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Research Article

ENHANCEMENT OF DROUGHT TOLERANCE OF *VICIA FABA* L. VIA LOW STRENGTH AQUEOUS EXTRACT OF *NICOTIANA GLAUCA* L.

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ABSTRACT
The aim of the present study was to investigate the induction of drought tolerance of *Viciafaba* (recipient species) by applying low concentrations of *Nicotiana glauca* (donor species) extract revealed by some growth and metabolic observations. Applying 3% *Nicotiana glauca* shoot aqueous extract (NGSAE) increased the plant height, number of branches and dry weight of the recipient species compared to the control at 25 and 50% water field capacity (WFC). Likewise, the crop productivity represented by number of pods, number of seeds, weight of 100 seed and economic yield upon applying the same NGSAE concentration and under the abovementioned field capacities. On the other hand, total chlorophyll attained its maximum content at 6% NGSAE compared to other treatments at 25% WFC. Compared to the control, insignificant difference in proline content of *V. faba* was noticed along the 3% and 6% NGSAE concentrations under different water conditions.

Keywords: *Allelopathy, Broad Bean, Crop Productivity, Growth Parameters, Water-use Efficiency*

INTRODUCTION
Agricultural productivity worldwide is subject to increasing environmental constraints, particularly to drought and salinity due to their high magnitude of impact and wide distribution (Bartels and Sunkar, 2005). Plants are limited to protect themselves against environmental stresses including drought. Thus, plants develop a wide range of strategies to cope with stress situations. Under conditions of water deficiency, drought escape and drought tolerance are two important strategies to ensure plant growth (CheolKim *et al*., 2012).
Drought is the most restrictive factor that diminished agricultural production in arid and Semi-arid regions of the world which covers more than 40% of global land. The rapid growing world population and the adverse impacts of climate change have led to growing competition for water use by industrial and urban users for agriculture to secure enough food. Irrigated agriculture is an important role in total agriculture and provides humanity with a wide range of agricultural products, including fruits, vegetables, grains and cereals. Different approaches have been used to reduce the damages caused by drought such as selection of high water use efficiency and drought resistant cultivars, use of growth regulators GA3, IAA and CK3) and seed treatments with osmoprotectants (Rathinasabapathi, 2000; Blume, 2005).
Recently, few studies indicated the possibility of using natural crude of chemical compounds to reduce the adverse effect of drought stress on plant. Makkar and Becker (1996) found that using of *Moringa oleifera* L. leaves water extract was found to be a potential source to mitigate the deleterious effect of drought on maize. They further indicated that the water extract contains growth promoters which may reduce the adverse effect of drought by delaying the leaves senescence and scavenging the Relative oxygen molecules. Others found that the lower concentrations of water extract have stimulatory effect on plant growth (Cheema *et al*., 2012). Maqbool (2010) found that foliar application of lower concentration of the water extract of sorghum significantly stimulated growth and productivity of maize. Furthermore,
Viciafaba L. is cultivated during winter season in Nile Delta of Egypt (rainy places) and in some desert ecosystems which dominates by drought and high temperature environment. The productivity of this crop is reported to reduce significantly by drought conditions (El-Darier and Elsayed, 2013). Therefore, searching for improve growth and yield of this crop when grow under drought condition would be a vital assignment. There is limited reported information dealing with the role of allelopathy on the improvement

of drought tolerance. Therefore, we provide a study on the general features of the induction of drought tolerance of *V. faba* (recipient species) by applying low concentrations of *Nicotiana glauca* (donor species) extract revealed by classic physiological and morphological observations. Overall, the application of low strength plant extract provides new insights into novel protocols to improve plant defense responses to drought, an important component of agricultural production systems affected by a changing climate.

MATERIALS AND METHODS

Plant Materials and Soil

Samples from the flowering branches of *N. glauca* were harvested from different locations at Burg El-Arab area (48 km west of Alexandria). The plant parts (leaves and flowers) were air-dried in shade under laboratory conditions for ten days after that chopped into pieces of about 1 cm long and kept under appropriate conditions until use.

Ten soil samples from the natural sites where the alleged materials of allelopathic action are not deposited were used to execute the growth experiment. The samples were analyzed for some of their chemical and physical properties according to Allen *et al.,* (1984).

Qualitative Phytochemical Screening Analysis

The collected materials were dried in shade then grinding and qualitatively analyzed to determine the presence of alkaloids (Harborne, 1999), saponins (Farnsworth, 1966), steroids and tannins (Harborne, 1973), as well as flavonoids and coumarins (Harborne, 1998). The leaves were also analyzed to detect the presence of phenolic compounds (Evans *et al*., 1996), glycosides (Lewis and Smith, 1967), Tri-terpenoids were determined according to Lawand and Gandhi (2013).

Quantitative Phytochemical Screening

The powder of *N. glauca* shoots was also analyzed to determine the total flavonoids (Marinova *et al*., 2005) and total phenolics (Singleton *et al*., 1999). The results of tannins are expressed in terms of gallic acid mg/g of the extract (Sultana *et al*., 2012). In addition, total ash was determined according to Allen *et al.,* (1984).

Preparation of N. Glauca Aqueous Extracts and Water Stress Levels

One hundred of the chopped plant materials were added to one liter of distilled water for 24 hours and occasionally shacked. The extract was filtered by cheese cloth to remove the plant debris. The filtrate was diluted with distilled water to obtain final concentrations of 3 and 6 % (W/V). One treatment was run as a control using distilled water.

The treatments were comprised of three stress levels and three *N. glauca* water extracts. The water stresses were applied by irrigating the pots to known field capacity until reaches 25%, 50% and75% beside the control. Field soil water capacity was determined by weight basis method (Nachabe *et al*., 2003). Water stress levels and *N. glauca* water extracts (0 “control, 3 and 6 % were applied alone. The experiment was conducted in randomized complete block design under split plot arrangement with three replications. The drought levels were kept in the main plots while donor species extract concentrations were assigned to subplots.

Parameters Recorded

Growth Criteria

Five randomly selected individuals from the studied species from each pot were used to measure plant height (cm), number of branches and dry weight per individual. As well, the number of pods, the number of seeds per pod, the weight of 100seed and economic seed yield were estimated.

Pigments and Proline Content

To determine the photosynthetic pigments, a sample of 0.25 g of fresh leaves was extracted with 10 ml acetone. The extract was centrifuged at 3000 rpm for one minute and the filtrate was taken and completed to 10 ml of appropriate amount of acetone. The leaf photosynthetic pigments were determined as described by Moran (1982). On the other hand, free proline content was determined following the method of Bates *et al*., (1973).

Statistical Analysis

Data collected on different parameters were analyzed statistically by using the COSTAT 2.00 statistical analysis software manufactured by Co-Hort Software Company. For analysis of variance and means data were separated using Fisher's protected least significant difference (LSD) test at 5% probability level (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Results

Soil Analysis

The analysis for soil applied in the current study is presented in Table 1.

Table 1: Variation in some physical and chemical characteristics of the soil applied in the pot experiment. Considering that, a: dSm^{-1}, b: % and c: $mg\ g^{-1}$

Chemical Character	Range
Electrical conductivity [a]	2.72 – 2.82
Organic matter [b]	9.08 – 10.00
pH	7.82 – 9.01
Na [c]	1.95 – 2.08
K [c]	3.50 – 3.98
Ca[c]	15.40 – 17.34
Mg [c]	1.40 – 1.42
Cl[c]	15.25 – 16.45
CO_3[c]	35.00 – 37.21
Physical character	
Sand [b]	58.00 – 61.28
Silt [b]	18.00 – 19.23
Clay [b]	24.00 – 26.90
Soil Texture	Sandy loam

Phytochemical Screening of the Donor Species

The phytochemical screening of the donor species is presented in Table 2 and 3. The data showed that the donor species was found to contain alkaloids, flavonoids, glycosides, phenolic compounds, coumarins, saponins, sterols/triterpenes, and tannins. In addition, the data clearly demonstrated that the total flavonoids (9.9 /100g dm), polyphenols (2.03 /100g dm), tannins (2.09 /100g dm) and the total ash (13.23%) were found in the donor species.

Table 2: Phytochemical screening of the donor species during the year of 2015

Component (s)	*Nicotiana glauca*
Alkaloids	+++
Coumarins	+ +
Flavonoids	+++
Glycosides	+++
Phenolic compounds	+
Saponins	+
Sterols and / or triterpenes	++
Tannins	+

The notations, +++, ++ and + refer to appreciable amounts (positive within 5 min); moderate amounts (positive after 5 min but within 10 min); trace amounts (positive after 10 min but within 15 min), respectively.

Table 3: Total flavonoids, total polyphenols, tannins and total ash estimated in the donor species during the year of 2015

Donor species	Total flavonoids* (% /100g dm)	Polyphenols ** (% /100g dm)	Tannins (% /100g dm)	Total ash content (%)
Nicotiana glauca	9.91	2.03	2.09	13.23

** Total flavonoids were determined as routin*
*** Totalpolyphenols were determined as chlorogenic acid*

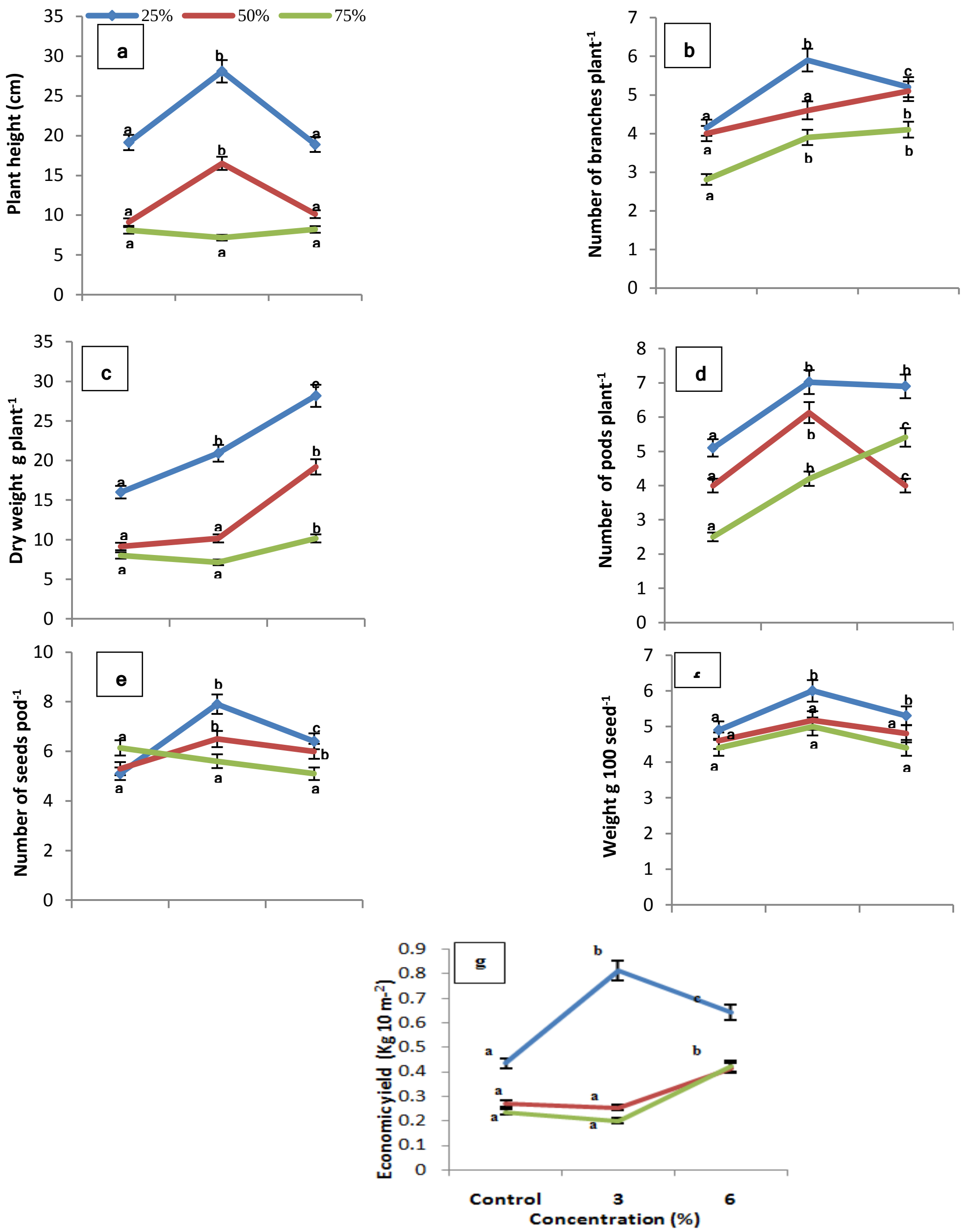

Figure 1: Effect of *Nicotiana glauca* shoots aqueous extracts under different water field capacity on some growth parameters of *Vicia faba.* Data points are the mean ± SD. Different letters for each water field capacity level indicate significance at probability level 0.05

Biological Activity of Low Strength of Nicotiana Glauca Shoots Aqueous Extracts (NGSAE) on Some Growth Parameters of Vicia faba

The effects of NGSAE under different water regimes on plant height, number of branches and dry weight of *V. faba* are represented in Figure 1a – 1c. Applying 3% NGSAE increased the PH compared to the control at 25 and 50% water field capacity (WFC) (Figure 1 a). However, the same treatment exhibited the minimum plant height at 75% WFC. On the other hand, 6% NGSAE treatment achieved higher PH values compared to control. Data showed a maximum value of the number of branches per plant (NBP) at 25% WFC with 3% NGSAE treatment (Figure 1 b). The lowest NBP was recorded at control under 25%, 50% and 75% WFC. 6% NGSAE treatments attained the maximum NBP at both 50 and 75% WFC. 3% NGSAE attained less NBP compared to 6% NGSAE at both water regime 50 and 75 %. Plant dry weight (PDW) achieved its maximum at 6% NGSAE treatment under all water conditions (Figure 1 c).

Likewise, the crop productivity represented by number of pods, number of seeds, weight of 100 seed and economic yield upon applying NGSAE under different water regimes of *V. faba* are demonstrated Figure 1d – 1g. The data demonstrated that the number of pods produced per plant (PP) of *V. faba* was significantly increased by applying the different concentrations of *N. glauca* aqueous extracts (Figure 1 d). Under low water field capacity (LWFC= 25%) the maximum number of PP was attained at 3% NGSAE followed by 6%NGSAE, while the minimum PP was attained at control. Half water field capacity (HWFC) conditions have modified the effect of 6% NGSAE on PP to become equal to control value, but the 3% NGSAE treatment achieved the maximum value of PP. Maximum PP was achieved at 6% followed by 3% whereas, control value was the minimum under high water holding capacity of 75%. In parallel, the maximum number of seeds per pod (NSP) was attained at 3% under 25% and 50% WFC (Figure 1 e). While, the minimum NSP was recorded at control under both 25% and 50% water field capacity. At 75% WFC, the highest NSP was reported at control followed by 3% NGSAE then 6% NGSAE treatments. The observed weight for 100 seeds (WS) at 25%, 50% and 75% WFC attained a maximum value at3% NGSAE (Figure 1 f). In addition,6% NGSAE treatment achieved higher WS values compared to control at 25% and 50% WFC. Treatment of *V. faba* with 3% and 6% NGSAE significantly increased the economic yield of the plant at 25% WFC (Figure 1 g).

Biological Activity of Low Strength of Nicotiana glauca Shoots Aqueous extracts (NGSAE) on Pigment and Proline Contents in Vicia faba

Values of total chlorophyll contents were notably different in response to changing in the concentrations of *N. glauca* aqueous extracts (Figure 2a).

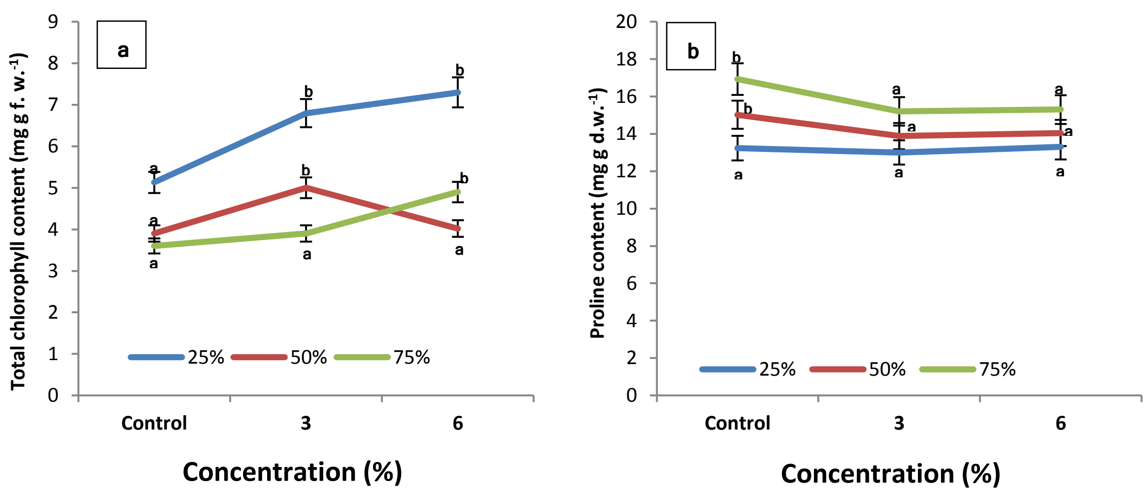

Figure 2: Effect of *Nicotiana glauca* shoots aqueous extracts under different water field capacity on total chlorophyll and proline content of *Vicia faba.* Data points are the mean±SD. Different letters for each water field capacity level indicate significance at probability level 0.05

Using 6% NGSAE recorded the highest total chlorophyll contents compared to other treatments at 25% and 75% WFC. At 50% WFC plus 3% NGSAE treatment, the maximum total chlorophyll content was recorded.

Compared to the control, insignificant difference in proline content of *V.faba* was noticed along the 3% and 6% NGSAE concentrations under different water conditions (25%, 50% and 75% WFC) (Figure 2b). Unexpectedly, the recorded proline content was increased with increasing of WFC from 25% to 75%.

Discussion

Water scarcity is the most significant challenge that meets agriculture worldwide, since most of the water resources (about 90%) of dry areas including many developing countries are consumed in irrigation (FAO, 2011).

In addition, global warming will make the problem of water shortage more sever and consequently will affect agriculture ecosystems and crop production.

In view of challenges that meet today's researchers which are increase crop productivity and sustainable water use, new strategies should be applied to solve and prevent the predicted negative consequences from water deficiency (WWDR, 2015).

Based on several studies (Makkar and Becker, 1996; Cheema *et al*., 2012; Maqbool, 2010; Al-Hussaini, 2013; Ahmed *et al*., 2014) in allelopathic field it was noticed that lower concentrations of the allelopathic species have stimulants that may affect on the recipient species. Therefore, the main goal of the current work is to analyze the role of low strength concentrations of *N. glauca* shoots aqueousextract (NGSAE) under different water stress conditions on some growth and physiological parameters of *V.faba;* a common economic crop in Egypt.

Recently, phenomenon of allelopathy is fruitfully applied in crop science field for biological weed management as well as improvement of crop growth (Farooq *et al.*, 2011).

Rinez *et al.*, (2011) reported that, seed germination and root length of lettuce and radish plants were inhibited in response to high concentrations of *Nicotiana glauca* aqueous extracts as compared to control and the inhibition was attributed to the presence of phenolics.

On the other hand, results of the present study have proved enhancement of some growth parameters of *V.faba* upon exposure to low strength concentrations of 3% NGSAE plus 25% WFC compared to control.

Likewise, the economic yield of *V. faba* at 3% NGSAE plus 25% WFC treatment was significantly increased compared to control with higher WFC conditions. Total chlorophyll content also has shown positive response to the use of 3% and 6% NGSAE plus 25% WFC compared to control.

Phiri (2010) reported that use of *M. oleifera* leaf extract to enhance the seed germination of sorghum crop. It is also documented that 0.05% of *Moringa oleifera* leaf aqueous extract significantly promoted the growth and grain yield of crops (Basra *et al*., 2009). Furthermore, foliar application of sorghum water extract proved to be effective in mitigating the drought effect on *Vigna radiata* (Al-Hussaini, 2013).

Application of *N. glauca* in the present study at low strength concentration has proven its worth as an excellent source of plant growth-promoting substances.

Practically, *N. glauca* is a rich source of phenolics and flavonoids as well as many essential plant compounds.

These compounds perform as signaling molecules via activation of node gene essential in the initiation of legume-rhizobia symbioses and can act as agents in plant defense against soil borne pathogens (Mandal *et al*., 2010).

In the future, it may be tried either as foliar spray or seed priming agent for growth promotion which may behaves differently with the type of crop and change in its dilution The higher concentrations of growth promoters including *N. glauca* are generally considered to suppress the plant growth of some crop and leafy vegetable species (Shaklool, 2015).

The result will help in improving the crop productivity under drought or low water condition which will match the global aim of having the best crop productivity with sustainable use of water. It is thus essential to improve water use efficiency in agriculture. This will require integrated strategies for effective and reasonable investment of water resources and to guarantee sustainability.

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