

Antioxidant Enzyme Responses and Metabolite Functioning of *Pisum Sativum* L. to Sewage Sludge in arid and Semi-arid Environments

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Abstract

The productivity of plants is a direct variant of the countless biotic and abiotic stresses to which a plant is exposed in an environment. This study aimed to investigate the capabilities of leguminous plant garden pea (*Pisum sativum* L.) to resist water deficit conditions in arid and semi-arid areas when applied with varied doses of sludge for growth response. The effect of sludge doses was evaluated on crop yield, antioxidant enzymes viz., ascorbate peroxidase APX, dehydroascorbate reductase (DHAR), superoxide dismutase (SOD), glutathione reductase (GR) and metabolites (ascorbic acid, glutathione and total protein content). The effective sludge concentrations with respect to seed weight and crop yield were found to be in the following trend: D_2 (6.25%) > D_3 (12.5%) > D_1 (2.5%) > D_0 (control) under organic amendment (OA). Conversely, a high dose of the sludge reduced the seed weight and total crop yield. The sludge doses D_2 under arid and semi-arid conditions along with organic amendments (OA) significantly enhance the antioxidant enzyme activity whereas, sludge dose D_3 with OA ominously regulates the activity of these enzymes. Besides, seeds depicted a considerable increase in ascorbic acid, glutathione and total protein content in arid and semi-arid conditions upon the application of sludge with OA. Sewage sludge as a source of nutrients indirectly enhances crop yield, antioxidant enzymes and antioxidant metabolites. Thus, it improves the defence mechanism, reduces abnormal protein glycation, and depletes susceptibility of protein to proteolysis.

1. Introduction

Most of the plants are prone to adapt to biotic and abiotic stresses, causing prominent variation in their productivity. These plants modify their internal and external factors to cope up with environmental stresses (Rouached et al., 2015). Their adaptive strategies determine their stress tolerance capability (Costa et al., 2011), and consequently survival in these conditions (Kramer and Boyer, 1995). Plants growing in stressful environments have the potential to adapt by way of changing their morphological, molecular and biochemical characteristics (Jeuffroy et al., 2012; Khaleghi et al. 2019). The anti-oxidative protection components in plants destruct reactive oxygen species (Pau and Lawson, 2002). The stability stuck between manufacture and reduction of reactive oxygen species could be troubled by numerous factors which rapidly enhance the production of reactive oxygen species within the cells (Pau and Lawson, 2002; Delfini et al., 2010) and may be responsible for the destruction of metabolites. Plants enhance the production of antioxidants to neutralize the oxidative stress effects within the cells (Lawson and Smith, 2002; Meyer and Hell, 2005; Colville and Kranner, 2010; Delfini et al., 2010).

Among the crop plants, leguminous plants are very sensitive to environmental stresses (Voisin and Gastal, 2015), prominently water shortages. The alteration in molecular and as well as in biochemical characteristics in legumes could be vital for growing these kinds of plants in any stressful environments as they activate the mechanism of stress tolerance by way of enhancing the production of antioxidants (Guilioni et al., 2003; Lejeune-Hénaut et al., 2008). Therefore, the changes both intrinsic and extrinsic adapted by these plants under stressful environments are pivotal for their survival and productivity.

Considering the problems associated with a shortage of water in many regions of the world the present research proposal shall be based on the assumption that leguminous plants having a high potential to resist water deficit environments can be grown in arid and semi-arid conditions with varied doses of sludge for growth-response. It is also assumed that at different stress doses, plants shall respond efficiently at a certain concentration with respect to the physiological characteristics, antioxidant molecules and metabolites.

Antioxidants significantly decrease the concentration of reactive oxygen species in plants and protect from the damages caused due to oxidative stress (Matamoros et al., 2010). Among the crop plants, leguminous plants are a good source of proteins and other important metabolites and have been placed on top of the economical scale (Graham and Vance, 2003). Numerous research studies confirmed that the antioxidants play a crucial role to protect internal tissues of crop plants and have enough capabilities to diminish the likely impacts of reactive oxygen species, thereby take part in tolerance from any kind of environmental stresses (Mittler et al., 2004; Van Breusegem et al., 2008). Due to oxidative stress fruit-bearing plants reduce the crop yield due to low shelf life which is associated with low production of antioxidants (Davey and Keulemans, 2004; Malacrida et al., 2006; Halliwell and Gutteridge, 2007; Stevens et al., 2008). Antioxidants and antioxidant enzymes particularly (superoxide dismutases, catalases, peroxiredoxins and glutathione peroxidases) amend the concentrations of reactive oxygen species and neutralize their toxic effects in plant cells (Dietz, 2003; Matamoros et al., 2003; Mittler et al., 2004; Navrot et al., 2006). The water-soluble antioxidants especially glutathione and ascorbate create redox buffers in cells of plants and provide stress responses (Bouvier et al., 1998; Arrigoni and De Tullio, 2002; Noctor et al., 2002) and growth (Matamoros et al., 2003; Palma et al., 2006; Hicks et al., 2007). Various research studies have been done globally on different crop plants viz., *Pyrus communis*, *Lycopersicon esculentum* and *Amelanchier alnifolia* and reported that the antioxidant and antioxidant enzymes are very crucial for fruit development, maturation and ripening under any stress (Jme 'nez et al., 2002a; Jime 'nez et al., 2002b; Reddy et al., 2004; Gill and Tuteja, 2010). Furthermore, fruit tissues are protected by antioxidants from reactive oxygen species and thereby resist against any kind of environmental disturbance (Mittler et al., 2004; Van Breusegem et al., 2008; Davey and Keulemans, 2004; Malacrida et al., 2006; Stevens et al., 2008). The use of seed inoculation and RDF with plant growth-promoting rhizobacteria (PGPR) increase the yield of leguminous crops (Mishra et al., 2010).

Water accessibility is the most vital factor for plant growth and crop productivity as well as an important factor for determining the species distribution in varied climatic zones around the world. Considering the problems associated with a shortage of water in many regions of the world the present research proposal shall be based on the assumption that leguminous plants having a high potential to resist water deficit environments can be grown in arid and semi-arid conditions with varied doses of sludge for growth-response. It is also assumed that at different stress doses, plants shall respond efficiently at certain concentrations with respect to the physiological characteristics, antioxidant molecules and metabolites.

2. Materials And Methods

Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications for each environmental condition (arid and semi-arid) to evaluate different sewage sludge treatments. Besides, an experimental unit with respect to environmental condition was further divided into two blocks *viz.*, organic amendment (OA) and without OA. Each experimental unit consisted of 9 plants, planted in three columns and two rows with a spacing of 50 cm × 25 cm (size of experimental unit 1.50 × 1.25 m²). Four-week old pea seedlings were transplanted on the fourth week of April with proper care.

Sludge dose

D₀ (Control); without sludge treatment

D₁ (2.5% sludge); 100g of pure sludge + 4Kg of soil

D₂ (6.25% sludge); 250g sludge + 4Kg of soil

D₃ (12.5% sludge); 0.5 Kg sludge + 4Kg of soil

The nutrient concentration of sewage sludge are presented in Fig. 1

Collection of seed samples

The seed samples were collected from randomly selected plants from each treatment of every replication for analysis.

Preparation of samples

After collecting fresh seed samples, they were washed thoroughly with tap water then dipped in dilute HCl and further washed with single and double-distilled water. The moisture was whipped with filter paper and muslin cloth. Treatment wise samples from each replication were then analyzed for quality parameters *viz.*, antioxidant enzymes and essential metabolites.

Antioxidant enzymes

Total superoxide dismutase (SOD) concentration was analyzed on the spectrophotometric method adopted by Rubio et al. 2002. About 40µl of enzyme extract was transferred in test tubes to which 50 mM phosphate buffer (pH 7.8), 55 µM NBT, 9.9 mM L-methionine, 2 mM EDTA and 0.02% Triton X-100 was added. To this reaction, the mixture riboflavin was added lastly in complete dark condition. The activity of

SOD depends upon its ability to decrease the photochemical reduction of nitro blue tetrazolium. SOD activity was calculated by reading the OD at 560 nm for 2 min at 25°C.

Ascorbate peroxidase (APX)

The plant sample tissue was extracted in 20 mM potassium phosphate (pH 7.4). The mixture was homogenized with Polytron, incubated on ice for 20 min, and vortexed for every 2-min interval. Next, the mixture was centrifuged at 15,000 × g at 4°C for 15 min. The resultant supernatant was collected and dialyzed before enzyme assay. The final reaction mixture consists of 1.0 ml that is comprised of 20 mM of potassium phosphate buffer (pH 7.0) and 2.5 mM ascorbic acid. The 10 µl of enzyme extract is added to initiate the reaction. Due to ascorbate oxidation, the decrease in absorbance is monitored for 3 min at an absorbance rate of 265 nm and calculated by using extinction coefficient, 14 mM⁻¹ cm⁻¹

Glutathione reductase

The procedure for determining the concentration of glutathione reductase in plant tissues was analyzed by the oxidation of NADH and NADPH (Schaedle and Bassham, 1977). The enzyme extract was prepared prior to enzyme assay. 200 mg of fresh samples were homogenized by mortar and pestle in 5 ml of 50 mM Tris-HCl buffer at pH 7.6. The resultant supernatant was collected after being centrifuged at 22,000 × g for 4 min and dialyzed prior to enzyme assay.

The final reaction mixture (1 ml) was composed of 200 µl enzyme extract, 50 mM Tris-HCl buffer (pH 7.6), 1 mM glutathione disulfide (GSSG), 0.15 mM NADPH, and 3 mM MgCl₂. A decrease in NADPH absorbance was observed at 340 nm. The specific activity of the enzyme is expressed as a unit per milligram of protein.

Antioxidant metabolites

Ascorbic acid estimated from fresh green seeds using 2, 6-dichlorophenolindophenol dye and expressed as per the procedure as outlined by (AOAC, 1995; Nielsen, 1998). Glutathione was measured as per the method (Matamoros et al., 1999) using HPLC fluorescence detection. The reduced and oxidized concentrations of glutathione were measured spectrophotometrically by utilizing enzymatic cycling (Griffith, 1980). Proteins were determined by the extraction method (Loscos, 2008).

Statistical analysis

Data for calculating the sludge and organic amendment effects were evaluated with one-way ANOVA followed by a significance test using Tukey's posthoc test. The level of significance was set $p \leq 0.05$ for

all the evaluating plant attributes. All other table, graphics and calculations were created using Microsoft Excel 2010. Statistical analysis was performed using GraphPad Prism Version 8.01 (SanDiego USA).

3. Results

Physiological characteristics

Seed weight

The recorded observations on seed weight exhibited different values with respect to sludge concentration. Moreover, environmental conditions (arid and semi-arid) and organic amendments significantly influence the seed yield. Plants responded quite effectively and exhibited the highest seed weight (1.13 g FW) at sludge concentration D₂ (6.25 %) under organic amendment (OA). However, some reverse effects were also observed at very high sludge concentration D₃ (12.5%) in both environmental conditions and depicted the values with a range of 0.70–0.90 g FW in arid conditions and 1.00-1.12 g FW in the semi-arid environment (**Fig. 2**). Overall, the effective sludge concentrations with respect to seed weight were found to be in the following trend; D₂ (6.25%) > D₃ (12.5%) > D₁ (2.5%) > D₀ (control).

Crop yield

The results indicated that crop yield per plant showed different values with the supplementation of different sludge doses. Moreover, the data signposted that plants amended with sludge dose of D₂ (6.25%) under organic amendment (OA) exhibited higher yield per plant in both arid as well as semi-arid environs (Fig. 3). Among the sludge doses, D₂ proved to be a better concentration, while D₃ significantly declines the crop yield per plant in both conditions. Organic amendments (OA) more precisely influence crop production in both arid and semi-arid conditions. However, the effect was observed dominantly in the semi-arid environment (623.20 g) at D₂ (OA). Interestingly, a decrease of crop yield exhibited at the high dose of sludge (D₃), mostly in arid conditions (205 g FW) than semi-arid environment (299.10 g FW).

Antioxidant metabolites

Total Protein content

Total proteins content (TPC) fluctuated considerably in seeds but variability was found low at dose concentration D₁ and D₃ with or without OA in all conditions. However, high concentrations (23 ± 2.01 and 26.5 ± 2.4) accounts 70–80% increase in TPC in comparison to control (Table 1). The overall trend of TPC

in seeds follows the trend $D_3 > D_1 > D_2 > D_0$. Less pronounced capriciousness in seed TPC could be due to a low supply of essential nutrients (D_1).

Ascorbic acid

The effect of different sludge doses on ascorbic acid content (AAC) both in arid and semi-arid environs with or without OA depicted in Table 1. The findings revealed that sludge significantly raises the levels of AAC in seeds up to 104.43 ± 12.23 mg/100g at D_2 . However, the negative effect was noticed at a high concentration of sludge (D_3), which decreases the 30% AAC level with respect to D_2 .

Reduced glutathione (GSH)

Treatment of sludge at three different doses D_1 , D_2 , and D_3 with organic amendments (OA) under arid and semi-arid conditions. These doses of sludge showed a decrease in GSH content, however, D_2 was found more significant and effective in both arid and semi-arid conditions to modulate the GSH content (27.23 ± 4.3 and 30.23 ± 3.3), which accounts 70–80% increase in GSH content in comparison to control. The overall trend of GSH follows the trend $D_3 > D_1 > D_2 > D_0$.

Table 1
Interactive effects of sludge and organic amendment on ascorbic acid, glutathione, and total protein content of pea

Sludge Doses	Arid		Semi-Arid	
	Ascorbic acid (mg per 100g)			
	Without BFP	With BFP	Without BFP	Without BFP
D ₀	30.5 ± 5.4 ^a	33.8 ± 4.5 ^a	35.50 ± 4.3 ^a	44.32 ± 5.3 ^a
D ₁	65.87 ± 6.6 ^b	69.76 ± 7.5 ^b	68.76 ± 7.1 ^b	74.56 ± 5.5 ^b
D ₂	90.82 ± 7.9 ^c	99.73 ± 10.2 ^c	97.65 ± 9.1 ^c	104.43 ± 12.23 ^c
D ₃	62.6 ± 5.7 ^b	64.71 ± 7.01 ^b	68.34 ± 7.2 ^b	73.54 ± 5.4 ^b
Glutathione (µmoles/mg protein)				
D ₀	9.3 ± 2.4 ^a	8.05 ± 2.3 ^a	7.82 ± 0.89 ^a	7.98 ± 2.1 ^a
D ₁	15.2 ± 3.2 ^a	16.62 ± 1.3 ^b	14.32 ± 2.3 ^b	19.23 ± 2.3 ^b
D ₂	26.83 ± 3.8 ^b	28.21 ± 2.3 ^c	27.23 ± 4.3 ^c	30.23 ± 3.3 ^c
D ₃	19.3 ± 2.8 ^a	20.87 ± 3.2 ^b	22.12 ± 2.2 ^b	22.11 ± 1.98 ^b
Total Protein content (mg/mg tissue)				
D ₀	5 ± 0.98 ^a	5.8 ± 1.1 ^a	6.2 ± 1.2 ^a	6.5 ± 0.88 ^a
D ₁	10 ± 2.1 ^b	13 ± 1.7 ^b	15 ± 2.1 ^b	18.7 ± 1.3 ^b
D ₂	20 ± 2.6 ^c	23 ± 2.01 ^c	22 ± 2.8 ^c	26.5 ± 2.4 ^c
D ₃	13 ± 1.3 ^b	12 ± 1.6 ^b	16 ± 1.8 ^b	18.5 ± 2.3 ^b

Data represent mean ± SD (n = 03). The alphabetical letters are significantly different within each group at p ≤ 0.05 using Tukey's posthoc test.

Antioxidant enzymes

Ascorbate peroxidase (APX)

The current study revealed that environmental stress affects the enzyme activity in garden pea (*Pisum sativum* L.). The sludge doses *viz.*, D₁, D₂ and D₃ under arid and semi-arid conditions along with organic amendments (OA) significantly enhance the ascorbate peroxidase (APX) activity. Among the sludge

concentrations, D₂ proved to be the best effective dose to exhibit high value (36.7 ± 9.8) in semi-arid conditions with OA and lowest (19.5 ± 2.2) in arid environment (Table 2).

Dehydroascorbate reductase (DHAR)

Whereas treatment of sludge with OA under arid and semi-arid conditions, ominously regulates the activity of dehydroascorbate reductase (DHAR). However, D₂ showed significantly higher enhancement of DR (1997 ± 12.7) under semi-arid conditions with OA.

Superoxide dismutase (SOD)

Pisum sativum showed a considerable increase in the activity of SOD under the joint influence of sludge and OA, more in a semi-arid environment. However, the influence of D₂ dose (with OA) showed the highest increase SOD activity (156.81 ± 18.9) under semi-arid condition.

Glutathione reductase (GR)

The sludge concentration exposure in *Pisum sativum* showed a significant increase in the activity of GR enzyme. The D₂ dose showed significantly higher restoration under semi-arid conditions (29.87 ± 6.2).

Table 2

Interactive effects of sludge and organic amendment on ascorbate peroxidase, dehydroascorbate reductase, superoxide dismutase and glutathione reductase of pea

Sludge Doses	Arid		Semi-Arid	
	Without BFP	With BFP	Without BFP	Without BFP
Ascorbate peroxidase APX activity (nmol AsA⁻s⁻¹g⁻¹FW)				
D ₀	19.5 ± 2.2 ^a	21.4 ± 1.9 ^a	15.7 ± 2.9 ^a	21.8 ± 3.01 ^a
D ₁	25.8 ± 3.4 ^b	28.8 ± 4.2 ^b	26.7 ± 4.8 ^b	27.8 ± 6.6 ^b
D ₂	35.7 ± 7.3 ^c	36.8 ± 8.9 ^c	34.5 ± 7.7 ^c	36.7 ± 9.8 ^c
D ₃	23.7 ± 6.8 ^b	26.7 ± 8.1 ^a	22.3 ± 6.8 ^b	24.9 ± 8.7 ^b
Dehydroascorbate reductase (DHAR) activity (nmol AsA⁻s⁻¹g⁻¹FW)				
D ₀	556 ± 12.3 ^a	565 ± 12.3 ^a	562 ± 9.3 ^a	604 ± 12.4 ^a
D ₁	765 ± 16.5 ^b	965 ± 11.23 ^b	1100 ± 11.2 ^b	970 ± 10.2 ^b
D ₂	1450 ± 45.5 ^d	1654 ± 23.2 ^d	1876 ± 21.2 ^d	1997 ± 12.7 ^d
D ₃	1010 ± 14.4 ^c	1342 ± 20.1 ^c	1545 ± 12.3 ^c	1454 ± 23.7 ^c
Superoxide dismutase (SOD) activity (Units/min/mg of protein)				
D ₀	89.87 ± 9.8 ^a	90.67 ± 10.2 ^a	97.98 ± 10.6 ^a	112.78 ± 8.7 ^a
D ₁	102.66 ± 12.4 ^b	126.45 ± 11.7 ^b	120.34 ± 13.6 ^b	140.65 ± 16.7 ^b
D ₂	134.56 ± 16.7 ^c	137.12 ± 15.7 ^c	145.54 ± 16.7 ^c	156.81 ± 18.9 ^c
D ₃	90.88 ± 9.2 ^a	115.12 ± 8.8 ^b	115.61 ± 9.9 ^b	137.98 ± 8.9 ^b
Glutathione reductase (GR) (Units/min/mg protein)				
D ₀	16.34 ± 2.4 ^a	14.12 ± 1.3 ^a	14.54 ± 3.2 ^a	12.76 ± 1.8 ^a
D ₁	18.23 ± 3.9 ^a	19.29 ± 2.4 ^b	18.55 ± 4.3 ^b	19.76 ± 2.8 ^b
D ₂	25.34 ± 5.7 ^b	27.01 ± 5.1 ^c	24.56 ± 5.7 ^c	29.87 ± 6.2 ^c
D ₃	17.23 ± 2.9 ^a	21.12 ± 2.7 ^b	21.65 ± 3.2 ^b	21.76 ± 2.1 ^b

Data represent mean ± SD. The alphabetical letters are significantly different within each group at $p \leq 0.05$ using Tukey's posthoc test.

4. Discussion

Physiological characteristics

Nevertheless, garden pea (*Pisum sativum* L.) is an imperative leguminous crop and significantly enriching the soil nutrient environment by fixing atmospheric N₂ (Soumare et al., 2020). However, arid and semi-arid conditions predominantly oppose plant production due to the lack of essential nutrients. The prudent supplementation of varied sewage sludge doses and organic amendments (OA) effectively change the scenario of pea production in arid and semi-arid environs. High nutrient and water supply in arid and semi-arid conditions are very important for the overall development of plants. Subsequently, sludge doses add an adequate amount of nutrients to garden pea and enhance the vegetable yield with high nutritional value. Plants require a constant supply of nutrients (Reyes et al., 2008; Mukherjee, 2014) as their availability determines the crop yields and quality by directly affecting photosynthesis, accumulation, transfer, and distribution of biomass (Zou et al., 2015; Lorenzoni et al. (2016)). Pertinently, at some stage of sludge concentration along with OA, the plant responds proportionally with respect to seed weight and overall crop yield (Choi, 2020). Conversely, a high dose of sludge decreases the seed weight and total crop yield. High nutrient supply supports enormous stem length and decreases the root length (Razaq et al., 2017; Balawejder et al., 2020). Besides, it decreases the pH of the soil to more acidic (Bloom et al., 2006)

Antioxidant metabolites

Total Protein content

Moreover, high sludge concentration (D₃) in all environmental conditions causes' toxicity and suppress plant growth. Nutrient supply enhances soil physical environment and pertinently enhance the plant metabolites than un-amendment ones (Dheeba et al. 2015). The reduction in the nutrient content may be due to the inhibition of enzymes involved in protein synthesis (Balashouri and Devi 1994).

Ascorbic acid

Significantly higher levels of ascorbic acid content in garden pea could be due to increased accumulation of carbohydrates and conversion of more organic acids into sugars with sludge doses. Similar findings were reported by (Aminifard et al., 2012; Dhotre et al. (2018)).

Reduced glutathione (GSH)

High sludge concentrations D₃ causes toxicity and under all environmental conditions. Less prominent capriciousness in seed GSH might be due to less supplementation of important nutrients and toxicity suppression due to high sludge concentrations D₃ under all environmental conditions. Glutathione itself a vital non-enzymatic antioxidant, it keeps other antioxidant components active and improves their activities (Hasanuzzaman et al. 2017). Nutrient doses (sludge) increased glutathione concentration in fruits (Gutiérrez-Gamboa et al., 2017).

Antioxidant enzymes

Ascorbate peroxidase (APX)

The activity of APX gets modulated by various environmental factors including OA. Sludge doses supply essential nutrients and cause an alteration in nutrients uptake. The present study coincides with earlier findings of (Han et al., 2009; Maheshwari and Dubey, 2009), who reported that APX activity increases with abiotic stresses.

Dehydroascorbate reductase (DHAR)

This up-regulation of DHAR activity in *Pisum sativum* might be due to the suppression of the production of free radicals under the influence of sludge and OA (Fig. 4). This increase in DHAR activity in *Pisum sativum* might be due to the constant up-regulation of the DHAR encoding genes with sludge dose in OA, which may increase the tolerance in *Pisum sativum* against the environmental stress. DHAR plays an important role in suppressing the oxidant (H₂O₂) and its expression is activated by several abiotic stress factors (Ali et al., 2005; Lu et al., 2008; Fan et al., 2014). Additionally, DHAR plays an important role in plant growth and development (Chen and Gallie, 2006). The lack of DHAR can swiftly decrease the ascorbic acid content and led to a slower rate of leaf expansion consequently affecting plant growth and development (Ye et al., 2000).

Superoxide dismutase (SOD)

This increase in the SOD activity might be the nutritive potency of sludge metallic minerals and nitrogen as a source for amino acid biosynthesis and the production of proteins. Sludge treatment containing various metallic ions increased SOD activity (Devi and Prasad, 2005; Yadav, 2010).

Glutathione reductase (GR)

This up-regulation in GR activity might be due to the scavenging of ROS because sludge contains various nutrients especially nitrogen components which is important for the synthesis of proteins/enzymes,

whereas D₃ showed an abnormally decline of GR due to toxicity of high sludge. Modulation in the expression profile of various GR isoforms have been known to occur under various stress conditions (Yousuf et al., 2012; Gill et al., 2013).

Conclusion

Based on the findings, sludge treatment in association with organic amendments (OA) contributes to conferring resistance and adaptation of garden pea (*Pisum sativum L.*) to reduced water stress and has the potential role in solving the effects of environmental inflections. Moreover, sludge doses given to the garden pea acts as a source of nutrients, which enhances crop yield, antioxidant enzymes (SOD, CAT, APX & GR) and antioxidant metabolites (GSH) in arid and semi-arid conditions. Besides, it increases the reductive powers such as NADPH of plant cells to neutralize the free radicals and also suppress the ROS down-regulatory pathways. Thereby, it improves the defence mechanism, reduces abnormal protein glycation, and diminishes programmed cell death in arid and semi-arid environs. Consequently, optimum sludge treatment (6.25%) in association with OA showed a significant reduction in protein or lipid peroxidation as well as inhibits the generation of free radicals. Therefore, the primary conclusion is that the sludge treatment (6.25%) can work as an adequate source of nutrients and may support the cultivation program of this essential crop to increase productivity, especially under arid and semi-arid conditions.

Declarations

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Contributions

Khalid Rehman Hakeem and Rouf Ahmad Bhat have designed and performed the experimentation. They have also written the original manuscript and analyzed the data. Hesham F. Alharby and Khalid M. Al-Ghamdi have analyzed the data, interpreted the results, and critically edited the manuscript.

Ethical approval

Not applicable.

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Consent to participate

All authors confirm consent to participate for this journal.

Consent for publication

All authors accept to publishing.

Conflict of interest

The authors declare no competing interests.

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Figures

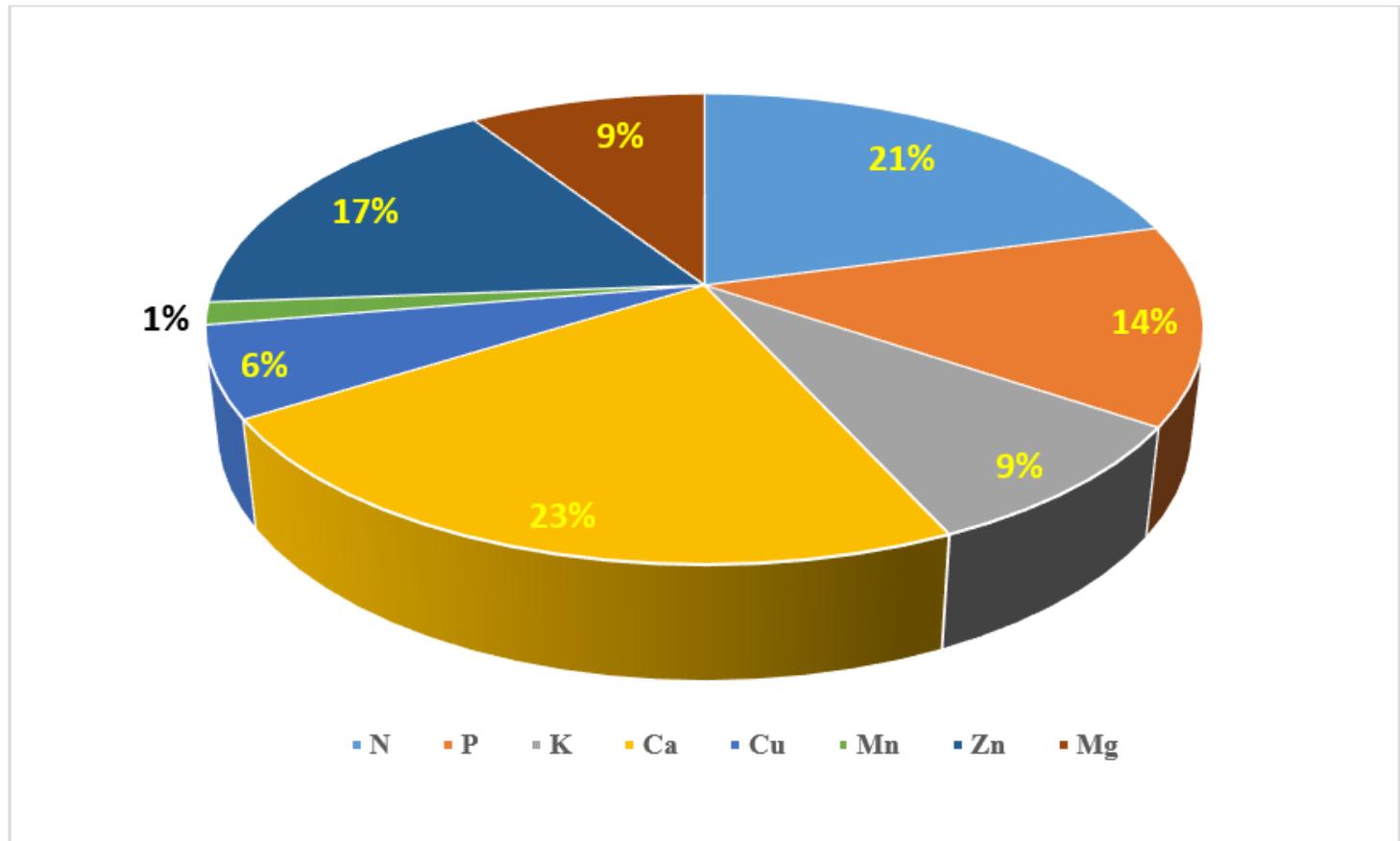


Figure 1

Nutrient characteristics of sewage sludge

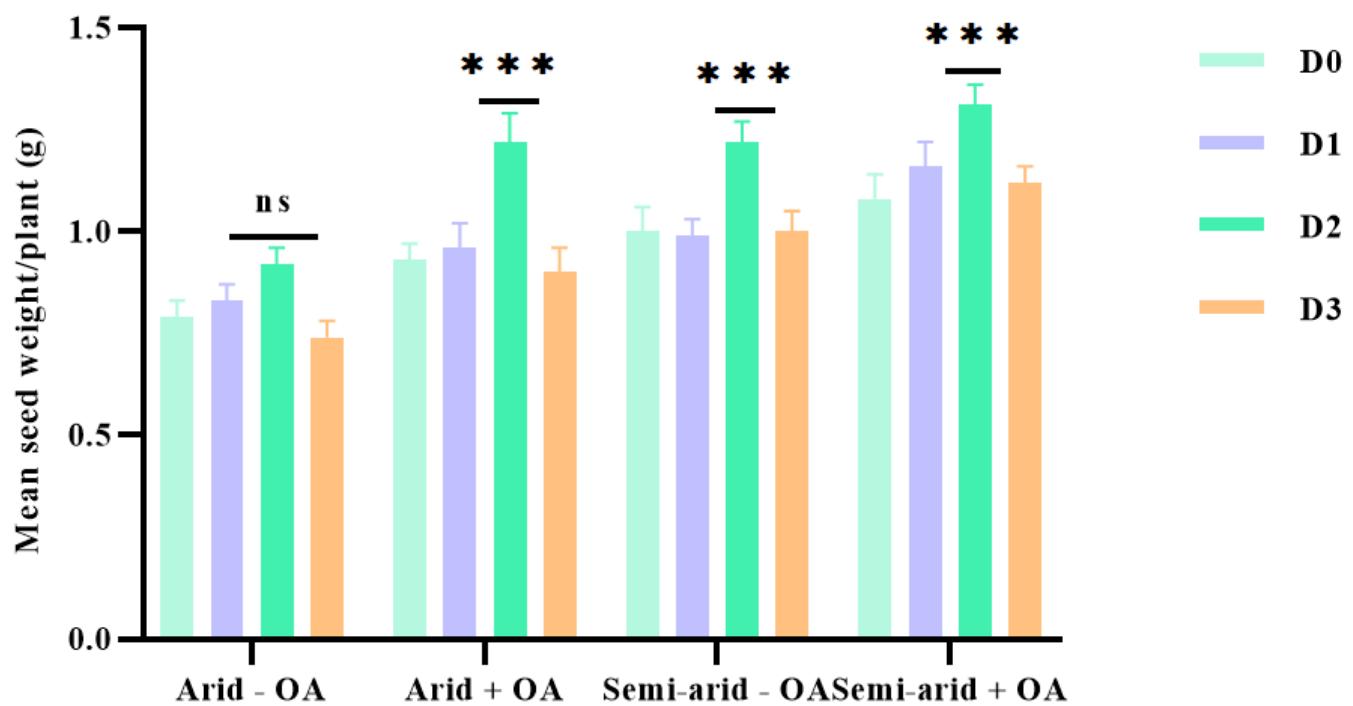


Figure 2

Effects of various concentrations of sludge on the mean seed yield/plant under arid and semi-arid conditions. ns- Non-significant; and ***P < 0.001.

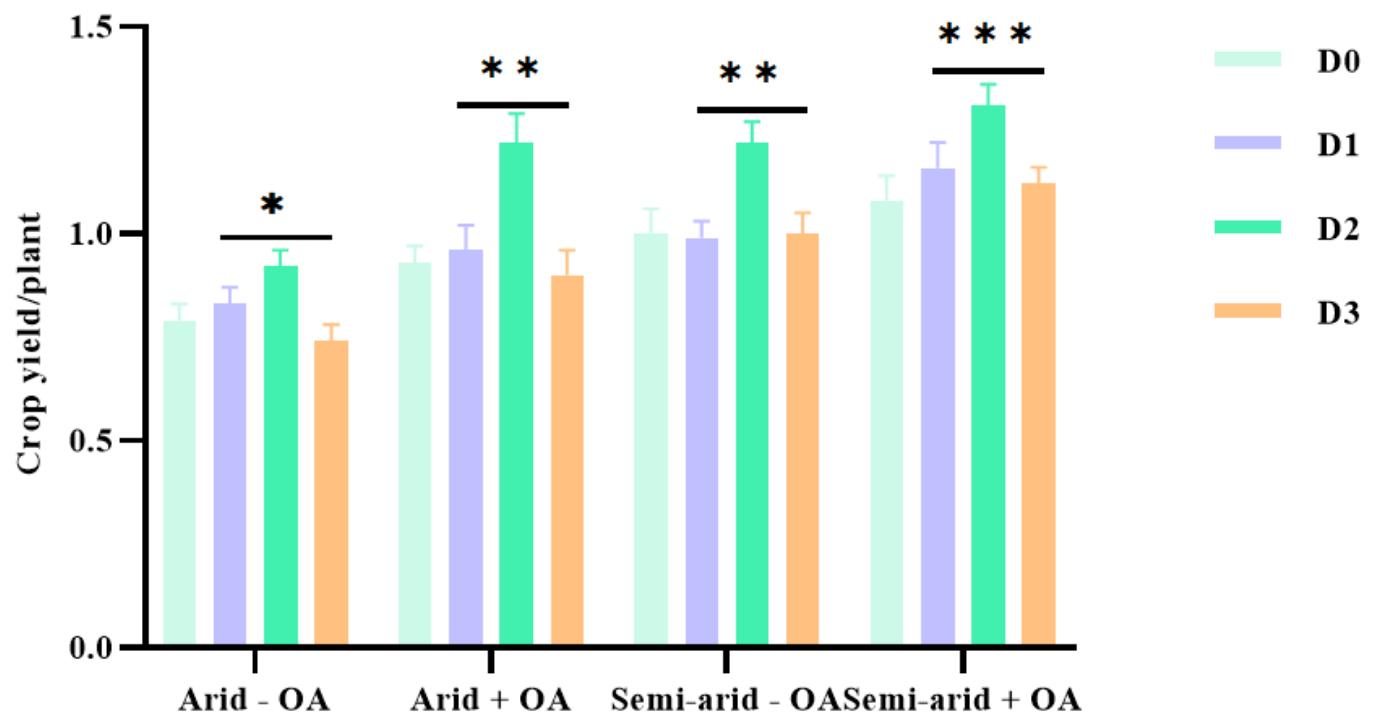


Figure 3

Effects of various concentrations of sludge on the crop yield/plant under arid and semi-arid conditions.
ns- Non-significant; *P < 0.05; **P < 0.01 and ***P < 0.001.

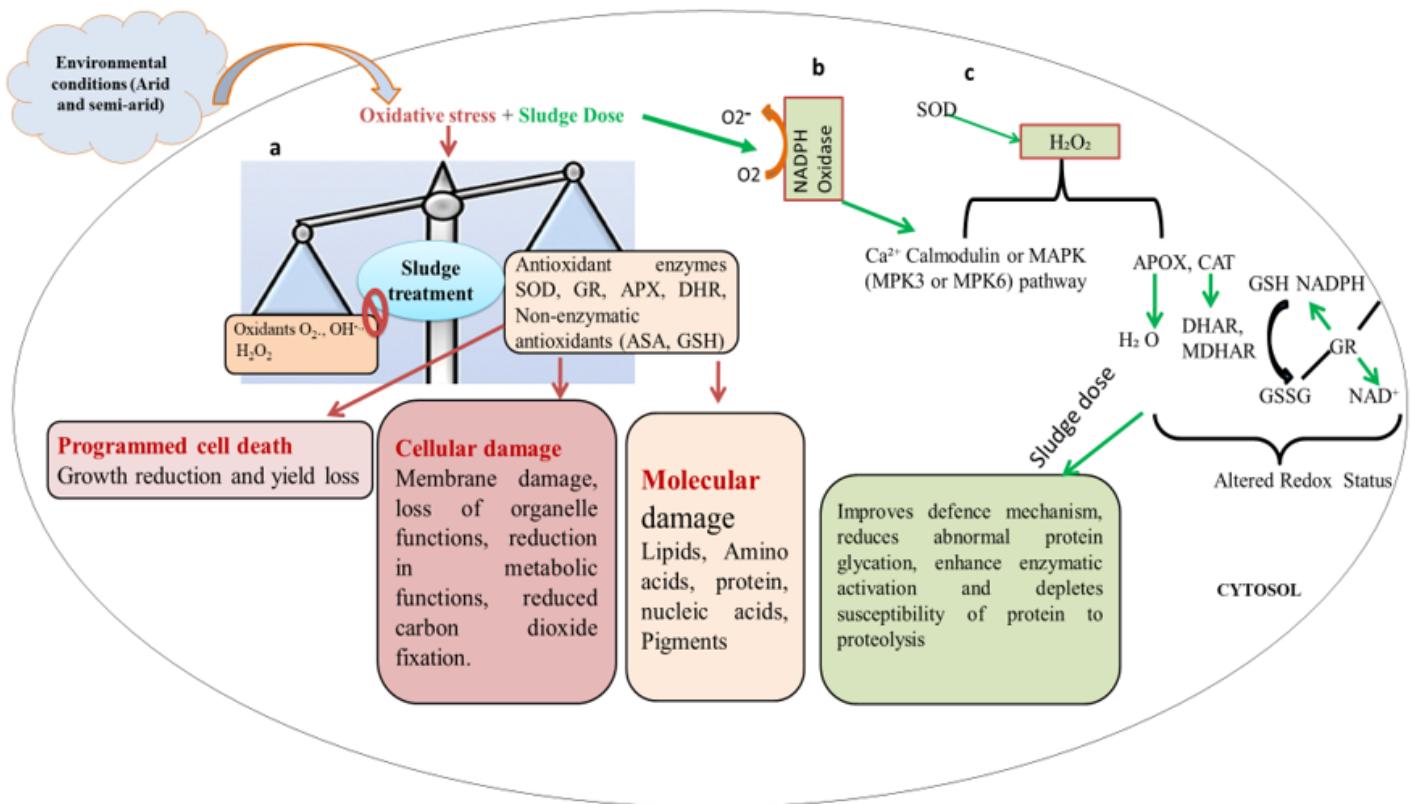


Figure 4

Environmental conditions induce modulation in various physiological systems including the defense system. Oxidative stress is one of the conditions induced due to the changed environment, a. shows the oxidative stress leads to the modulations in the defence systems (enzymatic as well as non-enzymatic) which decreases the efficiency of antioxidative enzymatic and non-enzymatic systems and leads to the molecular, cellular and programmed cells deaths. However, these alterations can be combated by supplying the nutrients such as sludge (nutrients rich) doses to the *Pisum sativum*. Sludge dose supplies nutrients to reactive the antioxidative enzymes, metabolites and also non-enzymatic ROS scavenging biomolecules (such as GSH) b. Sludge dose can also increase the reductive powers such as NADPH of plant cells to neutralize the free radicals and also suppress the ROS down-regulatory pathways (Ca^{2+} -Calmodulin or MAPK (MPK3 or MPK6)). Hence, can protect the plant cells against the oxidative stress induced damages c. Superoxide dismutase (SOD) is one of the primary antioxidative enzymes, which helps in the neutralization of free radicals, however, stress conditions inhibits the SOD activity. Sludge treatment as a source of nutrients can indirectly enhance the activity of SOD and can also up-regulate the other reductive power generating sources such as GSH. Thus, it improves the defence mechanism, reduces abnormal protein glycation, enhances enzymatic activation and depletes susceptibility of protein to proteolysis.