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# Effects of Imidacloprid and Pendimethalin on Carbohydrates, Lipid and Protein Constituents of *Pheretima posthuma*

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**ABSTRACT**: **The study was carried out for estimating the effect of pesticides imidacloprid and pendimethalin on biochemical constituents of *Pheretima posthuma* exposed for 90 days. Earthworm samples were collected on 1st and 90th days of treatment with imidacloprid and pendimethalin. Thereafter, earthworm powder was made which was then used for estimation of carbohydrate by Standard Phenol Sulphuric method, crude lipid by Soxhlet Extraction method and crude protein by Micro-Kjelhdal method respectively. Significant dose dependent reduction in biomolecules compositions observed in pesticides treated earthworms and maintaining the proper environment for properly measuring the carbohydrates, lipids, and protein constituents of *Pheretima posthuma* estimation was extremely difficult due to the rapid variation of these earthworm biomolecules.The combination dose of imidacloprid and pendimethalin were found to be more toxic than individual exposure of both chemicals. The maximum decrease in carbohydrate (40.88%), Lipid (52.04%) and protein (22.44%) has been observed in earthworms which are exposed to highest concentrations of combination dose of imidacloprid and pendimethalin (0.60+2.50 µl/kg). It was concluded that, Imidacloprid was found to be more harmful when individual exposure of both chemicals applied. It is critical to investigate the effect of imidacloprid and pendimethalin on earthworm biomolecule concentrations in order to reduce pesticide overuse and ensure the future conservation of soil invertebrate flora. As a result, similar studies should be conducted in situ and ex-situ in various areas on a regular basis to ensure biodiversity conservation and sustainable use.**

**Keywords:** *Pheretima posthuma*, Soxhlet Extraction, Standard Phenol Sulphuric method.

## INTRODUCTION

The human population explosion has compelled the agriculturist to increase the supply of food so farmers are now using more and more agrochemicals to increase the harvest. In spite of good crop yields, there are many adverse effects of these agrochemicals on both macro and micro fauna of soil leading to the degradation of environment. They prove harmful to non-target species like earthworm due to similarity in their physiology with pests which ultimately effect the environment sustainability (Wang *et al.*, 2012). It has been recorded that earthworms accumulate chemicals in their tissues at higher level than those substrate in which they live (Ali *et al.*, 2002). Insecticide imidacloprid being an strong insecticide, have selectively more toxicity to wide range of insects and more used in crop protection (Xia *et al.*, 2016; Renaud *et al.*, 2017) having structurally similarity with nicotine (Si *et al.*, 2018) whereas pendimethalin also an broadly used dinitroanaline herbicide, which have used about nearly 25-30 million pounds during 1997 in agricultural and non-agricultural sites (Belden *et al.*, 2005). Due to continuous use such pesticides results in environmental pollution and also harm the non-target organisms present in soil (Dittbrenner *et al.*, 2011). The study of Gill *et al.*, 2021 showed imidacloprid has detrimental effect on antioxidant peroxidase activity in Earthworm *Eisenia fetida.* Earthworm is one of them which is largely effected by these pesticides. Earthworm have crucial role in sustainability because they play important role in soil fertility and plant growth. Earthworm is a very important crucial indicator species in ecotoxicological and ecological risk assessment of soil pollution that are caused by pesticides and other toxics substances (Zhang *et al.*, 2014; Wang *et al.*, 2015; Huang *et al.*, 2018) and because of absence of protective covering on their body they are easily accessible to agrochemicals. Carbohydrate, lipid and protein content reduced after the exposure of *P. posthuma* to the pesticides contaminated soil. It is very well known fact that protein is used mainly as alternate energy source during stress conditions. So, the earthworms species like *E. fetida* and *P. excavates* must have used protein content for production of energy for tolerating the stress conditions due to presence of herbicides in soil. (Lanno *et al.,* 2004; Nahmani *et al.,* 2009). Due to use of imidacloprid and pendimethalin feeding efficiency was highly reduced. Pesticides effects the earthworm survivability rate, growth, behavioral response and reproductive rate was significantly reduced. It is observed that these pesticides have badly impact on earthworm length, biomass, skin, digestive system, biochemical parameter, DNA etc. *P. posthuma* being an endogeic species, bring pollutants on the soil surface from deeper layer of the soil (Zorn *et al.*, 2005). Therefore, the present study was carried out for estimating the effect of pesticides on biomolecules like carbohydrate, lipid and protein constituents of *P. posthuma*.

## MATERIALS AND METHODS

*A. Collection of test animal*

The earthworm species *P. posthuma* was collected from the virgin soil of the CCS, HAU Hisar campus in the month of June and July, 2018. Mature and clitellated earthworms were used for assessing the impact of imidacloprid and pendimethalin on worms.

*B. Experimental set up*

Thirty healthy and fully clitellated earthworm were selected and released in each tub of 90 L capacity after proper washing. Further, earthworms were checked to ensure that all earthworms had burrowed into the soil in tubs, thereafter each tub was covered with gunny bags to maintain moisture level by preventing water loss. Two chemicals imidacloprid and pendimethalin of different concentration (Table 1) were sprayed in each tub except control. Triplicates of each treatment were maintained and the experiment runs for duration of 90 days.

**Table 1: Description of treatments given to earthworms along with control.**

| Sr. No. | Treatments | Concentration (µl/kg of substrate) |
|---|---|---|
| 1. | Control | Without Pesticides |
| 2. | Imidacloprid (48% FS) | 0.6, 0.9 and 1.2 |
| 3. | Pendimethalin (30%EC) | 2.50, 3.75 and 5.00 |
| 4. | Imidacloprid+Pendimethalin | 0.30+1.25, 0.45+1.87, 0.60+2.50 |

*C. Biochemical assays*

After 1$^{st}$ and 90$^{th}$ days of exposure to imidacloprid and pendimethalin earthworms were taken for various biochemical studies. About 20 clitellated earthworms were washed with running tap water to remove any dirt from the body surface. The earthworm were fed with tissue paper, thus the alimentary canal of the living earthworm is substantially freed of soil by their own excretory power. After that they are kept in oven (45ºC) for a period of 72 hours. Thereafter, the dried earthworms were ground in homogenizer to make earthworm powder which was then used for estimation of carbohydrate by Standard Phenol Sulphuric method, crude lipid by Soxhlet Extraction method and crude protein by Micro- Kjelhdal method respectively.

*D. Data analysis*

The experimental design for screen house and laboratory studies were completely randomized block (CRD) with three replicates. Critical difference (CD) was calculated between the various treatments by using Software 'OPSTAT', developed at the Computer Centre, College of Basic Sciences and Humanities, CCSHAU, Hisar.

## RESULTS AND DISCUSSION

Conventional physiological changes were not so better indices to study the change in animal tissue by pollution. The biochemical studies similar to present investigation have been observed at the level of whole animals, organ, tissue and sub-cellular organelles (Bustos *et al.*, 2002) and such type of investigation helped in defining the dose response relationship, threshold limit value, revisable and inversely pattern of pollutant effect (Morgan and Morgan,1999). Data on the carbohydrate content of earthworm exposed to different concentration of imidacloprid and pendimethalin are presented in Table 2. Total tissue carbohydrate contents decreased gradually during the 90 days experiment and maximum decrease was (40.88%) observed in the *P. posthuma* exposed to highest dose of imidacloprid and pendimethalin in combination followed by (32.39%) in imidacloprid at 1.2µl/kg. However when earthworms were treated with pendimethalin at 2.50µl/kg, 3.75µl/kg and 5.00µl/kg carbohydrate decreased up to 7.4%, 19.67% and 27.65% respectively.

**Table 2: Effects of different concentrations of Imidacloprid and Pendimethalin on total carbohydrate content of *P.posthuma.***

| Sr.No. | Treatments (µl/kg of substrate) | Total Carbohydrate (%) DW | |
|---|---|---|---|
| | | 1$^{st}$ day | 90$^{th}$ day |
| 1. | Control | 15.02 ± 0.01 | 15.34 ± 0.04 |
| 2. | Imidacloprid (0.6) | 14.65 ± 0.03 | 13.73 ± 0.03 |
| 3. | Imidacloprid (0.9) | 14.52 ± 0.01 | 12.51 ± 0.04 |
| 4. | Imidacloprid (1.2) | 14.20 ± 0.03 | 9.60 ± 0.03 |
| 5. | Pendimethalin (2.50) | 14.50 ± 0.02 | 13.43 ± 0.02 |
| 6. | Pendimethalin (3.75) | 14.44 ± 0.02 | 11.60 ± 0.04 |
| 7. | Pendimethalin (5.00) | 14.32 ± 0.01 | 10.36 ± 0.04 |
| 8 | Imidacloprid + Pendimethalin (0.30+1.25) | 14.45 ± 0.03 | 13.56 ± 0.03 |
| 9. | Imidacloprid+ Pendimethalin (0.45+1.87) | 14.36 ± 0.01 | 11.27 ± 0.02 |
| 10. | **Imidacloprid +Pendimethalin (0.60+2.50)** | **14.14 ± 0.03** | **8.36 ± 0.04** |
| | SE (m)±<br>CD(P=0.05) | 0.02<br>0.06 | 0.03<br>0.09 |

The observed depletion in carbohydrates may have occurred due to hypoxia, as hypoxic conditions tend to increase the utilization of carbohydrates (Dezwaan and Zandee, 1972). Pollutants cause several types of environmental hypoxia which results in rapid depletions of stored carbohydrates (Heath and Pritchard, 1965). The effects of imidacloprid and pendimethalin on the crude lipid content of earthworms exposed to different concentration are presented in Table 3. Significant dose dependent decrease in the crude lipid content was observed after 90 days of exposure as compared to 1st day of treatment. Doses of imidacloprid and pendimethalin in combination were more toxic than individual exposure to both chemicals, as maximum decrease in crude lipid content (52.04%) was noted in earthworms exposed to highest concentration (0.60+2.50 µl/kg) of imidacloprid + pendimethalin followed by (40.57%) in imidacloprid alone at 1.2 µl/kg. Similarly when earthworms were treated with pendimethalin at 2.50µl/kg, 3.75µl/kg and 5.00 µl/kg dose dependent decreases in crude lipids content was noted up to 13.65%, 29.19% and 34.92% respectively. Decrease in crude lipid may occur due to efforts of the organism for the fulfilment glycogen deficiency, which is caused by any undesired factor or it may be due to neutralisation of the multi-furious toxicity of the xenobiotics present in animals (Rao *et al.*, 1981).

Observations on the effect of imidacloprid and pendimethalin on the crude protein content of the earthworms exposed to different concentrations are presented in Table 4. Doses of imidacloprid and pendimethalin in combination were more toxic than individual exposure to both chemicals, as maximum decrease in crude protein content (22.44%) was noted in earthworms exposed to highest concentration (0.60+2.50µl/kg) of imidacloprid+ pendimethalin followed by (19.33 %) in imidacloprid @1.2 µl/kg . However when worms were treated with pendimethalin @2.50µl/kg, 3.75µl/kg and 5.00µl/kg crude protein decreased up to 13.64 %, 15.84% and 16.47% respectively. Depletion in protein content may be due to interference of soil pollutants and pesticides at some certain level during protein synthesis. The another possible reason for decrease in protein content may be due to increase in proteolytic activities after exposure to soil pollutants. Oxidation of protein may be another reason for protein loss in worms exposed to agrochemicals (Takahashi *et al.*, 1991; Cakmak *et al.*, 2006; Akkas *et al.*, 2007).

**Table 3: Effect of different concentration of Imidacloprid and Pendimethalin on the crude lipid content of *P. posthuma.***

| Sr. No. | Treatments (µl/kg of substrate) | Crude Lipid (%) DW | |
|---|---|---|---|
| | | 1st day | 90th day |
| 1. | Control | 9.04 ± 0.02 | 9.31 ± 0.13 |
| 2. | Imidacloprid (0.6) | 8.87 ± 0.04 | 7.56 ± 0.06 |
| 3. | Imidacloprid (0.9) | 8.74 ± 0.02 | 6.32 ± 0.06 |
| 4. | Imidacloprid (1.2) | 8.48 ± 0.02 | 5.04 ± 0.04 |
| 5. | Pendimethalin (2.50) | 8.72 ± 0.01 | 7.53 ± 0.04 |
| 6. | Pendimethalin (3.75) | 8.70 ± 0.03 | 6.16 ± 0.09 |
| 7. | Pendimethalin (5.00) | 8.62 ± 0.02 | 5.61 ± 0.01 |
| 8. | Imidacloprid + Pendimethalin (0.30+1.25) | 8.81 ± 0.03 | 7.53 ± 001 |
| 9. | Imidacloprid + Pendimethalin (0.45+1.87) | 8.64 ± 0.04 | 6.22 ± 0.05 |
| 10. | **Imidacloprid + Pendimethalin (0.60+2.50)** | **8.32 ± 0.04** | **3.99 ± 0.01** |
| | SE (m)±<br>CD(P=0.05) | 0.03<br>0.08 | 0.06<br>0.18 |

**Table 4: Effect of different concentration of Imidacloprid and Pendimethalin on the crude protein content of the earthworm *P. posthuma.***

| Sr. No. | Treatments(µl/kg of substrate) | Crude Protein (%) DW | |
|---|---|---|---|
| | | 1st day | 90th days |
| 1. | Control | 60.02 ± 0.01 | 61.39 ± 0.08 |
| 2. | Imidacloprid (0.6) | 59.86 ±0.02 | 56.58 ±0.16 |
| 3. | Imidacloprid (0.9) | 59.64 ±0.02 | 53.76 ±0.16 |
| 4. | Imidacloprid (1.2) | 59.29 ±0.05 | 47.83 ±0.05 |
| 5. | Pendimethalin (2.50) | 59.68 ±0.02 | 51.54 ±0.06 |
| 6. | Pendimethalin (3.75) | 59.60 ±0.03 | 50.16 ±0.03 |
| 7. | Pendimethalin ( 5.00) | 59.44 ±0.03 | 49.65 ±0.05 |
| 8. | Imidacloprid + Pendimethalin (0.30+1.25) | 59.80 ±0.03 | 55.19 ±0.06 |
| 9. | Imidacloprid + Pendimethalin (0.45+1.87) | 59.51 ±0.02 | 54.47 ±0.16 |
| 10. | **Imidacloprid+ Pendimethalin (0.60+2.50)** | **59.09 ±0.02** | **45.83 ±0.05** |
| | SE (m)±<br>CD (P=0.05) | 0.03<br>0.08 | 0.10<br>0.29 |

## CONCLUSION

This study showed the degrading effect of Imidacloprid and Pendimethalin on carbohydrate, lipid and protein constituent of *Pheretima posthuma*. Imidacloprid was found to be more harmful than Pendimethalin when applied individually.

So information provides by results, will be useful in estimating the effect of pesticides on *Pheretima posthuma.*

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**Conflict of Interest.** None.

## REFERENCES

Akkas, S. B., Severcan, M., Yilmaz, O., Severcan, F. (2007). Effect of lipoic acid supplementation on rat brain tissue: an FT-IR spectroscopic and neutral network study. *Food Chemistry*, *105*: 1281-1288.

Ali, A. S., Chadha, K., Dev, I. C., Kumar, S., Vyas, V., Bee, R. (2002). Histopathological studies of some organs of earthworms treated with lead nitrate. *Oriental Journal of Chemistry*, *18*(1): 121-124.

Belden, J. B., Phillips, T. A., Clark, B. W., Coats, J. R. (2005). Toxicity of Pendimethalin to Nontarget Soil organisms. The *Bulletin of Environmental Contamination and Toxicology*, *74*: 769-776.

Bustos, O. E., Goicochea, R. I., (2002). Pesticides soil contamination mainly affect earthworm male reproductive parameters. *Asian Journal of Andrology*, *4*(3): 1995-1999.

Cakmak, G., Dogan, I., Severcan, F., (2006). 17b- Estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FTIR spectroscopy: a comparative study with nonylphenol. *Aquatic Toxicology*, *77*: 53-63.

Dezwaan, A., Zande, I. D., (1972). The utilization of glycogen and accumulation of some intermediates during Anaerobiosis in Mytilus edulisl. *Comparative Biochemistry and Physiology*, *43*(b): 47-54.

Dittbrenner, N., Moser, I., Triebskorn, R., Capowiez, Y., (2011). Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) by using 2D and 3D post-exposure techniques. *Chemosphere*, *84*: 1349-1355.

Gill, P., Singh, D. Sharma, B. L., Gupta, R. K., Dahiya, T., Rao, D. S., Lather, D. and Kumar, N. (2021). Effect of Neonicotinoid Acetamiprid and Imidacloprid Insecticides on Antioxidant Peroxidase Activity in Earthworm *Eisenia fetida*. *Biological Forum – An International Journal*, *13*(2): 171-174.

Heath, A. G., Pritchard, A. W., (1965). Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of freshwater fish. *Physiological Zoology*, *38*: 325.

Huang, M., Zhao, C., Zhou, X., Chen, G., Zou, Y., Uphoff, N., (2018). Earthworm responses to cropping rotation with oilseed rape in no- tillage rice fields and the effects of earthworm cast on human-essential amino acid content in rice grains. *Applied Soil Ecology, 127*: 58-63.

Lanno, R., Wells, J., Conder, J., Bradham, K., Basta, N., (2004). The bioavailability of chemicals in soil for earthworms. *Ecotoxicology and Environmental Safety*, *57*: 39-47.

Morgan, J. E., Moragn, A. J., (1999). The accumulation of metals (Cd, Cu, Pb, Zn and Ca) by two ecologically contrasting earthworm species (*Lumbricus rubellus* and *Aporrectodea caliginosa*): implications for ecotoxicological testing. *Applied Soil Ecology*. *13*: 9-20.

Nahmani, J., Hodson, M. E., Devin, S., Vijver, M. G., (2009). Uptake kinetics of metals by earthworm *Eisenia fetida* exposed to field- contaminated soils. *Environmental Pollution*. *157*: 2622-2628.

Rao, K. S. P., Ahamed, I. K., Rao, K. V. R., (1981). Impact of methyl parathion on the tissue $NH_3$.Changes in the fish *Tilapia mossambica* (peters). *Proceedings of the Indian National Science Academy*, *47*(3): 394-397.

Renaud, M., Akeju, T., Nataldalzu, T., Leston, S., Rosa, J., Ramos, F., Sousa, J.P., Azevedopereira, H., (2017). Effects of the neonicotinoids acetamiprid and thiocloprid in their commercial formulation on soil biota. *Chemosphere*, *194*: 85-93.

Si, F., Zou, R., Jiao, S., Qiao, X., Guo, Y., Zhu, G., (2018). Inner filter effect-based homogeneous immunoassay for rapid detection of imidacloprid residue in environmental and food samples. *Ecotoxicology and Environmental Safety*, *148*: 862-868.

Takahashi, H., French, S., M., Wong, P. T. T., (1991). Alterations in hepatic lipids and proteins by chronic ethanol intake: a high- pressure Fourier transforms infrared spectroscopic study on alcoholic liver disease in the rat. *Clinical and Experimental Research*, *15*: 219-223.

Wang, K., Mu. X. Qi. S., Chai, T., Pang, S., Yang, Y., Wang, C., Jiang, J., (2015). Toxicity of a neonicotinoid insecticide, guadipyr, in earthworm (*Eisenia fetida*). *Ecotoxicology and Environmental Safety*, *114*: 17-22.

Wang, Y. T., Cang, X., Zhao, R., Yu, L., Chen, C., Wu. Q. Wang,. (2012). Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicology and Environmental Safety*, *79*: 122-128.

Xia, X., Xia, X., Huo, W., Dong, H., Zhang, L., Chang, Z., (2016). Toxic effects of imidacloprid on adult loach (*Misgurnus anguillicaudatus*). *Environmental Toxicology and Pharmacology*, *45*: 132-139.

Zhang, Q., Zhang, B., Wang, C., (2014). Ecotoxicological effects on the earthworm *Eisenia fetida* following exposure to soil contaminated with imidacloprid. *Environmental Science and Pollution Research*, 12345-12353.

Zorn, M.I., Gestel, V. C. A. M., Eijsackers, H., (2005). The effect of *Lumbricus rubellus* and *Lumbricus terrestris* on zinc distribution and availability in artificial soil columns. *Biology and Fertility of Soils*, *4*: 212-215.

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