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QUANTITATIVE CHANGES IN PROTEINS, PIGMENTS AND SENNOSIDES OF *CASSIA ANGUSTIFOLIA* VAHL TREATED WITH MANCOZEB

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Abstract

Seeds of *Cassia angustifolia* Vahl treated with different concentrations (0%, 0.1%, 0.15%, 0.2%, and 0.25%) of a fungicide, Mancozeb, were sown in field conditions in order to study the effect of the stress caused by this fungicide on pigment concentration and the protein and sennoside contents of the plant. Protein content and pigment concentration increased at 0.10% dose of Mancozeb, but higher doses caused a significant decline in comparison with the control. Application of Mancozeb resulted in a considerable dose-dependent reduction in the contents of sennoside *a* (nearly 7-32% reduction in young leaves, 14-36% in mature leaves and 8-24% in pods) and sennoside *b* (about 11-36% reduction in young leaves, 5-38% in mature leaves and 5-30% in pods), thus affecting indirectly the therapeutic property of the plant.

Introduction

Agriculture suffers from activities of pests like fungi, weeds and insects, resulting in reduced yields. Chemical pesticides resolved the crisis to a great extent, but their excessive use proved hazardous as they cause stress in plants (Edvreva, 1998). The degree of damage varies with plant species, their active ingredients, and the dosage and frequency of pesticide application. Plant performance is an outcome of integration of a wide range of processes, and is strongly modified by environmental influences. The increased use of pesticides over the past few decades has resulted in a more intensive farming on cultivable lands, but the plants exposed to pesticides exhibit growth declines. Among the modern pesticides, fungicides are extensively used in agriculture, and Mancozeb is a broad-spectrum contact fungicide, which is used to control a variety of plant diseases. The present study examines its effects on the production of primary as well as secondary metabolites in *Cassia angustifolia* Vahl, a leguminous plant with great medicinal value, by analyzing the contents of protein, green pigments and sennosides. The medicinal importance of the species is due largely to its sennoside content, which is used to cure intestinal disorders, jaundice, anaemia, typhoid and dermal diseases. It is a powerful cathartic used in the treatment of constipation, as it stimulates intestinal peristalsis (Majid, 2010).

Materials and Methods

Field experiments were conducted in the kharif season (July–October) at the experimental field of Jamia Hamdard New Delhi. Seeds of Indian Senna (*Cassia angustifolia*), procured from Indian Agricultural Research Institute, New Delhi, were sown in plots (4m X 1.5m each) having 4 rows with a row to row distance of 15 cm and plant to plant distance of 10 cm. The number of plants per m^2 was 15. The field was duly ploughed and leveled prior to sowing, and plots were made with proper bunds along with necessary irrigation channels . Irrigation was done as and when required. The crop was kept free from weeds by manual weeding operation done regularly. In each plot, $1m^2$ area was earmarked for harvest analysis of seed yield and its components. The remaining rows (except the border rows) were used for the periodic sampling. The seeds were treated prior to sowing with 0% (Control), 0.10% (T_1), 0.15% (T_2), 0.20% (T_3) and 0.25% (T_4) concentrations of mancozeb. The pesticide solution was prepared by dissolving the required amount of pesticide in double distilled water. Each experiment was repeated three times. The sampling was done at three developmental stages i.e., at the pre-flowering (45 DAS), flowering (75 DAS) and post-flowering (90 DAS) stages. Samples were stored in deep freezer (-80°C) or used immediately. The control and the treated plants were maintained at a uniform water supply.

Soluble protein content was estimated following the method of Bradford (1976). A fresh clean and chopped leaf material (0.2 g) was homogenized in 2.0 ml of 0.1 M-phosphate buffer (pH 7.2) with the help of a pre-cooled mortar and pestle. The homogenate was transferred to the pre-cooled centrifuge tube and centrifuged at 5000 rpm for 10 min. One ml of supernatant was taken in a microfuge tube, to which an equal amount of chilled 10 % TCA was added. It was centrifuged at 3300 rprn for 10 min. The resulting supernatant was discarded and pellet was washed with acetone. It was then dissolved in 1.0 ml of 0.1N NaOH. To a 0.1 ml aliquot, 0.5 ml of Bradford's reagent was added and vortexed. The tubes were kept for 10 min for the maximum colour development. Absorbance was then recorded at 595 nm on uv-vis spectrophotometer (Model DU 640 B, Beckman, USA). Protein concentration was calculated using BSA (Sigma) as the Standard. The protein content was expressed in mg g^{-1} fr. wt.

Pigment concentration was estimated by the method of Hiscox & Israelstam (1979), which involves estimation of plant pigments without maceration. Leaves were washed with DDW and chopped. 100 mg of the chopped leaf material was taken in vials in triplicates and 10 ml of DMSO was added to each vial. The vials were then kept in oven at 65º C for 30 min. The absorbance of the solution was recorded at 663, 645, 510 and 480 nm on UV-vis spectrophotometer (Model DU 640 B, Beckman, USA).

Concentrations of chlorophyll a, chlorophyll *b* and carotenoids were calculated in mg g^{-1} fw by using the following formulae given by MacLachlan & Zalik (1963), Duxbury & Yentsch (1956) and Barnes *et al*., (1992), respectively.

$$\text{Chlorophyl a (mg g}^{-1}\text{ fw)} = \frac{12.3(A_{663}) - 0.86(A_{645})}{d \times 1000 \times W} \times V$$

$$\text{Chlorophyl b (mg g}^{-1}\text{ fw)} = \frac{19.3(A_{645}) - 3.60(A_{663})}{d \times 1000 \times W} \times V$$

$$\text{Carotenoids (mg g}^{-1}\text{ fw)} = \frac{7.6(A_{480}) - 1.49(A_{510})}{d \times 1000 \times W} \times V$$

where, d = distance travelled by the light path, W = weight of the leaf material taken, V = volume of the extract, and A = Absorbance.

Sennoside concentration: Quantitative analysis of sennosides *a* and *b* in the young and mature leaves and pods at pre-flowering, flowering and post-flowering stages was done by HPLC.

The sennoside of leaves was extracted using the method of Lemmli *et al.,* (1985). One hundred milligram of dried (60°C/72h) leaf taken in vials was extracted three times with 20, 20 and 10 ml of double distilled water. The extract was pooled and vials re-kept in boiling water for 15 min. After cooling, the extract was filtered through the Whatman's filter paper no. 1, and the final volume was made to 50 ml by adding double-distilled water. Five milliliters of cooled extract was dded with 10 ml of 15% ferric chloride, and incubated at 80 °C for 20 min. Thereafter, 0.1 ml of concentrated HCl was added and extracted three times with 20, 20, and 10 ml of ether using a separating funnel. The aqueous layer was collected and its volume made up to 50 ml by adding double-distilled water. High performance liquid chromatography (HPLC delta 600, Waters, USA) was employed for the quantitative analysis of sennosides *a* and *b*, using the method followed by Srivastava *et al.,* (1983). The solvent used in HPLC was a mixture of tetrahydrofurane with 2% glacial acetic acid and HPLC water (1:3) with the flow rate of 1ml min^{-1} through the column C_{18} using UV detector (280nm) set at the ambient temperature.

The sennoside concentrations (mg g^{-1} d.wt.) were calculated as follows:

$$\text{Sennoside concentration} = \frac{\text{Peak area of the sample x Amount of sennoside in 10 µl}}{\text{Peak area of sennoside x Sample weight}}$$

Statistical analysis: The data obtained were analyzed by two-way classification of ANOVA (Cochran & Cox, 1957), to confirm whether the values were significantly different, taking $p<0.05$ as significant level.

Results

Soluble protein content: The soluble-protein content of the control leaves enhanced significantly with growing age of the plant, reaching the maximum at the flowering stage and thereafter declining steadily. In the treated samples, an increase was observed with 0.10% dose of Mancozeb, whereas the other treatments (0.15-0.25) caused a significant reduction in the protein content (Fig. 1). The extent of variation from the control due to different Mancozeb treatments ranged approximately from 8-26% at pre-flowering stage, 3-14% at flowering stage and 12-45% at post-flowering stage of plant development (Fig. 1).

Pigment concentration: The chlorophyll '*a*' content in the control leaves increased significantly with growing age of the plant to attain the maximum at the flowering stage, beyond which it declined steadily. In the treated plants, chlorophyll '*a*' content increased with 0.10% Mancozeb and then declined significantly with higher treatments. The variation from the control, caused by the different Mancozeb treatments ranged between 5-30 at pre-flowering stage, 5-23 at flowering stage and 7-30 at post-flowering stage of the species studied (Table 1).

The Chlorophyll '*b*' content of the leaves also showed a similar variation trend with growing age of the plant in control as well as treated plants, but the extent of change was relatively small. The percent variation, with reference to the control, ranged between 1-3.5 at the pre-flowering, 1-2 at the flowering and 0.5-12 at the post-flowering stages (Table 2). Thus, the chlorophyll content increased initially with 0.10% Mancozeb concentration, but declined significantly with higher concentrations.

Carotenoid content in the leaves enhanced significantly with the age of the plant, attained its peak in the flowering stage and then showed a significant reduction thereafter. Compared with the control, the treated plants showed increase in the carotenoid content with 0.10% Mancozeb dose, but a consistent decline was recorded with higher doses. The extent of variation from the control due to fungicide treatments was nearly 2-9% at pre-flowering, 1-6% at flowering, and 3-11% at post-flowering stages (Fig. 2).

Sennoside concentration: The sennoside (*a* and *b*) contents were the maximum in pods (Table 3), followed by immature leaves (Table 4), and then by mature leaves (Table 5). Application of Mancozeb caused a significant decrease in both the sennosides. The effect was dose-dependent and hence the maximum reduction was observed with 0.25% Mancozeb treatment (Tables 3-5). Compared with the control, percent variation of sennoside *a* in the treated plants ranged from 8-24 in pods, 6-32 in immature leaves and 22-41 in mature leaves. Likewise, for sennoside *b*, the variation was 5-30% in pods, 10-36% in immature leaves, and 5-38% in mature leaves.

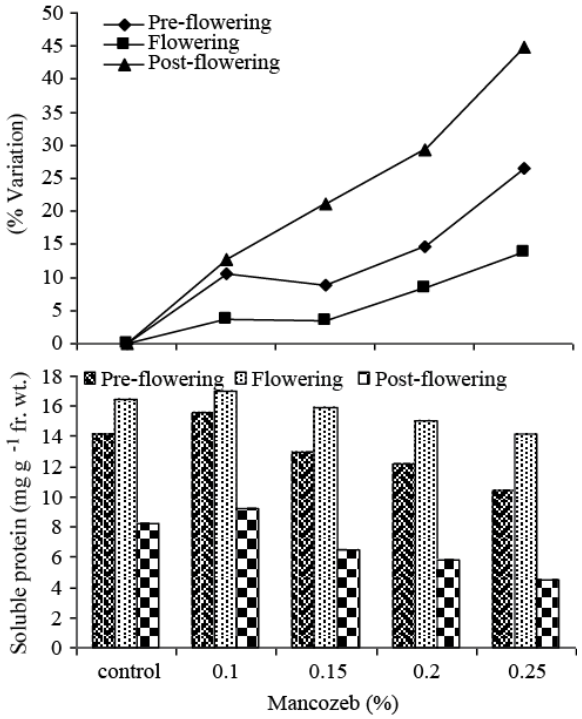

Fig. 1. Variation in the foliar soluble-protein content of the control and the Mancozeb-treated samples of *Cassia angustifolia*, as observed at the pre-flowering, flowering and post-flowering stages of plant growth.

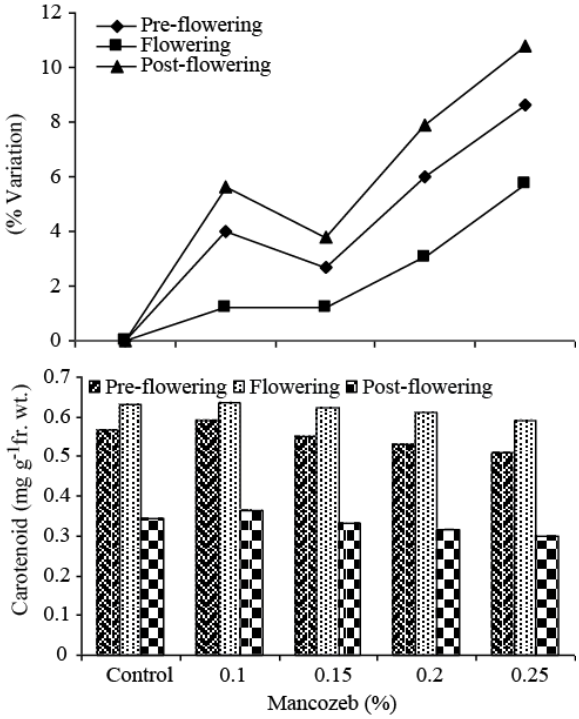

Fig. 2. Variation in the carotenoid content of the leaves of the control and the Mancozeb-treated *Cassia angustifolia*, as observed at the pre-flowering, flowering and post-flowering stages of plant growth.

Table 1. Variation in Chlorophyll 'a' content (mg g^{-1} fr. wt) in the leaves of the control and the Mancozeb-treated *Cassia angustifolia*, as observed at various stages of plant growth (Mean ± SE in µm). Parentheses indicate percent variation.

Treatments (%)	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	1.83 ± 0.02 (0.0)	2.23 ± 0.02 (0.0)	1.63 ± 0.01 (0.0)
0.10 %	2.04 ± 0.02 (11.47)	2.37 ± 0.02 (6.26)	1.85 ± 0.02 (13.67)
0.15 %	1.73 ± 0.11 (5.46)	2.11 ± 0.01 (5.37)	1.52 ± 0.01 (6.93)
0.20 %	1.45 ± 0.02 (20.58)	1.94 ± 0.01 (13.13)	1.32 ± 0.01 (18.77)
0.25 %	1.26 ± 0.02 (30.96)	1.72 ± 0.01 (22.83)	1.14 ± 0.01 (30.00)

CD at 5%: Developmental stages: 0.041, Treatments: 0.053, Treatment x Developmental stages: 0.047

Table 2. Variation in Chlorophyll 'b' content (mg g^{-1} fr. wt) in the leaves of the control and the Mancozeb-treated *Cassia angustifolia*, as observed at various stages of plant growth (Mean ± SE in µm). Parentheses indicate percent variation

Treatments (%)	Developmental stages		
	Pre-flowering	Flowering	Post-flowering
Control	0.75 ± 0.003 (0.0)	0.88 ± 0.002 (0.0)	0.71 ± 0.002 (0.0)
0.10 %	0.76 ± 0.001 (1.2)	0.89 ± 0.001 (1.24)	0.71 ± 0.001 (0.97)
0.15 %	0.74 ± 0.002 (1.58)	0.87 ± 0.002 (1.17)	0.70 ± 0.002 (0.53)
0.20 %	0.74 ± 0.001 (1.87)	0.87 ± 0.001 (1.6)	0.70 ± 0.003 (0.85)
0.25 %	0.73 ± 0.002 (3.45)	0.86 ± 0.003 (2.39)	0.62 ± 0.001 (12.7)

CD at 5%: Developmental stages: 0.0003, Treatments: 0.0004, Treatment x Developmental stages: 0.001

Table 3. Variation in the sennoside content of pods (mg g^{-1} dr. wt) of the control and the Mancozeb-treated *Cassia angustifolia*, as observed at various stages of plant growth (Mean ± SE in µm). Parentheses indicate percent variation.

Treatments (%)	Sennoside 'a'	Sennoside 'b'
Control	33.82 ± 0.16 (0.0)	11.84 ± 0.09 (0.0)
0.10 %	31.02 ± 0.14 (8.27)	11.19 ± 0.05 (5.48)
0.15 %	30.34 ± 0.17 (10.29)	10.76 ± 0.12 (9.12)
0.20 %	28.64 ± 0.14 (15.31)	9.51 ± 0.16 (19.67)
0.25 %	25.62 ± 0.17 (24.23)	8.28 ± 0.01 (30.03)
CD at 5%:	**0.021**	**0.144**

Table 4. Variation in the sennoside content of immature leaves (mg g^{-1} dr. wt) of the control and the Mancozeb-treated *Cassia angustifolia*, as observed at various stages of plant growth (Mean ± SE in µm). Parentheses indicate percent variation.

Treatments (%)	Sennoside 'a'	Sennoside 'b'
Control	26.01 ± 0.05 (0.0)	7.25 ± 0.06 (0.0)
0.10%	24.25 ± 0.16 (6.76)	6.46 ± 0.13 (10.89)
0.15%	23.49 ± 0.15 (9.68)	5.85 ± 0.13 (19.21)
0.20%	21.64 ± 0.02 (16.8)	5.32 ± 0.15 (26.62)
0.25%	17.69 ± 0.13 (31.98)	4.66 ± 0.14 (35.72)
CD at 5%:	**0.009**	**0.015**

Table 5. Variation in the sennoside content of mature leaves (mg g^{-1} dr. wt) of the control and the Mancozeb-treated *Cassia angustifolia*, as observed at various stages of plant growth (Mean ± SE in µm). Parentheses indicate percent variation.

Treatments (%)	Sennoside 'a'	Sennoside 'b'
Control	18.64 ± 0.16 (0.0)	3.77 ± 0.06 (0.0)
0.10 %	16.29 ± 0.16 (41.04)	3.58 ± 0.14 (5.03)
0.15 %	14.53 ± 0.06 (22.04)	3.25 ± 0.17 (13.79)
0.20 %	13.49 ± 0.15 (27.62)	2.80 ± 0.05 (25.72)
0.25 %	12.01 ± 0.13 (35.56)	2.32 ± 0.03 (38.46)
CD at 5%:	**0.009**	**0.015**

Discussion

Plants often harbor a variety of microbes, mainly bacteria and fungi, which often spoil plant-derived pharmaceuticals (Khattak, 2012). Use of pesticides, including pre-sowing seed treatment, is thus very common. Protein turnover, an indispensable feature of living systems, was negatively affected in *C. angustifolia* by high dose (0.15-0.25%) treatments of Mancozeb. Earlier, Aamil *et al*., (2005) observed that application of fungicides Carbendazim, Captan, Thiram and Mancozeb on chickpea seeds had no significant effect on plant vigor, seed yield, and the N and protein contents at a dose of 1 and 1.5 g a.i./kg seeds, but caused a significant decline in these parameters at 2 g a.i./kg seeds. In another study, systemic fungicide Benlate reduced the total protein content of *Triticum aestivum*, as compared with the control (Siddiqi & Ahmed, 2002). It is suggested that the toxicants produced by the application of systemic fungicides inhibit protein synthesis by binding to larger ribosomal subunits, thus affecting the enzyme system (Person *et al*., 1975) and inhibiting the ATP and NADP formation (Mishra & Waywood, 1968; Siddiqui, 1997). The decreased leaf protein and amino acids contents indicate that pesticide application disturbs nitrogen metabolism (Saladin *et al*., 2003).

Chlorophyll synthesis in plants is indicative of their growth potential and photosynthetic capability (Jain & Gadre, 1998). Chlorophyll loss is often correlated to stress injury (Ali *et al*., 2008; Iqbal *et al*., 2010). The significant reduction in chlorophyll contents in response to Mancozeb treatments in the present study is in line with findings of Mostafa & Helling (2002) in *Chlorella* cultures, where biosynthesis of chlorophylls *a* and *b* declined significantly with increase in Lindane concentrations. Khan *et al*., (2006) also reported a consistent decrease in chlorophyll contents in chickpeas with increased application of herbicides Linuron, Methabenzthiazuron and Terbutryn. Chlorophylls *a* and *b* have different degradation pathways and chlorophyll *a* might degrade under stress prior to chlorophyll *b*, which could account for greater loss of the former than the latter, as observed in the present study (Shaukat *et al*., 2013).

The reduction in chlorophyll content may be due to changes in chlorophyll fluorescence associated with inhibition of electron transfer (Matouskova *et al*., 1999). Pesticides may act as photo-oxidants (Bridge, 2000), leading to chlorophyll destruction and thereby causing a decrease in the total chlorophyll contents. As the chlorophyll pigments are found in thylakoids within chloroplasts, the denaturation of these pigments correlates to changes in these structures. The observed decrease in chlorophyll content at higher pesticide concentrations may be due to the breakdown of the thylakoids and chloroplasts envelop (Dodge & Lawe, 1974). Chlorophyll content may also be reduced due to decrease in leaf area, as observed in many earlier studies (Iqbal *et al*., 2010; Umar *et al.,* 2011).

Our observations, that the chlorophyll and carotenoid contents in the controlled as well as treated plants were highest at the flowering stage, followed by the pre-flowering and post-flowering stages, duly substantiate the findings of Fu *et al*., (2000), who observed that the contents of these pigments attained their maximum 15 days after flowering and decreased rapidly 33 days after flowering. Significant dose-dependent reductions at all growth stages of plants treated with pesticides were also reported by Tort & Turkyilmaz (2003) who applied the fungicide Captan on pepper leaves. In the present investigation, the maximum enhancement in chlorophyll concentration was observed at 0.10% treatment of Mancozeb and later it declined with increase in pesticide concentration. In a similar study, Bikrol *et al*., (2005) observed a significant increase in the chlorophyll (*a* and *b*) and protein contents up to l00 µg/ml of Thiram beyond which a consistent fall was recorded with growing Thiram concentration, the maximum loss occurring at 700 µg/ml.

The stereoisomers *a* and *b* of the sennoside, the former being a dextrorotatory and the latter a meso form, are the glycosides of rhein-dianthrons and glucose. In the present investigation, the highest sennoside concentration was recorded in pods, followed by immature leaves, while the lowest occurred in the mature leaves. Arshi *et al*., (2006) obtained similar results with reference to salinity stress. The onset of reproductive growth phase in *Cassia angustifolia* and *C. acutifolia* was concomitant with a decline in the leaf sennoside content (Lohar *et al*., 1979). A similar variation with season and age was exhibited by the leaf sennoside content in *Cassia fistula*; the older trees had lower sennoside content than the younger ones (Cano *et al*., 1990).

The quality and quantity of active substances from the wild growing and the cultivated plants often fluctuate depending on environmental conditions, infestation and diseases. Application of pesticides causes additional adverse impact on the active substances of plants. In the present study, the reduction in the sennoside content of leaves and pods of *Cassia angustifolia* could be due to interference of the pesticide ions with enzymes associated with the sennoside-biosynthesis pathway. Our observations are in agreement with those of Lydon & Duke (2006), who found that Alachlor reduces flavonoid synthesis, while Glyphosate blocks the synthesis of all cinnamate derivatives. The inhibitory effects of Glyphosate and Chlorsulfuron on the phenylalanine, tropinone and tropine levels in *Datura stramonium* suggest that these herbicides might cause a decrease in the overall tropane-alkaloid biosynthesis (Deng, 2005).

Conclusion

Application of Mancozeb in low (0.10%) concentration enhanced the protein content and pigment concentrations in *Cassia angustifolia* (Indian Senna), but high concentrations caused a reduction, in comparison with the control, thus showing that the pesticide used had a positive impact in low concentration, but became toxic for the biochemical processes in plants at high concentrations. A dose-dependent reduction in the sennosides *a* and *b* content in the leaves indicates that pesticide stress altered the levels of secondary metabolites, possibly by affecting the biosynthetic pathway and converting the sennoside precursors to sennosides.

References

Aamil, M., A. Zaidi and M.S. Khan. 2005. Fungicidal Impact on Chickpea- *Mesorhizobium* Symbiosis. *J. Environ. Sci. Health* B39: 779-790.

Ali, S.T., Mahmooduzzafar, M.Z. Abdin and M. Iqbal. 2008. Ontogenetic changes in foliar features and p*soralen content of Psoralea corylifolia Linn. exposed to SO_2 stress. J. Environ. Biol*., 29: 661-668.

Arshi, A., M.Z. Abdin and M. Iqbal. 2006. Sennoside content and yield attributes of *Cassia angustifolia* Vahl. as affected by NaCl and $CaCl_2$. *Sci. Hort.*, 111: 84-90.

Barnes, J.D., L. Balaguer, E. Manrigue, S. Elvira and A.W. Davison. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. *Environ. Exp. Bot.*, 32: 85-100.

Bikrol, A., N. Saxena and K. Singh. 2005 Response of *Glycine max* in relation to nitrogen fixation as influenced by fungicide seed treatment. *African J. Biotechnol.,* 4(7): 667-671.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72: 248-259.

Bridge, E.L. 2000. The mode of action of RH-1965. *Pest Manag. Source*., 56(3): 245-248.

Cano, L.M., A. Odilia, H. Hernandez and J.R. Sanchez. 1990. Seasonal variations in the content of sennosides in leaves and pods of two *Cassia fistula* populations. *Phytochem.* 29: 3095-3099.

Cochran, W.G. and G.M. Cox. 1957. *Experimental Design*. (2nd Ed) Wiley, New York, pp 611.

Deng, F. 2005. Effects of glyphosate, chlorsulfuron and methyl jasmonate on growth and alkaloid biosynthesis of jimsonweed (*Datura stramonium* L.). *Biochem. Physiol.,* 82: 16-26.

Dodge, J.D. and G.B. Lawe. 1974. Some effects of the herbicide diquat and morfamquat on the fine structure of leaf cells. *Weed Res.,* 14: 45-49.

Duxbery, A.C. and C.S. Yentsch. 1956. Plankton pigment monography. *J. Air Poll. Cont. Assoc.,* l6: 145-150.

Edvreva, A. 1998. Stress physiology, definitions and concepts of stress. *Symposium on Molecular Basis of Stress Physiology in Plants*, 22-26 June, EBILTEM Bornova-Izmir, Turkey.

Fu, J., B. Huang and G. Zhang. 2000. Physiological and Biochemical changes during seed filling in relation to leaf senescence in soybean. *Biol. Plant.,* 43: 545-548.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for extraction of chlorophyll from leaf tissue maceration. *Can. J. Bot.,* 57: 1332-1334.

Iqbal, M., Mahmooduzzafar, F. Nighat and P.R. Khan. 2010. Photosynthetic, metabolic and growth responses of *Triumfetta rhomboidea* to coal-smoke pollution at different stages of plant ontogeny. *J. Plant Interact.,* 5(1): 11-19.

Jain, M. and R. Gadre. 1998. Inhibition of chlorophyll synthesis and enzymes of nitrogen assimilation by selenite in excised maize leaf segments during greening. *Water Air Soil Pollut.,* 104: 161-166.

Khan, M.S., A. Zaidi and P.Q. Rizvi. 2006. Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity, and seed production in chickpeas. *Commu. Soil Sci. Plant Anal.,* 37: 1783-1793.

Khattak, K.F. 2012. Microbiological quality assessment of commercially available medicinal plants in Peshawar city, Pakistan. *Pak. J. Bot.,* 44(4): 1203-1208.

Lemmli, J., E. Verhaeren and J. Cuveele. 1985. Research on drugs containing anthraquinone principles. A note on alcoholic washing of Senna leaflets. *Plant Med. Phytotherapie,* 19: 57-61.

Lohar, D.R., P.K. Bhatia, S.P. Gard and D.D. Chawan. 1979. Seasonal variation in the content of sennoside in Senna leaves. *Pharm. World Sci.,* 1(1): 206-298.

Lydon, J. and S.O. Duke. 2006. Pesticide effects on secondary metabolism of higher plants. *Pest Manage. Sci.,* 25(4): 361-373.

Mac-Lachlam, S. and S. Zalik. 1963. Plastid structure. Chlorophyll concentration and free amino acid composition of a chlorophyll mutant of Barley. *Can. J. Bot.,* 41: 1053-1062.

Majid, U. 2010. Effects of agrochemical pollution on growth, structure and some physiochemical aspects of *Cassia angustifolia* Vahl. PhD (Botany) Thesis, Jamia Hamdard, New Delhi, India.

Matouskova, M., J. Naus and M. Flasarova. 1999. Long-term response of chlorophyll fluorescence induction to one-short application of cyanazine on Barley plants and its relation to crop yield. *Photosynthetica,* 37(2): 281-294.

Mishra, D. and E.R. Waywood. 1968. The effect of BZI and kinetin on the nicotinamide and nucleotide content of senescence wheat leaf. *Can. J. Bot.,* 46: 167-178.

Mostafa, F.I.Y. and C.S. Helling. 2002. Impact of four pesticides on the growth and metabolic activities of two photosynthetic algae. *J. Environ. Sci. Health.,* B37(5): 417-444.

Person, C.D., J. Sambroski and F.R. Forysth. 1975. The effect of BZI on detached wheat leaves. *Can. J. Bot.,* 180: 1294-1295

Saladin, G., C. Magne and C. Clement. 2003. Stress reactions in *Vitis vinifera L.* following soil application of the herbicide flumioxazin. *Chemosphere.,* 53: 199-206.

Shaukat, S.S., M.A. Farooq, M.F. Siddiqui and S. Zaidi. 2013. Effect of enhanced UV-B radiation on germination, seedling growth and biochemical responses of *Vigna mungo* (L) Hepper. *Pak. J. Bot.,* 45(3): 779-785.

Siddiqui, Z.A. and S. Ahmed. 2002. Effects of systemic fungicides on protein, carbohydrate, amino acids and phenolic contents of susceptible (Mexipak) and esitant (Povan) varieties of *Triticum aestivum* L. *Turk. J. Bot.,* 26: 127-130.

Siddiqui, Z.S. 1997. Effect of systemic fungicides on total protein, carbohydrate and phenolic contents of *Solanum melongena* and *Avena sativa*. *Appl. Ent. Phytopath.,* 64: 17-22.

Srivastava, V.K., M.L. Maheshwari and S. Mandal. 1983. A rapid HPLC method for analysis of sennosides in Senna. *Indian J. Pharm. Sci.,* 45: 230-231.

Tort, N. and B. Turkyilmaz. 2003. Physiological effects of captan fungicide on pepper (*Capsicum annum* L.) plant. *Pak. J. Biol. Sci.,* 6(24): 2026-2029.

Umar, S., I. Diva, N.A. Anjum, M. Iqbal, I. Ahmad and E. Pereira. 2011. Potassium-induced alleviation of salinity stress in *Brassica campestris* L. *Centr. Eur. J. Biol.,* 6(6): 1054-1063.

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