Biolife 1(3):-116-122
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE ORIGINAL ARTICLE

Studies on the Persistence and Degradation of Endosulfan in the Soil Ecosystem of Tropical Climate

Chanda Mallaiah

Deportment of Zoology, Kakatiya Degree & Post Graduate College, Warangal-506 009, Andhra Pradesh, India.

E-mail: chandamallaiah@gmail.com

ABSTRACT

The meteorological, adaphic, physical, physico-chemical and chemical properties have been observed at the experimental site. A detailed study of sub-soil inhabiting micro and macroarthropod population was made with the application of Endosulfan at lower and higher doses as well as at different time intervals with definite distances (i.e.1'.0ft;1.6';2.0';2.6';3.0'; 3.6' ; 4.0' ;5.0';6.0';8.0';10.0';12.0'; and 13.0' ft;) and different time periods from the time of toxicant application, different time intervals (48 hrs;6th;9th;15th;21st;30th;45th;60th; and 290 days) recorded. Maximum population of microarthropod fauna was found after 45 days at 4.0ft. distance while least population after 60 days at 6.0ft. distance was recorded. A significant increase of this population was found after 290 days at 8.0ft. distance. A three (3) fold increase was found after 290 days at 8.0ft. Further variation in the population in relation to time and distance are discussed. Among the microarthropod fauna the highest population was that of *Acarina* found at 15 cm. core depth. A total absence of collembolan population was found at several times clearly showing high susceptibility of the population to 'Endosulfan'. A maximum of coleopteron population was found on 6th day at 1.6 ft. and a total elimination of population at lower dose of 'Endosulfan was noticed at every point of study. The *Myriapoda* population was maximum at 8.0 ft. distance 290 days after the treatment; at other points of observation Myriapoda population was insignificant in the soil.

Key words : Endosulfan, tropical climate, microarthropod fauna.

INTRODUCTION

Study of soil microarthropod fauna in general has attracted many soil ecologists in recent years. Information on the biology, ecology and seasonal fluctuation studies on soil microarthropods was mainly from European regions. Studies on soil microarthropods from India are only of recent interest.

The arthropods constitute a major component of the invertebrate organisms. The activities of arthropods such as ingestion, egestion, movement in search of food, mates, etc. help in various ecosystem processes viz., litter decomposition and nutrient cycling (Edwards et al., 1975) and soil infiltration, aeration and soil turn over (Kevan, 1962 and Lee, 1972). Nevertheless, studies on these arthropods are useful in defining and analyzing the ecosystems and their component parts (Huffman and Harding, 1980). Different species occupied varied habitat of preference in an ecosystem which require more intensive investigation.

The soil microarthropods serve as indicators for variation of some of the factors which influence chemical, physical as well as

biological change in a habitat (Reddy, 1986). The species composition and abundance of these microarthropods are influenced by the geographical location, climate, physical and chemical properties of the soil, type of vegetational cover, nature and depth of the litter humus and variety of other environmental factors (Price , 1973). Nevirtheless, the fauna of the soil may very considerably from one place to the other and season to season. Several workers reported the influence of abiotic factors on the structure of microarthropod populations of soil (Weis-Fogh, 1948; Sheels, 1957; Poole, 1961 & 1963; Vatsauliya and Alfred, 1981).

Pesticides are used to a large extent in the agricultural sector for the protection of crops by killing the harmful and destructive pests. During the process major part of the toxicants will fall on the surface of these soil which due to various external factors will enter into the environment.

Organophosphate compounds such as parathion, sevin and diazinon cause general reduction in numbers of soil animals, whereas, chlorofenvinphos apparently upsets the predator prey balance in a manner similar to that produced by DDT and BHC , by reducing the predatory mite population and allowing the numbers of prey species, such as Collembola and detritivorous mites to increase (Edwards *et al.* 1968). These effects are usually temporary and recovery is more rapid than that occurs after the use of organochlorine pesticides. There is a consistent increase in the use of soil insecticides for the control of white grubs, termites, cut worms and nematodes in this country. However, little attention has been given to the effect of soil insecticides on the non-target soil fauna of our country. The overall effect of organochlorine insectisides (e.g DDT, lindane, dieldrin) applied to cropland is a net reduction in the carabid population and a change in the species composition. Applications of DDT decreased the numbers of carabids and staphylinids in crop fields (Edwards and Thompson, 1973).

The effects of organophosphorus compounds on soil arthropods have been reviewed in detail by Edwards and Thompson (1973) and Thompson and Edwards (1974). Baring (1957) also reported that predaceous bvgftr54mites were susceptible to a mixture of BHC and DDT. The lethal effects of DDT and BHC on soil microarthropod population (Collembola and Acarina) have been recorded by Sheals (1956).

Thus investigations are required particularly in semi-arid agricultural areas of Telangana region of Andhra Pradesh, where several types of pesticides are used but a systematic study on their affects have not been conducted so far In the present study, Endosulfan an organochlorine pesticide is employed to investigate its effects on the qualitative and quantitative composition of the soil fauna and the seasonal population structure in relation to distance traveled by the toxicant and time intervals.

Therefore, an attempt has been made to present by selecting among research findings the soil-inhabiting macro and micro arthropod fauna; in relation to the effect of Endosulfan and evaluate the persistence of its toxic residues with time and distance in the soil followed by Bio-assay studies on the residues.

MATERIAL AND METHODS

Experimental Site:

The experimental site is located at University Campus, Kakatiya University, Warangal district at an altitude of 263.7 m. above mean sea level with geographical bearing of $18^0$31" Latitude and $79^0$29' 5" longitude in Telangana, the semi-arid region of Andhra Pradesh in South India. The soil of the experimental site is a red soil, brownish yellow in colour and sandy loam in texture.

Air temperature:

Air temperature was observed at meteorological observatory at 7:00a.m and air temperature at height of 1m. from the soil was measured at 7:00 a.m and 2:30 p.m. with the

help of Thermometer during the investigation period.

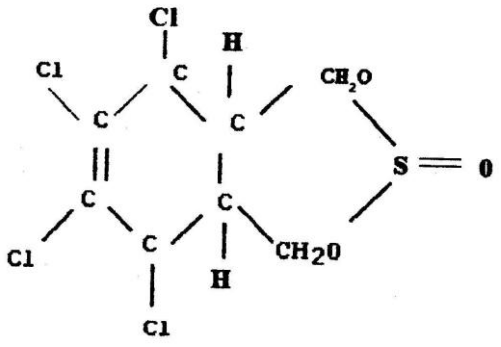
Soil temperature:
Soil temperature at a depth of 15 cm deep in the soil at 3 or 4 spots was measured by inserting specially designed soil Thermometer at 7:00 a.m. and 2:30 p.m.

Determination of soil moisture:
At the time of every soil sample taken, moisture content of soil was determined gravimetrically by using oven and a cylindrical Aluminum tin. The weight of the wet soil in grams was calculated and kept inside the oven. After 16 hours dried soil tins were taken out and its weight was calculated to find out the difference (i.e. moisture weight).

Studies were carried out in experimental plots after insecticidal (Endosulfan) treatment for February 2012 to December 2012. The experiment was laid out in a randomized block design in a 4 Sq.ft plot size to study the insecticidal effect of 'Endosulfan', its persistence and degradation in the soil in relation to time and distances in the soil ecosystem of tropical climate.

Test Insecticide-Endosulfan
Endosulfan (Technical Grade 98.4 %) Trade name of formulation "Thiodan ". Commercial grade of 35 EC was obtained from Hoechst Schering Agricultural Evo. Limited, Bombay. The chemical name Endosulfan is: 6,7,8,9,10,10 - hexachloro 1,5,5a,6,9,9a-hexa hydro-6,9–methano -2,4,3 - benzodioxathiepin 3-oxide (IUPAC).

Fig-1: Structural formula of Endosulfan



The technically active substance consists of two stereo isomers viz ; - α and β 'Endosulfan' both possessing similar insecticidal properties. It is a contact and stomach poison , highly toxic to humans and other warm blooded animals.

Dose and Application:
The insecticide (Endosulfan) treatment was done during February 2002. Both lower and higher doses were sprayed on the soil surface of prescribed size, 2 ft. x 2 ft. (4 Sq.ft) plots separately.

(i) Lower Dose (L.D.): 8ml of Endosulfan (35 E.C) dissolved in two (2) liters of water , which was treated at the rate of 50 ml in first plot

(ii) Higher Dose (H.D) : It was two times more than the lower dose i.e. 16 ml of 'Endosulfan' (35 E.C.) dissolved in 2 Liters of water, treated at the rate of 50 ml in second plot .

(iii) Control Plot :It was treated with water alone (without insecticide) at the rate of 50 ml., in 3rd plot.

Collection of soil inhabiting micro-arthropods using Soil Core Sampler:
The device used for sampling soil micro-arthropods in experimental field was an "Iron core - soil sampler" 5 cm diameter and 15 cm depth. This sampler was similar to the one used by Edwards and Lofty (1971 and 1974), and V. V. N. Hanuma Kumar (1998).

The soil samples were uniformly collected during early hours between 6:00 a.m. to 7:00 a.m. The experimental plots pre-treated with 'Endosulfan' (at lower & higher doses) were demarcated on four sides into different distances and the samples were collected at different intervals as follows 48 hours. (1.0 ft), 6th day (1.6 ft); 9th day (2.0 ft); 15th day (2.6 ft); 21 th day (3.0 ft); 30th day (3.6 ft) 45th day (4.0 ft) and 60th day (4.6 ft , 5.0 ft and 6.0 ft). After gap of 9 months 20 days and completion of the rainy season.

Extraction of Soil Fauna:

The soil samples thus collected , were brought to the laboratory and put into the Tullgren funnel apparatus of "Macfadyen" (1953) with some modification in an air -conditioned room, within one hour of their collection for the extraction of micro-arthropods.

Extraction of Micro-arthropods:
Soil samples were placed in canisters in the same position as they were collected from the field (Macfadyen, 1953). The electric bulbs (25 watts) present in the top baffle board were brought near the sample. Temperature near the samples was gradually increased by adjusting the voltage of bulbs with the help of an electronic dimmer stat. The temperature at the top of the canister was gradually increased from $30 \pm 2^{o}C$ to $40 \pm 2^{0}C$ and 45^{0} C for 12, 24 and 36 Hours of exposure respectively.

Temperature at the bottom of the funnels was maintained at 15 to $20^{0}C$ by forcing the cool air with the help of an air cooler temperature was checked with mercury thermometer at intervals. The steep gradients of temperature i.e., heating the soil sample is gradual, otherwise many arthropods, particularly immature stages, will succumb before they are able to escape. The apparatus was run for 2 to 3 days depending upon the moisture content of the soil samples.

Extraction of Insecticide:
Simultaneously, another set of soil samples were collected from various distances in relation to time. They were taken by the "Iron circular soil sampler "for extraction of insecticide residues and persistence and degradation of Endosulfan in the soil ecosystem.

Thus collected sub samples were put together and brought to the laboratory. These bulk samples taken from identified places were air dried at room temperature, ground and thoroughly mixed by passing several times through a 2 mm sieve to remove stones, organic debris and also to ensure even distribution of the insecticide in the soil sample.

Sample Preservation:
The total weight of sieved soil samples was recorded, and the sieved samples were transferred into thick polythene covers and strongly tie with hard thread (not allowing air in to the pocket) and labeled. Fraction of each soil sample was taken for extraction of residues. If the sample could not be extracted immediately after sampling, they were stored in a deep freeze at 10^{0} C (Williams, 1975).

On line Method for Extracting and Partitioning Insecticides:
After soaking, aqueous sample was poured into high speed blender jar and after addition of acetone 200 ml, the mixture was blended at high speed for 1-2 minutes. To this about 30 gr of Nacl and 150 ml of petroleum ether were added, again it was blended for 0.5 - 1.0 minutes.

The upper organic phase was transferred carefully into a 500 ml beaker and dried over anhydrous sodium sulfate : about 200 ml of organic phase was taken in a 250 ml beaker and reduced the volume to 3 to 5 ml on a vacuum rotatory evaporator. To this about 5 ml of petroleum was added and re-concentrated. Repeated the evaporation process twice with acetone (25 ml at each time) to ensure the complete removal of those solvents that affect the further procedures.

The final residue traces were transferred into 5 ml graduated flask with acetone and stored at 5^{o} C for use in residue analysis. The procedure as explained above was adopted for extraction of all the soil samples collected. Three points of special interest for this extraction method were properly maintained.

1. The ratio of acetone to water was about 2 : 1 (v /v).
2. For partitioning of the insecticides into the organic phase , about 30 gr of Nacl was added.
3. To remove the water from organic phase 150 ml of petroleum ether was

added , which forced the residual pesticides if any into the organic phase.

Qualitative and Quantitative Analysis (Determination) of Insecticide Residues:
In general, several methods for pesticide residue analyses are used. Among which thin layer chromatography (T. L. C.), bio-assay method and early fourth instar silk worms were selected for the treatment.

Thin Layer Chromatography (T.L.C):
This method is used in several organic, drugs, different types of pesticides and their residues analyses. This method is used in pharmaceutical research laboratories first of all ethyl acetate and benzene solutions are taken (ratio of 80 : 20) and mixed solution was prepared in the small (50 ml) beaker, and standardized. Silica gel layered (Aluminum) TLC plate was taken and divided equal fraction of distances and marked with pencil on the bottom of the TLC plate with the help of the micro pipette glass rod the Endosulfan (35 E.C) Technical grade was kept on the prescribed place , from this spot to every 2 cm distances, already prepared soil extraction pesticide residue samples are taken with the help of micro pippet glass tube / rod and was kept on the horizontal line of the TLC plate.

The TLC plate was kept into the ethyl acetate & Benzene solution beaker the solution run through the on test samples of the plate vertically and the beaker mouth was covered with petridish (lid). After some time , when the solution traveled some distance and stop , their moves on the top of the TLC paper. The TLC paper taken out from beaker and kept for some time at noon temperature to dry. The TLC plate was observed under the U. V. scan and results are recorded.

Calculation of R_f value:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent front}}$$

Principle of the Bio-assay:
In this assay frog rectus abdominis is used as Biological Indicator. Rectus abdominis is a skeletal muscle , contains nicotinic receptors. Acetylcholine acts as an against at nicotinic receptors and causes contraction of the skeletal muscle. This contraction is dose, dependant, increases with increase in concentration of Acetylcholine until the ceiling dose. This response can be recorded on the smoked drum of the kymograph.

Collection of Healthy Human Volunteers Serum:
5 ml of the blood is drawn from healthy human volunteers by Disposable syringe. Blood is centrifuged, serum separated and stored at - 4^0 C. It is used whenever required for the estimation of 'Endosulfan'

Preparation of Endosulfan Stock Solution:
Stock solution of 1mg / ml of 'Endosulfan' is prepared with methanol. From the stock solution various concentration (20,40,60,80,100μg) one prepared prior to the experiment.

Preparation of Acetylcholine Stock Solution:
Stock solution of 1 mg / ml of Acelyilcholine bromide is prepared with distilled water from the stock solution various concentration (10, 30, 100, 300 μg) were prepared prior to the experiment.

Preparation of Standard Graph:
A series of concentrations of pure 'Endosulfan' (2,4,6,8,10 μg) were prepared from stock solution. Each concentration is added to 0.5 ml of the serum in effindruf tubes mixed and kept a side for 15 minutes from this 0.1 ml of the serum containing 'Endosulfan' was taken and is added to the submaximal dose of Acetylcholine (0.1 ml Ach). After 15 minutes of incubation, the mixture was injected in to the organ bath containing rectus abdominis and the response noted on the smoked drum. The same procedure is repeated for each concentration of pure Endosulfan , and the response noted.

RESULTS AND DISCUSSION

Soil inhabiting Arthropod Population Under Normal Conditions:

Before the treatment of the insecticide ' Endosulfan' in the experimental site, different groups of soil inhabiting Arthropods were identified. Different types of soil inhabiting Arthropod fauna of the investigation area were extracted and differentiated in to taxonomic groups.

In different plots of the study area the Arthropods inhabited during the investigation period belong to Arachnida (Pseudoscorpionida) and Acarina, Myriapoda, (Chilopoda, Pauropoda, Symphyla), different orders of class Insecta (Diplura,proturan, Collembola, Isoptera, psocoptera, Lepidopluran, Formicidae, Larvae and Coleoptera) Carabidae, Tenebrionidae, Scarabidae, Staphylinidae and other beetles. Acarina was represented by Mesostigmata, Cryptostigamata, Prostigmata. Collembolans were represented by Entoncobryidae, Isotomidae, Onychiuridae and Sminthuridae.

Toxicity studies on Soil Inhabiting Arthropod Population following Endosulfan treatment:

After the application of lower and higher doses of 'Endosulfan' at the rate of 8 ml / 2 liters water and 16 ml/2 liters water respectively were applied to the respective plots of the experimental field.

Total Microarthropods Fauna:

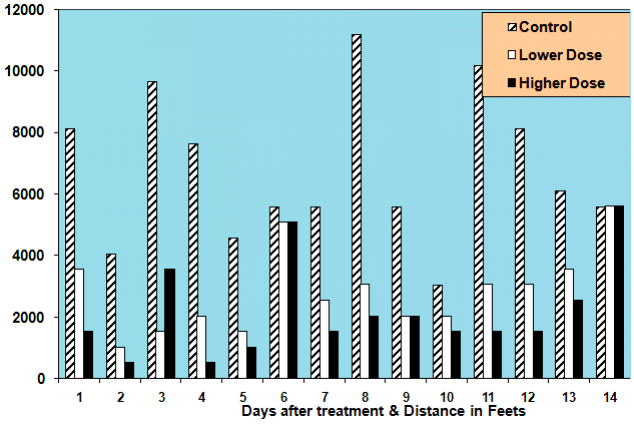
The total population of treated plots compared to that of microarthropods fauna of control plots was recorded after the treatment of 'Endosulfan' (lower and higher doses). The total population after 48 hours of , treatment with lower dose of insecticide at 0.6 ft distance was reduced to 3563 m-2, showing a decrease of population >2 times , the difference was more significant. Whereas at 1.0 ft distance the abundance of population was reduced to 1018 m-2 showing a decrease of population > 4 times and it was more significant as compared to that of control plots.

In control plots after 48 hours the density of population recorded at 0.6 ft distance was 8144 m-2 which decreased at 1.0 ft distance to 4072 m-2 it was suddenly increased after 6 th day at 1.6 ft distance, and reduced after 9 th day and 15 th day at 2.0 ft and 2.6 ft distance respectively. After 21st day at 3.0 ft distance it was slightly increased, and similar population was recorded after 30 th day at 3.6 ft distance also . It was suddenly increased to 11,198 m-2, after 45 days at 4.0 ft distance and decreased continuously till 60 days after treatment . However there was an increase in the density of population after 290 days at 8.0 ft distance followed by a decrease along with the distance (10.0 ft , 12.0 ft and 13.0 ft).

Total Acarina - arthropods Fauna:

The total Acarina population of treated plots compared to that of Arthropods fauna of control plots was recorded after the treatment of 'Endosulfan' (Lower and Higher doses). The data were presented in figure-1.

Figure-1: The total Acarine-arthropods fauna population of treated plots compared control plots

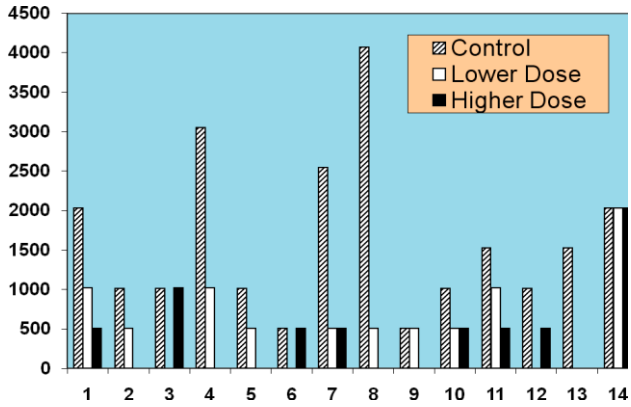


In control plots 48 hours after treatment of insecticide in adjacent plots the density of population was recorded at 0.6 ft distance was 2036 m-2 which was decrease at 1.0 ft distance to 1018 m-2 and similar population was recorded at 1.6 ft distance after 6 th day of treatment. It was suddenly increased to 3054 m-2 after 9 th day at 2.0 ft distance , and decrease continuously till 21 st day after treatment . After 30 th day at 3.6 ft distance

it was slightly increased continuously till 45 days at 4.0 ft distance. After 60 days at 5.0 ft distance the population was reduced. After 60 days at 6.0 ft distance the population was slightly increased till 60 days at 8.0 ft distance following by a decrease at 10.0 ft distance and also followed by a increase along with the distance (12.0 ft and 13.0 ft).

Total Collembola (Microarthropods Fauna): The total collembola population of treated plots compared to that of control plots was recorded after the treatment of 'Endosulfan' (lower and higher doses). The data were presented in Figure-2.

Figure-2: The total Collembola-arthropods fauna population of treated plots compared control plots

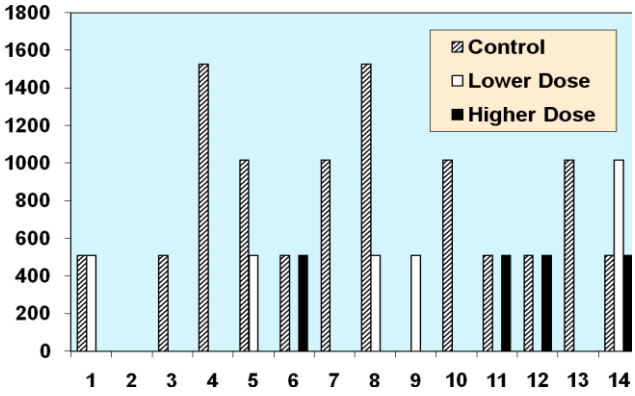


Total Myriapoda (Microarthropods) Fauna :

The total Myriapoda population of treated plots compared to that of control plots were recorded after the treatment of ' Endosulfan ' (lower and higher doses). The data were depicted in Figure-3.

The total Myriapoda population after 48hours of treatment with lower dose of insecticide at 0.6 ft distance was recorded to 1018 m-2 it was similar to compared to that of control plots. Whereas at 1.0 ft distance the population was recorded to nil. After 6 th day of treatment the population was recorded 509 m-2 at 1.6 ft distance. After 9 th day at 2.0 ft distance the population was recorded nil. It was reduced > 2 times compared to that of control population. After 15 th day and 21st day at 2.6 ft and 3.0ft distance the population was recorded to nil respectively compared to that of control of respective control plots.

Figure-3: The total Myriapoda -arthropods fauna population of treated plots compared control plots



In control plots 48 hours after treatment of insecticide in adjacent plots the density of population was recorded at 0.6ft distance was 1018 m -2, which was decreased at 1.0 ft distance to 509 m -2 and after 6 th day at 1.6 ft distance the population was recorded nil, it was suddenly increased after 9th day at 2.0 ft distance the population was recorded to 1018 m -2 and decreased after 15 th day at 3.0 ft distance. It was slightly increased and similar population recorded after 21 st day and 30 th day at 3.0 ft and 3.6 ft distance also. After 45 days at 4.0 ft distance up to at 6.0 ft distance after 60 days similar population was recorded. It was suddenly increased after 290 days at 8.0ft distance the population was recorded in control plot 1527 m -2 and similar population recorded after 290 days at 10.0 ft and 12.0 ft distance also. Whereas at 13.0ft distance the population was recorded nil in control plots.

CONCLUSION

A significant increase of this population was found after 290 days at 8.0ft. distance. A three (3) fold increase was found after 290 days at 8.0ft. Further variation in the population in relation to time and distance

are discussed. A maximum of coleopteron population was found on 6 th day at 1.6 ft. and a total elimination of population at lower dose of 'Endosulfan was noticed at every point of study. The Myriapoda population was maximum at 8.0 ft. distance 290 days after the treatment, at other points of observation Myriapoda population was insignificant in the soil. They are also severely affected by 'Endosulfan'. Treatment. It is concluded that Endosulfan which is known to be a persistent broad spectrum pesticide found causing a loss of soil micro & macroarthropod fauna which are the essential biotic component of the soil ecosystem.

REFERENCES

1. **Edwards , C.A., and Lofty, J.R. (1975)** The influence of cultivation's on soil animal population, In: **Progress in Soil Zoology, (ed).** J.Vanek. Academia Publishing House; pp : 399 - 405.
2. **Hanuma Kumar V. V. N (1998)** Studies on persistence and degradation of some pesticides in soil in relation to arthropod fauna. **Ph.D. Thesis.**
3. **Kevan, D.K. McE., (1955)** A practical key to the orders and subor ders of soil and litter inhabiting animals. In : **Soil Zoology (ed.) D.K. McE, Kevan.,** Butterworths Scientific Publications, London. pp: 452-479.
4. **Lee, K. E. (1985)** Earthworms. Their Ecology and relationship with soil and land use. Acad. Press. **Australia.**
5. **Macfadyen, A., (1952)** The small arthropods of Molinia fen at Cothili. **J. Animal. Ecology. Vol. 21 ;** pp : 87-117.63.
6. Macroarthropods , In ; Biology of Plant Litter Decomposition, (eds.) C.H. Dickinson and G.J.E. Pugh. Academic Press, London, Vol. 11; pp: 533 - 553.
7. **Poole,T.B. (1963).** The effect of some environmental factors on the pattern of distribution of soil Collembola in a coniferous woodland. **Pedobiologia; Vol. 2;** pp: 169 - 82.
8. **Reddy , M.V., and Venkataiah, B (1990)** Seasonal abundance of soil surface arthropods in relation to some meteorological and edaphic variables of the grassland and tree planted areas in a tropical semiarid savanna. **Int. J. Biometeorol. Vol. 34;** pp : 49-59.
9. **Sheals, J. G. (1956)** " Soil Population Studies". I - The effects of cultivation and
10. **Thompson , A.R., and Edwards , C.A., (1974)** Effects of pesticides on non-target invertebrates in fresh water and soil. In : **Pesticides in soil and water.** Soil Science Society of America , Inc. pp : 341 - 386.
11. **Wallace, M.M.H. (1959)** Insecticides for the control of the lucerne flea, *Sminthurus*
12. **Waterhouse , J.S., (1959)** A laboratory method for evaluating the toxicity of
13. **Wibo, C., (1973)** Etude del'action d'un insecticide organophosphore surquelques

DOI: https://dx.doi.org/10.5281/zenodo.7188312
Received: 16 July 2013;
Accepted; 29 August 2013;
Available online : 10 September 2013