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Yield, Fatty Acids and Antioxidant Enzymes of Two Canola (*Brassica napus* L.) Cultivars in Response to Stigmasterol

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ABSTRACT

Field experiments were carried out at the Agricultural Experimental Station of the National Research Center at Nobaryia, Egypt during two successive winter seasons (2006/7 and 2007/8) to study the effect of stigmasterol (SS) application (0, 200 and 400 ppm) on yield and its components, antioxidant enzymes, as well as seed quality of two canola varieties 'Serw 4' and 'Serw 6'. Plant height and dry weight/plant increased when SS was increased up to 400 ppm in both cultivars. Also, seed yield/plant, number of seeds/pod, 1000-seed weight, seed and oil yield (kg/ha) increased significantly after application of 400 ppm SS. The highest oil percentage was obtained when 200 ppm SS was applied, which also resulted in the highest oleic acid content in both canola cultivars, but decreased linoleic and linolenic acid contents. The erucic acid content of 'Serw4' plants treated with 400 ppm SS reduced slightly from 0.40 to 0.18%, while in 'Serw 6' the application of 200 ppm SS resulted in an increase in the erucic acid content from 0.16 to 0.46%. Glutathione reductase (GR) and ascorbate peroxidase (APX) gradually increased in 'Serw 4' and 'Serw 6' as SS increased from 0 to 200 and/or 400 ppm. GR and APX contents were highest in the 400 ppm SS treatment.

Keywords: ascorbate peroxidase (APX), glutathione reductase (GR), growth, fatty acids, oil percentage

INTRODUCTION

The cultivated area of canola in Egypt was relatively small this past decade. This is due to the strong competition between canola and other strategic winter season crops such as wheat (1,227,052 ha) and Egyptian clover (916,667 ha) on the limited arable land in the Nile Valley and the Nile Delta. The government's policy to meet the increasing demand of oil is to rely on winter rapeseed crops. Canola (*Brassica napus* and *Brassica campestris*) is the major edible rapeseed oil crop. Canola seeds are not only a rich source of oil (40-45%), but also a source of good quality protein (25%) (Scarisbrick and Daniels 1986). In Egypt, canola (spring types) can be successfully grown in winter (Sharaan 1987).

Stigmasterol (SS) is a structural component of the lipid core of cell membranes and is a precursor of numerous secondary metabolites, including plant steroid hormones or serves as a carrier in sugar and protein transport (Genus 1978). The biological functions of sterol conjugates such as fatty acids or glucoside esters sterol and sterylacyglucoside are as sterol storage forms, e.g. oil bodies of chamomile plant, *Chamomilla recutita* (Abd-El Wahed and Gamal El-Din 2005). The interaction of sterols with phospholipids may stabilize membrane permeability (Grunwald 1982) whereas sterols play a role in plant development, including cell expansion, vascular differentiation, etiolating and reproductive development of wheat, *Triticum aestivum* (Abd El-Wahed *et al.* 2000). Both sitosterol and SS, typical sterols, are similarly involved in the regulation of plant development and gene expression. Sterols are essential for normal plant growth and development (He *et al.* 2003). Sterols are known to regulate transcriptional and post-transcriptional events, which in turn affect lipid synthesis, meiosis, apoptosis, developmental patterning, protein cleavage and protein degradation (Edwards and Ericsson 1999). Practically, sterols affect carbohydrate distribution in maize, *Zea mays* (Abd-El Wahed 2001) and free amino acids, phenols and indoles in soybean, *Glycine max* (Abd-El Wahed 2008). In addition, brassinosteroids are able to generate erect leaves in rice, *Oryza sativa* (Morinaka *et al.* 2006).

Therefore, this investigation was carried out to study the effect of SS application on yield, fatty acid and antioxidant enzymes content of two canola cultivars grown in newly reclaimed sandy soils in Egypt.

MATERIALS AND METHODS

Two field experiments were carried out at the Agricultural Experimental Station of the National Research Center at Nobaryia during two successive winter seasons (2006/7 and 2007/8) to study the effect of SS application on yield and its components, as well as antioxidant activities and seed quality of two canola (*Brassica napus* L.) varieties. Both cultivars were obtained from the Serw Experimental Station, Damietta, Agriculture Research Center, Egypt. 'Serw 4' was produced via anther culture from var. 'Fido' (Sweden) while 'Serw 6' is a haploid plant selected from var. 'Premier' (Germany). The experimental design was a split-plot with four replications. The main plots were devoted to the canola varieties 'Serw 4' and 'Serw 6' while SS (0, 200 and 400 ppm) were randomly distributed in sub-plots. SS (3β-hydroxy-24-ethyl-5,22-cholestadiene-5,22-stigmastadien-3β-ol) was applied as a foliar spray at 60 days after planting (DAP). The experimental unit area was 10.5 m^2 consisting of 10 rows (3.5 m long and 30 cm between rows). Seeds were sown at a rate of 7.5 kg/ha on November 20th in 2006/7 and 2007/8 growing seasons. Potassium fertilizer was added before sowing at a rate of 240 kg/ha as potassium sulphate (48-50% K_2O), while nitrogen fertilizer was added at a rate of 150 kg N/ha as ammonium nitrate (33.5%N) in two equal doses at 21 and 35 DAP. Normal cultural practices of growing canola were conducted in the usual manner followed by the recommendations of this district (Saudi 2004). Physical and chemical properties of the soil are listed in **Table 1**.

At 75 DAP a random sample of 5 plants from each plot was

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Table 1 Physical and chemical properties of the soil.

Properties	(0-30 cm)
Coarse sand %	62.67
Fine sand %	34.71
Total sand %	97.68
Silt % + clay	2.32
Soil texture	Sandy
pH	8.43
E.C dS/m	0.22
Organic matter %	0.92
Calcium carbonate %	5.85
Total N (ppm)	392
Available P (ppm)	5.8
Cations (meq/ 100 g. soil)	
Ca^{+2}	3.0
Mg^{+2}	2.0
Na^{+}	1.5
Anions (meq/ 100 g. soil)	
HCO_3^{-}	1.72
Cl^{-}	0.70

taken to determine plant height (cm) and dry weight/plant (g). At the same time, a representative sample of leaves was taken and immediately deep frozen for estimating the activity of two antioxidant enzymes: *Glutathione Reductase* (GR, EC 1.6.4.2) and *Ascorbate Peroxidase* (APX, EC 1.11.1.11). GR and APX were extracted as follows: 5 g of frozen leaf tissues were homogenized in a pre-chilled mortar in 10 ml of 50 mM potassium phosphate buffer (pH 7.0) with 1% (w/v) insoluble poly(vinylpyrolidone)(Sigma Chemical Co., St. Louis, MO) and 0.1 mM EDTA (Sigma). The extraction procedures were repeated twice and supernatants were pooled, raised to a certain volume and referred to as crude enzyme extract. All operations were carried out at -4°C and enzyme extract was kept at -20°C for further analysis. The activity of GR was determined spectrophotometrically at 25°C following the decrease in absorbace at 340 nm according to the method described by Zanetti (1979). APX activity was assayed according to the method of Nakano and Asada (1981) by recording the decrease in ascorbate content at 290 nm as ascorbate was oxidize The reaction mixture contained 1.5 cm^3 of 100 mM potassium phosphate buffer (pH 7.0), 0.5 cm^3 of 3.0 mM ascorbic acid, 0.1 cm^3 of 3.0 mM EDTA, 0.2 cm^3 of 1.5 mM H_2O_2 and 0.1 cm^3 of diluted enzyme extract in a total volume of 3.0 cm^3. The reaction was started with the addition of H_2O_2 and absorbance was recorded at 290 nm spectrophotometrically. The activity was expressed as a change in the optical density/g fresh weight/min under the experimental conditions.

At harvest time, a random sample of 10 plants from each plot was taken to determine some yield attributes such as number of pods/plant, number of seeds/pod, seed yield/plant (g) and 1000-seed weight (g). Plants of 1 m^2 from the middle rows of each plot were harvested. These plants were dried under sunshine for 1 week and seeds were cleaned after separated from the pods, then the seed, straw and biological yields (kg/ha) were estimated.

Crude oil percentage in the seeds was determined according to AOCS (1982) using a Soxhlet apparatus and petroleum ether as a solvent at 40-60°C. Fatty acid composition of oil was also determined by using gas-liquid chromatography (HP-6890 GC Method, HEWLETT Packard Hp-6990 series). The fractionation of fatty acid methyl ester was conducted using coiled glass, column (30 m × 320 mm diameter × 0.25 mm film thickness). The column oven temperature was programmed at 8°C/min from 70-270°C, then isothermally at 270°C for 10 min with N at 30 ml/min. The methyl esters were prepared according to Stahl (1967) using benzene: methanol: sulphuric acid at a ratio of 10: 86: 4 (v/v/v).

Statistical analysis

The analysis of variance procedure of split-plot design was done according to Snedecor and Cochran (1980) and the combined analysis of two seasons was done according to Steel and Torrie (1960). Treatments means were compared using the LSD test at $P = 0.05$.

RESULTS

Effect of SS and canola cultivars on growth and yield

Application of SS as a foliar spray significantly increased plant height and dry weight/plant at 75 DAP and also significantly increased all the studied yield components, except for the number of pods/plant (**Table 2**). Plant height and dry weight were significantly increased by increasing SS up 400 ppm, resulting in the highest values for these traits, while the lowest values were recorded with 200 ppm SS or in untreated plants (control). **Table 2** also indicates that application of 200 or 400 ppm SS produced the highest seed yield/plant in comparison with untreated plants, the differences between treated plants and control being significant; differences were insignificant between 200 and 400 ppm SS.

Plant height and dry matter accumulation of 'Serw 4' and 'Serw 6' increased by increasing SS from 0 to 200 and/or 400 ppm (**Fig. 1A, 1B**). Plant height in 'Serw 4' increased by an estimated 40.78 and 48.56%, respectively, while in 'Serw 6' this increase was estimated at 21.11 and 27.33%, respectively in comparison with the untreated plants.

Dry weight also gradually increased in 'Serw 4' and 'Serw 6' when up 400 ppm SS was applied. The increment of dry matter accumulation due to the application of 200 ppm was estimated at 35.81% in 'Serw 4' and 36.05% in 'Serw 6' while the increase due to the addition of 400 ppm was estimated at 55.43 and 58.05%, respectively compared with the untreated plants. However, when 'Serw 4' and 'Serw 6' plants were sprayed with 400 ppm SS they recorded a slight increase in dry matter accumulation than plants that received 200 ppm (**Fig. 1B**).

Seed yield and yield components were significantly influenced by the application of SS. **Table 2** shows that application of 200 or 400 ppm SS resulted in the highest seed yield/plant; a significant increase was noticed in comparison with the untreated plants while insignificant differences were noticed between 200 and 400 ppm. The number of pods/plant was also significantly affected by the application of SS; however, 200 ppm produced more pods/plant than 400 ppm SS or untreated plants (**Table 2**). The same trend was observed in 1000-seed weight, oil percent and also oil yield.

On the other hand, increasing the SS concentration up to 400 ppm significantly increased the number of seeds/pod, as well as seed yield. A gradual increase in both the number of seeds/pod and seed yield was recorded when using high levels of SS. The highest number of seeds/pod (19.53) and also seed yield (2655.503 kg/ha) were recorded when 400 ppm SS was used. The increase in seed yield/ha was 801.665 and 884.997 kg/ha at 200 and 400 ppm SS compared with untreated plants, respectively, although the dif-

Table 2 Plant height, dry weight and yield in response to stigmasterol application.

Stigmasterol Ppm	Plant height cm	Dry weight / plant g.	Seed yield / plant (g)	Number of pods / plant	Number of seeds / pod	1000- seed weight (g)	Seed yield (kg/ha)	Oil yield (kg/ha)	Seed oil %
0.0	58.50	4.09	6.59	87.89	15.47	2.91	1770.52	689.09	38.92
200	50.00	5.56	10.12	98.47	17.53	3.22	2572.19	1063.86	41.36
400	52.67	6.41	10.20	84.80	19.53	3.03	2655.50	1039.10	39.13
LSD 5%	3.67	1.43	0.89	NS	1.17	0.08	182.50	61.80	0.54

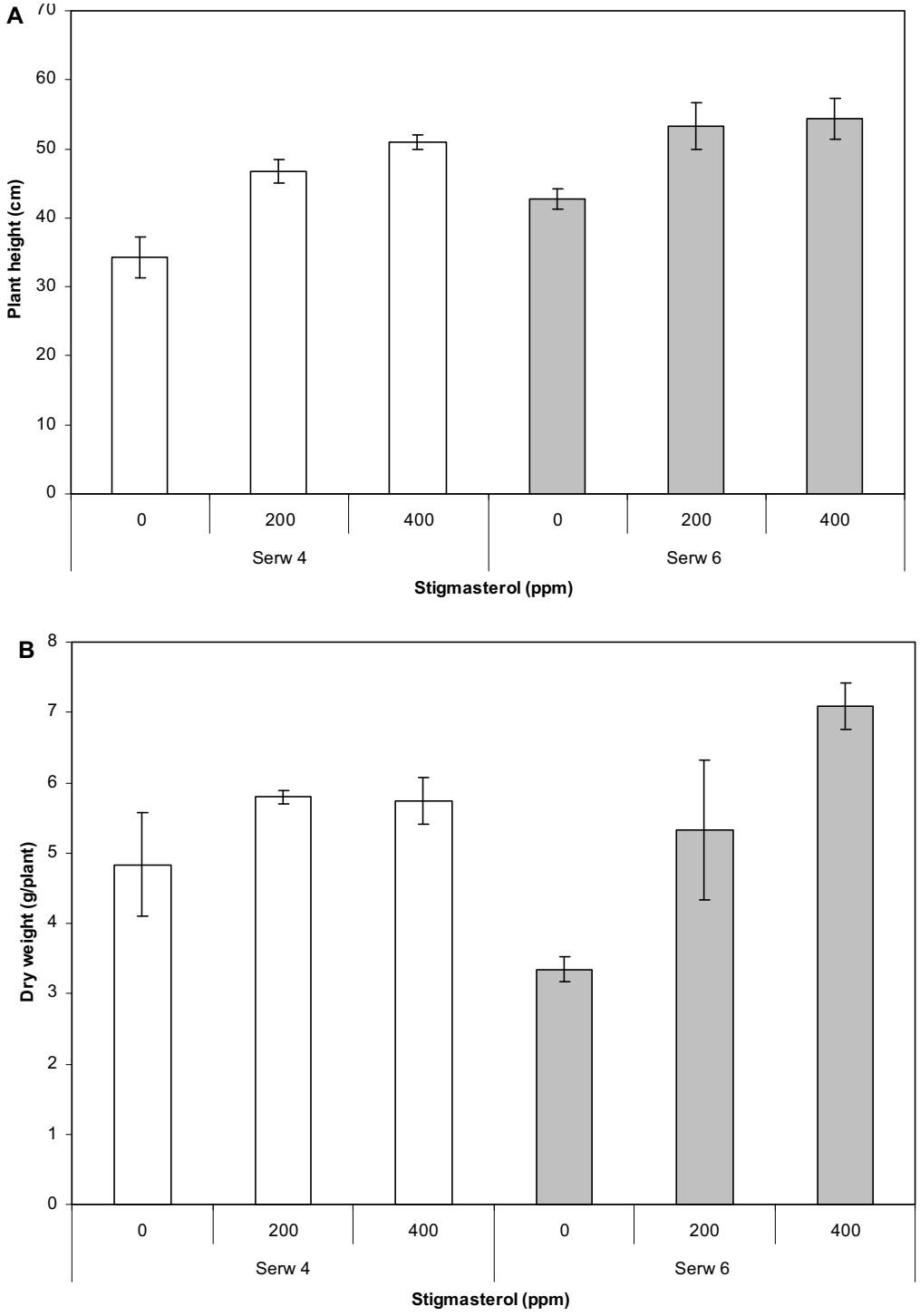


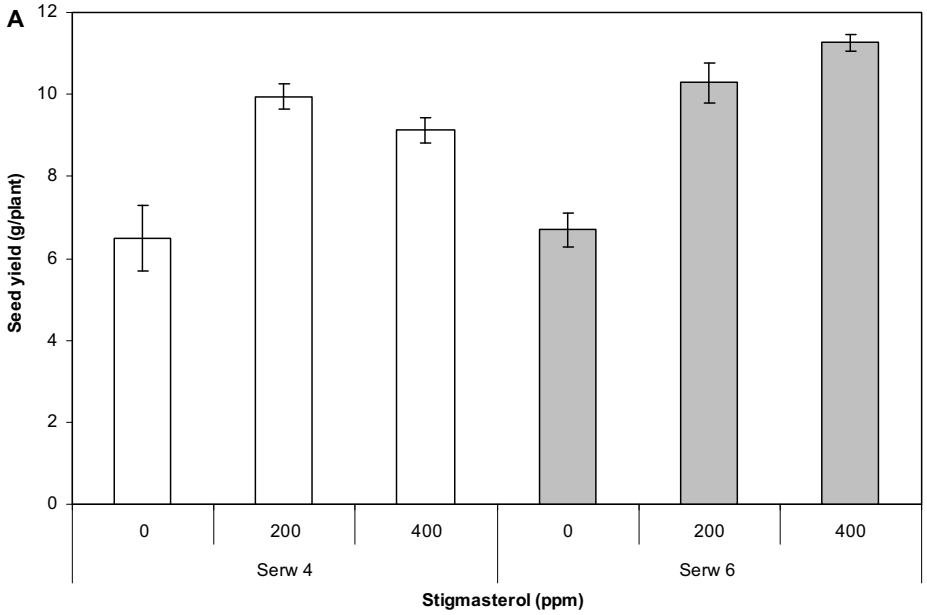
Fig. 1 Effect of application of stigmasterol on plant height (A) and dry weight/plant (B) of two canola cultivars.

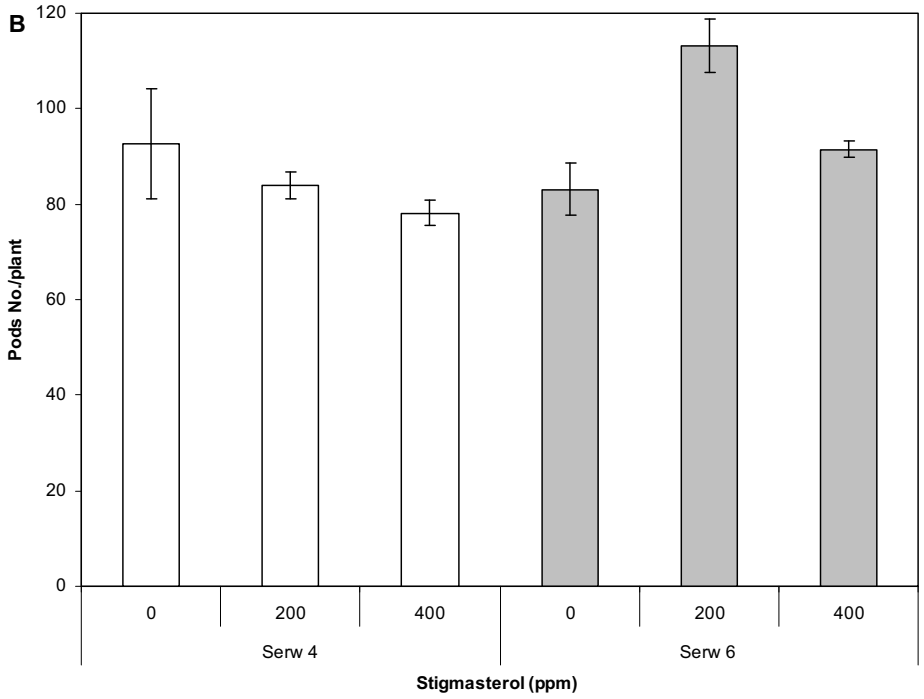
ferences between 200 and 400 ppm were insignificant.

Regarding the interaction between the two canola cultivars and SS application, yield and its components were not significantly affected. **Fig. 2A-G** illustrates that application of SS up to 200 ppm produced more seeds/plant in 'Serw 4' while application of 400 ppm increased the seed yield/plant in 'Serw 6'. In both canola cultivars 200 ppm SS resulted in an increase in number of pods/plant, 1000-seed weight, oil percentage as well as oil yield in comparison with plants treated with 400 ppm SS or with untreated plants. On the other hand, the number of seeds/pod gradually increased up to 400 ppm. A similar trend was observed in seed yield and the highest seed yield (2699.550) was produced by 'Serw 4' at 400 ppm SS (**Fig. 2 E**).

Effect of SS and canola cultivars on fatty acid composition

In both canola varieties the content of all fatty acids gave approximately the same values while the oleic acid was higher in 'Serw 4' than in 'Serw 6' (**Table 3**). The application of 400 ppm SS increased the palmitic acid content in 'Serw 4', although a slight decrease in this acid was obtained in 'Serw 6' at the same concentration (**Table 3**). Application of 200 ppm resulted in the highest oleic acid content (63.35%) in both canola cultivars while 'Serw 6' produced the lowest oleic acid (59.92%) in untreated plants. The linoleic acid content seemed to be the same in the control and in 'Serw 4' plants treated with 200 ppm SS with a slight decrease at 400 ppm. However, untreated 'Serw 6' plants produced the highest linoleic acid content (20.39%),





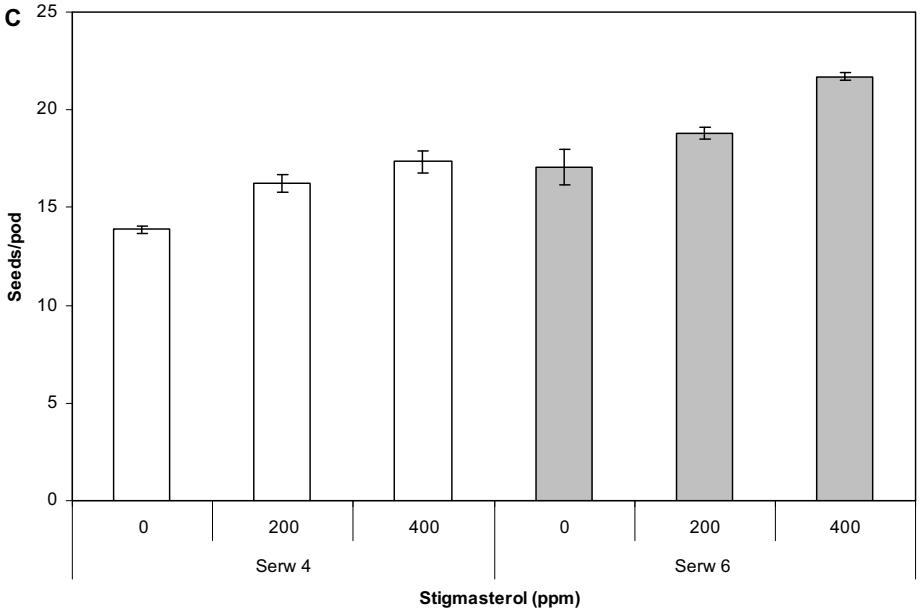
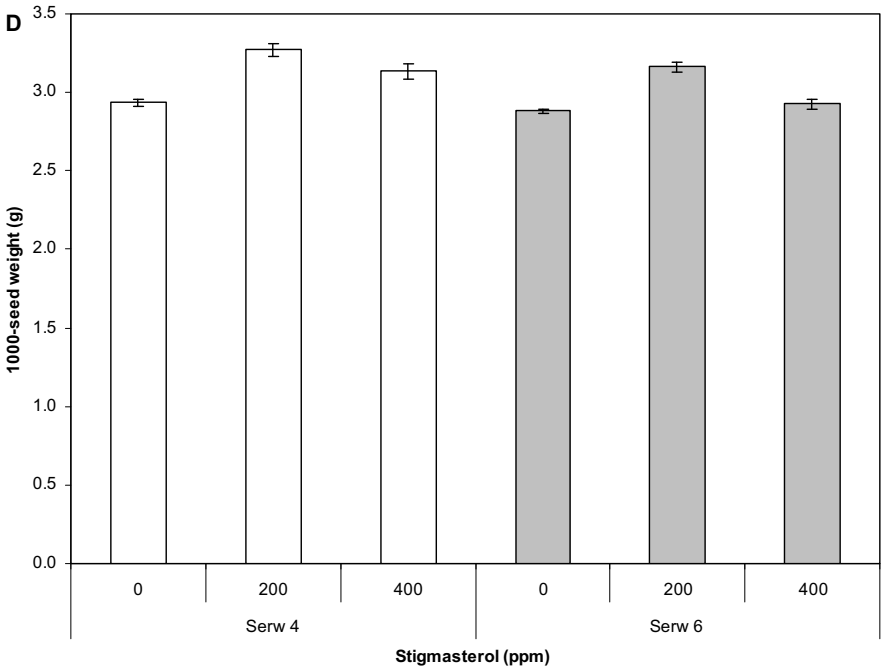
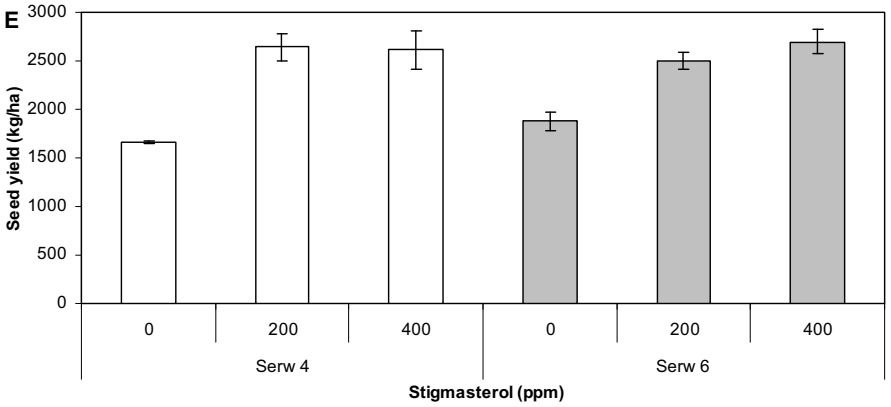
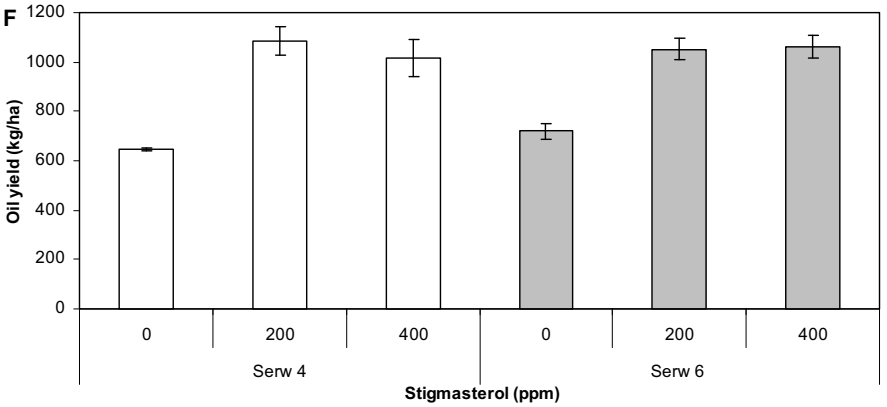


Fig. 2A-G Effect of application of stigmasterol on yield and yield components of two canola cultivars.


D
1000-seed weight (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
200
400
0
200
400
Serw 4
Serw 6
Stigmasterol (ppm)


E
Seed yield (kg/ha)
3000
2500
2000
1500
1000
500
0
0
200
400
0
200
400
Serw 4
Serw 6
Stigmasterol (ppm)


F
Oil yield (kg/ha)
1200
1000
800
600
400
200
0
0
200
400
0
200
400
Serw 4
Serw 6
Stigmasterol (ppm)

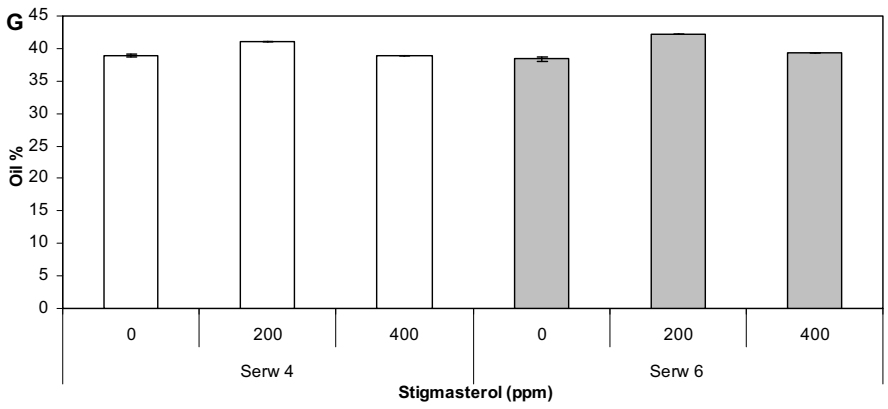

G
Oil %
45
40
35
30
25
20
15
10
5
0
0
200
400
0
200
400
Serw 4
Serw 6
Stigmasterol (ppm)

Table 3 Effect of stigmasterol application on fatty acids composition (%) of two canola cultivars.

Fatty acids	Serw 4				Serw 6			
	Stigmasterol (ppm)							
	0.0	200	400	Mean	0.0	200	400	Mean
Palmitic (16:0)	3.98	3.10	4.11	3.73	3.89	3.85	3.65	3.80
Oleic (18:1)	61.13	63.35	62.57	62.35	59.92	63.35	61.45	61.57
Linoleic (18:2)	19.33	19.72	18.24	19.10	20.39	18.60	19.44	19.48
Linolenic (18:3)	11.10	9.10	10.24	10.15	12.17	9.18	11.00	10.78
Erucic (22:1)	0.34	0.40	0.18	0.31	0.15	0.16	0.46	0.26

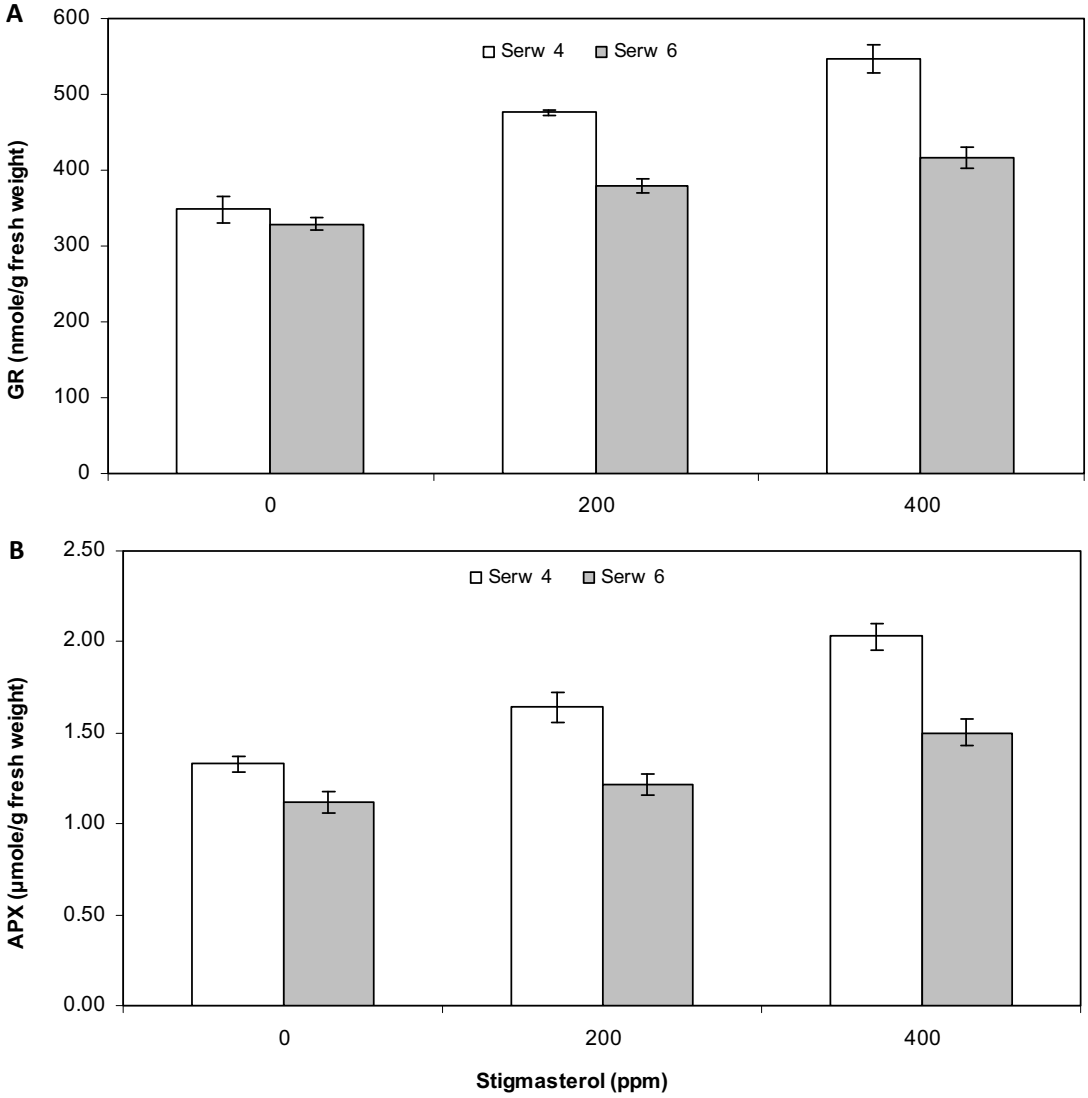


Fig. 3 Effect of application of stigmasterol on glutathione reductase (GR) and ascorbate peroxidase (APX) of two canola cultivars.

while adding 200 ppm SS resulted in a decrease in linoleic acid content. Linolenic acid content decreased only following the application of 200 ppm SS in both canola cultivars but it increased up to 12.17% in untreated 'Serw 6' plants (control). The erucic acid content of 'Serw 4' plants treated with 400 ppm SS reduced slightly from 0.40 to 0.18%. In 'Serw 6', the application of 200 ppm SS resulted in an increase in erucic acid content from 0.16 to 0.46%, but it decreased to 0.26% with 400 ppm SS (**Table 3**).

Effect of SS and canola cultivars on antioxidant enzymes

Figs. 3A, 3B indicated that 'Serw 4' had a higher GR (457.19 ± 13.33) and APX (1.67 ± 0.073) than 'Serw 6' (374.39 ± 10.69 and 1.28 ± 0.064, respectively). In this regard, application of SS to both canola cultivars increased the content of GR and APX in both cultivars. GR gradually increased in 'Serw 4' and 'Serw 6' by increasing the SS from 0 to 200 and/or 400 ppm. The highest GR content (547.07 ± 19.08) was recorded with 400 ppm SS, followed by the application of 200 ppm. At the same time, a similar trend was also noticed in APX content under the same SS application. In both 'Serw 4' and 'Serw 6', adding 400 ppm as a foliar spray resulted in the highest APX content (2.03 ± 0.086 and 1.50 ± 0.073, respectively) in comparison with 200 ppm or the treatment not sprayed with SS.

DISCUSSION

Some growth traits and yield and yield components of two canola varieties 'Serw 4' and 'Serw 6'were not significantly affected. This may be attributed to the suitable Egyptian agro-ecological conditions for growing local varieties. These results are in agreement with those obtained by Ahmed *et al.* (1999) and Keshta and Leilah (2003) who

pointed out that sowing 'Serw 4' was recommended for growing canola plants successfully in winter in Egypt so as to increase the yield and its components as well as seed quality. Mekki (2007) also revealed that 'Serw 4' was superior in growth, yield and yield components than 'Serw 6' under Egyptian conditions. Moreover, the favourable effect of SS on growth could be attributed to the stimulative action of SS on phytohormones such as auxins and cytokinins, which in turn induced cell elongation and division (Gregory and Mandava 1982). Gregory and Mandava (1982) also reported that the application of brassinosteroids to plants allows auxins and cytokinins to stimulate growth of whole plants. The increase in dry weight following the application of SS may have been due to the fact that SS improved photosynthetic activity in turn increasing dry matter accumulation of canola plants. These results are in agreement with Fernandes *et al.* (1992) and Rashad *et al*. (2009) who pointed that application of 80 mgL^{-1} β-sitosterol increased the dry weight of marigold plants, *Caledula officinalis* L. Nassar (2004) also reported that dry weight was significantly increased with the application of 100 ppm SS as a foliar spray, and then the highest dry weight was recorded at 100 ppm being 30.7% more than untreated soybean plants. This means that sterols play an important role in plant development, including cell expansion, vascular differentiation, etiolating and reproductive development (Abd-El Wahed 2001). Sterols are essential for normal plant growth and development (He *et al.* 2003). Also, Abd-El Wahed *et al.* (2000) on wheat, *Triticum aestivum*, Nassar (2004) on soybean, *Glycine max* and El-Greedly and Mekki (2005) on sesame, *Sesamum indicum*, reported that plant height and dry weight of sesame significantly increased following the application of 200 ppm SS compared to the 100 ppm treatment. In addition, the increase in yield and its components attributed to foliar application of SS might be a result of the beneficial effect of the SS enhancement of photosynthetic apparatus, growth parameters, cell division and enzymatic activity (Wang 1997; Clouse and Sasse 1998). A similar trend was observed by El-Greedly and Mekki (2005) on sesame; they indicated that plants that received 200 ppm SS showed an increased in number of seeds/plant in comparison with 100 or 150 ppm. In contrast, Nassar (2004) found a sharp significant decrease in seed yield/plant of soybean when SS was applied at a relatively high concentration of 200 ppm. Also, Nassar (2004) reported that number of pods/plant and number of seeds/plant of soybean gradually increased up to 100 ppm SS, but decreased sharply by using 200 ppm SS. In this respect, the increase in oil percentage with 200 ppm SS may be due to the increase of 1000-seed weight at the same application which consequently increased the oil yield. A similar trend of decrease of oil % with 150 ppm sitosterol was reported by Abd-El Wahed *et al.* (2000) on maize, while El-Greedly and Mekki (2005) on sesame concluded that plants treated with 200 ppm SS produced the highest seed oil content.

Application of 200 ppm showed the highest oleic acid (63.35%) in both canola cultivars, while 'Serw 6' produced the lowest oleic acid (59.92%) in untreated plants. Linoleic acid had similar values in the control and 200 ppm SS treatment in 'Serw 4', while a slight decrease was noticed up to 400 ppm. However, the untreated plants in 'Serw 6' produced the highest linoleic acid content (20.39%), whereas adding 200 ppm SS resulted in a decrease in linoleic acid. Linolenic acid content decreased only after application of 200 ppm SS in both canola cultivars, while it increased up to 12.17% in 'Serw 6' in untreated plants (control). The second oil quality breeding objective is to reduce the percentage of linolenic acid from 8-10% to less than 3%, while maintaining or increasing the level of linoleic acid (Downey and Röbblen 1989). Lower linolenic acid is desired to improve the storage characteristics of the oil, while higher linolenic acid content may be nutritionally desirable. Farag *et al*. (1986) reported that linoleic acid is the second major unsaturated fatty acid its content ranged from 19.2% in 'SEMU AX' to 13.8% in 'SEMU RT', while linolenic acid was present in low content (4.3%) of local rapeseed varieties. Also, Getinet *et al*. (1997) reported that the high levels of linolenic acid and zero erucic acid in *B. carinata* are undesirable for high quality vegetable oil. Sterols are the major constituents of the unsaponifiable fractions of the most vegetable oils, whose composition in oils and fats is useful in an oil's identity (Johansson and Croon 1981). Oxidative stability of oils is very important for consumers. When oils rich in nutritionally valuable linoleic and linolenic acids are stored, they easily react with oxygen and form hydroperoxides that decompose and give rise to till-tasting aldehydes and ketones (Wagner *et al.* 2004). Phytosteroles are also known to inhibit absorption of dietary cholesterol (Eskin *et al.* 1996). Mourtuza (2006) pointed out that a wide variation in the amount of sterols and tocopherols was observed among mustard/rapeseed cultivars and some cultivars of *Brassica napus* showed considerably high oxidative stability. These results are in agreement with those reported by Davik and Heneen (1993), who pointed out that the concentration of linoleic and linolenic acids were negatively correlated and a high oleic acid concentration (> 50%), which was always associated with a low erucic acid concentration (< 4%). Other studies, such as those by Getinet *et al*. (1994) and Raney *et al*. (1995) reported that in zero erucic acid *Brassica carinata*, the concentration of the polyunsaturated fatty acid linoleic and linolenic were very high and oleic acid was low compared to levels of these fatty acids in concentration of *B. napus* canola.

The application of SS of both canola cultivars increased the content of GR and APX. GR was gradually increased in 'Serw 4' and 'Serw 6' by increasing the SS from 0 to 200 and/or 400 ppm. Overexpression of antioxidant enzymes such as GR and APX conferred protection against reactive oxygen species (ROS) in plants which result from the exposure of plants to different environmental stimuli (O'kane *et al*. 1996). The increase of GR activity under SS application helps canola plants to be more tolerant to unfavorable conditions such as salt, drought and atmospheric stresses, especially when grown in newly reclaimed sandy soils. GR also can remove H_2O_2 via the ascorbate glutathione cycle to maintain a high level of reduced ascorbate within chloroplast. H_2O_2 is eliminated by APXs (Chen and Asada 1989). For instance, antioxidants intercept the free radical chain of oxidation and to contribute hydrogen from the phenolic hydroxyl groups themselves thereby forming stable free radicals which do not initiate or propagate further oxidation of lipids (Dziezak 1986). APX plays an important role in enzymatic ROS scavenging systems (Orabi 2004). Foyer *et al.* (1997) stated that overexpression of GRase in chloroplasts doubled the concentrations of ascorbate and glutathione in leaves and conferred increased resistance to oxidative stress, where it completed the ascorbate glutathione cycle by regenerating the reduction of glutathione using NADPH which in turn stabilize the ascorbate pool and increase the plant tolerance (Hakam and Simon 1996). Application of SS resulted in an increase of both GR and APX antioxidants, which play an important role for protecting canola plants from environmental stresses. According to these results the increment of these antioxidants enzymes activities could help the plants to destroy H_2O_2 and maintain the ascrobate pool which in turn elevates the plant tolerance against different environmental stresses (Lappartient and Touraine 1997).

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