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Influence of fungal elicitors on *Striga hermonthica* (Del.) Benth. and *Sorghum bicolor* (L.) growth under *in vitro* conditions

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Abstract: In the present investigation fungal elicitors (0.0, 1.0, 5.0, 10, 20 and 50 mL/L) were evaluated for ability to induce germination of *Striga hermonthica* and sorghum growth. Fungal elicitors induced *Striga* germination in a concentration dependent manner. In presence of sorghum, the germination inducing activity of fungal elicitors was lowest at the lower elicitor concentrations. The activity, invariably, increased on increasing concentrations level (10-50 mL/L). At 50 mL/L elicitor, germination was increased to 63% and 75% irrespective to time interval. In absence of sorghum, fungal elicitors at 10 mL/L sustained the highest *Striga* germination then declined with increasing elicitor concentrations. In absence of the sorghum, fungal elicitors at 5-10 mL/L induced *Striga* germination. However, 50 mL/L of the fungal elicitor induced maximum germination in presence of the sorghum. In terms of *Striga* length, the maximum height was recorded at 5 and 50 mL/L elicitor in presence and absence of the sorghum, respectively. Sorghum height, in presence or absence of *Striga*, was considerably affected by fungal elicitor compared to the control. Among the fungal elicitor concentrations, irrespective to presence of *Striga*, 5 mL/L proved to be more ability to stimulate the growth of sorghum. Furthermore, fungal elicitor at 1.0 mL/L

sustained the maximum root and root/shoot ratio in presence or absence of *Striga*. The improvement of root growth over the control was occurred only at the least fungal extract concentration and significantly decreased with increasing doses. The differential germination displayed by *S. hermonthica* in response to fungal elicitor merit further research and has to be taken into account in designing management strategies.

Keywords: Fungal elicitor, *In-vitro* co-culture, *Striga*, Sorghum.

INTRODUCTION

Plants are challenged by a variety of biotic stresses like fungal, bacterial, insect, weeds or viral infections which lead to a great loss to plant yield. There are various options available for the farmers to protect their crop from the pests. Some options include development of resistant cultivars, biological control, and crop rotation, tillage, and chemical pesticides. The better understanding of plant signaling pathways has led to the discovery of various natural and synthetic elicitors that induce similar defense responses in plants as induced by the pathogen infection[1]. Plants respond in a complex array of defense after detection of microorganism, via release of elicitor molecules. Elicitor was originally used to describe molecules capable of inducing phytoalexins, but it is now commonly used for compounds stimulating any type of plant defense[2]. Eventually, the induction of defense responses may lead to enhanced resistance. This broader definition of elicitors includes both substances of pathogen origin (exogenous elicitors) and compounds released from plants by the action of the pathogen (endogenous elicitors)[3]. Elicitation in plants cell culture as a strategy to enhance secondary metabolite production was widely used[4]. Fungal cell-wall fragments, yeast extract and proteins or carbohydrates have previously been used as elicitors to enhance the production of secondary metabolites in whole plant and plant cell cultures[5]. Most of the elicitors used in earlier studies originated from fungal [6], bacterial[7] and yeast cell extracts[8] or their purified fraction.

Striga species, obligate root parasites, are prodigious seed producers. A single *Striga* plant could produce up to 40,000 - 90,000 of minute dust-like seeds with prolonged viability. In nature, the germination stimulant is exuded by roots of hosts and some non-host plant species. The response of seeds to germination stimulants increases with conditioning period and then declines with time and eventually the seed enters into a stage of secondary or wet dormancy which breaks on drying [9]. The aim of the present work was to study the effect of fungal elicitors (Ai41) on *Striga hermonthica* incidence and sorghum growth when *in-vitro* co-cultured.

MATERIAL AND METHODS

Series of laboratory experiments were undertaken to investigate the effects of fungal elicitor Ai41 on *S. hermonthica* and sorghum Wad Ahmed. All experiments were carried out in the laboratory of Plant Cell and Tissue Culture, Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan.

Seeds collection and surface disinfection: *Striga* seeds were collected from infested sorghum plants in Gezira Scheme, Sudan. *Striga* seeds were surface disinfection as described by[10]. Briefly, seeds placed in a measuring cylinder (1000 mL/L) containing tap water, and any debris and immature seeds that floated were discarded. The seeds were washed several times with tap water and tween 20 to remove sand. Under

aseptic conditions, seeds were soaked for 5 min in 20% sodium hypochlorite solution and rinsed five times with sterile distilled water.

Sorghum seeds (Wad Ahmed cultivar) were surface sterilized, under aseptic conditions, with 15% sodium hypochlorite for 15 min. and then washed five times with sterile distilled water.

Media preparation: The effective fungal isolate Ai41 (selected based on their ability to suppress *Striga* germination) [11] was cultured on Potato Dextrose Agar (PDA) amended with chloramphenicol. The medium was prepared by boiling 200g of sliced potato in 1 liter distilled water until the potato was soft. Twenty grams of dextrose and 20g agar powder were added to the medium and the volume was adjusted to 1 liter then sterilized by autoclaving for 15 minutes at 121 °C and left to cool. The fungal isolate (Ai41) was maintained on PDA. Cultures on solid medium were stored at 5°C until use.

Wheat flour medium (WFM) was prepared as described by Ahmed *et al*. [11]. Briefly, ten grams of wheat flour were placed in 500 mL/L conical flask and 400 mL/L distilled water were added followed by hand shaking for five minutes. From the resulting extract, 250 mL/L were placed in 500 mL/L conical flasks, then autoclaved for 15 minutes at 121^{o}C and left to cool for 24 h.

B5 basal medium [12] adjusted to the desired pH 5.5 using HCl or NaOH and solidified with 7 g/L agar. The medium was dispensed into the culture tubes before autoclaving at 121 °C and 15 psi for 15 min. The medium was used in all co-cultures of *Striga* and sorghum.

Elicitors were prepared from cultures of Ai41 received from the Environment, Natural Resources and Desertification Research Institute (ENRDI), National Center for Research, Khartoum, Sudan. The fungal filaments were grown in 1000 mL/L conical flasks containing 250 mL/L of potato dextrose broth for 15 days at room temperature. Fully grown mycelia with spores were homogenized and centrifuged at 4000 rpm and the supernatants were autoclaved for 20 min at 121 °C. The mycelium fragments were filtered using Whatman filter paper No. 1 and used as elicitors[13]. Different concentrations of Ai41 elicitor's viz. 0.0, 1.0, 5.0, 10, 20 and 50 mL/L were prepared in stock solutions.

Bioassay: *In vitro* culture technique was used to evaluate the effect of fungal elicitors on germination and seedlings development of sorghum and *Striga*. Disinfected seeds of sorghum and *Striga* were cultured separately or in co-culture on B5 medium supplemented with different concentrations (0.0–50 mL/L) of fungal elicitor. All procedures were carried out under strictly aseptic conditions. Thereafter, all cultures were kept in incubation room with a constant temperature of 26±2 °C under cool fluorescent light of about 5000 lx and a photoperiod of 16-h light and 8-h dark.

Interaction effects on growth of the co-cultured sorghum and *Striga* seedlings were monitored simultaneously. The effect of fungal elicitor on *Striga* incidence was examined. Data on *Striga* germination and seedling length were recorded at three time intervals, each 7 days. The effect of fungal elicitor on sorghum growth was studied. Data of sorghum shoot length, root length, root/shoot ratio, shoot dry weight and root dry weight were recorded at three time intervals, each 7 days.

All the experiments were conducted in a Completely Randomized Design with five replicates. The data was subjected to statistical analyses following standard procedures. Means were separated using Duncan's new multiple rang test at 5% level and presented as means ± standard error (SE).

RESULTS AND DISCUSSION

***Striga* germination:** *Striga* seed cultured in B5 medium supplemented with different concentrations (0.0–50 mL/L) of fungal elicitors in presence or absence of sorghum seedlings showed differential response (**Figure 1**). Fungal elicitors induced *Striga* germination in a concentration dependent manner. In presence of sorghum, germination inducing activity of fungal elicitors was lowest at the lower concentrations. The activity, invariably, increased on increasing elicitors' concentrations level (10-50 mL/L) (**Figure 1A**). At the higher concentration of fungal elicitor, germination was increased to 63% and 75%, irrespective to time interval (**Figure 1A**).

In absence of sorghum, fungal elicitors at 10 mL/L sustained the highest *Striga* germination (**Figure 1B**). It increased germination to 56.5%, 63.5% and 73.5%, during 2, 3, and 3 weeks, respectively. Fungal elicitor at 20 and 50 mL/L decreased *Striga* germination.

***Striga* length:** Result displayed that fungal elicitors enhance *Striga* significantly (**Figure 2**). In presence of sorghum, increasing fungal elicitor's level to 10-50mL/L increased *Striga* length significantly. However, minimum and maximum length was achieved by 1 and 50 mL/L, respectively (**Figure 2A**). In absence of sorghum (**Figure 2B**), *Striga* length increased significantly with time, irrespective to the fungal elicitors. However, at 4 weeks, *Striga* length was improved significantly to 3.38±0.45 in response to elicitor at 5 mL/L. These confirmed the influence of the fungal elicitor extracts exposure-time on seedling length. It could be assumed that, *Striga* length improved with increasing elicitor concentrations from 5.0 to 50 mL/L in presence of sorghum, in comparison with lowest concentrations from 1.0 to 5.0 mL/L in absence of sorghum. Rebeka et al.[14] reported that *Fusarium oxysporum* have markedly suppressed the number of emerged *Striga* and *Striga* growth under greenhouse condition. Different extracts of *F. solani* was caused complete inhibition of *Striga* germination[15]. Root exudates from arbuscular mycorrhizal crops induced lower germination of *S. hermonthica and S. gesnerioides* seeds compared to exudates from non-mycorrhizal [16]. Suppression of *Striga* seedling growth with application *Fusarium oxysporum* was also reported [14]. Ahmed et al. [15] assumed that the inhibition activity was due to fungal metabolites rather than to the parasitic or saprophytic fungal growth. Therefore, as only fungi extract used in present study, it could implied that the reduction of *Striga* development due to secondary metabolites. In the present study, stimulation of *Striga* germination by biotic elicitors (fungal isolate A41) was observed. It improved greatly with increasing elicitor concentrations. Elicitors are one of the important factors that can act as a switch for increasing the yield of secondary metabolites of useful bioactive compounds in plant cell cultures [17-18]. Most of the elicitors used in earlier studies originated from fungal [6-18], bacterial [7] and yeast cell extract [8-19] or their purified fraction. The use of elicitors in crop protection and pest management is still in the very early stages of use as a new control method. However, the enhancement of *Striga* development in presence of sorghum in cultures may be because of the competition or sorghum root exudation. Stimulation of *Striga* seed germination by exudates produced *in vitro* by sorghum seedlings was reported[20].

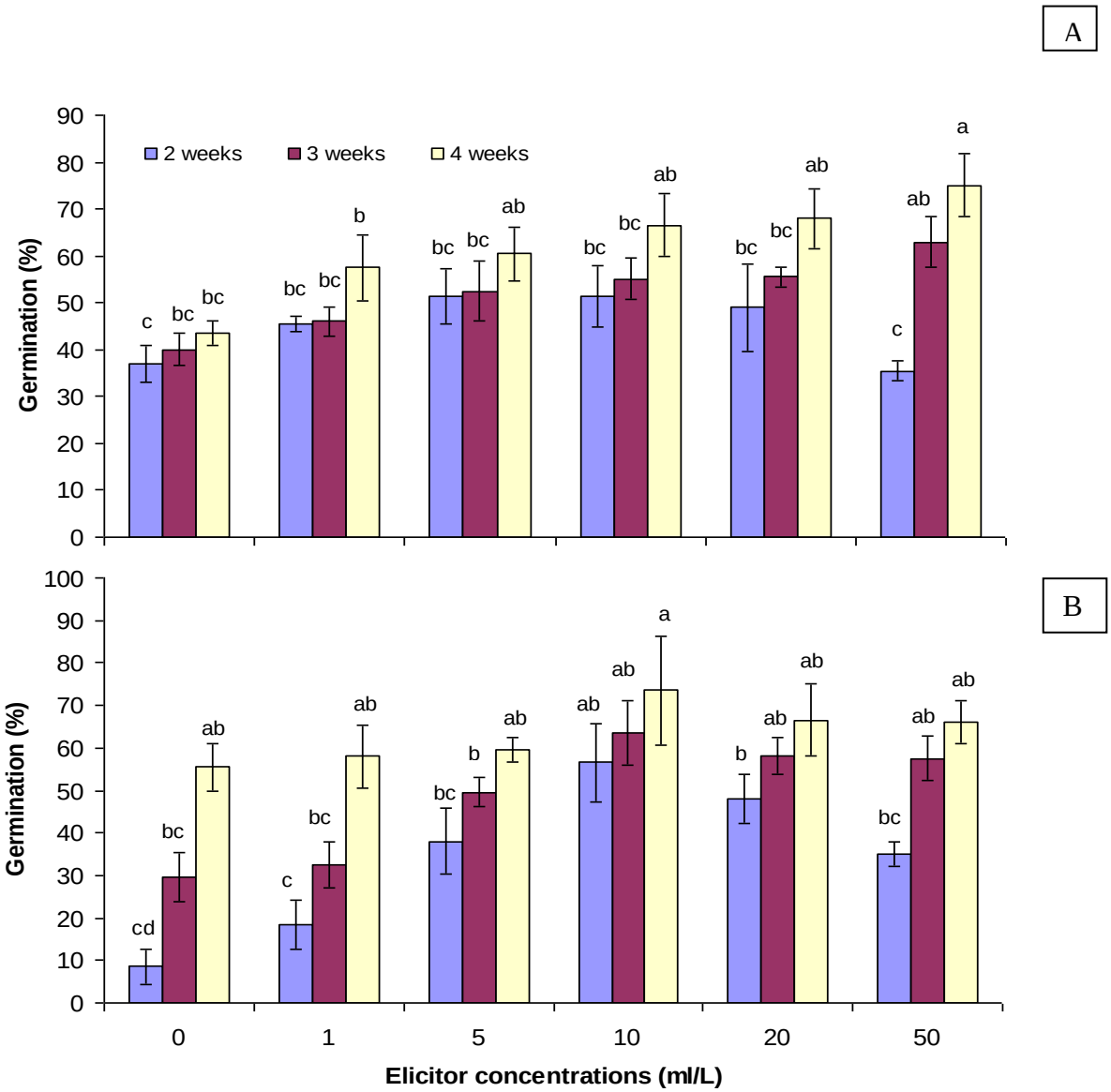

Figure 1: Effect of fungal elicitors (0-50 mL/L) on *Striga* incidence in (A) presence or (B) absence of sorghum after three periods (2, 3 or 4 weeks) of exposure. Means followed by the same superscript letter are not significantly different (*P*=0.05) using Duncan's Multiple Range Test (DMRT). Vertical bars indicated standard errors.

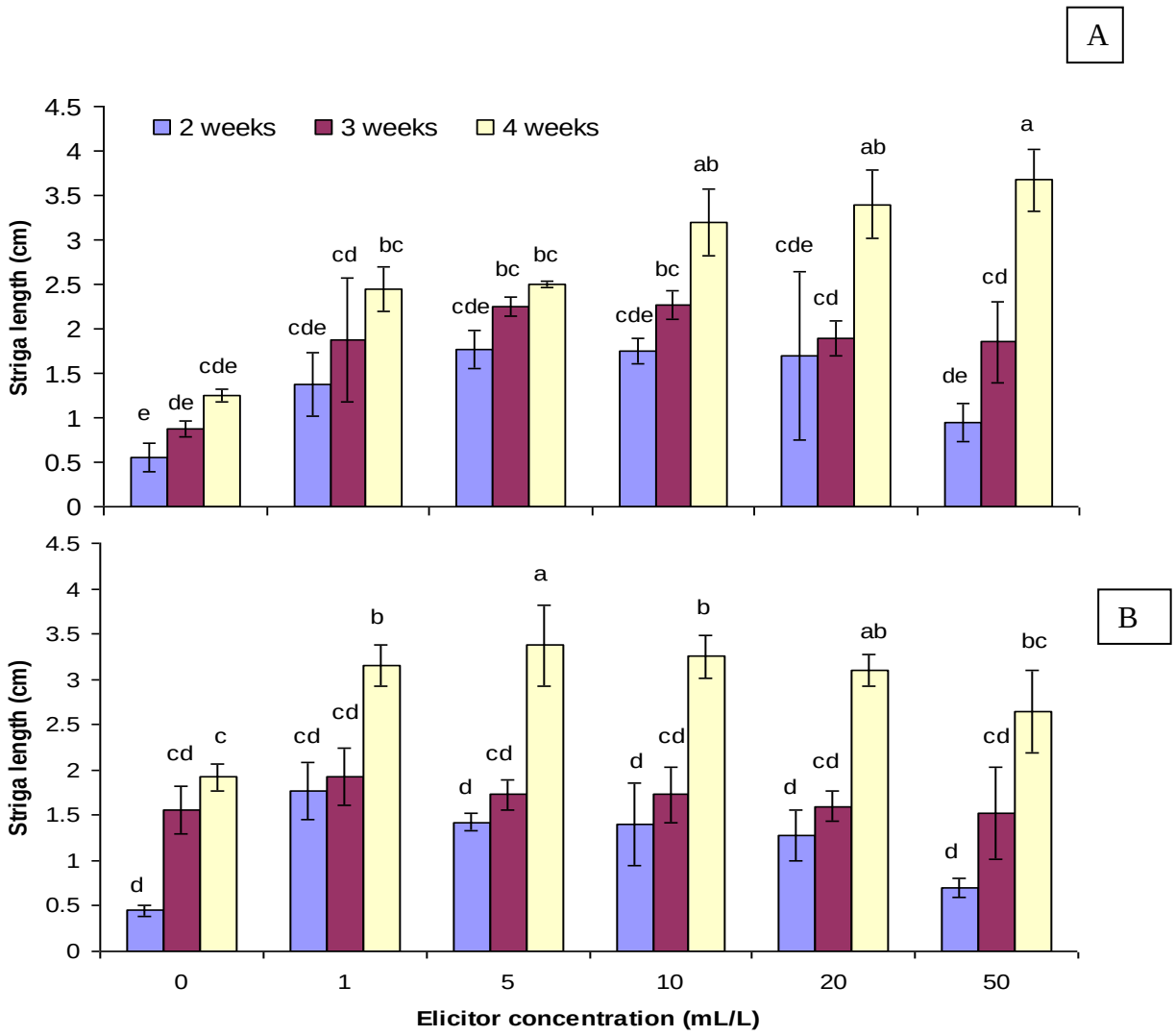

Figure 2: Effect of fungal elicitors (0-50 mL/L) on *Striga* length in presence (A) or absence of sorghum (B) after three periods (2, 3 or 4 weeks) of exposure. Means followed by the same superscript letter are not significantly different (*P*=0.05) using DMRT. Vertical bars indicated standard errors.

Sorghum growth: Results showed that sorghum height was considerably affected by elicitor application compared to the control in presence or absence of *Striga* through times **(Figure 3**). Among the fungal elicitor concentrations, irrespective to the *Striga*, 5 mL/L proved to be more ability to stimulate the growth of sorghum.

On the other hand at 5 mL/L of the fungal elicitor, sorghum cultured alone increase shoot length in a range from 14.78 cm to 16.75 cm, irrespective to the time (**Figure 3B**). Elzein et al. [21] and Rebeka et al. [14] reported that sorghum seeds coated with chlamydospores of *F. oxysporum* have no adverse effects on the sorghum seed germination and emergence.

Root length: In term of sorghum root length, fungal elicitor at 1.0 mL/L elicitor sustained the maximum length in presence or absence of *Striga* **(Table 1)**. The improvement of root growth over the control was

occurred only at the least fungal extract concentration and significantly decreased with increasing doses. Such elicitations could lead to significant modifications in the metabolism of plant cells [22].

Dry weight: The results indicated that sorghum dry weight was negatively affected by fungal extracts application in presence or absence of *Striga*. Sorghum dry weight decreased significantly with increasing of fungal elicitor concentration **(Table 2)**.

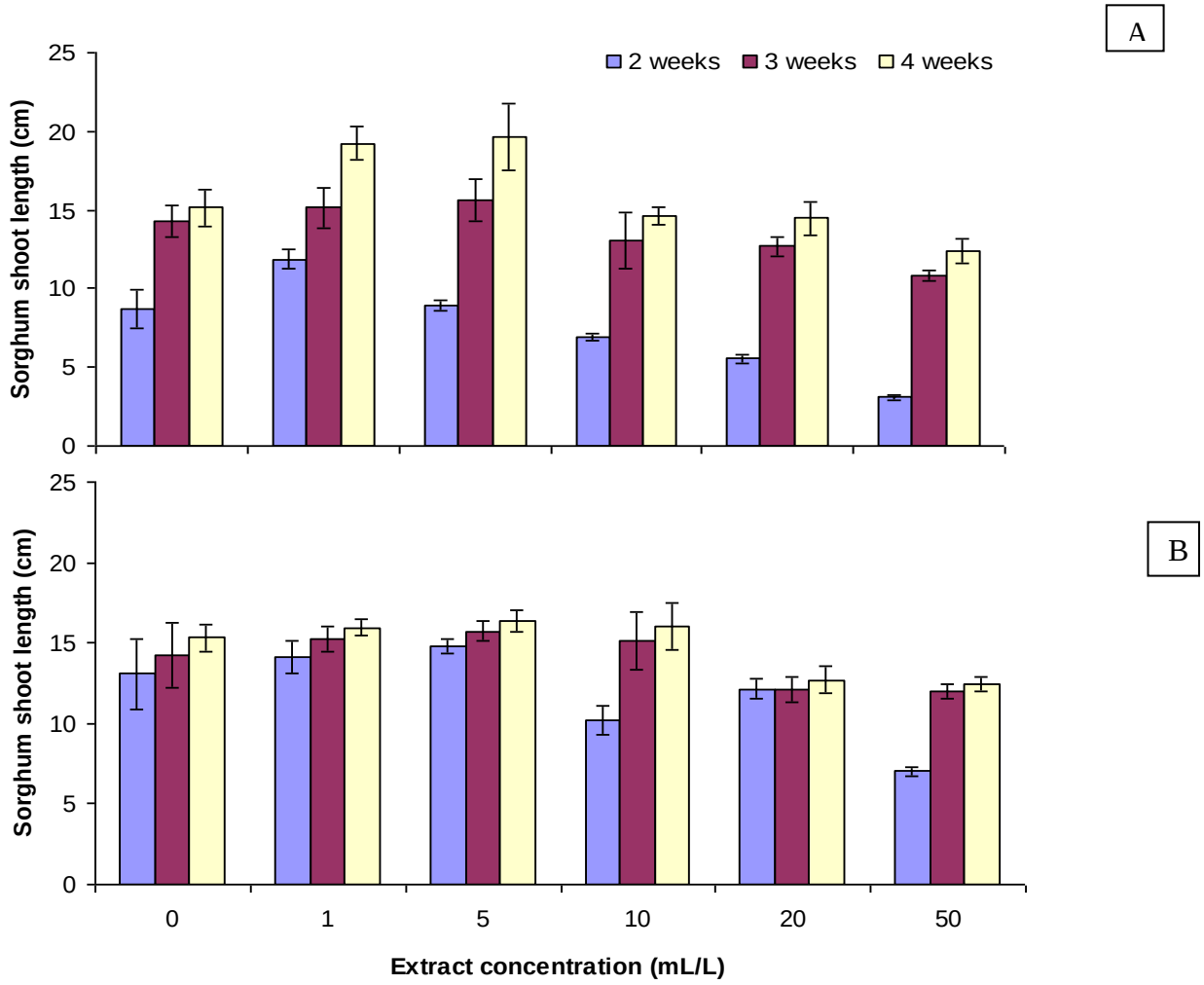

Figure 3: Effect of fungal elicitors (0-50 mL/L) on sorghum shoots length in presence (A) or absence of *Striga* (B) after three periods (2, 3 or 4 weeks) of exposure. Vertical bars indicated standard errors.

Table 1: Effect of fungal elicitor (0-50 mL/L) on sorghum root length and roots/shoots ratio cultured with or without *Striga* after 4 weeks of exposure.

fungal elicitor concentration (mL/L)	Root length (cm)		Root/shoot ratio	
	Existence of *Striga*		Existence of *Striga*	
	+	-	+	-
0.0	7.81±0.48ab	7.65±0.57b	0.53±0.06a	0.52±0.05b
1.0	8.41±0.31a	9.13±0.34a	0.44±0.02ab	0.57±0.03a
5.0	7.24±0.35abc	7.0±0.52c	0.38±0.04b	0.43±0.05cd
10	6.8±0.79bc	6.58±0.81d	0.47±0.05ab	0.41±0.04d
20	5.85±0.41c	5.68±0.44e	0.41±0.03ab	0.46±0.05c
50	4.13±0.24d	4.3±0.22f	0.34±0.02b	0.36±0.03e
Mean	6.71±0.6	6.72±0.5	0.43±0.03	0.46±0.04

Values represent means ± standard errors. Existence of *Striga* (+): presence, (-): absence.

Table 2: Effect of fungal elicitor (0-50 mL/L) on sorghum dry weight cultured with or without *Striga* after 4 weeks of exposure.

fungal elicitor concentration (mL/L)	Shoot dry weight (mg)		Root dry weight (mg)	
	Existence of *Striga*		Existence of *Striga*	
	+	-	+	-
0.0	28.7 ± 3.2^{a}	34.4 ± 0.7^{a}	10.1 ± 2.0^{a}	10.1 ± 0.4^{a}
1.0	25.7 ± 1.8^{a}	27.9 ± 1.1^{b}	7.4 ± 0.7^{ab}	7.4 ± 0.2^{b}
5.0	22.3 ± 2.3^{a}	22.6 ± 2.6^{c}	6.6 ± 1.2^{b}	5.6 ± 0.1^{c}
10	22.6 ± 3.4^{a}	22.6 ± 0.6^{c}	6.4 ± 0.6^{b}	5.1 ± 0.2^{d}
20	20.8 ± 2.3^{a}	20.2 ± 2.6^{d}	5.3 ± 1.1^{b}	4.5 ± 0.4^{de}
50	10.5 ± 1.5^{b}	15.5 ± 1.3^{e}	4.6 ± 0.5^{b}	4.8 ± 0.3^{e}
Mean	21.83±2.4	23.9±1.5	6.7±1.0	6.3±0.2

Values represent means ± standard errors. Existence of *Striga* (+): presence, (-): absence..

CONCLUSION

The results showed that fungal extract concentrations enhanced *Striga* germination and seedlings length as compared to the control. The activity of fungal elicitor on *Striga* germination, invariably, increased on increasing concentrations level (10-50 mL/L). Generally, fungal elicitor improved growth of *Striga* and sorghum compared to control.

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