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Original Research

Growth and Acclimatization Response of Sunflower Plantlets Treated with Naphthyl Acetic Acid-Based Environment Friendly Plant Growth Promoters

Ali Ahmad Sabir[1], Firdaus-e-Bareen[2]*, Muhammad Zia-ur-Rehman[3], Sajid Rashid Ahmad[1]

[1]College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan
[2]Department of Botany, University of the Punjab, Lahore, Pakistan
[3]Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan

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Abstract

Preparing novel compounds using environment-friendly protocols for plant growth has remained a major goal for plant scientists. In this research, effect of a novel plant growth promoter on sunflower (*Helianthus annuus* L.) was assessed hydroponically as well as in soil acclimatized plantlets at 1, 10, and 100 µM. The plants were also assessed for antioxidant enzymatic and antifungal activities. The synthesized derivative 2 produced significantly better results in terms of overall growth as compared with standards at 1 µM concentration among all compounds. Increased catalase and superoxide dismutase levels in plant seedlings treated with synthesized compounds were observed when grown in hydroponics, but these values were found to be significantly low as compared to soil-acclimatized plants. No significant zone of inhibition was found on agar plates in antifungal bioassay.

Keywords: antifungal bioassay, antioxidant enzymatic activities, soil acclimatization, hydroponics, substituted naphthalene hydrazinyl ethyl acetate

Introduction

Although the development of organic products have been a decisive factor in rapid development for the last two centuries, hazardous solvents, non-biodegradable catalysts, and long reaction times have always been a serious issue in organic synthesis. The solution to all issues highlighted above lies in the environment-friendly synthesis and green chemistry. In such synthesis, reaction time is decreased by using energy-efficient methods of heating (like microwave irradiation), high yield, biodegradability, good selectivity of solvents, good performance of catalysts, and usage of environment-friendly greener solvents like water, ethanol, acetone, etc. in an all-in-one approach [1]. Auxins, a class of naturally organic compounds, are plant growth hormones found in and required by plants in very little amounts, yet their presence is very critical. This class of compounds contain acetic acid-based

*e-mail: fbareen@gmail.com

moieties that are very helpful in enhancing plant growth via increasing cell elongation [2]. Exogenous application of synthetic plant growth hormone interacts and affects the endogenous presence of hormones. Naphthyl acetic acid (NAA) has been found to be important in increasing mass of seed and chlorophyll contents and evokes various other positive physiological parameters in plants [3].

Many compounds chemically and structurally unrelated to existing plant growth regulators are being synthesized in laboratories. They have shown considerably good phytohormonal response and many other compounds are under check [4]. Some reports suggest that the presence of carboxylic acids and their esters, diverse substituents, space configuration, etc. are mainly responsible for increase or decrease in the activity of synthesized derivatives, and the presence of double carboxyl moiety sometimes enhances and sometimes retards growth response [2]. Similarly, ester and amide derivatives of Indole acetic acid (IAA) and Naphthyl acetic acid (NAA) been synthesized in a laboratory and their application has proven to be effective on *Ceratopteris richardii* gametophytes exhibiting auxin-like activity [5].

Sunflower (*Helianthus annuus* L.), mainly cultivated for oil production, food, medicine, and ornamental purposes [6-7], but has recently been used as a bio-renewable energy source. As sunflower is a short-duration crop, it has become an integral part of cropping in many parts of the world. Moreover, due to its wide adaptability and high edible oil contents, it has gained much importance in modern agriculture [8].

Growth promoter hormones like auxin are well known to induce growth of sunflowers by increasing cell division, extension, and inducing lateral growth of root [9]. Different plant growth regulators, like indole butyric acid (IBA), indole acetic acid (IAA), naphthyl acetic acid (NAA), 2,4 dichloro phenoxy acetic acid (2,4 D), benzyl amino purine (BAP) and their combination, are employed for organogenesis of sunflower, and it proved to be the best explants to monitor growth [10]. The effects of different concentrations of Hoagland solutions has also been studied as it is the source of minerals and may provide different results when used in different concentrations [11]. Similarly, various plant growth regulators and their combinations at 10-15 ppm increased the chlorophyll content of tomato plant grown in Hoagland solution by 51-135% [12].

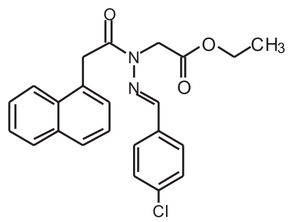
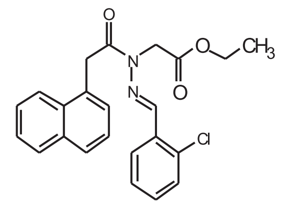
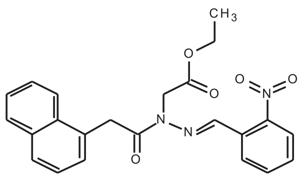
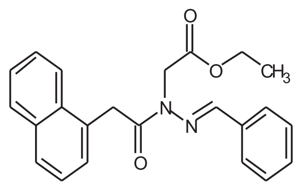
Reactive oxygen species (ROS) have also become an important tool to determine plant environmental stresses and development of plants. ROS can interact with other molecules like plant growth regulators and can modify their activity [13]. Similarly, ethyl ester-based derivatives of β-Naphthol have been synthesized and show considerable antifungal activity [14]. Various schiff bases were synthesized by reacting naphthyl acetic acid hydrazide with substituted indole 3 aldehydes and were studied for their antiviral ability. Some of the synthesized compounds exhibited good antiviral activity [15]. Various derivatives of 2-(2-benzyl-4-chlorophenoxy) acetohydrazide were also prepared and studied for their antibacterial and antifungal activity against various pathogens [16].

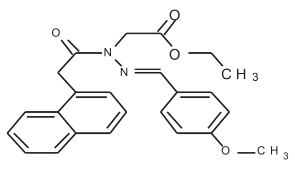
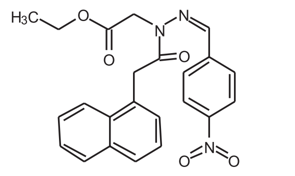
The aim of this study was to assess the growth impact of environment-friendly synthesized plant growth regulator-based compounds on sunflower seedlings (*Helianthus annuus* L.) grown hydroponically in half strength of Hoagland solution and their acclimatization response in soil. Antioxidant activities like catalase and superoxide dismutase were also calculated for plantlets grown in both Hoagland solution and soil. Antifungal activity of synthesized compounds was also assessed by using agar tube dilution protocol.

Material and Methods

Synthesis of Novel Compounds Using Green Protocols and their Chemical Characterization

All compounds were prepared using greener protocols of chemistry, including microwave irradiation (Orient eNNe781JF household microwave oven) that was producing fixed frequency radiations at 2450 MHz throughout the required time at multiples of operating at 100-1000 watts in the presence of green solvents. Detail methodology and chemical characterization has been published in another study (10.15244/pjoes/70854). The structure of compounds along with their IUPAC name is as follows:

	
Ethyl [2-(4-chlorobenzylidene)-1-(naphthalen-1yl acetyl) hydrazinyl] acetate	Ethyl [2-(2-chlorobenzylidene)-1-(naphthalen-1-yl acetyl) hydrazinyl] acetate
1	2
	
Ethyl 1-[(naphthalen-1-yl) acetyl]-2-[(2-nitrophenyl) methylidene] hydrazinyl acetate	Ethyl [2-benzylidene-1-(naphthalen-1-ylacetyl) hydrazinyl] acetate
3	4

	
Ethyl {2-[(4-methoxyphenyl) methylidene]-1-[(naphthalen-1-yl) acetyl] hydrazinyl} acetate	Ethyl {1-[(naphthalen-1-yl) acetyl]-2-[(4-nitrophenyl) methylidene] hydrazinyl} acetate
5	6

Preparation of Stock Solution for Plant Growth Bioassay

The synthesized compounds were dissolved in the minimum quantity of DMSO in order to prepare 10^{-3} M stock solutions that were added to 50% Hoagland solution in order to prepare 1, 10, and 100 μM 50% Hoagland solution.

Seed Dormancy

Sunflower (*Helianthus annuus* L.) Hysun-33 hybrid seeds were taken from Punjab seed corporation and sterilized with 1% sodium hypochlorite for 5 minutes and washed with running tap water to remove sodium hypochlorite residues. The surface-sterilized seeds were soaked in double-distilled autoclaved hot water for about 15 minutes. After that, ten seeds were placed between two layers of blotting paper in petri dishes having adequate water at 25ºC in the dark for about 36-48 hours [17].

Seedling Growth in Hydroponics Using Hoagland Solution

Seedlings having uniform weight and hypocotyles were taken for further process and were immersed in 50% Hoagland solution having synthetic plant growth regulators at 1, 10, and 100 μM concentrations. The seedlings were placed under 16 hours light at intensity of 2000-3000 lux with 5 replicates each [18]. After 7 and 14 days, fresh weight, dry weight, root length, shoot length, leaf surface area, number of leaves, and chlorophyll content were monitored.

Plant Growth in Soil

After 14 days of growth in hydroponics the plants were transferred to soil to check their acclimatization ability in pots with three replicates each. These plants were placed under sunlight in a greenhouse. They were watered with 100 ml water three times a week. Synthesized plant growth regulators were provided after 7 days and 21 days of their transfer in soil at 1 μM concentration via foliar spray. Fresh weight, dry weight, root length, shoot length, chlorophyll content, leaf area index (LAI), CAT, and SOD activity was monitored after 14 and 28 days. Plant stem girth was also measured.

Antioxidant Enzymes

Approximately 100 mg of fresh leaf tissue was randomly taken from cultured plants, frozen in liquid nitrogen, and stored at -80ºC until further analysis. Extraction of SOD and CAT was performed as described by He et al., (2001) with little change. Briefly, frozen leaves were mixed with 3 ml of 150 mM cold phosphate buffer (pH 7.0) with a mortar and pestle and centrifuged at 12,000 rpm for 20 minutes at 4ºC in a temperature-controlled centrifuge [19]. The extract was used for estimating enzyme activities.

Estimation of Superoxide Dismutase (SOD) Activity

SOD activity was calculated by the method of Ginnapolis and Ries (1977) with minor modifications. [20].

Estimation of Catalase (CAT) Activity

CAT activity was calculated based on the oxidation of hydrogen peroxide using the method of Chance and Maehly (1955) with few changes [21].

Agar Tube Dilution Protocols for Antifungal Activity

Antifungal activity of synthesized ethyl esters was evaluated by the method of Choudhary et al. (1995). The tubes containing media and synthesized compounds after inoculation were incubated at 27-29ºC for 3-7 days. Cultures were checked twice weekly during incubation. Growth in the compound-containing media was observed by measuring linear growth (mm) and inhibition of growth calculated with reference to the negative [22-23].

Results and Discussion

Synthesized esters at 1 μM concentration proved more effective in promoting growth of sunflower seedlings and will be discussed in detail here. Out of the six synthesized esters, the best growth was produced by plants treated with synthesized ethyl ester 1 and 2 at 1 μM concentration. Structurally both compounds resemble each other because in synthesized ethyl ester 1 chloro substituent is present at para positon and in synthesized ethyl ester 2 at ortho position appears to be responsible for growth. This chloro group is not present in any other synthesized compounds. The shoot growth of 7-day-old hydroponically grown sunflower seedlings was better than control and NAA but comparable to Thidiazuron (TDZ), as shown in Fig. 1. Although shoot growth is better in plantlets treated with synthesized ethyl ester 1 and 2 at 1 µM concentration, it is clearly observable that NAA produces better root growth as this compound is the master hormone in controlling root growth.

Synthesized ethyl ester 1 and 2 treated hydroponically grown plants showed better results in all growth parameters like root and shoot length after 7 days as shown in Fig. 3(a-b). Chlorophyll content, fresh and day biomass, another criterion to determine plant growth, were also found higher in treated plants with growth regulator as shown in Table 1. These values are considerably greater than control and NAA, and are comparable with TDZ after 7 days.

Sunflower seedlings after treatment with compound 2 on the 14th day in hydroponics (Fig. 3a) showed a greater root length among all synthesized ethyl esters, which is comparable to standard NAA. Compound 2 also exhibited the maximum shoot growth among all synthesized compounds. The order of shoot growth was compound 2 > TDZ > compound 1 > compound 4 (as shown in Fig. 3b). The remaining synthesized compounds did not show good growth response. The highest fresh and dry biomass was obtained when plant seedlings were treated with compound 1 and 2 at 1 µM concentration than other compounds, and these biomasses were found to be comparable to TDZ as shown in Table 1. On the other hand, TDZ-treated seedlings were found to have the highest chlorophyll content.

Fig. 1. Growth response of sunflower plantlets treated with compound 1 (602 old name) at 1 µM after seven days in hydroponics. (Control indicates Hoagland solution only, NAA indicates naphthyl acetic acid at 1 µM in Hoagland solution, 602 indicates synthesized plant growth regulator 1 at 1 µM in Hoagland solution, TDZ indicates thidiazuron at 1 µ M in Hogland solution).

Fig. 2. Growth response of sunflower plant treated with compound 1 (B-02 old name) at 1 µM after twenty eight days in soil. (Control indicates water only, B-02 indicates synthesized plant growth regulator 1 at 1 µM in water, STD indicates thidiazuron (TDZ) at 1 µM in water).

When plants were shifted into the soil from hydroponics, foliar spray of the synthesized compound was applied after 7 days of their settlement in soils. Parameters like root, shoot length, number of leaves, fresh and dry biomass, stem girth, leaf area index, chlorophyll contents, CAT, and SOD levels were again monitored after 14 days of soil transfer. It was surprising to observe that none of the compounds was able to trigger any root growth response better than NAA. Comparatively better shoot growth was obtained within plants treated with compound 1, which is less as compared to standard TDZ but the highest among all synthesized derivatives. It is interesting to observe that compound 5 which was unable to produce any significant results in growth parameters producing the maximum number of leaves, even greater than the commercial standard (TDZ). Fresh and dry biomass of plants treated with compounds 1 and 2 (3429.7, 620.9 mg), (3380.8, 593.9 mg), respectively, were the highest after 14 days, as shown in Table 2. Plants treated with compound 5 at 1 µM concentration were found to have the maximum stem girth and leaf area index as compared to other treatments. These all suggest better adaptation of plants treated with compound 5 in soil than the rest.

A foliar spray of compound was applied again on plants after 3 weeks (21 days) of their transfer from hydroponics into soil and growth parameters were measured on the 28th day of their transfer. It was found that compound 1 (6.33±0.17 cm), which was showing less growth in the beginning, showed the best root growth as shown in Fig. 2. Order of root growth is NAA > compound 1 > compound 2 as shown in Fig. 3a. The maximum shoot length was observed

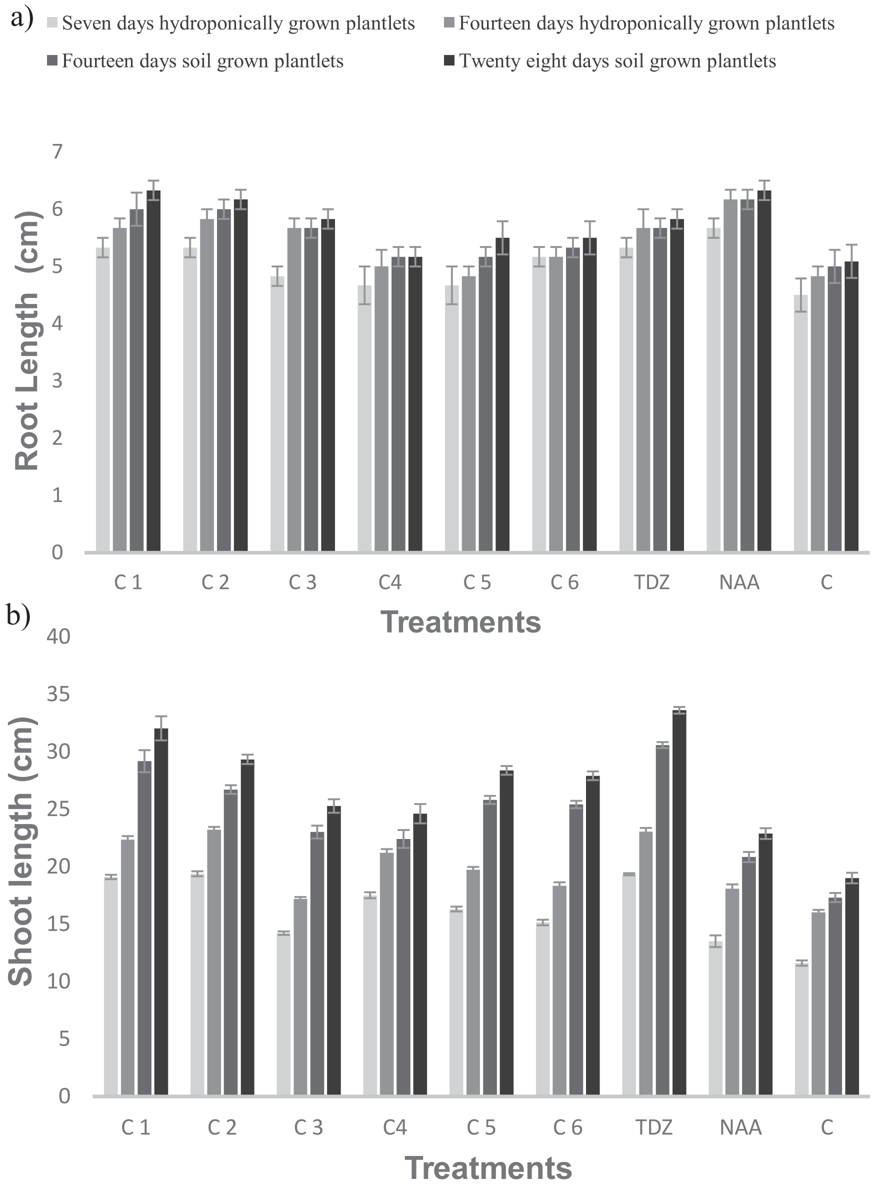


Fig. 3. a) Root length comparison of hydroponically grown and soil acclimatized sunflower plantlets after treatments with various synthesized plant growth promoters, b) Shoot length comparison of hydroponically grown and soil acclimatized sunflower plantlets after treatments with various synthesized plant growth promoters.

with TDZ (33.60±0.29 cm) after 28 days (Fig. 3b). Plants treated with compound 5 (14.33±0.88 cm) were able to produce the maximum number of leaves and also increased the stem girth as shown in Table 3. The highest biomass (fresh and dry) was obtained after treatment of 1 μM concentration of compounds 1 (4603.7±53.5, 868.4±14.8 mg) and 2 (4538.0±64.0, 830.6±19.3 mg) among all synthesized compounds. The maximum leaf area index was calculated for plants treated with compound 5, followed by TDZ. The highest chlorophyll content was calculated in plants treated with compound 1 after 28 days among all synthesized compounds as shown in Table 3.

Our results are in accordance with Stilts et al. [5] as their synthesized compounds butyl 2-(naphthalene 1-yl) acetate and ammonium 1-naphthoate-based plant hormones also increased the length and width of *Ceratopteris richardii* gametophyte after 14 days. Similarly, Ernst et al. also found an increase in oil contents and sunflower seed yield by foliar spray of free amino acids containing plant growth regulators [24]. Agarwal et al. [25] found positive correlation between growth, development, and yield attributes of sunflower (*Helianthus annuus L.*) with different concentrations of (5,10,15 ml/L) nitrobenzene. They observed different parameters like plant height, number of leaves, head diameter, oil content and yield. They found 10 ml/L concentration more effective than others in promoting growth.

Elaleem et al. [26] also studied the effect of different plant growth compounds such as 2,4 dichlorophenoxy acetic acid and α Naphthalene acetic acid on callus

Table 1. Effects of various synthesized derivatives of ethyl substituted 1-(naphthalen-1-ylacetyl) hydrazinyl] acetates on growth of 7- and 14-day-old hydroponically grown sunflower seedlings.

Deriv.	Number of leaves	Fresh biomass (mg)	Dry biomass (mg)	Chlorophyll Content (SPAD value)	Number of leaves	Fresh biomass (mg)	Dry biomass (mg)	Chlorophyll Content (SPAD value)
	After seven days				After fourteen days			
1	4.00±0.00a	1027.0±4.9b	70.35±0.34ab	46.30±0.25ab	4.00±0.00a	1227.0±4.93b	84.05±0.34b	46.30±0.25c
2	4.00±0.00a	1018.0±13.9bc	69.73±0.95ab	44.97±0.49b	4.00±0.00a	1218.0±13.8b	83.43±0.95b	47.97±0.49bc
3	4.00±0.00a	974.7±24.9cd	66.76±1.71bc	32.97±0.23d	4.00±0.00a	1141.3±9.33c	78.18±0.64c	42.93±0.20d
4	4.00±0.00a	940.3±7.0d	64.41±0.48cd	33.47±0.38d	4.00±0.00a	1040.3±6.98d	71.26±0.48d	43.47±0.38d
5	4.00±0.00a	887.3±31.7e	60.78±2.17de	33.50±0.21d	4.00±0.00a	1054.0±11.2d	72.20±0.77d	43.50±0.20d
6	4.00±0.00a	842.0±6.4ef	57.68±0.44e	28.03±0.38e	4.00±0.00a	939.0±8.96e	64.32±0.61e	38.27±0.49e
TDZ	4.00±0.00a	1265.3±11.1a	73.47±1.25a	49.80±1.82a	4.00±0.00a	1265.3±11.1a	86.53±0.76a	55.73±2.06a
NAA	4.00±0.00a	822.3±8.7f	52.43±1.23f	44.23±0.18b	4.00±0.00a	929.3±4.91e	63.66±0.34e	49.50±0.21b
Control	4.00±0.00a	683.7±4.1g	42.30±1.48g	25.40±0.60f	4.00±0.00a	782.0±5.51f	53.57±0.38f	28.43±0.67f

Means sharing similar letter in a column are statistically non-significant according to DMRT (P>0.05)

Table 2. Effects of various synthesized derivatives of ethyl substituted 1-(naphthalen-1-ylacetyl) hydrazinyl] acetate on growth of 14-day-old soil-grown (acclimatized) sunflower seedlings.

Deriv.	Number of leaves	Fresh biomass (mg)	Dry biomass (mg)	Girth (cm)	Leaf area Index (cm^2)	Chlorophyll content (SPAD value)
1	8.00±0.00cd	3429.7±39.8a	620.9±10.6ab	0.263±0.009c	33.00±6.03ab	42.97±0.97ab
2	8.67±0.67bc	3380.8±47.7a	593.9±13.8b	0.293±0.007a	33.00±1.73ab	39.80±1.15bc
3	9.00±1.00bc	2708.3±20.8c	482.5±9.9d	0.270±0.012bc	32.41±4.62ab	27.93±1.83d
4	8.67±0.67bc	1870.7±70.6e	419.6±12.4e	0.233±0.007d	33.67±0.88ab	27.07±1.12d
5	11.67±0.33a	3020.0±32.6b	554.9±10.2c	0.290±0.006ab	36.77±1.76a	36.63±0.54c
5	8.67±0.67bc	2766.2±69.3c	534.0±5.8c	0.240±0.006d	25.67±6.06abc	38.37±1.33c
TDZ	10.67±0.67ab	3488.1±29.0a	631.9±7.6a	0.297±0.003a	33.67±3.48ab	44.33±1.03a
NAA	8.67±0.67bc	2499.2±52.4d	420.1±3.2e	0.227±0.003d	22.33±1.86bc	36.93±0.47c
Control	6.00±1.15d	1401.8±39.8f	329.6±10.5f	0.123±0.007e	17.00±2.08c	27.20±1.32d

Means sharing similar letter in a column are statistically non-significant according to DMRT (P>0.05) induction *of Helianthus annuus L,* and found that almost all PGR promote callus, but maximum response was generated by 1.5 mg/L 2,4 dichlorophenoxy acetic acid on MS basal medium. Similarly, Inoka et al. [27] studied shoot regeneration of in vitro grown seedlings of three different explants by using MS basal medium. They found the highest number of shoots by using a combination of 0.1 mg/L NAA and 0.1 mg/L BAP. Ahmed et al. [28] found salicylic and carbonic acid to be effective in alleviating adverse effects of drought in two sunflower hybrids at 2000 mg/L. They found a maximum increase in growth parameters in the case of Hysun-33 hybrid. Yeremenko et al. found that, although both hybrid and plant growth promoters affect yield of sunflower. They found influence of hybrid (85%) is much much more than the PGR (7.5%) [29].

Naik et al. [30] observed the effect of 2,4-Dichlorophenoxyacetic acid and its lanthanide complexes, and indole 3 acetic acid-derived Schiff base and their lanthanide complexes on the germination and growth activity on wheat seeds, and found the best results at 10^{-6} M concentration. However, Podlesakova et al. [31] observed the good impact of some novel cytokinin derivatives on leaf emergence and lateral root branching in nano molar range of concentrations in *Arabidopsis* and maize. All these studies and their results are in accordance with our selected concentration of plant growth hormones and their positive correlation with the growth of plants.

Similarly, synthesized ethyl ester 1 and 2 treated plants at 1 µM concentration produced significantly higher CAT activity (40.37±1.19, 41.73±0.64 µmol min^{-1} mg^{-1} protein) than the control after 7 days in hydroponics (Fig. 4a). This indicates the generation of excessive oxygen-free radicals in these plants. This gives the clue that probably some growth activity has

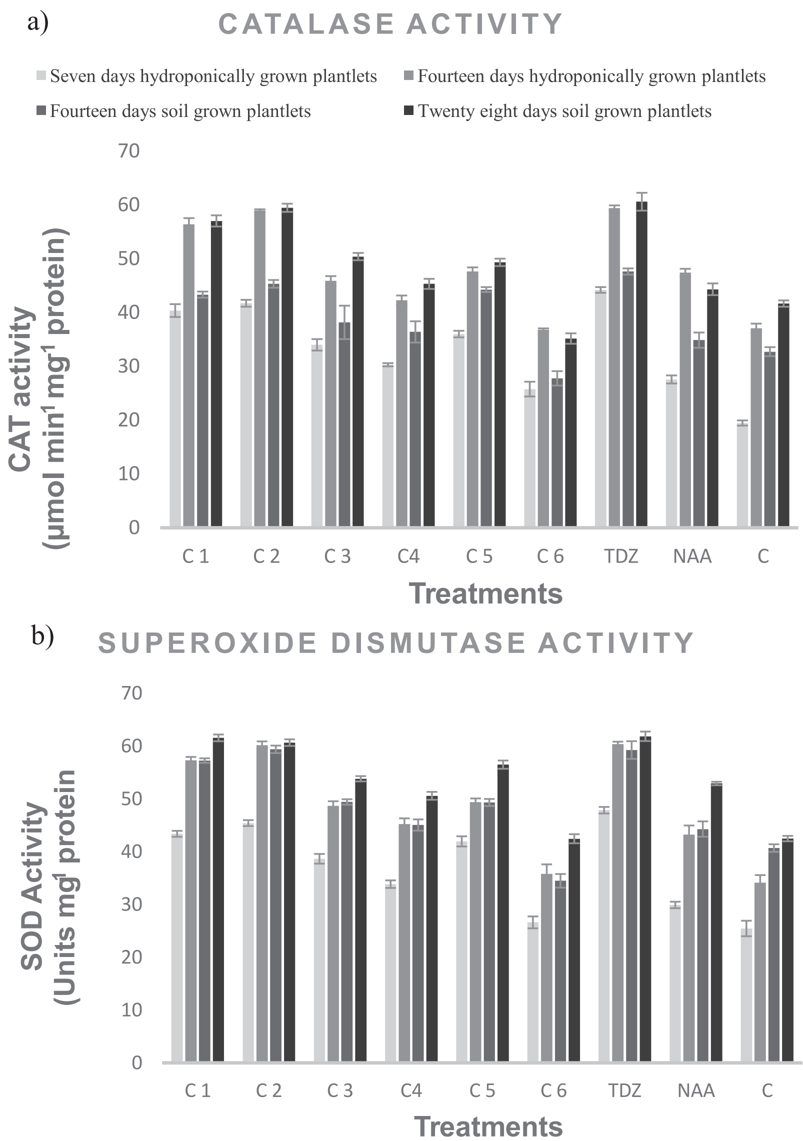


Fig. 4. a) Catalase activity comparison of hydroponically grown and soil acclimatized sunflower plantlets after treatments with various synthesized plant growth promoters, b) Superoxide dismutase activity comparison of hydroponically grown and soil acclimatized sunflower plantlets after treatments with various synthesized plant growth promoters.

occurred. Similarly, Fig. 4b indicates a high level of SOD activity in plants treated with 1 µM concentration of synthesized plant growth regulators. This shows that the plant is detoxifying a higher level of hydrogen peroxide into water. The highest level of SOD was observed in plantlets treated with synthesized ethyl ester 1 and 2 after 7 days among all synthesized compounds.

After TDZ, compound 2 and then compound 1-treated plant seedlings at 1 µM concentration after 14 days in hydroponics showed the highest CAT and SOD activity as shown in Fig. 4(a-b). Again the highest CAT and SOD were found in plants treated with compound 2 after 14 days of their transfer into soil. The order of CAT and SOD activities in plants was TDZ > compound 2 > compound 1 as shown in Figs 4(a-b).

The highest CAT activity was found for plants treated with compound 2 (59.70±0.76 µmol min^{-1} mg^{-1} protein), followed by compound 1 (57.03±1.04 µmol min^{-1} mg^{-1} protein) at the same concentration of synthesized compounds after 28 days, as shown in Fig. 4a). Order of CAT was TDZ > compound 2 > compound 1. More SOD activity was produced by compound 1 among others but less than TDZ. It is interesting to note that compound 5 also exhibited better CAT and SOD results after 28 days in soil, confirming its better acclimatization response in soil as shown in Fig. 4b).

Anushi et al. [32] found an increase in CAT and SOD activity in plantlets of *Cardiospermum halicacabum* L. in *in vivo* and *ex vitro* environment with the passage of time. They also found increases in CAT and SOD activity during the acclimatization period when plantlets were transferred into the soil. Probably the increase in CAT and SOD activity of plantlets when transferred to soil is due to struggling in oxidative stress. Gupta et al. [33] also found increased SOD activity during somatic embryogenesis while the activity of CAT and POX decreased significantly. Conversely, increased CAT and POX activity and a decrease in SOD activity were found during shoot organogenesis.

Alatar et al. [34] noted a decrease in chlorophyll a, b, and carotenoids, net photosynthetic pigment, and anti oxidative enzyme like (SOD, CAT) in leaves of rooted plants of *Ravolifa Serpentina* after 0, 7, 14, 21, and 28 days. But these parameters subsequently increased after 7 days of acclimatization. The increased values indicated some kind of adjustment when plants were shifted into soil. Our results are in accordance with Alatar et al. Similarly, Wojtania et al. [35] studied the relationship between meta topolins (mT) and 6 benzyl amino purine (BAP) with anti-oxidative enzyme activities in *Pelargonium hortorum* cultivars, and found the highest level of SOD and CAT at the beginning of subculture and during initiation of shoot formation in both geranium genotypes. Niczyporuk et al. [13] also observed that all auxins either natural or synthetic, stimulate an enzymatic and non-enzymatic antioxidants

Table 3. Effects of various synthesized derivatives of ethyl substituted 1-(naphthalen-1-ylacetyl) hydrazinyl] acetate on growth of 28-day-old soil-grown (acclimatized) sunflower seedlings.

Deriv.	Number of leaves	Fresh biomass (mg)	Dry biomass (mg)	Girth (cm)	Leaf area Index (cm^2)	Chlorophyll content (SPAD value)
1	9.33±0.67c	4603.7±53.5a	868.4±14.8ab	0.323±0.009c	37.67±6.89a	46.23±1.03ab
2	10.67±0.67bc	4538.0±64.0a	830.6±19.3b	0.357±0.009a	37.00±1.73ab	42.80±1.25bc
3	11.00±1.00bc	3635.3±27.9c	674.8±13.8d	0.330±0.012bc	35.62±4.62a	30.07±1.97d
4	10.67±0.67bc	2511.0±94.7e	586.8±17.4e	0.283±0.007d	37.00±1.15ab	29.10±1.21d
5	14.33±0.88a	4053.7±43.8b	776.0±14.2c	0.350±0.006ab	42.33±0.88a	39.43±0.59c
6	10.33±0.33bc	3713.0±93.0c	746.8±8.1c	0.290±0.006d	33.33±4.48ab	41.30±1.42c
TDZ	12.67±0.67ab	4682.0±38.9a	883.8±10.6a	0.347±0.003ab	41.33±1.45a	47.70±1.10a
NAA	10.67±0.67bc	3354.7±70.3d	587.5±4.5e	0.277±0.003d	26.00±3.06bc	39.73±0.52c
Control	8.67±0.67c	1881.7±53.4f	461.0±14.7f	0.153±0.007e	20.33±2.60c	29.27±1.44d

Means sharing similar letter in a column are statistically non-significant according to DMRT (P>0.05)

Table 4. Detail of antifungal activity of synthesized derivatives of ethyl substituted 1-(naphthalen-1-ylacetyl) hydrazinyl] acetate after seven-day incubation at 27ºC.

Compound number	Concentration (mg/ml)	*Candida albicans*	*Trichophyton rubrum*	*Aspergillus niger*	*Microsporum canis*	*Fusarium lini*	Inference
		% Inhibition					
1	200 µM	14	16	15	09	21	Inactive
2	200 µM	12	15	10	06	13	Inactive
3	200 µM	15	20	18	10	16	Inactive
4	200 µM	10	14	13	08	17	Inactive
5	200 µM	12	16	11	07	12	Inactive
6	200 µM	16	22	18	16	15	Inactive

Standard drug	Name of fungus	MIC µg/ml
Mincazole	*Candida albicans*	97.8
Mincazole	*Trichophyton rubrum*	113.5
Amphotrecin B	*Aspergillus niger*	20.70
Mincazole	*Microsporum canis*	98.1
Mincazole	*Fusarium lini*	73.50

system in *C. vulgaris* and helps in reducing the H_2O_2 gathering. Vivancos et al. [36] found a decrease in SOD activity due to the use of TDZ while BAP and isopentyl adenine (2 ip) increased the SOD activity in cultures of Saffron. Similarly, Ahmed et al. [37] found an increase in SOD and CAT activity in wheat seeds after treatment with 100 mM IAA, NAA, Kinetin H_2O_2, and 2.8 mM GA_3.

Chemistry of the naphthyl ring is unique as some of its derivatives like naphthol, schiff bases, furan carboxamide, etc. showed good antimicrobial activity (antibacterial and antifungal) against pathogens, particularly related to humans with minimum toxicity [38]. Similarly, 2-naphthyl pyrazoline moiety-containing compounds has been synthesized by reaction of phenyl chalcones with phenyl hydrazine, and have been evaluated for their antimicrobial activities [39]. These illustrations indicate that naphthyl moiety with diverse substituents have considerable antimicrobial activities as well.

In vitro antifungal activity in agar tubes indicated that these compounds do not have a significant antifungal activity as shown in Table 4. They have a 6-22% zone of inhibition in tubes containg synthesized compounds at 200 µg/ml concentration. Like other structurally related compounds synthesized and evaluated by various scientists, these results are little different. Comparatively higher inhibition was found with synthesized ethyl ester 1 and 2 due to the presence of chloro substituent with nucleus at positions 2 and 4 against *Fusarium lini* and *Trichophyton Rubrum*. The highest inhibition among all was found with synthesized ethyl ester 6 (22%) against *Trichophyton rubrum* due to the presence of nitro substituent at position 4. The lowest inhibition was calculated against *Microsporum canis* by all synthesized ethyl esters.

Novel Manich bases and 1,3,4-oxadiazole-containing compounds having 6-bromonaphthalene moiety were prepared. These compounds were characterized by IR, ^{1}HNMR, and mass spectroscopy. The derivatives were also screened for their antifungal and antibacterial activities. Some compounds with fluorophenyl, chlorophenyl, and methylphenyl substituents on oxadiazole showed good antimicrobial behavior [40]. Mohammad et al. [41] synthesized derivatives of 3-Aryl-1-(2-naphthyl)-prop-2-en-1-ones and found them to be effective against fungi like A. niger and Penicillium. Derivatives of Azetidinone were synthesized from beta naphthol by reacting beta naphthol with ethyl chloroacetate. The resultant compounds were reacted with hydrazine hydrate and finally with aldehydes to get desired products. The structures of these derivatives were confirmed with FTIR, ^{1}HNMR, and elemental analyzer. These compounds were screened for their antifungal and antibacterial activity as well and found almost equipotent to that of standard [42]. Liu et al. [43] synthesized novel chloro containing 1-aryl-3-oxypyrazoles derivatives and confirmed them with various spectroscopic techniques. They also screened these compounds against *Rhizoctonia solani* and found these compounds to be active. They also found that the increase in the number of chloro groups decreases the fungicidal ability. Bhor et al. [16] synthesized 2-(2-benzyl-4-chlorophenoxy) acetohydrazide derivatives and also screened these derivatives for various antimicrobial activities. They found these derivatives less to moderately active against bacterial and fungal strains than standard drugs. They found that the compound having 4 nitro substituents to be more active than the rest. Our results are in accordance with them.

These compounds may be taken as good plant growth promoters with slight antifungal behavior for diverse fungal pathogens. However, further research is required in this regard.

Conclusions

Among six eco-friendly synthesized naphthyl acetic acid-based ethyl esters, best growth was observed with compounds 1 and 2 at 1 µM concentrations in both hydroponics and in soil acclimatized plants of sunflower. The growth promoted by these compounds was better than control and was comparable with commercial standard TDZ in all parameters, such as root growth, shoot growth, fresh weight, dry weight, chlorophyll contents, SOD, and CAT levels, etc. These synthesized ethyl esters also exhibited less to moderate antifungal behavior against various fungal pathogens in agar tube dilution protocol. Thus, these compounds can prove strong plant growth promoters along with mild anti-fungal agents.

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Conflict of Interest

The authors declare no conflict of interest.

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