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Ameliorating Potential Of Silver Nano-Particles On Growth And Physiology Of Carrot (*Daucus Carota L.*) Under Hydrocarbon Stress (Kerosene Oil)

[1]Aamir Iqbal, [2]Aamir Ali, [3]Sajida Shabbir, [4]Muhammad Waqas, [5]Faisal Iqbal Jafri, [6]Aafaq Ali, [7]Asif Abbas Shah [8]Mian Jahan Zaib Rasheed

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Aamir Iqbal
Department of Botany, University of Sargodha, Sargodha,Pakistan.
aamirmughal924@gmail.com
Aamir Ali
Department of Botany, University of Sargodha, Sargodha,Pakistan. aamirali73@gmail.com
Sajida Shabbir
Department of Botany, University of Sargodha, Sargodha, Pakistan.
sajida.shabbir11@gmail.com
Muhammad Waqas
Institute of Chemistry, University of Sargodha, Sargodha, Pakistan.
waqasmalik1697617@ gmail.com
Faisal Iqbal Jafri
Department of Botany, University of Gujrat, Gujrat, Pakistan. faisal.bhs@gmail.com
Aafaq Ali
Department of Botany, University of Sargodha, Sargodha,Pakistan. Aafaqalibio@gmail.com
Asif Abbas Shah
Department of Botany, University of Sargodha, Sargodha, Pakistan. aasgd86@gmail.com
Mian Jahan Zaib Rasheed
Department of Botany, University of Sargodha, Sargodha Pakistan.
jahanzaibrasheedgc@gmail.com

ABSTRACT

Kerosene (crude oil) is one of the most common persistent organic pollutant that is very harmful for plants morphologically and physiologically. Therefore, present study is aimed to investigate the ameliorating potential of AgNPs on the growth and physiology of carrot (Daucus carota L.) under hydrocarbon stress (kerosene oil). Kerosene oil stress caused a significant reduction in all morphological parameters such as root diameter, root length, root weight, shoot weight, shoot length, number of leaves and fresh weight of leaves. Also remarkable reduction in the activity of nitrate reductase and peroxidase (NRA and POX). Application of AgNPs ameliorated the kerosene oil stress by scavenging reactive oxygen species (ROS) as well as decreasing the level of malondialdehyde (MDA). Additionally, AgNPs enhanced the activities of antioxidant enzymes peroxidase, phenyl alanine ammonia lyase, polyphenol oxidase as well as increased nitrate reductase activity to mitigate kerosene stress. .

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INTRODUCTION

Now a day's crude oil pollution is considering as a major challenge and global issue especially for crops. Crude oil stress interacted with physiological and biochemical processes which ultimately results in the change of morphological (yield contributing parameters). Among all the abiotic stresses, crude oil stress causes enormous loss in production of crop because oil spillage produces layers on the water, it inhibits oxygen penetration into the water and causing abiotic stress (Ohanmuet al., 2017).

Crude oil pollution has also been shown to harm plants by inhibiting germination, lowering plant development (stem girth and height), lowering photosynthetic rate, causing ultimately death. The chemical and physical properties of soil can be affected by crude oil pollution. Crude oil affect air-water balance, changes the physicochemical properties of the soil, disrupt enzymatic activity in soil, restrict microbial proliferationand have negative impact on plant development and growth (Skrypnik et al., 2021)

In case of root vegetable, connected with this abiotic stress on the carrot and also weakens the cell, as a results in membrane leakage (cell damage) subsequently greater weight loss in the storage. In stem and root crude oil enters in cell and reduce intercellular spaces as well as in root (Bellout et al., 2016). Kerosene oil is the major factor which is negatively effect on the plant development, decrease photosynthetic rate and even plant death under severe conditions (Ohanmuet al., 2017). The most hazardous disorder is oxidative stress which is caused by crude oil pollution which leads to the formation of ROS (reactive oxygen species) with higher oxidizing capacity in plant cells (H_2O_2, superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), etc.). these reactive oxygen species destroy cell membranes and inhibit plant growth activity (Zaid, and Wani. 2019).Kerosene oil stress limits the availability of nutrients particularly phosphorus and nitrogen. Nutrients are very essential for plants because without these nutrients plant cannot complete their life cycle (Otitoju et al., 2017; Achubaand Ja-Anni, 2018).Crude oil stress will affect the crop yield, authenticity of the food and vegetable quality, supply and safety (Ambreen et al., 2016).

Vegetables are very essential component of our diet providing vital nutrients such as minerals, vitamins, protein and fiber that are essential for growth and development of human life. That is consumed in different forms i.e. fruits, stems, roots, leaves both by man and animals (Aderinola and Abaire, 2019). Carrot is among the top ten most economically vital vegetable crop worldwide (Tegen andJembere, 2021).

Carrot (*Daucus carota* L.), a biennial herbaceous plants that belong to Apiaceae family, is one of the chief vegetable consumed and produced in all over world. Carrot roots that originate from the hypocotyls have greater storage capacity (Que et al., 2019).The use of carrot is increasing day by day due to having pleasant taste and superficial health benefits related to, minerals, vitamins and rich source of β-carotene, a dimer of Vitamin A. Carrots are also high source of minerals, antioxidants and dietary fibres, as well as being an alkaline food.A significant amount of carbohydrates is present in taproots (Que et al., 2019). Carrot are good source of natural phyto-pigments, such as β-carotene lycopene, anthocyanin and xanthophyll, found in numerous colors, such as black, pink, yellow, orange, red and yellow. Carrots have number of health benefits, including regulate metabolism, boosting the immune system, preserving good vision and skin, and lowering the risk of stroke, high blood pressure, cardiovascular disease and several cancers (Ahmad et al., 2012).

As a result of long term effects of crude oil on the environment a large variety of plant species have been recorded to be extinct. One of the most severe problems about crude oil contamination in the ecosystem is the danger of contamination of crops. To overcome the effect of crude oil stress, there is a dire need to introduce silver nanoparticles with improving yield potential and ameliorating potential against crude oil stress (Kim et al., 2012).

Silver nanoparticles (AgNPs) are now most investigated and utilized nanoparticles in agricultural sector to improve agricultural crops efficiency, sustainability and productivity. It has been recognized for a long time to have strong antifungal, pesticidal, bactericidal, antiviral effects (Khan and Rizvi, 2014).AgNPs have potential to enhance quality and quantity of food, plant protection, world's food production, disease detection and control, plant growth monitoring and pest management for sustainable agriculture due to their wide ranging spectrum of antimicrobial actions (Prasad et al., 2017). In aqueous solution, AgNPs are extremely stable as well as dispersive. It is used as foliar spray to prevent growth of fungus, rot, molds and many other diseases in plants (Singh et al., 2015). Furthermore, AgNPs are an effective plant growth stimulator. It provides an innovative tool for the disease control, quick disease diagnosis, and nutrient loss minimization in fertilization by improve nutrient management (Hussain et al., 2019).

Nanoparticles increased plant height due to increase the gibberellic acid because GA is responsible for plant elongation (Aroraet al., 2012). AgNPs enhance the reproductive capacity and remarkably improved morphology as well as protective role against hydrocarbon stress (Wahid et al., 2020). AgNPs reduced the oxidative stress (Wahid et al., 2020), under oxidative

stress hydrogen peroxide produced which is a harmful cellular metabolite and ROS (Reactive Oxygen Species). After exposure of AgNPs, plant defense mechanism activated that remove hydrogen peroxide as well as inhibit the production of ROS (Thuesombatet al., 2016). Therefore, present study was planned to study the ameliorating potential of AgNPs in alleviation of kerosene oil stress in carrot. Furthermore, to investigate the adverse effect of hydrocarbon toxicity on the growth of *Daucus carota* L.

MATERIALS AND METHODS

Certified seeds of T-20 variety of carrot were used in present study were obtained from germplasm collection of carrot, Plant tissue Culture Laboratory, Department of Botany, University of Sargodha, Pakistan. Seeds were treated with sodium hypochlorite (0.5%) for sterilization which further washed with distilled water. For 24 h at 25 °C seeds were imbibed in distilled water. The research work was conducted in Plant Tissue Culture Laboratory, University of Sargodha, Pakistan (32.0737° N, 72.6803° E).Experiments were performed in pots, each pot containing 3kg autoclaved (121° C and 1.5 bar) soil and then 5 sterilized seedsof carrot were sown in each pot were placed in green house at 18/23 ± 3 ° C (day/night) and 16 h photoperiod.

After 30 days of germination kerosene oil was used for hydrocarbon stress. Different concentrations (0%, 2% and 4%) were applied on allocated pots. AgNPs were used to check their amelioratory role against hydrocarbon toxicity. Silver nanoparticles with 5.0 m2g-1 surface area and particle size is <100 nm were obtained from Sigma Aldrich. The stock solution of 1000ppm was prepared by adding double distilled water. Different concentrations 0ppm, 20ppm and 40ppm were prepared from stock solution. After 15 days of kerosene oil stress, foliar application of AgNPs were applied on allocated pots.

MORPHOLOGICAL CHARACTERIZATION

After harvesting plants were collected, washed with distilled water, dry with filter paper and different morphological parameterssuch as root diameter, root length, root weight, shoot weight, shoot length, number of leaves and fresh weight of leaves were recorded withscale, analytical balance, vernier caliper carefully.

BIOCHEMICAL CHARACTERIZATION

Quantitative analysis (total amino acid contents, total soluble protein content, total soluble sugar contents and enzymes PAL, POD and nitrate reductase) were carried out at Tech comp UV-1100 absorption spectrophotometer. For this purpose, a weighed amount of leaves tissues was taken

and crushed into pestle mortar with the help liquid nitrogen. Add 0.1M phosphate buffer at pH 7.0 was added. The obtained slurry was centrifuged at 1200 rpm for 10 minutes. After centrifugation the supernatant was obtained and used for various biochemical tests.

ESTIMATION OF TOTAL SOLUBLE PROTEIN

The method described by Bradford (1976) was used for the estimation of total protein contents. For this purpose, 0.02 ml plant extract, 0.2 ml Bradford reagent and 0.8 ml distilled water were added in test tube. Shaken vigorously and left for 15 minutes for completion of reaction. Optical density was taken at 570 nm UV -Spectrophotometer.

SPECIFIC ACTIVITY OF PHENYL ALANINE AMMONIA LYASE ACTIVITY (PAL)

The procedure used to check PAL activity described by Zucker (1965) and modified by Pendharker and Nair (1975). For this estimation 1.5 ml of 200 µM borate buffer and 1.5 ml of 30 mM phenylalanine were added into 0.3 ml of plant extract and incubated for one hour at 40 ° C. Then 0.2 ml of 5N HCL was added to terminate the reaction and volume was increased up to 4ml with distilled water. The activity of PAL was recorded at 270 nm with the help of UV-spectrophotometer.

SPECIFIC ACTIVITY OF POLYPHENOL OXIDASE

The method for the analysis of PPO is proposed by Decker (1977).For this purpose, 0.1 ml of plant extract, 1 ml phosphate buffer, 1 ml of 0.001 M tyrosine and 0.9 ml of distilled water were added in test tube and kept 10-15 min then optical densities were measured by UV-Spectrophotometer at 280 nm.

SPECIFIC ACTIVITY OF PEROXIDASE

David and Murray (1965)proposed method for the estimation of peroxidases. For absorbance, 0.2 ml extract, 0.2 ml of % guaiacol, 2.5 ml of 0.1 M Phosphate buffer (pH 7.2), 0.1 ml of 0.3% H_2O_2 were mixed. The optical density was taken at 470 nm using UV-Spectrophotometer.

NITRATE REDUCTASE

Specific activity of nitrate reductase was determined by Sym (1984). For this activity, 0.5 ml extract was added in 5 ml of 0.2 M phosphate buffer+0.02 M KNO_3 and incubate for 30 minutes at 30 °C. Secondly, 0.5 ml sulphanilamide and 0.5 ml of 0.02% of N-ethylene diamine dihydrochloride were added and wait for 20 minutes to complete the reaction. Optical density was recorded at 542 nm at UV-Spectrophotometer.

ESTIMATION OF TOTAL AMINO ACID CONTENTS

Method described by Hamilton and Van Slyke (1943) was used for estimation of total amino acids

contents. For the estimation of total soluble amino acid, 1 ml plant extract, 1 ml of 10% pyridine, 1 ml of 2% Ninhydrin were mixed in test tubes. Then tubes were heated for 30 minutes in water bath and optical density was taken at 570 nm using UV-Spectrophotometer.

ESTIMATION OF TOTAL SOLUBLE SUGAR CONTENTS

The method for analysis of total sugar content described by Yemm and Willis (1954). For absorbance, 3 ml Anthrone reagent was added to 1ml of plant extract in test tubes. After that test tubes were placed into water bath for 10 minutes at 30-35 °C to complete the reaction. Then left for cooling and optical density was taken at 620 nm.

ESTIMATION OF REDUCING SUGAR

For estimation of reducing sugar is described by Dubowski (2008). 0.5 ml extract was mixed with 1.5 ml of 6% Orthotoluidine. Then test tubes were kept in water bath for 10 minutes at 100 °C. Optical densities were recorded by UV-Spectrophotometer at 630 nm.

STATISTICAL ANALYSIS

Statistical analysis was performed by using statistic software Statistical, XL-Stat and ANOVA (Analysis of Variance).

RESULTS

Ameliorating potential of AgNPs on the growth attributes of *Daucus carota* L. under kerosene oil stress.

Kerosene oil adversely affected all of studied morphological attributes of carrot. Under 2% kerosene reduced root diameter, root weight, root length, shoot length, shoot weight, number of leaves and fresh weight of leaves in carrot by -88.25%, -95.21%, -78.50%, -65.85%, -77.39%, -74.19%, -76.24% as compared to control, respectively (Table 1). AgNPs tried to mitigate the effect of kerosene by enhancing all morphological parameters. After application of 20ppm and 40ppmof AgNPs increased root diameter (71.12%and 100.81%), root weight (77.40% and 95.89%), root length (75.58% and 89.53%), shoot length (22.68% and 38.21%), shoot weight (73.61% and 93.40%) number of leaves (62.50% and 100%) and fresh weight of leaves (48.55% and 59.09%) as compared to 2% of kerosene oil (Table 1).

Under 4% of kerosene oil all growth attributes were further decreased root diameter, root weight, root length, shoot length, shoot weight, number of leaves and fresh weight of leaves in carrot by -91.77%, -98.49%, -78.75%, -77.44%, -85.68%, -83.87%, -91.62% as compared to control respectively (Table 1).

Moreover, application of 20ppm and 40ppmof AgNPs improved root diameter (88.75%and

99.38%), root weight (91.30% and 97.83%), root length (55.29% and 95.29%), shoot length (88.38% and 100.54%), shoot weight (82% and 94.14%) number of leaves (100% and 100%) and fresh weight of leaves (51.55% and 76.80%) as compared to 4% of kerosene oil (Table 1).

Treatments	Root Diameter (mm)	Root weight (g)	Root Length (cm)	Shoot Length (cm)	Shoot Weight (g)	Leaves Number (no.)	Fresh weight (g)
T_0	10.53 ± 0.94^{b}	7.59 ± 3.81^{b}	12.62 ± 0.89^{bc}	38.01 ± 15.26^{b}	15.97 ± 7.99^{b}	8.00 ± 2.23^{c}	5.81 ± 2.19^{b}
T_1	2.04 ± 0.51^{a}	0.55 ± 0.07^{a}	4.12 ± 1.17^{a}	17.84 ± 3.77^{a}	5.36 ± 0.73^{a}	3.22 ± 0.66^{ab}	2.13 ± 0.61^{a}
T_2	1.31 ± 0.27^{a}	0.25 ± 0.13^{a}	3.31 ± 0.66^{a}	13.80 ± 3.20^{a}	3.95 ± 0.74^{a}	2.11 ± 0.60^{a}	1.10 ± 0.76^{a}
T_3	15.17 ± 0.62^{c}	15.69 ± 3.33^{c}	11.46 ± 6.65^{abc}	57.66 ± 16.43^{c}	26.24 ± 4.23^{c}	14.66 ± 3.32^{d}	11.52 ± 2.65^{c}
T_4	17.53 ± 0.82^{d}	17.82 ± 1.93^{c}	14.41 ± 8.68^{c}	65.61 ± 22.95^{c}	30.52 ± 6.23^{c}	17.50 ± 3.14^{d}	13.43 ± 1.63^{c}
T_5	2.73 ± 0.64^{a}	0.79 ± 0.08^{a}	11.45 ± 7.04^{abc}	22.68 ± 0.53^{ab}	8.76 ± 0.44^{aa}	4.33 ± 0.51^{ab}	2.83 ± 0.21^{a}
T_6	3.24 ± 0.42^{a}	0.96 ± 0.01^{b}	13.40 ± 8.73^{bc}	25.21 ± 0.92^{ab}	10.03 ± 0.65^{ab}	5.33 ± 0.51^{bc}	3.11 ± 0.34^{a}
T_7	2.24 ± 0.42^{a}	0.27 ± 0.02^{a}	4.03 ± 0.64^{a}	22.33 ± 1.10^{ab}	5.78 ± 0.41^{a}	3.33 ± 0.51^{ab}	0.94 ± 0.04^{a}
T_8	2.53 ± 0.44^{a}	0.29 ± 0.01^{a}	5.66 ± 0.36^{ab}	23.93 ± 3.65^{ab}	6.65 ± 0.52^{a}	3.83 ± 0.75^{ab}	1.13 ± 0.01^{a}

TABLE 1: EFFECT OF KEROSENE OIL AND AMELIORATING POTENTIAL OF AGNPS ON GROWTH ATTRIBUTES OF CARROT.

Values are means ± SD of three replicates. T_0, control; T_1, 2% kerosene oil; T_2, 4% kerosene oil; T_3, 20 ppm AgNPs; T_4, 40 ppm AgNPs; T6, 2% kerosene oil + 20 ppm AgNPsT6, 2% kerosene oil + 40 ppm AgNPs; T_7, 4% kerosene oil + 20 ppm AgNPs, T_8, 4% kerosene oil + 40 ppm AgNPs.

Ameliorating potential of AgNPs on total carbohydrates contents of *Daucus carota* L. under kerosene oil stress

In present study kerosene enhanced total soluble sugar including reducing and non-reducing sugar when compared to non-stress plant.A significant increase in total soluble sugar content, reducing sugar and non-reducing sugar in carrot by 34.50%, 20.19% and 15.10% of 2% K.O treated plant and 49.65%, 37.32% and 71.04% increased respectively of 4% K.O treated plant as compared to control. AgNPs played a synergistic role to enhance the total soluble sugar content, reducing sugar as well as non-reducing sugar as compared to kerosene oil treated carrot.

After application of 20ppm and 40ppmof AgNPs enhanced TSS (24.92%and 32.51%), RS (35.60% and 41.84%) and NRS (8.98% and 30.13%)as compared to 2% of kerosene oil (Table 2).When 4%

of kerosene oil carrot treated with 20ppm AgNPs and 40ppm AgNPs further enhanced TSS (23.88%and 33.59%), RS (34.64% and 43.70%) and NRS (6.22% and 15.85%)

Treatments	TSS (mg/gm of tissue)	RS (mg/gm of tissue)	NRS (mg/gm of tissue)
T_0	0.09 ± 0.01^{a}	0.009 ± 0.002^{a}	0.07 ± 0.02^{a}
T_1	0.11 ± 0.03^{ab}	0.011 ± 0.002^{ab}	0.08 ± 0.02^{ab}
T_2	0.13 ± 0.03^{abc}	0.012 ± 0.003^{abc}	0.12 ± 0.03^{cd}
T_3	0.14 ± 0.01^{bc}	0.015 ± 0.001^{bcd}	0.10 ± 0.00^{abc}
T_4	0.16 ± 0.01^{cd}	0.016 ± 0.001^{cd}	0.11 ± 0.01^{bc}
T_5	0.16 ± 0.02^{cde}	0.017 ± 0.001^{de}	0.11 ± 0.00^{abc}
T_6	0.18 ± 0.01^{de}	0.018 ± 0.001^{de}	0.13 ± 0.00^{cd}
T_7	0.19 ± 0.01^{de}	0.019 ± 0.002^{de}	0.16 ± 0.00^{de}
T_8	0.20 ± 0.02^{e}	0.021 ± 0.001^{e}	0.17 ± 0.01^{e}

TABLE 2: EFFECT OF KEROSENE OIL AND AMELIORATING POTENTIAL OF AGNPS ON CARBOHYDRATES CONTENTS OF CARROT.

TSS, total soluble sugar; RS, reducing sugar, NRS, non-reducing sugar. T_0, control; T_1, 2% kerosene oil; T_2, 4% kerosene oil; T_3, 20 ppm AgNPs; T_4, 40 ppm AgNPs; T6, 2% kerosene oil + 20 ppm AgNPs T6, 2% kerosene oil + 40 ppm AgNPs; T_7, 4% kerosene oil + 20 ppm AgNPs, T_8, 4% kerosene oil + 40 ppm AgNPs.Values are means ± SD of three replicates.

Ameliorating potential of AgNPs on total amino acid and total soluble protein contents of *Daucus carota* L. under kerosene oil stress.

Kerosene caused enhancement of total amino acid and total soluble protein contents when compared to non-treated plant. A significant increase in total amino acid and total soluble protein contents in carrot by 17.53% and 25.89% of 2% kerosene treated carrot and 20.77% and 45.71% increased respectively of 4% kerosene treated carrot as compared to control. A synergistic role of AgNPs was observed to enhancement of total amino acid and total soluble protein contents as compared to kerosene oil treated carrot. After application of 20ppm and 40ppmof AgNPs increased AA (24.94%and 34.14%) and TSP (30.07% and 42.51%) as compared to 2% of kerosene oil (Table 2).

When 4% of kerosene oil carrot treated with 20ppm AgNPs and 40ppm AgNPs further increased AA (54.86%and 64.56%) and TSP (30.17% and 37.02%) respectively (Table 3).

Treatments	AA (mg/gm of tissue)	TSP (mg/gm of tissue)
T_0	2.36 ± 0.41^{a}	0.10 ± 0.01^{a}
T_1	2.69 ± 0.49^{ab}	0.12 ± 0.02^{ab}
T_2	3.44 ± 0.48^{abc}	0.14 ± 0.03^{abc}
T_3	3.23 ± 0.60^{abc}	0.14 ± 0.01^{bc}
T_4	3.87 ± 0.83^{cde}	0.17 ± 0.01^{cd}
T_5	3.57 ± 0.86^{bcd}	0.19 ± 0.01^{de}
T_6	3.76 ± 0.69^{bcd}	0.21 ± 0.01^{def}
T_7	4.61 ± 0.65^{de}	0.22 ± 0.01^{ef}
T_8	4.94 ± 0.64^{e}	0.23 ± 0.01^{f}

TABLE 3: EFFECT OF KEROSENE OIL AND AMELIORATING POTENTIAL OF AGNPS ON AA AND TSP CONTENTS OF CARROT.

AA, amino acid; TSP, total soluble protein. Mean followed by different letters in the same column differ significantly at $P\leq0.05$ according to Tuckey's new multiple range tests. T_0, control; T_1, 2% kerosene oil; T_2, 4% kerosene oil; T_3, 20 ppm AgNPs; T_4, 40 ppm AgNPs; T6, 2% kerosene oil + 20 ppm AgNPs T6, 2% kerosene oil + 40 ppm AgNPs; T_7, 4% kerosene oil + 20 ppm AgNPs, T_8, 4% kerosene oil + 40 ppm AgNPs.Values are means ± SD of three replicates.

Ameliorating potential of AgNPs on phenol synthesizing and oxidizing enzymes of *Daucus carota* L. under kerosene oil stress.

The current investigation depicted that stress of kerosene oil (2%) boosted the activities of PAL and PPO by 26.94% and 39.84 and decreased the activities of POX and NRA by -34.40% and -68.84% as compared to control. Similar remarkable enhancement the activities of PAL and PPO by 33.81% and 67.91% respectively and remarkable reduced the activities of POX and NRA by -42.97% and -82.81% under 4% of kerosene oil as compared to control. However, supplementation of AgNPs induced further increased at 20ppm and 40ppm of AgNPs PAL (33.88% and 40.56%) and PPO (21.64% and 29.67%) as well as improved the activities of POX (68.65% and 93.29%) and NRA (79.12% and 98.70) as compared to 2% of kerosene oil (Table 4). Moreover, further AgNPs showed ameliorated potential in carrots which were subjected to 4% of kerosene. Increased the activities of PAL (41.39% and 45.14%) and PPO (11.21% and 17.64%) and boosted the activities of POX (36.49% and 63.15%) and NRA (39.61% and 77.73% after

application of 20ppm and 40ppm of AgNPs as compared (Table 4).

Treatments	PAL (units/mg of tissue)	PPO (units/mg of protein)	POX (units/mg of protein)	NRA (µM NO_2/hr/gm of tissue)
T_0	0.018 ± 0.005^{a}	0.015 ± 0.003^{a}	0.017 ± 0.001^{cd}	0.407 ± 0.261^{d}
T_1	0.023 ± 0.006^{ab}	0.020 ± 0.003^{ab}	0.012 ± 0.000^{ab}	0.168 ± 0.088^{ab}
T_2	0.025 ± 0.005^{ab}	0.023 ± 0.005^{bcd}	0.011 ± 0.001^{a}	0.108 ± 0.054^{a}
T_3	0.030 ± 0.004^{bc}	0.021 ± 0.001^{bc}	0.020 ± 0.001^{e}	0.745 ± 0.053^{e}
T_4	0.032 ± 0.004^{bcd}	0.025 ± 0.003^{cde}	0.023 ± 0.002^{f}	0.831 ± 0.034^{e}
T_5	0.035 ± 0.004^{cde}	0.027 ± 0.001^{def}	0.018 ± 0.001^{de}	0.329 ± 0.022^{bcd}
T_6	0.037 ± 0.003^{cde}	0.029 ± 0.001^{efg}	0.020 ± 0.002^{ef}	0.370 ± 0.018^{cd}
T_7	0.039 ± 0.004^{de}	0.031 ± 0.000^{fg}	0.014 ± 0.001^{bc}	0.185 ± 0.039^{abc}
T_8	0.041 ± 0.003^{e}	0.033 ± 0.000^{g}	0.017 ± 0.000^{cd}	0.218 ± 0.030^{abcd}

TABLE 4: EFFECT OF KEROSENE OIL AND AMELIORATING POTENTIAL OF AGNPS ON ANTIOXIDANT ENZYMES OF CARROT.

PAL, phenylalanine ammonium lyase; PPO, Poly phenol oxidase;POX, peroxidase; NRA, nitrate reductase activity. Values are means ± SD of three replicates.T_0, control; T_1, 2% kerosene oil; T_2, 4% kerosene oil; T_3, 20 ppm AgNPs; T_4, 40 ppm AgNPs; T6, 2% kerosene oil + 20 ppm AgNPs T6, 2% kerosene oil + 40 ppm AgNPs; T_7, 4% kerosene oil + 20 ppm AgNPs, T_8, 4% kerosene oil + 40 ppm AgNPs.

DISCUSSION

Morphological parameters i.e. shoot length, root length, root weight, shoot weight, surface area of leaf, number of leaves and fresh weight of leaves are crucial factors that are directly related to yield of the crop and are particularly caused by hydrocarbon stress.Growth retardation is one of the most visible symptoms and can be useful index for assessment of toxicity by visual inspection of different changes at morphological as well as physiological (Hussain et al., 2019).

FIG. 1.EFFECT OF SILVER NANOPARTICLES UNDER 2% AND 4% OF KEROSENE OILIN CARROT.

FIG. 2. MORPHOLOGICAL ATTRIBUTES UNDER DIFFERENT CONCENTRATIONS OF K.O AND AGNPS

Growth retardation in the plant may be due to several hazardous substances in petroleum, particularly hydrocarbon with low molecular weight as well as polycyclic aromatic component (Adieze et al., 2012). In current work, significant reduction in morphological parameters was recorded after crude oil stress.

Root length is the major morphological marker which is associated with the yield of crop. Abiotic stress causes decrease in soil moisture contents which leads to disturbance in the mechanism of nutrients and minerals (i.e. Sulphur, nitrogen and phosphorous) uptake causing the reduction of root length of the plant (Chuku et al., 2017). Root length was drastically affected under crude oil stress (Castro-Mancilla et al., 2019). This may be due to reason that due to hydrophobic attributes of kerosene oil and metabolic processes of plant such as inhibition of cell division causes disturbance of cellular redox control in the root tip zone causing damage root tip that ultimately leads to reduction in root elongation (Rusin, 2015).

Root diameter is also an important parameter associated with yield of crop. Abiotic stress causes increase in the production of hydrogen peroxide (H_2O_2). Hydrogen peroxide is a ROS which can cross the membrane easily because H_2O_2 has larger pKa and can harmful for subcellular structures which leads to the disturbance of membrane integrity, increased lipid peroxidation which ultimately causes the reduction of root diameter. (Wang et al., 2013).

Root weight is also vital role yield contributing parameter. It is very important trait which is associated with final yield of carrot. Result of present investigation depicts that root weight is affected by kerosene oil. This may be due to the fact that hydrophobicity of kerosene oil affects the seepage of water into the soil causing depletion of soil moisture contents, as a result of this depletion rate of transpiration and stomatal conductance is decreased leading to stress condition (Khairi et al., 2015). Under such stress plants usually close their stomata to prevent loss of water which correspondingly leads to less CO2 intake into the plant. As a result, remarkable reduction in photosynthetic activity takes place that consequently cause great reduction in root weight (Wang et al., 2013).

Photosynthesis under hydrocarbon stress on crop can reduce ability of cells to gain reducing power, resulting in the production of ROS. These can produce oxidative stress in the cells and ROS enter in the cell to damage chloroplast and decreases the chlorophyll contents which leads to the disturbance in metabolic processes like photosynthesis causing the retardation of photosynthetic activity (Rezvani et al., 2012). In current research work, remarkable reduction in chlorophyll contents was recorded under kerosene oil stress.

Number of leaves is also major morphological marker. Kerosene oil can change the electrical conductivity, increase total organic carbon, increase salinity and increase pH value which may disturb growth and development of plants which leads to decreasing the number of leaves (Anienye et al., 2015; Castro-Mancilla et al., 2019; Akpokodje and Uguru, 2019). The decrease in number of leaves may be due to decrease water potential and turgor potential of the leaves, decrease photosynthetic pigments and reduce carbon dioxide assimilation rate (Ambreen et al., 2016). Lowering the water and turgor pressure, minerals cannot travel toward leaves consequently leaves will become chlorotic due to the deficiency of minerals in leaves as a result remarkable reduction in the leaves size and number of leaves (Oyem and Oyem, 2013).

One of the most severe problems about crude oil contamination in the ecosystem is the danger of contamination of crops. To overcome the effect of crude oil stress, there is a dire need to introduce silver nanoparticles with improving yield potential and ameliorating potential against crude oil stress (Kim et al., 2012). Nanotechnology is now the most promising new technology in one in the world of agronomy and plants biotechnology. Silver nanoparticles (AgNPs) are now most investigated and utilized nanoparticles in agricultural sector to improve agricultural crops efficiency, sustainability and productivity (Prasad et al., 2017). AgNPs contains novel features of nanomaterial that facilitate agronomic research in crop development

programmes as well as stress relief. AgNPs increases the expression of genes (photosystem marker) which leads to the formation of chlorophyll which ultimately lead to enhance the yield of crop (Kokina et al., 2020; Krishnaraj et al.,2012). Nanoparticles increases the concentration of gibberellic acid which leads to plant elongation (Arora et al., 2012). In presenet investigation, AgNPs enhanced reproductive capacity and remarkably improved morphology also protective role against hydrocarbon stress (Ikhajiagbe, 2019; Wahid et al., 2020).

Abiotic stresses influence on the growth and development of crop. Plants undergo many physiological and biochemical changes as a result of these stressor. Amino acids contribute to plant stress tolerance by assisting the detoxification of ROS (Reactive Oxygen Species), pH regulation and osmotic adjustment (Khan et al., 2020). Under abiotic stress, plant species accumulate large amount of amino acid. This may be due to increase in amino acid contents under hydrocarbon stress conditions by degradation of protein or may be due to inactivation of nitrate reductase activity (Li et al., 2012; Salama, 2012). Results of present study also show that silver nanoparticles mitigate the crude oil stress and inhibit degradation of protein (Ikhajiagbe, 2019). The activation of de novo synthesis of proteins to be one of most essential mechanism associated with cell defense against stress. Under abiotic stress, specific stress tolerance genes activate and accelerate the mechanism of transcription and translation as result large amount of protein is produced which play its role to combat stress (Achuba, 2014). In leaves, there is strong correlation between chlorophyll pigment and total soluble protein. Higher level of chlorophyll contents increases photosynthetic rate which leads to formation of total soluble protein. Current investigations focus on the role of silver nanoparticles, AgNPs increase the chlorophyll contents which ultimately leads to increases total soluble protein contents (Kumar et al., 2020).

Soluble sugars play an important role in a variety of metabolic pathway, acting as signal to control gene expression that are involved in the process of photosynthesis, sucrose metabolism and osmolyte production. (Khan et al., 2020). Total soluble sugar increase in the cell to provide C-skeleton used to maintain mechanism of the plant during stress conditions. Sugar protects the cells because the OH group may substitute for water to maintain interaction in membranes and protein during dehydration. In present study it was found out that under kerosene oil stress, total soluble sugar contents increased (Sami et al., 2016). This remarkable increase in that carbohydrates contents under hydrocarbon stress may be due to phyto-lignification in roots (Achuba, 2014).By application of AgNPs photosynthetic rate is increased that brings higher level of chlorophyll contents which leads to more carbon dioxide fixation. As a result, higher level of

carbohydrates is produced in plants which is evident from the result of present study (Mahakham et al., 2017; Kumar et al., 2020).

Plant development, growth and productivity are seriously affected by different environmental stresses (both abiotic and biotic). These stresses frequently disrupt ion distribution and homeostasis in plant cell causing osmotic stress and increase the formation of reactive oxygen species. These reactive species are toxic for plant metabolism which leads to degradation of different biological molecule which ultimately leads to the cell death. The ability of the plants to scavenge the harmful effects of reactive oxygen species appears to be most critical factor for their stress tolerance. Antioxidant enzymes play a key role in assisting growth and development of plant by using different mechanism (Rajput et al.,2021). There are two types of antioxidant enzymes i- phenol synthesizing enzymes (PAL) ii- phenol oxidizing enzymes (PPO and POX).PAL is a phenol synthesizing enzyme. It catalyzes the deamination of phenylalanine to generate cinamic acid which is then converted into phenylpropanoids, such lignins, coumarins and flavonoids (Zhang et al., 2017). Under abiotic stress, specific activity of PAL is the key which enhance the antioxidant protection. This key factor promotes phenolic metabolism which leads to the production of lignin precursors and antioxidant phenolics (Kong, 2015; Zhang and Sun, 2021) PPO and POX (peroxidase) are correlated because both are phenol oxidizing enzyme and are involved in the formation of phenol containing cell components like lignin. Both have the ability to catalyze the oxidation of phenol to quinone (Sabarre and Yagonia-Lobarbio, 2021). Quinones are responsible for the decrease of hazardous ROS and block the entry of hydrocarbon into cellular compartments (Ahammed et al., 2013; Zhang and Sun, 2021).

Exposure of kerosene oil on growing plants, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents increases as a result oxidative loss occur. These are harmful cellular metabolites as well as ROS (Reactive Oxygen Species). These ROS crosses the membranes easily due to having larger pKa and have potential to damage the subcellular structure and different cellular membranes (Rajput et al.,2021; Achuba, 2014). To overcome this stress, plants have natural defense mechanism (antioxidant enzymes). Under such stress conditions in plant, antioxidant enzymes are activated which are involved in the degradation of H_2O_2 into H_2O (Sachdev et al., 2021). Increase in the specific activity of PAL and PPO is due to elevated level of ROS while decline in the specific activity of POX was recorded in conducted experiments.By application of AgNPs, decreases the production of hydrogen peroxide and malonaldehyde levels which causing enhance the redox reaction and which ultimately lead to increase the specific

activity of antioxidant enzymes because silver nanoparticles reduce the oxidative stress as well as low malonaldehyde levels are beneficial for maintaining the function and structure of plasma membrane (Thuesombat et al., 2016; Wahid et al., 2020). In this current investigations, increase the specific activity of antioxidant enzymes with the application of AgNPs was recorded by stabilizing the antioxidant enzyme under stress conditions.

Nitrogen is key component of many biomolecules including amino acid, protein and nucleic acid. Plants cannot directly absorb nitrogen, so it must be converted into nitric oxides. Nitrate reductase is one of the most important enzyme in plants which is involved in nitric oxide production (Singh et al., 2019). Nitrate reductase is responsible for the conversion of nitrate into nitrite to assists in nitrogen assimilation. Nitrate reductase play an important role in the formation of amino acid. Nitrate reductase is an enzyme that participates in two electron transfer process in all Sulphur, carbon and nitrogen cycles and has molybdenum as a cofactor (Chamizo-Ampudia et al., 2017; Singh et al., 2019). Kerosene oil causes hydrocarbon toxicity. Results revealed that due to hydrophobic attributes of kerosene oil decreases the availability of nitrogen in the soil which leads to significant reduction in the specific activity of nitrate reductase (Achuba and Erhijivwo, 2017). Inhibition of nitrate reductase may be due to replacement of molybdenum as the ligand of hydrocarbon.By application of AgNPs enhance the uptake of nutrients and minerals such as nitrogen and molybdenum which are essential for the formation of nitrate reductase (Wahid et al., 2020; Manickavasagam et al., 2019). Because nanoparticles activate the specific genes which are involve in nitrogen metabolism and nutrition storage have been extensively upregulated (Babajani et al., 2019; Kumar et al., 2020). In this current study, enhance the specific activity of nitrate reductase by upregulation of genes with the applications of silver nanoparticles was recorded.

CONCLUSION

Kerosene oil has been proved to have harmful impact on yield contributing attributes i.e. root length, root diameter and root weight which ultimately reduces the final yield of carrot.Successfully established correlation between biochemical characters and kerosene oil stress in carrot. Application of AgNPs has been proved in increasing the yield contributing attributes in carrot that ultimately leads to increase final yield of crop.

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