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## Editorial

### Could soil- sustainability be prolonged!

Today the soil sustainability is in great danger. Our farmers are taking out more from the soil than putting into it throughout the year. The concept of crop-rotation is no more acceptable by the farming communities now. Sowing of seeds, growing and harvesting of crops is a regular feature. Our farmers are not giving the resting phase to their farming lands to regain sustainability of various macro and micro nutrients, ions and minerals but are applying excessive and imbalance chemical fertilizers for the maximum production of crops. This regular practice has adversely affected the sustainability of our agricultural soil.



There are few more reasons responsible for lowering the soil-sustainability. Regular application of various pesticides (insecticides, weedicides, fungicides and rodenticides) is one of them. It causes killing of our soil flora and fauna which have a great role in increasing soil-sustainability. Our earthworms are one of the major components of soil animals. It is known that earthworm rich soil has more sustainability and produces better crops than the soil devoid of earthworms.

It is a very serious and alarming situation. We should work hard in knowing the adverse effects of all the commonest pesticides on earthworm's life used by the farmers world-widely and suggestions should be formulated for their appropriate and minimum usage so that the infestation of the pests could be controlled on one side and the death of earthworms and other soil organisms on the other.

The present issue of the *VERMECO* is very special as both the articles entitled, "Phorate toxicity on two species of earthworms & Carbaryl avoidance behavior of earthworms" represents the exhaustive work on the impact of common pesticides on different species of earthworms with the hope that our energetic researchers will carry out more work in saving the life of our earthworms- the farmers friends and for maintaining the soil-sustainability.

*Prof. S.M. Singh*

## Message from the President



Soil, water and air are three bases of life. Soil fills our hunger, water our thirst and air, the breaths. Life is not possible if any one of three is not available. Life becomes diseased if any one or two or all the three change their normal constitution. In today's scenario, it is happening. The soil is toxicated, the water is not clean and the air we breathe is not pure.

Most of us are diseased. How can we save our life! It is an alarming question for all of us. The biological, agricultural and environmental scientific community could take a progressive step that might be a milestone for the entire human society by doing research in the areas that will help in maintaining the sustainability of soil, cleanliness of water and purity of the air.

Toxicological unit of Earthworm Research Laboratory of the Department of Animal Science of the University is doing exhaustive work on the adverse effects of common pesticides on earthworms which are the indicators of soil-physical index. Such studies will surely be of immense importance in knowing the minimum dose of a particular pesticide in killing the crop-pests but not the earthworms and other beneficial soil-organisms.

I wish all the success to the team of the research workers in the University Department and urge to the entire scientific community to work more on the applied aspects so that the sustainability of soil may be prolonged in the years to come.

**Prof. Mushahid Husain**  
Vice Chancellor

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## Phorate Toxicity on Two species of Earthworms

Earthworms are important regulators of soil structure and dynamics of soil matter. They are major component of soil fauna communities in most ecosystems and comprise a large proportion of micro fauna biomass. Expansion of agriculture is heavily relying on the use of pesticides; these are generally toxic to non-target soil organisms by causing anatomical, physiological and morphological changes in the vital organs. Pesticide residues enter the environment through industrial and agricultural activities, reaching the earthworm's room soil and water. Extensive usage of organo-phosphorous (OP) compounds in agriculture has resulted in a widespread distribution in the environment. In present study, phorate was selected for assessment of its toxic effect on two indigenous earthworm species, *Metaphire posthuma* (Vaill.) and *Lampito mauritii* (Kinberg) to determine the morphological and histo-pathological changes on both species of earthworms. Although, a very little study had been made on the histo-pathological effects of pesticides on earthworms.

The acute toxicity experiments were conducted by direct contact test (48h) through a filter paper method recommended by OECD Guideline, 207 (1984). The histology of the body wall of *Metaphire posthuma* and *Lampito mauritii* was studied adopting the routine paraffin method (Humason, 1979) and prepared slides were observed under the Olympus research microscope (make-CKX-41).

The toxic effects of phorate on two species of earthworms, *Metaphire posthuma* and *Lampito mauritii* were recorded at 48h using filter paper contact test. Morphological changes in the experimental worms showed the constriction and swelling in the anterior region with in 24h of exposure and degenerative changes appeared at the posterior end of the exposed earthworms after 48h of exposure (Fig.1A & B); while multiple ruptures at body length and oozing of coelomic fluid, nod like glandular appearance on the clitellum and posterior region in the earthworm, *Lampito mauritii* (Fig. 2A). On the other hand, changes in the earthworm, *Metaphire posthuma* showed coiling of body with release of copious amount of mucous with partial disruption of segment and degradation of body wall (Fig. 2B).



Fig.1. *Lampito mauritii*



Fig.1. *Metaphire posthuma*



Fig.2. *Lampito mauritii*



Fig.2. *Metaphire posthuma*

Histo-pathological examination of body sections from control group of earthworms revealed normal architecture of body wall and intact nature of circular and longitudinal muscles (Fig. 3A & B). Earthworms exposed to LC<sub>50</sub> for 48h revealed loss of architecture, showed a tendency to develop excess changes in the body wall structure in the both the worms. Further, sections also showed disintegration of ectodermal layer, neighboring cells in circular and longitudinal muscles appeared discontinuous (separated by narrow to large gap junctions) may be due to necrosis depending upon the effect of toxicant and also appeared enlargement of ectodermal cells and expansion of spaces between the longitudinal muscles with proliferation of glandular cells and erosion of ectodermal of body wall of *Lampito mauritii* (Fig. 3C). Damage in circular and longitudinal muscles and tissue erosion were prominent at 48h of exposure, which may lead to the fragmentation of the body with cloudy swelling of longitudinal muscles, damage in circular and longitudinal muscles and loss of structural integrity in longitudinal muscles and internal & ectodermal tissue erosion leading to total damage of body wall of the *Metaphire posthuma* (Fig. 3D).

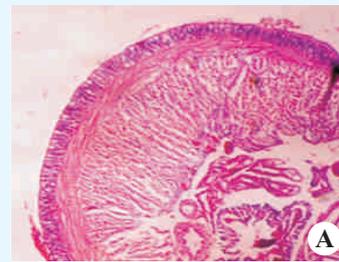


Fig.3. *Lampito mauritii*

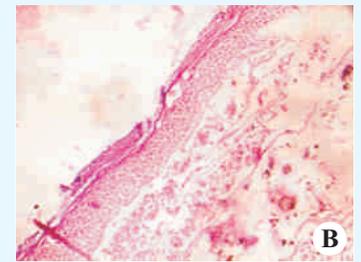


Fig.3. *Metaphire posthuma*



Fig.3. *Lampito mauritii*

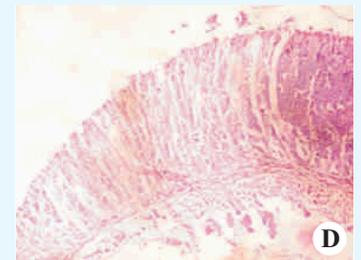


Fig.3. *Metaphire posthuma*

Earthworms are standard test organism in soil toxicity testing. They have been broadly used in assessing environmental impact from heavy metal pollution; however, the knowledge on toxic effects of pesticides upon these organisms is still very limited (Castellanos and Hernandez, 2007). Earthworms are affected by pesticides either through skin contact or by feeding on contaminated litter in soil. Primarily, these toxicants passed through the skin throughout the body wall. Previous studies have suggested that earthworm's skin has direct contact to the contaminated soils and is considered as a significant route to uptake of toxicants (Saxe *et al.*, 2001; Jager *et al.*, 2003; Vijver *et al.*, 2003). Epidermis and cuticle represent a primary barrier that protects earthworm's body from the environment and are also responsible for the transport of ions, thus allowing/blocking xenobiotics to enter the body (Clauss, 2001).

The current investigations on the earthworms, *Lampito mauritii* and *Metaphire posthuma* revealed that the contact toxicity of phorate through its integument was increased with increasing concentration and /or exposure time of the given toxicant. It is evident from the results that phorate can be rated as highly toxic to these earthworms with LC<sub>50</sub> value 20-40  $\mu\text{l}/\text{cm}^2$  at 48h of exposure. The present toxicant is comparatively several folds less toxic to *Eisenia fetida* in

comparison to the earlier studies on other organophosphate (OP) insecticides (Venkateswara Rao *et al.*, 2003b; Venkateswara Rao and Kavitha, 2004). However, work of Zang *et al.* 2000 revealed that these worms were highly sensitive to Chloronicotinoid insecticide, Imidacloprid with an LC<sub>50</sub> of 0.1 and 0.034 µg cm<sup>-2</sup> after 24h and 48h of exposure, respectively.

The anatomical symptoms like coiling, abnormal swelling, fragmentation, rupture of body skin and loss of mucus etc., were noticed by the effect of phorate proved to be identical with the effects of lead acetate, tetra ethyl lead (TEL), methyl tetra-butyl ether (MTBE) (Venkateswara Rao *et al.*, 2001, 2003a) and it was found quite similar in other species of earthworms, *Perionyx excavates* and *Eisenia andrei* too after the exposure to MTBE and Imidacloprid (Youn, 2005; Yvan *et al.*, 2005). These progressive changes, such as hyperplasia and hypertrophy can be considered as general defense mechanism against toxicants (Baynes and Hodgson, 2004), which increase the distance between external environmental and the internal organs and thus serve as a barrier for the entrance of xenobiotics

(Poleksic *et al.*, 2010). A similar kind of autolysis from the posterior region was observed in earthworms, *Polypheretima elongate*, exposed to textile dyes (Ramaswami and Subbram, 1992).

The histo-pathological evaluation revealed that the cuticular membrane and ectodermal layers were completely disintegrated and profusion of glandular epithelium given a protection to the muscle layers of the body wall. It is evident from earlier reports that the morphological and histological changes were prominent when earthworms were exposed to different toxic metals and other OP pesticides (Amaral *et al.*, 2006).

In conclusion, the results of the study suggested that the toxic effects of phorate are mediated through its effect on the morphological and structural integrity of the tissues. Its effect on other biological indicators of stress and pollution (Gobi *et al.*, 2004; Xiao *et al.*, 2006) could play a major role in determining its lethal impact. It further shows that the pesticide had adverse effect on non target organisms particularly the earthworms that are critical in the evaluation of soil fertility.

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## Carbaryl avoidance behavior of earthworms

Earthworms have a number of characteristics which make them one of the most suitable soil animals to be used as key bio-indicator organisms to assess soil pollution by chemicals. Though, *Eisenia fetida* is widely used species for assessing pesticide toxicity, there have been doubts about the sensitivity and concern about it representing typical earthworms. As a result of this, other species have been promoted as test species, the important ones being, *Lumbricus terrestris*, *L. rubellus*, *Aporrectodea caliginosa*, *A. rosea*, *A. longa* and *Allolobophora chlorotica*.

One of the pre requisite for selecting a particular species of earthworm for toxicity testing is that it could be cultured and bred easily, besides its sensitivity to chemicals. *A. caliginosa* is the most widespread epigeic earthworm in temperate countries.

Although pesticides may directly affect earthworms by killing them, they may have more subtle effects on earthworms by adversely affecting growth and reproduction, neurologic function, or by causing behavioural manifestations. Acute toxicity commonly used to test for evaluating pesticide effects on earthworms provide relatively little information on actual pesticide effects on a population in the natural environment. These are delayed effects which are not easily accounted for. The question of environmentally acceptable concentrations of a chemical under conditions of chronic exposure cannot be answered from the results of acute toxicity tests.

Adverse effects on the organisms such as disturbances in behavior, retarded development, lowered fertility, teratogenic effects etc. may cause population changes in the field although animals do not show acute mortality. The information on this count has a greater ecological relevance than those obtained from measurement of acute toxicity. Therefore, there has been a growing interest in conducting

studies on sub-lethal effects of pesticides on earthworms so that a sound tool would be added to the battery of tests currently available to risk assessors.

In addition to toxicity, earthworms may avoid surface litter and soil layers contaminated by certain pesticides. The presence of chemo-receptors in the prostomium and anterior segments of the earthworm body render them highly sensitive to chemicals in the environment. This acute chemical sensitivity coupled with its locomotory abilities enables the earthworm to avoid unfavourable environments such as pesticide treated soil. Significantly, this is also relevant to risk assessment as it may reduce exposure to pesticides and mortality.

The experiments were conducted to assess the carbaryl pesticide avoidance behavior of earthworms in general and two species with varying vertical stratification, and to assess the implications of avoidance behavior in risk assessment in the laboratory at MAFF, CSL, York, England in soil mesocosms by simulating natural conditions with reference to organic matter contact, environmental factors and food by using the pesticide assessed for toxicity was Carbaryl 50 W.P. The experiment was conducted for an exposure period of three months.

The results showed that (i) *L. terrestris*, a deep soil dweller with burrows was exposed to pesticide more than *A. caliginosa*, an epigeic earthworm. (ii) *L. terrestris* appeared to avoid contact with the pesticide by moving deeper into the soil, but to a lesser degree. (iii) *A. caliginosa* exhibited a certain degree of avoidance response by moving deeper into the soil. (iv) Sub-lethal effects of carbaryl were more on *L. terrestris* than *A. caliginosa*. (v) No horizontal migration from treated to untreated surfaces was noticed indicating that earthworms prefer to move down in the soil layers.

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## Indian Earthworm Biologist-10

### Prof. Rudrappa Somappa Giraddi (b.1958)

Dr. Giraddi- presently Dean (Agriculture), College of Agriculture Dharwad (Karnataka) did his UG (1980) and PG (1982) from the University of Agricultural Sciences, Bangaluru and PhD (1992) in Agricultural Entomology from the University of Agricultural Sciences (UAS) from the serving University.

Prof. Giraddi started his carrier as an Instructor at the UAS, Bangalore from 1984 to 87; Assistant Professor, ARS, Bidar from (1987-92); Assistant Entomologist (MARS) from 1992-1995); Associate Professor (MARS) from(1995-2003); Professor of Entomology (MARS) from (2003-2010) and Prof.& Head, Institute of Organic Farming from 2010-12 at the UAS, Dharwad and Dean (Agric.) AC from 2012-15 at UAS, Bheemarayanagudi, Raichur.

He has owned the crown of International Post-Doctoral Fellowship by British Council and Commonwealth Scholarship Commission in UK (1997) and Alumni of CSL, Ministry of Agriculture Fisheries and Foods, York, England (1998); while he was awarded Junior and Senior Fellowship of ICAR, GOI, New Delhi for meritorious performance at National level Exam (1981& 1983) at Post-graduation level. He bestowed for the Award for Excellence by the Society for Applied Biotechnology (SAB) in 2012; ICAR and UAS Award for significant research and presentation in the “National Seminar on Current Trends and Future Prospects in Production and Exports in Special References to Chillies” (2007); as Fellow of SAB, Chennai, India in recognition of outstanding work on Municipal Solid Waste Management (MSWM); as Facilitator and Evaluator by CAPART, GOI, New Delhi for evaluation and support of projects in Agriculture in South India. At the University level, he was appointed as a Nodal Scientist for research, teaching and extension on Vermitechnology for promotion of this branch of science in the University.

He also worked as a member of teams on establishment of Organic Farming Institute and Hitech Horticulture Centers in UAS, Dharwad; Member of UAS, Dharwad for State Pollution Control Board, Bangaluru, for addressing issues pertaining to environment management; Member of UAS, Dharwad teams on Bt cotton monitoring, pigeon pea Roving Survey program in Gulbarga district and chilli crop development. Prof. Giraddi worked as a Team member of UAS, Dharwad for development of GM crops Foreg-Brinjal, Chickpea, and Pigeon pea. He has been nominated as Internal

Expert of Institutional Bio-safety Committee (IBSC) of UAS, Dharwad by Government of India, Ministry of Science & Technology, Department of Biotechnology, New Delhi in October 2015; Member of Peer Review Team of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, by the Assistant Director General (EQR), ICAR, New Delhi in November 2015; as External Member of BOS for UG Degree program at UAS, Bangalore in Feb. 2016; Member of the Board of Management of the University of Agricultural Sciences, Dharwad in December 2016.



He has visited Cardiff University, Wales (United Kingdom) in the International Symposium on Earthworm Ecology in 2002; Taichung, Taiwan and Hong Kong University to participate in the “Expert Consultation and General Assembly of the APAAR” in 2016.

Prof. Giraddi has 29 years of research and 24 years of teaching experience. He supervised 03 Ph.D. and 10 M.Sc. (Agri.) students. He has competed six research projects of different funding agencies like CAPRT, ICAR, Hatti Gold Mines, GOK; DBT and NABARD, Mumbai.

He edited 03 books on earthworms and published 05 International papers and 60 papers in National Journals of repute and presented 56 scientific papers in Symposium/Seminar at the International and National level.

Recently he has delivered Dr. M. Puttarudraiah Memorial Endowment Lecture on “Organic and Integrated Crop Pest Management Strategies” in the Institute of Agricultural Technologists (IAT), Bangalore in November, 2016; Oral presentation in the International Conference on “Climate change adaptation and biodiversity; Ecological sustainability and resource management for livelihood security” in Port Blair, Andaman & Nicobar Islands from December 8-10, 2016 and given a lead presentation on Integrated Pest Management of Chilli Insect Pests with special reference to SAP feeding insects and virus vectors at UAS, Dharwad on Decemember.15, 2016.

Presently, besides serving as Dean (Agric.), College of Agriculture, University of Agricultural Sciences, Dharwad (Karnataka), he is engaged on different toxicological aspects of earthworms.

READER'S OPINION IS SOLICITED

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