priming with Growth Regulators and Bio-stimulants

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ABSTRACT

Lime is of widest distribution in Sudan compared to the other members of the citrus group. As it is conventionally propagated by seeds, this study aimed to investigate the possibility of enhanced germination and seedling growth attributes upon lime's seed priming with growth regulators and bio-stimulants in two separate tests under the conditions of the nursery. The regulators test was for gibberellic acid (GA_3) and naphthalene acetic acid (NAA) each in concentrations of 0, 5, 10, 15 and 20 mg/l. In the second test, the priming potential was tested for solutions of the hot water extracts of Argel shoots (*Solenostemma argel* Del Hayne) in concentrations: 0, 5, 10, 15 and 20 g/l, and the gel extracted from the leaves of Aloe (*Aloe vera*) plants in concentrations: 0, 10, 20, 30 and 40%. In both trials, the completely randomized design was used with 4 replicates. Except the 5 mg/l treatments of both regulators –in some cases- the other concentrations resulted in significant increases in germination and seedling growth parameters compared to the control. In particular, GA_3 was most enhancive for germination %, leaf length and width, while seedling height was promoted by the 15 mg/l NAA treatment. The results elucidate the bio-stimulating potential of Argel and Aloe applications at certain level. Although the 15% Argel and 20% Aloe were best enhancers for germination %, the highest concentrations from each were of poorest effect. The longest seedlings resulted at an equal statistical level from the 10 and 20% Aloe and the 5 and 10 mg/l Argel concentrations. The lower Argel concentrations (5 and 10) were inductive to leaf attributes, whereas Aloe at 30 and 40% were of particular impact on length. The findings of the study propose the use of the two plants as seed primers for enhanced germination and seedling growth attributes of lime. Yet, confirmatory trials with seeds of other horticultural crops might be justified in efforts towards organic farming.

INTRODUCTION:

Lime (*Citrus aurantifolia* Swingle) is the most cultivable citrus species in Sudan due to its tolerance to diverse soils and agro-climatic conditions. The self pollination of the hermaphrodite flower rarely results in genetically variable seeds and therefore seeds are the main propagation means of this species. Like other citruses, the seeds of lime lose viability rapidly under ambient conditions. Seed germination is the most critical phase in plant life (ElKeblawy and ElRawi, 2005). Germination is a quantile process characterized by active cell division and elongation, and is influenced by inherent, biotic and a-biotic factors (Grewal, 2016). During germination, catabolic processes result in degradation of reserved food within cotyledons by hydrolytic enzymes that provide energy and nutrients for embryo and seedling growth and therefore viable large large seeds with adequate food reserve are advantageous for enhanced germination and seedling growth (Grewal, 2016). However, the germination process is influenced by light (Grey *et al.,* 2003) and temperature (Jacobsen and Beach, 1998). Besides, the inhibition and promotion of this process is also influence by a balance between abscissic acid (ABA) and gibberellins (GA) (White *et al.,* 2000). A high ABA: GA ratio delays germination and the reverse is enhancive (Yang *et al.,* 2004). The commencement of germination is accompanied by initiation of growth that results in radicle emergence (Finkelstein and Lynch, 2000). Emergence is the appearance of the first aerial organ above soil level and can be influenced by seeding depth, soil texture or compaction and the strength or length of the coleoptyl or hypocotyl which is affected by seed size (Gupta, 1984). Low germination percentage, heterogeneous emergence and unbalanced seedlings growth are among problems facing farmers in developing countries, and seed priming might result in enhanced germination and emergence (Sidghi *et al.,* 2010). Priming refers to pre-sowing seed soaking treatment with water (hydro-priming), chemical solution (osmo-conditioning), hormones or bio-stimulants (Idris and Modawi, 2016). Plant growth stimulation based on natural materials received considerable attention by the scientific community in the last two decades (Yakhin *et al.,* 2017). Bio-stimulants offer a potentially novel approach to enhance physiological processes in plants and mitigate stress-induced limitations to increase performance and yield (Khan *et al.,* 2009; Du Jardin, 2015; Yakhin *et al.,* 2016). Therefore, this study aimed to explore the effect of seed priming with growth regulators and bio-stimulants on the germinability and post emergence growth of lime (*Citrus aurantifolia* Swingle).

MATERIALS AND METHODS:

In an effort to improve lime seeds germination and the subsequent seedling growth, this study which was composed of 2 separate tests was conducted under the conditions of the nursery of the Department of Horticulture, Sudan University of Science and Technology at Shambat, Khartoum North, Sudan. The seeds were extracted from mature lime fruits and immediately washed under running tap water until the remains of juice were removed. They were left over-night to dry on paper under shade and then packed in air –tight plastic container under ambient conditions. Seeds of uniform size were used for experimentation a week later.

Growth regulators concentrations test: Concentrated solutions of gibberellic acid (GA_3) and naphthalene acetic acid (NAA) were prepared in the laboratory using a digital precision balance. NAA was dissolved with the aid of I N NaOH. Further dilutions were made for the preparation of test concentrations where each of the growth regulators was tested as seed priming solution in concentrations of: O, 5, 10, 15 and 20 mg/l.

Bio-stimulants test: The priming potential was tested for solutions of the hot water extracts of Argel shoots (*Solenostemma argel* Del Hayne) in concentrations: 0, 5, 10, 15 and 20 g/l, and the gel extracted from the leaves of Aloe (*Aloe vera*) plants in concentrations: 0, 10, 20, 30 and 40%. In all tests, 40 seeds were used per treatment. Seeds were submersed in priming solutions for 24 hrs. At planting, seeds were divided into groups of 10 seeds to get 4 replicates per treatment and were sown in 15X20 cm plastic bags containing 1 sand : 1 silt soil mix and were irrigated immediately. Thereafter irrigation was applied at 3 days intervals. The complete randomized design was used under the condition of the nursery. The commencement date of the tests was 22 December 2016, and the final data were recorded on March 22nd 2017. Data were collected for germination, seeding height (from soil level to tip bud with a tape-meter), the number of leaves per seedling, the length and width of leaf. Data were subjected to analysis of variance, followed by means separation according to Duncan's multiple rang tests with the aid of M-Stat-C computer program.

RESULTS:

A. Growth regulators: The impact of growth regulators on germination percentage is presented in Figure (1). GA_3 at concentration of 20 mg/l was the most enhancive for germination. Statistically equal germination percentages were obtained from the NAA 15 mg/l and GA_3 at 10 and 15 mg/l that shared the second rank. Nevertheless, except the 5 mg/l NAA and GA_3 treatments, all other levels of either regulator resulted in significant increase in germination percentage compared to the control.

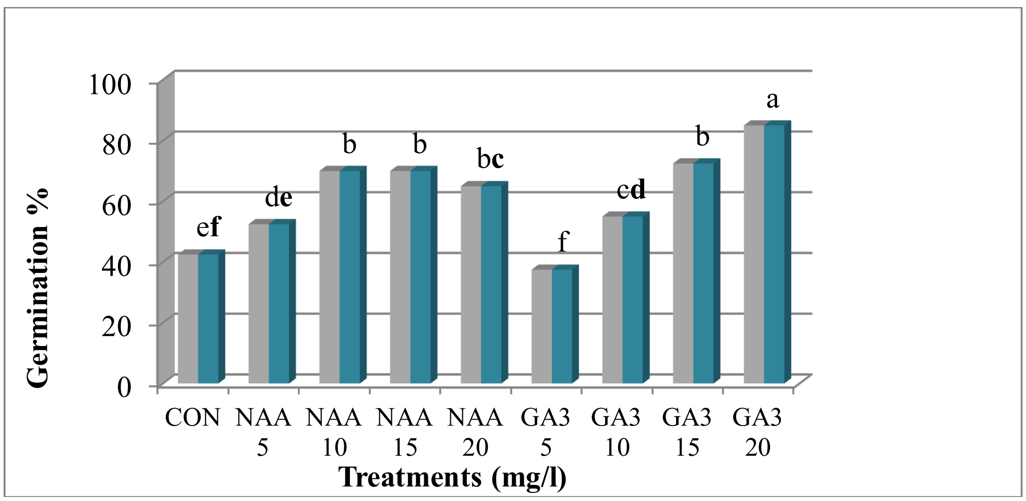

Figure (1): Impact of seed priming with NAA and GA_3 on germination of lime

Besides, all treatments with regulators increased the seedlings height significantly over the control. GA_3 in concentration of 15 mg/l was the most enhancive for this parameter (Figure 2).

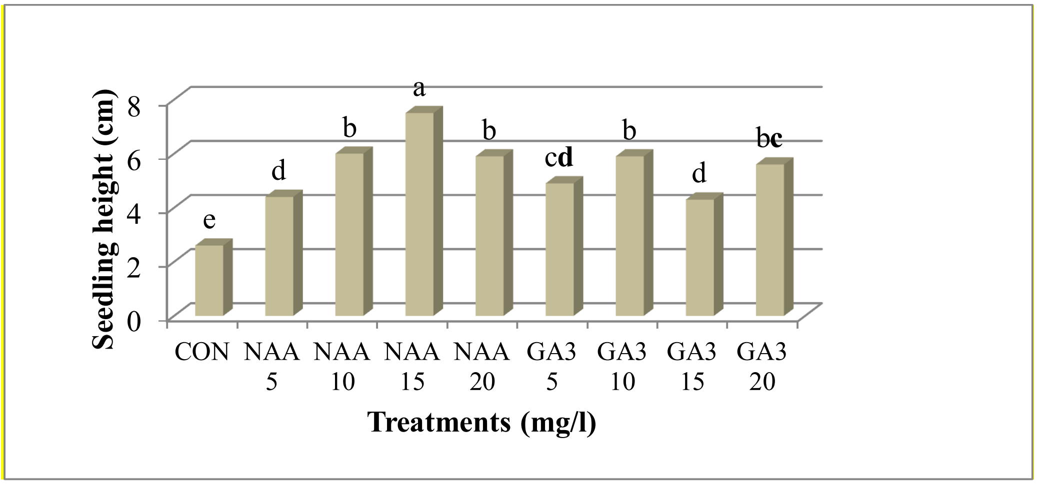

Figure (2): Impact of seed priming with NAA and GA_3 on height of lime's seedlings

Regarding the number of leaves per seedlings, this characterwas improved significantly over the control by all growth regulator treatments.The top rank was recorded for the 10 mg/l GA_3 and 5 mg/l NAA treatments. However, increases in concentration of both regulators resulted in gradual decrease in the number of leaves (Table 1). The impact of growth regulators on leaf length was positive as all treatments resulted in significant length increasecompared to the control. The longest leaves were obtained from the 20 mg/l $GA_3$and the 15 mg/lNAA treatments (Table 1). However, expect NAA in concentration of 5 mg/l, all growth regulators priming treatments increased leaf width significantly over the control andthe best leaf width resulted from the 20 mg/ l GA_3 treatment (Table 1).

Table (1): Impact of seed priming with GA_3 and NAA on the leaves of lime seedlings

Treatments (mg/l)	No. of leaves per seedling	Leaf length (cm)	Leaf width (cm)
Control	4.750d	1.675c	1.276c
GA_3 05	8.000b	2.550b	1.250c
$GA_3$10	9.000a	2.875ab	1.675b
$GA_3$15	8.500ab	3.125ab	1.675b
$GA_3$20	7.250bc	3.850a	1.975a
NAA05	9.500a	2.500b	1.750ab
NAA 10	8.000b	2.425b	1.750ab
NAA 15	7.250bc	3.575a	1.675b
NAA 20	6.750c	3.075ab	1. 625b

*Means within column with the same letter(s) are not significantly different at P=0.05 according to DMRT.

B. Plant Bio-stimulants: As shown in Figure (3), the germination percentage of lime's seeds was affected by the various treatments with Argel and Aloe. The best values were recorded equally for the 15 g/l Argel and the 20% Aloe gel treatments, but without significant difference from the 5 and 10 g/l Argel and the 10% Aloe gel treatments. It is noteworthy to recognize the deterioration in germination as a result of the high doses where Argel at 20 g/l ranked below the control while Aloe gel at 40% equaled the control.

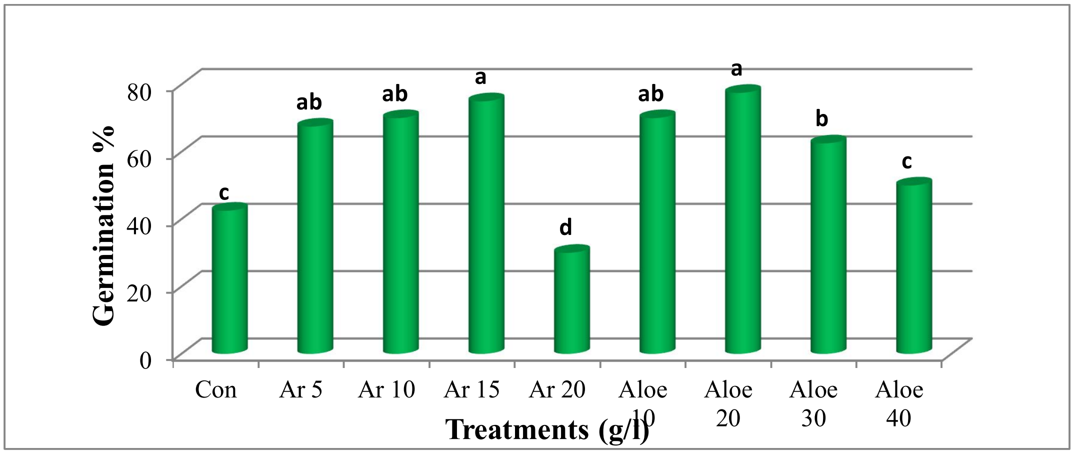

Figure (3): Impact of priming with *Solenostemma argel* hot water extracts (g/l) and *Aloe vera* gel (%) on lime's germination

As illustrated in Figure (4), all botanicals increased seedling height significantly over the control. The highest seedlings resulted from the 10% Aloe treatment, but without significant difference from the 5 and 10 g/l Argel and 20% Aloe treatments.

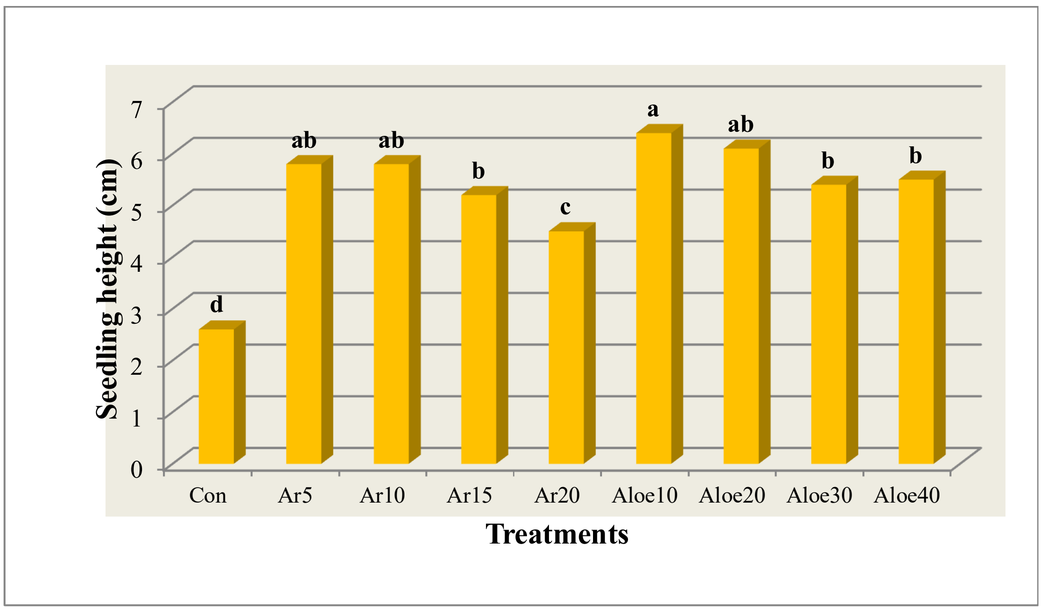

Figure (4): Impact of priming with *Solenostemma argel* hot water extracts (g/l) and *Aloe vera* gel (%) on lime's seedling height

Regarding leaves characteristics, all Argel and Aloe treatments increased the number of leaves per seedling significantly over the control. The highest number resulted from the 5 g/l Argel treatment without difference from the 10% Aloe treatment, whereas the weakest results for this parameter were obtained from the two botanicals at their highest rates (Table 2). Likewise, the applications of the two plants were of substantial benefit over the control for enhanced leaf length and the most outstanding levels were equally obtained from the 10 g/l Argel and 30% Aloe treatments (Table 2). Similarly both plants increased leaf width significantly compared to the control and the widest leaves resulted from the 5 g/l Argel which was double the width of the control. However, Aloe at 10% equaled the 5 g/l treatment statistically and at highest concentrations the impact of both plant was poor (Table 2).

Table (2): Impact of seed priming with Argel hot water extracts (g/l) and Aloe gel (%) on the leaves in lime's seedlings

Treatments	No. of leaves per seedlings	Leaf length (cm)	Leaf width (cm)
Control	4.750d	1.675c	4.750d
Argel 05	9.500a	3.050ab	9.500a
Argel 10	8.000b	3.275a	7.000bc
Argel 15	8.000b	3.000ab	8.000b
Argel 20	7.000bc	2.825b	7.000bc
Aloe 10	8.750ab	3.000ab	8.750ab
Aloe 20	8.00b	3.000ab	7.500b
Aloe 30	7.50b	3.500a	8.000b
Aloe 40	6.500c	3.200a	6.500 c

*Means within column with the same letter(s) are not significantly different at P=0.05 according to DMRT.

DISCUSSION:

The results of the test of plant growth regulators elucidated remarkable enhancements in germination percentage and seedlings growth expressed in height, number, length and width of leaves upon seed priming with these regulators. Likewise, similar enhancements were also observed in the second test where the stimulating potential of botanicals was tested. The substantial increments in growth attributes are indicative of their potential use as substitutes for synthetic growth regulators for seed priming. It is noteworthy to recognize the common delay of germination in both tests which might be attributed to the environmental effect as sowing was made in the last week of December; a period of cool weather and short day length. According to Ochuodho (2005), temperature and light are among factors influencing germination, and the plasma membrane is the first site in seed cells which detects the growth stimulus from the external environment. High and low temperatures reduce the speed and percentage of germination (Hills *et al.,* 2001). However, thermo-inhibition of germination was attributes to both high ABA content and high sensitivity to ABA (Gonai *et al.,* 2004). Therefore, GA_3 might have encountered the negative impact of ABA which might have resulted from the cool winter temperature. Besides, synergism of juvenility hormones i.e. (gibberellins, auxins and cytokinins) might be among reasons of enhanced germination and seedling growth as the sufficiency and activity of either hormone necessitates the activation of the others. Cytokinins induce cell division, auxins expand and enlarge cell wall, while gibberellins elongate cells and it is noteworthy that growth develops by their combined effects (George *et al*., 2008). According to Thomas *et al*., (2003), hormones at optimum levels accelerate growth and development. Nevertheless, the enhanced germination percentage obtained in this study due to priming with growth regulators is in line with findings of Sidghi *et al*., (2010) who reported enhanced germination percentage and seedling growth when GA_3 was tested as seed primer for Mary-gold plants. The accelerated germination and seedling growth attributes upon priming with Argel and Aoe, is indicative of their potencies as growth bio-stimulants. The results are in conformity with findings of Idris and Modawi (2016) who reported enhanced germination percentage and seedling growth attributes upon priming the seeds of 'Kitchener' mango cultivar with extracts of Argel or Haza (*Haplophyllum tuberculaum*). The yield and quality of date palm were stimulated by low doses of Argel applied as soil dressing (Idris *et al.,* 2011), but the cause of stimulation was not fully defined and was attributed to growth-regulator-like effect of the phyto-chemical constituents of Argel. Likewise, growth bio-stimulation effect of Argel was also reported on mango (Idris *et al.,* 2014) and Golden Duranta (Hamed, 2016). The findings of this study are confirmatory to the preceding reports, but the difference and addition is the bio-stimulating property of *Aloe vera*; as no reports on its impact on plant growth was available according to our intensive literature search. In depth chemical and bio-chemical analysis of both plants might give solid interpretations for their bio-stimulating constituents. Yet, further confirmatory tests on botanicals as seed priming solutions might enlarge the base of flora with enhancive and growth accelerating properties; a practice that may boost crop agronomy coupled with the benefits of organic production which is environmentally friendly.

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