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ORIGINAL PAPER

# Toxicity of methyl parathion on growth and reproduction of three ecologically different tropical earthworms

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**Abstract** This work illustrates the toxicological impact of pesticide methyl parathion (MP) (dust—2 % active ingredient, a.i.) on growth and reproduction performance in tropical earthworms: *Metaphire posthuma* (endogeic), *Lampito mauritii* (anecic) and *Allolobophora parva* (epigeic). A total of three concentrations (a.i. g $kg^{-1}$ dry test soil), 1.00 ($T_1$), 1.125 ($T_2$) and 2.25 ($T_3$) of MP, were applied in test substrate to examine the impact on mortality, individual live weight changes and reproduction patterns in test species over 60 days under laboratory conditions. MP caused significant mortality in all tested species, and median lethal dose ($LD_{50}$) for *L. mauritii*, *M. posthuma* and *A. parva* was 24.85, 23.64 and 22.67 mg a.i., respectively. The individual live weight loss was 27.0–37.0 % in *L. mauritii*, 36.0–57.1 % in *M. posthuma* and 1.2–11.0 % in *A. parva* in different test concentrations. The pesticide-exposed worms produced less cocoons than control, but in *L. mauritii*, an unusual reproduction (hormesis) was recorded. Results suggested the species-specific toxicity of MP against tropical earthworms.

**Keywords** Ecotoxicology · Earthworm · Soil biology · Cocoon · Organophosphate pesticide

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## Introduction

Earthworms are considered the main part of belowground food web as they affects soil biological functioning through litter decomposition and nutrient mineralization (Fernández-Gómez et al. 2011; Suthar and Singh 2008) in upper soil layers. The interrelationship between earthworm communities and soil ecosystem functionality is well documented in scientific literature (Brussaard et al. 2006; Rodriguez-Castellanos and Sanchez-Hernandez 2007). The earthworm communities are also recommended as important bioindicator of soil ecosystem health (Suthar et al. 2008; Suthar 2009). The presence or absence, abundance, species richness and reproduction rate are few key biological signals by earthworm under disturbed land systems. Also, earthworm is included in the list of five key indicator organisms for eco-toxicological testing of industrial chemicals by organizations like European Economic Community (EEC), Organization for Economic Co-operation and Development (OECD), International Organization for Standards (ISO) and the Food and Agriculture Organization of the United Nations (FAO). According to Spurgeon et al. (2003), the earthworm communities can play an important role in terrestrial ecotoxicological risk assessment programmes because of its direct relations with soil biological community structure and soil fertility issues. The direct impact of agro-chemicals on earthworm communities has been included as a topic of research priority by researchers engaged in green and sustainable farming practices.

The organophosphate pesticides (parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos and phosmet) have been used widely in commercial crops by farmer communities in India. The pesticides of this family is considered to be the second largest consumed pesticides (about 40–45 % of the total consumption of pesticide) in

the country (Abhilash and Singh 2009). Methyl parathion is a broad-spectrum pesticide and mainly used to control chewing and sucking insects in a wide range of crops, including vegetables, rice and cotton crops. The World Health Organization classifies methyl parathion as a class Ia 'extremely hazardous' pesticide (WHO 1996). Like other organophosphate insecticides, methyl parathion is a cholinesterase inhibitor.

According to EPA (1999), methyl parathion also poses a high risk to birds, aquatic invertebrates and honey bees. Few previous researchers had reported the toxicological impact of pesticides of parathion family on soil-dwelling faunal communities (Kula and Kota 1992; van Gestel et al. 1992; Suthar 2009). However, specific impacts of the parathion family may differ as some causing significant impacts while others not (Van Rhee 1972; Kula and Kota 1992; van Gestel et al. 1992) on soil animals. Therefore, studies on ecotoxicological impact of parathion on non-target organic especially earthworm seems a sound topic for ecotoxicological research programme.

The earthworms are available abundantly in all types of land-use systems of western arid and semiarid lands of India. Few earthworm species, *Lampito mauritii*, *Metaphire posthuma*, *Ocnerodrilus occidentalis* and *Allolobophora parva* are widely distributed in arable lands of north-western parts of Rajasthan State, India (Suthar 2009). However, both *L. mauritii* and *M. posthuma* are commonly distributed earthworms in both conventional and mixed farming systems of this region (Suthar 2009). After reviewing the scientific literature, it is realized that ecotoxicological sensitivity of pesticide like methyl parathion against local earthworm fauna needs to be explored extensively under some laboratory trials.

The aim of this study was to estimate the toxicological impact of pesticide methyl parathion on survival, biomass and reproductive performance of three tropical earthworms: *Metaphire posthuma* (Vaillant), *Lampito mauritii* Kinberg and *Allolobophora parva* Eisen through lab-based toxicological trials. The species-specific susceptibility of earthworms against methyl parathion and pesticide sensitivity in earthworms in respect to earthworm's vertical distribution in soils was also investigated in this study.

## Materials and methods

### Earthworms and pesticide

The individuals of all test species, *M. posthuma*, *L. mauritii* and *A. parva*, were collected from local soils by hand sorting method. The collected earthworms were then brought to the laboratory in plastic containers along with native soils. In laboratory, earthworms were washed under tap water to remove adhering materials and transferred to contamination free organic-rich soils filled in large-sized circular pot containers of 2.5 L capacity. To avoid the possibility of earlier exposure of earthworms to any kind of contamination, the second generation of test species was used for further toxicological experimentation.

Akodol, methyl parathion (MP) dust having 2 % a.i. (active ingredients) was procured from local authentic pesticide distributer, formulated by Akola Chemicals (India) Ltd., Mumbai, India.

**Table 1** Chemical characteristics of soil used as test substrate

| Parameter | Range |
|---|---|
| pH | 7.9 ± 0.01 |
| Organic C | 8.67 ± 0.05 g $kg^{-1}$ |
| Total N | 2.18 ± 0.01 g $kg^{-1}$ |
| Available P | 0.69 ± 0.002 g $kg^{-1}$ |
| Exchangeable K | 0.87 ± 0.001 g $kg^{-1}$ |
| C:N ratio | 3.98 ± 0.03 g $kg^{-1}$ |

### Test soil collection and chemical analysis

For experimentation, the soil was collected from a non-contaminated site of a newly developed ornamental garden. The soil was sandy loam belonging to Torripsamments (P) subgroups as per soil classification. The garden soil was homogenized by passing it through a 0.5 cm sieve. Sieved soil was dried at 60 °C for 24 h and thereafter stored in airtight containers for further experimentation. Prior to experimentation, the test container soil was analyzed for its different physico-chemical parameters. The chemical characteristic of soil is described in Table 1.

### Toxicity screening procedure

For toxicity studies, the plastic containers of 2.5 L capacity with lids were used. The homogenized and dried soil weighing 1 kg was filled in each test container (25 cm height). The methyl parathion dust (with 2 % active ingredient, a.i.) was used for toxicity screening. The ready-to-use concentration of methyl parathion in crop is 0.05–0.1 %. A total of three different concentrations of MP (g $kg^{-1}$ test soil), 1.00 (20 mg/kg soil a.i. = $T_1$), 1.125 (22.5 mg/kg soil a.i. = $T_2$) and 2.25 (25 mg/kg soil a.i. = $T_3$), were used for toxicity screening experiment. The

testing substance, i.e. MP, was homogeneously mixed in soils of experimental test container to achieve total contamination level. For safety purposes, disposable gloves were worn during pesticide handling. The experimental test pots were kept in triplicates for each testing dose for each earthworm species (a total of 27 test containers for three tests doses and three spp.). A separate soil container without testing substance MP was also kept in triplicate for each dose and each species which acted as experimental control. The moisture level of all containers was maintained up to 35 % by periodic sprinkling of adequate deionized water. All experimental test containers were incubated in moist and shady place at the room temperature of 26.5 °C (SD = 0.75). Twenty healthy and mature pre-weighted earthworms (weighted: *L. mauritii* = 0.910–0.970 g; *M. posthuma* = 0.980–1.02 g and *A. parva* = 0.690–0.710 g) were introduced on soil surface of each experimental container. Finely grinded cattle manure (100 g, dry weight basis), as food for earthworms, was spread over the surface of test soils of each experimental container. The food was supplied in test containers after each 15 days interval for 60 days. The test containers were monitored regularly after each 15 days interval up to 60 days to measure mortality rate, biomass change and cocoon production in testing earthworms for each testing dose. After certain interval (15 days), the inoculated earthworms and cocoons (produced during the experimental interval) were separated from the test soil by hand sorting, after which worms were washed in petri plates using deionized water to remove adhering material from their body, and subsequently weighed on a live-weight basis. No correction for gut content was applied to any of the data. To avoid contaminations, disposable gloves washed with HCl 1 N were worn during water sampling. Then, all measured earthworms were returned to the same container along with body-wash water of Petri plate. The separated cocoons from test containers were counted and then inoculated in separate beddings containing sieved garden soil mixed with cow dung.

The $LD_{50}$ was calculated using probit plot method as described by Finney (1971).

### Soil chemical analysis

The pH was measured using a digital pH meter (Systronics made) in 1:10 (w/v) aqueous solution (deionized water). Organic carbon was determined by the partial oxidation method Walklay and Black (1934). Total nitrogen was measure by following micro Kjeldahl method (Jackson 1975). Extractable phosphorous was determined by following Olsen's sodium bicarbonate extraction method (Olsen et al. 1954). Exchangeable K was determined after extracting the sample using ammonium acetate extractable method (Simard 1993).

### Statistical analysis

One-way ANOVA was used to analyze the significant difference among different treatments. Tukey's *t* test was also performed to identify the homogeneous data sets of different observed parameters. Two ways ANOVA with two factors, i.e., pesticide dose and earthworm species, was used to evaluate the role of pesticide dose and earthworm species on earthworm growth parameters (biomass and cocoon production rate). The statistical analysis in this study was conducted using SPSS® (Windows Version 8.0). All statements reported in this study are at the $P < 0.05$ level.

## Results and discussion

### Mortality

The earthworm mortality was recorded in all test containers for different concentrations of MP. On the basis of initial mortality (during 96 h) median lethal dose, i.e. $LD_{50}$, was calculated 24.85 mg a.i. for *L. mauritii*, 23.64 mg a.i. for *M. posthuma* and 22.67 mg a.i. for *A. parva*. The mortality rate among different concentration of MP was significantly different (ANOVA, $P < 0.05$). *A. parva* showed the highest sensitivity against MP and it could be possibly due to its small size and/or little body weight than other test species of earthworm. Results, thus suggested that MP has significant toxicity against soil-dwelling earthworms even at low concentration. MP is considered as a neurotoxic agent and it cause direct toxicological impact in testing organisms even at low doses in surrounding habitat or feedstuffs (Leblance 2004). It irreversibly inactivates acetylcholinesterase, which is essential to nerve function in insects, humans, and many other animals. The earthworm mortality among different test species of earthworm for different doses is described in Fig. 1. The overall mortality in earthworms was in the ranges of 10 (*L. mauritii* with $T_1$) to 90 % (*A. parva* with $T_3$) in different test substances. The total mortality rate in earthworms was in the order: $T_3 > T_2 > T_1$. The mortality rate was 10–46 % in *L. mauritii*, 30–56 % in *M. posthuma*, and 18–90 % in *A. parva* during experimentation as compared to the control. The difference between $T_1$ and $T_3$ (for *L. mauritii*) as well as $T_1$ and $T_2$ (for *A. parva*), for total mortality, was not statistically significant (ANOVA: Tukey's *t* test; $P = 0.109$ for *L. mauritii* and $P = 0.189$ for *A. parva*).

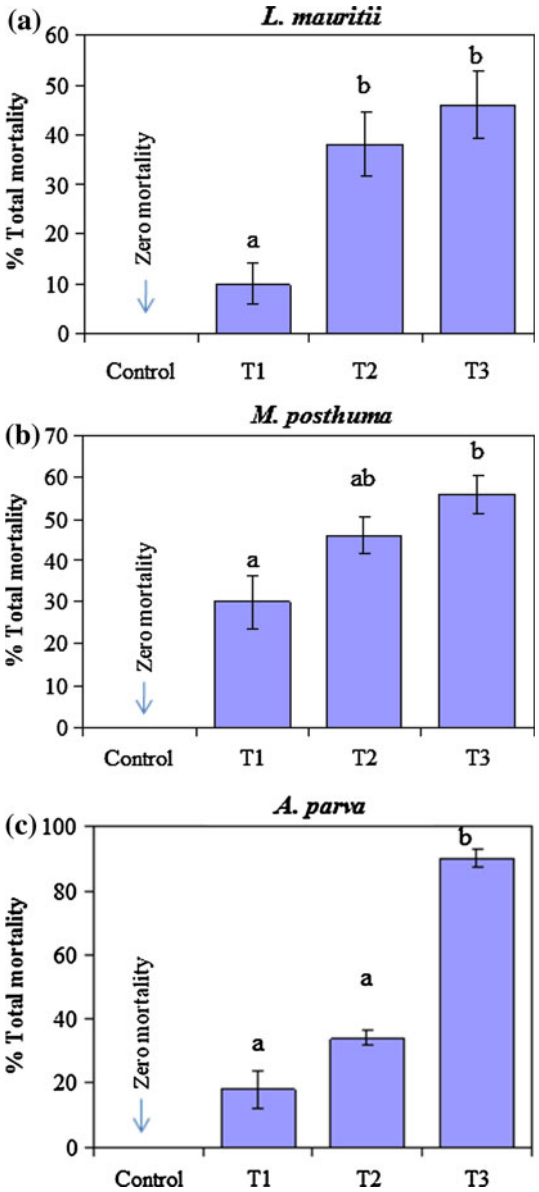


**Fig. 1** Total mortality in *L. mauritii* (**a**), *M. posthuma* (**b**) and *A. parva* (**c**) population (mean ± SEM, $n = 5$), after 60 days of exposure. Significant differences (ANOVA: Tukey's *t* test; $P < 0.05$) are indicated by *different letters*

Results, thus clearly indicates the species-specific mortality patterns among testing species of earthworms for this study. Also, few previous workers have claimed a high mortality pattern in laboratory trials of earthworms against MP (Roberts and Dorough 1984; Haque and Pflugmacher 1985; Bauer and Rombke 1997). A positive correlation between earthworm mortality rate and pesticides concentration for *L. mauritii* ($r = 0.767$, $P < 0.01$) *M. posthuma* ($r = 0.847$, $P < 0.01$) and *A. parva* ($r = 0.945$, $P < 0.01$) was also recorded for this study.

The species-specific sensitivity of pesticides has been reported by various workers (Haque and Ebing 1983; Heimbach 1985) for *Eisenia fetida*, *Aporrectodea longa* and *Lumbricus terrestris.* According to Tomlin (1992), the earthworm niche structure and its vertical distribution in soil layers (e.g. epigeic, endogeic, anecic etc.) has direct role in degree of earthworm susceptibility against soil toxicants. In a lab trial, Holmstrup (2000) also reported different sensitivity patterns of *A. longa* (anecic) and *A. rosea* (endogeic) against a pesticide. The ecological distribution of earthworms in soil system could render some species more susceptible to toxicants in the substrate than others. In general, epigeic *A. parva* do not constructs burrows and mainly inhabits in surface humus-rich soil layers while *M. posthuma* and *L. mauritii* are considered to be horizontally burrowers—mineral soil feeder (endogeic) and vertically burrowers—surface soil feeder (anecic), respectively. Holmstrup (2000) under laboratory trails demonstrated that vertical position of earthworm in soil profile also alters the toxic sensitivity in earthworms against xenobiotics. The observed difference between *M. posthuma* and *L. mauritii* for sensitivity against MP clearly suggested the species-specific pesticide metabolism and/or ecological adaptation. The cutaneous absorption and dietary intake are two main mechanisms of pesticide uptake by soil organisms and in both cases epigeics may have advantage over burrowing earthworms mainly due to their surface-dwelling mode of life (Langdon et al. 2001).

## Effect on biomass

There was statistically significant difference among experimental concentration for biomass changes in *L. mauritii* (ANOVA, $F = 25.69$; $P < 0.05$) and *M. posthuma* (ANOVA, $F = 542.78$; $P < 0.05$) at the end. As compared to the experimental control, the live weight of individual worm was lower: 27.02 ($T_1$)–37.02 % ($T_3$) in *L. mauritii*, 35.98 ($T_1$)–57.14 % ($T_3$) in *M. posthuma* and 1.22 ($T_1$)–42.0 % ($T_3$) in *A parva*, after 60 days of MP exposure (Table 2). The patterns of live weight of individual earthworm in different treatment are described in Table 2.

The loss in individual live weight was lower in *A. parva* than other two species, except in $T_3$ (Table 2). Statistically, the difference among treatment doses was not significant for final live weight in experimental worms (ANOVA/Tukey's; $P > 0.05$), except in *M.*

**Table 2** Individual biomass (g) (mean ± SEM) by earthworms with different concentration of methyl parathion, after 60 days

| Treatment (dose, g/kg dry soil) | *L. mauritii* | | *M. posthuma* | | *A. parva* | |
|---|---|---|---|---|---|---|
| | 0 days | 60 days | 0 days | 60 days | 0 days | 60 days |
| Control | 0.930 ± 0.08 | 1.81 ± 0.05b | 0.980 ± 0.02 | 1.89 ± 0.03c | 0.710 ± 0.02 | 0.820 ± 0.09b |
| $T_1$ (1.00) | 0.956 ± 0.06 | 1.32 ± 0.06a | 1.00 ± 0.04 | 1.21 ± 0.03b | 0.680 ± 0.02 | 0.810 ± 0.02b |
| $T_2$ (1.125) | 0.910 ± 0.04 | 1.28 ± 0.06a | 1.02 ± 0.02 | 0.87 ± 0.81a | 0.700 ± 0.01 | 0.730 ± 0.01b |
| $T_3$ (2.250) | 0.970 ± 0.05 | 1.14 ± 0.02a | 1.01 ± 0.04 | 0.81 ± 0.02a | 0.690 ± 0.01 | 0.475 ± 0.03a |
| ANOVA | | | | | | |
| | $df^b$ | *F* value | *P* | | | |
| Earthworm species (*E*) | 2 | 10.61 | <0.001 | | | |
| Pesticide dose (*D*) | 2 | 77.98 | <0.001 | | | |
| $E \times D$ | 4 | 24.81 | <0.001 | | | |
| Total | 44 | | | | | |

Mean values followed by different letters are significantly different (ANOVA/Tukey's test; $P < 0.05$)

*ND* not determined

[b] Error $df = 36$

*posthuma* ($T_1$). *M. posthuma* showed the maximum loss in individual live weight than other two species studied. According to Tomlin (1992), the population reduction is considered to be interrelated to the health of individuals in population. However, both $T_1$ and $T_2$ doses did not show significant impact on individual biomass of *A. parva*, in comparison with other test species. This suggests the least impacts of pesticides on epigeic (*A. parva*) population than other two, endogeic (*M. posthuma*) and anecic (*L. mauritii*). Probably, the surface-dwelling mode of life of epigeics facilitates them to avoid direct toxicity of pesticides through cutaneous uptake. Tomlin (1992) has concluded that the ecological characteristics and niche structure of an earthworm species may alter the susceptibility against xenobiotics. Furthermore, species-specific borrowing and feeding behavior patterns may render some species more and/or less susceptible to soil toxicants (Tomlin 1992; Tripathi et al. 2010) under field (Spurgeon and Hopkin 1995) as well as laboratory (Edwards and Coulson 1992) conditions. Both anecic and endogeic have different niche structure and feeding habits. *M. posthuma* (endogeic) is a geophagous earthworm and feeds upon sub-soil layers, while *L. mauritii* (anecic) mostly feed upon surface debris layers on soils. The high susceptibility of *M. posthuma* against MP could be due to its feeding habits. The observed low live weight in endogeic than anecic earthworm further extends this hypothesis.

## Effect on cocoon production

There was statistically significant difference among experimental test containers for total cocoon production (Table 3).

But in *L. mauritii*, the difference among treatment was not statistically significant (ANOVA/Tukey's *t* test; $P > 0.05$). Surprisingly *L. mauritii* showed 17 % more cocoon numbers in $T_1$ treatment than experimental control (container without pesticide). But the same pattern was not observed for other two treatments ($T_2$ and $T_3$) and total cocoon numbers were about 27.1 and 42.5 %, respectively, lower than experimental control. MP caused significant reduction in cocoon production rate in *M. posthuma* (ANOVA, $F = 33.96$, $P < 0.05$). In contrast to control, *M. posthuma* produced 30.12, 68.67 and 76.69 % less cocoons in $T_1$, $T_2$ and $T_3$ test containers, respectively. In *A. parva*, different doses of MP caused significant impact (ANOVA, $F = 9.44$, $P < 0.05$) on reproducing pattern (Table 3).

*A. parva* showed 49.65, 71.72 and 99.31 % less cocoon numbers in $T_1$, $T_2$ and $T_3$ treatment, respectively. Few earlier studies have also claimed a similar type of

**Table 3** Total cocoon numbers and reproduction rate (mean ± SEM) by earthworms in different treatments of methyl parathion

| Treatment (dose, g/kg dry soil) | *L. mauritii* | | *M. posthuma* | | *A. parva* | |
|---|---|---|---|---|---|---|
| | Total cocoon numbers | Reproduction rate[c] | Total cocoon numbers | Reproduction rate | Total cocoon numbers | Reproduction rate |
| Control | 9.4 ± 0.73a | 0.0157 ± 0.001a | 16.8 ± 1.11c | 0.0280 ± 0.02a | 39.0 ± 1.79b | 0.0650 ± 0.002c |
| $T_1$ (1.00) | 11.0 ± 1.85a | 0.0197 ± 0.003a | 11.6 ± 1.15b | 0.276 ± 0.001a | 14.6 ± 2.49a | 0.290 ± 0.003b |
| $T_2$ (1.125) | 7.40 ± 0.87a | 0.0203 ± 0.002a | 5.40 ± 0.40a | 0.170 ± 0.001a | 8.20 ± 1.82a | 0.0389 ± 0.006b |
| $T_3$ (2.250) | 5.60 ± 0.61a | 0.173 ± 0.001a | 3.80 ± 0.40a | 0.0160 ± 0.008a | 0.20 ± 0.18a | 0.0016 ± 0.001a |
| ANOVA | | | | | | |
| | *df*[b] | *F* value | *P* | | | |
| Earthworm species (*E*) | 2 | 19,378.58 | <0.001 | | | |
| Pesticide dose (*D*) | 2 | 1,615.55 | <0.001 | | | |
| $E \times D$ | 4 | 326.5936 | <0.001 | | | |
| Total | 44 | | | | | |

Mean values followed by different letters are significantly different (ANOVA/Tukey's test; $P < 0.05$)

[b] Error $df = 36$

[c] Cocoon/adult worm/day

observation that parathion significantly affects the reproducing capabilities in soil-dwelling earthworms (Reddy and Reddy 1992; Landrum et al. 2006). In general, the soil moisture and temperature regime could play an important role in reproduction activity of earthworms (Lee 1985). It is hypothesized that in addition to the pesticides stress, the ambient environmental conditions could suppress the reproducing rate in epigeic *A. parva*, as they inhabits in top layer of substrates in test containers. Holmstrup et al. (2010) and Holmstrup (1999) have reviewed that climatic conditions and moisture levels in top layers of soils directly alters the cocoon production rate in soil-dwelling worms. Also, Bauer and Rombke (1997) have remarked that species-specific toxic sensitivity of earthworms is directly affected by moisture regimes of the test substrate. The cocoon production rate was relatively lower in both *M. posthuma* and *L. mauritii* than epigeic *A. parva*. Surprisingly, *L. mauritii* showed more cocoon numbers (ANOVA/Tukey's *t* test; $P > 0.05$) in $T_1$ treatment than control. This type of mysterious reproduction behavior is called hormesis (providing stimulus by nontoxic amounts of a toxic agent) in general terminology. The biochemical mechanism of hormesis is not well understood but it is hypothesized that low doses of toxins or other stressors might activate the repair mechanisms of the body and expressed in opposite effect. Few earlier researchers have also claimed the enhanced reproduction rate in experimental earthworms, reared in test soils spiked with Benzene hexachloride (BHC) (Gregoreva 1952), Aldrin (Griffiths et al. 1967), and Malathion (Senapati et al. 1992). The science of such behavior in earthworm is still unsolved. Probably, xenobiotic stress in earthworms could trigger the starting of cocoon production in experimental earthworms at low doses of pesticides. However, the same reproduction rate in *L. mauritii* was not observed for other doses of MP. Further detailed studies are required to explore the hypothesis of hormesis.

## Conclusion

Results of this study have revealed that surface-dwelling mode of life guarded the epigeic earthworm against their exposure to pesticide. Overall, endogeic (*M. posthuma*) appeared as most sensitive against pesticide and showed high population mortality as well as live weight loss. The niche structure and feeding habitats of test species directly affect the sensitivity of earthworms against methyl parathion. Results, thus suggested the species-specific sensitivity in earthworms against MP.

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