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Effect of Seasonal Variations and Different Organic Wastes on Growth Parameters and Fecundity of *E. fetida*

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Abstract

Earthworms are major contributors to soil biodiversity; they are the basic driver of abiotic and biotic soil properties, also known as important ecosystem engineers in terrestrial soils. Their feeding, damming and casting affects pedogenesis, soil structure, water regulation, bioremediation of toxins, distribution of organic matter and soil structure. *E. fetida*, an exotic species also known as the red wriggler, has been shown to have wide international potential for converting organic waste into high-value compost useful for plant growth media called vermicomposts. These red worms are active year-round and can tolerate a wide range of temperature and humidity fluctuations. These worms have strong capacity to survive due to its regenerative capacities and this capacity depends on the amputation site, ambient conditions and developmental stages involved. Worm growth patterns in different types of organic wastes have been investigated by various authors in laboratory studies. Animal manure is used as the main substrate for *E. fetida*. They can easily multiply on a variety of organic wastes in a short generation time and are therefore a standard test organism used in terrestrial ecotoxicology.

Key words : Bioremediation, E. fetida, ecotoxicology, pedogenesis, vermicomposts.

Earthworms are categorized into 23 families, representing more than 700 genera and more than 7,000 species worldwide, however the actual number of earthworm species is significantly larger than what is reported. Earthworms are members of the phylum annelida, and they are assigned to the class oligachaeta. Exotic earthworms drastically alter the composition of the soil's microbial community by reducing the amount of fungal growth and increasing the amount of bacteria, according to Dempsey *et al*. (2011). In soil, earthworms and microbes work together in harmony. Vermicomposting refers to the ability of earthworms to transform organic wastes into useful compost, which is advantageous for environmentally friendly farming methods. Many nutrients in vermicompost are available to be used by earthworms, such as exchangeable phosphorous, nitrate, and soluble potassium, magnesium, and calcium. By considerably increasing the amount of minerals like nitrogen, phosphorus, and potassium and lowering the pH, electrical conductivity, and carbon to nitrogen ratio, earthworms increase biofertility. Utilizing *Eisenia fetida* reduces the pollution risk brought on by the degradation of organic wastes. It serves as the standard earthworm in tests for global toxicity (Nahmani *et al*. 2007). At higher temperatures, temperate species are frequently more susceptible than tropical ones, and conversely at lower temperatures (Lee, 1985). A temperature beyond 40 °C will entirely halt the development of juvenile earthworms and also diminish the formation of cocoons. Below 10 °C, earthworm feeding activities have been found to decrease (Edwards and Bohlen 1996., Satchell 1967) The activity of earthworms may be restricted at temperatures of 5°C or below, depending on the species. However, after reaching reproductive maturity, Presley *et al*. (1996) found that *E. fetida*'s

maximum growth and survival rates occurred at moderate/high humidity and low temperatures. At higher temperatures of more than 40 °C, temperate species are frequently more susceptible than tropical ones, and conversely at lower temperatures (Lee.1985).

Materials and Methods

Collection of the test animal : Earthworm culture of *E. fetida* was maintained for use of third generation earthworms to avoid pre-exposure or residual effects of agrochemicals at vermicomposting unit of Institute of Zoology, CCSHAU, Hisar. Mature and fully clitelate earthworms were used for experiments to evaluate the effect of herbicides on growth and reproductive potential.

Collection of Substrate : The dung used as substrate was obtained from the Biogas Station of the Department of Microbiology, CCS HAU, Hisar. To exclude the harmful effect of noxious gases and temperature rise during vermicomposting, cow dung was pre-decomposed for 15 days prior to the study.

A total of three organic wastes viz. cow dung, buffalo dung and a mixture of both were used as substrates after pre-decomposition for 15 days. Three replicates of each treatment were maintained in 90 liter containers at the Vermicompost Unit of the Department of Zoology and Aquaculture, CCSHAU Hisar. Tubs with different finishes have been marked for recording.

Twenty healthy clitellated earthworms (*E. fetida*) were randomly selected and released into each bath after washing and weighing. Moisture content in all treatments was maintained regularly by sprinkling water as needed.

The experiment took place in the summer and winter seasons. Earthworms were sampled after 30 days and regularly up to 90 days. Length, total number of adults/cocoons and total weight of earthworms were recorded.

Results

Survivability of earthworms : Current research has revealed the effect of seasonal changes and different organic wastes on the growth and reproduction of earthworms. The substrate mixture of cow and buffalo dung showed maximum growth in both seasons as shown in Table 1 and Fig. 1. Relatively maximum numbers of adult earthworms, 48 and 19.3 were recorded in summer season and winter season, respectively on day 90. Individually, buffalo dung showed better growth in summer season with 39.3 earthworms on day 90 and cow dung in winter with 13.6 earthworms. The correlation effect of temperature and humidity on earthworm survival was found to be insignificant in both seasons. (Table 2)

Number of cocoons : Maximum numbers of cocoons were found in combined substrate of cow and buffalo dung during summer season than winter. *E. fetida* produced 69.6 and 24 cocoons during the summer season and 24 cocoons. Individually, *E. fetida* produced 48.6 and 36 cocoons in buffalo and cow dung, respectively, during the summer season. But the reproduction rate decreased to 18 and 20.3 cocoons during the winter season in buffalo and cow dung, respectively, on the 90th day of the experiment.

Length of earthworm : Maximum increase in worm length was observed with buffalo + cow dung combination in summer season as compared to winter. A low increase in length (7.2 cm) was recorded in cow dung during the summer season and 5.9 cm in buffalo dung during the winter season on day 90 of the experiment.

Body weight of Earthworm : Maximum

Table 1. Effect of different seasons and substrate on adult earthworms, *E. fetida*

Treatments	Season							
	Summer				Winter			
	Time Interval							
	30 days	60 days	90 days	Mean	30 days	60 days	90 days	Mean
Cow + buffalo dung	19.6	36.6	48	34.7	12.6	18	19.3	16.6
Buffalo dung	18.6	28.6	39.3	30.6	5.6	10.3	9.0	7.2
Cow dung	17.6	20.6	27.6	21.9	8.6	10.3	13.6	10.8
Mean	18.6	28.6.	36.3		8.9	11.7	18.6	
Overall Mean	28.5	11.5						

Factors	C.D.
Season	1.571
Time interval	1.924
Interaction (Season x Time interval)	2.72
Substrate	1.924
Interaction (Season x Substrate)	NS
Interaction (Time interval x Substrate)	3.332
Interaction(Season x Time interval x Substrate	4.712

mean body weight was achieved by the adult earthworms in combination of cow + buffalo dung in both seasons but comparatively maximum (0.82 gms) gain was recorded in summer season and (0.52 gms) in winters on 90th day of experiment. Individually, gain in body weight was 0.61gms and 0.54gms in buffalo dung and cow dung respectively, in summer season and it was 0.37 gms and 0.44 gms respectively on 90th day of experiment in winter season.

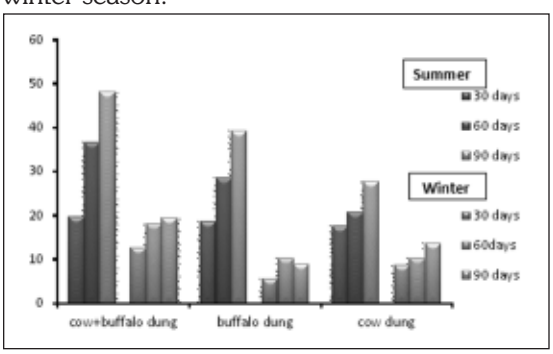

Fig. 1. Effect of different seasons and substrate on adult earthworms, *E. fetida*.

Discussion

E. fetida showed a significant increase in the mixture of buffalo and cow dung during the present research and this mixture initiated pupa production, clitellum development, length and weight gain during summer and winter season, but our observations showed that summer

Table 2. Correlative effects of temperature and humidity on survival of earthworms, *E. fetida*

Treatments	Season			
	Summer		Winter	
	Climatic factors		Climatic factors	
	Humidity	Tempe-rature	Humidity	Tempe-rature
Cow+buffalo dung	0.968^{NS}	-0.937^{NS}	0.938^{NS}	-0.743^{NS}
Buffalo dung	0.958^{NS}	-0.924^{NS}	0.992^{NS}	-0.874^{NS}
Cow dung	0.970^{NS}	-0.940^{NS}	0.999^{*}	-0.911^{NS}

Cow dung is significant at (P = 0.05) with respect to humidity in winters

Table 3. Effect of different seasons and substrate on reproductive potential of earthworms, *E. fetida*

Treatments	Season							
	Summer				Winter			
	Time Interval							
	30 days	60 days	90 days	Mean	30 days	60 days	90 days	Mean
Cow + buffalo dung	33.6	54.6	69.6	52.6	18	20.3	24	20.7
Buffalo dung	27.3	41	48.6	38.9	9.6	12.3	18	13.3
Cow dung	19.3	29	36	28.1	14.3	16.3	20.3	16.9
Mean	26.7	41.5	51.4		13.9	16.3	20.7	
Overall Mean	39.8	16.9						

Factors	C.D.
Season	1.501
Time interval	1.838
Substrate	1.838
Interaction (Season x Time interval)	2.599
Interaction (Season x Substrate)	2.599
Interaction (Time interval x Substrate)	3.184
Interaction(Season x Time interval x Substrate)	4.502

season was more favorable for all growth parameters. Binary combination of cow and buffalo dung showed superior performance in all aspects of growth parameters and fertility compared to individual cow and buffalo dung. When comparing the effect of the season, the summer season showed a significant growth of earthworms compared to winters and non. cocoons were produced by 69.6 and 24 on the 90th day of the experiment. Season and substrate therefore have a significant effect on earthworm growth and survival. A mixed substrate (cow + buffalo dung) among the three was found to be the most suitable for rearing earthworms. Although seasonal variations in temperature and humidity during this study had a negligible effect on worm growth and survival, the cumulative effect of season can affect earthworm growth and survival. Presley *et al* in

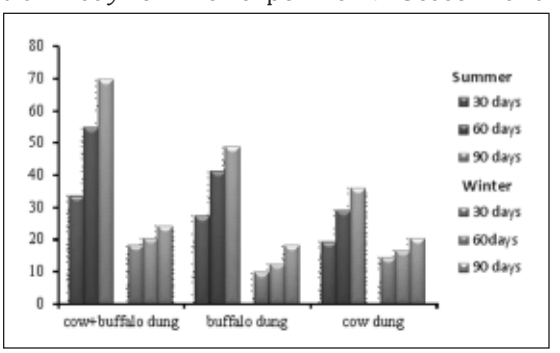

Fig. 2. Effect of different seasons and substrate on reproductive potential of earthworms, *E. fetida*

Table 4. Correlative effects of different seasons and substrate on reproductive potential of earthworms, *E. fetida*

Treatments	Season			
	Summer		Winter	
	Climatic factors		Climatic factors	
	Humidity	Temperature	Humidity	Temperature
Cow+buffalo dung	0.989^{NS}	-0.968^{NS}	0.979^{NS}	-0.834^{NS}
Buffalo dung	0.974^{NS}	-0.945^{NS}	0.996^{NS}	-0.957^{NS}
Cow dung	0.981^{NS}	-0.957^{NS}	0.956^{NS}	-0.780^{NS}

Table 5. Effect of different seasons and substrate on the body length (cm)of earthworms, *E. fetida*

Treatments	**Season**							
	Summer				**Winter**			
	Time Interval							
	30 days	**60 days**	**90 days**	**Mean**	**30 days**	**60 days**	**90 days**	**Mean**
Cow + buffalo dung	7.0	7.6	7.9	7.5	6.7	6.9	7.2	6.9
Buffalo dung	6.8	7.3	7.5	7.2	5.8	6.0	5.9	5.9
Cow dung	6.3	6.8	7.2	6.7	6.4	6.5	6.7	6.5
Mean	6.7	7.2	7.5		6.3	6.5	6.6	
Overall Mean	7.1	6.4						

Factors	**C.D.**
Season	0.092
Time interval	0.113
Substrate	0.113
Interaction (Season x Time interval)	0.159
Interaction (Season x Substrate)	0.159
Interaction (Time interval x Substrate)	N/A
Interaction (Season x Time interval x Substrate	N/A

1996 also observed the effects of various climatic factors on earthworm growth and reported that the reproductive fitness and growth of *E. fetida* is affected by changes in temperature and humidity conditions. The observations of Chauhan and Singh 2012 also matched our findings that the binary combination of buffalo dung with wheat bran showed significantly better results for the growth and development of *E. fetida* earthworm.

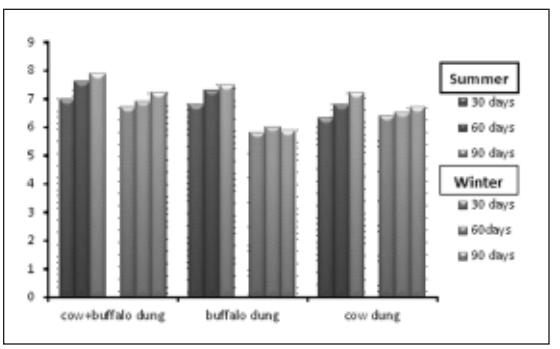

Fig. 3. Effect of different seasons and substrate on the body length of earthworms, *E. fetida.*

Beyginiya *et al*. in 2013 reported that the best growth of *E. fetida* was observed in 80% sheep manure + wheat straw 20% in spring season. Their results evaluated that the maximum activity, biomass increase and greater number of cocoons were produced during the spring season and the reproductive ability of *E. fetida* varies in different seasons. Biradar *et al*, 2015 evaluated the effect of seasonal environmental factors and different organic

Table 6. Correlative effects of different seasons and substrate on the body length of earthworms, *E. fetida*

Treatments	**Season**			
	Summer		**Winter**	
	Climatic factors		**Climatic factors**	
	Humidity	**Tempe-rature**	**Humidity**	**Tempe-rature**
Cow+buffalo dung	0.993^{NS}	-0.975^{NS}	0.967^{NS}	-0.803^{NS}
Buffalo dung	0.998^{*}	-0.986^{NS}	0.619^{NS}	-0.866^{NS}
Cow dung	0.970^{NS}	-0.940^{NS}	0.945^{NS}	-0.756^{NS}

Table 7. Effect of different seasons and substrate on weight (gms) of earthworms, *E. fetida*

Treatments	Season							
	Summer				Winter			
	Time Interval							
	30 days	60 days	90 days	Mean	30 days	60 days	90 days	Mean
Cow + buffalo dung	0.58	0.69	0.82	0.69	0.41	0.45	0.52	0.46
Buffalo dung	0.47	0.56	0.61	0.54	0.32	0.38	0.37	0.35
Cow dung	0.42	0.46	0.54	0.47	0.35	0.40	0.44	0.39
Mean	0.49	0.57	0.65		0.36	0.41	0.44	
Overall Mean	0.57	0.40						

Factors	C.D.
Season	0.015
Time interval	0.018
Substrate	0.018
Interaction (Season x Time interval)	0.025
Interaction (Season x Substrate)	0.025
Interaction (Time interval x Substrate)	0.031
Interaction (Season x Time interval x Substrate	NS

waste on life activities of Perionyx excavations. The study revealed that environmental factors had a significant effect on the growth, maturity and reproduction of Perionyx digs.Maximum growth was recorded in monsoon season for all wastes. Mixed organic waste and soft (straw-based) wastes were found to be more favorable to the overall life activity of the Perionyx excavation than hard (pod-based) wastes. Findings Vodounnou *et al*., 2016 on the effect of plant compost and various organic wastes on the growth and survival of *E. fetida* support our observations and the highest growth rate was observed in cow dung among all organic wastes, *viz*., pig, cattle, rabbit, sheep, poultry and vegetable compost). They observed all physico-chemical parameters of growth and survival for 90 days. To promote vermiculture biotechnology as an approach for ecological livestock

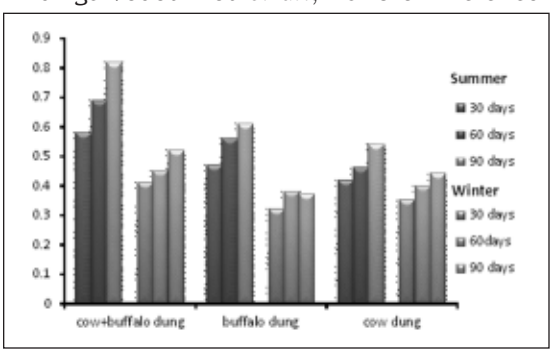

Fig. 4. Effect of different seasons and substrate on weight (gms) of earthworms, *E. fetida.*

Table 8. Correlative effects of different seasons and substrate on weight (gms) of earthworms, *E. fetida*

Treatments	Season			
	Summer		Winter	
	Climatic factors		Climatic factors	
	Humidity	Temperature	Humidity	Temperature
Cow+buffalo dung	0.936^{NS}	-0.896^{NS}	0.955^{NS}	-0.778^{NS}
Buffalo dung	0.989^{NS}	-0.969^{NS}	0.997^{NS}	-0.896^{NS}
Cow dung	0.877^{NS}	-0.824^{NS}	0.860^{NS}	-0.988^{NS}

enhancement and sustainable production of earthworm biomass as a source of protein and vermicompost, Kabi *et al*., 2020 attempted to understand fecundity, survival and growth patterns of earthworms using four organic substrates including cattle manure (CM), slaughterhouse waste (AW), soybean post-harvest residue (SBCR) and a binary mixture of manure and soybean crop residue (CM + SBCR) for 2 weeks. They reported that the fecundity, growth and survival of *E. eugeniae* were affected by the different type of organic substrate and the rate of cocoon production (0.41 cocoons/ worm/day) was highest in earthworms fed CM + SBCR. A study by Esmaeili *et al.* (2020) on a combined (composting - vermicomposting) process with *E. fetida* for the treatment of pistachio waste (PW) mixed with cow dung (CD) in different proportions (100% CD (T1), 25% PW (T2), 50% PW (T3), 75% PW (T4) and 100% PW (T5)) support our results. Their results showed that the combined process significantly increased the quality of the final product and the fertilizer produced. Physico-chemical analysis showed a significant decrease in the C:N ratio, total organic carbon and total potassium and an increase in total nitrogen, available phosphorus and pH. The number of adult worms during the procedure also increased in all treatments, and the net weight and growth rate of worms in T2 and T5 were higher than in other treatments.

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