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Effect of putrescine and pyridoxine (vitamin B6) on the antioxidant defense systems and free radical scavenging activity in canola plants

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Abstract : A pot experiment was conducted in the screen of the National Research Centre during two successive seasons (2013/2014 & 2014/2015) to study the response of canola plant seeds to soaking in pyridoxine (vitamin B6) or putrescine at the concentrations of (50, 100 and 200 mg/l). The obtained results revealed that presowing treatments of canola seeds with pyridoxine (vitamin B6) and putrescine significantly promoted plant growth criteria (shoot length, shoot dry weight, root length, root dry weight) and yield criteria (seed yield/pod, weight of seeds yield and yield of pods number / plant). Presowing treatment of canola seeds with putrescine, especially at 100 mg/l and pyridoxine at 200 mg/l significantly increased the ascorbic acid content in the seeds, reduced glutathione in seeds, total phenols in seeds. PAL enzyme activity in leaves, Polyphenol oxidase (PPO) activity in leaves, ascorbate peroxidase (APX) activity in leaves and DPPH free radical scavenging activity in seeds (%) were significantly increased as a result of putrescine and pyridoxine treatments.
Keywords: putrescine and pyridoxine (vitamin B6), antioxidant defense systems, free radical scavenging, canola plants.

Introduction

Canola (*Brassica napus* L.) is one of the most important oil crops in the world which is newly introduced in Egypt. The oil extracted from Canola seeds is of high quality with low erucic acid and glucosinolate contents[1].

Pyridoxine (Vitamin B6) acts as a coenzyme for numerous metabolic enzymes. It is an essential metabolite in all organisms which has recently been shown to be a potent antioxidant. It plays an important role in amino acid metabolism and antibiotic biosynthesis. Pyridoxine is considered as a requirement for development of some plant species [2]. It has been established that it promotes the growth of underground parts[3,4] and improve nutrient absorption and yield efficiency[5]. Corns growth and yield criteria were enhanced due to pyridoxine treatments[6]. The nutritional quality and stress tolerance of crop plants could be increased by applying this essential micronutrient[7].

Polyamines are small ubiquitous molecules that have been involved in nearly all developmental processes, including the stress response[8]. Polyamines are biologically active compounds involved in various physiological activities. They are positively charged molecules under intracellular pH, which are essential for plant development, related to aging and senescence, and usually involved in plant responses to stress[9,10]. Diamine putrescine (Put), the triamine spermidine (Spd) and the tetramine spermine (Spm) are the most studied polyamines in plants[11]. They also reported that, polyamines are low-molecular weight polycations essential for nitrogen metabolism and involved in the regulation of growth and stress, might bind to negatively charged macromolecules[12,13,14].

Material and Methods

Seeds of canola plant (*Brassica napus* L., cv. pactol) were secured from Egyptian Ministry of Agriculture. The seeds were soaked for 12 hours in different concentrations of vitamin B6 and putrescine (i.e., 0.0, 50, 100 and 200 mg/l), and were sown in plastic pots 30 cm top diameter in November, 15th 2013 and 2014, respectively. Each pot contained 8 kg loamy clay soil.

Treatments were distributed in complete randomized block design with five replications comprised with five pots for each replicate. Twenty days after sowing, the seedlings were thinned to the most three uniform plants in each pot. Each pot received equal and adequate amounts of water and fertilizers. Phosphorous as calcium superphosphate was mixed with the soil before sowing at the rate of 4.0 g / pot. Three grams of nitrogen as ammonium sulphate in three applications (one g for each) with two weeks intervals started 30 days after sowing, also, two grams of potassium sulphate were added as soil application. Other agricultural processes were performed according to normal practice.

Two plant samples were taken during the growing season, at vegetative growth stage (20 days after sowing) and the second one was at 150 days after sowing (yield).

Biochemical Constituents Determination

Activity of ascorbate peroxidase was measured according to [15] by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 mm cm-'). The reaction mixture contained 25 mm phosphate buffer (pH 7.0), 0.1 mm EDTA, 1.0 mm H_2O_2, 0.25 mm AsA, and the enzyme aliquot.

The activity of PAL was assayed as described by[16]. The method applied for total phenols extraction was described by [17] and recommended by[18]. Levels of AsA were measured according to the procedure described by [19]. Total glutathione was determined according to [20]. PPO activity quantification was based on the method described by [21] with some modifications.

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) ASSAY

The scavenging ability of the natural antioxidants of the plant seeds extract towards the stable free radical DPPH was measured by the method of [22]. Briefly 2 ml aliquot of DPPH methanol solution [25μg/ml] was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. L-Ascorbic acid was used as the standard. Where AC = absorbance of the control and AS = absorbance of reaction mixture [in the presence of sample]. All tests were run in triplicates [n = 3], and the average values were calculated.

Statistical Analysis

The data obtained were subjected to standard analysis of variance procedure according to [23]. The values of LSD were calculated whenever F values were significant at 5% level.

Results and Discussion

Data illustrated in Figures (1-7) indicate that presowing treatment of canola seeds with vitamin B6 and putrescine significantly promoted plant growth and yield criteria.

It is obvious that soaking canola seeds in V B6 and putrescine significantly increased shoot length (cm) and shoot dry weight (g). The most effective treatments in this respect were that of putrescine at 100 mg/l followed by 200 mg/l, then 200 mg/l pyridoxine (VB6) (Figs., 1 & 2). Data illustrated in Figures (3-6) indicate that root length (cm), root dry weight (g), seed yield/pod and weight of seeds yield (g) followed the same trend.

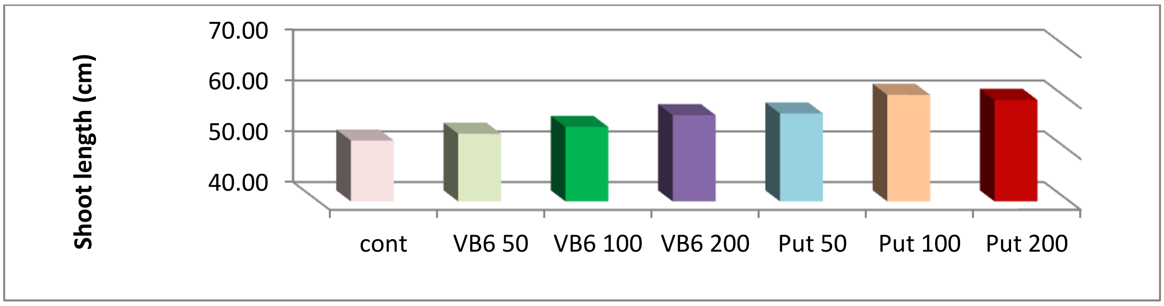

Fig. (1): Effect of vitamin B6 and putrescine on shoot length (LSD: 2.92)

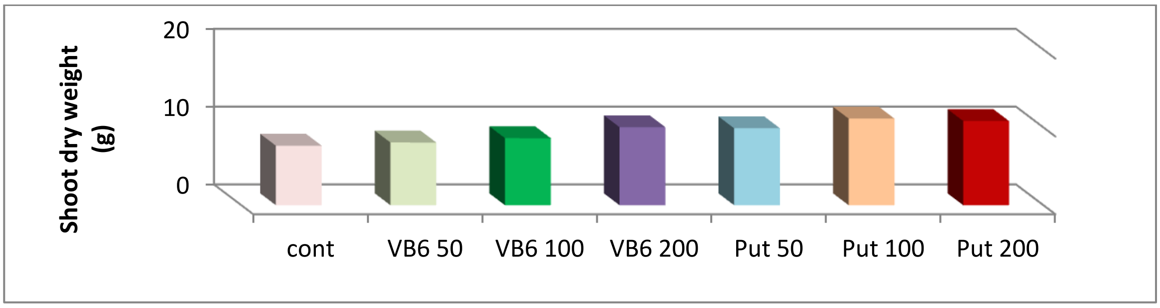

Fig. (2): Effect of vitamin B6 and putrescine on shoot dry weight (LSD: 0.73)

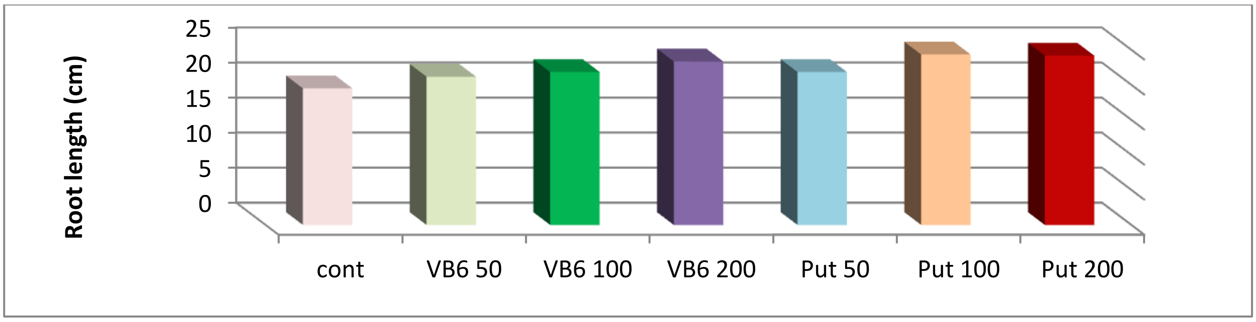

Fig. (3): Effect of vitamin B6 and putrescine on root length (LSD: 1.39)

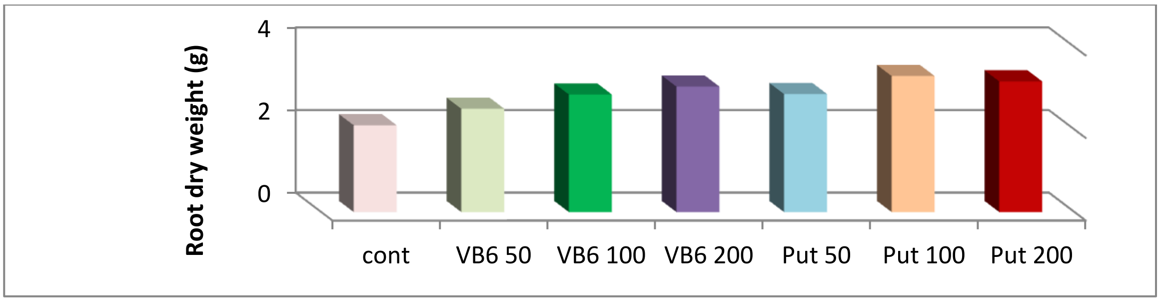

Fig. (4): Effect of vitamin B6 and putrescine on root dry weight (LSD: 0.25)

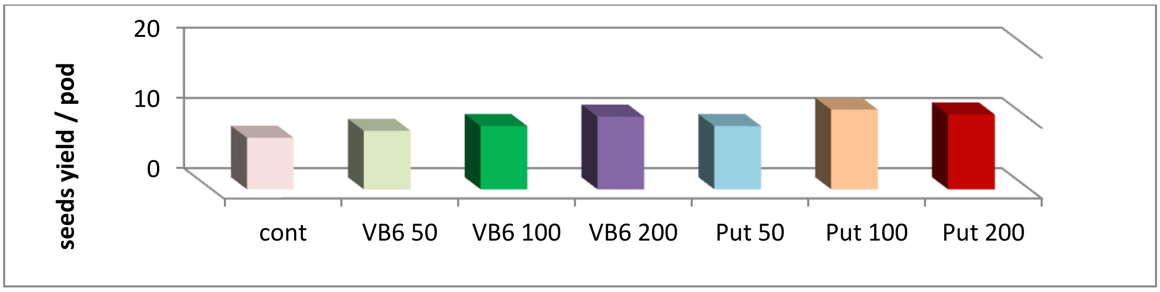

Fig. (5): Effect of vitamin B6 and putrescine on seeds yield / pod (LSD: 0.9)

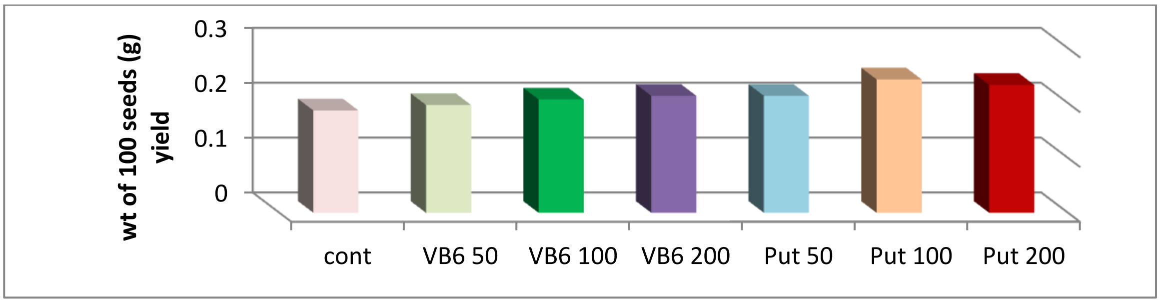

Fig. (6): Effect of vitamin B6 and putrescine on weight of 100 seeds yield (g) (LSD: 0.01)

Pods number / plant was significantly increased as a result of presowing soaking the seeds in putrescine and pyridoxine (Figure, 7). The highest recorded values were obtained in plants treated with 100 mg/l putrescine followed by 200 mg/l vitamin B6 then 200 mg/l putrescine.

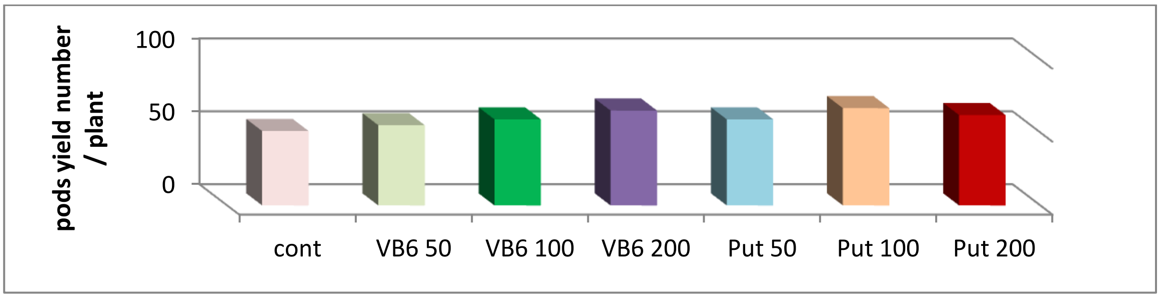

Fig. (7): Effect of vitamin B6 and putrescine on pods yield number / plant (LSD: 4.31)

Supporting these results, it has been established that pyridoxine enhances the growth of root system [3,4] and lead to better nutrient uptake and higher economic yield [5]. Treatment with pyridoxine has been shown to improve growth and yield parameters in corn plants [6]. Pyridoxine (V B6) significantly increased growth of *Cymbopogon citrates* L. plants, especially in plants treated with 200 mg/l [24]. These results are also in agreement with those obtained by [25] on periwinkle. They reported that exogenous application of putrescine on periwinkle transplants considerably increased plant growth at successive developmental stages. Application of putrescine on basil plants considerably increased plant growth at both cuttings. It was also recognized that the most promising results of vegetative growth criteria (i.e., plant height, number of branches, fresh and dry weights of herb) were obtained from plants treated with 150 mg/l putrescine [26]. Similar results were recorded by [27] on *Antirrhinum majus* plant, and [28] on corn plant.

Effect on biochemical constituents

Ascorbic acid content in the seeds of canola plant (µmol/g) was significantly increased as a result of putrescine and pyridoxine treatments. The highest recorded values were obtained in plants treated with 100 mg/l putrescine, followed by 200 mg/l putrescine and 200 mg/l pyridoxine, respectively (Figure, 8).

It is clear from the obtained results that reduced glutathione in seeds (Figure, 9), total phenols in seeds (Figure, 10), followed the same trend.

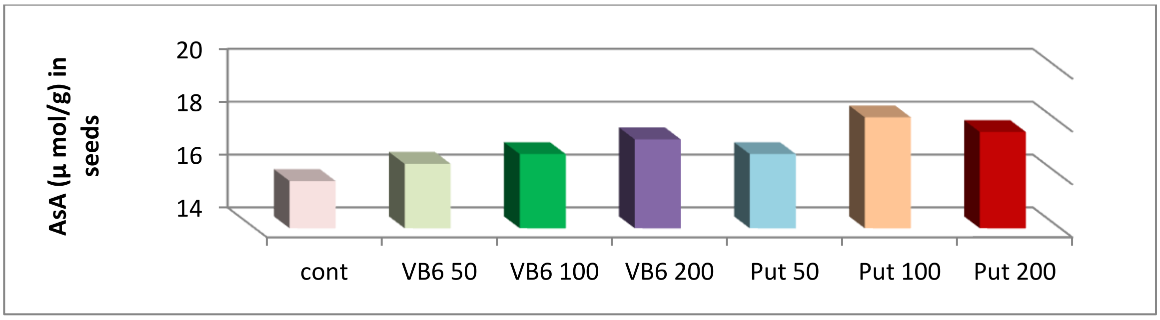

Fig. (8): Effect of vitamin B6 and putrescine on AsA in seeds (LSD: 1.08)

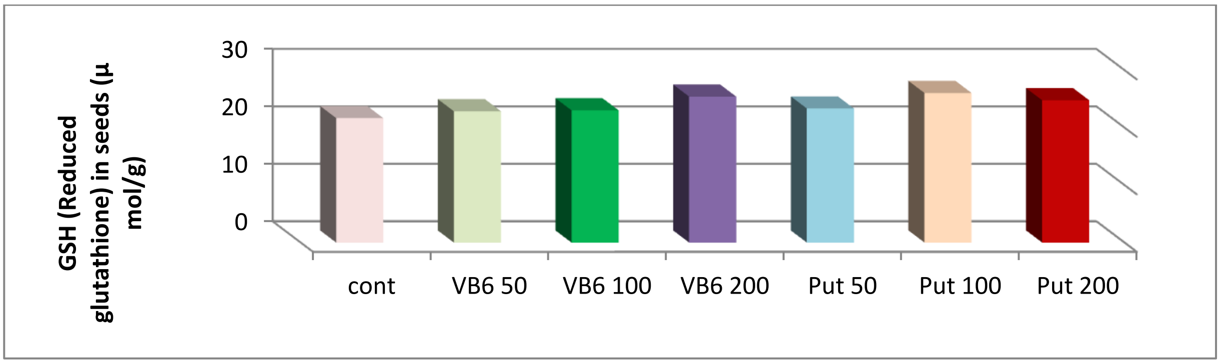

Fig. (9): Effect of vitamin B6 and putrescine on GSH (Reduced glutathione) in seeds (LSD: 2.48)

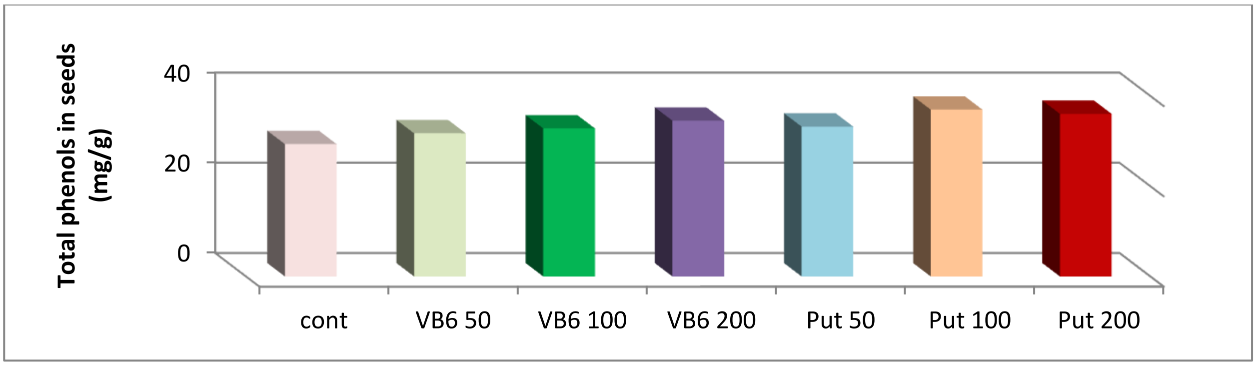

Fig. (10): Effect of vitamin B6 and putrescine on total phenols in seeds (LSD: 3.08)

Data illustrated in Figure (11) indicate that treatment of canola plants with 100 mg/l putrescine significantly increased PAL activity in leaves (µg/g f. wt) followed by application of 200 mg/l VB6 and 200 mg/l putrescine, respectively. Polyphenol oxidase activity in the leaves (unit/min/g f. wt) followed the same trend (Figure, 12).

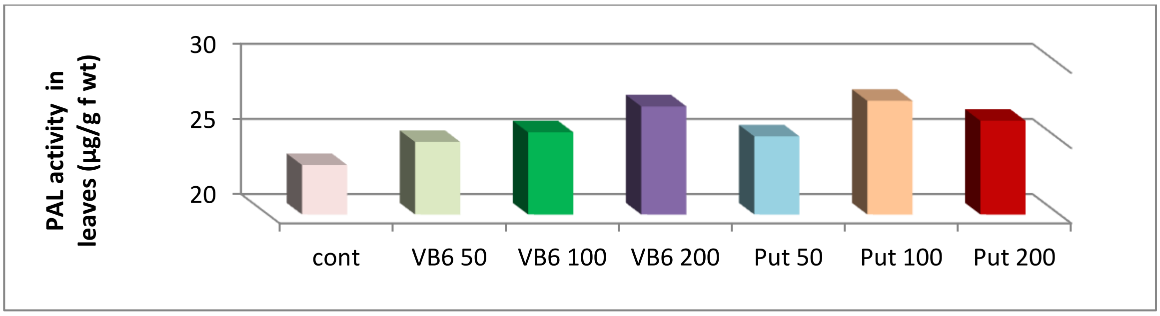

Fig. (11): PAL activity in leaves (LSD: 1.72)

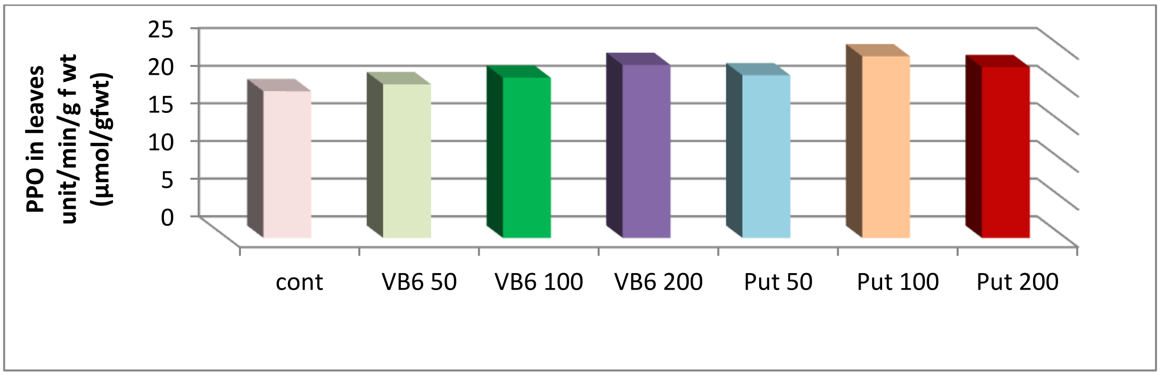

Fig. (12): Effect of vitamin B6 and putrescine on PPO activity in leaves (LSD: 0.72)

Meanwhile, ascorbate peroxidase activity in leaves and DPPH free radical scavenging activity in seeds (%) were significantly increased as a result of putrescine treatments, especially at 100 mg/l followed by 200 mg/l. Similar results were also recorded in plants treated with 200 mg/l pyridoxine but to a lesser degree (Figures, 13 & 14).

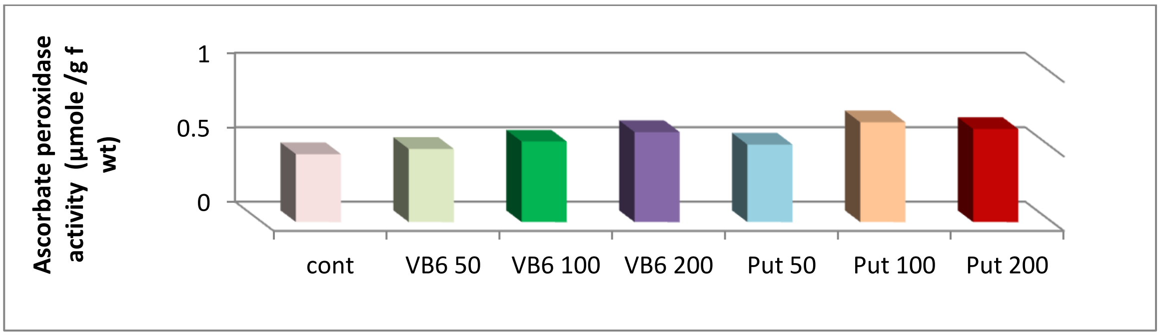

Fig. (13): Effect of vitamin B6 and putrescine on ascorbate peroxidase activity (LSD: 0.09)

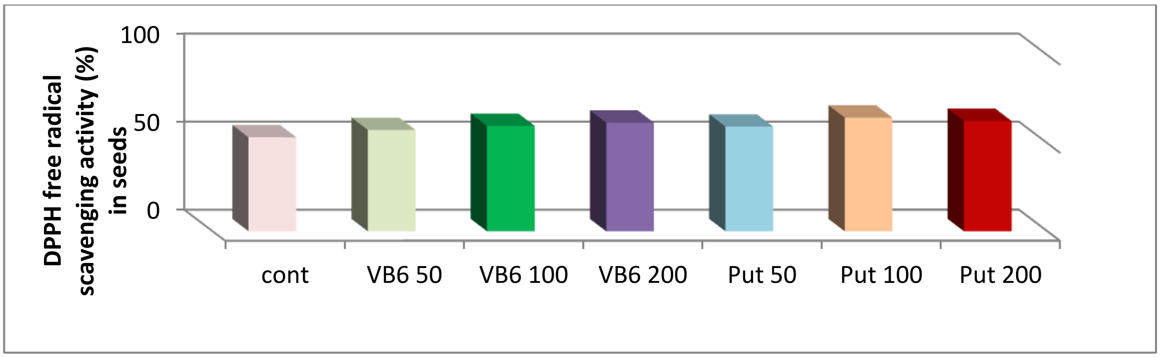

Fig. (14): Effect of vitamin B6 and putrescine on DPPH free radical scavenging activity in seeds (LSD: 3.19)

In this concern, [29] stated that spermidine treatments significantly increased phenols content in the leaves of chamomile plant. [30] reported that spermine application to two wheat cultivars significantly increased total phenols and PAL enzyme avtivity. [24] reported that pyridoxine and spermine treatments significantly affected the scavenging ability of the natural antioxidants of lemongrass plant extract towards the stable free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and application of spermine at the concentration of 100 mg/l recorded the highest increment in phenylalanin**e** ammonia lyase (PAL) enzyme activity (µg/g FW).

In respect to the antioxidant detected in canola seeds, phenols are nonenzymatic secondary metabolites synthesized by higher plants which have an antioxidative role in scavenging free radicals [31,32]. In addition, under oxidative stress glutathione and ascorbic acid are the most important antioxidants of the defense system in the process of scavenging ROS [33].

Conclusion

In conclusion, by comparing the effect of presowing seed soaking treatments of canola plants with pyridoxine and putrescine, it is apparent from the previous results that treatment with putrescine (100 mg/l) or pyridoxine (200 mg/l) have beneficial influence for increasing plant growth, yield and antioxidant enzyme activities. It is also worth to mention, that putrescine (100 mg/l) was more effective in influencing canola growth than pyridoxine (200 mg/l).

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