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PROTECTION OF SOYBEAN FROM AMBIENT UV RADIATION THROUGH FOLIAR SPRAY WITH *LAWSONIA INERMIS* LEAVES EXTRACT

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Abstract

Use of green technology for the crop improvement in place of harmful chemical agents could not only provide protection of plants from biological and non-biological agents but also minimize the consequent health hazards for the consumers. Many studies have shown the deleterious effects of ambient UV radiation on crop plants. Present study was conducted with Soybean variety JS-335 to assess the effect of foliar spray with *L. inermis* leaves aqueous extract on vegetative growth (plant height, leaf area, total biomass), UV absorbing substances (UAS), photosynthesis (chl-a, chl-b, chl-a/b ratio, carotenoids), nodule parameters (number of nodules and nodule fresh weight) and yield. The results showed significant changes in vegetative and physiological parameters. JS 335 showed improved performance after foliar spray with *L. inermis* leaves extract. It indicates the extract has the potential of protecting plants from ambient UV radiation. Treatment of plants with *L. inermis* extract could enhance the performance of soybean. The application could be used to improve the performance of other plants as well and could replace the use of chemical reagents for the betterment of crops.

Key words : *Lawsonia inermis,* UV radiation, soybean, foliar spray.

Introduction

Organic farming, Bio-stimulants and Green technology are the words which have attracted the attention of researchers working in the field of crop improvement. Conventional mineral fertilizers and other type of chemical sprays for the protection of crop plants from different kinds of biotic and abiotic stresses could result in their protection but could have quite harmful consequences for the health of consumers and the harmful residues could enter the food chain. Biostimulants from plants have many beneficial effects, in the terms of quality and yield enhancement (Blunden 1991, Crouch and Van Staden, 1994). Earlier studies have shown the positive effect of sea weed extracts foliar spray on crop improvement on flowers grasses, cereals and vegetable species (Crouch and Van Staden, 1994). According to Mooney and Van Staden, 1986 Seaweed extracts contains many growth stimulating ingredients. These Stimulants have been reported to enhance the growth and yield of plants plants (Rama Rao, 1991), enhance antioxidant properties (Verkleij, 1992) and help to cope with different kinds of stress (Zhang and Schmidt, 2000, Zhang *et al.*, 2003). Besides harmful biological agents plants have to cope-up with the non-biological agents such as temperature, draught, and UV radiation. In the process of making food by the process called as photosynthesis they are unavoidably exposed to the harmful UV rays present in the sunlight. Plants need protection from these UV rays to minimize the damage and foliar spray could be applied as a possible technique to protect plants with biological extract.

Use of biological extracts have increased in acceptance due to their potential use in organic and sustainable agriculture in recent years (Russo and Beryln, 1990). Positive effect of *L. inermis* leaf extract could be due to synergistic effect of growth regulating substances present in the extract, though the mode of action still remains unclear (Fornes *et al.*., 2002).

India is rich in its biodiversity with medicinal plants. *Lawsonia inermis* (syn. *Lawsonia alba*), commonly known as 'mehndi'. This plant is small tree or, much branched shrub with greyish-brown bark. According to Muhammad & Muhammad, 2005 Leaves are arranged in opposite phyllotaxy, small petiole i.e. sub-sessile, shape is elliptic or broadly lanceolate, entire margine, acute or obtuse apex, length 2–3 cm long and 1–2 cm wide. This plant is frequently cultivated in India. Besides its use in cosmetics for staining hands and as a hair dye, the leaves are used as a prophylactic agent against skin diseases (Ahmed *et al.*., 2000). According to Hsouna *et al.*, 2011 *L. inermis* has good antioxidant volumes and this species could be used as a probable source of new natural antioxidants.

Soybean (*Glycine max* (L.) Merrill) cultivation is about four decades in India. It is considered to be the most important sources of protein and can be a potential source of high protein food for the majority of Indian population. In terms of its use in human foods and livestock feeds, Soybean (*Glycine max* L.) is said to be the most important grain legume crop in world. Since 1970, soybean production has been almost doubled that of any other oilseed crop. The contribution of soybean to world oilseed production increased from 32% in 1965 to over 59% in 2020 (Soy Stats, 2020).

The ozone layer absorbs about 90% of the UV-B radiation reaching the Earth from the sun (WMO, 1995). Due to different kinds of pollution and human activities its thickness is getting reduced. India is situated in a low ozone belt and accepts more UV-B radiation compared with temperate higher latitudes (Mitra, 1991). There has been a significant decline in the total ozone column (TOC) at many stations in India (Sahoo *et al.*. (2005). Many studies have shown that current levels of UV are high enough to impart changes in the structural and physiological components of plants. In our earlier study we found Soybean veriety JS-335 is susceptible to the current levels of ambient UV radiation (Baroniya *et al.*., 2011)

The present study was conducted to evaluate the UV protective effect of *L. inermis* leaves extract application on growth, UAS, nodule parameters, photosynthetic pigments and yield in soybean (*Glycine max*) under experimental conditions.

Materials and methods

Plant material

Soybean variety JS-335 seeds were obtained from DSR Indore and were sown in pots under natural conditions. Plants growing under ambient UV radiation and treated with foliar spray of leaf extract from *L. inermis* were compared with the control plants growing in similar conditions with equal amount of DW spray. Plants were grown in pots (30-cm in diameter and 50-cm deep).

Collection and preparation of *L. inermis* leaves extract

The extract used in this study was obtained from *L. inermis* leaves belonging to the family Lythraceae. *L. inermis* leaves were collected from Dewas (India, 22°57'12''_N and 76° 2'47''_E) in June 2018. After the botanical identification of the species with the help of Flora of Madhya Pradesh a specimen was preserved for future reference. The samples were prepared according to the method of (Eswaran *et al.*, 2005).

Experimental design and treatments

In present experiment control i.e. water sprayed plants were compared with three treatments .5g l^{-1}; 1g l^{-1}; 2g l^{-1} (weight/volume; w/v) of *L. inermis* leaves in water. Two sprays of *L. inermis* leaves extract were applied, one at the seedling stage (10 days after sowing) and the second at the third trifoliate stage (30 days after sowing), thereafter the test plants were sprayed after every fifteen days, (*i.e.*, at 45 DAE and 60 DAE).

Plant height and leaf area

Plant height was measured from the soil level to shoot tip in five plants in each replicate at third trifoliate stage. The areas of the third trifoliate leaves were assessed by drawing the outlines on graph graph paper and weighing the cut paper outlines. A calibration curve was prepared by weighing 0–500 mm2 sections of the graph paper. The mean of the 5 leaves was taken as the measured value for each treatment.

Total biomass accumulation at third trifoliate stage

15 plants (five plants from each replicate) were randomly selected to measure the total dry weights after oven drying at 60 °C for 72 h.

Root length and root biomass

The roots were removed carefully from the pots and washed prior to measuring the lengths. The roots without the nodules were dried on a filter paper, then dried at 60

°C for 72 h before weighing. Number of root nodules root length, and nodule fresh weight per plant, were measured for all the investigational plants.

Number of nodules/fresh weight of nodules

The number of nodules on the roots were counted and recorded, then weighed for all treatments after 45 days.

Yield parameters

At maturity fifteen plants (five plants from each replicate) were randomly selected and pods were separated and the total number of pods were recorded. Number and dry weights of seed components were recorded after drying at 35–40°C for 10 days.

Extraction and estimation of enzymes

All operations were performed at 4°C. The enzyme extract was prepared by homogenizing 0.5 g leaves with 10% (w/v) polyvinylpolypyrrolidone and 10 ml of 0.1 mol/ L phosphate buffer (pH 7.0) for SOD, APX, GR and GPX. The homogenate was ûltered through four layers of cheesecloth, centrifuged at 15,000 rpm for 30 min and the supernatant obtained was used to determine the activity of those enzymes described.

Superoxide dismutase (SOD) [EC 1.15.1.1] activity was assayed as described previously by Beauchamp and Fridovich (1971). The reaction mixture contained 0.24 mM riboûavin, 2.1 mM methionine, 1% Triton-X 100, 1.72 mM nitroblue tetrazolium chloride (NBT) in 50 mM sodium phosphate buffer (pH 7.8) and 200 ll of enzyme extract (in 50 mM Tris–HCl buffer, pH 7.8). The activity was expressed as Units/milligram protein. One unit of SOD was deûned as the amount of enzyme required to cause 50 % inhibition in the rate of NBT photoreduction.

Guaiacol peroxidase (GPX) [EC 1.11.1.7] was assayed as described by Maehly (1955). The reaction mixture contained 0.5 ml enzyme extract (in 0.02 M, phosphate buffer, pH 6.4), 1 ml 20 mM guaiacol and 3 ml 0.02 M phosphate buffer. The reaction was started by the addition of 0.03 ml of H2O2 (88.2 mM). The initial and ûnal absorbance was recorded at 475 nm for 2 min. The activity was calculated as the change in OD/min/mg protein.

UV absorbing substances

UV absorbing substances (UAS) were monitored by the procedure of Mirecki and Teramura (1984) in fully matured third trifoliate leaves at fifth node. Leaf discs of 100mm^2 were taken and extracted in 10 ml of acidified methanol (79:20:1 v/v methanol, water, HCl) for measuring UV absorbing compounds. The absorbance was taken at 305 nm using Shimadzu spectrophotometer. Absorbance was expressed on a mass basis (A mg^{-1} fw).

Chlorophyll content

The total chlorophyll content was determined by dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam, 1979). For the extraction of chlorophyll 50 mg well cleaned fresh third trifoliate leaves were chopped and transferred to a test tube containing 10 ml of DMSO. The contents were incubated at 65^{0}C for 3 h and volume was made up to 10 ml with DMSO. The content was allowed to settle down and the absorbance was recorded at 470, 646 and 663 nm with Systronics UV/VIS spectrophotometer. Equations of Wellburn and Lichtenthaler (1984) were used to calculate the chl *a,* chl *b,* total chl and carotenoid concentrations.

Results and discussion

Plant height, leaf area total biomass accumulation

The plant height, the leaf area (third trifoliate leaves) and the total biomass accumulation were enhanced by the foliar spray in soybean. A maximum enhancement of the plant height after the foliar spray was obtained in 1g l^{-1} treatment 29.14% and a minimum enhancement was recorded for .5g l^{-1} (6.78%) as compared with control plants (Table 1).

Similar trend was observed for the increases in the leaf area and the total plant biomass accumulation (Table 1)

Yield parameters

The foliar spray increased the number of pods per plant, seeds per plant and seed weight per plant in all of the treatments (Table 1). Maximum increase was recorded in 1g l^{-1} treatment as compared to the control.

Growth parameters of the below ground parts

Root length in control plants growing under control resulted in less root growth. The root length of the plants grown under 1g l^{-1} was found highest. (Table 3).

Number of nodules/fresh weight of nodules

The foliar spray increased the number of nodules and fresh weights of nodules in all of the treatments (Table 3). Maximum increases were recorded in 1g l^{-1} treatment as compared to the control. A similar trend was obtained for the fresh weights of nodules. (Table 3)

AOS activity

Foliar spray with *L. inermis* leaves extract decreased the activities of SOD and POD in soybean. Maximum reduction was observed in 1g l^{-1} treatment as compared to the control. (Fig. 1)

TABLE 1: Effect of *Lawsonia* leaves extract on plant height, No. of Pods plants^{-1}, No. of Seeds plant^{-1}, 100 Seed Weight, Biomass (without Pod & Shell) plant^{-1}, Seed yield g plant^{-1}, Leaf Area in soybean var. JS-335.

Treatment (%)	Plant height (cm)	Leaf area (cm^2)	No. of Pods plants^{-1}	No. of Seeds plants^{-1}	100 Seed Weight	Shoot Biomass (FW)	Seed yield g plant^{-1}
Control	41.30 (±0.45)	47.00 (±0.54)	17.40 (±0.21)	42.30 (±0.33)	8.27 (±0.11)	6.90 (±0.12)	3.50 (±0.07)
T1	44.10 (±0.31)	51.32 (±0.63)	19.90 (±0.17)	47.00 (±0.35)	8.47 (±0.12)	8.41 (±0.14)	3.98 (±0.04)
T2	53.33 (±0.52)	66.00 (±0.43)	20.20 (±0.23)	51.00 (±0.43)	8.50 (±0.21)	8.79 (±0.11)	4.01 (±0.05)
T3	52.1 0(±0.43)	56.42 (±0.51)	20.00 (±0.25)	50.00 (±0.41)	8.48 (±0.13)	8.75 (±0.12)	3.97 (±0.06)

TABLE 2: Effect of *Lawsonia* leaves extract on photosynthetic pigments and UAS in soybean var. JS-335.

Photosynthetic pigments	Control		Treated		T2		T3	
Chl-a	3.65	±0.035	4.31	±0.038	4.33	±0.038	4.21	±0.038
Chl-b	0.57	±0.013	0.74	±0.023	0.76	±0.023	0.73	±0.023
Chl a/b ratio	6.32	±0.045	5.76	±0.018	5.68	±0.018	5.72	±0.018
Total Chlorophyll	4.21	±0.104	5.03	±0.224	5.09	±0.224	4.94	±0.224
UAS	1.32	±0.104	0.92	±0.101	0.67	±0.101	0.73	±0.101

TABLE 3: Effect of *Lawsonia* leaves extract spray on root length, number of nodules, root fresh weight in soybean var. JS-335.

Treatments	Root length (cm)	No. of nodules	Root FW (g)	Root DW (g)	Nodule FW (mg)
Control	17.21±0.13	10.20±0.09	0.94±0.01	0.31±0.012	64.52±0.54
T1	21.23±0.13	12.31±0.09	1.15±0.01	0.37±0.011	72.85±0.55
T2	22.61±0.12	13.41±0.08	1.21±0.01	0.39±0.013	75.37±0.63
T3	22.24±0.13	13.21±0.09	1.20±0.02	0.38±0.011	74.45±0.66

Chlorophyll content

There was an enhancement in Chlorophyll pigments after foliar spray in all of the treatments (Table 2). Maximum enhancement was recorded in 1g l^{-1} treatment as compared to the control.

UV absorbing substances

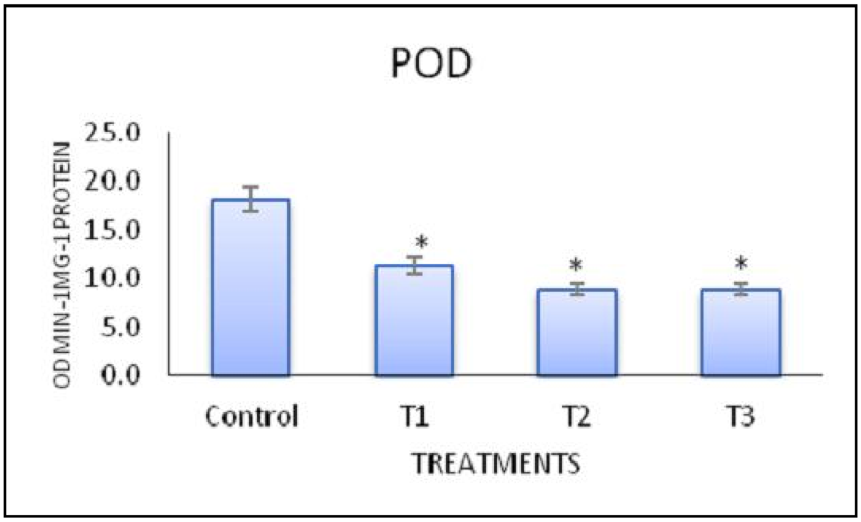
Foliar spray decreased the amount of UAS in all of the treatments (Table 2). Maximum reduction were recorded in 1g l^{-1} treatment as compared to the control.

Statistical analysis

All data are presented as mean of 15 plants. The data are expressed as mean ± SEM and analyzed by the analysis of variance (ANOVA) followed by post hoc Newman–Keuls Multiple Comparison Test (*P\0.05) using Prism 5 software for window, Graf Pad Software, Inc, LaJolla, CA, USA.

Green technology is the demand of present time. Due to overuse of artificial and chemical agents on plants the productivity and quality are getting reduced. Techniques which exert least damage to the quality and could improve the crop productivity are being researched these days. Crop production could be improved with the help of green technology. Due to the depletion of stratospheric ozone layer amount of UV reaching the earth surface has increased. Many studies have shown damaging effects of ambient levels of UV-B on Soybean plants, Baroniya *et al.*, 2013. UV-B radiation is presently at its maximum levels and is anticipated to revert to the pre-1980s level at the mid-latitudes by 2040–2070, if all member countries implement the Montreal Protocol (WMO 2003). According to Mitra, 1991 India lies in a low ozone belt and thus obtains more amount of UV-B radiation as compared with temperate higher latitudes. Sahoo *et al.,* 2005) also found a reduction in TOC and enhanced UV reaching the earth surface.

Sunlight is used by the Plants for photosynthesis and during this they are also exposed to solar ultraviolet (UV) radiation. Variety of morphological alterations in higher plants are reported in many studies in response to UV-B. Several recent field studies have shown, however, that near-ambient UV-B can reduce root length, number of nodules and rhizome internode elongation (Zaller *et al.*, 2002; Chouhan *et al.*, 2008). Morphologically plants show significant reduction in the stem elongation and leaf area



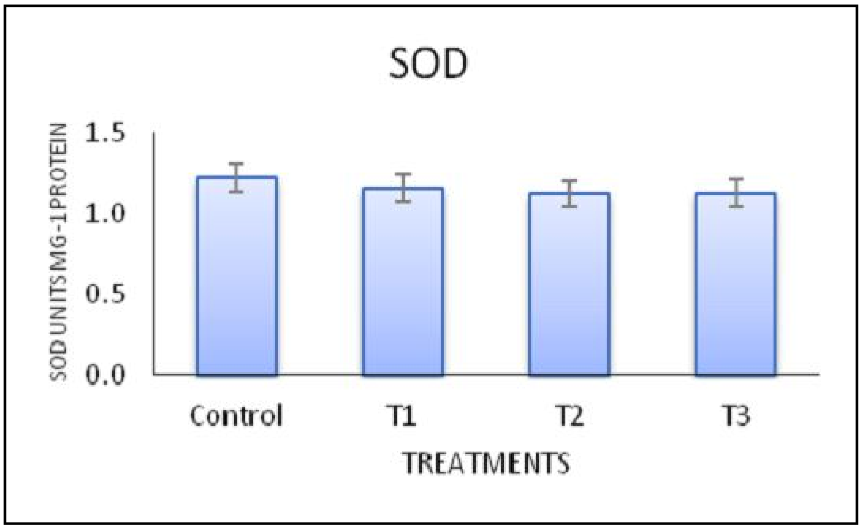


Fig. 1: Effect of foliar spray of soybean with *L. inermis* leaves extract on peroxidase and superoxide dismutase activity in the third trifoliate leaf of soybean variety JS-335. Values are signiûcantly different at (*P\0.05) from control (Newman–Keulis Multiple Comparison Test)

by exposure to the ambient levels of UV radiation. There is a need of protecting crop plants from these ambient UV radiations. Herbs could provide an effective option for protecting crop plants from these harmful UV rays as they have been used in medicines and cosmetics from centuries. Their potential to treat different skin diseases, to adorn and improve the skin appearance is well-known. Effective botanical antioxidant compounds are widely used in traditional medicine and include tocopherols, flavonoids, phenolic acids, nitrogen containing compounds (indoles, alkaloids, amines, and amino acids), and monoterpenes.

Till now there has not been any study been conducted to assess the protective role of these herbs for crop plants. The potential of herbal plant could be tested for protection of crop plants against ambient levels of UV radiation. In the present study we observed significant morphological and physiological change in soybean plants treated with *L. inermis* leaves extract as compared to compared to control plants.

DNA protective activity of water extract of L. *inermis* L. leaves was reported by Manish Kumar *et al.*, 2016. Presence of different polyphenolic compounds such as ellagic acid, catechin, quercetin, kaempferol etc. were reported in HPLC profile of water extract of *L. inermis* L. leaves and these compounds were attributed for the antioxidant activity Manish Kumar *et al.*, 2016. Also stress generated oxyradical scavenging potential of this extract was reported by Manish Kumar *et al.*, 2016.

Spray with water extract of *L. inermis* L. leaves on soybean had a positive effect on all growth parameters. Growth parameters were comparatively found better in 1g l^{-1}treatment (Table 2). Plant height, leaf area and total biomass yield were enhanced significantly after foliar spray. Root length, root biomass and number of nodules per plant enhanced significantly after the foliar application. Number of seeds per plant and pods number per plant increased after foliar application. Enhancement in all these parameters was more significant at 1g l^{-1}treatment. As per the literature available this is the first study conducted on foliar application of *L. inermis* leaves extract. As per the available literature our results are similar to studies conducted (Aldworth and Van Staden, 1987, Russo *et al.*, 1994) on marigold, where they found an enhancement vegetative growth of marigold by seaweed extract application. Sivasankari *et al.*, 2006 found similar results with *Vigna sinensis* L. According to Mooneyand VS, 1986 the betterment of growth in plants after foliar spray could be due to the presence of growth promoting substances in the extract. Our results suggest that, along with the growth promoting substances the extract consist of UAS and phenolic compounds which formed a layer on the leaf surface and protect plants from ambient UV radiations. Also radical scavenging potential of extract could be responsible for lowering the levels of oxyradicals in the treated plants which could induce better growth and other physiological parameters.

Overall improvement in production of seeds is due to increased vegetative growth, number of pods per plant and number of seeds per pod after spray with water extract of *L. inermis* L. leaves. Rama Rao (1991) also found Similar results in Zizyphus *mauritiana* Lamk. after foliar applications of seaweed extract.

Environmental stress like ambient UV radiation can also cause oxidative stress (Yannarelli *et al.*, 2006, Yang *et al.*, 2005). In the present study we found a reduction in activity of SOD and POD after foliar application of *L. inermis* leaves extract (Fig. 1). This could be due to the free radical scavenging properties of extract. The AOS include not only free radicals such as superoxide (O_2^-) and hydroxyl radicals (.OH), but also hydrogen peroxide (H_2O_2) and singlet oxygen (O_2). These AOS can cause oxidative damage to membrane lipids, nucleic acids, and proteins (Foyer *et al.*, 1994). To keep this damage to a minimum, plants possess enzymatic and nonenzymatic antioxidative defense systems. Among the latter flavonoids (UV absorbing substances) are very important. The enzymatic antioxidants include enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), guaiacol peroxidase (POD; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11), glutathione reductase (GR; EC 1.6.4.2), and others. O_2^- is rapidly converted to H_2O_2 by the action of SOD (Noctor and Foyer, 1998). An alternative mode of H2O2 destruction is via peroxidase which is found throughout the cell (Jimenez *et al.*, 1997). In the present study control plants are having higher amount of SOD and POD enzyme activity to combat the free radicals generated by the ambient UV radiation in soybean varieties. On the other hand, foliar spray with *L. inermis* extract provides a radical scavenging potential and hence the reduced enzyme activities. resulting in the enhanced primary metabolism in soybean.

Our results suggest that soybean plants grown after foliar spray with *L. inermis* leaves extract show enhanced plant growth and biomass. This increased growth and biomass appear to be due to the better harvesting of light and lower level of free radicals in treated plants. foliar spray with *L. inermis* leaves extract triggered carbon sequestration in biomass, which seems directly linked to enhancement in photosystem components in soybean plants. Since the increase in biomass could be linked to the enhancement of photosynthetic components, the Chl *a* and *b* content, total Chl, and Chl *a/b* ratio were measured in the mature third trifoliate leaves in soybean. The Chl *a* and *b* content per unit FW of leaf showed enhancement by the foliar spray with L. *inermis* leaves extract (Table 2). An increase in total chlorophyll after the reduction of solar UV-B has also been reported in cotton (Dehariya, 2011), Beech samplings (Laposi *et al.*, 2008) *Helianthus annuus* (Cechin *et al.*, 2007) and Cymopsis (Amudha *et al.*, 2005).

Plants in tropical regions are exposed to high ambient levels of UV-B radiation compared to the temperate zones. Plants are protected against the penetration of UV-B into internal tissues by accumulating phenolic compounds to absorb the excess UV-B radiation (Rozema *et al.*, 1997). Flavonoids are produced primarily in the epidermal layers of the leaves and absorb UV-B radiation effectively while transmitting PAR to the chloroplasts. In addition to their role as sunscreens, flavonoids are also known to have an antioxidant function and can help dissipate UV-B radiation within the leaf. In our results we found reduced amount of UAS after foliar spray (Table 2). It could be due to reduced penetration of ambient UV into the epidermal layer due to the presence of UAS and phenolic compounds in the extract which have made a layer on the leaves of Soybean.

Whole plant extracts have proven to be better UV protectants as compared to a specific component of the extract. Use of herbal extracts for protection of crop plants against UV radiation present in the sunlight is a better solution for UV protection. These herbal extracts could prove as UV protectants against ambient levels of UV radiation and plant protected by these extracts may perform better in terms of growth, development and yield parameters.

Conclusions

From the results of present study, it could be concluded that *L. inermis* leaves water extract has protective potential for plants against ambient UV radiation because of presence of phenolic compounds which have potential as antioxidant and role in DNA protection. Further detailed analysis of compounds present in the extract is needed to be studied to validate its plant protective potential against ambient UV radiation.

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