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RESEARCH ARTICLE

EFFECT OF AQUEOUS EXTRACT OF NEEM (AZADIRACHTA INDICIA) LEAVES ON PLANT DEVELOPMENT AND LIVER ENZYMES IN RATS.

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Abstract

Background: Herbicides have been used widely and intensively in agricultural areas around the globe to enhance crop yield. However, many biocides are causing serious environmental problems and may possess critical risks on treated agricultural crops.

Materials and Methods: The study was designed to investigate the effects of biocides (azadirachta) treatment at different concentrations (0, 5, and 10) on few chemical constituents in plant leaves. Furthermore, the study also illustrated the extent to which an organic fertilizer could enhance deleterious impact of azadirachta indicia on plant growth. Lastly, in this study, lab experiments were performed on rats to examine influence of leaf on their liver function.The results were determined by one way Anova analysis of variance .

Results: This study exhibits that low and high of Azadirachtin produced significant increase in total and soluble sugar content, compared with normal and fertilizer treated groups. Synchronized treatment of fertilizer with Azadirachtin in low and high dose showed significant increase in total and soluble content in contrast to normal and fertilizer groups. However, there is an exception of significant decrease in total sugar with high dose of Azadirachtin. Proline contents significantly increased in fertilizer treated group, while contents decrease significantly in other treated groups compared with normal and fertilizer. Chlorophyll A and chlorophyll B significantly increased in fertilizer treated groups. Low and high dose of Azadirachtin induced significant decrease in chlorophyll A and chlorophyll B content when compared with fertilizer treated group. High dose of Azadirachtin and low dose with fertilizer treated groups induced significant decrease in carotein content. Rats in all treated groups were observed with significant level of increase in AST activity with high dose of Azadirachtin group. LDH activity increased significantly in high dose of Azadirachtin . Total protein level, significant decrease was recorded in low dose of Azadirachtin group . Significant increase was observed in high dose of Azadirachtin alone, and in both high and low doses with fertilizer groups

Conclusions: The study argued that biocides have their impacts on plant leaves. Hence, it is necessary to stay aware of chemical types applied on crops. Awareness should not limit to environmental contamination and toxicity, but their effects on crops should also be considered to obtain good quality of agricultural products for healthy consumption. The current investigation adequately proves that azadirachta indicia leaves are an effective heptaprotective agent at low dose.

Introduction:-

Chemical pesticides have been used successfully for many decades, but their effects on groundwater pollution, residues on food crops, effects on non-targeted organisms, and the development of resistance to chemicals have gained significant attention on alternative control methods. Thus, pesticides with high toxicity to humans are targeted for rationalization, irrespective of the quantity used.

Monitoring procedure of pesticide residues in vegetables for evaluation of their quality is a prime objective in pesticide research today in order to avoid potential risks to human health [1-2]. Food contamination monitoring programs in the Middle East regions have been conducted for several years now by innumerable investigators and researchers [3-4-5]. The results of these studies revealed that possibility of various pesticides from different chemical groups and contents are high.

Azadirachtin and Azadirachtin-Containing Neem (Azadirachtin indica) seed extracts results multiple health effects in insects. The effect on insect development is extremely significant, since several hypotheses are proposed and existing on this aspect. However, pesticide-removing activity of insects is a potential alternative for using natural products from plants, because they found with minimum mammalian toxicity, minor harm to ecology and broad public acceptance [6-7].

Azadiractha indica is a tree, which belongs to Meliaceae family. Its origins were found in Southern and South-East Asia. Currently, it also grow in tropical and subtropical regions of Africa, America and Australia. In recent years, this specie has successfully gained radical attention, particularly as a component of integrated pest management program, since it has been found to be more beneficial and less toxic for humans rather pests [8].

Azadiractha indica is a tetranortriterpenoid or limonoid, which is familiar to steroids [9]. Extracts from neem leaves, roots, fruits, seeds and barks comprise with properties of biocids properties and utilize for soil amendments. These amendments are done in order to control and prevent soil from soil borne and contaminated pathogens such as bacteria, fungi, nematodes and insects that harm crops [10].

On contrary, effects of Azadiractha indica on biological properties of soil are another area of subjective argument. Different parameters of soil biology are widely used for monitoring distinct organic chemicals. Biochemical parameters like enzyme activities, microbial biomass and their activities are constructive indicators for essential biological activities.

The residual effect usually exists for approximately 4 to 8 days, which is completely dependent on environmental aspects and the treatment given to plant species. However, systematic effects are longer than residual in context to lasting with similar conditions [11].

Discussing further, leaf is one of the most essential segment in plant, because it has multiple purposes. Significant role of a leaf consists of food production, also known as photosynthesis, food storage, water transportation, exchange of environmental gases that helps flow of respiratory, and prevention from vegetation and floral buds. Proceeding besides plants, leaves are even essential for other organisms since photosynthesis procedure generates oxygen into ecology. Furthermore, leaves are prime source of food for both, humans and animals.

Plants containing green leaves, their quality is determined by chlorophyll contents, which are found in leaves. Since chlorophyll is responsible of providing natural green color to vegetables and fruits, which makes it a quality measuring medium. However, the green pigment is criticized to form other types of molecules with changes in colors. The molecules found in green pigment are phaeophytin, phaeophorbide and olive-brown compounds. According to [12] and [13], the criticized molecules are even found to metabolize into colorless compounds.

Several other factors are constantly influencing loss of chlorophyll namely normal aging, inadequate natural sunlight, variations in temperature, deficiency in essential nutrients and viral infections [14]. Nevertheless, this study aims to identify and examine the potential effects on lettuce plant and liver functioning of rats, which is caused by growing parameters of a biological pesticide called azadirachtin.

Materials and Methods:-

Plant experimental methods:-

Chemicals:

- Azadirachtin (EC) 10 grams/liters acquired from Astrachem Company, Astra Industrial Group. Low and high doses are used at 0.25 ml per 100 liters of water and 250 ml per 100 liters of water respectively.
- Acadian, a fertilizer, is used at a dose level of 100 ml per liter. Natural content was acquired from Acadian Agrich Company, Canada.

Methods:

Lettuce plants were used for testing and segmented in six groups:
Group I: Untreated plant (normal control group)
Group II: Lettuce exposed to organic fertilizer only
Group III: Lettuce exposed to low concentration of Azadirachtin
Group IV: Lettuce exposed to high concentration of Azadirachtin
Group V: Lettuce exposed to low concentration of Azadirachtin and mixed with organic fertilizer
Group VI: Lettuce exposed to high concentration of Azadirachtin and mixed with organic fertilizer

This study was designed to investigate potential impact of Azadirachtin at different levels of concentrations namely low, high, and organic fertilizers in lettuce plant. The samples of the study will be gathered at different stages of plant growth. Further, when concluding the experimental period, which is three months, sample extracted from plant will be studied for chemical estimations. The estimations are:

- Proline content, which is amino acid according to the method of [15] based on the usage of Spectrophotometer
- Total soluble sugars by using Spectrophotometer
- Total sugars according to the study method of [16] by using Spectrophotometer
- Photosynthetic pigments, which are chlorophyll A and chlorophyll B, and carotien according to the study method of [17] by using Spectrophotometer model namely Spectro UV-VIS RS.

Animal experimental methods:

The present study was conducted on experimental animals to examine the effect of lettuce ingestion before and after the exposure to deltamethrin only, or with organic fertilizers. To carry out this process, mature rats were used for experimental purposes. Rats will be domiciled under the prevailing atmospheric period provided in laboratory of physiology, Faculty of Science and Arts, Al-Qassim University.

The study involved 60 albino rats that are adult and male by age and gender, weighing 200 grams, ± 50 grams. The acquired rats were housed under prevailing atmospheric conditions throughout experimental period, which lasted after four weeks, conducted in the laboratory of physiology. Similar to Lettuce, Rats were also divided into six equal groups.
Group I: Normal control group fed on ordinary rat chow received 0.5 ml saline vehicle daily
Group II: Rats fed on Lettuce exposed to organic fertilizer only
Group III: Rats fed on Lettuce exposed to low concentration of Azadirachtin
Group IV: Rats fed on Lettuce exposed to high concentration of Azadirachtin
Group V: Rats fed on Lettuce exposed to low concentration of Azadirachtin mixed with organic fertilizer
Group VI: Rats fed on Lettuce exposed to high concentration of Azadirachtin mixed with organic fertilizer

At the end of experimental period, fasting blood samples were collected from orbital sinus. It was later centrifuged and serum used for the measurement was AST, ALT and LDH activities, total proteins and albumin concentration.

Statistical analysis:-

The generated data were analyzed through 'Statistical Package for Social Sciences (SPSS), version 16' software program. The analysis of covariance or one way ANOVA was applied to identify differences in mean between treated groups and control groups. Similar application was also done to identify mean difference between fertilizer treated groups against all treated groups. The mean difference is significant at $P < 0.05$.

Results:-

Plant field results:-

The results of this study exhibits that carbohydrate T.R.S and D.R.V. increased significantly in fertilizer treated group, while soluble sugar decreased in contrast to normal group. Low and high of Azadirechin comprised with significant increase in total and soluble sugar content, compared with normal and fertilizer treated groups. Synchronized treatment of fertilizer with Azadirachtin in low and high doses showed significant increase in total and soluble content in contrast to normal and fertilizer groups. However, in similar circumstance, there is an exception of significant decrease in total sugar with high dose of Azadirachtin.

Proline contents significantly increased in fertilizer treated group, while contents decrease significantly in other treated groups compared with normal and fertilizer. Chlorophyll A and chlorophyll B significantly increased in fertilizer treated group when compared with normal group. Low and high dose of Azadirachtin induced significant decrease in chlorophyll A and chlorophyll B content when compared with fertilizer treated group. However, when normal group was compared with fertilizer treated group, an insignificant decrease was observed.

Discussing carotein contents, insignificant changes were observed in fertilizer and low dose of Azadirachtin treated groups compared to normal. There was, however, a significant increase in low dose of Azadirachtin compared with fertilizer treated group. On the other hand, high dose of Azadirachtin and low dose with fertilizer treated groups induced significant decrease in carotein content versus both normal and fertilizer treated groups.

Laboratory rats feeding results:-

In this study, rats in all treated groups were observed with significant level of increase in AST activity with high dose of Azadirachtin group compared with normal and fertilizer treated groups. ALT activity showed insignificant variations in all treated groups. LDH activity increased significantly in high dose of Azadirachtin treated group when compared with normal and fertilizer treated groups.

In context to total protein level, significant decrease was recorded in low dose of Azadirachtin group compared to fertilizer group. Furthermore, a significant increase was observed in high dose of Azadirachtin alone, while both high and low doses with fertilizer also showed a significant increase when compared with normal and fertilizer treated groups. Albumin contents showed insignificant changes in all treated groups except when high dose Azadirachtin combined with fertilizer. Albumin level significantly increased when compared to normal and fertilizer treated groups.

Discussion:-

The public concerns regarding the applications of environmentally hazardous agrochemicals increased radically in past few years. This concern is triggering significant research activities to develop more friendly strategies for plant and disease control that will contribute towards agricultural sustainability. While testing specimens or groups, the higher dose of Azadirachtin was observed with considerable reductions in photosynthetic pigments in contrast to untreated groups. On the other hand, the lower doses, 0.38-ppm and 0.76-ppm specifically, had a positive influence on biological competencies of soil [18]. Similarly, some researchers even reported that Azadirachtin with low concentrations possesses properties of fertilizer [19], since it contains few essential nutrients (N, P, K) and high levels of organic compounds [20]. The results of the present study reveled that total sugars increased significantly fertilizer treated group . However, soluble sugar reduced when compared to normal group. Low and high dose of Azadirachtin stimulated significant increase in both total and soluble sugar content when compared to normal and fertilizer groups except high dose of Azadirachtin, which had a significant decrease in total sugar compared to normal group.

The transformations observed in soluble sugar contents were dose-dependent, exhibiting a strong correlation with a tendency of treatment due to short-term and annual cropping duration of lettuce and green leaves. The short seasoning is because of intense vulnerability from insect damage and environmental contamination, which makes consideration of biological control generally unacceptable.

Higher expectations from consumers for aesthetically appeal fabricates free of pesticide residues. Furthermore, it forces farmers and vegetable producers to execute chemical control tactics that are not just effective, but safe as well. Consequently, scientists have been researching and developing integrated pest management programs for

lettuce that are help to reduce economic, occupational and dietary risks caused by chemical controls in past [21] .Proline contents significantly increased in fertilizer treated groups than in other treated groups. Its contents significantly reduced when compared with normal and fertilizer treated group. A reduction in growth, measured as chlorophyll content, accompanied with reduction in proline content in each group treated with Azadirachtin plants. Such results may occurred since total phenolics and radical-scavenging constituents of Azadirachtin along with the ability of extracts to catch carbon-centered hydroxethyl radicals indicates towards pro-oxidative activity [22].

In alignment with our results, [23-24], discussed that the intracellular proline content increased when environment comprises with presence of heavy metals, pesticides and high salt concentration. The intercellular-cyano bacterial proline accumulation was enunciated under salt stress than in the presence of pesticides and heavy metals. Chlorophyll A and B increased significantly in fertilizer treated group rather in normal group. Low and high dose of Azadirachtin persuaded with significant decrease in chlorophyll A and chlorophyll B content when compared with fertilizer treated group rather found in normal group. The significant level of decrease occurred with 0.0061.

In context to carotein contents, insignificant changes were found in fertilizer and low dose of Azadirachtin treated group compared to normal group. However, low dose of Azadirachtin compared with fertilizer group denotes a significant increase. High dose of Azadirachtin and low dose of fertilizer treated groups persuaded with significant decrease in carotein content when compared to both, normal and fertilizer treated groups.

Neem extract products have been used very frequently as a potential substitute to synthetic pesticides, since they comprise with insecticidal, insect antifeedant and growth regulating effects. Moreover, new formulations and solutions are continuously researched and developed; therefore, they are evaluated for their efficacy and persistency.

[25] argued that Azadirachtin (AZA) was used with a dose rate of 10 mg and 1.2 mg 3-tigloyl-azadirachtol (azadirachtin b) on each treated bean plant. Later on, the translocation and persistence of AZA and 3-tigloyl-azadirachtol were studied in detail. Variable amounts of residues of the active components in relation to parts of plants and period of analysis.

[11] examined and studied the potential effects of various concentrations of aqueous neem leaf extract on growth and aflatoxin production at different incubation intervals. The inhabitation of aflatoxin synthesis by plant extracts was found to be highly dependent on time and dose.

The residual effects of Azadirachtin usually lasted for approximately 4 to 8 days, based on environmental conditions and species to which a treated plant belongs [11]. In relation to chlorophyll, degradation or loss in leaves is conveniently used as an indicator for plant senescence, since the changes in green color is observed feasibly.

Nonetheless, there were various factors, which significantly influenced senescence in plants. These factors have potential to either enhance or inhibit senescence in plants widely. Herbicides and insecticides are chemicals that are broadly consumed in agricultural activities to increase productivity of crop with huge significance. However, some biocides have some effects in growth, photosynthesis and chlorophyll synthesis of plants.

Azadirachtin falling in the range of 2.16 grams to 10.80 grams per liter seems to increase degradation rate of chlorophyll during the early stages of contact, once Azadirachtin is directly combined with chlorophyll extracts. This is a result when contents of Ca and Cb are decreased significantly compared to control group. However, after several hours of contact, there was no obvious difference between chlorophyll content in treated and control extracts.

This indicates that contact time between biocide and chlorophyll extract is a critical factor resulting in changing rate of chlorophyll degradation. Azadirachtin will radically increase rate of degradation of chlorophyll in plants to an extent before slowing it down. The study conducted by [26] argued that biocides had an adverse effect on photosynthesis. It was revealed through a significant reduction in rate of chlorophyll and carotenoid contents, which resulted in a strong inhibition of plant growth. On the other hand, soluble sugar accumulated in all organic components of plant suggesting that stimulation of sugar uptake from the medium.

Moreover, photosynthetic activity recovered in 3 weeks of treatment with low dose of herbicide concentration. Agricultural crops are in general, contaminated with biocides. Hence, in order to observe noticeable effects of

biocides on the change of chlorophyll content in crops, insecticides were applied to plants. In the case of Azadirachtin, either submerging or spraying on the detached leaves with such solutions will result in approximately 50 percent loss of Ca and Cb.

Proceeding, increasing concentrations Azadirachtinup to 5 times of the recommended figures resulted in similar level of chlorophyll loss in plants, as per recommended concentration. Azadirachtin may even influence other sources of chlorophyll degradation, not only through direct impact on chlorophyll molecule, but indirect as well. Application of Azadirachtin on leaves exhibited slight increase in amounts of chlorophyll than found in control group. Therefore, it can be argued that Azadirachtin comprises with a property that inhibit senescence or chlorophyll degradation in lettuce leaves.

Since Azadirachtin is a pesticide, they are not expected to result adversely to plant growth. The corresponding results were observed with non-decreasing chlorophyll contents compared to the control done after treating plants with pesticides.

This study is providing and playing role of evidence that some biocides are having huge impact on chlorophyll degradation. In growing plants, loss of chlorophyll in small leaves may not harm plant growth or quantity of leaves. It is mainly resulted because plants are able to recover chlorophyll through photosynthesis process, which is not possible in post-harvest crops. Henceforth, it is critical to say if contaminated chemicals cause an increase in rate of chlorophyll degradation.

It is necessary to be aware of the application of chemicals on crops, not only in terms of their effects on ecology, such as contamination and toxicity, but even in context to their effects on crops required to extract good quality of agricultural productivity. The pesticide plays a significant role of a substrate for the soil microflora. In fact, its degradation may supply certain organisms with carbon and related energies like nitrogen, which are essential for growth. In any case, the most critical consequence of these aspects appears essentially in relation to pesticide degradation [27].

There are innumerable studies conducted in past and each proposed distinct results to this field. [28] discussed negativity of effects of application of pesticides on biological characteristics present in soil. Conversely, other studies pointed the biological properties under similar context [29]. The differences occurred due to various methodological factors, for instance, laboratory and field ecotoxicology studies, environmental monitoring. Further, the variations in pesticide factors such as types, concentrations, degradable properties, and soil environmental factors namely clay, organic matter and pH even contributed to reach distinct results of researches conducted. A brief summary of extracted results generated from studies like [30] argued that there are enormous disparities between findings of various studies where pesticide concentrations are toxic.

In this study, rats belonging to all treated groups revealed significant increase in the activity of AST with high dose of Azadirachtin treated group versus normal and fertilizer treated groups, where ALT activity denoted insignificant changes in all treated groups. LDH activity rose significantly in high dose of Azadirachtin treated groups compared to normal and fertilizer treated groups.

In contrast to total protein levels, significant reduction was observed in low dose of Azadirachtin treated group compared with fertilizer group. On the other hand, high dose of Azadirachtinalone, and low and high dose with fertilizer, a significant increase was observed versus normal and fertilizer treated groups. Albumin contents denoted that insignificant changes in all treated groups were present, except in high dose of Azadirachtin combined with fertilizer albumin level, which increased significantly with normal and fertilizer treated group.

The liver is a center for detoxifying any external compounds penetrating into a body. Hence, it uniquely exposed a wide range of exogenous and endogenous products. These products include ecological toxins and chemicals present in food or drink[31].

The liver is prominent for disorders, which is a serious health state. In allopathic medicinal practices, reliable liver protecting drugs are not available, which provides herbs an opportunity to play an important role in managing and curing liver disorders. Numerous medicated plants are used for similar purposes, in medical practices and conventional system of medicines, specifically in regions of India.

Schmutterrer[32] evaluated the influence of Azadirachtin indicia leaves powder in contrast to carbon tetrachloride (CC14) induced liver damage. The evaluation determinants were ALT, AST, Alkaline, Phosphate, Cholesterol and Total Protein. The biomedical parameters were found to change significantly due to single dose of CC14; however, the treatment of aqueous slurry powder extracted from leaves of Azadirachtin significantly recovers all markers to normal levels.

Observation of markers along with light and electron microscope photographs supports the regeneration of liver parenchyma. This suggests and is an evidence for overall promising effect against liver disorders.

Azadirachtin Indicia is fastest growing evergreen popular tree, which is commonly found in India, Africa and America. The leaves of this tree has ability to expel worms from body and use for cough, ulcers, inflammation of liver and various skin diseases. It is used as a purgative to treat urinary issues, tumors, piles and toothache and hepatoprotective activity-1. Total tissue protein significantly increase after treating with CC14 but its level significantly decreased after neutral recovery and silymarin treatment.

Plant slurry treatment caused marginal reduction in total tissue proteins in rats. The liver of rats, after combined treatment of CC14 and Azadirachta Indicia leaves shows mild congestion , dilatation of some sinusoids is evident in centrilobular areas and vacuolation was observed after removing CC14 treatment significantly [33].

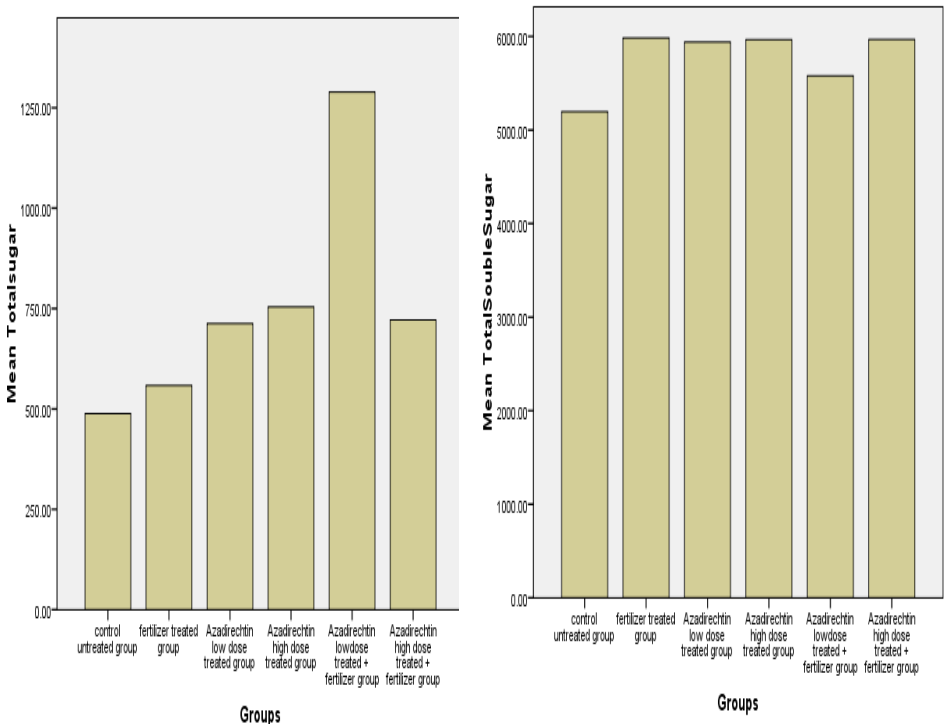

Figure 1: Effects of Azadirechtin, fertilizer alone or combined on total sugar, and soluble sugar (u/gm) in different treated groups.

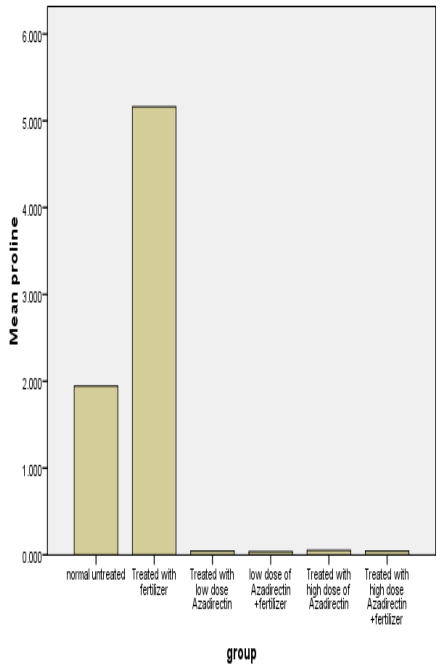

Figure 2: Effect of different doses azadirachtin, fertilizer alone or combined on proline content (u/gm).

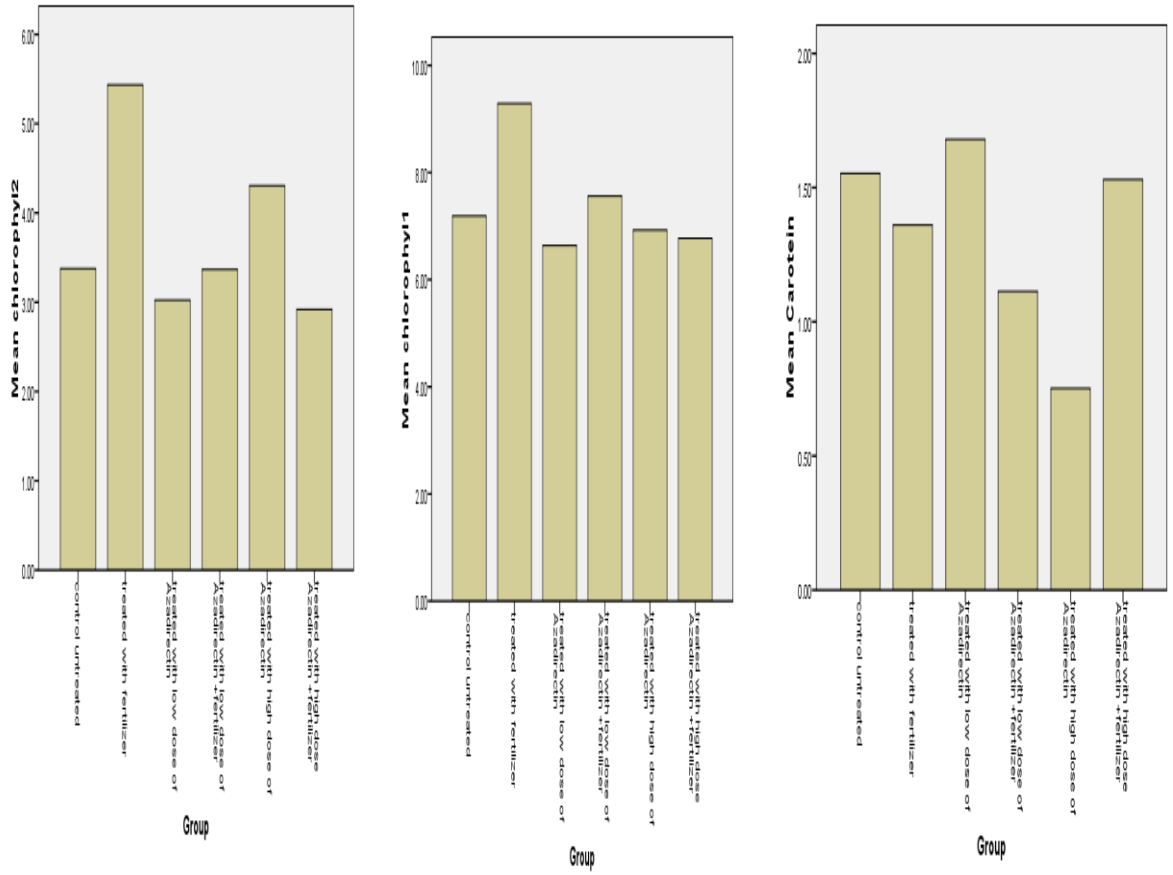

Figure 3 : Effect of different doses azadirachtin,fertilizer alone or combined onchlorophyll(A),(B)and carotein (u/gm) in different groups

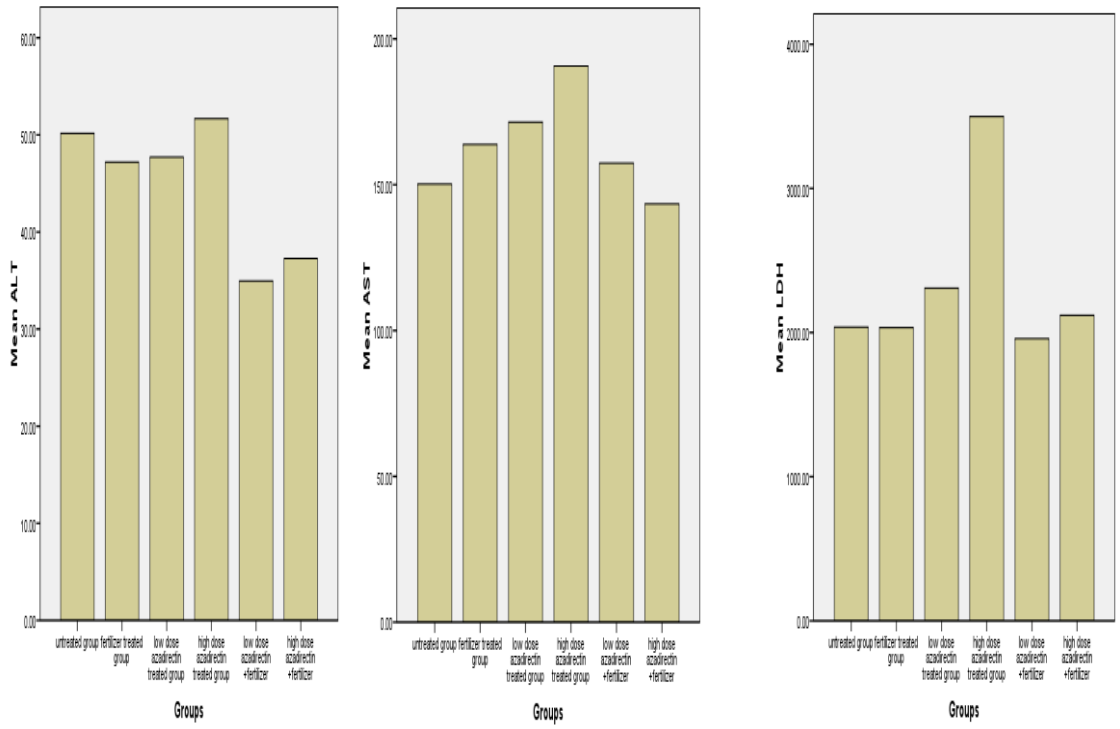

Figure 4 : Effect of different doses azadirachtin,fertilizer alone or combined onserum AST, ALT and LDH (U/L)activities in different treated groups.

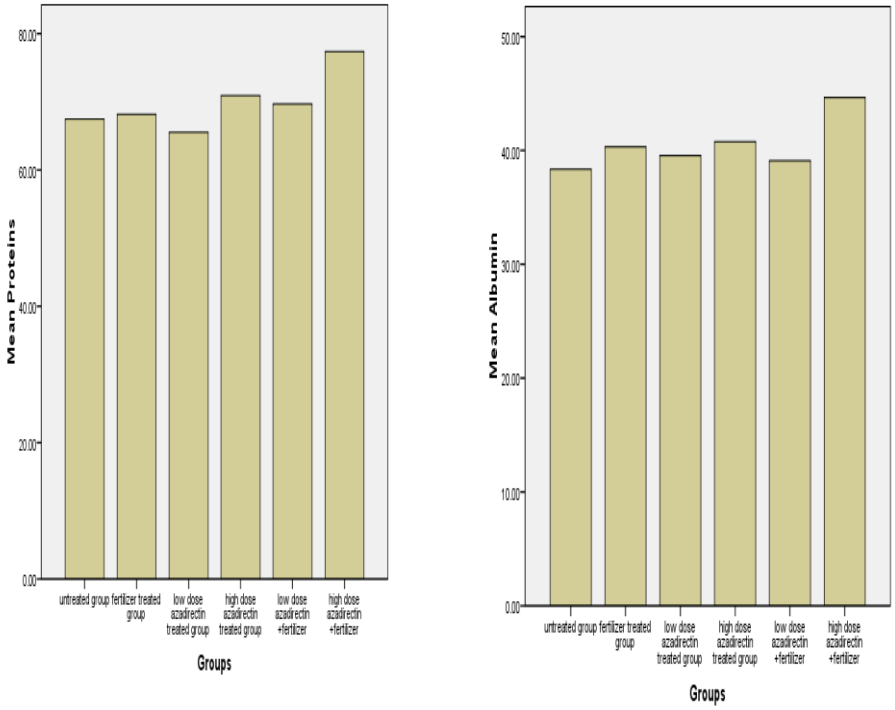

Figure5: Effect of different doses azadirachtin, fertilizer alone or combined on serum total protein and albumin (U/L)concentrations in different groups

Table 1: Effects of Azadirechtin, fertilizer alone or combined on total sugar, and soluble sugar (u/gm)in different treated groups.

Parameter Group	Total sugar (u/gm)			Soluble sugar(u/gm)		
	Mean±S.E.	(Ta) significant test	(Tb) significant test	Mean±S.E.	(Ta) significant test	(Tb) significant test
Group 1	**488.5±20.70**	**-----**	**.000***	**5196.37±86.52**	**---**	**.000***
Group 11	**558.17±34.28**	**.000 ***	**——**	**5984.42±21.65**	**.000***	**——**
Group 111	**712.83±22.03**	**.000***	**.007***	**5940.77±16.17**	**.000***	**.007 ***
Group 1V	**753.83±12.14**	**.000***	**.021***	**5965.55±30.30**	**.000***	**.021***
Group V	**1289.0±22.532**	**.000***	**.041***	**5580.05±11.89**	**.000***	**.041***
Group VI	**721.60±1.013**	**.000***	**.000 ***	**5754.86±16.62**	**.000 ***	**.000***

The mean difference is significant at P<0.05 (*)
(Ta): Significant, compared with normal control group.
(Tb): Significant, compared with fertilizer treated group.

Table 2: Effect of different doses azadirachtin, fertilizer alone or combined on proline content. (u/gm)

Parameter Group	proline (u/gm)		
	Mean±S.E.	**(Ta) significant test**	**(Tb) significant test**
Group 1	**1.943 ±.1692**	**---**	**.000***
Group 11	**5.162 ±.111**	**.000***	**---**
Group 111	**.0443 ±.005**	**.000***	**.000***
Group 1V	**.037 ±.0016**	**.000***	**.000***
Group V	**.052 ±.001**	**.000***	**.000***
Group V1	**.043±.002**	**.000***	**.000***

The mean difference is significant at P<0.05 (*)
(Ta): Significant, compared with normal control group.
(Tb): Significant, compared with fertilizer treated group

Table 3: Effect of different doses azadirachtin,fertilizer alone or combined on chlorophyll(A),(B)and carotein (u/gm)in different groups

Parameter Group	Chlorophyll(A) (u/gm)			Chlorophyll(B) (u/gm)			Carotein(u/gm(		
	Mean±S.E.	(Ta) significant test	(Tb) significant test	Mean±S.E.	(Ta) significant test	(Tb) significant test	Mean±S.E.	(Ta) significant test	(Tb) significant test
Group 1	**7.189±.128**	**——**	**.000 ***	**3.377±.124**	**——**	**.000***	**1.553±.039**		**.139**
Group 11	**9.290±.363**	**.000 ***	**——**	**5.434±.157**	**.000***	**——**	**1.360±.050**	**.139**	
Group 111	**6.637±.204**	**.188**	**.000 ***	**3.024±.306**	**.230**	**.000***	**1.681±.163**	**.325**	**.017**
Group 1V	**7.559±.511**	**.374**	**.000***	**3.367±.154**	**.973**	**.000***	**1.111±.040**	**.001***	**.060**
Group V	**6.924±.172**	**.524**	**.000***	**4.306±.163**	**.003***	**.000***	**.751±.108**	**.000***	**.000***
Group VI	**6.771±.152**	**.317**	**.000 ***	**2.919±.257**	**.121**	**.000***	**1.530±.068**	**.859**	**.191**

The mean difference is significant at P<0.05 (*)
(Ta): Significant, compared with normal control group.
(Tb): Significant, compared with fertilizer treated group.

Table 4: Effect of different doses azadirachtin,fertilizer alone or combined onserum AST, ALT and LDH (U/L)activitiesin different treated groups.

Parameter Group	Aspartate Aminotransferase (U/L)			Alanine Aminotransferase (U/L)			Lactic Dehydrogenase (U/L)		
	Mean±S.E.	(Ta) significant test	(Tb) significant test	Mean±S.E.	(Ta) significant test	(Tb) significant test	Mean±S.E.	(Ta) significant test	(Tb) significant test
Group 1	150.18±11.42	-	0.33	50.1667±915ز	-	0.749	203.77±15.92	-	0.984
Group 11	163.82±1.26	o.33	-	47.2000±1.18	0.749	-	203.30±21.11	0.984	--
Group 111	171.47±11.61	0.13	0.58	47.7167±6.17.	0.791	0.955	230.66±2.14	0.255	0.247
Group 1V	190.70±14.53	0.006	0.059	51.6667±9.80	0.871	0.630	349.88±1.60	0.000*	.0.000*
Group V	157.40±2.69	0.602	0.643	34.9500±2.38	0.108	0.192	195.82±33.08	0.735	0.750
Group V1	143.37±8.77	0.622	0.146	37.2667±5.27	0.170	0.288	211.93±2.53	0.728	0.713

The mean difference is significant at P<0.05 (*)
(Ta): Significant, compared with normal control group.
(Tb): Significant, compared with fertilizer treated group

Table 5: Effect of different doses azadirachtin, fertilizer alone or combined on serum total proteine and albumin concentrations in different groups.

Groups	Protein (U/L)		Albumin (U/L)	
	Mean ± S E	Ta) significant test	Mean ± S E	significant test
Group 1	67.45 ±1.03	---0.541	38.35 ±1.36	0.157--
Group 11	68.17 ±0.59	0.541	40.32 ±0.78	0.157
Group 111	65.52 ± 0.84	0.106 0.029*	39.55 ± 1.29	0.3830.576
Group 1V	70.90 ± 0.77	0.006* 0.025	40.77 ± 0.89	0.085 0.742
Group V	69.68 ± 0.62	0.064 0.201	39.10 ± 0.66	0.584 0.377
Group V1	77.37 ± 0.96	0.000*0.000*	44.65 ± 0.43	0.000* 0.003

The mean difference is significant at P<0.05 (*)
Significant, compared with normal control group.

Conclusion:-

The study successfully demonstrated that biocides have a significant influence on chlorophyll degradation in plants. The loss of chlorophyll at minor levels in growing plants may not influence plant growth and plant quantity, since it can easily recover adequate rate of chlorophyll through process of photosynthesis. The study even argued that biocides have their impacts on plant leaves. Hence, it is necessary to stay aware of chemical types applied on crops. Awareness should not limit to environmental contamination and toxicity, but their effects on crops should also be considered to prevent and obtain good quality of agricultural products for healthy consumption.

The study reiterates the importance of standardization while formulating herbal-based phenomena. Thus, it may act in humans as potential liver disorder as well. The current investigation adequately proves that azadirachta indicia leaves are an effective heptaprotective agent at low dose, which is used in present investigation earlier.

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References:

1. Specht, Pelz, and Gilsbach (1995): Determination of pesticide residue in food matrices using the Quechers. J. Anal. Chem.,353, 183-190.
2. Jones DR, Sutton AJ, Abrams KR, Fenty J, Warren F, Rushton L. (2009).Systematic review and meta-analysis of mortality in crop protection product manufacturing workers. Occup Environ Med. : 66(1):7-15.
3. Mansour SA, BelalMH,Abou-Arab,Ashour HM, Gad MF (2009). Evaluation of some pollutant levels in conventionally and organically farmed potato tubers and their risks to human health. Food Chem. Toxicol.47:615-24.
4. Mukharjee I, Gopal M (2003) .Pesticide residues in vegetable. In proceedings of symposium on risk assessment of pesticide residues in water and food .By IL SI Washington DC, ITRC Lucknow, pp, A1-8
5. B. Lozowicka, P. Kaczynski, A.E. Paritova, G.B. Kuzembekova, A.B. Abzhalieva (2013). Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticide. Food and Chemical Toxicology. 64,238–248
6. Arnason, J.T.; Plilogene, R.J.R.; Morand, P. (1998) .Insecticides of Plant Origin, ACS SymposiumSeries 387. (American Chemical Society: Washington, DC.
7. Isman, M.B. (1994): Botanical insecticides. Pesticides Out, 5, 26–31.
8. Karanja J, Poehling HM, Pallmann P.(2015). Efficacy and Dose Response of Soil-Applied Neem Formulations in Substrates with Different Amounts of Organic Matter, in the Control of Whiteflies, Aleyrodesproletella and Trialeurodesvaporariorum (Hemiptera: Aleyrodidae).J Econ Entomol. 108(3):1182-90. Doi: 10.1093/jee/tov047.
9. Thoeming G, Draeger G, Poehling HM.(2006). Soil application of azadirachtin and 3-tigloyl-azadirachtol to control western flower thrips, Frankliniellaoccidentalis (Thysanoptera: Thripidae): translocation and persistence in bean plants. Pest Manag Sci.;62(8):759-67
10. Quelemes PV, Perfeito ML,Guimarães MA, Dos Santos RC, Lima DF, et al .(2015). Effect of neem (Azadirachtaindica A. Juss) leaf extract on resistant staphylococcus aureus biofilm formation and Schistosoma mansoniWorms.JEthnopharmacol.Volume 175, Pages 287–294
11. Scheumann, V., Schoch, S. and Rüdiger, W.(1998). Chlorophyll a Formation in the the Chlorophyll b Reductase Reaction Requires Reduced Ferredoxin. The Journal of Biological Chemistry. 273, 35102-35108.
12. Heaton, J.W. and Marangoni, A.G. (1996). Chlorophyll degradation in processed foods and senescent plant tissue. Trends in Food Science & Technology. 7, 8-15.
13. Eckhardt, U., Grimm, B. and Hörtensteiner, S. (2004). Recent advances in chlorophyll biosynthesis and breakdown in higher plants. Plant Molecular Biology. 56, 1-14.
14. Wann, F. B. 1930. "Circular No. 85 - Chlorosis Yellowing of Plants: Cause and Control" UAES Circulars. Paper 77.
15. Bates et al., (1973).proline for water-stress studies . Plant and Soil. , Volume 39, Issue 1, pp 205-207.
16. E. W. Yemm and A. J. Willis (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochem J.: 57(3): 508–514.
17. Lichtenthaler, H.K. (1987). Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembrnes. Methods in Enzymology. 148, 350-382.
18. IzzetAK , NazlDideKutluk Yılmaz and RıdvanKızılkaya. (2005). Effects of Azadirachtin on Beet SoilbornePomovirusand Soil Biological Properties on Sugar Beet .Journal of Environmental Science and Health, 40:285–296. 14'Anonymous).
19. Anonymous. Neem, a Tree for Solving Global Problems (1992). Report of an Ad Hoc Panel of the Board on Science and Technology for International Development National Research Council. National Academy Press: Washington, DC, 141.
20. Pandey, R. (1994). Comparative performance of oil seed cakes and pesticides in the management of root knot disease of davana. Nematol. Medit. 22, 17–19
21. Palumbo JC, Castle SJ. (2009): IPM for fresh-market lettuce production in the desert southwest: the produce paradox. -Pest Manag Sci. Dec; 65(12):1311-20.
22. PongtipSithsarn . Charlotte U. Clrlsen,Mongens L. Andersen,WandeeGritsanapan, and Leif H .Skibsted(2007). Antioxidative effects of leaves from Azadirachta species of different provenience .Food Chemistry, 104,1539-1549.
23. Fatma T, Khan M A and Choudhary M (2007) .Impact of environmental Pollution on cyano bacterial proline content. Journal of Applied Phycology. 19 (6):625 – 629.
24. G.V.N.S.Deviram and R.GyanaPrasuna (2012). Effect of fungicides on proline content of NostocSP .Int J Pharm Bio Sci.3 (4): (B) 152-157.

25. Ayyangar, G.S.G.; Rao, P.J. (1990). Cganges in haemolymphconistituents of Spodoptralitura under the influence odazadirachtin. Indian Journal of Entomology, 52(1) pp69-83.
26. Houimli, S.I.M., Denden, M. and Mouhandes, B.D. (2010). Effects of 24-epibrassinolide on growth, chlorophyll, electrolyte leakage and proline by pepper plants under NaCl-stress. EurAsian Journal of BioSciences. 4, 96-104.
27. Simon-Sylvestre, G.; Fournier, J.C. (1979). Effects of pesticides on the soil microflora. InAdvances in Agronomy; Brady, N.C., Ed. Academic Press: New York, USA. 31,1–92.
28. Stark, J.D.(2001) . Population level effects of the neem insecticide, neemix, on Daphnia pulex. J. Environ. Sci. Health B, 36 (4), 457–465.
29. Agbenin, J.O.; Agbaji, E.B; Suleiman (1999) I. Assessment of nitrogen mineralization potential and availability from neem seed residue in a savanna soil. Biol. Fertil. Soils, 29, 408–412.
30. Liao, M.; Abid, S.; Huang, C.; Xie, Z.(2002). Impact of triazophos insecticide on paddy soil environment. J. Environ. Sci., 14 (3), 309–316.
31. Weaver, L.M., Gan, S.,Quirino, B. and Amasino, R.M. (1998). A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. Plant Molecular Biology. 37, 455-469.
32. Schlentner, R.E.; Van Cleve, K. Relationships between CO2 evolution from soil, substrate temperature, and substrate moisture in four mature forest types in interior Alaska. Can. J. For. Res. 1985, 15, 97–106.
33. PingaleShirishS.(2010). Hepatoprotection study of leaves powder of Azadirechta Indica A. Juss. International Journal of Pharmaceutical Sciences Review and Research Volume 3, Issue 2,page 37 .Article 007 ISSN 0976 – 044X.