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Effect of Endectocides and Antibiotic Dung Poisoning on Mortality of Dung Beetle Species

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Abstract. We explored the effect of endectocides (deltamethrin and trichlorophenol) and antibiotics (enrofloxacin and oxytetracycline) on dung beetle species, *Onthophagus gazella* and *Onitis excavatus* under laboratory conditions. *O. gazella* and *O. excavatus* were collected from pastures and crop lands of Jhelum, Punjab, Pakistan. The selected endectocides and antibiotics were applied on dung pats placed over soil @ 0.25, 0.50, 0.75 and 1 ppm and kept in glass containers of 2' x 2' x 1.5' size. The laboratory bioassay was carried out at 28 ± 2 °C temperature and 65 ± 5 % RH with 12:12 of light to darkness ratio. Our results demonstrated that deltamethrin, trichlorophenol and oxytetracycline were toxic to both species, *O. gazella* and *O. excavatus* after two weeks of the exposure. Comparatively higher dung beetle mortality was recorded in both deltamethrin and trichlorophenol when applied @ 1 ppm as compared to 0.25, 0.50 and 0.75 ppm concentrations in *O. gazella* and *O. excavatus* showed significantly higher mortality against oxytetracycline (53.3 % and 93.3 %, respectively), trichlorophenol (80 % and 94 %, respectively) and deltamethrin (88 % and 76 %, respectively) at 1 ppm concentrations, whereas no mortality was recorded in all concentrations of enrofloxacin. *O. gazella* and *O. excavatus* responded negatively to the presence of different concentrations of veterinary parasiticides. In addition to the hazardous effect of *O. gazella* and *O. excavatus,* the study reports that these dung beetle species can be exploited as bio-indicators for environmental assessment in ecosystem studies.

Keywords: veterinary medicines, *O. gazella*, hazardous effect, non-target species, *O. excavatus*

Introduction

Endectocides treated animals excrete residue of parasiticides primarily in the feces, thus making it poisonous for dung dwelling or eating arthropod fauna, reducing insect abundance associated with the dung decomposition resulting in severe ecological and economic costs (Baena-Díaz *et al*., 2018). Owing to low mammalian toxicity the endectocides and veterinary parasiticides, are globally used against arthropods and nematode parasites (Floate, 2006). Environmentalists show grave concerns about the deleterious effects of endectocides on the dung-inhabiting fauna that decomposes dung (Foster *et al*., 2014; Floates 2006). Significant larval mortality of dung beetles occur in the brood balls of endectocides treated cattle (Iwasa and Sugitani, 2014).

Many drugs are excreted in the feces of treated animals that affect the beneficial insects involved in the decomposition of dung pats (Lumaret *et al*., 2012; Lumaret *et al*., 2005) primarily dung beetles and flies (Chihiya *et al*., 2015). Irrespective of the animal species or method of administration most of the number of drugs applied is excreted infrangible and the insecticidal activity remains in the dung for days (Jacob and Scholtz, 2015; Iwasa *et al*., 2007).

Higher larval and pupal mortality, delayed development and lest allure to drug contaminated dung was observed by the coprophilous arthropods. The deleterious effects of anti-parasitic drugs result in the population decline of non-target dung dwelling species by halting basic biological happenings, thus, contributing to biodiversity loss (Suarez *et al*., 2003). Population index of dung beetles has a directrelationship with the intensity of the endectocides contaminated dung pats (Floate, 2006). Dung beetles represent one of the key insect environmental indicators of biodiversity loss and ecosystem instability due to anthropogenic interventions (Davis *et al*., 2004). Thus, there is a need for constant biological monitoring of dung beetle populations in pastures, croplands and agro-ecosystems.

The current study was conducted *in vivo* bioassay to determine the effect of endectocides and antibiotics on two common species of dung beetle, *O. gazella* and *O.*

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excavatus with the hypothesis that veterinary drugs affect these non-target beneficial species which could be environmental pollution indicators. *Onthophagus gazella* and *O. excavatus* have not been yet explored in the context of environmental indicators of anti-parasitic and antibiotic drugs excreted in the feces of cattle. Our study would provide an insight to conclude the role of endectocides and antibiotics on the biodiversity of non-target species.

Materials and Methods

Study area. The study on the systematics of dung beetles species collected from pastures and croplands of Jhelum, Punjab, Pakistan was conducted in the Laboratory of Systematics and Pest Management, Department of Zoology, University of Gujrat during 2015-2016 (Fig. 1). Owing to diversity and distribution patterns of dung beetle fauna in the study area, the effect of antibiotic and anti-parasitic drugs on mortality of *O. gazella* and *O. excavatus* was assessed in the laboratory bioassay (Ghazanfar *et al*., 2017).

Experimental specimens. Adult specimens of *O. gazella* and *O. excavatus* were collected from untreated cattle dung by hand picking and homogenized method and subjected to identification in the laboratory (Ghazanfar *et al*., 2017).

Collection of fresh dung. Cattle dung was used to rear the beetles which were collected from dairy farms adjacent to the University of Gujrat after ensuring the non-presence or mixing of any material having a detrimental effect to the dung beetles.

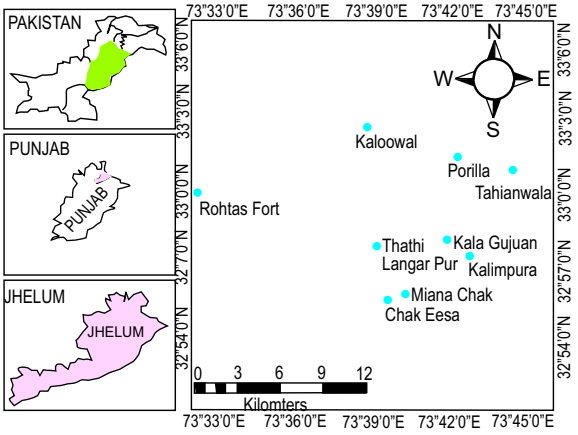

Fig. 1. Map showing of sampling sites for the collection of specimens of *O.gazella* and *O.excavatus* from Jhelum, Punjab, Pakistan.

Soil sampling. The soil samples were collected from the study sites from where beetle fauna was collected. The sample were and treated in the incubator at 70 °C for 2 days before using in the experiment to kill microbes and parasites (Boleas *et al*., 2005).

Preparation of different concentrations. The different concentrations (0.25 ppm, 0.5 ppm, 0.75 ppm and 1 ppm) of the selected antiparasitic and antibiotics were prepared to evaluate their effect on dung beetles (Lumaret, 1986).

Laboratory bioassay procedure. Adults of *O. gazella* and *O. excavatus* were reared on dung pats treated with 0.25 ppm, 0.5 ppm, 0.75 ppm and 1 ppm of each antiparasitic drug and antibiotics were applied by using Potter Tower Sprayer. The experimental units were kept at room temperature of 28 ± 2°C and 65 ± 5 % RH with 12:12 of light to darkness ratio. Each experimental unit was comprised of five male and five female adult dung beetles placed in a 2' x 2' x 1.5' size glass containers provided with soil collected from their natural habitat.

Data collection and analysis. The experiment was laid down in Completely Randomized Design (CRD) with 17 treatments each replicated thrice and data was collected on mortality of beetles after two weeks of the exposure. Dung beetles which were unable to move any part or segment of their body were considered as dead. Dung beetles were counted and mortality was adjusted by applying Abbott's Formula (Verdú *et al*., 2015). The data were subjected to statistical analysis for the comparison. LC_{50} and LC_{90} values for different drugs were calculated by using Probit Analysis (Abbott, 1925). The statistical analysis was performed by using SPSS 21.

Results and Discussion

In the laboratory bioassay, LC_{50} and LC_{90} values were calculated by applying a series of doses of different concentrations on dung pats inhabiting *O. gazella* and *O. excavatus*. The lower values of LC_{50} and LC_{90} of endectocides for the mortality of *O. gazella* and *O. excavatus* showed their greater toxicity to dung beetle species where as higher values of antibiotics suggested less toxicity against non-target *O.gazella* and *O. excavatus* (Table 1).

Effect of oxytetracycline on O. *gazella and O. excavatus* . The significant variations in the mortality of adult dung beetles, *O.gazella and O. excavatus,*were recorded after two weeks exposure to the different

Table 1. LC_{50} and LC_{90} values of different treatments against *O. gazella* and *O. excavatus* after two weeks of application in the laboratory bioassay

	O. gazella		*O. excavatus*	
	LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)
Antibiotic drugs				
Enrofloxacin	15.594	48.50	2.42	5.45
Oxytetracycline	01.120	3.488	0.50	1.13
Antiparasitic drugs				
Trichlorophenol	0.23	0.721	0.21	0.49
Deltamethrin	0.24	0.761	0.44	1.002

concentrations of oxy-tetracycline i.e. 0.25, 0.50, 0.75 to 1 ppm. Increasing trend in the mortality was observed with the increase in the concentration from 0.25 ppm to 1 ppm, whereas no mortality was recorded in the control showed significant differences ($F_{3, 95}$ = 75.52, P <0.05) in the mortality of both species at different concentrations of oxy-tetracycline (Fig. 1). *O. excavatus* indicated significantly higher mortality (93.3 %) as compared to *O. gazella* (53.3 %) at 1 ppm. The data also showed significant differences in the mortality of both species at different concentrations of oxytetracycline (Fig.2).

Effect of trichlorophenol. The data indicated higher mortality of *O.gazella* and *O.excavatus* at all concentrations, whereas no mortality in control. Both species showed greater mortality at 0.75 and 1 ppm as compared to 0.25, 0.5 ppm and control. The data depicted significant differences in the mortality of two species ($F_{1, 95}$ = 5.14, P <0.05) at different concentrations (Fig. 3).

Effect of deltamethrin. The exposure of dung beetle species to different concentrations of deltamethrin resulted in the mortality of both species at all concentrations, whereas no mortality was observed in control where no treatment was applied. The higher mortality was observed at higher concentrations of deltamethrin (Fig. 4). The results showed significant differences between drugs ($F_{3, 95}$ = 273.24, P <0.05).

Effect of enrofloxacin. The results indicated no mortality when dung was treated with different concentrations of enrofloxacin.

Comparative effects of anti-parasitic drugs and antibiotics on *O. gazella.* *O. gazella* indicated significant variations in the mortality against anti-parasitic drugs after an exposure of two weeks. Effect of different concentrations (0.25, 0.5, 0.75 and 1 ppm) in each treatment group (oxytetracycline, trichlorophenol and deltamethrin) indicated greater mortality of *O. gazella* with the increase in the concentration(0.25 to 1 ppm). Significant differences were observed between concentrations of endectocides and antibiotics for each treatment group ($F_{1, 95}$= 5.14, P <0.05) where as, no mortality was recorded in control (Fig. 5).

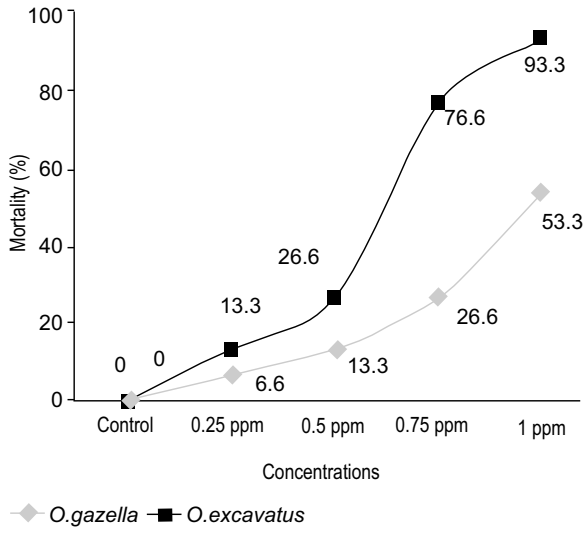

Fig. 2. Effect of oxytetracycline treated dung on the mortality of *O. gazella* and *O. excavatus* after two weeks of exposure.

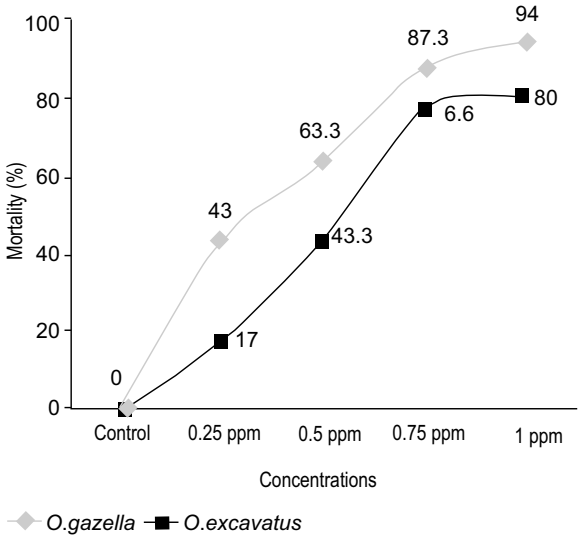

Fig. 3. Effect of trichlorophenol treated dung on the mortality of *O. gazella* and *O. excavatus* after two weeks of exposure.

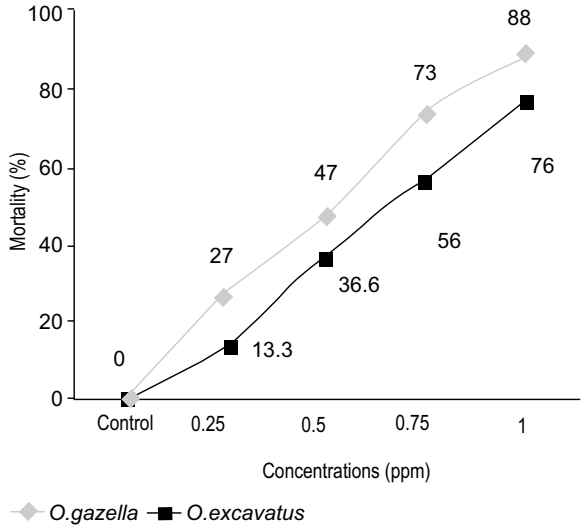

Fig. 4. Effect of deltamethrin treated dung on the mortality of *O. gazella* and *O.excavatus* after two weeks of exposure.

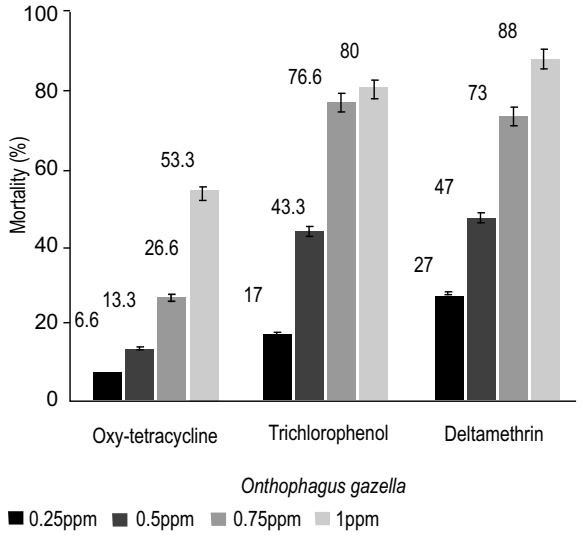

Fig. 5. Comparative effect of various concentrations of antiparasitic drugs and antibiotics on the mortality of *O. gazella* after two weeks of exposure.

Comparative effects of anti-parasitic drugs and antibiotics on *O. excavatus*. Mortality of *O. excavatus* indicated significant variations in the mortality of dung beetles against antiparasitic drugs and antibiotics. Significant differences ($F_{1, 95}$ = 5.14, P <0.05) were observed between concentrations of each treatment group i.e. oxy-tetracycline, trichlorophenol and deltamethrin (Fig. 6). The data indicated that higher

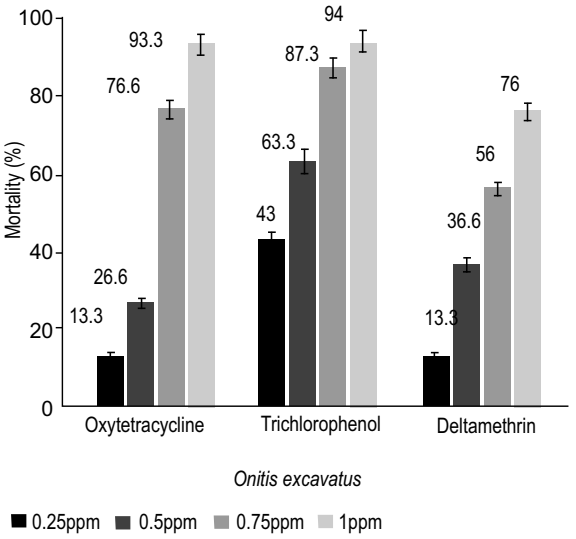

Fig. 6. Comparative effect of various concentrations of antiparasitic drugs and antibiotics on the mortality of *O. excavatus* after two weeks of exposure.

mortality was recorded at 1 ppm concentration of all treatment groups, whereas no mortality was recorded in the control of each treatment group**.**

Dung beetles mortality was assessed by exposing adults of *O. gazella and O. excavatus* to anti-parasitic and antibiotic treated dung pats. Our results showed that both species exhibited mortality against the anti-parasitic drugs, whereas no mortality was recorded in groups exposed to Enrofloxacin treated dung pats. Earlier studies reported that the presence of hazardous material in the dung may lead to feeding non-preference, ingesting hazardous material, and change in the brooding behaviour (Blume, 1975). Ingesting anti-parasitic drugs may have led towards the greater mortality of dung beetles. The studies explored that the volatile compounds emitted by dung are utilized by the dung beetles to locate fresh sources of dung which may be masked by the presence of antibiotic and anti-parasitic drugs, thus, may result in slowing down the growth and development of dung beetles (Dormont *et al*., 2007; Dormont *et al*., 2004; Burger, 1975).

The studies on the toxicity of endectocides (Ivermectin, Eprinomectin, Methoprene and Abamectin) against dung beetles and dung flies reported adverse effects (Iwasa *et al*., 2007; Hooper and Wolfson, 1985). Veterinary anti-parasitic drugs have undesirable effects on dung beetle species as our study reported that drugs

like trichlorophenol negatively influenced the dung beetles species i.e. *O. gazella* and *O. excavatus*. However, in our study we experienced no dung beetle mortality in case of enrofloxacin treated pats at all concentrations which coincide with the results reported by Chandra *et al*. (2012) who studied the acute toxic effect of enrofloxacin on the growth, reproduction and development of *Eisina festidia* and concluded that non target fauna like dung beetles remained unaffected by antibiotics. Our results are also similar to Li *et al*. (2015) who reported no mortality of dung beetles when exposed to enrofloxacin. Both species of dung beetles under consideration showed sensitivity even at the lower concentration (0.25 ppm) of deltamethrin which is in line with Iwasa *et al*. (2014) who reported that Scarabaeide beetles were sensitive to 0.01mg per litter of deltamethrin.

The study documented that veterinary drugs have lethal and sub-lethal effects on non-target species belonging to scarabaeide beetles. Our findings are in line with those of earlier researchers who reported negative effects of such products on the scarabaeid beetles that depend on the dose, concentration, species and application method (Lumaret *et al*., 2012; Lumaret *et al*., 2005). Pest management drugs used against parasites of veterinary animals negatively affect immature and adult forms of beetles reducing their chances of survival, disturbing physiological processes lowering their capacity in the dung decomposition. The study asserts its application in the use of selected anti-parasitic drugs for livestock pest management. The study further reports that dung beetle community structure may be affected by the presence of residue of drugs administered to the livestock for parasite management, thus, lowering the ability of fauna inhibiting the dung.

Conclusion

This research work provided evidence that the population of dung beetles belonging to two representative genera Onthophagus and Onitis collected from pastures may be adversely affected by the use of veterinary drugs especially anti-parasitic drugs. The result demonstrated that the population of *Onthophagus* and *Onitis* species may be adversely affected, thus may lead to community disturbance and contribute in biodiversity loss. Trichlorophenol and deltamethrin showed highly toxic effect on *O. gazella*and *O. excavatus* and reducing their population by about 80 % when the concentration of treated pats was higher. The descending order of mortality observed in this work was trichlorophenol > deltamethrin > oxytetracycline > enrofloxacin. The judicious use of these drugs in addition to sanitary measures may help in preventing biodiversity loss, better nutrient recycling and improving the decomposition rates.

Conflict of Interest. The authors declare that they have no conflict of interest.

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