

Studies on the Tolerance of *Vigna Sinensis* L. And *Oryza Sativa* L. To The Application of Pesticides

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Abstract: In the present investigation, an attempt has been made to study the effect of two pesticides (DDT and Bordeaux mixture) commonly used in the agricultural production landscape, on seed germination and seedling growth of *Vigna sinensis* L. and *Oryza sativa* L. The objective of this study is to determine the rate of germination, radicle emergence, plumule elongation, biomass and the chlorophyll content of the two cultivar seeds exposed to five concentrations of the pesticides viz., 0.01%, 0.1%, 1%, 2% and 4%. In general the seeds of both *Vigna sinensis* and *Oryza sativa* showed a marginal increase in the rate of germination, radicle elongation, plumule growth, biomass and chlorophyll content in 0.01% over the control, when treated with both the pesticides. There was gradual decrease in rate of germination, radicle emergence, plumule elongation, biomass and the chlorophyll content at 0.1%, 1% and 2% concentrations of DDT and Bordeaux mixture. The maximum inhibition was observed in higher concentration of both the pesticides (4%). Though the total chlorophyll and chlorophyll a content decreased as the concentration of the pesticide increased, the chlorophyll b recorded an increase in the DDT treated seeds of *Vigna sinensis*. This research contributes to the phytotoxicity assessment database, and besides to lay the foundation for the use of *Oryza sativa* and *Vigna sinensis* as a phytoremediation tool for agricultural pesticide runoff.

Key Words: DDT, Bordeaux mixture chlorophyll, seed germination, Seedling

I. Introduction

The intensive and multiple cropping systems recommended in modern production technology are unavoidably associated with intensification of disease incidence. Pesticides represent the only group of chemicals that are purposely applied to the environment with an aim to suppress plant and animal pests and to protect agricultural and industrial products. Use of enormous quantities of pesticides also spoils the ecosystem directly or indirectly (Mozumder and Hossain, 2013).

Agricultural 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 (Siddiqui and Ahmed, 2006). The toxic impact of the pesticides endosulfan and kitazin on the germination and enzyme activities of *Solanum melongena* (Sammaiah et al. 2011) and the effect of 4 pesticides during soil treatment in winter wheat trials (Petróczi et al. 2002) were carried out.

The effects of pesticides on seed germination, growth, survival, yield and residue quantification of crop plants have been investigated by several researchers (Olofinboba and Kozlowski 1982, Ahmed and Khan 2011). The organochlorinated pesticides are a constant danger to non-target organisms and are known to alter the behavioral changes, growth, nutritional value, cellular and physiological properties (Sarnaik et al. 2006). They affect the normal functions of specific cell and tissues of plants and animals and make their survival difficult by the application of insecticides (Singh 1990). The toxic effect of many fungicides, herbicides and insecticides on germination and seedling growth was carried out (Sengupta et al. 1986).

Oryza sativa L. is the staple cereal, consumed as a grain almost exclusively by humans, supplying 20% of daily calories. *Vigna sinensis* L. are one of the most important food legume crops and has the useful ability to fix atmospheric nitrogen through its root nodules. DDT, a pesticide, with low water solubility, has relatively high solubility in fats. Bordeaux mixture (also called Bordo mix) is a mixture of copper sulphate CuSO₄ and slaked lime used as a fungicide in vineyards. Pesticides can be considered useful to eliminate pests, but can also induce negative effects to the processes of germination, growth and development of plants or disrupt some physiological and metabolic processes. By studying the effect of pesticides on treated seeds on germination and determination of growth indices, it is possible to evaluate the positive or negative influence or toxicity of the chemical compounds. In the present study a preliminary attempt was to determine more exactly the impact of these pesticides on seed germination and early seedling growth of *Oryza sativa* L. and *Vigna sinensis* L.

II. Materials And Methods

The seeds of *Oryza sativa* L. and *Vigna sinensis* L. purchased from Ernakulam District Agri-Horticultural society, were taken for this study. A stock solution of DDT and Bordeaux mixture was prepared by

dissolving 4 g of in 100 mL of distilled water. From this, solutions of different concentrations viz., 0.01% ,0.1%, 1%, 2% and 4%, were prepared. The seeds of *Oryza sativa* and *Vigna sinensis* were surface sterilized with 0.1% mercuric chloride and washed thoroughly with distilled water 3 times. hundred seeds each were soaked in the respective concentrations of DDT and Bordeaux mixture solution for 6 hrs. Distilled water was used in place of pesticides in control. Twenty seeds were uniformly spread on each Petri plate and were allowed to germinate at room temperature. The filter papers lined in Petri plate were moistened with respective concentration of pesticides. Petri plates containing moistened filter paper soaked different concentrations of 0.01% ,0.1%, 1%, 2% and 4%, and with distilled water (control). Three replicates for each of the four treatment including control were used. The soaked seeds were incubated in Petri plates lined with moist blotter at $28 \pm 2^{\circ}\text{C}$ in dark. The percentage of germination was calculated from the second day itself by counting the number of seeds germinated in each Petri plate. On the 2nd day the seedlings were taken out, and the radicle and plumule length were measured. The chlorophyll was estimated in the 15 day old seedlings (Arnon,1949).The fresh and dry weights of 15 day old seedlings were measured. For dry weight seedlings were kept in a oven for 48h at 60°C .The data was average of three replicates. .

III. Results And Discussion

Different concentrations of the pesticides revealed different influences on the seed germination of *Vigna sinensis* and *Oryza sativa* (Fig.1to 4). Control, 0.01% and 0.1% concentration of DDT recorded 100% germination after 24 hours of treatment. The effect of the pesticide DDT on seed germination recorded a decrease of 50% (*Vigna sinensis*) and 20% (*Oryza sativa*) in the highest concentration studied. The delay in germination was noticed more in 2% and 4% concentration of both DDT and Bordeaux mixture. In the treatment with 4% concentration of Bordeaux mixture, the lowest of 50% (*Oryza sativa*) and 40% (*Vigna sinensis*) was recorded on the second day. The seeds treated with different concentrations of DDT showed a remarkable decrease in germination percentage over the control. (Venkataramaiah, 1982). On the contrary the application of triazole fungicides resulted in a yield increase of 5-10% which is an indication of a cytokinin-like effect of these compounds (Stevens and Palmer, 1980). However The overdose sprayings of 2,4- D herbicide could reduce the yield by 8-20 % (Dutta et al. 1992).

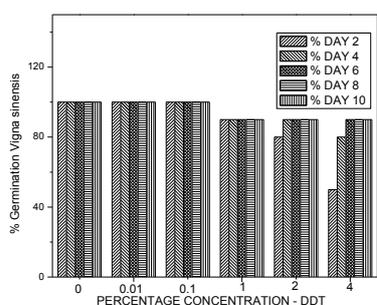


Fig.1: Effect of DDT on the rate of Seed germination in *Vigna sinensis*.

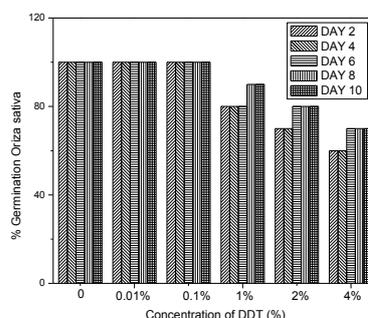


Fig.2: Effect of DDT on the rate of Seed germination in *Oryza sativa*.

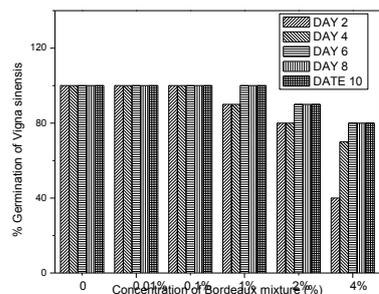


Fig.3: Effect of Bordeaux mixture on the Rate of Seed germination in *Vigna sinensis*.

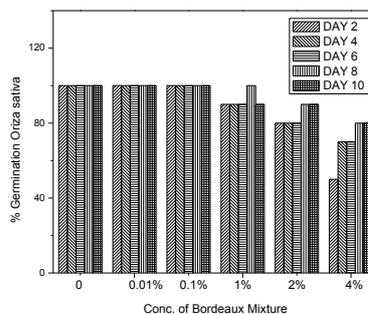


Fig.4: Effect of Bordeaux mixture on the rate of seed germination in *Oryza sativa*.

Results displaying the effect of pesticides on the radicle elongation of *Vigna sinensis* and *Oryza sativa* (Fig.5-8) recorded its maximum in 0.01% concentration (3.78cms) in *Vigna sinensis* treated with Bordeaux

mixture on the second day of germination. The radicle length showed a decreasing trend with increase in pesticide concentration. The radicle emergence recorded an increase from the fourth day onwards. On the sixth day, the radicle length in *Vigna sinensis* showed a 24% decrease when treated with DDT and 61.7% with Bordeaux mixture in treatments with 4% dilution over the control.

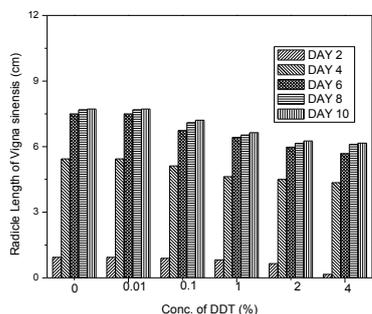


Fig.5: Effect of DDT on the radicle length of *Vigna sinensis*.

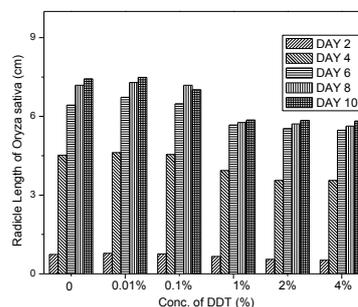


Fig.6: Effect of DDT on the radicle length of *Oryza sativa*.

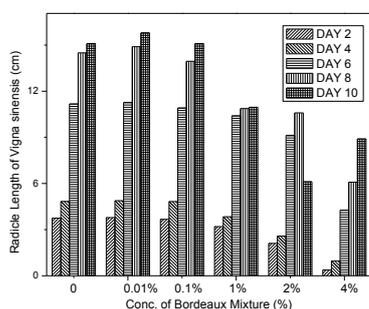


Fig.7: Effect of Bordeaux mixture on the Radicle length of *Vigna sinensis*.

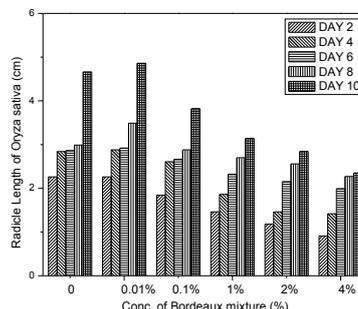


Fig.8: Effect of Bordeaux mixture on the Radicle length of *Oryza sativa*.

Results displaying the effects of the varying concentrations of the pesticides on the growth of the seedlings did not show any inhibition with 0.01% pesticide concentration. With increase in concentration of DDT and Bordeaux mixture, the elongation of the hypocotyls showed a decrease. Data from Fig. 9 -12 displayed maximum inhibition of 21.26% (DDT treated) and 49.5% (Bordeaux mixture treated) in the hypocotyls of *Vigna sinensis* on the 10th day over the control. Similar results were obtained by Daniela et al. 2013 in a study of the changes in growth on wheat plantlets induced by the action of thiamethoxam and thiophanate-methyl. Dubey and Fulekar (2011) reported notable decrease in germination a study of three pesticides on the germination of grass seeds using pesticide spiked soil. On the tenth day of study, the 4% dilution of DDT treated seeds showed a marked decrease of 52% (*Vigna sinensis*) and 22% (*Oryza sativa*). Coleoptile growth was significantly lowered in pesticide treatments (Misra & Mani, 1994). On the contrary, treatment with Bordeaux mixture showed 50% inhibition in *Oryza sativa* and 28% in *Vigna sinensis* in the same dilution. Among the two cultivars treated with Bordeaux mixture at 4% concentration a decrease in the hypocotyl length was recorded which was more pronounced in *Vigna sinensis* (65%) than the coleoptiles in *Oryza sativa* (6.5%) in 10 day old seedlings.

Treatment with the varying concentration of DDT showed similar results. Moore and Kroger (2010) reported inhibition of germination, radicle (root) and coleoptile (shoot) growth of rice seeds exposed to three insecticides and two herbicides, commonly used in agriculture. Triadimenole and triticonazole, applied as seed treatments at various rates affected plant growth, shoot development and root axis production adversely. (Montfort et al. 1996). The complete inhibition of germination and seedling growth of *Oryza sativa* L. were observed in higher concentration of Sivic solutions (Sathees et al. 2014). Higher concentrations of dimecron showed adverse effect more on root than on the growth of shoot in *Pennisetum americanum* (Siddiqui et al. 1999).

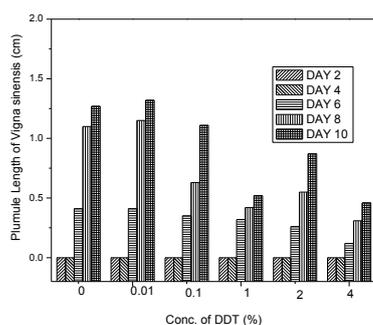


Fig.9: Effect of DDT on the plumule length of *Vigna sinensis*.

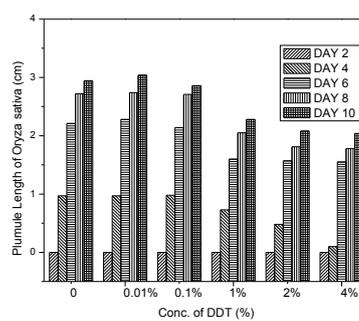


Fig.10: Effect of DDT on the plumule length in *Oryza sativa*.

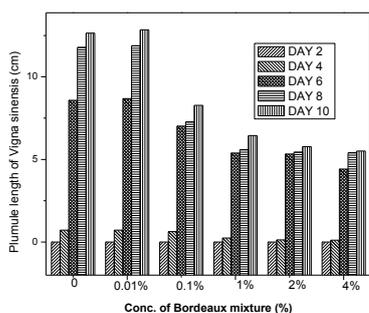


Fig.11: Effect of Bordeaux mixture on the plumule length in *Vigna sinensis*.

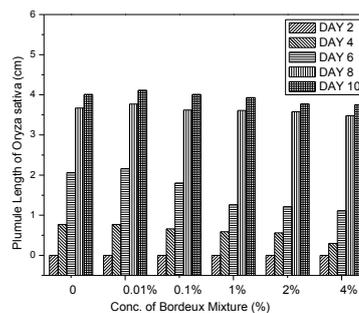


Fig.12: Effect of Bordeaux mixture on the plumule length in *Oryza sativa*.

Results displaying the effect of pesticides on the chlorophyll content of *Vigna sinensis* and *Oryza sativa* is given in Fig.13-16. The total chlorophyll, chlorophyll *a* and the chlorophyll *b* content recorded the highest in control and .01% treated seeds in both the pesticides. The total chlorophyll and the chlorophyll *a* content of seeds treated with DDT and Bordeaux mixture showed a marginal decrease with increase in the pesticide concentrations, the 4% treatment with DDT recorded a 15% decrease over the control. In *Oryza sativa* chlorophyll *b* content showed a decreasing trend with increase in DDT concentration and a maximum reduction of 53.8% was recorded in 4% dilution of DDT. On the contrary in seeds treated with 4% concentration of DDT, ten day old seedlings of *Vigna sinensis* recorded a remarkable increase (67%) in the concentration of the chlorophyll *b*. 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.. 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 (Siddiqui and Ahmed 2002).The effect of systemic fungicides and insecticides showed significant ($P < .001$) deviation on absorption spectra of both chlorophyll *a* and *b*.(Siddiqui and Khan 2001).They observed chlorophyll and phenolic contents stimulated by the action of systemic fungicides and insecticides at lower concentrations.

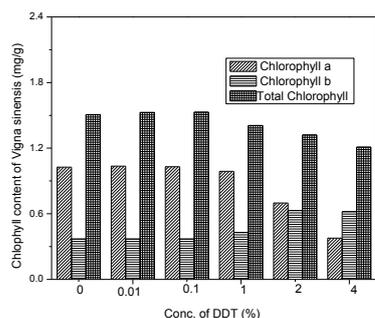


Fig.13: Effect of DDT on the chlorophyll content in *Vigna sinensis*.

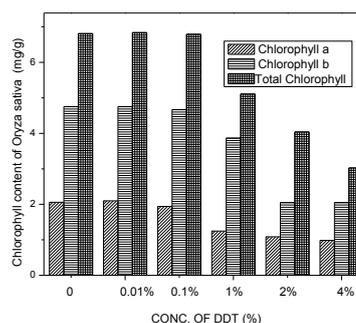


Fig.14: Effect of DDT on the Chlorophyll content in *Oryza sativa*.

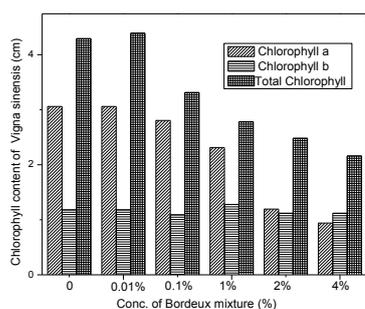


Fig.15: Effect of Bordeaux mixture on the chlorophyll content in *Vigna sinensis*.

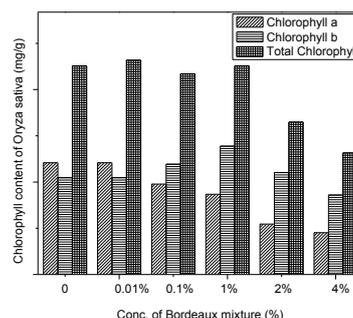


Fig.16: Effect of Bordeaux mixture on the chlorophyll content in *Oryza sativa*.

The Fresh and dry weights of 15 day old seedlings for both the pesticide treatments showed the maximum value in the 0.01% concentration of DDT and Bordeaux mixture (Fig. 17&18). The effect of the pesticides on the biomass of 15 day old seedlings recorded a 52% decrease in the fresh and dry weight in treatment with 4% concentration of DDT, while a marginal decrease of 7 % was observed in treatment with Bordeaux mixture in the above concentration. A significant decrease in seed germination and seedling vigour under green house conditions was observed by Aveling et al. (2013) in maize seeds treated with four pesticides. In the present study 0.01 and 0.1% concentration of both the pesticides did not show any inhibition on the biomass of *Vigna sinensis* and *Oryza sativa*. Lower dose (0.2%) of endosulfan stimulated the activity of amylases (Vidyasagar et al. 2009). The presence of pesticide residues (solutes) in soil distress the water potential which reduces uptake of nutrients from the surrounding soil (Rengel and Wheal, 1997), depolarizes the plasma membrane of the root cells (Shimabukuro & Hoffer 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 (Wahengbam et al. 2013)

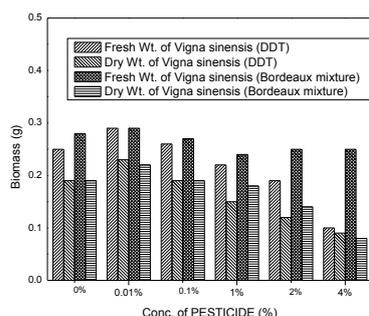


Fig.17: Effect of DDT and Bordeaux mixture on the on the biomass in *Vigna sinensis*.

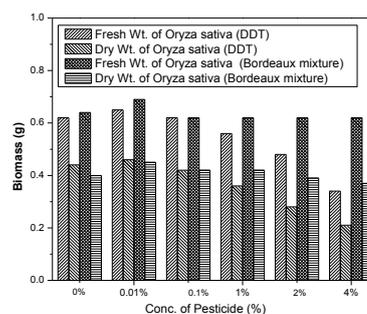


Fig.18: Effect of DDT and Bordeaux mixture on the biomass of *Oryza sativa*.

IV. Conclusion

The data obtained from the present study indicates that germination percentage of seeds, growth of the seedlings and biomass production were affected at varying levels in *Vigna sinensis* and *Oryza sativa*, at five concentrations investigated. As the concentration of DDT and Bordeaux mixture increased, the rate of germination, shoot length, root length and biomass decreased. The highest value was observed in the control and in 0.01% concentration of the pesticides (DDT and Bordeaux) studied. The amount of total chlorophyll and chlorophyll *a* decreased gradually with the increase in the pesticide concentration, whereas chlorophyll *b* recorded a marginal increase. DDT at 4 % concentration was the most toxic and among two plants studied and *Vigna sinensis* was more affected than *Oryza sativa*. Pesticide concentration of 0.01% was found to be ideal for seed treatments. By studying the effect of pesticides on treated seeds germination, and determination of growth indices, it was possible to evaluate the toxicity of these pesticides.

References

- [1]. S.N. Mozumder, and M.M. Hossain, Effect of seed treatment and soaking duration on germination of *Eryngium foetidum* L. seeds, *International Journal of Horticulture*, 2013, 3 (10) , 46-51.
- [2]. Siddiqui Zamin Shaheed and Ahmed Soaliha, Combined effects of pesticide on growth and nutritive composition of soybean plants, *Pak. J. Bot*, 38(3), 2006, 721-733.
- [3]. D. Sammaiah, C.H. Chandrashekar, V. Ramakrishna Prasad, and K. Jaganmohan Reddy, Pesticides induced alterations in physiological responses in *Solanum melongena* L, *International journal of Pharma and Biosciences*, 2(1), 2011, 374-384.
- [4]. Petróczy István, M. János Matuz, and Csaba Kótai, Study of pesticide side-effects in winter wheat trials, *Acta Biologica Szegediensis. Proceedings of the 7th Hungarian Congress on Plant Physiology*, 46 (3-4), 2002, 207-208.
- [5]. M.O. Olofinboba, and T.T. Kozłowski, Effects of three systemic insecticides on seed germination and growth of *Pinus halepensis* seedlings, *plant and soil*, 64, 1982, 255-258.
- [6]. M. Ahmad, and M. S. Khan, Plant growth promoting fungicides-tolerant *Rhizobium* improved growth and symbiotic characteristics of lentil (*Lens esculentus*) in fungicide applied soil, *J Plant growth Regul*, (23), 2011, 32-39.
- [7]. S. S. Sarnaik, P. P. Kanekar, V. M. Raut, S. P. Taware, K. S. Chavan, and B. J. Bhadbhade, Effect of application of different pesticides to soybean on the soil microflora, *J. Environ. Biol*, 27, 2006, 423-426.
- [8]. M. Singh, Effect of some insecticidal on the alimentary canal of surface grasshopper, *Chrotogonus trachypeterus* Blanchard (Pyrgomorphide: Orthoptera), *Ind. J. Entomol*, 52, 1990, 89-99.
- [9]. P. Sengupta, K. A. Chakrabarti, and S. K. Banerjee, Biochemical changes induced by Toxic concentration of Malathion in germinating Wheat seeds, *Curr Sci*, 55 (10), 1986, 492-494.
- [10]. D. I. Arnon, Copper enzymes in isolated chloroplasts polyphenyl oxidase in *Beta vulgaris*, *Plant Physiol*, (24) , 1949, 1-15.
- [11]. C.Venkatramaiah, Effect of DDT on seed germination and Seedling growth of *Phaseolus mungo* L. *Com ,Physiol.Ecol*, 7, 1982, 27-28.
- [12]. D. B. Stevens, and G. M. Palmer, Winter wheat disease control 1977-79. *Norfolk Agric Stat Ann Report*, 72, 1980, 20-23.
- [13]. B. K. Dutta, S. Debnath, S. K. Pradhan, and R.M. Gurung., Role of systemic fungicides on the growth and phenolic content of *Vigna radiata*, *Pak. J. Bot*, 1992, (28), 191-193.
- [14]. Daniela Nicuta, Nicoleta Badaluta, Gabriel Lazar, Iuliana Mihaela Lazar, Changes in growth of wheat plantlets induced by the action of thiamethoxam and thiophanate-methyl on seeds, *Environmental Engineering and Management Journal*, 12(1), 2013, 85-96.
- [15]. Dubey Kriti Kumari and M.H. Fulekar, Effect of pesticides on the Seed Germination of *Cenchrus setigerus* and *Pennisetum pedicellatum* as Monocropping and Co-cropping System: diseased and healthy plant of *Triticum aestivum* L, *Pak. J. Biol. Sci.* (3), 2011, 2148-2150.
- [16]. S. G. Misra, and D. Mani, Adverse effects of Pesticides, In: *Agricultural pollution II*. (Eds.): S.G. Misra and D. Mani Ashish Publisher, New Delhi. 1994.
- [17]. M. T. Moore, and R. Kroger, Effect of Three Insecticides and Two herbicides on Rice (*Oryza sativa*) Seedling Germination and Growth *Archives of Environmental contamination and Toxicology*, *Arch Environ Contam Toxicol*, 2010, 59(4), 574-581.
- [18]. F.Montfort, B. L. Klepper, and R.W. Smiley, Effects of two triazole seed-treatments, triticonazole and triadiminol, on growth and development of wheat, *Pest. Sci*, 1996, (46), 315-322.
- [19]. T. M. Sathees Kannan, M. Rajesh, and Kaliyamoorthy Jayakumar, Effect of Fungicide Sivic on Seed germination and Seedling Growth of *Oryza sativa* L, *International Journal of Modern Biology and Medicine*, 5(1), 2014, 1-4.
- [20]. S. Siddiqui Zamin, Soaliha Ahmed and S.S. Shaukat, Effect of systemic fungicide (Topsin-M) and insecticide Dimecron on germination, seedling growth and phenolic content of *Pennisetum americanum* L, *Pakistan Journal of Biological science*, 2(1), 1999,182-184.
- [21]. Z.S. Siddiqui, and S. Ahmed, Effects of systemic fungicides on protein, carbohydrate, amino acids and phenolics contents of susceptible (Mexipak) and resistant (Poven) varieties of *Triticum aestivum* L. *Turk. J. Bot*, 26, 2002, 127-130.
- [22]. Siddiqui Zamin Shaheed and Khan Sadeef, Effect of systemic fungicides and insecticides on Absorption spectra, chlorophyll and phenolic content of *Vigna radiata* (L.)Wilczek, *Pakistan Journal of Biological science*, 4(7), 2001, 812-814.
- [23]. T. A. S. Aveling, V. Govender, D. S. Kandolo, and Q. Kritzinger, The effects of treatments with selected pesticides on viability and vigour of maize (*Zea mays*) seeds and seedling emergence in the presence of *Fusarium graminearum*, *Journal of Agricultural Science*, 151, 2013, 474-481.
- [24]. G. M. VidyaSagar, D. Kotresha, N. Sreenivasa, and Ramesh Karnam, Role of endosulfan in mediating stress responses in *Sorghum bicolor* (L.), *Moench J. Environ. Biol* , 30(2), 2009, 217-220.
- [25]. Rengel Zdenko and Wheal Matthews. Kinetic parameters of Zn uptake by wheat are affected by chlorsulfuron, *Journal of Experimental Botany*, , 48 (309), 1997, 935-941.
- [26]. R. H. Shimabukuro, and B.L. Hoffer, Effects on transmembrane proton gradient and lipid biosynthesis in the mode action of diclofop-methyl Pest, *Biochem. & Physiol*, 48, 1994, 85-97.
- [27]. M. Tester, Plant Ion Channels: whole cell and single channels studies, *New Phytol*, 114, 1990, 305-340.
- [28]. Wahengbam Dhanamanjuri, Romila Thoudam and B.K. Dutta, Effect of Some Pesticides (Fungicides) on the Germination and Growth of Seeds/Seedlings of Some Crop Plants, (i.e. *Cicer arietinum* and *Zea mays*) *Middle-East Journal of Scientific Research*, 17 (5), 2013, 627-632.