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POTENTIAL EFFECTS OF GROWTH REGULATOR AGENTS ON ANTIOXIDANT ACTIVITY OF TWO VERITIES OF FABA BEAN (*VICIA FABA*L.,)

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ABSTRACT

Faba bean (*Vicia faba L.*) as the most cultivated leguminous species in the world, is an excellent source of protein, dietary fiber, micronutrients and phytochemicals. A field experiment was conducted at Ghazala, Zagazig University, Sharkia Governorate during 2015, to study the effect of plant Growth Regulator such as; Gibberellic acid (GA3) and Inidol acetic acid (IAA) on antioxidant contents of two genotype seed extracts Nubaria 1 and Miser 3. Both two varieties were treated with both GA3 and IAA at two levels 50 and 100 ppm and 100ppmGA3 +100ppm IAA as foliar treatments. The Nubaria 1 seeds extracts have highest contents of phenolics (13.51, 13.64, 16.94, 17.97 and 20.53 GAE/g and flavonoid (8.07, 8.06, 9.12, 10.28 and 11.86 mg/QE/g, respectively. Also genotype seed extracts (Nubaria 1) has have highest antioxidant capacity (82.82% DPPH). Plant growth regulators such as GA3 and IAA are improving biosynthesis of phenolics and flavonoids, therefore improve antioxidant capacity and nutrition value of seeds.

KEYWORDS: **Faba bean, Growth Regulator, Antioxidant, Phenolic Compound, Foliar treatment, Flavonoid.**

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INTRODUCTION

As agriculture becomes mechanizal and science increases the possibilities for using inputs to enhance production, the role of plant growth regulators(PGRs)becomes more vital. Plant growth regulators function as chemical messengers for intercell communication. They are currently five recognized group of plant hormones: auxins, gibberellins, cytokinins abscisic acid and ethylene. The principle auxin in plants is indol-3-acetic acid(IAA), which stimulate cell elongation and influence a host of other developmental responses, such as root initiation, vascular differentiation, tropic responses, apical dominance and the development of auxiliary buds, flowers and fruits. Also the main effect of gibberellins in plants is to cause stem elongation and flowering, as well as, they are prominently involved in mobilization of endosperm reserves during early embryo growth and seed germination (yoman et al 1977, Luckwill; 1981 and Osbomeand Mc Manus;2005) [1-3]. There is currently much interest in phytochemicals as bioactive components of food. Antioxidants are a molecule that inhibits the oxidations of other molecules. Oxidation is a chemical reaction that can produce free radicals leading to chain reaction that my damage cells. Antioxidants such as thiols or vitamin C (Ascorbic acid) terminate these chain reactions. Oxidative stress is defined as imbalance between the generation of free radicals in particular reactive oxygen species (ROS) and end-ogenus antioxidant defense mechanism (Sies,1986)[4]. The deactivation of antioxidant species by phenolic antioxidants is based, with regard to food systems that deteriorated by peroxyl radicals ($R^{\cdot}$) on the donations of hydrogen, which interrupts chain reactions:-

$$R^{\cdot} + PhOH \rightarrow RH + PhO^{\cdot}$$

Phenoxyle radicals ($PhO^{\cdot}$) generated according to thio reaction may be stabilized through resonance and/or intermolecular hydrogen bonding, as proposed for quercetin, or combine to yield dimerization products, thus terminating the reaction: -

$$PhO^{\cdot} + PhO^{\cdot} \rightarrow PhO + OPh$$

The antioxidant compound (such as flavonoids, tannins, cumarins, curcumaanoids, xanthons, phenolics, lignin's, and terpenoids) sources are fruits and vegetables, as well as products derived from plants. Some good choices include cabbage, eggplant and legumes like red kidney beans or hack beans. Usually the presence of colour indicates there is a specific antioxidant in that food. Recent studies have shown that many dietary phenolic constituents derived from plants are more effective antioxidants in vitro than vitamins E or C, and thus might contribute significantly to the protective effect in vivo; (Demming-Adams and AD ams;2002, Jeong et al ;2004 and Aoun and Markis, 2012)[5-7]. Faba bean *(Vicia faba* L.) is one of the most important legumes in the Middle East countries and its cultivation leads to the increase of soil nitrogenous compounds. Faba bean seeds are excellent sources of proteins (20-40%), carbohydrates (50-60%) and fairly good sources of thiamin, niacin, calcium and iron Alghamdi, S.S., (2009) [8]. Many phytochemicals found in whole plant foods can help preserve vascular health and diminish cancer risk; direct antioxidant activity may mediate much of their benefit Halliwell, B etal(1992) and Mccarty, M(2004) [9-10]. Natural antioxidants are compounds that detoxify reactive oxygen species and prevent their damage to cellular macromolecules and organelles through different mechanisms Shahidi, F.(2000) [11.8]Response of plant to PGRs may vary with species, varieties, environmental conditions, physiological and nutritional status, stage of development and endogenous hormonal balance Naeem, M etal(2011) [12]. Plants have the ability to store excessive amounts of exogenously supplied hormones in the form of reversible conjugates which release active hormone when the plants need them during the growth period Davies,P.J.,(2004) [13.]Natural food contains bioactive compounds that protect liver cells and improve liver functions (Azevedo et al., 2003)[14].As well as DNA from damage (Jung et al., 2008)[15].The antioxidant capacity of plant foods is derived from the cumulative synergistic action of a wide variety of antioxidants such as vitamins C and E and polyphenols, mainly phenolic acids and flavonoids, carotenoids, terpenoids, Maillard compounds and trace minerals Pérez-Jiménez, J et al(2008)[16].Therefore, effect of (PGRs)on phenolic content and flavonoids contents as well as to determine antioxidant capacity of extracts for two genotypes Nubaria 1 and Misr 3.

MATERIALS AND METHODS

Chemicals: Gallic acid, quercetin hydrate, 2, 2-Dipheny1- Picrylhydrazyl (DPPH), Folin Ciocalteu reagent, sodium carbonate, sodium nitrite , aluminum chloride and quercetin hydrate , sodium hydroxide , methanol, were purchased from Sigma- Aldrich, Germany. Instruments: cyclotel 1093 sample mill(swden), Unipan vacuum rotary evaporateor.350P(Poland) and UV-vis spectrophotometr,UV2505, LabomedInc,(USA).

Source and preparation of faba bean seeds

Seeds of tow genotypes tested cultivars faba bean (*V.faba*) cultivars namely Nubaria 1 (Nu1) and Misr 3 (M 3) were obtained from Agronomy farm of Faculty of Technology and Development, Zagazig University (Ghazala, Zagazig, Sharkia). Experiment was conducted in the Agronomy farm of Ghazala (2015). A complete randomized block design was applied. Each treatment was replicated three times. The area of each plot was 3 x 3m. Two foliar growth regulators besides control were applied as follow: 1-50 and 100 ppm Indole acetic acid, 2- 50 and 100 ppm Gibberellic

acid,3- 100 ppm Indole acetic acid + 100 ppm Gibberellic acid, Foliar hormones were applied after 25 and 50 days from sowing data at rate 50,100 ppm GA3, 50,100 ppm IAA and 100 ppm GA3+100 ppm IAA. Mature seeds were fully developed and hand-harvested after 110 days and store in separate plastic bags at 25 ^{0}C prior to analyses.

Seed extraction

In order to measure antioxidant activities, total phenolics (TP), and total flavonoids (TF) of raw materials, beans were grounded with an analytical laboratory mill. The seed extract was prepared as follow. Briefly,20 grams of crushed and powdered seeds were soaked in 100 ml of methanol (LR grade, Merck, India) separately and kept on a rotary shaker for 24 h. Each extract was filtered under vacuum through a Whatman No. 1 filter paper and the process repeated until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint color. The extracts were evaporated to dryness The extracts were evaporated to dryness under reduced pressure in a rotary evaporator. A portion of the residue was subjected for analysis.

Estimation of total phenolics

Phenolic compounds were determined based on a method described by Singleton VL et al (1999)[17]Briefly,1 ml of methanolic extract was mixed with 1 ml of Folin Ciocalteu reagent. After 3 min, 1 ml of saturated sodium carbonate solution (20%) was added to the mixture and adjusted to 10 ml with distilled H_2O. The reaction mixture was kept in the dark for 1 h with intermittent shaking. The absorbance was measured at 725 nm using UV visible spectrophotometer. Phenolic contents were calculated on the basis of the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per g of dry extract.

Estimation of total flavonoids

The methanolic extract (250 µl) was mixed with 1.25 ml of distilled H2O and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of a 10% $AlCl_3$•H2O solution was added and filtered for 6 min. About500 µl of 1 M NaOH and 275 µl of distilled H_2O were added to the mixture, mixed well and the intensity of pink color was measured at 510 nm. The level of total flavonoid concentration was calculated using quercetin (QU) as a standard Jia Q etal(1999)[18].The results were expressed as mg of quercetin equivalents per g of dry extract.

Estimation of antioxidant activity

Radical scavenging ability using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical.The antioxidant activity of plant methanol extracts was determined based on the radical scavenging ability in reacting with a stable DPPH free radical according to Ayoola , GA et al (2008)[19]. Methanolic Solutions of concentrations 0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml, and 0.1 mg/ml were prepared for each extract. Freshly prepared 10 ml DPPH solution (1 mM) was mixed with 20 ml of different samples (0.02 – 0.1) mg/ml. Ascorbic acid solutions of same concentrations 0.02 mg/ml ─ 0.1 mg/ml were prepared and used as positive control for the radical scavenging activity test. Fifteen minute later, the absorbance was measured at 517 nm. The radical scavenging activities of ascorbic acid were also determined as positive controls. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Purple colored stable free radicals were reduced to the yellow colored diphenylpicrylhydrazine when antioxidant was added. The corresponding blank readings were taken and the capability to scavenge the DPPH radical was calculated using the following equation

$$\text{DPPH}^{\cdot}\ \text{scavenging effect (\%)} = [(A0 - As/ A0)]\ X100$$

Where,

Abs control (A0) = Absorbance of DPPH solution

Abs sample (As) = Absorbance of extracts and ascorbic acid solutions Mothana RA etal(2009)[20].

The simplest estimate of IC50 is to plot x-y and fit the data with a straight line (linear regression). IC50 value is then estimated using the fitted line

$$Y = a * X + b\ ,$$
$$IC50 = (0.5 - b)/a.$$

RESULTS AND DISCUSSIONS

Effect of PGRs on total phenolic contents of tow genotypes seeds (Nu1 and M3).

Phenolic compounds are secondary metabolites have repeatedly been implicated as natural antioxidants in fruits, vegetables, and another plants Larson RA (1988)[21]. Polyphenols play a vital part in the protection of plant against UV radiation, pathogens and herbivores, and help maintain structural integrity for the cell wall Klepacka J, Fornal L (2006) and Inglett GE (2011)[22-23]. The present study reveals the phenolics contents of the raw seeds of faba bean (Nu1 and M3) extracts in terms of mg gallic acid equivalent/g of dry weight, (standard plot: y=0.0039x+0.0439, R2=0.9892). The values are found between 13.52 to 20.53 mg gallic acid equivalent/g in Nubaria 1 and 12 to15.79 mg gallic acid equivalent/g in Misr3Total phenolics contents of Nu1 was more positively affected with PGRs foliar application than those of M 3 (table:1). These were attributed to genotype and PGRs concentrations. Where total phenolic compounds were more affected with gibberellic acid treatments, at 50ppm content was 13.64mgGAE/g and 17.47mgGAE/g at

100ppm.Alsoit can be noticed gibberellin was synergized effect of IAA (combine application gave 20.53mgGAE/g of phenolic compounds, table:1). Results tabulated on table1 showed that concentration of PGRs slightly improved phenolic content either applied individually or in combination genotype M3.

Table 1
Effect of foliar PGRs application on the phenolic content of two genotypes (Nu 1 and M 3)

Cultivars Treatments	Nubaria1 Total phenolic (mg GAE/g DW)	Misr3 Total phenolic (mg GAE/g DW)
Control	12.79	11.05
50 ppm IAA	13.51	12.0
50 ppmGA3	13.64	12.74
100 ppm IAA	16.94	13.13
100 ppmGA3	17.97	13.56
100 ppmGA3+IAA	20.53	15.79

Effect of some PGRs agents on total flavonoids contents of two genotypes seeds (Nu1 and M 3).
Results illustrated on table,2 showed that total flavonoid as mg Qu/g of genotypes Nu1 was ranged between 8.07 mg Qu/g and 11.86mgQu/g,that more than control(7.02mgQu/g, while it ranged between 7.23gQu/g and 10.71mgQu/g for genotype M3,also that was higher than control(6.55mgQu/g). Thus showed that flavonoid content was affected by GPRs foliar applications, concentrations of PGRs, as well as by genotypes (table,2). Also it can be noticed GA3 was more pronounced than IAA and was synergized IAA effect, when combined with IAA (11.86mgQu/g, (table 2). Flavonoid content of M3 was slightly affected with PGRs type or concentration, but it can be noticed that the gibberellin effect was more than effect of IAA, since mode of actions for both GA3 and IAA, surly differ Jules, Jetal (1981) and Naeem, M (2011) [24,12].

Table 2
Effect of foliar PGRs application on the flavonoids content of two genotypes (Nu1 and M3).

Cultivars *Treatments*	*Nubaria1* Total flavonoid (mg QU/g)	*Misr3* Total flavonoid (mg QU/g)
Control	7.02	6.55
50 ppm IAA	8.07	7.23
50 ppm GA3	8.6	8.28
100 ppm IAA	9.12	8.81
100 ppm GA3	10.28	9.55
100 ppm GA3+IAA	11.86	10.71

Effect of some PGRs on antioxidant capacity of faba bean genotypes (Nu1and M3) seeds DPPH assay.
Recently the modern researches proved that the natural plants have high capacity to curative many diseases. This makes the importance for returning depend on herbal and medical planets in medicine instead of chemical drugs which have fast functional effects in many diseases. This illustrates that important to use natural plants (extracts, oils and seeds) in preparing medicine. Phenolic and flavonoids compounds in plan and its extracts play impotent role as natural sources for antioxidant activity.The effect of growth regulator Agents GA3 and IAA on the scavenging antioxidant activity of two genotypes seeds extracts, (*Nu1*) and (*M3*) were evaluated using DPPH assay. The scavenging activity of the DPPH radical was tested by reduction of the stable radical DPPH to the yellow- colored diphenylpicrylhydrazine. The free radical scavenging activity of DPPH free radical in comparison to the standard ascorbic acid is shown in fig [1].

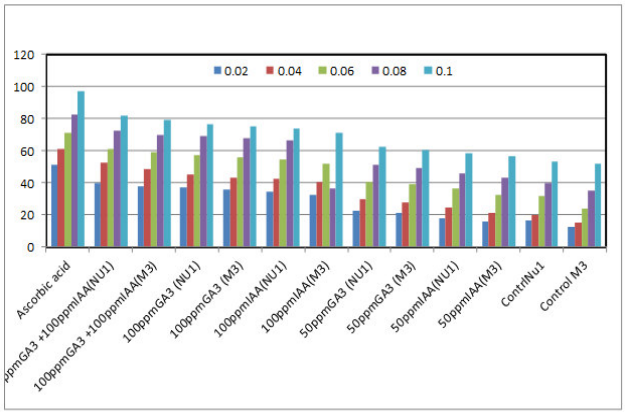


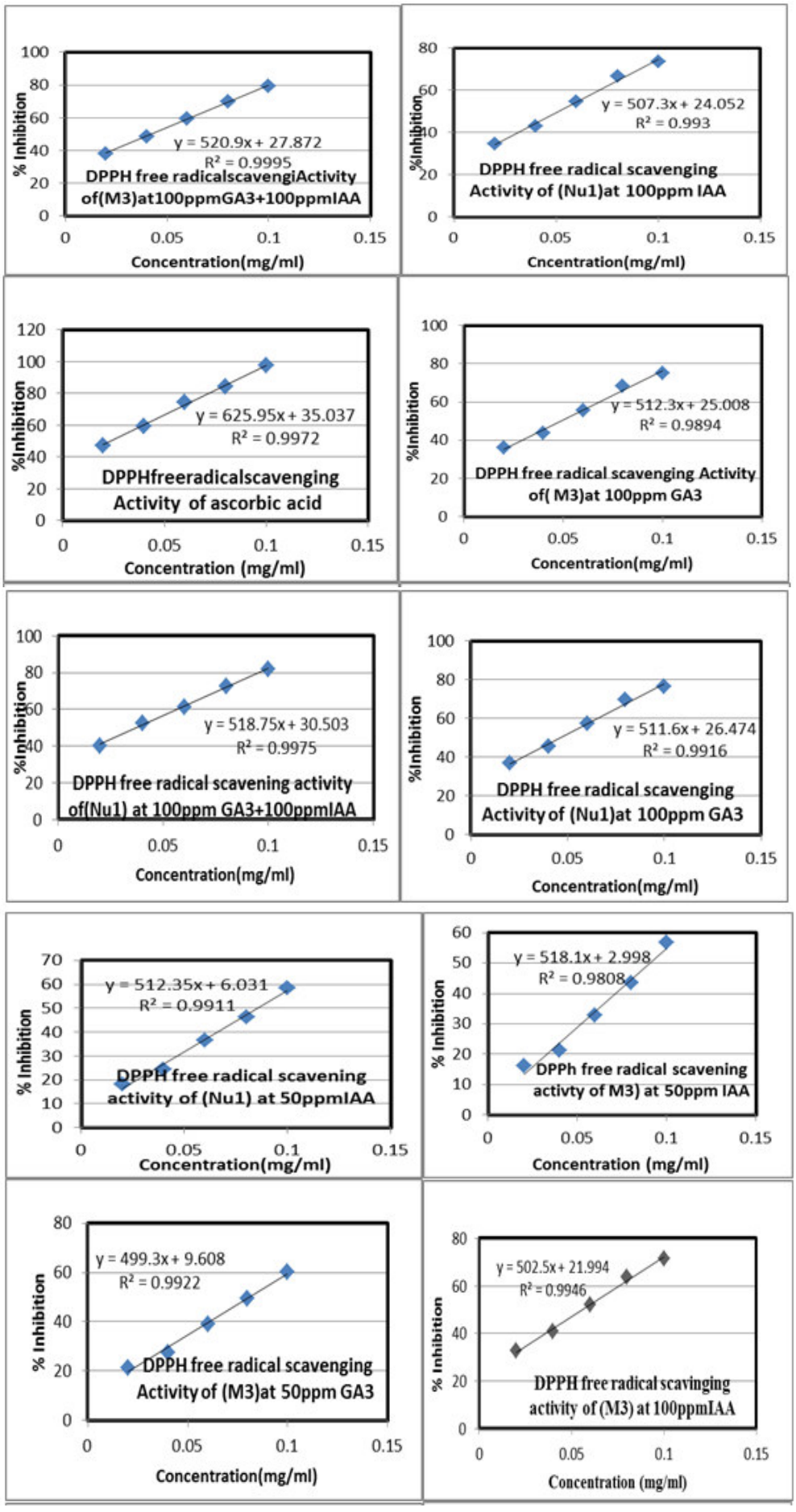
Figure 1
DPPH free radical scavenging activity of different concentrations of two genotypes seeds extracts (Nu1 and M3.

The effect of antioxidants on DPPH· radical scavenging was thought to result from their hydrogen or electron donating ability (Shimada et al., 1992)[25]. The decrease in absorbance of the DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Data showed on (fig 1) stated that antioxidant capacity is correlated with the concentration of both phenolics and flavonoids contents of two genotypes seeds extracts, Also, data showed that extracts obtained from GA3 foliar application have antioxidant activates more than those obtained from IAA foliar application as well as, antioxidant activity is affected with genotypes. Thus extracts of genotypes seeds Nu1 have antioxidant capacity higher than those of M3 (82.82%) and 79.52% respectively) at100ppmAG3+100ppmIAA application, the lowest antioxidant capacity (52.59%)noticed at 50ppm IAA applications and its M3 extract. Generally, it was clear that synergize the effect of IAA, science their mixture at 100ppm of both GA3 and IAA gave seed extract has highest antioxidant capacity. *In vitro* the DPPH radical scavenging activity assay is one of the important methods for the measurement of the capacity of an antioxidant to reduce free radicals. DPPH free radical scavenging activity of Vicia faba cultivars extract in various concentrations ranging from 0.02 – 0.1 mg/ml was depicted in fig(1) and compared with standard ascorbic acid Ayoola GA(2008)[19].DPPH scavenging activities of the methanol extract were found as 81.82, 72.5, 61.43 , 52.39 and40.00% respectively at various concentrations when treated by 100ppmGA3 +100ppmIAA, 76.29 ,69.5, 57.48, 45.4 , 37.18% respectively at various concentrations when treated with 100 ppmGA3, 73.67,66.48, 54.7, 42.84, 34.76 %respectively at various concentrations when treated with 100 ppm IAA, 62.9, 51.36, 40.76, 29.84, 22.34 % respectively at various concentrations when treated with 50 ppmGA3 and 58.43, 46.22, 36.67, 24.47, 18.07 % respectively at various concentrations when treated with 50 ppm IAA in comparison with ascorbic acid 97.4,90.45,81.65,72.67,61.67 % respectively at various concentrations 0.02,0.04.0.06,0.08 and 0.1. DPPH scavenging activities of the methanol extract of M3 cultivar were found as 79.52, 70.06, 59.35, 48.48, 38.22% respectively at various concentrations when treated by 100ppmGA3 +100ppmIAA, 75.12, 68.23, 55.62, 43.51, 36.25% respectively at various concentrations when treated with 100ppmGA3, 71.35, 36.8, 52.19, 40.76, 32.62% respectively at various concentrations when treated with 100ppmIAA, 60.28, 49.58, 39.03, 27.6, 21.34% respectively at various concentrations when treated with 50ppmGA3, 56.81, 43.51, 32.76, 21.17, 16.17% respectively at various concentrations when treated with 50 ppm IAA in comparison with ascorbic acid mention latter.

Table 3
***IC50 values of the crude extract of genotype seeds (Nu1 and M3) against DPPH scavenging capacity*.**

		% of Scavenging											
		Nubaria1						Misr3					
Extract Concentration mg/mL	Ascorbic acid	100ppmGA3+ 100ppmIAA	100ppmGA3	100ppmIAA	50ppmGA3	50ppmIAA	Control	100ppmGA3+ 100ppmIAA	100ppmGA3	100ppmIAA	50ppmGA3	50ppmIAA	Control
0.02	51.36	40	37.18	34.76	22.34	18.07	16.43	38.22	36.25	32.62	21.34	16.43	12.59
0.04	61.56	52.39	45.4	42.84	29.84	24.47	19.71	48.48	43.51	40.76	27.6	19.71	15.01
0.06	71.18	61.43	57.48	54.7	40.76	36.67	31.88	59.35	55.62	52.19	39.03	31.88	23.9
0.08	82.45	72.5	69.5	66.48	51.36	46.22	39.64	70.06	68.23	36.8	49.58	39.64	34.99
0.1	97.4	81.82	76.29	73.67	62.9	58.43	53.59	79.52	75.12	71.35	60.28	53.59	51.73
IC_{50}	0.020	0.038	0.046	0.051	0.077	0.086	0.0968	0.043	0.049	0.056	0.081	0.091	0.098

The IC50 value, ,is a parameter widely used to measure antioxidant activity and defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. *In vitro* the DPPH radical scavenging activity assay is one of the important methods for the measurement of the capacity of an antioxidant to reduce free radicals. In comparison to the standard ascorbic acid, IC50 of two cultivars both methanolic extracts (Nu1) and (M3) seed and there treated with growth hormones, depicted in table (3).Where a smaller IC50 value corresponds to a higher antioxidant activity of the methanolic seed extract of two cultivars.The experimental results fig (6) showed that, antioxidant activity of methanolic extract for (Nu1) seeds were found to be 81.82% at 0.1 mg/ml with IC50 = 0.038 mg/ml (r2 = 0.9975), 76.29% with IC50 = 0.046 mg/ml (r2 = 0.9916), 73.67%with IC50 = 0.051 mg/ml (r2 = 0.9930), 62.9% with IC50 = 0. 0.077 mg/ml (r2 = 0.9950) and58.43% with IC50 = 0.086 mg/ml (r2 = 0.9911) respectively for 100ppm GA3 +100ppmIAA, 100ppm GA3, 100ppmIAA, 50ppm GA3, 50ppm IAA. However free radical scavenging activity of methanolic extract for (M3) seeds were found to be 79.52% at 0.1 mg/ml with IC50 = 0.043 mg/ml (r2 = 0.9995), 75.12% with IC50 = 0.049 mg/ml (r2 = 0.9894), 71.35%with IC50 = 0.056 mg/ml (r2 = 0.9946), 60.28% with IC50 = 0. 0.081 mg/ml (r2 = 0.9922) and 56.81% with IC50 = 0.091 mg/ml (r2 = 0.9808) respectively for 100ppm GA3 +100ppmIAA, 100ppm GA3, 100ppmIAA, 50ppm GA3, 50ppm IAA. DPPH free radical scavenging activity and IC50 values of faba bean cultivars seeds compared to stander DDPH free radical of ascorbic acid as synthetic antioxidant source.


% Inhibition
y = 520.9x + 27.872
R² = 0.9995
DPPH free radicalscavengiActivity of(M3)at100ppmGA3+100ppmIAA
Concentration(mg/ml)
%Inhibition
y = 507.3x + 24.052
R² = 0.993
DPPH free radical scavenging Activity of (Nu1)at 100ppm IAA
Cncentration(mg/ml)
%Inhibition
y = 625.95x + 35.037
R² = 0.9972
DPPHfreeradicalscavenging Activity of ascorbic acid
Concentration (mg/ml)
%Inhibition
y = 512.3x + 25.008
R² = 0.9894
DPPH free radical scavenging Activity of(M3)at 100ppm GA3
Concentration(mg/ml)
%Inhibition
y = 518.75x + 30.503
R² = 0.9975
DPPH free radical scavening activity of(Nu1) at 100ppm GA3+100ppmIAA
Concentration(mg/ml)
%Inhibition
y = 511.6x + 26.474
R² = 0.9916
DPPH free radical scavenging Activity of (Nu1)at 100ppm GA3
Concentration(mg/ml)
% Inhibition
y = 512.35x + 6.031
R² = 0.9911
DPPH free radical scavening activity of (Nu1) at 50ppmIAA
Concentration(mg/ml)
% Inhibition
y = 518.1x + 2.998
R² = 0.9808
DPPh free radical scavening activty of M3) at 50ppm IAA
Concentration (mg/ml)
% Inhibition
y = 499.3x + 9.608
R² = 0.9922
DPPH free radical scavenging Activity of (M3)at 50ppm GA3
Concentration(mg/ml)
% Inhibition
y = 502.5x + 21.994
R² = 0.9946
DPPH free radical scavinging activity of (M3) at 100ppmIAA
Concentration (mg/ml)

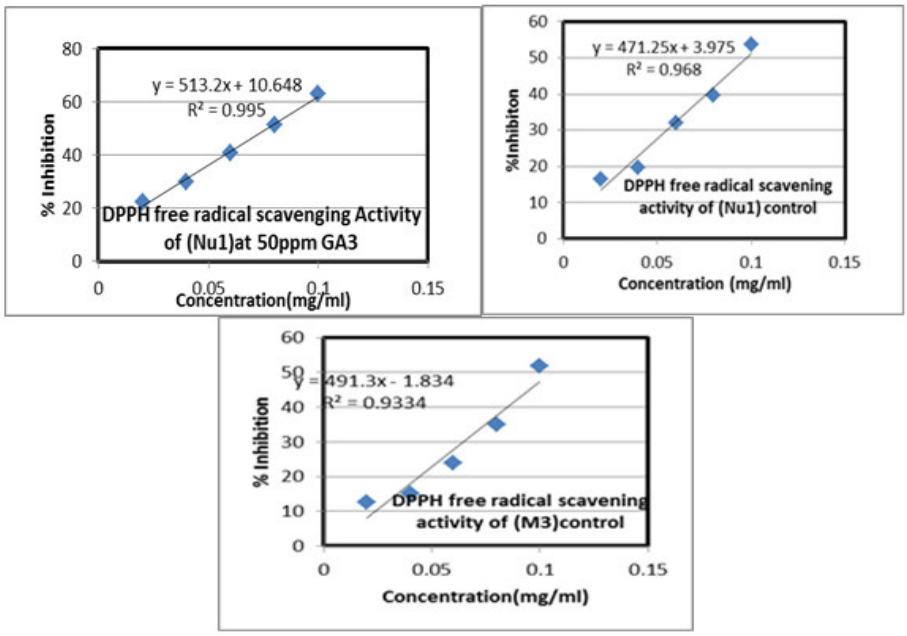


Figure 6
IC50 (mg/ml of extracts gain of two genotypes seed against DPPH scavenging capacity

Vicia faba (Nu1) highest antioxidant activity because of highest content of phenolic and flavonoids; *Vicia faba (M3)* showed lowest reducing property, may be due to lowest phenolic content. Antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 2, 2-diphenyl-1-picryl hydrazine resulting in de coloration. All the seed extracts significantly scavenged DPPH. Naturally occurring phenolic compounds exert their beneficial health effects mainly through their antioxidant activity Fang, Y.Z(2002)[26]. These compounds are capable of scavenging free radicals; chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases Heim, K.Eet al(2002)[27]. The results strongly suggest phenolic compounds are present in different tissues of this plant, however, more abundantly in seeds which could be attributed possibly in *vivo* scavenging reactive oxygen species capability.

CONCLUSION

In conclusion based on the results obtained,growth regulators i.e. GA3 and IAA increase total phenolic and flavonoid and so improvement the antioxidant activity of *faba been* (Nu1 and M3) seeds. Methanolic extracts of two genotypes Nu1 and M3 showed antioxidant and free radical scavenging activity and major antioxidative component seems to be phenolic and flavonoids. Therefore, it can be concluded that the methanolic extract *of faba bean* (NU1 and M3) seeds could be considered for prevention and treatment of diseases and its complications as potent antioxidant.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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