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EFFECTS OF SOME SOIL TREATED PESTICIDES ON GROWTH CHARACTERISTICS OF FABA BEAN AND WHEAT PLANTS

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Abstract— The present study was conducted during December 1st, 2009 to October 1st 2010 to determine the effects of Treflan, Glyphosate, Ridomil and Cyrin C, on germination, vegetative growth characteristics, nodulation (in faba bean), and nutrient status as well as pesticide residues in Wheat (*Triticum aestivum* L.) and Faba bean (*Vicia faba*) plants. Results showed that Treflan caused significant decrease in number of nodules per plant, number of branches, plant height, shoot fresh and dry weight in bean plants, and significantly reduced plant contents of potassium, sodium, zinc, manganese and calcium of bean plants. Treflan also reduced the number of tillers per plant, plant height, shoot fresh and dry weight, root dry weight and plant contents of total nitrogen, phosphorus, potassium, sodium, iron, zinc and manganese in wheat plants significantly. Cyrin C reduced the plant contents of nitrogen and manganese in bean plants. Ridomil decreased number of branches per plant in bean, root dry weights of both beans and wheat plants, and reduced the plant contents of nitrogen, sodium and manganese in bean plants and increase phosphorus, and manganese contents of wheat plants significantly. Glyphosate caused significant decrease in number of tillers per plant, plant height, shoot fresh and dry weight in bean and wheat plants and increased the contents of N, P, Na and decrease Fe contents significantly in wheat plants. Treflan was reduced the wheat seed germination percentage significantly and delayed the germination to 15 days. Cyrin C caused reduction in wheat seed germination, but Ridomil and Glyphosate were increased wheat seed germination percentages. All pesticide treatments delayed bean seed germination. After three months of pesticide treatments, residues of Metalaxyl (Ridomil) and chlorpyrifos were detected in samples of bean and wheat plants.

Keywords— Wheat, faba bean, Pesticides, Plant Growth.

I. Introduction

Pesticides have been extensively used in agrochemical practice ^[1]. Pesticides are found as common contaminants of soil, air, and water, and on non-target vegetation in our urban landscapes. Once there, they can harm plants and animals ranging from beneficial soil microorganisms and insects, non- target plants, fishes, birds, and human beings. So, the use of large amount of pesticide is the main reason for agricultural soil pollution ^{[2] - [3]}. The increasing world population demands a continually growing supply of food and food products. Application of pesticides can improve crop yields, but soil adsorption may occur as well, and these chemicals accumulate in the soil, hence the pesticide residues may be toxic and may disturb the biotic and abiotic components of soil ecosystem and thus the fertility of the soil decrease ^{[4]- [5]- [6]}. Pollution considered as one of the most serious Problem that faces human societies in the whole world especially in the developing countries ^[7]. Modern anthropogenic activities, including energy production, manufacturing, and agriculture have resulted in toxic effects in biota and ecosystems which are investigated in close connection to their environmental chemistry and fate in the environment ^[8].

The environmental contamination by toxic xenobiotic chemicals, arising mainly from agricultural and industrial sources ^[9], as a result of the increase in population, industrial activities, and the modernization of agricultural practices, such as the increasingly widespread use of pesticides, resulting in an increase of the amount of effluents thrown into the air, water, and soil ^[10], and have consequences on food quality and human health which are very serious worldwide problems. The extensive use of pesticides (fungicides, herbicides, insecticides, etc.) in various parts of the world for years without any control was responsible for a dramatic increase of pollution level in soil and water ^[9]. Soil pollution is an undesirable alteration in its physical, chemical or biological characteristics that harmfully affect the organism's life ^[11]. Pollutants from agrochemical sources include fertilizers, manure, growth regulators, defoliant and pesticides. Organic compounds used as pesticides, however; have more far reaching effects for the whole community depending on soil ecology ^[12], and may lead to toxic accumulation in arable products and in herbage for grazing animals, thus having important implications for human health. Xenobiotic levels in soil are generally low (<100 mg.kg⁻¹) particularly those of the pesticides ^[13]. Soil is the main accumulator of pesticides and it is the main source of secondary contamination of the components of the biosphere. Some pesticides transfer from the soil into plants through their root system ^{[14]- [15]}.

Pesticides have various characteristics that determine how they act once in soil. Mobility refers to how much a pesticide will move around in the soil. The half-life of a pesticide refers to the length of time it takes for half of the pesticide to degrade. Persistence refers to the length of time until all measurable residues of pesticide are gone^[2].

The use of pesticides in agriculture has been steadily increasing in the last 40 years^[12]. Besides agricultural applications to increase the production of food and fiber, large amounts of pesticides are in use for maintaining urban plantings, hygienic handling and storage, control of vegetation beneath power lines and along railways and roadways, mosquito and fly control, preservation of wood and control of mould growth in paper mills has led to an increase in the use of pesticides and, more recently, transgenic products^{[10] - [16] - [17]}.

A glasshouse Factorial (4*2) experiment (pesticides and plant species) was conducted using Completely Randomized Design (CRD) with four replications to determine the impact of some commonly used pesticides in the region on some plant physiological parameters.

II. Materials and Methods

Soil samples were air-dried and sieved through 2 mm sieve and mixed well. A sample of the soil was analyzed to determine the physical and chemical characteristics. Five kilograms of dried soil was poured into each of similar plastic pots of 22 cm in diameter and 20 cm height. Pesticides used in this study [Treflan (herbicide), Cyrene C (insecticide), Ridomil (fungicide) and Glyphosate (herbicide)] were obtained from EAGD (Erbil Agricultural General Directorate). Seeds of broad bean *Vicia faba* L. and wheat *Triticum aestivum* L. were obtained from EARC (Erbil Agricultural Research Centre).

On December 1st, wheat seeds were implanted inside each of the first 20 pots randomly at 5 cm depth and seeds of broad bean in the remaining 20 pots at 8 cm depth. On December 15, seedlings were thinned to five seedlings per pot. The pots were irrigated whenever needed with tap water to bring the soil moisture to the field capacity. After 45 days, plants were harvested by means of hand-cutting using scissors, so the plants were cut at the soil surface from each experimental unit (pot), and placed into paper bags and weighted immediately to obtain their fresh weights. Plant samples were oven-dried at 70°C for 72 hours^[18]. The roots of bean were excised and the loose soil was crumbled and removed using sieves to retrieve and detach the nodules^[19].

Plant samples were digested using 1:1 conc. H₂SO₄ and H₂O₂^[20]. Nitrogen content was measured by micro-Kjeldahl digestion method; phosphorus content was determined by the molybdenum blue colorimetric method (using ammonium molybdate with SnCl₂)^[21]. Mineral contents including iron, zinc and manganese were determined by atomic absorption spectrophotometer; flame photometric method was used to determine sodium and potassium^[21].

Vegetative parts of plants (75 g for each sample) were cut into small pieces, then ground and homogenized by means of a kitchen blender. The blended plant samples were mixed with anhydrous sodium sulphate (50 g) and extracted with ethyl acetate (200 ml) in conical flasks using an Ultra-Turrax for 4-5 minutes. The contents then allowed settling down for about half an hour and the ethyl acetate extracts were then filtered through a Buchner-funnel fitted with a filter paper covered by 20 g of anhydrous sodium sulphate. Extracts were evaporated until dryness and redissolved in 5 ml of acetonitrile (MeCN) and finally the volumes were made up to 2 ml using rotary vacuum evaporator (RVE). The extracts were then transferred to graduate test tubes and their final volumes were adjusted to exactly 2 ml by adding few drops of acetonitrile and solutions were centrifuged and filtered. The clean organic layers were taken and analyzed by high performance liquid chromatography (HPLC) having UV/Visible detector^[22]. Tested pesticides were extracted from plant samples as follow. Representative unwashed samples (20 g) were blended with 100 ml methanol for two minutes (three replicates). Samples were extracted three times with 100 ml methanol for each time. Methanol extracts were combined and transferred quantitatively to 500 ml separating funnel for liquid-liquid partition with dichloromethane (100 ml × 3) and 40 ml of saturated solution of sodium chloride was added to each. Dichloromethane layers were collected, dried over anhydrous sodium sulfate and then evaporated using RVE at 35-40 °C until dryness^[23].

A. Glyphosate Extraction Procedure:

Finely chopped and mixed fresh plant materials (10 g) were added to 50 ml of 1:2 acetones: hexane mixture then macerated in a high speed homogenizer. The extracts were separated by filtration and centrifugation and the residues were washed with 20 ml of solvent mixture and combined with first extracts. Finally, water was removed by shaking with anhydrous sodium sulfate^[21].

B. Preparation of Pesticide Standards:

For preparation of pesticide standards, stock solution of Chlorpyrifos (chlorpyrifos Analytical Standard %99.8 purity was obtained from Cheminova Als-Denmark), 0.1 g of the standards were dissolved in acetonitrile and 5 levels of intermediate standard solutions were prepared maintaining the same matrix concentration for the preparation of calibration curve and stored at 4 °C in dark condition^[22].

The aqueous stock solution of Metalaxyl of 100 ml l⁻¹ was prepared by dissolving 0.01 g in 100 ml de-ionized water. The stock solution was refrigerated and kept in dark condition. The prepared standard (stock) solution of Metalaxyl of 100 mg L⁻¹ was analyzed by HPLC to compare its peak and retention time with that of Metalaxyl from the standard of Sigma- Aldrich High Performance Liquid Chromatography (HPLC) method. Four working standard solutions with Metalaxyl concentrations of 2, 5, 10, and 15 mg l⁻¹ were used throughout the study. An aqueous stock solution of Glyphosate (purity %99.8 from Monsanto Europe, NV) of 1000 mg l⁻¹ was prepared by dissolving 0.1 g in 100 ml de-ionized water. The stock solution was kept in dark and refrigerated. Three working solutions with Glyphosate concentration of 50, 75, 100 mg l⁻¹ were used throughout the study^[24].

C. HPLC Analysis Systems:

HPLC having UV/ visible detector (Perkin Elmer) was used for identification and quantification of pesticides. Separation was performed on reversed phase C-18 column. Samples were injected manually through an Injector. The working condition of HPLC was binary gradient, mobile phase was acetonitrile: water; (70:30), flow rate was 1 ml min⁻¹, injection volume was 20 µL and the wavelength of the UV/visible detector was fixed at 230 nm for the residual analysis of chlorpyrifos^[22]. HPLC was used to determine the residues of Metalaxyl in plants. The analysis was conducted with HPLC (having UV/ visible detector (Perkin Elmer), with a mobile phase of 50 % acetonitrile and 50 % water, which was estimated by HPLC, equipped with a 20 µL fix loop, C18 reversed phase column, flow rate 1.4 ml/min, UV detector and peak area responses used in quantification were measured at wavelength 220 nm^[24]. Separation of Glyphosate was achieved with a mobile phase of 12% methanol and 88% water. Glyphosate was estimated by HPLC, equipped with a 20 µL fix loop, C18 reversed phase column, flow rate 1.4 ml/ min UV detector and peak area responses used in quantification were measured at wavelength 196 nm^[25].

Data was analyzed statistically using SPSS version 11.5 and treatment means were compared using Duncan Multiple Range Test at the level of 0.05 of significant.

III. Results and Discussion

A. Effect of Pesticides on Nodule Numbers of Faba bean:

Pesticide treatments affected significantly on number of nodules per plant (Fig. 1). No nodules on the roots of faba bean plants were found in both Treflan and Glyphosate treated pots.

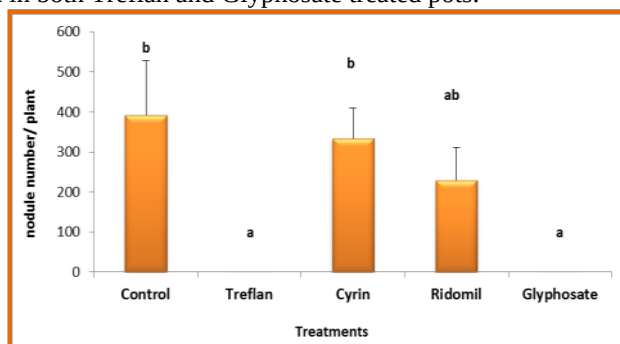


Fig. 1 Effects of pesticides on number of nodules per plant (mean±S.E.). (Note: different letters indicate significant differences ($P<0.05$) between treatments for all figures in the present study).

Reports outlined by different researchers^{[26] - [27] - [28]} showed that Glyphosate and Treflan causes decrease in legume nodulation. *Rhizobium* infects plant roots through root hairs and thus it was hypothesized that herbicides affecting root hair development might interfere with nodulation. Glyphosate, caused root hair deformations that apparently resulted in fewer nodules being formed^{[29], [30]} also reported that Treflan reduced nodulation of soybeans and seemed to inhibit utilization of cotyledonary reserves and redistribution of organic and mineral constituents of unifoliolate leaves and as a consequence, nodulation could be reduced. Further studies^{[29] - [31] - [32]} suggested that herbicide induced reduction in nodulation are due to effects upon the plant such as reduction in photosynthate transport to the roots and disruption of root hair infection by *Rhizobium* which are often accompanied by root stunting or other damage caused by the herbicide. On the other hand, the results disagreed with^[33] who showed that Treflan at 4.76 L ha⁻¹ 21 days after sowing stimulated plant growth and increased nodulation. Low dose (800 g ha⁻¹ a.i.) of Treflan has stimulated nodule numbers, while highest rate has decreased number of nodules^[34]. Researchers found that Metalaxyl (Ridomil) has negatively affected on nodulation^[25]. Moreover, the studied pesticides have shown variable patterns of nodule distribution in each treatment. Most nodules in control plants were located on the upper 5cm of the primary root (Fig. 4), whereas in

Cyrin C treated plants, the nodules were distributed along the roots (Fig. 5). While Ridomil treated pots showed restricted location of nodule formation (Fig. 6) when compared with control and nodules were rarely present in the Treflan treated zone^[30].

B. Effects of Pesticides on Fresh and Dry Weights of Nodules:

Fig. 2 shows that both Glyphosate and Treflan treatments were affected significantly the fresh weight of nodules per plant, and caused reduction in fresh weight to 0.0 compared with 2.79 for the control.

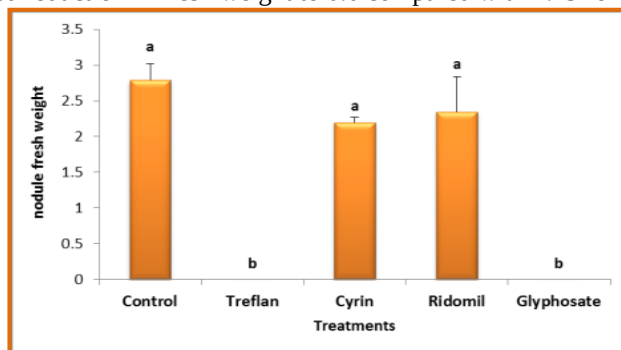


Fig. 2 Effects of pesticides on fresh weight of nodules of faba bean plants.

Glyphosate and Treflan decreased the dry weight of nodules significantly (to 0.0 mg plant⁻¹ for the both) comparing with control (0.72 mg plant⁻¹) (Fig. 3). No nodules were formed in Glyphosate and Treflan treated faba bean (Fig. 7 and 8 respectively) these results are in agreement with^{[19] - [34] - [35]}. In most cases the Rhizobium remain viable, but they are not capable to make nodules in the host plants or their ability to fix nitrogen is reduced. Some herbicides affect nodulation, by reducing growth of root system while others interfere with the process of nodule initiation or establishment of the nodules^{[36] - [37]}.

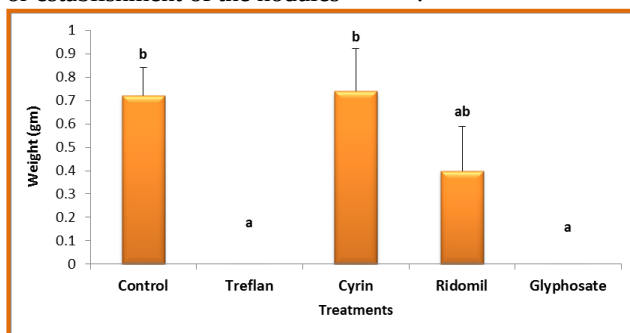


Fig. 3 Effects of pesticides on dry weight of nodules of faba bean plants.

C. Effect of Pesticides on Number of Tillers per Plant:

Plants treated with pesticides for eight weeks have produced significant decreases in number of tillers plant⁻¹ in case of both Treflan and Glyphosate treated pots (Fig. 9). There were significant differences between faba bean treated with Treflan, Glyphosate and Ridomil with their mean values of 1.825, 7.188 and 9.3 tillers respectively compared with control (11.750). In the case of wheat plants, significant differences were found between pots treated with Treflan and Glyphosate (2.5 and 0.00 tillers respectively) and control 7.100 tillers). The results were in disagreement with previous studies, which concluded that Glyphosate at high levels increase number of branches plant⁻¹ in faba bean^[38], while agreed with^[39], regarding the phytotoxic effects of pesticides on different physiological processes such as distortion of leaves and growing points. Also reduction in number of heads was reported as disadvantageous side effects of herbicides applied to wheat plants^[40].



Fig. 4 Faba bean roots (control) after two months of pesticide application.



Fig. 5 Nodules of faba bean plants treated with Cyrin C after two months of pesticide application.



Fig. 6 Nodules of faba bean plants in Ridomil treatment after two months of pesticide application.

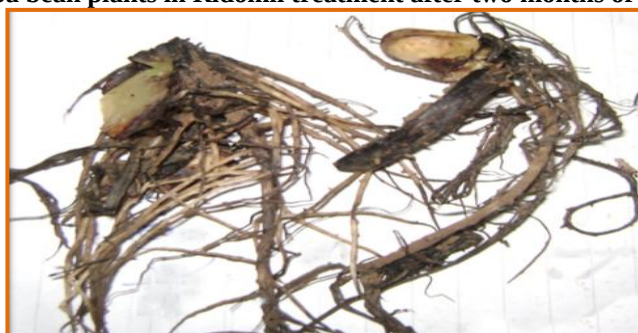


Fig. 7 Faba bean roots of plants treated with Glyphosate(no nodules) after two months of pesticide application.



Fig. 8 Faba bean seeds of plants treated with Treflan after two months of pesticide application.

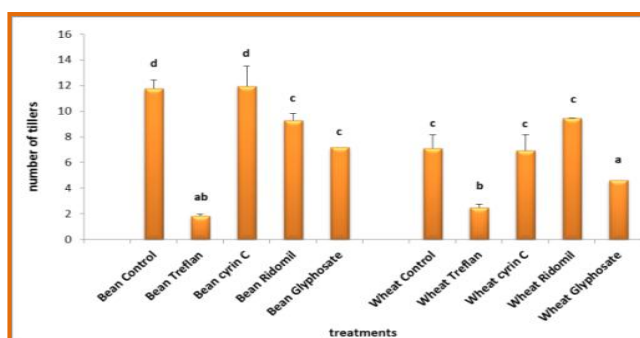


Fig.9 Effects of pesticides on tillers number per plant (mean±S.E.) of faba bean and wheat.

D. Effects of Pesticides on Plant Height of Wheat and Faba bean:

Fig. 10 shows that there are significant reductions in plant height of bean treated with Treflan and Glyphosate (3.375 and 17.012 cm respectively) when compared with control (42.950 cm). In the case of wheat treated with Treflan and Glyphosate also showed significant reduction in plant heights (6.875 and 0.00 cm respectively) between the two herbicides and significant differences between them and control (46.975 cm).

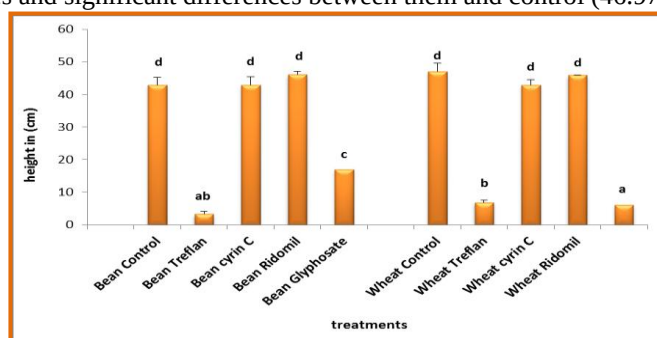


Fig. 10 Effect of pesticides on plant height (cm) of faba bean and wheat.

Previous reports showed that Glyphosate decreased the plant height of faba bean [38], also they suggested that Glyphosate may increase the level of ethylene, others [41] reported that ethylene inhibited cell division of meristematic tissues and noticed that plants exposed to ethylene induced inhibition of stem height, so as a result, plant height may be decreased when treated with Glyphosate. While it could be hypothesized that the morphological responses observed at low Glyphosate doses were due to an increase in activation of auxin, because Glyphosate interferes with the auxin pathway by blocking some of the precursors of auxin synthesis [42], [43]. Reports showed that Glyphosate has decreased the height of winter wheat [40], [44], others detected that pesticides reduce plant growth [39]. These results of Glyphosate action on morphological characteristics may be due to that Glyphosate is a wide spectrum herbicide (non-selective), as grasses are often more susceptible than broad leaved plants. Application of high rates of Treflan disturbs growing [4], as a result of disruption of mitosis [45].

E. Effects of Pesticides on Shoot Fresh Weight:

Fig. 11 shows that Treflan and Glyphosate decreases the fresh weight of both bean plants (20.00 and 37.50 gm) compared with control (136.25 gm) and wheat plants (2.07 and 16.76 gm) compared with control (59.813gm) respectively. These results in agreement with reports who determined that herbicide application to the soil was adversely affected physiological characteristics in crop plants [4]-[39] - [40]-[44] - [46] - [47] and [48]. In pots treated with Glyphosate after 5-7 days of plant emergence the color of shoots were converted to light green.

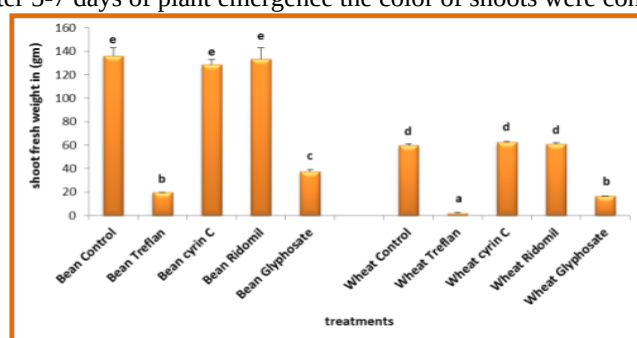


Fig. 11 Effects of pesticides on shoot fresh weight (mean±S.E.) of bean and wheat.

The most common symptoms of Glyphosate when foliage applied on treated plants are chlorosis which appears after 7-14 days of application followed by necrosis [49] - [50]. Soy bean plants top growth was reduced when Treflan was incorporated into the bottom 10 cm of soil while shoot and root weight ratios of corn were positively correlated to concentration of Treflan in the soil [51]. However, reports showed that Glyphosate decreased shoot growth proportionately the same for plants supplemented with N fertilizer [52].

F. Effects of Pesticides on Shoot Dry Weights of Faba bean and Wheat Plants:

Treflan and Glyphosate caused a significant reduction in shoot dry weight in both bean and wheat plants (Fig. 12). Faba bean shoot dry weight in pots treated with Treflan and Glyphosate were 17.50 and 20.0 mg respectively, compared with 36.25 mg for control, while Shoot dry weight of wheat plants in pots treated with Treflan and Glyphosate were 1.98 and 16.09 mg respectively, compare with 31.49 mg for control. These results agreed with those observed by [19], [27], [38], [52] and [53].

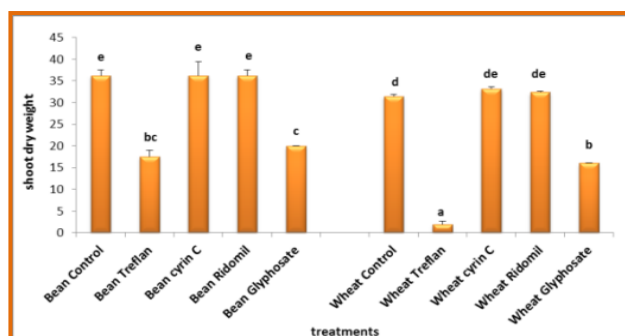


Fig. 12 Effect of pesticides on shoot dry weight of bean and wheat (mean±S.E.).

G. Effect of Pesticides on Root Fresh Weight:

Root fresh weights of bean plants in pots treated with Treflan and Glyphosate were decreased to 28.750 and 26.250 mg respectively compared with 106.250 mg for control (Fig. 13). Treflan and Glyphosate application to wheat plants caused marked decreases in root fresh weight from 86.273 mg in control to 14.198 and 3.387 mg respectively, While, Ridomil increased root fresh weight to 211.908 mg. Results agreed with reports in which the herbicidal action of Treflan is related to the impairment of root growth by inhibition of microtubule polymerization from free tubulin subunits^[54]. Also reported that fresh and dry weights adversely affected as the duration and concentration of Glyphosate increased^{[52], [55]}. In case of grasses, the leaves may not fully emerge from the coleoptiles, while Treflan inhibits mitosis in the root tips of wheat seedlings^[45].

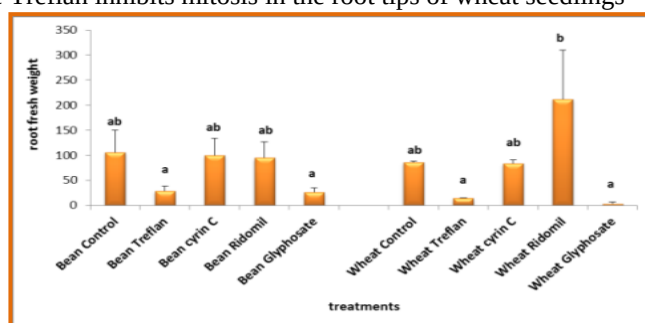


Fig.13 Effects of pesticides on root fresh weight (mean±S.E.) of bean and wheat plants.

H. Effect of Pesticides on Root Dry Weight:

Fig. 14 indicated that root dry weight in bean plants treated with both Ridomil and Glyphosate were reduced (15.00 and 15.00 mg respectively) compared with control (25.00 mg). While in wheat plant grown in pots treated with Treflan, Glyphosate and Ridomil, root dry weights were decreased significantly to 11.990, 3.092 and 27.655mg respectively compared with control (37.005 mg). Reports showed that ethylene and ammonia accumulated by Glyphosate in faba bean resulted in reduction in total dry weight plant⁻¹^[38], and roots were reduced as a result of pesticide treatments^[19]. Others discovered that dry weight was adversely affected as the duration and concentration of Glyphosate increased^[55].

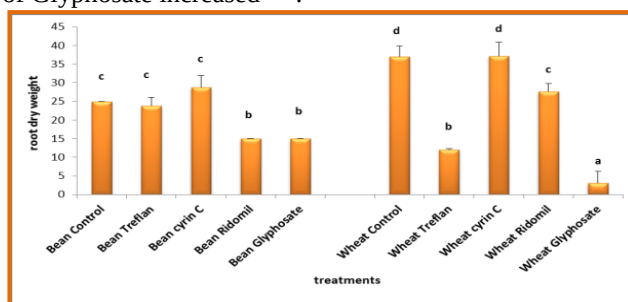


Fig. 14 Effects of pesticides on root dry weight (mean±S.E.) of bean and wheat.

I. Effects of Pesticides on Wheat Seed Germination:

Treflan was delayed seed germination to seven days; and the seedlings were remained in lower percentages till 15 day after sowing (8%) compared with control (95%) (Fig.15). Cyrin decreased the percentage of seed germination, so after 7 days the germination percentage was 60% compared with 78, 80 and 68% for control, Glyphosate and Ridomil respectively. After 2 weeks of planting, seed germination were 8, 75, 82, 82 and 95% for seeds treated with Treflan, cyrin, control, Glyphosate and Ridomil respectively. The results of Glyphosate

treatment disagreed with ^[39] - ^[56] who observed Glyphosate toxic symptoms in wheat. Reports showed that Glyphosate has been stimulated plant growth in a range of species when applied at low doses on barley plants ^[57]. Seedling poisoned with Treflan is severely stunted ^[58]. Mitotic activity of meristematic cells in Treflan treated plants was suppressed due to the retardation of metaphase progression-alteration that can be caused by cytoskeleton disorder ^[4].

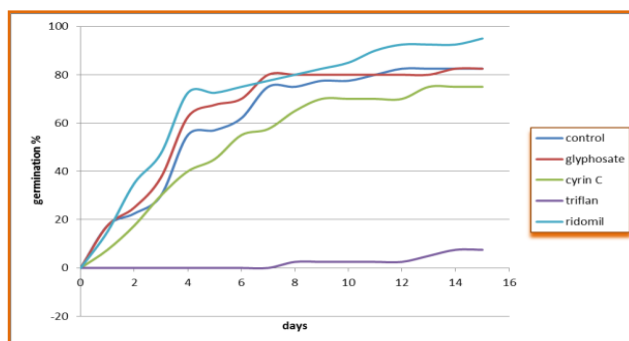


Fig. 15 Effects of pesticide treatments on wheat seed germination %.

J. Effects of Pesticides on Faba bean Seed Germination:

All pesticide treatments were reduced germination of bean seeds significantly comparing with control (Fig. 16). After 7 days of sowing, seed germination percent were 20, 28, 44, 68 and 72% for Glyphosate, Treflan, cyrin, control and Ridomil treatments respectively. After two weeks the seed germination percentages were 35, 68, 68, and 72% for Treflan, Glyphosate, cyrin and Ridomil treatments compared with 72% for control. These results are in agreement with observations made by ^[27] who proposed that germination of alfalfa seeds were reduced significantly when treated with herbicide. In contrast, dressing with Euparen (fungicide) and Gaucho (insecticide) were decreased the germination significantly ^[59], ^[60].

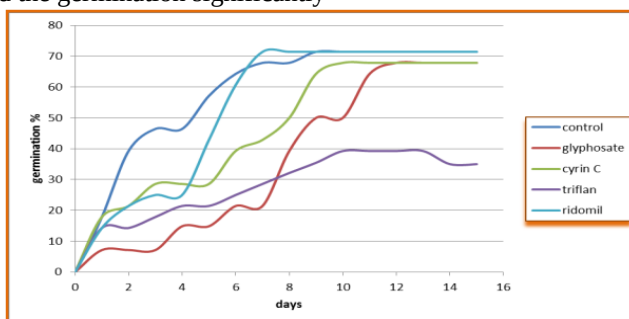


Fig. 16 Effects of pesticide treatments on faba bean seed germination %.

K. Effects of Pesticides on Total Nitrogen Contents of Plant Tissues:

Total nitrogen contents of bean plants in pots treated with Cyrin and Ridomil were significantly decreased (9.32 and 10.72 respectively) compared with 15.23 mg gm⁻¹ for control (Fig. 17). In the case of wheat plants the total nitrogen was significantly reduced to 0.00 mg gm⁻¹ in pots treated with Treflan compared with 2.72 mg gm⁻¹ for control. Total nitrogen contents in wheat plant tissues were lower significantly compared with bean plants. These results were predicted because it is clear that wheat plants need more nitrogen compared with bean plants, which fix the nitrogen by their root nodules using *Rhizobium* symbiotically and supply the soil with nitrogen. N uptake takes place mainly up to the time of flowering ^[61]. This statement disagreed with previous works that showed that herbicides increased shoot N content in pea plants ^[62]. While others mentioned that herbicides had no effect on nitrogen uptake in wheat plants ^[63]. It is possible that effects of herbicides on nutrient uptake may be resulted from their effects on plant cell membranes.

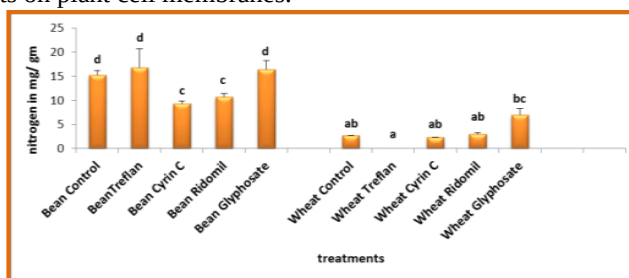


Fig. 17 Effects of pesticides on total nitrogen content (mean±S.E.) of bean and wheat plants.

L. Effects of Pesticides on Total Phosphorus in Plant Tissues:

Fig. 18 shows that in wheat plants grown in pots treated with Treflan, the total phosphorus decreased significantly to 1.980 mg gm^{-1} compared with control (6.4 mg gm^{-1}). While, total phosphorus content was increased significantly to 12.9 and 15.7 mg gm^{-1} in both Ridomil and Glyphosate treated plants respectively. Reports showed that pesticides significantly inhibited Arbuscular Mycorrhiza (AM) root colonization and the number of spores in all legumes and the accumulation of N, P and K in pesticide-treated plants were lower than in control plants [64].

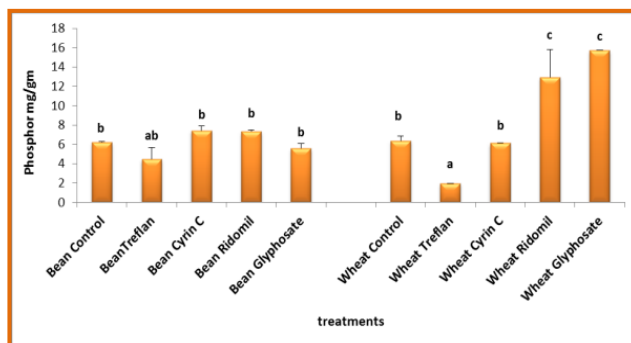


Fig. 18 Effects of pesticides on total Phosphorous content (mean±S.E.) of bean and wheat plants.

Glyphosate is chemical chelators which binds with mineral elements and can immobilize the minerals [65], thus it desorbed from soil by Phosphorous [66], and this statement confirms present results. The intense expression of root-induced mechanisms for P mobilization in the rhizosphere, reported by various plant species and cultivars [67], can similarly induce toxic effects by co-metabolization of Glyphosate adsorbed to P sorption sites [68], [69] and increased P significantly in young leaves that might be related to accumulation of P-containing Glyphosate in young plant parts.

M. Effects of Pesticides on Total Sodium

Fig. 19 showed that pesticide treatments affected significantly on total sodium in bean and wheat tissues. Bean plants grown in pots treated with Treflan and Ridomil were containing lower concentrations of sodium (0.47 and 1.96 mg gm^{-1} respectively), compared with control (2.78 mg gm^{-1}). Sodium contents of wheat plants grown in pots treated with Treflan had significantly decreased the total sodium to 0.01 compared with control (0.93 mg gm^{-1}). On the other hand, Glyphosate treated soils caused an increase in total sodium of wheat plant tissues to 1.97 mg gm^{-1} comparing with control (0.93 mg gm^{-1}).

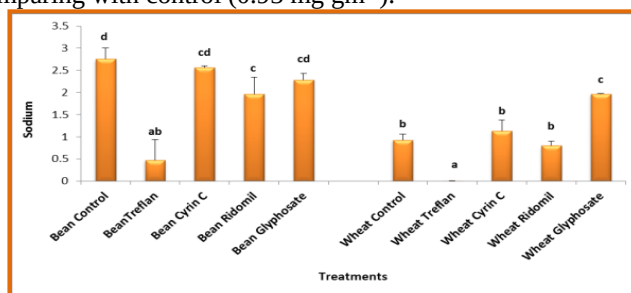


Fig. 19 Effects of pesticides on total Sodium content (mean±S.E.) of bean and wheat plants.

N. Effects of Pesticides on Total Potassium in Plant Tissues:

Treflan caused significant decrease in total potassium contents of tissues of bean plants to 25.89 mg gm^{-1} compared with $775.07 \text{ mg gm}^{-1}$ for control (Fig. 20). In case of wheat plants, Treflan was reduced the total potassium contents of plant tissues significantly to 0.02 mg gm^{-1} compared with 58.67 mg gm^{-1} for control. Glyphosate was reduced the availability or uptake of essential nutrients like potassium by affecting promotion of microbial nutrient sinks [66]. It was concluded that K-concentration of the shoots increased 30 % in relation to the non-fertilized absolute control plants in comparison with pesticide treated plants [70].

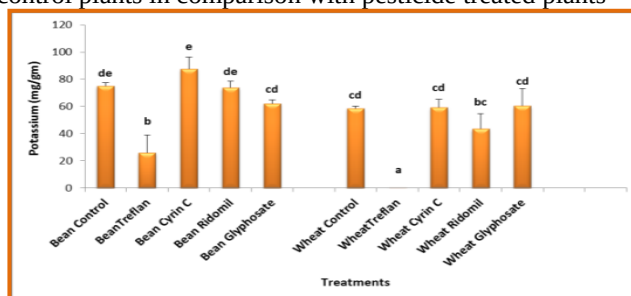


Fig. 20 Effects of pesticides on total potassium content (mean±S.E.) of bean and wheat plants.

O. Effects of Pesticides on Total Iron in Plant Tissues:

Glyphosate treatments lead to decrease total iron content to 0.056 ppm in treated bean compared with control (0.157 ppm) (Fig. 21). Wheat plants treated with Treflan and Glyphosate have reduced total iron to 0.00 and 0.212 ppm respectively, compared with control (0.412 ppm).

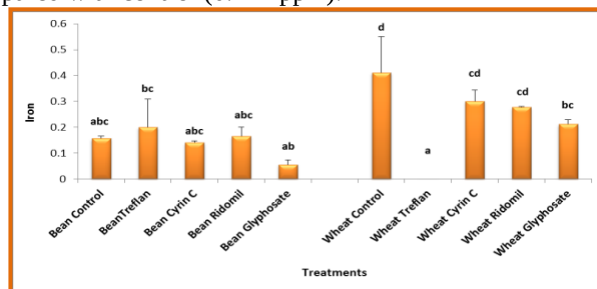


Fig. 21 Effects of pesticides on total Iron content (mean±S.E.) of bean and wheat plants.

P. Effects of Pesticides on Total Zinc Concentration:

Treflan treatments caused significant decrease in zinc contents of bean tissues (0.02 ppm) compared with control (2.13 ppm) (Fig. 22). Reports showed that Zn concentration in young leaves of some wheat species were depressed by herbicide (Chlorsulfuron) treatment [63]. The most nutrients affected by herbicide seem to be those transported Zn to the roots by diffusion.

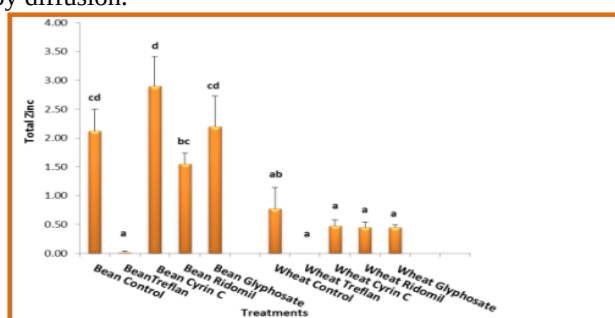


Fig. 22 Effects of pesticides on total Zinc concentration (mean±S.E.) of bean and wheat plants

Q. Effects of Pesticides on Total Manganese Content:

Treflan was caused a significant decrease in Mn concentration of bean tissues (0.3 ppm) compared with 2.52 ppm for control (Fig. 23). It was stated that Glyphosate affected Mn transformed microbial groups by affecting Mn transformation and plant availability [28]. Glyphosate intoxication of sunflower seedling was associated with an impairment of manganese nutritional status [68].

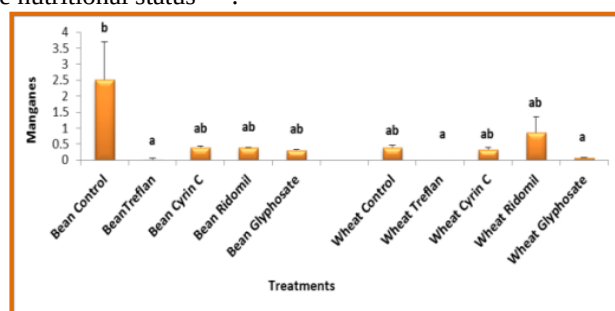


Fig. 23 Effects of pesticides on total Manganese content (mean±S.E.) of bean and wheat plants.

R. Effects of Pesticides on Total Calcium in Plant Tissues:

Fig. 24 showed that the studied pesticides were significantly affected on calcium Ca concentration in both plants. Total Ca was reduced to 26.667 ppm in bean plants treated with Treflan, compared with control (85.333 ppm). Calcium is a critical mineral nutrient affecting cell wall stabilization and cell elongation such as in shoot meristematic tissues [71]. Due to their low transpiration capacity, shoot tips are highly sensitive to small changes in Ca concentrations. Accumulation of Glyphosate in such sink organs with low Ca concentration would induce physiological Ca deficiency by making complex compounds. Glyphosate may also hinder Ca transport into reproductive organs (e.g., seeds) by immobilizing Ca in root or vegetative tissues.

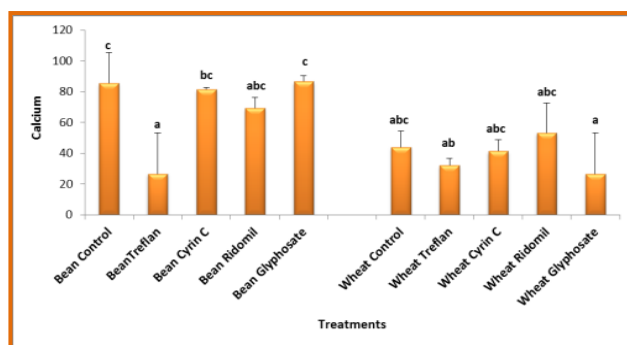


Fig. 24 Effects of pesticides on total Calcium content (mean±S.E.) of bean and wheat plants.

S. Pesticide Residues in Plant Samples:

Fig. 25 and 26 showed the residues of Metalaxyl in samples of wheat and bean plants respectively, that mean all detectable Metalaxyl in the soil is absorbed from the soil by plant roots, because it disappeared in the soil, to detect by HPLC and remained in plant tissues after 90 days of application. Fig. 27 and 28 showed that residues of chlorpyrifos is present in tissues of bean and wheat plants, means that chlorpyrifos remain in both, the soil and plant tissues after 90 days of application. It seems that Metalaxyl and chlorpyrifos were remain in the wheat plants grown in soils treated with these two pesticides and they need more time for destruction and degradation.

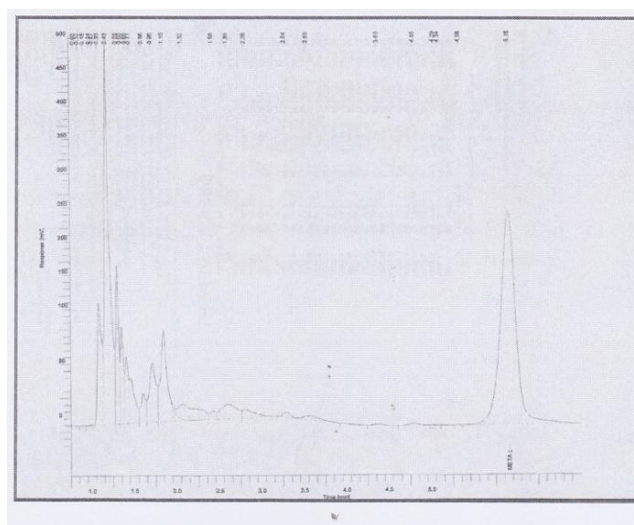


Fig.25 HPLC Chromatogram showing Metalaxyl residue in Wheat Plants

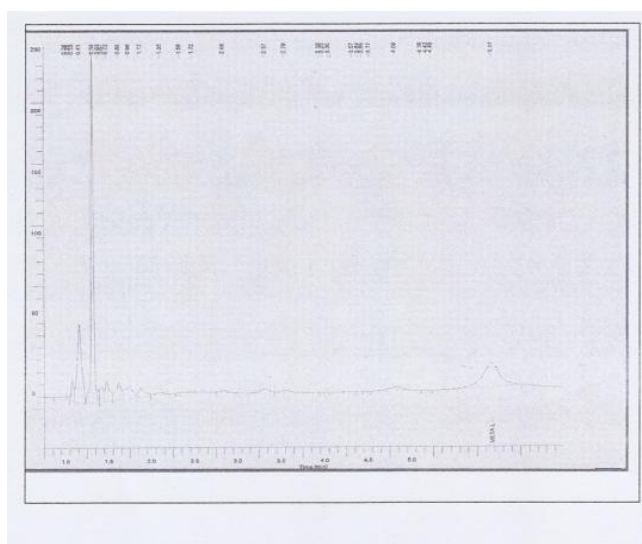


Fig.26 HPLC Chromatogram showing Metalaxyl Residue in Bean Plants.

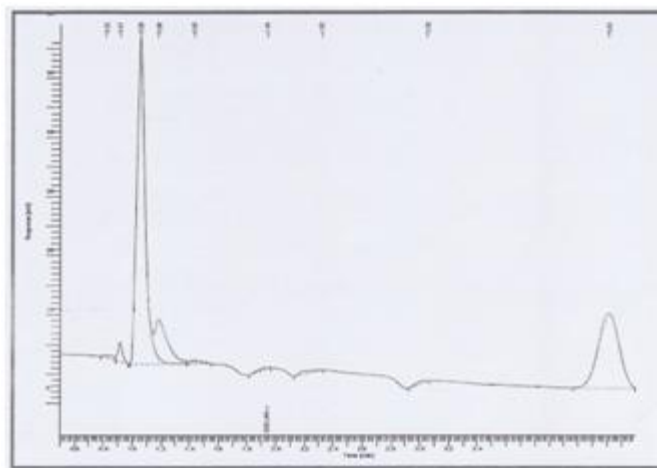


Fig. 27 Chromatogram showing Residue in Bean Plants.

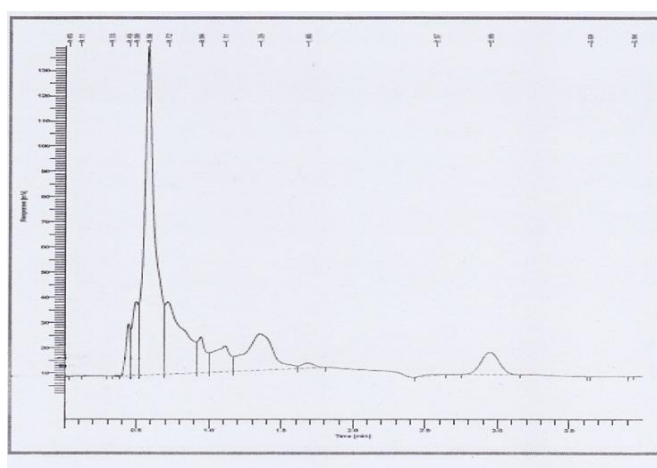


Fig. 28 HPLC Chromatogram showing Chlorpyrifos Residue in wheat plants.

Uptake of pesticides from the soil by plants is probably a major source of food chain bioaccumulation and an important route of exposure to humans and animals ^[72]. It was detected that Glyphosate prevents from being taken up by the roots of plants ^[73] because of adsorption to soil particles, but the obtained results showed that Glyphosate may be taken up by plants and decomposed in both soil and plant tissues. Organochlorine pesticide contamination in wheat from Konya region, seem to be the same as obtained in the present study with regard to most of the samples have been found to be contaminated with residues and some residues exceed MRLs ^[74]. Metalaxyl residues were detected in samples imported sesame and Yecheon sesame (0.014 ppm and 0.04 ppm, respectively) which confirm the present results ^[75]. Pesticide residues generally increased as the rate of application increased regardless the stage of the crop, and generally decreased when pesticide was applied at later stages of crop development ^[76], the physiological maturity of the crop at application, rate of pesticide application and environmental factors all play important roles in determining the magnitude of pesticide residues.

IV. Conclusions

According to results obtained from the present study we can conclude that:

1. Studied Pesticides especially the herbicides have affected nodulation in faba bean.
2. Plant vegetative characteristics were affected by pesticide application as well as nutrient contents.

V. Recommendations

According to results obtained from the present study we can recommend:

1. 1- Harvesting the crops and fruiting bodies after a sure time to reduce the side effects of pesticides.
2. Using proper soil microorganisms for degrading certain pesticides in the soil.
3. Extra studies should be performed on pesticides used in the country and studying its effect on soil, plant nutrition and pollution in all scientific branches.

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