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COMBINED EFFECTS OF PESTICIDE ON GROWTH AND NUTRITIVE COMPOSITION OF SOYBEAN PLANTS

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Abstract

The present study was carried out in a field located at Department of Botany, University of Karachi where soil was treated with various concentrations of pesticides before sowing. Combined effects of pesticides on soybean growth and nutritive composition of seeds were observed. Pesticide treated soil had significant effects on leaf growth components such as leaf area ratio, leaf area index, specific leaf area, net assimilation rate, leaf weight ratio and leaf area duration. Low concentration of pesticide enhanced leaf growth components at all the growth stages studied. The plants grown at the site treated with 0.25g L^{-1} pesticide, displayed maximum relative growth rate (RGR) and crop growth rate (CGR) compared to control. However, significant reduction in CGR and RGR were recorded at the sites treated with 0.5 and 0.75g L^{-1} concentration. Total phenols in leaf, shoot and fruit were used as a stress indicator to ascertain the possibility of chemical stress caused by systemic pesticide. 114 and 220% increase in total phenol at vegetative stage and 50, 166 and 163% at late fruiting stage were recorded in the sites treated with high pesticide concentration. Significant differences in nutritive values of seeds between treated and control plants were also observed. At the site treated with low pesticide concentration, lipid content was very high (28.9%) compared to the control. With increasing concentration, protein and lipids contents started to decline. Present study showed the combined effects of pesticides concentration on plant growth and nutritive composition of seeds.

Introduction

The intensive and multiple cropping systems recommended in modern production technology are unavoidably associated with intensification of disease incidence. The optimal doses of water and fertilizers in the high yielding varieties of crops and vegetational cover they provide to the land almost throughout the year have enhanced the survival ability and capacity for multiplication and dissemination of the pathogen. Therefore, it is possible that cultural practices including resistant varieties may fail to provide desired level of disease control and additional precaution in the shape of chemical protection may be necessary. Consequently, chemical control of harmful organisms of crop plants is firmly established measure at the present time. Nowadays, many modern pesticides are in use all over the world. Among the modern pesticides, systemic pesticides are being extensively used in agriculture. Most of them are applied through the soil in agricultural lands for the protection of soil borne diseases (systemic fungicides) and reduction in competition (herbicides) against unwanted plants. The tendency of pesticides which are applied through soil is determined largely by the characteristics of the soil to which they are added. The presence of certain functional groups such as –OH, $-NH_2$, -NHR, $-CO.NH_2$, -COOR and $–NR_3$, in the molecular structure of the pesticides has hasten adsorption, especially on the soil humus (Misra & Mani 1994) which subsequently may affect the plant growth through soil water plant

relationship. The presence of pesticide residues (solutes) in soil distress the water potential which reduces uptake of nutrients from the surrounding soil (Rengel & Wheal 1997), depolarizes the plasma membrane of the root cells (Hausler *et al.*, 1990; Shimabukuro, 1990; Shimabukuro & Hoffer, 1994; Wright, 1994) that would effect the uptake of cations including Zn, Cu and Mn etc (Tester, 1990). Thus the inability of plants to take up the essential micro nutrients may be due the presence of pesticide residues in soil and the nutrient deficiency so created might be reflected in the abnormality in the different growth parameters. It is therefore hypothesized that extensive and over use of systemic pesticides in agricultural land can exhibit negative effects on the growth of non-targeted host plant.

The objectives of the present study were 1) to know the combined effect of pesticides on soybean plant growth throughout the growing season and 2) subsequent changes on nutritive composition of soybean seeds harvested from the treated sites as well.

Materials and Methods

The present study was conducted in field aligned with Department of Botany, University of Karachi. The solution of 4 pesticides, *viz*., Topsin M, Benlate (benomyl), Demacron (phosphomedon), and chlorosuphuron, Cypermethrin and Cypermethrin dimethride (Lazer) were prepared in water with approximate values of 0.25 g L^{-1}, 0.50 g L^{-1} and 0.75 g L^{-1} (w/v). Individual proportion of each of the 4 pesticides in the solution has been listed in Table 1. Experiment was designed as completely randomized block. In the field, 12 plots each of $6m^2$ were prepared for the study. Three replicates for each of the four treatment including control were used. Three concentrations of pesticide were sprayed to the plots four times repeatedly before sowing while untreated plot served as control. Six rows were prepared in each plot having 100 cm distance between them and 25 randomly selected germinating seeds were sown in each row. Therefore 150 seeds were sown in each plot at a depth of 1 inch at the start of May and plants were allowed to grow. The inter plant distance was 24 cm. Irrigation was given twice in a month. Temperature was in the range of about 30°C to 35°C ± 2°C with 70-80% humidity throughout the growing season. Hoeing and weeding were done at the early stage of seedling establishment. The pH of the soil was in the range of 6.2 to 6.6, maintained by applying potassium salts at 150 pound/acre. Twenty-five plants per square meter were collected in each growth phase (Seedling, Vegetative, Flowering, Early Fruiting and Late Fruiting). The soil was removed from the plants manually and they were washed with tap and then with distilled water. The plants were then separated into leaves, stems, roots, flowers and fruits. The leaf area of plants was determined by the methods of Hunt (1990). Different plant parts were bagged and oven-dried at 65°C for biomass estimation. Growth analysis was carried out at different developmental stages of the plant using the different parameters as suggest by Hunt (1990) and modified accordingly:

Quantitative determination of total phenols: Leaf, shoot and fruit samples (1.0g each) were collected randomly at vegetative and late fruiting stage and plunged into 2N HCl with the result that the tissues were killed immediately to restrict the enzymes peroxidase activity. Tissues were then crushed in pestle and mortar using 10 ml of 2N HCl. The crushed material was taken in a tube and boiled for half an hour in a water bath at 60°C. Then it was filtered and the filtrate was left over anhydride $CaCl_2$ at room temperature

Table 1. Individual proportion of pesticides at three experimental sites aligned with department of Botany, University of Karachi.

	Pesticide (g)						
Location	**Benlate**	**Topsin-M**	**Dimecron**	**Cypermethrin**	**Cypermethrin-dimethride**	**Chlorosulfuron**	**Total Conc. (g L-1)**
Site 1	0.041	0.046	0.081	0.028	0.024	0.025	0.245
Site 2	0.081	0.062	0.182	0.021	0.022	0.125	0.493
Site 3	0.019	0.025	0.122	0.133	0.147	0.135	0.754

until dryness (Harbone, 1973). Quantitative determination was performed by the method described by Swain & Hillis (1959). Briefly, 0.5ml of pure ethanol was added to the dried extract. After 5 minutes, 0.1ml of extract was taken in another tube and 0.2 ml of Folin-Ciocalteu reagent (1:9) and 5ml of distilled water were added to the extract. Tubes were shaken in electric shaker (Shaker, Vibrax VXR) 1500 rpm for 10 min., and then saturated with $NaHCO_3$ solution. Tubes were shaken again and then incubated at 25ºC for 30 minutes. OD was recorded at 660nm using Shimadzu UV 1260 mini Spectrophotometer against reagent blank. Amount of total phenolic content was expressed as mg g^{-1} fresh weight using standard curve.

2.4. Extraction and estimation of total protein: Seeds (1.0 ± 0.002g) were randomly collected from the treated sites, then extracted in 10ml of 5%TCA (Tri-Chlorocetic acid), centrifuged at 4000rpm for 20minutes followed by the addition of 5ml of 0.5N NaOH in each respective residue, then incubated at 37ºC for 16h. This solution was then filtered through glass wool and OD was recorded at 260 and 280nm by using Shimadzu UV 1260 mini Spectrophotometer. Total protein in $mg.ml^{-1}$was calculated using the formula adduced by Boyer, (1993) as follows and expressed in g g^{-1} dry weight.

$$\text{Protein } (mg.ml^{-1}) = 1.55A_{280} - 0.76A_{260}$$

2.5. Extraction and estimation of total lipid: Finely crushed seeds (1.0 ± 0.002g) of soybean were transferred into a 125mL Erlenmeyer flask. Then 10ml of hexane-isopropanol (3: 2) was added and warmed on a hot-plate for 15 minutes. Mixing was thoroughly carried out while heating. Extraction mixture was then filtered rapidly thorough Whatman No. 3 filter paper and poured an additional 10ml of warm hexane-isopropanol. Removal of the solvent from the extract under vacuum on a rotary evaporator at 40ºC was performed to obtain a yellow oil or off-white solid. Total lipids were estimated on the basis of complex formation between lipids and ammonium ferrothiocyanate as an improved method described by Zhou & Arthur (1992). The value of total lipid was expressed as g g^{-1} dry weight using standard curve.

Statistical analysis and figure representation: Analysis of variance (ANOVA) was computed by SPSS version 11.0 for growth parameters. LSD and Bonferroni were tested. Graph illustrations for growth parameters are based on differences in mean values between the control and treated samples.

Results

Plant growth components and seed nutritive composition: Pesticide had significant effects on leaf growth components. At the site where 0.25 g L^{-1} were sprayed, LAR gradually increased up to the flowering stage and thereafter it came down to reach 6.33 cm^2 g^{-1} over control at late fruiting stage. At higher concentrations i.e., 0.5 g. L^{-1} and 0.75 g L^{-1}, LAR significantly decreased over control as is obvious from negative values (Fig. 1). Maximum decrease (-36.12 cm^2 g^{-1}) in LAR was found at the flowering phase of plants grown in the soil treated with the concentration of 0.75 g. L^{-1}. At lesser concentrations of pesticide, leaf weight ratio (LWR) or the amount of biomass devoted to leave material gradually increased until the flowering stage (Fig. 1) and from then on it stabilized showing non-significant ($p>0.05$) differences in mean values between control and treated plants. Likewise, maximum decrease (-0.1007 g g^{-1}) was recorded in LWR at 0.75 g kg^{-1}.

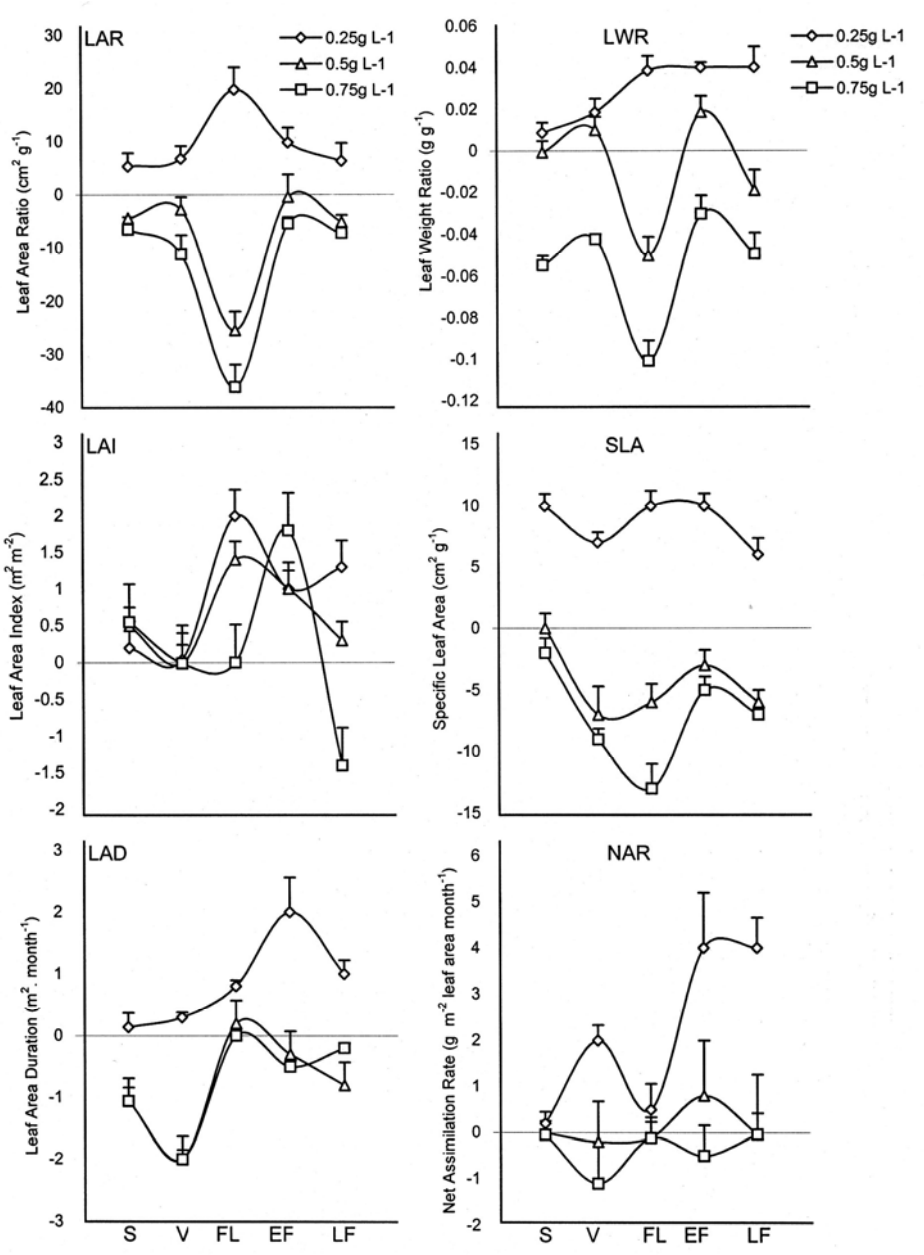

Fig. 1. Effects of pesticide on growth components of soybean plant at different stages. Symbols on horizental-axis stand for S= seedling, V= vegetative, FL= flowering EF= early fruiting, LF= late fruiting stages.

LAI values were affected by the pesticide concentration in soil showing maximum increases (2.0 m^{-2} m^{-2}) at vegetative stage. However, significant decrease in LAI was observed when plants were grown in the soil having 0.75g L^{-1} pesticides concentration (Fig. 1). Concentrations of 0.25 and 0.5 g. L^{-1} showed an increase in LAI from vegetative to flowering phase. However, at the soil treated with 0.75 g. L^{-1} the phase from flowering to fruiting was declined at the late fruiting stage.

Values of specific leaf area (SLA) at 0.25 g. L^{-1} were found to be higher than control and vary between 10 and 6 cm^2 g^{-1} (Fig 1). At the remaining concentrations, the considerable decreases in SLA over control were obtained. Increase in leaf area duration (LAD) from seedling to vegetative phase was not as pronounced as it was from vegetative to flowering stage (Fig 1) in the soil having concentration 0.25 g. L^{-1}. At early and late fruiting phases, lesser concentrations of pesticide caused significant increase in LAD over control while higher concentrations considerably decreased it when LAD compared to control treatment. Maximum decrease (-2.0 m^2 $month^{-2}$) was recorded at both the 0.5 and 0.75 g. L^{-1} treated soil. Net assimilation rate (NAR) peaked twice at vegetative (2.0 cm^2 g^{-1} leaf area/month) and early fruiting (4 cm^2 g^{-1} leaf area/month) phases (Fig. 1). At lesser concentration (0.25 g. L^{-1}), significant increase in NAR was noted. At seedling, flowering and late fruiting stages, the NAR values were as high as 0.2 to 4 $g.m^{-2}$ leaf area $month^{-1}$ as compared to control. Maximum decrease (-1.10 g. m^2 leaf area $month^{-1}$) was recorded when plants were subjected to high concentration at vegetative stage.

Like leaf growth components, maximum crop growth rate (CGR) was found in the plants cultivated in the site having 0.25 g. L^{-1} pesticide concentration. From seedling to flowering stage the CGR increased steadily and then up to the early fruiting phase steep rise (78.0 g m^{-2} ground area $month^{-1}$) was noted. Until the maturation of fruits the values again fell sharply to come as low as 8.04 g g^{-1} ground area/month (Fig. 2). The plants grown at the site having 0.25 g. L^{-1} concentration of pesticide, displayed maximum RGR values compared to those growing in control treatments (Fig. 2). At vegetative and early fruiting phases, highest RGR values were noted while at seedling, flowering and late fruiting phases, RGR values were merely within a range of 34.03 to 27.34 g $g^{-1}month^{-1}$. At the higher concentrations, RGR values were significantly decreased over control ($p< 0.05$).

Nutritive composition of seeds was also affected by the pesticide (Fig. 3). Plant seeds produced at control site or those at sites with lesser concentration had similar in protein contents. With an increase in concentration, significant decrease in protein was noted. Maximum decrease by 32.04% in protein was recorded in the seeds of plants from the soil sprayed with 0.75 g L^{-1} pesticides. However, seed lipids produced in plants grown in 0.25 g. L^{-1} treated soil, were in significant excess compared to the seed lipids at control and high concentration sites. A progressive decrease in lipids contents started to occur as the concentration increased, with maximum decrease (48.4%) was noted in plants grown at high concentration sites.

Total phenols: Total phenols were tested at the vegetative and fruiting (last) phase of the growth in leaf, shoot and fruit to examine by different pesticides treated sites. With increasing concentrations, total phenols also tended to increase (Fig. 4) in both the growth phases. Total phenols stayed more or less constant in plants grown in the soil treated with 0.25 g L^{-1} compared to control, although at fruiting stage, leaf phenols were notably decreased at 0.25 g L^{-1}. At vegetative stage, 114 in leaf and 220% increase in shoot phenols were recorded compared to 166% in shoot and 163% in fruit at late fruiting stage. Maximum increase (220%) in shoot was recorded at 0.75 g L^{-1} concentration.

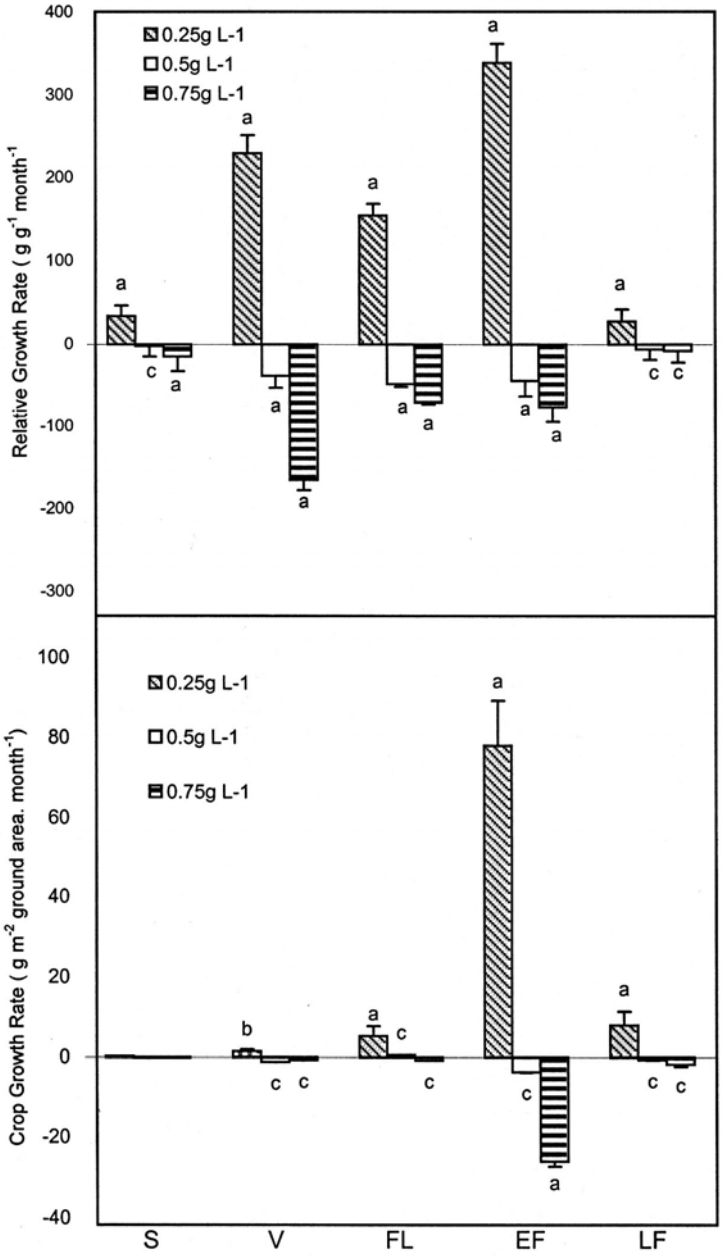

Fig. 2. Effects of pesticide on relative growth rate and crop growth rate of soybean plant at different phenophases.Symbol on X-axis stands for S= seedling, V= vegetative, FL= flowering, EF= early fruiting, LF= late fruiting stages.
a = LSD and Bonferroni are significant at ($p<0.05$)
b =LSD is significant but Bonferroni is not significant at ($p<0.05$)
c = LSD and Bonferroni are non-significant at ($p<0.05$)

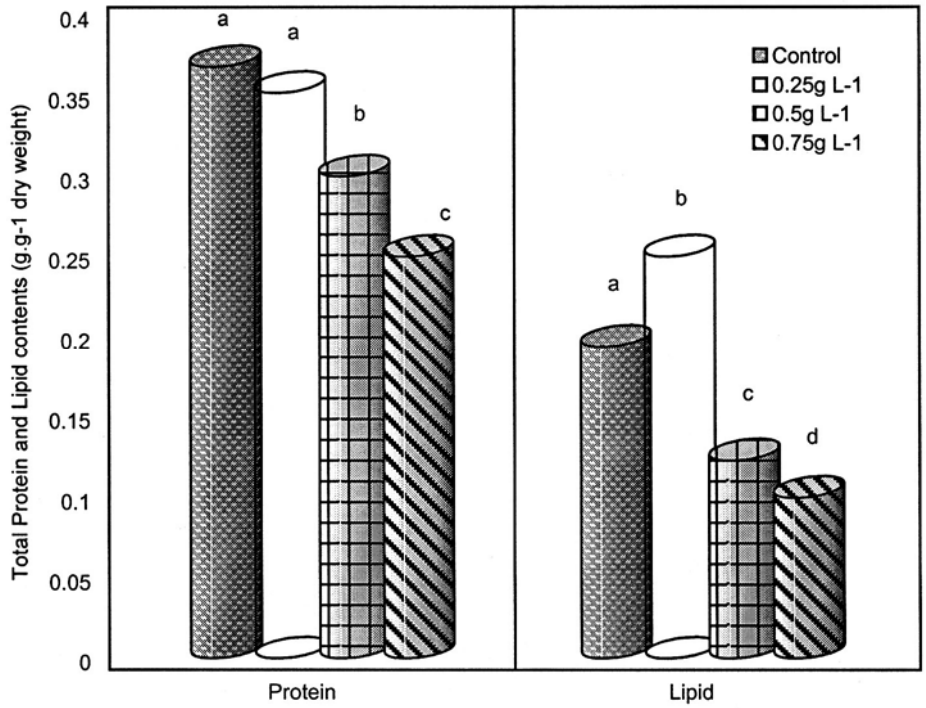

Fig. 3. Effect of pesticide on total protein and lipid contents of soyabean seeds after harvesting. Values at each concentration having the same letter are not significantly different ($p>0.05$).

Discussion

Different mechanisms have been suggested, how the higher concentration of pesticides retards the physiological and biochemical processes of the plants which could provide further insights into growth retardation as occurred in the present study. For instance, the presence of pesticide residues (solutes) in soil distresses the thermodynamic activity of water along with micro and macro nutrients in surrounding soil. The presence of pesticide residues in soil tends to decrease the uptake of water along with nutrients (Rengel & Wheal 1997; Taiz & Zeiger 2003) as pesticides residue gets attached with the soil particles affecting the nutrient uptake from the soil to root (Rengel & Wheal 1997). The presence of pesticides residues like chlorosulforon, dclofop, haloxyfop fluazifop have shown some specific effects on the micronutrient transport system and the plasma membrane of the root cells (Hausler *et al.,* 1990; Shimabukuro, 1990; Shimabukuro & Hoffer, 1994; Wright, 1994) which in turn affect the cations uptake including Zn, Cu and Mn (Tester, 1990). Besides this several studies reported considerable effects of pesticide residues on soil properties, plant growth, densities and yield of crop plants (Brixie & Boyd, 1994; Cox *et al.,* 1995; Murphy *et al.,* 1996; Pavlovic *et al.,* 1996; Celis *et al.,* 1999a, 1999b). The nature and amount of organic ion in the interlayer also influence systemic activity of pesticide in soil (Celis *et al.,* 2000).

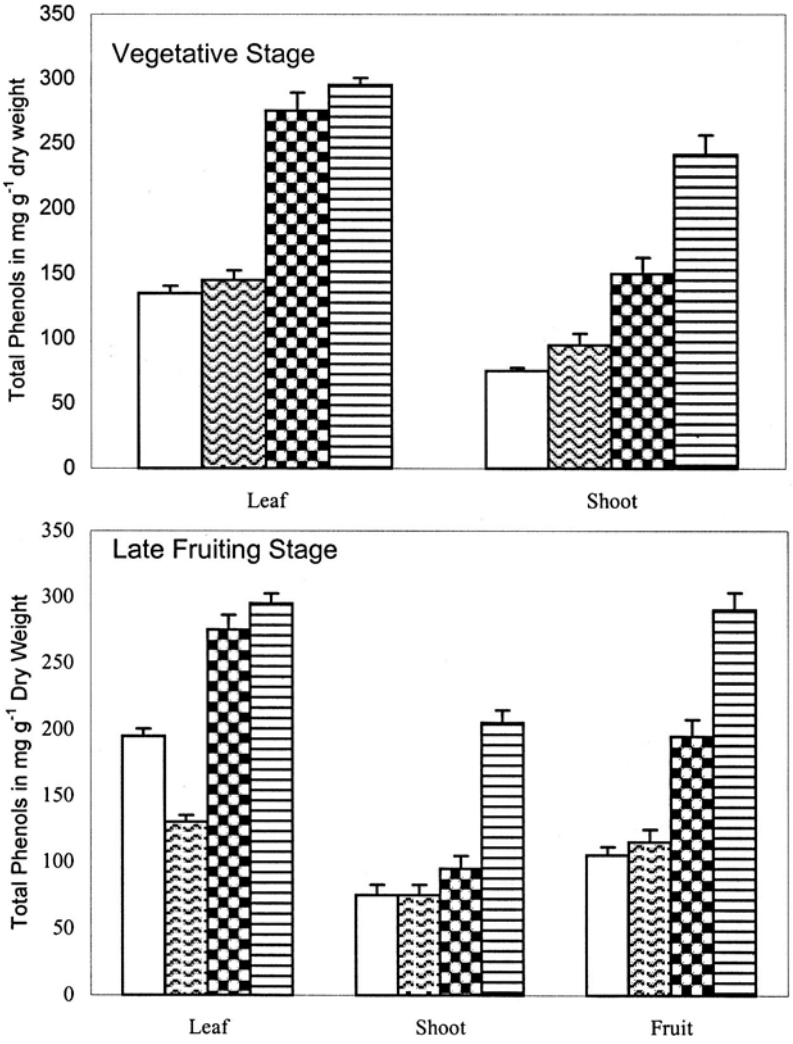

Fig. 4. Changes in total phenols in leaf, shoot and root at vegatative and late fruiting stages. Symbol on legend stands for Control = 0g L^{-1}, A = 0.25g L^{-1}, B= 0.50 g L^{-1} C = 0.75 g L-1 pesticide

The behaviour of some pesticides particularly organophosphate insecticides, herbicides and systemic fungicides in soil shows that higher concentrations requires more time to degrade and there are reports to show that higher concentrations of pesticides have harmful effects on various growth parameters of plants. (Reyes, 1975; Forster *et al.,* 1980; Buchenauer & Rohner, 1981; Sheinpflug & Duben, 1988; Gusta *et al.,* 1994; Montfort *et al.,* 1996; Siddiqui & Ahmed, 1996; Siddiqui *et al.,* 1997; Siddiqui & Ahmed, 2000).

Net Assimilation Rate (NAR) to a larger degree is determined by the quality and quantity of protein and carbohydrates synthesized within in the leave. The adverse effect of pesticide residues on proteins, lipids and carbohydrates metabolism may cause the

lowering of NAR values as well. At lower concentrations, pesticide residues seemed to elevate the growth parameters up to certain limit. Nevertheless, it was at the higher concentration that all the growth parameters, viz., LAR, LAI, LWR, SLA, RGA, LAD, NAR and CGR were remarkably reduced in all the growth phases under study. The phenomenon seems to corroborate the findings of Fletcher and Nath, (1984) and Gao *et al.,* (1988) who reported stimulatory effects on plant growth and enhanced tolerance to drought, heat, chilling, ozone and sulfur dioxide at lower rates of pesticide residues compared at higher rates.

Different suggestions have been proposed to elucidate the mechanism leading to biochemical changes brought about by pesticide which results in the change of nutritive composition. Amar and Reinhold (1973) had reported that plant growth is affected by an osmotic shock effect of systemic pesticides which causes release in structural protein and loss of transportability in the leave cells. Likewise, the possibility of the inhibition of protein synthesis, a decrease in the carbohydrate content cannot be ruled out as, the investigations by Person *et al.,* (1957) and Siddiqui & Ahmed (2002) have earlier shown. Earlier studies suggested that toxicant produced by pesticides application retarded the protein and carbohydrate synthesis by inducing alteration in cytochrome oxidase activity, blocking alternative respiratory pathways and accumulation of succinate (Berger & Cwick, 1990; Siddiqui & Ahmed, 2000). Contrary to the present study, systemic fungicides have been found to increase NAD and NADP ratios interfering electron transport system (Pillonel, 1993) and increases ATP levels (Mishra & Waywood, 1968) by inducing change in enzymes system which results in the conservation of leaf protein and chlorophyll in detached wheat leaves (Person *et al.,* 1957).

Until now most of the studies have described the individual effect of one or two pesticides on soil, seed germination, plant growth and nutritive composition. (Dyar, 1968; Clark *et al.,* 1978; Forster *et al.,* 1980; Buchenauer & Rohner, 1981; Gusta *et al.,* 1994; Brixie & Boyd, 1994; Cox *et al.,* 1995; Siddiqui & Ahmed, 1996; Siddiqui *et al.,* 1997; Celis *et al.,* 1999a; Siddiqui & Ahmed, 2000; Murphy *et al.,* 1996; Pavlovic *et al.,* 1996). It is assumed that these pesticides are applied in an agricultural land as per requirement depending upon the prevailing conditions in the field. It is therefore possible that the residues of these pesticides are accumulated and remains in the soil which may affect the plant growth synergistically as described in the present study.

It seems that cultivation of soybean plants in a soil treated with higher concentration of pesticide initiate some kind of abiotic stress (chemical stress) in plants triggering formation of phenolic compounds like iso-flavones (Genistein, diadzein), phenolic acid (elagic, tannic and vanilic acid) and hydroxycinnamic acid derivatives (ferulic acid, ρ-hydroxy benzoic acids and ρ-caumeric acid). These compounds are potential inhibitors of germination and plant growth (Einhellig *et al.,* 1985; Macias *et al.,* 1992; Gerald *et al.,* 1992; Mersie & Singh 1993). Few reports have elucidated the physiological mechanism of phenols induced inhibition on plant growth. Einhellig *et al.,* (1985) who proposed that Ferulic and ρ-coumaric acids reduce leaf water potential and stomatal diffusive conductance in sorghum and soybean. Another study by Einhellig (1995) found that a primary effect of phenolic acids is on the plasma membrane, and this perturbation contributes to a number of physiological effects causing growth reduction. High level of ρ-coumaric, Ferulic, Cinnamic and Vanillic acids and Coumarins severely suppressed the photosynthesis of soybean and *Lemna minor* L., (Patterson, 1981; Einhellig, 1986). Three phenolic acids, ρ-Coumaric acid, Ferulic and Vanillic acids, were also reported to severely inhibit photosynthesis and protein synthesis of isolated leaf cells of velvet leaf *Abutilon theophrasti* (Mersie & Singh, 1993). Friend (1977) is of the opinion that these very compounds may act as protective compounds against pest as well.

In addition, investigations on total phenols provide insight regarding abiotic stress by pesticide with higher doses adversely affecting the leaves, shoot and fruits. In the past, several studies have attempted to explain the possible mechanisms of phenol induced stress in plants. Yang *et al.,* (2002) reported that phenolic stress on plant growth can occur in two ways (1) by the blockage of biosynthetic pathway of chlorophyll (an inhibition of supply orientation) or (2) by stimulating degradative pathway (i.e. a stimulation of consumption orientation) or both leading to a reduction of chlorophyll accumulation, which in turn causes reduction of photosynthesis, retardation in plant growth and lowering of NAR. Likewise, Alsaadawi (1992), explicate the mechanism of some phenolic compounds such as syringic, caffeic, and protocatechuic acids on cowpea (*Vigna sinensis*) seedlings using sand culture medium showed significant reduction in seedling growth, chlorophyll a, b, total chlorophyll, chlorophyll a/b ratio, and N, P, K, Fe and Mo uptake. Pillonel (1993) opined that toxicant, i.e. phenols do negatively affects the activity of NADH cytochrome "C" oxidases in the respiratory chain and accumulation of succinate which causes blockage of alternative pathway of respiration. This study further suggests that it may also cause inhibition of photosynthesis, and alter the protein, carbohydrate and lipid metabolisms.

Nonetheless, the premise of the authors is that it is the amount/concentration of the compounds produced by the plants in response to pesticide application, which determines if the application is going to prove beneficial or disruptive for plant growth. The findings of some earlier studies (Fletcher & Nath 1984; Gao *et al.,* 1988; Reicher & Throsel, 1997; Siddiqui *et al.,* 1997; Siddiqui *et al.,* 2004) had shown that beyond a certain threshold concentration, the pesticide begin to act as growth inhibitor instead of growth promoter or regulator.

In most of the agro based economies, injudicious application of pesticides irrespective of concentration and the crop phenology, results in lower yields and incurring of losses to those associated with agriculture sector. Phytotoxicity in plants gains importance due to an ever-increasing demand for food crops worldwide. The study not only has attempted to suggest the suitable concentration of the pesticide to be applied in soil, it has also evaluated to arrive at the appropriate growth stage of soybean to apply the systemic pesticide at and likewise the most vulnerable one when due care should be taken before any chemical treatment. More insights are needed to further uncover the riddle of phytotoxicity associated with systemic fungicides like pesticide. It is concluded that pesticide residue at lesser concentration promote growth components at all phenophases. However, higher concentrations are substantially phytotoxic for the growth of soybean.

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