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Effect of UVC on allelopathic potential of lupine against metabolites and their biosynthetic enzymes of Portulacaolercea

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Abstract

The aim of this work is to study the influence of UVC on allelopathic potential of Lupinustermis L (lupine) for various time intervals against metabolites and their biosynthetic enzymes in Portulacaoleracea L.The results indicate that exposure of lupine seedlings to UVC resulted in the increase of total phenols and total flavonoids in lupine leaves. Treatment of P. oleracea seedlings with various irradiated and non-irradiated lupine leaf extracts at various concentrations (20, 40, 60, 80 and µg ml-1)resulted in reduction of total soluble carbohydrate and total soluble protein in a concentration-dependent manner. Also, the treatment enhanced H2O2 content, lipid peroxidation and the activity of NADH-oxidase (EC: 1.7. 99. 4) in P. olercaealeaves. However, the activities of ribulose 1,5-bisphosphate carboxylase (EC: 4.1. 1.39), glyceraldehyde 3-phosphate dehydrogenase (EC: 1.1. 1.8), nitrate reductase (EC: 1.7. 99. 4) and nitrite reductase (EC: 1.7.1.4) were reduced under the same treatment. The impact of allelopathic effect of irradiated lupine leaf extract was more pronounced compared to non-irradiated.

Keywords:

UVC; Allelopathy; Lupinus; Portufaly; Enzymes

Ozone depletion is now observed over large parts of the land, permitting increased penetration of solar UV radiation to the earth's surface. Much of reports indicate that increased UV affects the balance of competition among higher plants Mohamood et al. (2013). One of the important adaptive mechanisms to enhanced UV irradiation is the increase production of secondary metabolites in leaf tissues under UV irradiation (Li et al., 2009). It has been reported that most secondary metabolites such as phenolics and flavonoids are potentially allelopathic compounds (El-Shora et al., 2022), which are accumulated in epidermal layer for absorbing UV and shield the underlying tissues against harmful UV-radiation (Li et al., 2009).

Portulacaoleracea L. (Purslane) is a C3 plant and a common troublesome weed worldwide. This plant is used as herbal plant with wide pharmacological applications including, analgesic, antibiotic and antioxidant and anti-inflammatory (Gürsoyet al., 2013). Lupinustermis (lupine) is grown in Egypt and it is used as fodder crop (El-Shora and Abd El-Gawad, 2014). Lupine seeds contain great content of proteins, fibers

and carbohydrates and they are used for medical and industrial purposes (Abdel-Monaim et al., 2012).

The effects of plant compounds on neighbor plant physiology and processes are called allelopathy. In fact, allelopathy is a type of biotic stress that plays an important regulatory role in agro-ecosystems (Lara-Núñezet al., 2009).,(Merinoet al., 2018).Allelopathy of crops is considered as one of the most successful tool to manage weed infestations in 5agricultural production, if it can be exploited appropriately in a rotational cropping system (El-Shora and Abd El-Gawad, 2014).

Application of chemical herbicides is associated with environmental contamination and emerging of herbicide-resistant weeds (Gao et al., 2018). Thus, the use of allelochemicals is being encouraged to utilize this up tapped resource for weed control thereby reducing the ecological, environmental and health problems associated with synthetic pesticides (Cheema et al., 2013).

Allelochemicals enter to natural environment or agricultural systems in various ways, including evaporation of leaf volatiles, leaching of non-volatiles from leaf by rain, secretion from roots, and decomposition of dead plant parts by soil microorganisms (Wang et al., 2022). Allelochemical compounds are capable of changing physiological and biochemical processes, including water consumption and use, mineral uptake, leaf development, photosynthesis, amino acid metabolism, protein synthesis, glycolysis, mitochondrial respiration, and ATP synthesis (Mushtaq et al., 2020). Therefore, new approaches to weed control using plant-released compounds (allelochemicals) have expanded significantly due to their short half-life, high biodegradability, safety compared to synthetic compounds, and less damage to environment (El-Shora et al., 2022).

The allelopathic activity of lupine is poorly investigated up to now (El-Shora et al., 2022). Therefore, the present study aimed to evaluate the influence of UVC on allelopathic potential of lupine leaf on total soluble carbohydrates, total soluble proteins and their biosynthetic enzymes in P. oleracea leaves. In addition, the study aimed to evaluate the effect of UVC on allelopathic effect of lupine on hydrogen peroxide and lipid peroxidation as marker for allelochemical stress in P. oleracea leaves.

Materials and Methods

Experimental plants

The experimental seeds of bothLupinustermis L. and Portulacaoleracea were obtained from Egyptian Ministry of Agriculture.

Growth of Lupinustermis L.

The seeds of L. termis were surface sterilized in 10% sodium hypochlorite for 10 min and then soaked in running tap water for 24 h according to El-Shora and Abo-Kassem (2001). The seeds were then germinated between paper towels, moistened with distilled water for 7 days. The germinated seeds with well-grown roots were then supported on plastic bowls containing Hoagland′s solution and left to grow for 20-days. The pH of the nutrient solution was set to5.5, and it was replaced every 2 days. The system was kept in a growth chamber under the following conditions: at 22 ±1°C, 16 h light/8 h darks photoperiod, day/night temperature, 350 µmol m2s−1photonflux density and approximately 60%, relative humidity. Plants of 20-day-old from each con-centration, as well as control samples (treated with water) were used for analysis. The experimental design was carried out with three replications.

Treatment of lupine seedlings with UVC

Seedlings (20-day old) of Lupinustermis L. (lupine) were irradiated with UVC for various time intervals (20,40,60,80 and 100 min) according to El-Bediwi et al. (2021) using a system consists of fluorescent lamp (Type-C with λ from 2000-2800 Å), its power equal to 15 watt. Also, the system was covered totally with aluminum foil to illuminate the sample from all sides.

Preparation of the lupine leaf extracts

Leaves from 20-day old irradiated (100 min) and non-irradiated lupine seedlings were collected, washed, dried for 48 h in 50oC and ground to fine powder by grinder. Various concentrations (20, 40, 60, 80 and µg ml-1) of leaf extracts from irradiated and non-irradiated seedlings were prepared using distilled water. The control samples were carried out using distilled water.

Treatment of P. oleracea plants with lupine leaf extracts

The seeds of P. oleracea were surface sterilized in 10% sodium hypochlorite for 10 min and then soaked in

running tap water for 24 h according to El-Shora and Abo-Kassem (2001). The seeds were then germinated between paper towels, moistened with distilled water for 7 days. The germinated seeds with well-grown roots were then supported on plastic bowls containing.

Three groups of germinated Portulaca seeds with well-grown roots were then supported on plastic bowls containing Hoagland´s solution. The first group was control which was not treated with lupine extract. The second was treated with different concentrations (20, 40, 60, 80 and 100 µgml-1) of irradiated lupine leaf extract. The third group was treated with non-irradiated lupine leaf extract at the same concentrations. The three groups were vigorously aerated for 20 days under the same conditions described above for the growth of lupine.

Determination of total soluble carbohydrates in P. oleracea leaves

A sample of plant powder (300 mg) was mashed in a mortar, then extracted for 48 h in 20 mL of 80% (w/v) ethanol (Schortemeyer et al.1997). The extract was centrifuged and the supernatant was composed and evaporated. The residue was dissolved in 20 ml distilled water and utilized in estimation of total soluble carbohydrates. A sample of the obtained extract (0.2 ml) was combined with reagent of anthrone (7 mManthrone in 90% (w/v) H2SO4). The mix-ture was carefully heated for 15 min, cooled, and incubated for 30 min in an ice bath, with the absorbance measured at623 nm. The content of total soluble sugars was calculated from glucose calibration curve (Schlüter and Crawford, 2001).

Determination of total soluble protein in P. oleracea leaves

Determination of total soluble protein of P. oleracea leaves was carried out according to the method of Bradford (1976). In a test tube, a sample of leaf extract (50 µl) was made up to 100 µl using 150 mMNaCl. The solution was blended with one ml Bradford's reagent, and the absorbance was measured at 595 nm. The protein content was calculated using the standard curve of bovine serum albumin.

Determination of hydrogen peroxide content in P. oleracea leaves

Fresh leaves (500 mg) were homogenized in 10 mL phosphate buffer (50 mM, pH 7.5) with 25 mM 2-deoxyriboseand incubated at 35°C for 3 h. The mixture was centrifuged for 25 min at 10,000 g, and a sample (0.5 ml) of the produced supernatant was mixed with 5 ml of 0.5% (w/v) of TBA in 10 mMNaOH and 1 ml of glacial acetic acid (Kaur et al., 2012). This reaction medium was heated to 90°C for 25 min at 90°C and then cooled at 4°C for 20 min. The hydroxyl radical concentration was determined using red absorbance at 532 nm with non-specific absorbance correction at 600 nm. The values were expressed as µmol g−1FW using extinction coefficient of 0.155 mol−1cm−1.

Determination of lipid peroxidation P. oleracea leaves

Lipid peroxidation was measured by the method of Mandalet al. (2008) using the thiobarbituric acid (TBA) tests at 532 nm. The extract (0.2ml) was mixed then incubated with 2 ml of trichloroacetic acid (TCA) (20 %)containing 0.5 % TBA at 95°C for 40 min. The reaction was terminated by cooling for 10 min on ice then the product was centrifuged for 15 min at10,000 g. The produced malondialdehyde (MDA) was determined.

Preparations of enzyme extract from P. oleracea leaves

The enzyme extract was prepared according to Abd El-Gawad and & El-Shora (2017). Leaves of P. oleracea (5 g) were homogenized in 100 mL of 150 mM phosphate buffer (pH 7.0). The homogenate was then filtered through four layers of cheesecloth and centrifuged at 10,000 g for 25 min at 4°C. The supernatant was stored at 4°C as crude enzyme extract.

Determination of ribulose 1,5-bisphosphate carboxylase (EC: 4.1. 1.39) activity

Spectrophotometric assay of Rubisco was carried out according to Lan et al. (1991) in a cuvette containing 0.5 mL 50 mMBicine (pH 8.0), 15 mM MgCl2, 10 mM NaHCO3, 10 mMNaCl, 2 mMRuBP, 5 mM DTT, 5 mM ATP, 0.2 mM NADH, 5 mM phosphocreatine, 10 units ml-1creatine phosphokinase, 10 units ml-1 glyceraldehyde-3-phosphate dehydrogenase, 10 units ml 3-phosphoglycerate kinase, and leaf extract. The reaction was initiated by adding RuBP (0.66 mM) and the absorbance was recorded at 340 nm. These values were converted to NADH concentration (molar) using an extinction coefficient of 6.22 mM 1.

Determination of glyceraldehyde 3-phosphate dehydrogenase (EC: 1.1. 1.8)

activity

The activity of G-3-PDH was examined in a coupled assay by following the decrease in absorbance of NADH at 340 nm. The reaction mixture (1 ml) consisted of 50 mMTris·HCl (pH 7.5), 0.03 mM NADH, 5 mM magnesium chloride, 1 mM cysteine, 1 mM ATP, 3 unit ml-1 of 3-phosphoglyceric acid kinase, and 1 mM 3-phosphoglyceric acid as described by Wilson et al. (1981).

Determination of nitrate reductase (EC: 1.7. 99. 4) activity

Nitrate reductase (NR) activity was determined according to Evans and Nason (1953). A sample (0.2 ml) of the reaction mixture was mixed with 1 mL of 1% (w/v) sulphanilamide in1N HCl then 1 mL of 0.025% (w/v) N-(1-Napthyl)-ethylenediammonium dichloride in distilled H2O. The resulting pink color from diazotization was kept for 30 min for developing. The volume was diluted using distilled H2O up to 6 ml. The absorbance was recorded spectrophotometrically at 540 nm.

Determination of nitrite reductase (EC: 1.7.1.4) activity

The reaction was carried out in a 0.5 mL closed vial containing 100 mM potassium phosphate buffer (pH 6.5), 50 mM methyl viologen and 2.5 M sodium nitrite, 1 M sodium chloride and 0.02 mL enzyme (Gao et al., 2018). The reaction was initiated with the addition of 50 ml sodium dithionite (1 M) and the vial gently swirled. After incubation at 37 °C for 10 min, the reaction was terminated by vigorous shaking for 3 min. The nitrite remaining was measured spectrophotometrically.

Determination of NADH-oxidase (EC: 1.6.3.1) activity

NADH-oxidase was determined according to the method of Morret (1995). The assay mixture of 3 mL contained 0.5 ml of 0.5 mM NADH, 2 ml of 100 mMTris-Me Buffer (pH 7.5) and 0.5 ml of enzyme extract. The activity was measured at430 nm spectrophotometrically.

Results and Discussion

Lupinustermis (lupine) of 20-day old seedlings were exposed to UVC for various time intervals (20, 40, 60, 80 and 100 min). The total phenols (Fig. 1) and total flavonoids (Fig. 2) in lupine leaves from irradiated and non-irradiated seedlings were determined. It was observed that both total phenols and total flavonoids increased under treatment with UVC. UV affects plants through biochemical and physiological processes, such as enhancement of the antioxidant enzymatic system and accumulation of UV-absorbing phenolic compounds and flavonoids Plants respond to UV stress through biochemical, physiological and morphological acclimations, including the synthesis of secondary metabolites such as phenylpropanoids, which are involved in reactive oxygen species (ROS) scavenging (Falvo et al., 2012).

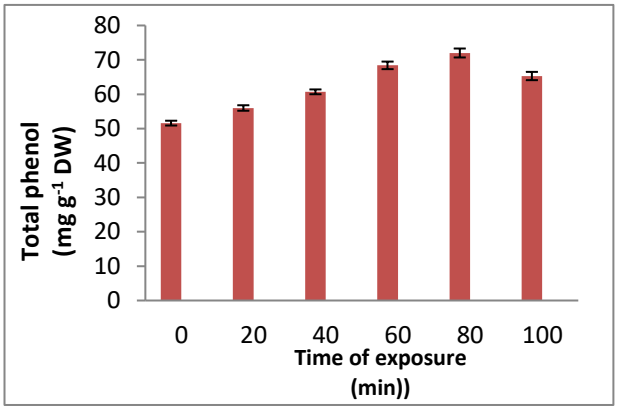

Fig.1: Effect of UVC on total phenols in lupine leaves.

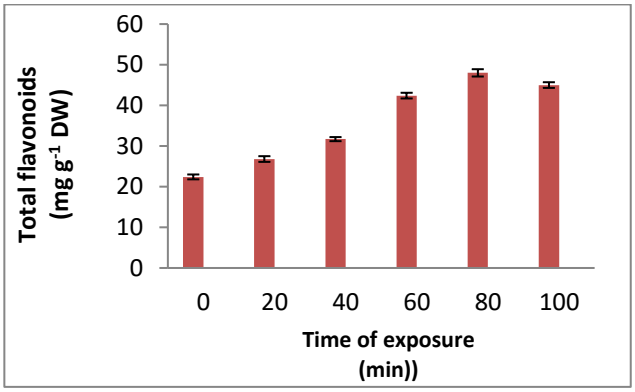

Fig. 2: Effect of UVC on total flavonoids in lupine leaves.

UV-C radiation effects on biological systems are considered more dramatic than UV-B effects, even though both UV wavelengths induce very similar photoproducts accumulation. For these reasons, results obtained from UV-C studies are indicative of plant response to ultraviolet stress (Danon&Gallois, 1996).

Quantifying the content of total soluble carbohydrates in P. olercea leaves is a way to confirm the

effect of allelochemicals in lupine on weed seedlings. The total soluble carbohydrate content in P. oleracea was reduced by allelopathic effect of lupine leaf extract (Fig. 3) whether irradiated or non-irradiated; however the irradiated extract expressed higher inducing effect. In support, the total soluble carbohydrate content was reduced in P. oleracea by Trichodesmaafricanum leaf extract (El-Shora et al., 2015). Allelochemicals in lupine leaf extract might inhibit plant photosynthesis and the inhibition of photosynthesis results in depletion of food reserve including carbohydrate.

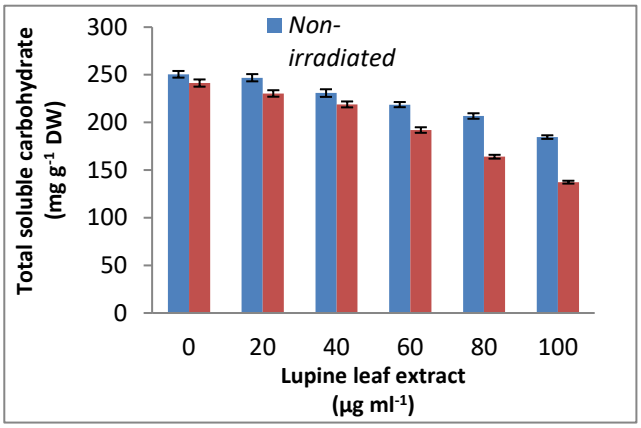

Fig. 3: Allelopathic effect of lupine leaf extract from irradiated and non- irradiated seedlings on total soluble carbohy`drate of Portulacaoleracea leaves.

The inhibition of ribulose 1,5-bisphosphate carboxylase (Rubisco) (Fig.4) and glyceraldehyde-3-phosphate dehydrogenase (G-3-P-DH) (Fig.5) by lupine leaf extract confirms depletion of the total soluble carbohydrate by lupine leaf extracts. Rubisco is a key enzyme in both photosynthetic carbon fixation and photorespiration. It catalyzes the first step of carbon fixation in the C3 pathway or Calvin cycle in higher plants (Kurepa&Smalle, 2019).

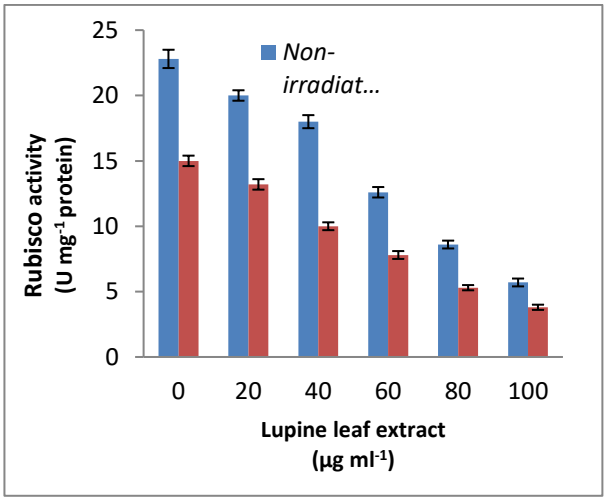

Fig. 4: Allelopathic effect of lupine leaf extract from irradiated and non- irradiated seedlings on ribulosebisPhosphate carboxylase (Rubisco) activity of Portulacaole racealeaves.

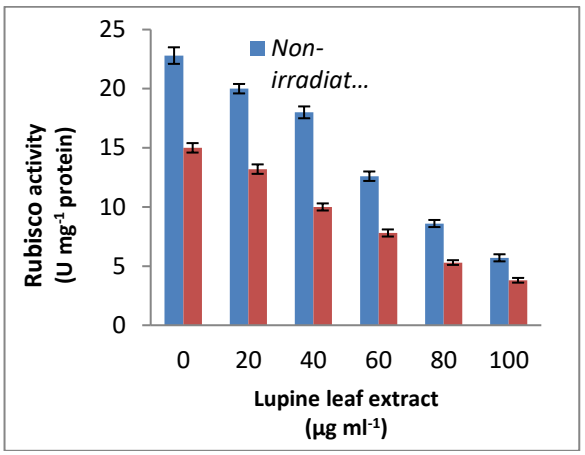

Fig. 5: Allelopathic effect of lupine leaf extract from irradiated and non- irradiated seedlings on 6-phosphogluconate dehydrogenase (6-PGDH) of Portulacaoleracealeaves.

This enzyme catalyzes the addition of CO2 onto ribulose 1,5-bisphosphate (RuBP), producing 3-phosphoglycerate which is then converted to sugars. G-3-P DH catalyzes an NADPH-consuming reaction, which is part of the Calvin cycle. This enzyme reduces 1.3—bisphosphoglycerate to glyceraldehyde -3-phosphate. NADPH for this reaction is supplied by photosynthetic electron transport (Merino et al., 2018).

Thus, the reduction of total soluble carbohydrate in Portulacaolercaea leaves is due to the inhibition of the activity of both Rubisco and G-3-P DH under allelopathy of lupine leaf extract at various concentrations. At the same time it reveals that the activity of Calvin cycle in Portulaca leaves is affected seriously by lupine extract. This could be an idea to use lupine leaf extract as bio-herbicide to remove such weed instead of using chemical herbicide.

It has been reported that allelochemicals can affect plant physiological processes including protein synthesis (AbouElGhit, 2016). The total soluble protein content in P. oleracea leaves decreased gradually by lupine leaf extracts (Fig.6). El-Shora et al. (2015) reported that the level of both total soluble protein and total amino acids were reduced under allelopathic effect. The plant allelochemicals can repress the absorption and transmission of amino acids and therefore interfere with the protein synthesis (Lee et al., 2016).

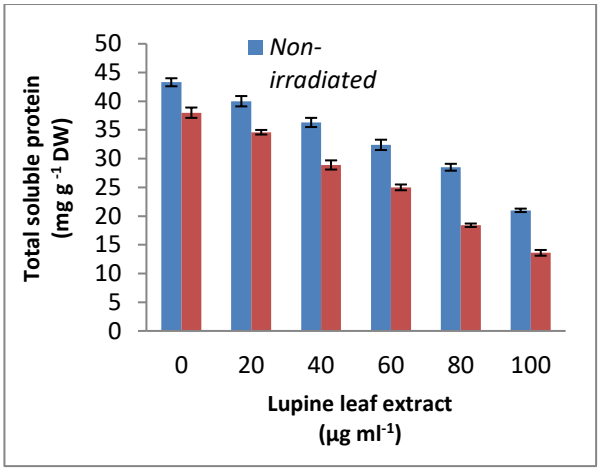

Fig. 6: Allelopathic effect of lupine leaf extract from irradiated and non- irradiated seedlings on total soluble protein of Portulacaoleracealeaves.

Hussain et al. (2010) attributed the reduction of the soluble protein content under allelopathic stress to the increase of phenolic acids such as vanillic acid and ferulic acid in leaf extract, which are known to reduce the incorporation of specific amino acid into proteins and subsequently reduce the level of protein synthesis. Allelochemicals such as cinnamic acid and ferulic acid were found to inhibit synthesis of protein (Li et al., 2010).

The protein degradation under stress such as allelochemical stress has been attributed to the decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acids and denaturation of enzymes involved in protein synthesis (Lakhdar et al., 2008). Inhibition of protein synthesis has also been reported in response to several phenolic acids through the reduction of the assimilation of specific amino acids into proteins (Hussain et al., 2010). Unlike the present results several previous publications have shown increase in protein content under allelopathic effect (Unal and Bayram, 2019; Alghamdi et al., 2022).

Nitrate is assimilated via a pathway involving nitrate uptake steps and by two reductive steps catalyzed by NR and NiR enzymes. Lupine leaf extracts inhibited the activity of NR (Fig.7) and NiR (Fig.8) in P. olerceas leaves. These results are in agreement with those reported by El-Shora and Abd El-Gawad (2014) who reported reduction of NR and glutamine synthetase as two key enzymes of nitrate reduction and protein synthesis under allelopathic effect. Also, the activity of NR was inhibited in P. oleracea leaves by allelopathic potential of Rumexdentatus (El-Shora et al., 2022). There is apparent scope for the NR inactivation by the allelochemicals (Shaik and Mehar, 2016). Thus, the inhibition of NR and NiR confirms the reduction in total soluble protein.

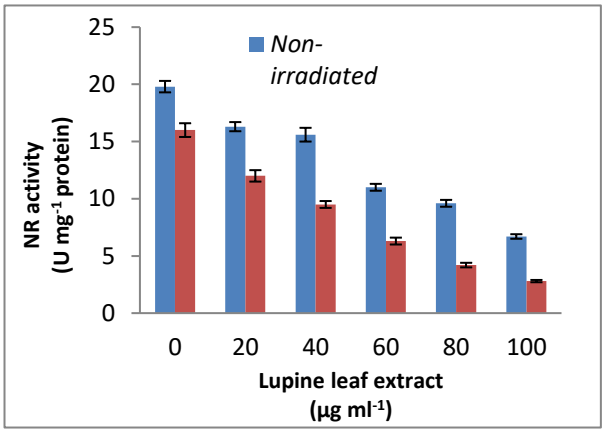

Fig. 7: Allelopathic effect of lupine leaf extract from irradiated and non- irradiated seedlings on nitrate reductase (NR) activity of Portulacaoleracealeaves.

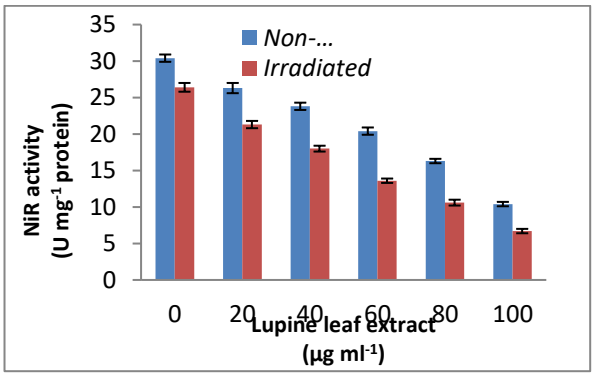

Fig. 8: Allelopathic effect of lupine leaf extract from irradiated and non-irradiated seedlings on nitrite reductase (NiR) activity of Portulacaoleracealeaves.

The observed increase in the production of H2O2 in P. oleracea leaves (Fig.9) indicated that lupine leaf extracts have caused oxidative stress in these plants. Generally, the accumulation of H2O2 in weeds causes oxidative stress and induces metabolic disorders (Shekari et al., 2022). Lipid peroxidation which is measured as malondialdehyde (MDA) production (Fig. 10) as a result of elevated levels of H2O2 in P. oleracea leaves as response to allelopathic effect of lupine leaf extracts. Many studies have shown that increased plasma membrane NAD(P)H oxidase activity was associated with increased H2O2 production following biotic and abiotic stresses (Lara-Nunez et al., 2006)

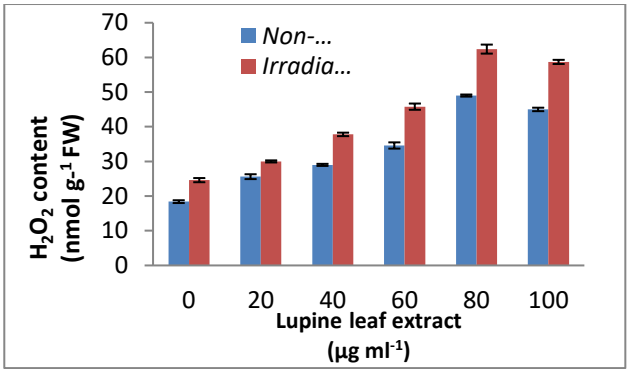

Fig. 9: Allelopathic effect of lupine leaf extract from irradiated and non-irradiated seedlings on H2O2 content of Portulacaoleracea leaves.

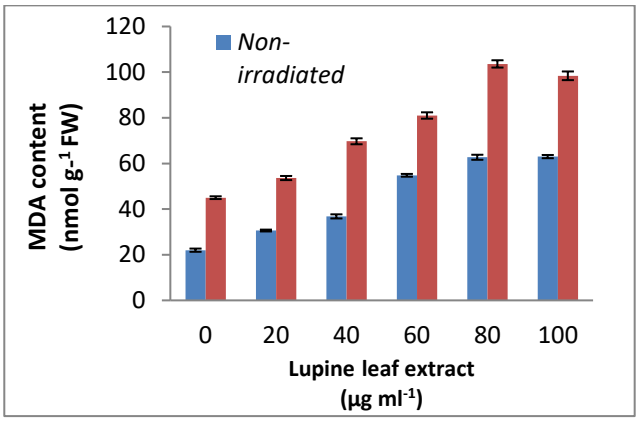

Fig. 10: Allelopathic effect of lupine leaf extract from irradiated and non- irradiated seedlings on malondialdehyde (MDA) content of Portulacaoleracea leaves.

The above results are confirmed by the increase in NADH-oxidase activity (Fig. 11), which catalyzes the oxidation of NADH to yield NAD+ and H2O, H2O2 or both. Under stress reactive oxygen species (ROS) such as H2O2 are produced and can affect membrane permeability, causing lipid peroxidation and ultimately trigger programmed cell death (Mittler et al., 2022). Studies have reported increased lipid peroxidation of membranes as a result of allelochemical compounds (Lara-Núñez et al., 2009).

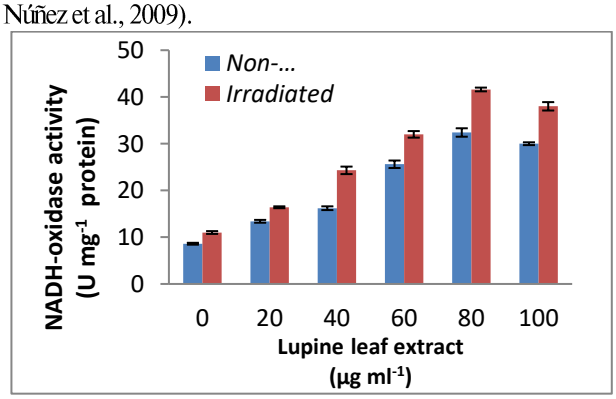

Fig. 11: Allelopathic effect of lupine leaf extract from irradiated and non-irradiated seedlings on NADH-oxidase activity of Portulacaoleracea leaves.

In conclusion, exposure of lupine seedlings to UVC irradiation enhance production of total phenols and total flavonoids in lupine leaves which resulted in disturbance of metabolism in P. oleracea leaves which was obvious from reduction in total soluble sugars and total soluble protein and their biosynthetic enzymes. In addition, treatment of P. olercea with irradiated and no-irradiated lupine leaf extracts enhance lipid peroxidation through increasing H2O2. The results suggest possible using of irradiated lupine extract as bio-herbicide against weeds.

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