1. Introduction

Intense use of herbicides has a number of detrimental impacts on human health and the environment, as well as raising herbicidal tolerance of several weeds (Li et al. 2021). Therefore, allelopathy plays major role in sustainable agriculture including biological control of pest and weed. Allelopathy is described as interaction between two plants that leads in the release of certain secondary components designated as allelochemicals (Rice 1984). Allelochemicals have beneficial or detrimental effects on recipient plants. Secondary compounds are freed into the environment through leaching, volatilization, root exudation and residue decay; accelerate allelopathic interactions that disturb agriculture, growth, and development (Cheema et al. 2013; Novakoski et al. 2020).

Many researchers evaluate the effect of allelopathy for weed control through aqueous extract of plant tissue. Allelopathic water extracts are utilized as a biological herbicide since they are less harmful to the environment than synthetic herbicides. Water-soluble allelochemical substances are convenient to use and apply without additional wetting agent (Hussain et al. 2020).

However, the effects of allelochemicals from different plants and the concentration of the extract on the target plants are variable (Bajwa et al. 2020).

Allelochemicals belong to various chemical groups, have a variety of sites and mechanism of action, and they achieve an important role in adaptation of many species (Cheng and Cheng 2015). They can be recognized in roots, seeds, fruits, pollen, stems, as well as leaves (Zeng 2008). Phenolics comprise hydroxyl groups which joined to aromatic ring. They contain flavonoids, tannins, aromatic phenol, quinones, benzoic acids, and cinnamic acid (Zeng 2008).

Allelopathic reactions are influenced by the formation and elimination of reactive oxygen species (ROS) and the reox state of the cell. After being exposed to allelochemicals, recipient plants may create ROS in the contact region and affect antioxidant enzyme activity (Bais et al. 2003; Ding et al. 2007). Allelopathic interactions resulted in changes in signal transduction as well as an imbalance between the formation of reactive oxidant species and antioxidant capacities, according to the findings by Shearer et al. (2012).

*Rumex dentatus* L. is a weed located in planted fields. It can be observed in various environments include neglected areas and river banks. This plant is sourced as a leafy vegetable (Anwar et al. 2018); numerous studies have confirmed the allelopathic activity of *R. dentatus* (Anwar et al. 2013), *R. crispus* (Pilipavicius et al. 2012), and *R. obtusifolius* (Zaller 2006).

*R. dentatus* covers several bioactive compounds such as emodin, paeonin, chrysophanol, and nepodine (Fatima et al. 2009; Choi et al. 2004), gallic, isovanillic, ferulic, p-hydroxy-cinnamic, vanillic, succinic, syringic acids, and anthraquinones (Zhang et al. 2012; Kucekova et al. 2011). Moreover, caffeic acid has been identified in *R. gmelini* (Lee et al. 2011).

Weed control is essential for increasing agricultural productivity and lowering weed seed generation. Weeds raise thinning and hoing costs, lower yield quality, and act as disease and insect pest alternative hosts (Prather 1996; Cudney et al. 2002). *P. oleracea* is an invasive weed that is a serious problem in several field crops (Mitichlitichl 1997). The ability of *P. oleracea* to reproduce vegetatively is one of the traits that makes it difficult to control as a weed (Proctor et al. 1996).
Because of its fast growth, *P. oleracea* produce a huge quantity of seeds within six weeks after germination (Miyanishi and Cavers 1980). As a result, *P. oleracea* has the ability to limit the production of summer crops and decrease their yield in commercial operations (Haar and Fennimore 2003).

Consequently, the present study intended to examine the responses of *P. oleracea* to the allelopathic influence of *R. dentatus* leaf extract by looking at the impact of *R. dentatus* aqueous leaf extract on metabolites, ROS, and enzymes activities on *P. oleracea*. Additionally, the study aimed to analyze *Rumex* extract by gas chromatography–mass spectrometry (GC-MS) that may explain the impact on *Portulaca* metabolites and enzymes activities. Furthermore, this study will contribute to recognize the mechanisms of allelopathic effect of *R. dentatus*.

2. Materials and methods

2.1. Germination of *P. oleracea* seeds

*P. oleracea* seeds were selected apparently healthy and uniformly and disinfected by using 0.1% HgCl₂ for 10 min, washed several times with tap water. The seeds were germinated for 7 days, in tiny plates, moisturized with distilled water and placed between wet wipes according to Hoagland and Arnon (1950).

2.2. Preparation of aqueous leaf extract of *R. dentatus*

The aqueous leaf extract of *R. dentatus* was prepared according to El-Shora and El-Gawad (2014). The leaves were washed three times with tap water before being dried in the open air. The dried material was ground into a fine powder, and a sample of (15 g) was soaked in distilled water and orbitally shaken for 48 h at 23–28°C for extraction. Various concentrations (2.5, 5, 10, 20, and 40 mg mL⁻¹) of *R. dentatus* leaf extract were prepared and mixed with Hoagland’s solution.

2.3. Treatment of *Portulaca* with *R. dentatus* extract

The germinated seeds (7-day-old) of *P. oleracea* with well-grown radicles were supported on plastic bowls containing Hoagland’s solutions mixed with the various doses (2.5, 5, 10, 20, and 40 mg mL⁻¹) of *R. dentatus* and vigorously aerated with air pump. The pH of the nutrient solution was set to 5.5, and then 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 m⁻² s⁻¹ photon flux density and approximately 60%, relative humidity. Plants of 20-day-old from each concentration, as well as control samples (treated with water) were used for analysis. The experimental design was carried out with three replicates.

2.4. Determination of metabolites in *P. oleracea* leaves

2.4.1. Determination of total soluble sugars

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 mM anthrone in 90% (w/v) H₂SO₄). The mixture was carefully heated for 15 min, cooled, and incubated for 30 min in an ice bath, with the absorbance measured at 623 nm. The content of total soluble sugars (TSSs) was calculated from glucose calibration curve (Schlüter and Crawford 2001).

2.4.2. Determination of total insoluble sugars

The remained residue after extraction process was dissolved in 1.5 M perchloric acid and incubated for 2 h in water bath at 70°C. The solution was centrifuged for 20 min at 1000 g and the concentration of non-soluble carbohydrates was estimated by the anthrone reagent as stated above (Schlüter and Crawford 2001).

2.4.3. Determination of total soluble protein

The determination of total soluble protein (TSP) 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 mM NaCl. 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.

2.4.4. Determination of total free amino acid

The determination of free amino acids was performed following the method adopted by Moore and Stein (1948). A sample (400 mg) leaf powder was extracted in 70% (w/v) ethanol, and then 10 mL of the extract was mixed with 3% (w/v) acetic acid, boiled, then cooled and retained in the refrigerator for 6 h for coagulation of the water-soluble protein. One mL of amino acid extract was combined with 2 mL of acetate buffer (pH 6.4), followed by the addition of 1mL of ninhydrin reagent. The mixture was later heated for 20 min in a boiling water bath, and after cooling glycine was used as a reference for total free amino acid (TFA) determination.

2.4.5. Determination of nucleic acid content

The leaf nucleic acid content was extracted by the method of Sadasivam and Manickam (2008). A sample (600 mg) of fresh leaves was crushed in 12 mL of 70% ethanol (w/v). The homogenate was filtered and diluted to 12 mL with ethanol before being treated with 12 mL of 1 N perchloric acid. The mixture was heated for 15 min at 90°C. The resulting homogenate was centrifuged at 1000 g for 25 min, and the supernatant was made up to 12 mL. A sample (1 mL) of DNA extract was diluted to 5 mL distilled water, mixed with 2 mL of diphenylamine reagent and then heated at 10 min 90°C. After cooling at room temperature, the absorbance of the developed blue color was recorded at 600 nm spectrophotometrically. Colorimetric procedure suitable for pentose determination has been used for measurement of RNA. The reaction of ribose in RNA with orcinol was used. One mL of RNA extract was diluted to 5 mL with distilled water, mixed with 5 mL of orcinol reagent followed by heating for 20 min in a boiling water bath.
2.5. Determination of antioxidants

2.5.1. Determination of total phenolic content
Total phenolic content in *P. oleracea* leaf extract was determined by the colorimetric method of Singleton and Rossi (1965) using Folin–Ciocalteu reagent. Leaf extract (1 mL) was mixed with 1 mL sodium carbonate solution and one mL of 10-fold diluted Folin–Ciocalteu reagent. The mixture was incubated for 30 min at 30°C and colorimetrically measured at 500 nm.

2.5.2. Determination of total flavonoids
Determination of the total flavonoid was performed by AlCl₃ method of Chang et al. (2002). One mL of leaf extract was combined with 1.5 mL methanol, 2.8 mL of distilled water, 0.1 mL of 10% (w/v) AlCl₃, and 0.1 mL of 1 M potassium acetate. The mixture was incubated for 30 min at 30°C and the absorbance was recorded spectrophotometrically at 415 nm. Total flavonoid content was calculated using a catechin standard curve and the results were expressed as mg catechin equivalent (CE)/g d.wt.

2.5.3 Determination of lipid peroxidation
Lipid peroxidation was determined by measurement of the formation of malondialdehyde (MDA) according to Hodges et al. (1999) which is a product of lipid peroxidation. The seedlings of *P. oleracea* were homogenized with 3 mL of 10% (w/v) trichloroacetic acid (TCA) in an ice bath, followed by centrifugation at 8000 g for 15 min. Then, 1 mL of the supernatant was mixed with 2 mL of 0.6% (w/v) thiobarbituric acid (TBA) in 10% TCA. The reaction was then stopped by immersing the tubes in an ice bath after 30 min of incubation in a boiling water bath. The mixture was centrifuged at 10,000 g for 20 min and the absorbance of the obtained supernatant was recorded at 440, 532 and 600 nm.

2.6. Determination of ROS

2.6.1. Determination of hydrogen peroxide
*P. oleracea* seedling leaves were pulverized in 1 mL cold acetone in an ice bath, then centrifuged at 8000 g for 15 min. The supernatant was mixed with 0.2 mL of 25% (w/v) titanium reagent and 0.2 mL of 20 mol ammonia solutions. The mixture was centrifuged at 4000 g for 15 min at 25°C and the supernatant was discarded. The residue was dissolved in 1 mL of 1 mol sulfuric acid and the absorbance of the solution was measured at 415 nm. The method of He et al. (2005) was used to determine hydrogen peroxide (H₂O₂) content in *P. oleracea* leaves, and the concentration of H₂O₂ was calculated using a standard curve.

2.6.2. Determination of superoxide anion (O₂⁻)
Superoxide anion in *P. oleracea* leaves was determined at 480 nm in terms epinephrine oxidation to adrenochrome (Misra and Fridovich 1972) and expressed as µmol g⁻¹ FW. The oxidation has a maximum absorption wavelength of 480 nm, with an extinction coefficient of 4020 M⁻¹cm⁻¹. Its oxidation resulted in the disappearance of its 480 nm absorption band, as well as an increase in absorbance at 300 nm and an isosbestic point at 415 nm. The extinction coefficient for adrenochrome at 415 nm was estimated to be 2500 M⁻¹cm⁻¹.

2.6.3. Determination of hydroxyl radical (OH)
Fresh leaves (500 mg) were homogenized in 10 mL phosphate buffer (50 mM, pH 7.5) with 25 mM 2-deoxyribose and 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 mM NaOH 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⁻¹ f.w using extinction coefficient of 0.155 mol⁻¹ cm⁻¹.

2.7. Preparation of enzyme extract
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.

2.8. Determination of the enzymes activities

2.8.1. Determination of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity
The glucose-6-phosphate dehydrogenase (G6PD) activity was assayed according to Betke et al. (1967) based on NADPH production that could be measured spectrophotometrically at 340 nm. The reaction mixture of 3 mL consists of 0.5 mL of 0.6 mM glucose-6-phosphate, 0.5 mL of 0.2 mM NADP, and 1.5 mL of 10 mM MgCl₂ in 150 mM Tris-HCl buffer (pH 8.0). This reaction assay was initiated by adding 0.5 mL of enzyme extract. One unit (U) of G6PD activity was expressed as the amount of enzyme needed to reduce one µmol of NADP per min.

2.8.2. Determination of 6-phosphogluconate dehydrogenase (EC 1.1.1.44) activity
6-phosphogluconate dehydrogenase (6PGD) activity was determined according to Papapetridis et al. (2016). The mechanism is based on the conversion of NAD+/NADP⁺ to NADH/NADPH and measured spectrophotometrically at 340 nm. The reaction mixture of 3 mL containing of 0.5 mL of 0.4 mM NAD⁺ (NADP⁺), 0.5 mL of 5 mM MgCl₂, 1.5 mL of 100 mM Tris-HCl buffer (pH 8.0). The reaction assay was initiated by adding 0.5 mL of enzyme extract. The increase in 340 nm absorbance as a measure of NADPH generation was recorded every 9 s for 10 min spectrophotometrically.

2.8.3 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 in 1N HCl then 1 mL of 0.025% (w/v) N-(1-Napthyl)-ethylene diammonium 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,
and a standard curve was created using sodium nitrite solution.

2.8.4. Determination of glutamine synthetase (EC 1.6.4.2) activity
Glutamine synthetase (GS) was determined according to Elliott (1953). The method was dependent on the biosynthetic activity of λ-glutamyl hydroxamate. The reaction mixture contained 0.25 mL of phosphate buffer (pH 7.0), 0.25 mL of 500 mM sodium glutamate, 0.05 mL of 1 mM MgSO4, 0.1 mL of 50 mM ATP, 0.15 mL of 100 mM hydroxyxylamine, 0.15 mL of the enzyme extract, and 0.05 mL of 100 mM cysteine. The reaction assay was done at 30°C and terminated after incubation period by adding 1 mL of 10% (w/v) FeCl3; 24% (w/v) TCA: 6 mM HCl, 1:1:1, resulting a brown-yellow precipitate. After centrifugation at 5000 g, the absorbance was recorded at 540 nm for determination of λ-glutamyl hydroxamate produced, using a standard calibration curve. The activity of GS was expressed as µmole mg⁻¹ of protein.

2.8.5. 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 mM Tris-Me Bu buffer, 0.05 mL of 0.5 mM NADH-oxidase was determined according to the method of Elliott (1953). The assay mixture of 3 mL contained 0.5 mL of 0.5 mM NADH, 2 mL of 100 mM Tris-Me Bu buffer, 0.05 mL of 0.5 mL of enzyme extract. The activity was measured at 430 nm spectrophotometrically.

2.8.6. Determination of phenylalanine ammonia-lyase (EC 4.3.1.24) activity
Phenylalanine ammonia-lyase (PAL) activity was determined according to the adopted method of D’Cunha et al. (1996) and modified by El-Shora (2002). The method was dependent on the production of cinnamate. One mL reaction mixture contained 0.5 mL of 150 mM Tris-HCl (pH 8.5) with 1 mL of 2-mercaptoethanol, 0.5 mL of 100 mM L-phenylalanine and 0.05 mL of enzyme extract was incubated for at 30°C for 15 min. 0.5 mL of 6 M HCl was used to end the reaction, which measured spectrophotometrically at 290 nm. The conversion of one mole substrate to product per minute was defined as one unit (U).

2.9. GC-MS analysis for identification of R. dentatus leaf extract

2.9.1. GC-MS analysis
The phytochemical profile of R. dentatus leaf extract were detected using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG−SMS (30 m x 0.25 mm x 0.25 µm film thickness). The temperature of the column oven was initially maintained at 50°C, then increased at a rate of 5°C/min to 250°C, kept for 2 min, and then increased at a rate of 30°C/min to 300°C, held for 2 min. The detector (270°C and 260°C, respectively, and helium was used as the carrier gas at a flow rate of 1 mL/min. An autosampler AS1300 and a split mode GC were used to inject 1 µL diluted samples automatically after a 3-minute solvent delay. The temperature of the column oven was initially maintained at 50°C, then increased at a rate of 5°C/min to 250°C, kept for 2 min, and then increased at a rate of 30°C/min to 300°C, held for 2 min. EI mass spectra were recorded at 70 eV ionization voltages and over the range of m/z 50–650. The ion source’s temperature was set to 250°C. The retention times and mass spectra of the components were compared with those of the WILEY 09 and NIST 14 mass spectral databases to identify them.

2.9.2. Identification of components
The components of the aqueous leaf extract of R. dentatus were identified by comparing the mass spectra of R. dentatus leaf extracts to those of a computer library or authentic compounds, and their retention index was confirmed by comparing them to those of actual compounds.

2.10. Statistical analysis
Data represent the mean ± (n = 3). Different letters indicate significant difference (p < 0.01) assessed by Tukey–Kramer’s test.

3. Results
The allelopathic activity of various concentrations of R. dentatus leaf aqueous extracts on P. oleracea was investigated by conducting biochemical analysis and assay of various key enzymes of the metabolism in P. oleracea leaves. Seedlings of P. oleracea that were 21-day-old were used for the various biochemical analyses. The effect of R. dentatus leaf aqueous extract on soluble and insoluble sugars content was found to be significantly increased at all concentrations tested as shown in Figure 1. The highest concentration of R. dentatus (40 mg ml⁻¹) recorded the highest accumulation of soluble and insoluble sugars by 77 and 35%, respectively over the control treatment.

The content of total soluble protein and TFA showed a reverse manner in their accumulation after application of R. dentatus leaf extract (Figure 1). The total soluble proteins in P. oleracea leaves showed continuous significant decrease with increasing the concentration of R. dentatus leaf aqueous extract. However, the TFA recorded continuous increase with increasing concentration up to 32 mg g⁻¹ DW at 40 mg mL⁻¹ which represented by 111% increase over control treatment.

The accumulation of phenolic compounds and flavonoids in P. oleracea leaves increased with the application of various concentrations of R. dentatus leaf extract (Figure 1). The impact of the highest concentration (40 mg ml⁻¹) of R. dentatus leaf extract on P. oleracea leaves resulted in induction of total phenolic compounds and total flavonoids up to 66 and 22 mg g⁻¹ DW, respectively. Moreover, such induction of phenolic compounds and flavonoids increased by 38 and 258%, respectively, than the control treatment.

The contents of DNA and RNA in leaves of P. oleracea were estimated spectrophotometrically, and these contents are shown in (Figure 2). These results indicate continuous decrease in DNA and RNA with the application of different concentrations of R. dentatus leaf extract. The greatest reduction was seen at the highest concentration of R. dentatus extract (40 mg ml⁻¹) and was 47 and 60% lower than the control treatment for DNA and RNA, respectively.

When the concentration of R. dentatus extract was raised, both superoxide and hydroxyl radicals increased progressively in P. oleracea leaves. (Figure 3). The superoxide radical
content increased and reached to 22.2 µmol g$^{-1}$ FW at 40 mg ml$^{-1}$. However, the hydroxyl radical content was 24 µmol g$^{-1}$ FW at 40 mg ml$^{-1}$.

The contents of both H$_2$O$_2$ and MDA progressively increased in *P. oleraceae* leaves under the influence of *R. dentatus* extract in concentration-dependent manner (Figure 3). The content of H$_2$O$_2$ and MDA after treatment with 40 mg ml$^{-1}$ were raised by 113 and 208%, respectively over the control.

The activities of PGDH and G6PD in leaves of *P. oleracea* were assessed, and a decrease trend was observed as the concentration of *R. dentatus* leaf extract was increased (Figure 4). When compared with the control treatment, the activity of these two enzymes at (40 mg ml$^{-1}$) decreased by 81% and 57%, respectively.

NR activities and GS were assessed and shown in (Figure 4). The tested concentrations of *R. dentatus* leaf extract caused continuous suppression in activities of NR and GS by 74 and 66%, respectively, than the control treatment at 40 mg ml$^{-1}$.

The activities of NADH-oxidase and PAL were estimated in *P. oleracea* leaves under allelopathic effect of *R. dentatus* leaf extract and revealed a steady increase in their activities with increasing the concentrations of *R. dentatus* leaf extract up to 40 mg ml$^{-1}$ (Figure 4). The maximum activity of NADH-oxidase and PAL enzymes were over the control treatment by 137% and 128%, respectively.

### 3.1. GC-MS analysis

The aqueous leaf extracts of *R. dentatus* was subjected to GC-MS analysis, and the results were represented in (Table 1). It was interestingly observed that some compounds such as 1-dodecanamine, N,N-dimethyl-, 1-tetradecanamine, N,N-dimethyl, 2,6,10-trimethyl, 14-ethyl-14-pentadecene, n-hexadecanoic acid, 3-(N-Benzyl-N-methyl-amino)-1,2-propanediol, and cis-vaccenic acid were the dominant compounds in the leaf extract. These compounds accumulated at range from 5 up to 18.7%. It’s worth mentioning that some fatty acids were detected in *R. dentatus* leaf extract such as oleic acid, octadecanoic acid, cis-vaccenic acid, hexadecanoic acid, 9,12,15-octadecatrienoic acid, 10-octadecenoic acid. In comparison to the detected fatty acids, n-hexadecanoic acid had the greatest concentration of 10.66%.

### 4. Discussion

Soluble sugars are accumulated in leaves of *P. oleraceae* under allelopathic stress. The accumulation of soluble carbohydrates in plants has been widely reported as a response to stress and the accumulation of sugars in plant was considered as one of the most marked consequences for osmotic adjustment under stress (Kameli and Lösel 1996). Bohnert and Jensen (1996) affirmed that carbohydrates may also play as ROS scavengers and act as an osmo-protectant that stabilizes cellular membranes and conserves turgor (Jouve et al. 2004). In addition to their function as cytosolic osmolytes, they may stabilize the structure and function of macromolecules such as enzymes in cells (Radi 2013).

The present results revealed that treatment of *P. oleracea* plants with aqueous leaf extract of Rumex progressed reduction in protein content and an increase in the TFA. In support, El-Shora and Abd El-Gawad (2015a) reported reduction of protein content in *P. oleracea* leaves under treatment with Lupinus terms leaf extract. However, 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). The decreased protein content after application of
allelochemicals may be due to the accumulation of phenolic glycine that can interfere with the cytoplasmic ribosomes and production of RNA, which in turn inhibited protein synthesis (Hegab and Ghareib 2010).

Decline of protein concentration in Portulaca leaves may imply an inhibitory effect of allelochemicals of Rumex extract on various pathways of protein synthesis in Portulaca leaves. 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, which are known to reduce the incorporation of specific amino acid into proteins and subsequently reduce the level of protein synthesis. Lakhdar et al. (2008) attributed the reduction of soluble protein content under stress to the proteolysis and the decrease in the enzymes activities that are involved in protein synthesis. Baziramakenga et al. (1997) found that allelochemicals reduced the uptake of methionine as well as its assimilation into protein. It has been reported that damage of nucleic acid causes reduction of protein synthesis (Britt 1999) since the protein synthesis depends on RNA synthesis (Einhellig 1996).

The increase in free amino acid content under allelopathic stress in the present investigation may be due to rapid hydrolysis of protein, which results in release of free amino acids.

The level of DNA and RNA in Portulaca leaves were reduced under allelopathic effect of Rumex leaf extract. These results are in harmony with previous findings (Betke et al. 1967) for allelopathic effect of Hyoscyamus muticus on Portulaca leaves. This reduction in nucleic acids may be due to the possible decline in the incorporation of phosphorus into DNA and the interaction of allelochemicals with nucleic acids metabolism (John and Sarada 2012). Also, the reduction in nucleic acid content could be attributed to the effect of allelochemicals on RNA translation and DNA transcription or combination of these factors (Jelassi et al. 2016). Kamal et al. (2013) reported a drastic increase in the level of nucleic acids in response to allelopathic effect and attributed this effect to enhancement of the enzymes of synthetic pathways.

High levels of ROS produced by allelochemicals in Rumex extract can cause damaging of the nucleic acids (Valko et al.
ROS cause damage to DNA such as cross-links, strand breaks, base deletion, pyrimidine dimers, and base modification including oxidation and alkylation (Tuteja et al. 2001). DNA damage can also cause replication errors and genomic instability (Cooke et al. 2003). The phenolics denote hydrogen and this makes them applicable antioxidants (Vinson et al. 2001). It has been proposed that the antioxidant property of phenolic compounds can be due to numerous mechanisms including: (i) up-regulating antioxidant defense, (ii) scavenging ROS, and (iii) prevention of ROS formation (Dai and Mumper 2010). In addition, the phenolics can stabilize the cell membranes by reducing the fluidity of membrane in concentration-dependent manner, interfering with free radical diffusion and preventing the peroxidative reactions (Blokchina et al. 2003). Generally, the phenolic compounds which hold higher number of hydroxy groups have strong ROS scavenging capability (Świętek et al. 2019).

The total flavonoids in Portulaca leaves increased under the allelopathic effect by Rumex leaf extract. It is commonly believed that allelochemicals enhance the content of non-enzymatic antioxidants (García-Sánchez et al. 2012). The flavonoids denote hydrogen and this makes them applicable antioxidants (Vinson et al. 2001). It has been proposed that the antioxidant property of phenolic compounds can be due to numerous mechanisms including: (i) up-regulating antioxidant defense, (ii) scavenging ROS, and (iii) prevention of ROS formation (Dai and Mumper 2010). In addition, the flavonoids can stabilize the cell membranes by reducing the fluidity of membrane in concentration-dependent manner, interfering with free radical diffusion and preventing the peroxidative reactions (Blokchina et al. 2003). Generally, the phenolic compounds which hold higher number of hydroxy groups have strong ROS scavenging capability (Świętek et al. 2019).

In our study it was observed that treatment of Portulaca plants with Rumex leaf extract led to an increase in MDA concentration. MDA, the end product of lipid peroxidation, is considered to be an important marker for assessing membrane lipid peroxidation, and its increase reveals an oxidative stress has increased too (Parvanova et al. 2004). The lipid peroxidation causes changes in fluidity and permeability of the membrane lipid bilayer and can considerably alter cell integrity (Yan et al. 2015).

H₂O₂ content significantly was increased in Portulaca leaves after treatment with Rumex extract. The allelochemical stress increases the concentration of ROS in plant cells (Sharma et al. 2012). The ROS consequently leads to oxidative damage, increase of lipid peroxidation in the membrane and eventually results in cell death (Goraya and Asthir 2016). H₂O₂ was described to disrupt the activities of sulfhydryl group-containing enzymes and reduces photosynthetic activity particularly at elevated concentrations (Takeda et al. 1997). The hydroxyl radical (·OH) is the most powerful short-lived of ROS. H₂O₂ was considered to be a deleterious byproduct of oxidative stress and as an agent of destruction (Richards et al. 2015). ROS do not have long lifetimes and cannot be transported for long distances in an organism. ROS do not have long lifetimes and cannot be transported for long distances in an organism (Dumanović et al. 2020), therefore they damage the cell structures that are adjacent to the site of their formation. The molecule that was attacked was destroyed, damaged, or lost its function (Szwed et al. 2019).

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The hydroxyl radical (·OH) is the most powerful short-lived of ROS. H₂O₂ was considered to be a deleterious byproduct of oxidative stress and as an agent of destruction (Richards et al. 2015). ROS do not have long lifetimes and cannot be transported for long distances in an organism (Dumanović et al. 2020), therefore they damage the cell structures that are adjacent to the site of their formation. The molecule that was attacked was destroyed, damaged, or lost its function (Szwed et al. 2019).

NADH-oxidase catalyzes the transfer of electrons to O₂ generating superoxide radical using NADH as an electron donor. The present results showed an apparent increase in NADH oxidase in Portulaca under treatment with aqueous Rumex leaf extract. These results are in agreement with the report of Lee et al. (2016) in Portulaca leaves under allelopathic effect of Trichodesma africanum extract. It was suggested that the increased activity of this enzyme is associated with increased ROS production particularly superoxide
Glucose-6-phosphate dehydrogenase is a principal enzyme of oxidative pentose phosphate pathway (OPPP) that catalyzes the transformation of glucose-6-phosphate to 6-phosphogluconolactone and thus offering NADPH for the biosynthetic routes and supplies pentose for nucleic acid synthesis. In addition, 6-phosphoglucuronate dehydrogenase (6PGDH) is another enzyme of OPPP catalyzes the transfer of 6-phosphogluconate into ribulose 5-phosphate. The activities of these two enzymes were reduced in Portulaca leaves under allelopathic effect of Rumex leaf extract. This reduction was positively associated with the increase in TSSs. Also, El-Shora and Abd El-Gawad (2015b) informed a reduction of Cucurbita 6PGDH in response to allelopathic effect of Portulaca extract. The plants growing in light can produce NADPH, through the photosynthesis, which is used in various routes including carbon fixation, fatty acid production, and nitrogen assimilation. When plants grow in the dark, however, NADPH in photosynthetic or non-photosynthetic tissues can be provided by OPPP.

Nitrogen is an essential element for plant growth and plays a vital role in the biosynthesis of amino acid, as well as synthesis of protein (El-Shora and Ali 2011). NR is a key enzyme in nitrogen metabolism. Nitrate is assimilated through a pathway comprising NR and nitrite reductase. In the present investigation, NR activity in Portulaca leaves was inhibited by Rumex leaf extract. It seems likely that NR is deactivated by allelochemicals in Rumex extract (Shaik and Mehar 2016). This inhibition may be ascribed to the interfering of allelochemicals with NR reaction or its synthesis.

The activity of GS of Portulaca leaves was reduced by Rumex leaf extract and the reduction in GS was concentration-dependent. The reduction of GS under allelopathy is in harmony with the finding of El-Shora and El-Gawad (2014) and may be due to protein oxidation through the free radicals produced by allelochemicals of Rumex extract.

PAL is the key enzyme of phenylpropanoid pathway and performs an important role in controlling the phenolics biosynthesis in plants (Gad et al. 2021). The results indicate an enhancement in PAL activity in Portulaca leaves and this proposes other reason for the increase of the total phenolic compounds. The enhanced activities of enzymes involved in phenylpropanoid pathway embracing PAL suggested a shift from sucrose production to processes that reinforce defense and repair (Madany and Saleh 2015; Kameli and Lösel 1996).

GC-MS analysis of R. dentatus leaf aqueous extract demonstrated the presence of several compounds. Among these compounds were 2-hexadecen-1-ol, 3,7,11,15-tetramethy-,[R-[R,R-(E)]]- which is a decomposition product of chlorophyll and it is cytotoxic (Kumar et al. 2010). Also, 9,12,15-octadecatnioic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-, hexadecanoic acid, and n-hexadecanoic acid were present in the extract, and these compounds are enzyme inhibitors (Abubakar and Majinda 2016; Yif et al. 2002). It is possible that the inhibition of the various tested metabolic enzymes in P. oleraceae was due to the presence of such compounds. In addition, the GC-MS analysis revealed the presence of oleic acid which is known by its apoptotic activity (Fontana et al. 2013).

Generally, the importance of allelopathy in agriculture is increasingly accepted. Allelopathic interactions are often used for weed inhibition or managing of weeds in different cropping systems (Weston and Mathesius 2013).

5. Conclusion

Under conditions of allelochemical stress by R. dentatus leaf extract an increase in the content of total phenolics and total flavonoids in Potulaca leaves were observed, which signifies an adaptation to the stressful environmental conditions. Also, the allelopaic effect on P. oleraceae resulted in reduction of metabolic enzymes activities, protein, carbohydrate, as well as nucleic acid contents. This reduction in metabolites and enzymes activities is considered as a mechanim of allelopathic effect and provides the base for more studies upholding the use of Rumex leaf extract as bioherbicde for weed management.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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