

Allelopathic effects of *Taraxacum officinale* L. and *Ricinus communis* L. leaves extracts on sunflower and maize

Saima Syed, Asghari Bano* Muhammad Naeem¹ and Nimisha Amist²

Department of Biosciences, University of Wah, Wah Cantt, Rawalpindi, Pakistan
E. Mail: asghari.bano@uow.edu.pk

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ABSTRACT

We evaluated the effects of *Taraxacum officinale* L. and *Ricinus communis* L. on growth and physiology of sunflower and maize plants. Seeds of sunflower (cv Parson 3) and maize (cv TP 1217) were surface sterilized and soaked in 0.3 % and 0.5 % aqueous extracts of *T. officinale* L. and *R. communis* L. prior to sowing, plants were grown in pots under natural conditions. Fresh and dry weight of shoot and root, chlorophyll, carotenoids, protein, proline, phenols and flavonoids and phytohormones contents in leaves of sunflower and maize were determined. The activities of antioxidant enzymes (Superoxide dismutase (SOD), Peroxidase (POD) and defense related enzymes viz. Phenylalanine ammonia-lyase (PAL) and Polyphenol oxidase (PPO) were also recorded in leaves of maize and sunflower at vegetative phase. Phytohormones indole acetic acid (IAA), gibberellic acid (GA) and abscisic acid (ABA) were detected in *T. officinale* L. and *R. communis* L. Extract of *T. officinale* L. and *R. communis* L. significantly enhanced the shoot biomass in maize and sunflower as compared to control. Proline, phenolics, flavonoids, protein, chlorophyll, carotenoids and terpenoids content of fresh leaves were enhanced in all the treatments as compared to control. Activities of PAL, SOD, PPO and POD were also enhanced. Among all the treatments 0.3 % aqueous extract of *T. officinale* L. and 0.5 % aqueous extract of *R. communis* L. were more effective in sunflower and maize plants. The extracts of *T. officinale* L. and *R. communis* L. may be implicated to improve the growth and defensive system of maize and sunflower and induce tolerance to stresses by augmenting osmoregulation and enhancing the antioxidant and defense related enzymes.

Key words: Allelopathy, maize, , *Ricinus communis*, sunflower, *Taraxacum officinale*

INTRODUCTION

T. officinale L. is commonly called Kukraundha, Kanphool and dandelion. Dandelion is perennial herb. It inhibits growth of neighboring plants by emitting ethylene gas, a hormone that promotes ripening of premature fruits. According to Biel *et al.* (3), dandelion leaves are a good source of carotenoids, phenolics, niacin, riboflavin, thiamine, phosphorus, protein, calcium, iron, zinc, phosphorus, fat, and coarse fibre. Saponins, flavonoids, triterpenoids, sesquiterpenes, glycosides, and phenols are all present in dandelion plants (51).

It has antioxidant, antimicrobial, antiviral and anti-inflammatory property (48). Total antioxidant level is also found to be very high (28). Phenolic content from the dandelion may be helpful for discovery of antibiotic medicines for many infections (48). Balah *et al.* (7) demonstrated the effect of allelochemicals on weeds growth, that dandelion aqueous extract inhibited weed germination and increased crop productivity. Extract of dandelion proved to be a best herbicide for pre and post seed inhibition of *Medicago minima* (L.) a weed (55).

*Correspondence author, ¹Department of Biotechnology, Mohi-ud-Din Islamic University, Nerian Sharif, AJ&K, Pakistan, ²Department of Botany, Ewing Christian College, Prayagraj, Uttar Pradesh, India.



Figure 1. *Taraxacum officinale* L collected from Wah Cantt which is 465.54 m above sea level, latitude 33.71541, longitude 72.751092, annual maximum temperature 36.6 °C and annual minimum temperature 24.17 °C at the time of collection

R. communis L. (castor) is a native of Africa. If ingested plant parts are poisonous. The plant is adapted in different habitats (15). *R. communis* L. grows well in sandy loam soil needs less nutrient soil with pH from 4.5 to 6.5. *R. communis* L. contains ricinine an alkaloid in leaves which paralyzes and kill insects (41). Castor constitutes flavonoids, alkaloids, phenolics and terpenoids (11). Aqueous extract of castor inhibit germination of seeds of lentil and can be used as bioherbicide (54). Allelopathic potential of *R. communis* L. and *Jatropha curcas* was determined (27), on growth of seedlings of lettuce, cress, alfalfa and Italian grass.

Zea mays L. (maize) is commonly called as corn is staple food. Indole acetic acid (IAA), abscisic acid, isopentyladenosine dihydrozeatin riboside and zeatinriboside are present in maize roots (12). In maize leaves phytohormones like ABA, gibberellin, indole acetic acid and zeatin have significant role (40). In maize, *Fusarium* fungi causes ear and kernel rot disease. Maize mosaic virus is caused by *Peregrinus maidis* insect (2), its hosts, *Convolvulus arvensis* L. and *Cynodon dactylon* L. are common weeds of maize. Sunflower (*Helianthus annuus* L., family: Asteraceae) contains many phytohormones (24) and antioxidants, flavonoids, phenolic acids, trace elements, alkaloids and steroids (45). In sunflower amount of chemicals is more in leaves followed by roots and stem respectively. Stress stimulates production of total phenols, alkaloid content, flavonoids and phenols content in high amount in leaves than roots and stem (31). Sunflower is an important oil yielding crop. Its seeds have about 30 pathogenic microbes (4), these causes major diseases [rust, verticillium wilt, sclerotinia stalk and head rot, charcoal rot, blight and leaf spot], which reduces crop yield.

Allelopathic potential of *Ricinus communis* and *Jatropha curcas* was determined (27), on growth of seedlings of lettuce, cress, alfalfa and Italian grass which revealed that aqueous and methanolic extract of *R. communis* leaves inhibit the growth of seedlings. Leaf extract effect of dandelion have antibacterial potential on different strains of bacteria (26).

It is among of those plants that have ability of removing microbial agents (1). Phenolic content from the dandelion may be helpful for discovery of antibiotic medicines for many infections (32).

This investigation aimed to determine the allelopathic effects of aqueous extract of *T. officinale* L. and *R. communis* L. leaf extracts on sunflower and maize and study their herbicidal potential.

MATERIALS AND METHODS

2.1 Sterilization of seeds

The seeds of sunflower (cv. Parson 3) and maize (TP1217) were sterilized in 95 % ethanol for 2-3 min, the seeds were soaked in 10 % chlorox with shaking for 3-4 min and subsequently washed 4-5 times with the autoclaved distilled water.

2.2 Preparations of leaf extract of *Taraxacum officinale* and *Ricinus communis*

Leaves of *T. officinale* L. and *R. communis* L. were collected from Wah Cantt located on 33.7° N and 72.7° E. The seeds were washed and shade dried and finally powdered by mortar. Then 10 g powdered leaves were mixed in 100 ml autoclaved distilled water, incubated for 48 h at 28-30 °C, centrifuged at 30,000 rpm. Supernatant was collected and used as stock solution to prepare 0.3 % and 0.5 % extracts of both *T. officinale* L. and *R. communis* L. separately.

2.3 Method of leaf extract application to sunflower and maize

Surface sterilized seeds of maize and sunflower were soaked for 36 hours at 28 °C in 0.3 % and 0.5 % extract of *T. officinale* L. and *R. communis* L. After soaking sterilized seeds at 28 °C these were shifted to pots under natural conditions. Leaves of sunflower and maize were collected after two months during vegetative growth for biochemical analyses.

2.4 Chlorophyll and Carotenoids Content of Leaves

Chlorophyll and carotenoids contents of maize and sunflower leaves were measured following the modified method of Arnon (1949) (14). For determining the carotenoid content, the absorbance of reaction mixture was measured at 470 nm and for the chlorophyll was measured at 644 nm and 662 nm using spectrophotometer

2.5 Flavonoids Content

Total flavonoids were detected according to AlCl₃ method of Shraim *et al.*, (2021) (54). The homogenate of leaves (1 g) was prepared in 80 % methanol and was centrifuged at 3000 rpm for 15min. AlCl₃ reagent was prepared by taking 133 mg crystalline AlCl₃ and 400 mg crystalline sodium acetate, dissolved in 100 ml of 80 % methanol. Water (400 µl) and 1ml of AlCl₃ reagent were added to 1 ml of supernatant. The absorbance was recorded against blank at 430 nm. The total flavonoids content was expressed as mg quercetin equivalent per gram of extract.

2.6 Phenolics content

Phenolics content of leaves were detected by Folin-Ciocalteu method of Maurya *et al.*, (38). Gallic acid was used as standard. Distilled water (9 mL), plant extract (2 mL) and 1 mL Folin-Ciocalteu reagent were mixed thoroughly. Sodium-carbonate (10 mL) solution (7 %) was added to the mixture. At room temperature the mixture was incubated

for 90 min. Absorbance was noted at 765 nm. The phenolics content were expressed as mg gallic acid equivalent per g of extract.

2.7 Phytohormones Analyses

Fresh leaves of maize and sunflower were collected from treated and control plants, and the Indole acetic acid (IAA), gibberellic acid (GA) and abscisic acid content were determined following the method of (5) extracted in 80 % methanol with butylated hydroxy toluene (10 mg/l). The extract was centrifuged after 72 h at 3000 rpm for 15 min. Supernatant was dried using RFE (37 °C at 45 rpm). Dried residue was suspended in water. The pH of the aqueous extract was adjusted to pH 2.5-3 using 1N HCL and partitioned 3 times with ethyl acetate (1/3 volume). The ethyl acetate phase containing the hormone was dried in rotary evaporator. The residue was redissolved in Methanol (100 %). Wavelength for IAA was 280 nm and for GA was 254 nm at 36 °C.

2.8 Terpenoids analyses

Quantitative test for terpenoids was made by adapting the method of Palta (44). In this method dried plant extract (100mg= w_i) was soaked in 9 ml of ethanol for 24 h. Filtration was done, extracted with 10ml of petroleum ether using separating funnel, which was pre-weighed and left for complete drying of ether (w_f). Total terpenoids yield was calculated as per the formula:

$$(W_i - W_f / W_i \times 100).$$

2.9 Proline Content

The proline content of leaves was recorded by the method of (19). Proline content (mg/g) = K value \times dilution factor \times Absorbance (O.D) /weight of the sample
K value =19.6.

Enzymes Assays

2.10 Phenylalanine Ammonia-lyase (PAL)

Enzyme Phenylalanine ammonia-lyase was determined by the method of Dos Santos *et al.* (13). After grinding of fresh leaves (2 g) at 4 °C in 6 mL of 0.1 M sodium borate buffer (pH 8.8), extract was centrifuged at 4 °C (12,000rpm for 15 min) and the supernatant was used as the enzyme extract. The reaction mixtures consisting of 500 μ L sodium borate buffer (pH 8.7) and 250 μ L enzyme extracts pre-incubated at 40 °C (6 min). Addition of 300 μ L of 50 mM l-phenylalanine started the reaction. Addition of 50 μ L of 5 N HCl stopped the reaction. Again, the mixture was centrifuged at 3000 rpm for 20 min and absorbance was recorded at 275 nm. The activity of PAL was expressed as; nmol *t*-cinnamate $\text{min}^{-1} \text{g}^{-1}$ of fresh mass in relation to the peak area of *t*-CA standard solution (1 mg 100 mL^{-1} sodium borate buffer, pH 8.7).

2.11 Poly phenol oxidase activity

Enzyme activity was detected according to Jung *et al.* (29) method. A homogenized mixture of phosphate buffer (pH 6.8) with fresh leaves (0.2g) was made then centrifuged (4°C) at 10,000rpm for 20 min. Reaction mixture containing 500 μ l of the enzyme, 1500 μ L of assay buffer and 50mM pyrogallol was incubated at 30°C for 5 min to determine enzyme activity. Addition of 500 μ l of sulphuric acid stopped the reaction. The absorbance of purpurogallin formed was recorded at 42nm with UV spectrophotometer (U-1500 spectrophotometer HITACH Germany).

2.12 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity was detected by the method of Sharma *et al.* (50). At 4°C fresh leaves (0.5 g) were ground in phosphate buffer (4 mL) (pH 7.8), 1 % PVP was added and centrifuged at 15000 rpm for 20 min. The supernatant (0.8 ml) was collected in test tube. The reaction mixture comprises riboflavin (1.17×10^{-6}), methionine (0.1 M), potassium cyanide (2×10^{-5}) and nitroblue tetrazolium (5.6×10^{-5}) dissolved in 0.3 ml 0.05 M sodium phosphate buffer. The reaction mixture (4 mL) was mixed with enzyme extract (2 ml). One sample was kept in light at 30°C for 1 h to start the reaction. Similar sample was placed in dark for blank. The absorbance recorded at 560 nm.

2.13 Peroxidase (POD) Activity

POD activity was recorded by using method of Sunpapao *et al.* (56). Enzyme extract (0.1 mL) was taken and 100Mm MES buffer (pH 5.5), 0.05 % H₂O and phenylenediamine (0.1 %) was added. Absorbance was recorded at 485 nm.

2.14 Method of preparation of leaf extract for TLC

Shade dried leaves powder (10 g) of *T. officinale* and *R. communis* were soaked in 100 ml of 80 % methanol stirred for 30 mins then incubated at 27 °C for 36 h, centrifuged for 30 min at 30,000 rpm. Supernatant was collected and dried using rotary evaporator at 35°C. The dried leaf extract was re-dissolved in methanol. The extract was spotted on TLC plate coated with Silica gel HF254 and was eluted in a solvent system (butanol, acetic acid and autoclaved distilled water in 12: 3: 5 ratios) in the tank in dark at room temperature (25 °C -30 °C). The spots were visualized under UV lamp detector (spectroline, model ENF-240/FE) and trans illuminator (Biostep, UST, 20 m-BK). The R_f value of the bands which appeared under UV light were calculated. The silica gel corresponding to the major bands visible under UV lamp was scrapped and suspended in methanol, incubated overnight and centrifuged at 3000 rpm, passed through Millipore syringe filter and analyzed on HPLC. The sample was injected in HPLC, High-pressure liquid chromatography (SYKME Company) equipped with UV detector and C-18 column at 35°C. Commercial grade IAA, GA, ABA (Sigma Chemical Company USA) were used as standard for identification and quantification of phytohormones standard. IAA and GA were evaluated at 280 nm and 254 nm respectively using methanol at flow rate of 1 ml /min. The ABA were eluted using linear gradient of methanol (30-70 %) at a flow rate of 0.8 ml min⁻¹ at 254 nm. The retention time of ABA was determined by using authentic standard.

2.15 Germination of maize and sunflower

Results presented in Table 1 indicated that germination percentage was increased by all the treatments as compared to control. Maximum increase (57.5 %) over control was recorded in 3C treatment of maize, which differ non significantly for the measurement made on 6th day, minimum increase (11 %) as compared to control was recorded in 3T for the measurements made.

Whereas, in sunflower maximum increase (44.9 %) over control was recorded in 5C which was further increased by 20 % more on 6th day, minimum increase as compared to control was recorded in 3T for the measurements made.

RESULTS AND DISCUSSION

Priming of seeds of both sunflower and maize with *T. officinale* L. and *R. communis* L. enhanced the physiological and biochemical parameters of receiver plants over control. Germination (%) increased in all the treatments of sunflower. The increase in germination (%) was much higher after priming (pre sowing soaking of seeds) in the leaves extract of either *T. officinale* L. and *R. communis* L. Sea weed (*Sargassum wightii*) liquid extract increased germination, shoot and root growth, number of kernels and yield of *Triticum aestivum* (34). The higher concentration (0.5 %) of both the plants extract was more stimulatory than that of the 0.3 % extract in sunflower. The rate of germination demonstrated slow pace of germination in 5C treatment in maize whereas, the same treatment was more stimulating in sunflower. The observed stimulation in germination and growth parameters may be attributed to the induction of growth promoting hormones e.g Indole acetic acid (IAA) and Gibberellic acid (GA) in the leaves of treated plants following priming. The *T. officinale* L. and *R. communis* L. also produce IAA and GA in leaves (25).

1. Germination and Seedlings Growth

Germination (%) of maize and sunflower

Results presented in Table 1 indicated that germination percentage was increased by all the treatments as compared to control. Maximum increase (57.5 %) over control was recorded in 3C treatment of maize, which differ non significantly for the measurement made on 6th day, minimum increase (11 %) than control was recorded in 3T for the measurements made.

Whereas, in sunflower maximum increase (44.9 %) over control was recorded in 5C which was further increased by 20 % more on 6th day, minimum increase as compared to control was recorded in 3T for the measurements made (Table 1).

Table 1. Effects of leaf extract of *Ricinus communis* (castor) and *Taraxacum officinale* (dandelion) on seeds germination (%) of maize and sunflowers

Seed soaking, Leaf Extract conc (%)	Maize		Sunflower	
	Days after germination (DAG)			
	3	6	3	6
0 (Control)	45	45.9	26	69
0.3 % <i>T. officinale</i>	50	50	43	88
0.5 % <i>T. officinale</i>	66.7	66.7	37	94
0.3 % Castor	70.9	70.9	31	92
0.5 % Castor	52.5	55	51	100

The effect of leaf extract of castor and dandelion on germination percentage of seeds of sunflower and maize measured after 3rd and 6th day of germination. The value represents the mean of three replicates. After 6th day of germination, seedlings were transferred to pots.

Fresh and dry weight of shoot of maize and sunflower

Table 2 shows the fresh and dry weight shoot of maize and sunflower. The fresh shoot weight was increased in all the treatments of maize and sunflower. In maize, maximum increase (52.9 %) as compared to control was recorded in 5T treatment the maximum increase (38.2 %) was recorded in 3T treatment. In sunflower, the maximum increase

(34.4 %) as compared to control was recorded in 5C treatment. Minimum increase (0.7 %) was noticed in 5T treatment.

Table 2. Effects of leaf extract of *T. officinale* and *R. communis*. on fresh and dry weight (g) of shoot of maize and sunflower, measurement made at harvest.

Seed soaking, Leaf Extract conc (%)	Maize		Sunflower	
	Fresh weight	Dry weight	Fresh weight	Dry Weight
0 (Control)	34 (\pm 0.2)	17 (\pm 0.03)	29 (\pm 0.21)	19 (\pm 0.05)
0.3 % <i>T. officinale</i>	47 (\pm 0.03)	27 (\pm 0.02)	37 (\pm 0.05)	22 (\pm 0.05)
0.5 % <i>T. officinale</i>	52 (\pm 0.02)	24 (\pm 0.01)	50 (\pm 0.05)	26 (\pm 0.07)
0.3 % Castor	49 (\pm 0.11)	23 (\pm 0.01)	25 (\pm 0.15)	20 (\pm 0.2)
0.5 % Castor	48 (\pm 0.2)	25 (\pm 0.05)	39 (\pm 0.29)	21 (\pm 0.2)

The data depicts mean of three replicates and \pm represents standard error value. Measurements for fresh and dry weight of shoot were made at harvest (103rd days after germination).

All the treatments showed increase in dry weight of shoot. Maximum increase (52.9 %) was recorded in 5T treatment in maize whereas minimum increase (76.4 %) as compared to control was noticed in 3C. Maximum increase (36.8 %) was recorded in 5T treatment of sunflower whereas minimum increase (5.26 %) as compared to control was noticed in 3C. The observed increase in the fresh and dry weight of shoot following treatment with the extract of *T. officinale* L. and *R. communis* L. may be attributed to the increased water uptake for which higher concentration (0.5 %) was more stimulating particularly in case of *T. officinale* L. The observed increase in the dry weight of plants following seed priming with the plant extracts may be attributed to the leaves extracts induced increase in IAA and GA level of plants. Application of 0.2 % and 0.4 % *Ecklonia maxima* leaves extract to *Phaseolus vulgaris* L. depicted increase in fresh and dry weight, number of pods and increased biomass (57).

2. Biochemical analysis

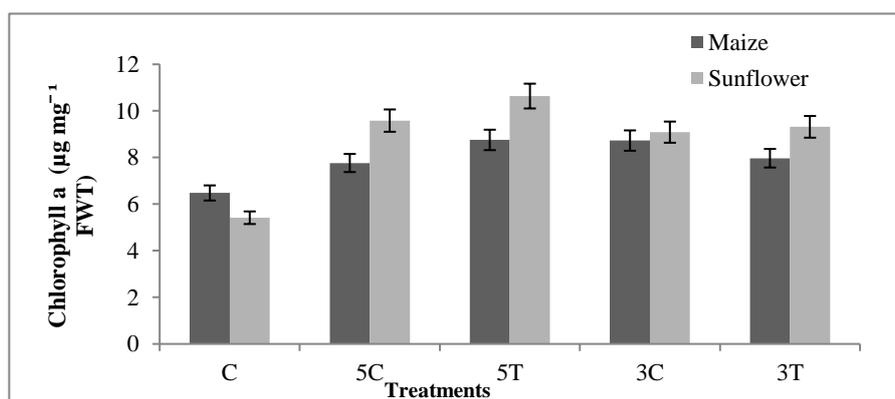


Figure 2. Effects of leaf extract of castor and dandelion on chlorophyll a content of leaves of maize and sunflower. Three replicates of each treatment were taken. The bar on graph depicts standard error. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

2.1. Chlorophyll a content of maize and sunflower

Chlorophyll a content was increased in all the treatments, both in sunflower and maize (Figure 2). In maize, chlorophyll a content was recorded maximum (35 %) over control in 3C and minimum increase (18 %) as compared to control was noted in 3T. In sunflower maximum increase (96.36 %) over control was noted in 5T. The least increase (67.62 %) as compared to control was recorded in 3C.

2.2 Chlorophyll b content of maize and sunflower

All the treatments of maize and sunflower showed increase in chlorophyll b content. In maize, maximum increase (719.5 %) over control was recorded in 3T and minimum increase (22.6 %) was noted in 5C. In sunflower, chlorophyll b content was maximum (246 %) over control in 5C, and minimum increase (15 %) was recorded in 3T (Figure 3).

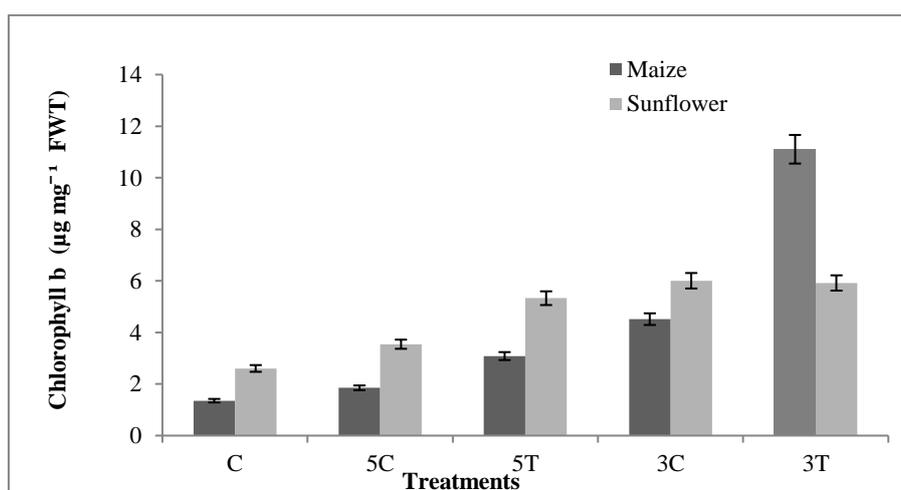


Figure 3. Effects of leaf extract of castor and dandelion on chlorophyll b content of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

Chlorophyll a influence the photosynthetic capacity of plants (42). Production rate of plants is strongly indicated by chlorophyll content in leaves (37). Higher concentration of *T. officinale* L. extract (5T) was more effective in increasing chlorophyll a content in both maize and sunflower. Fresh and dry weight of shoot being maximum in 5T as compared to control this increase was correlated with the augmentation of chlorophyll a. It is evident from the result that *T. officinale* L. and *R. communis* L. extracts were more responsive in sunflower than maize. Both chlorophyll a and b were detected in leaves of dandelion (53).

Chlorophyll b is accessory pigment supplying energy to chlorophyll a, both the pigments are involved in photo assimilation (52). The two plants differ in their response to dandelion and castor extracts for chlorophyll b. The increased carotenoids content was observed in 3C in maize and 3T (lower concentration of dandelion extract) treatment in sunflower. The carotenoids play significant role in energy transfer, photosynthesis and redox

reactions (17), Carotenoids are responsible for pigmentation and antioxidant property (59). Moreno *et al.* (39) studied increase in photosynthesis due to increase carotenoids in dandelion leaves. It was demonstrated that different plants use different mechanism for protecting the photosynthetic machinery. The higher carotenoids content was detected in 3C in sunflower in which chlorophyll b was also higher, this demonstrated that both chlorophyll b and carotenoids are used in 3C treatment for protecting the photosystem. Response of sunflower were concentration independent.

The higher value of protein content in maize and sunflower leaves were recorded in 5T and 5C treatments respectively. Lower concentration (3C treatment) of *R. communis* L. was stimulatory to protein production in maize but, higher concentration of *R. communis* L. (0.5 %) was more stimulatory in sunflower. Likewise, 5T, higher concentration of *T. officinale* L. extract showed greater protein content in maize. Higher protein production due to seed priming may assist the plant to combat stress. Proteins are responsible for speeding up chemical reactions (enzymes), membrane transport, energy producing reactions e.g electron transport and intracellular structure. Ricin protein present in castor leaves is a carbohydrate binding protein (49), *T. officinale* L. leaves contain protein (16). Colburn *et al.* (10) reported protein in dandelion leaves.

2.3 Carotenoids content of maize and sunflower

The carotenoids content showed increase in all the treatments of maize and sunflower. In maize, maximum increase (251.8 %) over control was recorded in 3T and minimum increase (33.3 %) in 5C treatment. In sunflower, maximum increase (119 %) over control was noted in 3T and minimum increase (71.5 %) was recorded in 5C treatment (Figure 4).

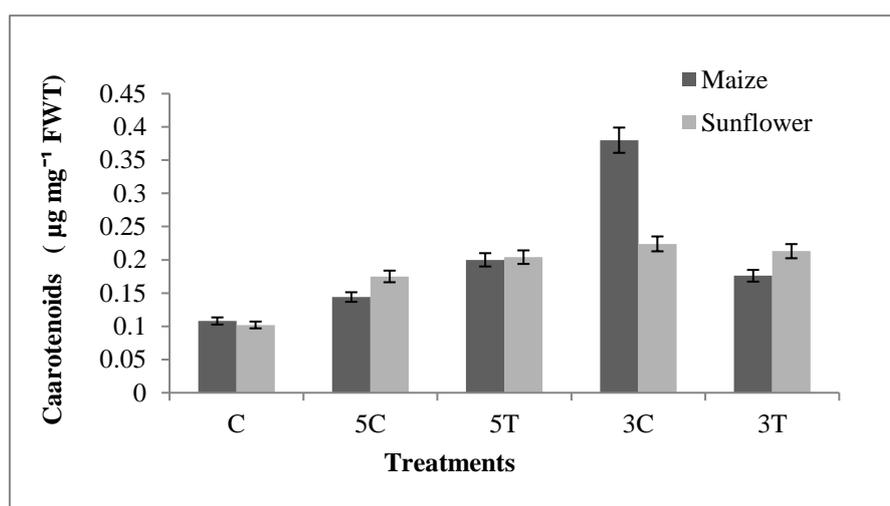


Figure 4. Effects of leaf extract of castor and dandelion on carotenoids content of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

2.4 Protein content of maize and sunflower

The protein content was increased in all the treatments (Figure 5). In maize the maximum (61 %) increase over control was in 5C. The minimum increase (23.8 %) was recorded in 3C. In sunflower maximum increase (51.8 %) was noted in 5T and minimum increase (15 %) was recorded in 3T.

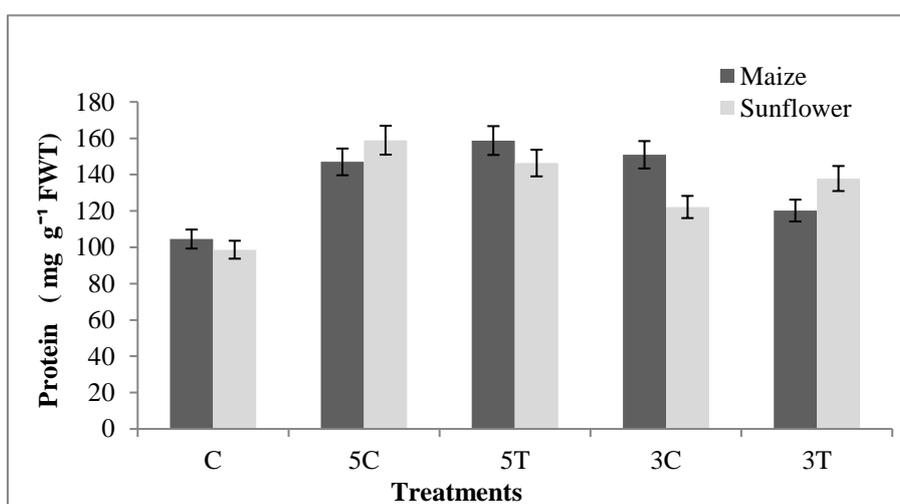


Figure 5. Effects of leaf extract of castor and dandelion on protein content of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

2.5 Proline content of maize and sunflower

In maize, proline content was recorded maximum (160 %) over control in 3T (Figure 6). The minimum increase (85 %) in proline content was recorded in 5C. In sunflower, maximum increase (109 %) was noted in 5T and minimum increase (11.99 %) over control was recorded in 3T.

2.6 Flavonoids content of maize and sunflower

In maize, flavonoid content was recorded maximum (163.22 %) over control in 3T and minimum increase (43.9 %) as compared to control was noted in 5C. In sunflower the maximum increase 324 % over control was noted in 3C. The least increase (50.5 %) as compared to control was recorded in 5T (Figure 7).

Flavonoids and phenolics are antioxidants and protect the cell from oxidation (18). Flavonoids play role in defense and signaling between plants and microorganisms (46). Phenolics are meant for protection against stress, involved in pigment and lignin biosynthesis provide structure stability and scaffolding support to plants. Phenolics repel microbes and pathogens (8). Phenolics protect plants from pathogens attack (6). Seed priming resulted in the apparent higher accumulation of flavonoids content in leaves of 3C treatment in maize and 3T treatment in sunflower. Maximum contents of phenolics in maize and sunflower were found at lower concentrations of *T. officinale* L. and *R. communis* L. extracts (3T and 3C).

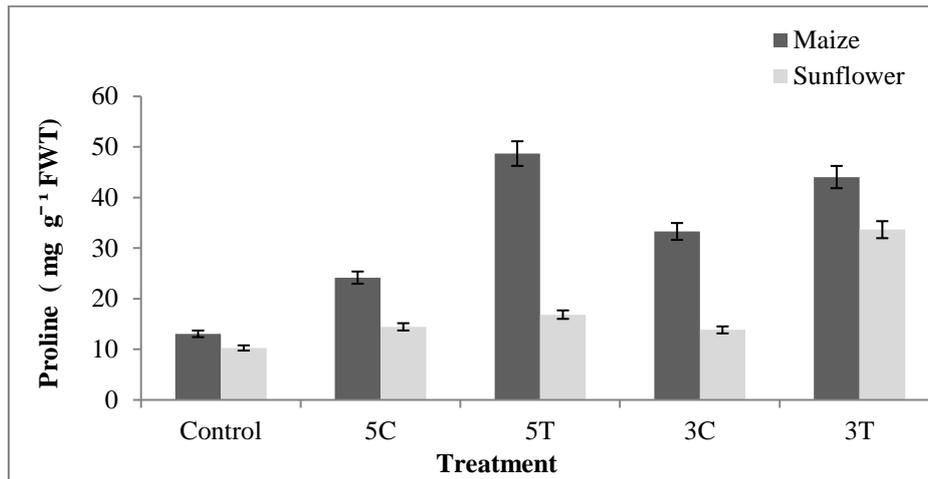


Figure 6. Effects of leaf extract of castor and dandelion on proline content of leaves of maize and sunflower. Leaves of two months old plants were harvested. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

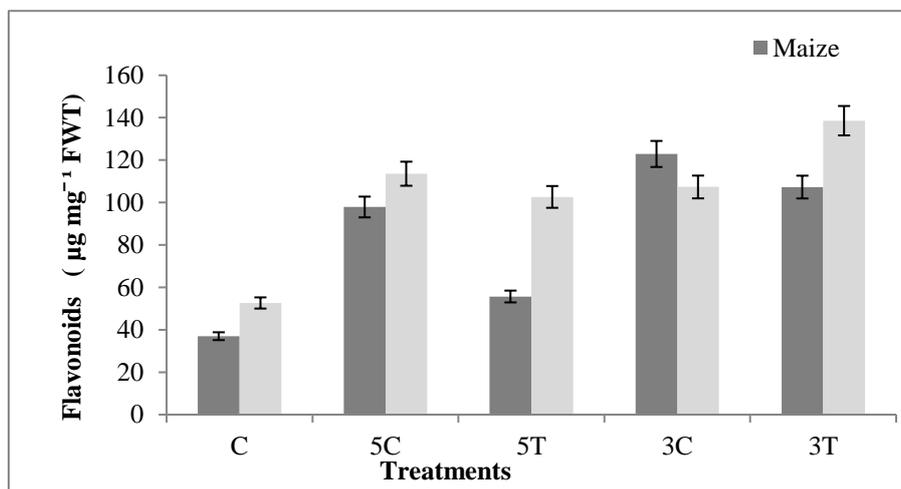


Figure 7. Effects of leaf extract of castor and dandelion on flavonoid content of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

Flavonoids, Phenolics and terpenoids are secondary metabolites. These phytochemicals can be antimicrobial, act as attractants/repellents, or as deterrents against herbivores. Plant defense system and stresses due to environment are controlled by phenolics (23).

2.7 Phenolic content of maize and sunflower

In maize leaves, phenolic content was recorded maximum (196.96 %) over control in 3T. The least increase (130.3 %) was recorded in 3C. In sunflower, the maximum increase (117.07 %) over control was noted in 3T. The least increase was recorded in 3C (Figure 8).

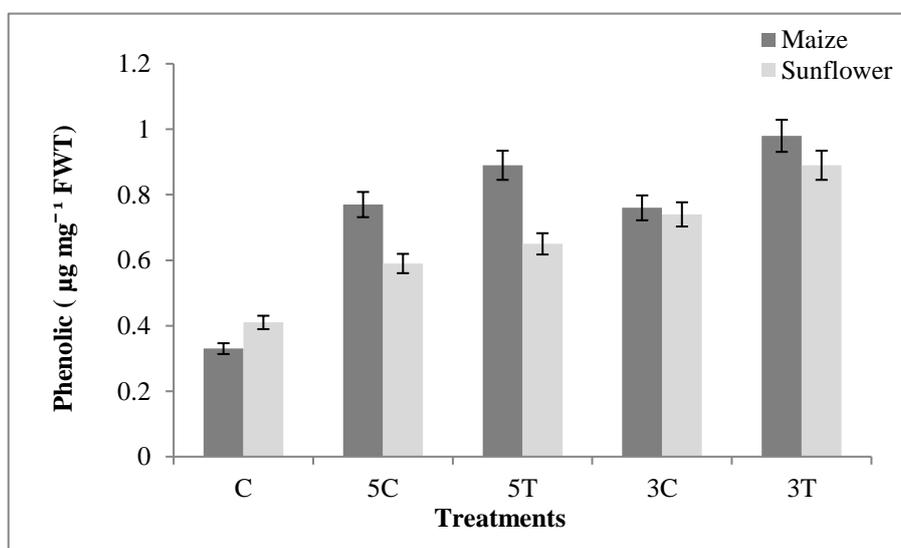


Figure 8. Effects of leaf extract of castor and dandelion on phenolic content of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

Proline is an osmoregulant under stress (30). Proline helps in osmotic adjustment and plays a protective role (21). Proline content was maximum in 5T (higher concentration of *T. officinale* L. extract) in maize and 3T (lower concentration of *T. officinale* L. extract) in sunflower.

On the contrary, sugar content was maximum in 3T in maize and 5T in sunflower. Possibly the higher concentration of proline in maize as compared to sunflower may demonstrate that maize used proline as osmoregulant whereas, sunflower used sugar as osmoregulant. Castor extract showed increase in proline content (33).

2.8 Terpenoids content of maize and sunflower

Terpenoids content was increased in all the treatments of maize and sunflower (Figure 9). In maize, maximum increase (80 %) over control was recorded in 3T and minimum increase (20 %) was recorded in 5C. In sunflower, maximum increase (77.7 %) was recorded in 3T, minimum increase (11.1 %) was recorded in 5T.

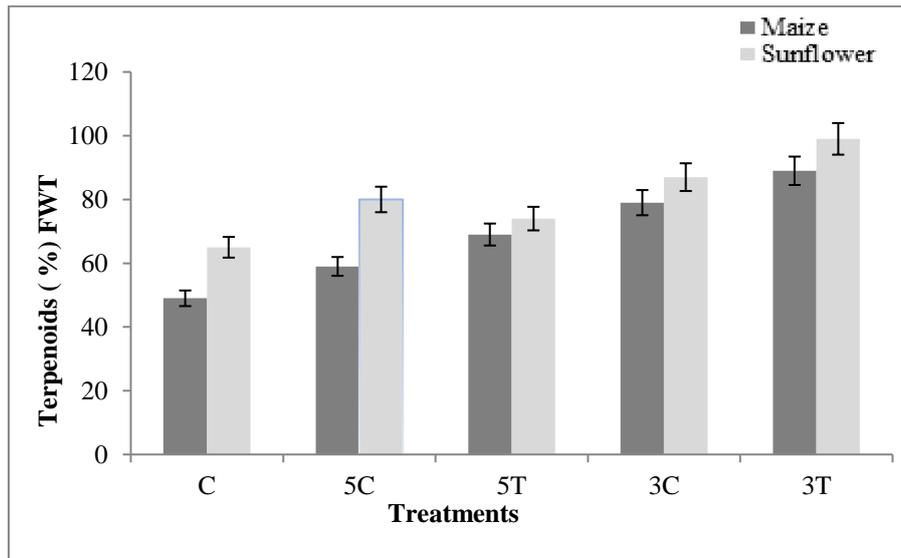


Figure 9. Effects of leaf extract of castor and dandelion on terpenoids content of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

Terpenoids are used in growth and development of plant and helpful in defense activities of plants. Terpenoids in Castor leaves were also detected. Terpenoids were isolated from ethanolic extract of dandelion leaves; having antibacterial activity. Presence of terpenes, phenolic compounds in leaves are potential source of useful nutrients and are used in antibiotics for treating different pathogenic strains (3). Terpenoids content was recorded maximum in 5C treatment in maize and 3T treatment in sunflower which might be attributed to phytochemicals present in the extracts applied. In maize and sunflower, the lower concentration (0.3 %) of *T. officinale* L. extract was more effective than higher concentration (0.5 %) of *T. officinale* L. extract (5T).

3. Enzymes Activity

3.1 PAL Activity of Maize and Sunflower

In maize, PAL activity was recorded maximum (278 %) over control in 5C (Figure 10), while the minimum increase (102 %) was recorded in 5T. In sunflower, maximum increase (150 %) over control was noted in 3C and minimum increase (33.33 %) was recorded in 3T.

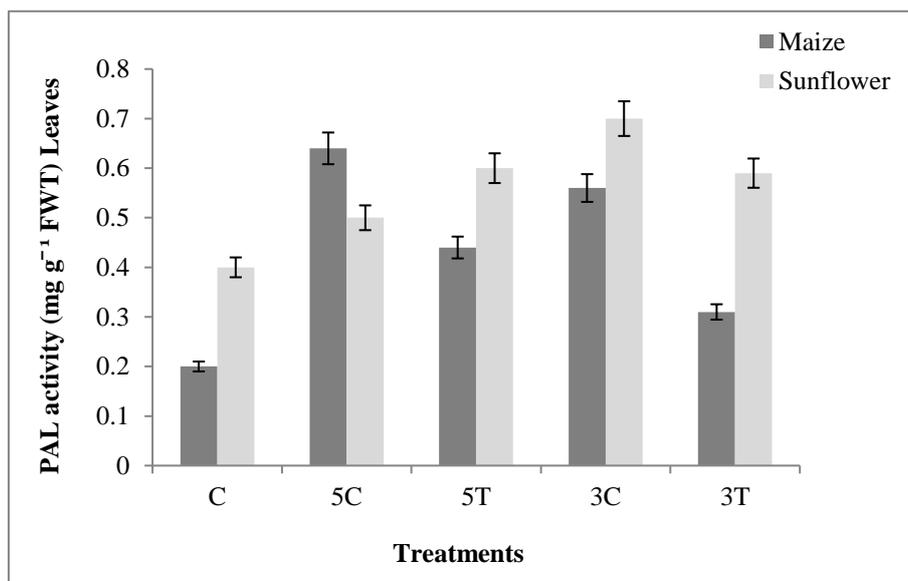


Figure 10. Effects of leaf extract of castor and dandelion on PAL activity of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

3.2 PPO Activity of maize and sunflower

The effect of leaf extract of castor and dandelion on PPO activity of leaves of maize and sunflower is presented in Figure 11. In maize, maximum increase (233 %) over control was recorded in 3T and minimum increase (33.33 %) was noted in 5T. In sunflower, PPO activity was maximum (75 %) over control in 5C and minimum increase (24 %) was recorded in 3T. Defense related enzyme PAL result in disease resistance in plants (36). PAL activity involved in lignin biosynthesis as demonstrated by (7), in *R. communis* L. (35), recorded several fold increase in PAL and PPO in leaves of castor exposed to drought stress. The PPO is responsible for many physiological functions in plants (58), PAL enzyme has important function during pathogen and insect attack and plants vigor is affected by PPO enzyme (47). Phenylalanine ammonia lyase (PAL) was greater in both the plants following application of *T. officinale* L. and *R. communis* L. extracts as compared to control. PAL activity was more enhanced in 5T in maize than in sunflower, in which it was concentration independent. The PPO enzyme activity was increased in 5C treatment of maize than that of 3C treatment of sunflower indicating that priming with *R. communis* L. extract induced PAL enzyme in sunflower and maize thereby imparting resistance. Polyphenol oxidase (PPO) catalyzes oxidation of phenolic compounds. In maize low concentrations of both extracts were effective (3T and 3C). In sunflower high concentrations of both extracts were effective (5T and 5C).

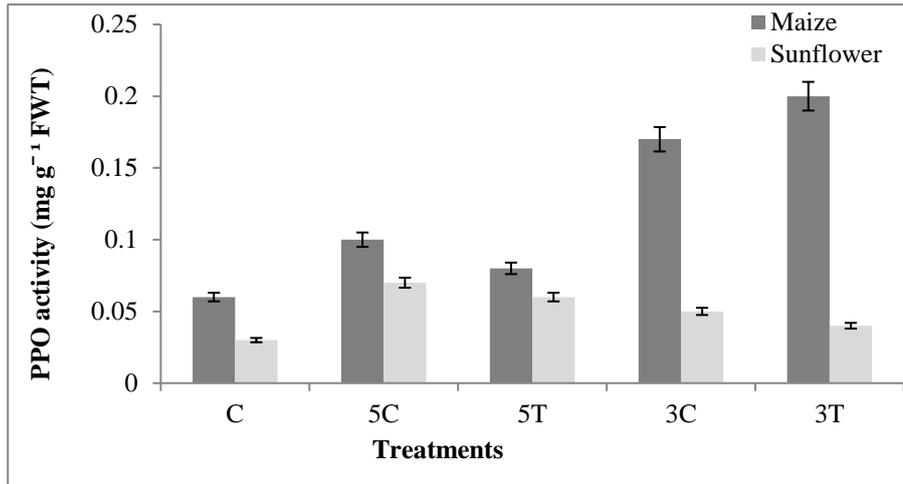


Figure 11. Effects of leaf extract of castor and dandelion on PPO activity of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

3.3 SOD Activity of maize and sunflower

The SOD activity in maize leaves enhanced in all the treatments (Figure 12). The SOD activity was recorded maximum (90.9 %) over control in 5C. The minimum increase (12.5 %) was recorded in 3T. Increase in SOD activity of sunflower was noted in all the treatments of sunflower. The highest increase (58.9 %) over control was noted in 5C. The least increase (23.2 %) as compared to control was recorded in 3T.

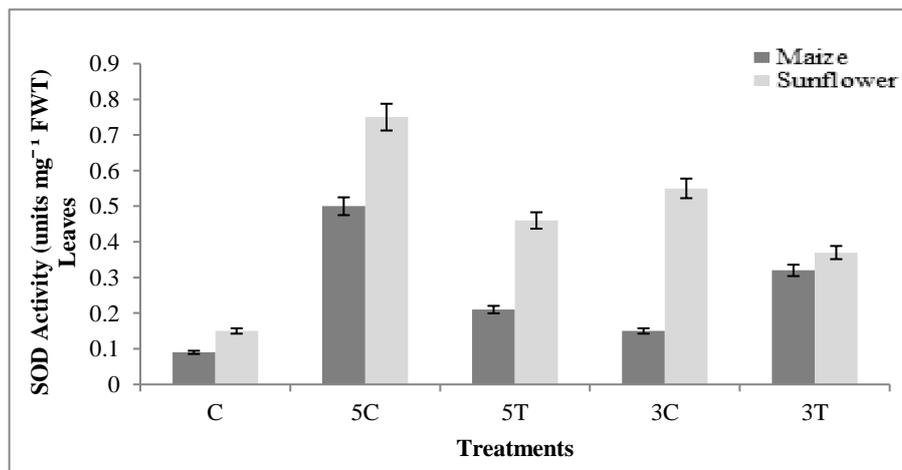


Figure 12. Effects of leaf extract of castor and dandelion on SOD activity of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

3.4 POD Activity of Maize and Sunflower

In maize and sunflower leaves, POD activity increased in all the treatments (Figure 12). The POD activity was recorded maximum (176.9 %) over control in 3T and minimum increase (53.8 %) was noted in 3C. In sunflower, the maximum increase (165.9 %) over control was noted in 5T. The least increase (42.5 %) was recorded in 3T.

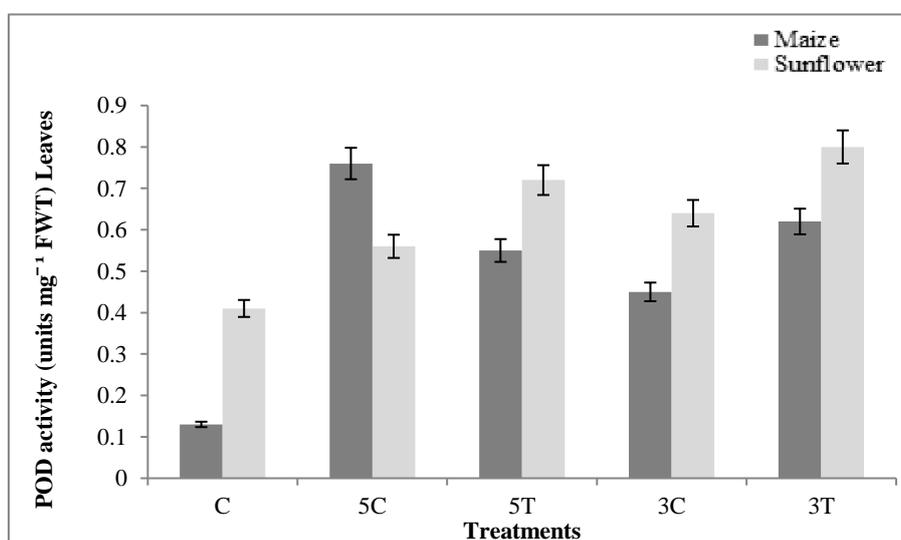


Figure 13. Effects of leaf extract of castor and dandelion on POD activity of leaves of maize and sunflower. Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. Bar on graph depicts standard error.

Superoxide dismutase (SOD) is the first scavenger of ROS (Reactive oxygen species) and act as antioxidant defense against oxidative stress in plants (20). SOD enzymes are antioxidant and protect cellular components from being oxidized. The 5C treatment in both the plants had shown increased SOD activity. In maize 3T and 5T induced SOD activity perhaps attributed to less scavenging activity. High concentration of *R. communis* L. extract (5C) has shown increased activity of SOD in maize and sunflower. Peroxidase enzyme (POD) play important role in eliminating superoxide and hydrogen peroxide, thus protecting cellular function and structure (9). POD activity was higher in 5C treatment in maize and 3T treatment in sunflower may be attributed to the phytochemicals present in *T. officinale* L. Higher concentration of *R. communis* L. extract (5C) in maize and (3T) lower concentration of *T. officinale* L. extract in sunflower were effective in detoxification of H₂O₂ and superoxide.

It is inferred from the present investigation that the two plants *T. officinale* L. and *R. communis* L. having allelopathic potential impart allelochemicals e.g. the secondary metabolites phenolics, terpenoids and flavonoids, when the seeds of sunflower or maize were soaked in these plant extracts prior to sowing. Further results indicate that the priming of seeds of plants with extracts of these allelopathic plant induce better potential in plant for oxidative and osmotic stresses as well as for defense against pathogen as observed by

enhanced activities of SOD, POD, PAL and PPO. In addition, these plant extracts also improve growth and development of plants by increasing the phytohormone contents of leaves of plants. Hence, the interacting plants can better adapt the biotic and abiotic stresses and competition with weeds.

4. Phytohormones analysis:

Phytohormones content were determined in fresh leaves of 2-months old plants of maize and sunflower.

4.1. IAA content of maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.)

The maximum increase (665.9 %) in IAA content of maize was observed in 5T over control. Least increase (39.5 %) over control was observed in 5C. The maximum increase (996.5 %) of IAA content of sunflower over control was exhibited in 5C whereas, least increase (76.57 %) was observed in 3T.

Table 3. Effects of leaf extract of *R. communis* L. and *T. officinale* L. on phytohormones content (mg g⁻¹) of maize and sunflower FWT leaves

Seed soaking, Leaf Extract conc. (%)	Maize		Sunflower	
	IAA	GA	IAA	GA
0 (Control)	4.71 (0.050)	14.1 (0.096)	1.75 (0.0345)	14.61 (0.06)
0.5 % <i>T. officinale</i>	38.52 (0.051)	237.6 (0.106)	22.31 (0.406)	37.75 (0.101)
0.3 % Castor	73.99 (0.239)	165 (0.836)	15.59 (0.448)	40.37 (0.084)
0.3 % <i>T. officinale</i>	40 (0.117)	204.5 (0.342)	3.09 (0.113)	18.23 (0.175)
0.5 % Castor	13.22 (0.064)	98.18 (0.300)	89.19 (0.072)	118 (0.024)

Test crops seeds soaking treatments: C: Control, 3T: 0.3 % *T. officinale*, 5T: 0.5 % *T. officinale*, 3C: 0.3 % Castor, 5C: 0.5 % Castor. FWT= Fresh weight. Data Mean of 3-replicates. The value in parenthesis represent standard error values of the mean data.

4.2 GA content of maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.)

Phytohormones IAA and GA content and ABA content in the leaf extract of *R. communis* L. and *T. officinale* L. TLC eluant of the leaf extract of *R. communis* L. revealed maximum (1.35 ± 0.85) IAA content at Rf 0.97. In *T. officinale* L. maximum IAA (mg g⁻¹ of dry weight of leaves) content (0.29 ± 0.052) recorded at Rf value 0.92. TLC eluant of the leaf extract of *R. communis* L. revealed the maximum GA (mg g⁻¹ of dry weight of leaves) content (0.61 ± 0.225) at Rf 0.5. In *T. officinale* L. maximum GA content (0.17 ± 0.008) was recorded at Rf value 0.64. TLC eluant of the leaf extract of *R. communis* L. revealed the maximum (10.54 ± 0.394) ABA (µg g⁻¹) content at Rf 0.5. In *T. officinale* L. maximum ABA content (7.54 ± 0.128) was recorded at Rf value 0.48. saponins, flavonoids, triterpenoids, sesquiterpenes, glycosides and phenols (22).

Table 4. IAA and GA content (mg g⁻¹ of dry weight of leaves) and ABA content (μg g⁻¹) of *Ricinus communis* and *Taraxacum officinale*.

	<i>Ricinus communis</i> L.	<i>Taraxacum officinale</i> L.
ABA	10.54 (±0.42)	7.54 (± 0.057)
IAA	1.35 (± 0.039)	0.29 (± 0.006)
GA	0.61 (± 0.02)	0.17 (± 0.008)

CONCLUSIONS

It is inferred from the present investigation that the two plants *T. officinale* L. and *R. communis* L. having allelopathic potential impart allelochemicals e.g. the secondary metabolites phenolics, terpenoids and flavonoids, when the seeds of sunflower or maize were soaked in these plant extracts prior to sowing. Further results indicate that the priming of seeds of plants with extracts of these allelopathic plant induce better potential in plant for oxidative and osmotic stresses as well as for defense against pathogen as observed by enhanced activities of SOD, POD, PAL and PPO. In addition, these plant extracts also improve growth and development of plants by increasing the phytohormone contents of leaves of plants. Hence, the interacting plants can better adapt the biotic and abiotic stresses and competition with weeds. Further investigations are required to have an insight into the molecular mechanism for detecting allelopathic potential of these plants. The compatibility of the treated plants may need to be checked in field under natural environmental conditions, to determine the allelopathic potential and response of the recipient plants.

CONFLICT OF INTEREST

The author(s) have no conflict of interest.

AUTHOR'S CONTRIBUTION

A.B., designed the research, reviewed the manuscript, and edited finally. S.S., performed the experiments. M.N and Nimisha revised the manuscript. All authors have read and approved the final version of the manuscript.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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