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***Research Article** (Open Access)*

EFFECT OF DIFFERENT COMBINATIONS OF FEED MATERIAL OF CATTLE DUNG WITH KITCHEN WASTE ON REPRODUCTION OF EARTHWORM *EUTYPHOEUS WALTONI* MICHAELSEN

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ABSTARCT

In this study the effect of different combinations of animal dung (buffalo, cow and goat) with kitchen waste (vegetable wastes and banana peels) on the reproductive potential of *Eutyphoeus waltoni* during vermicomposting has been investigated. Initiation of clitellum development, initiation of cocoon production, period of cocoon production, incubation period, cocoon/worm, hatchling/cocoon and number of hatchlings emerged out from cocoon of *Eutyphoeus waltoni* in feed materials of binary and tertiary combinations of animal dung with vegetable waste and banana peels were observed. The significant early development of clitellum, period of initiation of cocoon and cocoon/worm were observed in the binary combination of buffalo dung with vegetable waste in (1:1) ratio. Significantly high number of cocoon production was obtained in binary combination of (BD+VW) in (1:1) ratio (23.33±0.55SE cocoon/worm). The higher number of hatchlings per worm was observed in the binary combination of (BD+VW) in (1:1) ratio (62.05hatchling/worm). The results of this study indicate that the combination of buffalo dung with vegetable waste in (1:1) ratio is the most effective feed material for the reproduction of *Eutyphoeus waltoni* and production of better vermicompost. *Eutyphoeus waltoni* play an important role in recycling of organic wastes.

***Keywords**: Buffalo dung, Cow dung, Eutyphoeus waltoni, Goat dung, Kitchen waste, Reproduction, Vermicomposting.*

INTRODUCTION

One of the primary sources of municipal solid waste is household kitchen waste. Domestic waste, primarily organic wastes accounts for approximately 70% to 80% of urban solid waste in India (Kale, 1998). A significant amount of kitchen waste is generated annually in India (Mohite at al., 2024). These kitchen wastes can have negative effects on health and the environment if it is not decomposed properly (Kaviraj and Sharma, 2003). Every household generates a large amount of food scraps in the kitchen. When disposed of in garbage bins, these scraps can produce odours and increase the volume of waste sent to landfills. Although disposing of kitchen waste in a garbage disposal is convenient, it places a burden on waste treatment systems and discards a potentially valuable resource (Chavan *et al.,* 2017). India generates about 320 million tonnes of agricultural waste annually (Suthar *et al.*, 2005), with vegetable waste comprising a significant portion. Vegetable scraps collected from markets and dumped in municipal landfills pose environmental problems due to their high biodegradability (Bouallagui *et al.,* 2004). Vegetable waste is ideal for vermicomposting as it is purely organic and decomposes more easily than other types of waste. Common vegetable waste includes spinach, Chinese mustard, leaf mustard, cabbages, peas and other vegetables (Febriyantiningrum *et al.*, 2018). Bananas are the second-largest fruit crop after citrus fruits, accounting for about 16% of global fruit production. India is the largest banana producer, contributing 27% to global banana production. Bananas are highly nutritious, rich in potassium and calcium. Banana peels are rich in vitamins, starch, crude protein, crude fat, dietary fiber, polyunsaturated fatty acids, and micronutrients such as potassium, phosphorus, calcium, and magnesium (Mohapatra *et al.,* 2010; Siddiqui and Singh, 2023a).

Animal dung is a nutrient-rich substrate that provides essential nutrients and organic matter, promoting earthworm growth and activity. It is an abundant and renewable resource in many regions, making it a cost-effective option for vermicomposting (Kale *et al.,* 1992). Reports suggest that cattle dung can be utilized either independently or in combination with other organic wastes as a bulking agent to produce vermicompost that is more stable and richer in nutrients (Yadav *et al.,* 2013; Yuvraj *et al.,* 2020; Singh and Singh, 2024). Animal dung is rich in nitrogen, typically contains about 1.3% to 2.0% nitrogen on a dry weight basis (Kale *et al.,* 1992). Nitrogen is a vital component for protein synthesis, which is crucial for earthworm growth, reproduction, and enzyme production. It enhances microbial activity, which accelerates the decomposition of organic matter in vermicomposting, while kitchen waste are rich in organic matter and provide an additional source of carbon, which is crucial for maintaining a balanced carbon-to-nitrogen (C/N) ratio in the substrate (Suthar, 2009). The combination of these materials creates a nutrient-rich environment which supports the growth and accelerates the reproductive development in earthworms. Vermicomposting is an eco-friendly and efficient method for converting organic waste into nutrient-rich compost, providing a sustainable solution for waste management and soil fertility enhancement (Bhat *et al.,* 2018; Siddiqui *et al.,* 2022). This process utilizes earthworms to decompose organic materials, leading to the production of vermicompost that is rich in essential nutrients, such as nitrogen, phosphorus, and potassium, as well as beneficial microorganisms that enhance soil health (Tiwari and Yadav, 2023; Edwards and Bohlen, 1996; Singh and Singh, 2023 a, b, c). *Eutyphoeus waltoni* is predominantly found in India, where it occupies a range of habitats from forest to agricultural lands (Singh *et al.,* 2015). This species found in moist and humus rich soil. They are present in plant nurseries, garden, heaps of manure and cultivated field. It has been reported that anecic species are ideal for processing organic waste from sources such as canteens, homes, towns, and farms (Gergs*et al.,* 2022; Singh and Singh, 2024). The geophytic nature of anecics allows them to break tougher substrates than epigeics (Gajalakshmi *et al.,* 2001; 2002). It has been reported that anecic species do not only efficiently produced vermicompost but also generated more offspring during six-month trials (Gajalakshmi *et al.,* 2000). However, there is limited research on the use of *Eutyphoeus waltoni* with specific organic waste combinations, particularly in the context of animal dung and kitchen waste mixtures.The current study aimed to enhance the reproductive potential of *Eutyphoeus waltoni*in a specific combination that is important in increasing the conversion of organic waste into effective vermicompost.

MATERIALS AND METHODS

Collection and rearing of the earthworm Eutyphoeuswaltoni:

The cultured earthworm *Eutyphoeus waltoni* from the vermibiotechnology Laboratory, Department of Zoology, Deen Dayal Upadhyaya, Gorakhpur University, Gorakhpur were used for the experiment. For this, vermibed were prepared by using garden litter with buffalo dung on a cemented surface in the laboratory. Young cultured earthworms were used for the experiment.

***Collection of animal dung and kitchen wastes**:*

Animal dung was collected from the local farm houses and vegetable wastes (VW) as well as banana peels (BP) were procured from the local area of the Gorakhpur district. Buffalo dung (BD), cow dung (CD) and goat dung (GD) were spread in a layer and exposed to sunlight for 20 days to remove various harmful organisms and noxious gases. After pre-treatment these biological wastes were used in the experiment as feed material in vermicomposting (Garg *et al.,* 2005).

Experimental setup:

The experiment was conducted on cemented surface. Two kilograms (Kg) of each five different combinations of buffalo dung i.e. BD+VW (1:1), BD+VW (2:1), BD+BP (1:1), BD+BP (2:1), BD+VW+BP (1:1:1) and BD alone; Cow dung i.e. CD+VW (1:1), CD+VW (2:1), CD+BP (1:1), CD+BP (2:1), CD+VW+BP (1:1:1) and CD alone as well as goat dung i.e. GD+VW (1:1), GD+VW (2:1), GD+BP (1:1), GD+BP (2:1), GD+VW+BP (1:1:1) and GD alone were prepared in beds of (30 x 30 x10 cm^3) at room temperature (27±2 ^{0}C) in the dark. The experiment to study the growth and development of earthworm *Eutyphoeus waltoni* was performed by the method of Garg and Kaushik (2005). The vermicomposting beds were turned over manually every 24 hours for 2 weeks in order to eliminate volatile substances. Thereafter 20 young *Eutyphoeus waltoni* were inoculated into each bed. In order to provide optimal environmental conditions for worms, the moisture of all the treatments were maintained at 60-70% by sprinkling water during the experiment. Each experiment was replicated six times (Singh and Singh, 2024).

Reproduction of the earthworm Eutyphoeus waltoni:

Every week, cocoons were collected using a 0.5 mm mesh sieve and gently washed, and their numbers were recorded on an individual basis. Before weighing, the cocoons were lightly washed in distilled water to eliminate any debris stuck to the sticky hull. The culture sets were carefully observed daily for cocoons, if any. The cocoons were immediately isolated and incubated. In the experiment old cultural media was replaced with the same amount of fresh media on a weekly basis, thus food was not a limiting factor. After isolation, each cocoon was freshly put in a petri dish containing moist filtered paper at 30±2 ^{0}C and 70±5% RH. The hatching of cocoons were determined to estimate incubation, and the number of progenies emerged per cocoon were recorded (Singh and Singh, 2024).

Statistical Analysis:

All the experiments were replicated six times to ensure consistency in the results and figure out the mean with standard error. Analysis of variance was used to analyze the significant difference between the combinations and *t* test ($P < 0.05$) used to identify the homogeneous type of bedding in terms of reproduction and growth compared to the control (Sokal & Rohlf, 1973).

RESULTS

There was significant clitellum development, cocoon formation and cocoon emerged out of worm were observed in feed materials of binary and tertiary combinations of different animal dung (buffalo, cow, goat) with vegetable waste and banana peels during vermicomposting. Among all the binary and tertiary combinations of animal dung with vegetable waste and banana peels the significant early clitellum development was observed in the combination of buffalo dung with vegetable waste in (1:1) ratio (14.16±0.30 days) with respect to the buffalo dung alone (24.16±0.30 days) (Table 1-3, Figure 1-3). The significant period of initiation of cocoon production was observed in the binary combination of cow dung with banana peel in (1:1) ratio (24.66±0.49 days) with respect to the cow dung alone (31.83±0.40 days) (Table 1-3, Figure 1-3). The period of cocoon production as well as incubation period in all the combinations of animal dung with vegetable waste and banana peels is earlier than the dung alone (Table 1-3, Figure 1-3). Significantly higher number of cocoon production was observed in the binary combination of buffalo dung and vegetable waste in (1:1) ratio (23.33±0.55 cocoon/worm) in comparison to buffalo dung alone (14.83±0.90 cocoon/worm) among all the combinations of animal dung with vegetable waste and banana peels (Table 1-3, Figure 4-6). The higher number of hatchling from each worm were observed in the binary combination of buffalo dung with vegetable waste in (1:1) ratio (62.05 hatchling/worm) than buffalo dung alone (22.24 hatchling/worm) among all the combinations of animal dung with vegetable waste and banana peels (Table 1-3, Figure 4-6). It indicates that the combination of buffalo dung with vegetable waste in (1:1) ratio is the most suitable feed material for the reproduction of earthworm *Eutyphoeus waltoni*.

Table 1: Effect of different combinations of buffalo dung with kitchen wastes on the production of cocoon and hatchling of *Eutyphoeus waltoni*

Combination	Initiation of cliellum (days)	Initiation of cocoon production (days)	Period of cocoon production (days)	Incubation period (days)	Cocoon/worm	Hatchling/ Cocoon	Reproduct-ion rate (hatchling/ worm)
BD Control	24.16±0.30a	34.50±0.50a	19.33±0.42a	30.16±0.30a	14.83±0.90c	1.50± 0.22a	22.24
BD+VW (1:1)	14.16±0.30d	26.33±0.33c	14.50±0.56a	25.83±0.90a	23.33±0.55a	2.66± 0.40a	62.05
BD+VW (2:1)	18.33±0.66c	30.33±0.49b	17.16±0.30a	27.16±0.30a	19.50±0.71b	1.66± 0.21a	32.37
BD+BP (1:1)	16.83±0.74c	29.16±0.47b	15.83±0.40a	28.66±0.71a	17.66±0.42b	1.33± 0.32a	23.48
BD+BP (2:1)	17.33±0.49c	31.66±0.84b	18.66±0.71a	29.16±0.60a	16.33±0.21c	1.16± 0.17a	18.94
BD+BP+VW (1:1:1)	21.50±0.56b	30.16±0.87b	16.83±1.22a	27.83±0.70a	21.16±0.40b	1.85± 0.30a	39.14

Each value is the mean ±SE of six replicates. BD= Buffalo Dung, BP= Banana Peel, VW= Vegetable Waste
**Mean differences in column followed by common letter are not significant at P<0.05 (DMRT) in 30.0x30.0x10.0cm^{3}area of vermicompost bed.*

Table 2: Effect of different combinations of cow dung with kitchen wastes on the production of cocoon and hatchling of *Eutyphoeus waltoni*

Combination	Initiation of clitellum (days)	Initiation of cocoon production (days)	Period of cocoon Production (days)	Incubation period (days)	Cocoon/worm	Hatchling/ Cocoon	Reproduct-ion rate (hatchling/ worm)
CD Control	22.66±0.49a	31.83±0.40a	18.16±0.47a	23.33±0.21a	12.83±0.60c	1.50± 0.22a	19.24
CD+VW (1:1)	15.33±0.55c	27.83±0.60b	13.33±0.49a	18.83±0.40a	17.16±1.04a	2.83± 0.16a	48.56
CD+VW (2:1)	16.66±0.61c	29.16±0.30b	17.50±0.56a	22.50±0.80a	14.66±0.22b	1.66± 0.33a	24.33
CD+BP (1:1)	15.83±0.40c	24.66±0.49c	14.83±0.54a	17.66±0.42a	13.33±0.48c	1.33± 0.21a	17.72
CD+BP (2:1)	19.33±0.76b	28.83±0.30b	16.66±0.21a	20.33±0.60a	11.16±0.70c	1.16± 0.16a	12.94
CD+BP+VW (1:1:1)	16.16±0.16c	27.50±0.22b	15.16±0.60a	21.67±0.33a	15.83±0.40a	1.83± 0.40a	28.96

Each value is the mean ±SE of six replicates. CD= Cow Dung, BP= Banana Peel, VW= Vegetable Waste
**Mean differences in column followed by common letter are not significant at P<0.05 (DMRT) in 30.0x30.0x10.0cm^{3}area of vermicompost bed.*

Table 3: Effect of different combinations of goat dung with kitchen wastes on the production of cocoon and hatchling of *Eutyphoeus waltoni*

Combination	Initiation of clitellum (days)	Initiation of cocoon production (days)	Period of cocoon Production (days)	Incubation period (days)	Cocoon/worm	Hatchling /Cocoon	Reproduct-ion rate (hatchling/worm)
GD Control	28.50±0.50a	44.16±0.70a	22.66±1.08a	22.50±0.16a	11.16±0.91c	1.66± 0.33a	18.52
GD+VW (1:1)	24.30±0.33b	34.50±0.80c	20.16±0.30a	20.83±0.90a	13.33±1.11c	0.83± 0.16a	11.06
GD+VW (2:1)	25.33±0.21b	36.83±0.56c	18.66±0.79a	21.66±0.61a	12.33±0.98c	1.83± 0.47a	22.56
GD+BP (1:1)	23.33±0.20b	41.16±0.47b	17.83±0.60a	19.33±0.98a	17.66±0.21a	1.51± 0.22a	26.66
GD+BP (2:1)	24.17±0.83b	38.50±0.76c	17.50±0.21a	22.16±0.80a	16.32±0.32b	1.18± 0.47a	19.32
GD+BP+VW (1:1:1)	20.83±0.60c	32.83±0.60c	19.50±0.50a	21.20±0.60a	14.16±0.30c	1.17± 0.30a	16.56

Each value is the mean ±SE of six replicates. GD= Goat Dung, BP= Banana Peel, VW= Vegetable Waste
**Mean differences in column followed by common letter are not significant at P<0.05 (DMRT) in 30.0x30.0x10.0cm^{3}area of vermicompost bed.*

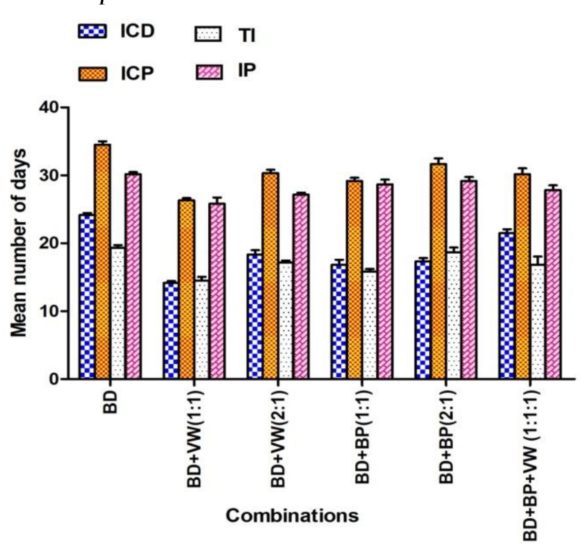

Figure 1: Effect of different combinations of buffalo dung with kitchen wastes on the initiation of clitellum development and cocoon production of *Eutyphoeus waltoni*. ICD= Initiation of clitellum development (in days), ICP= Initiation of cocoon production (in days), TI= Time taken for cocoon production after initiation of clitellum development, IP= Incubation period, BD= buffalo dung, VW= Vegetable waste, BP= Banana peel

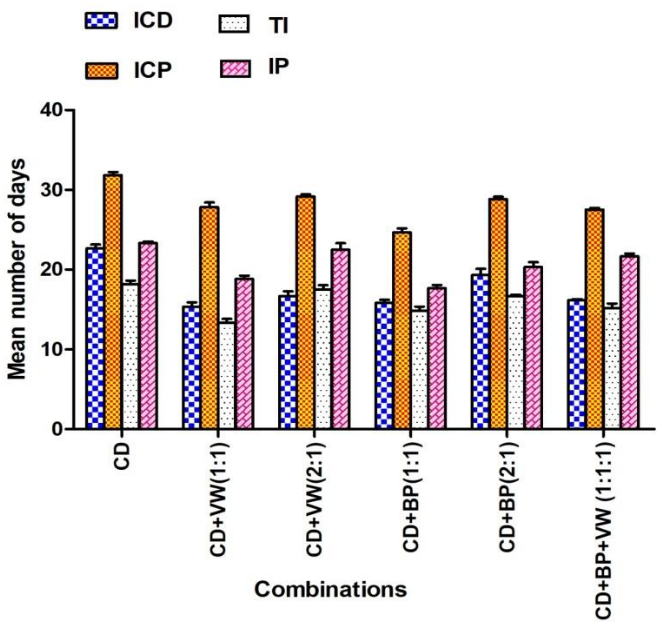

Figure 2: Effect of different combinations of cow dung with kitchen wastes on the initiation of clitellum development and cocoon production of *Eutyphoeus waltoni.* ICD= Initiation of clitellum development (in days), ICP= Initiation of cocoon production (in days), TI= Time taken for cocoon production after initiation of clitellum development, IP= Incubation period, CD= Cow dung, VW= Vegetable waste, BP= Banana peel

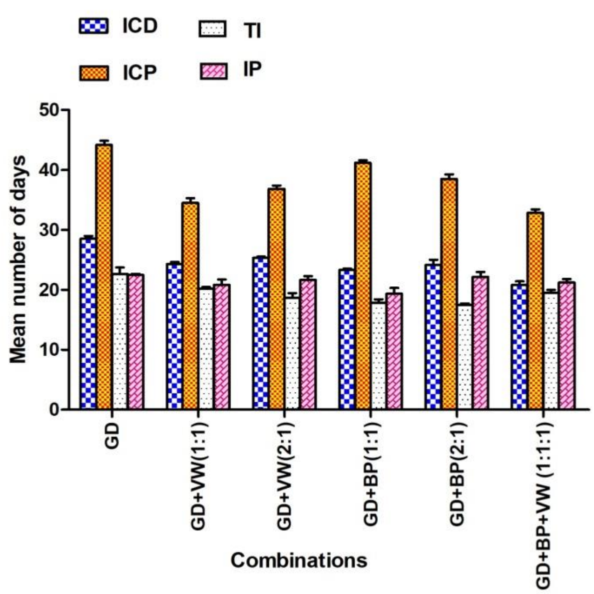

Figure 3: Effect of different combinations of goat dung with kitchen wastes on the initiation of clitellum development and cocoon production of *Eutyphoeus waltoni.* ICD= Initiation of clitellum development (in days), ICP= Initiation of cocoon production (in days), TI= Time taken for cocoon production after initiation of clitellum development, IP= Incubation period, GD= Goat dung, VW= Vegetable waste, BP= Banana peel

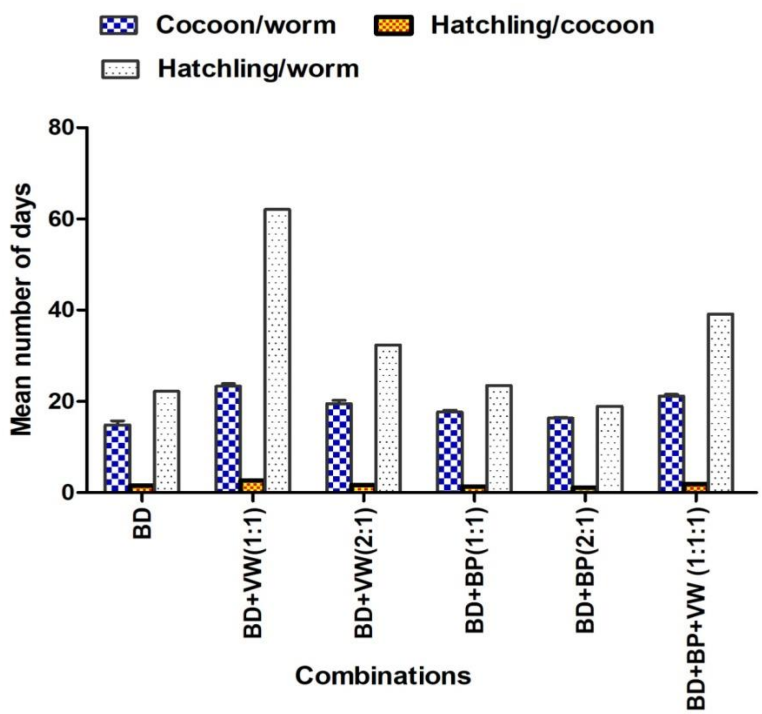

Figure 4: Effect of different combinations of buffalo dung with kitchen wastes on the production of cocoon and hatchling of *Eutyphoeus waltoni*. BD= Buffalo dung, VW= Vegetable waste, BP= Banana peel.

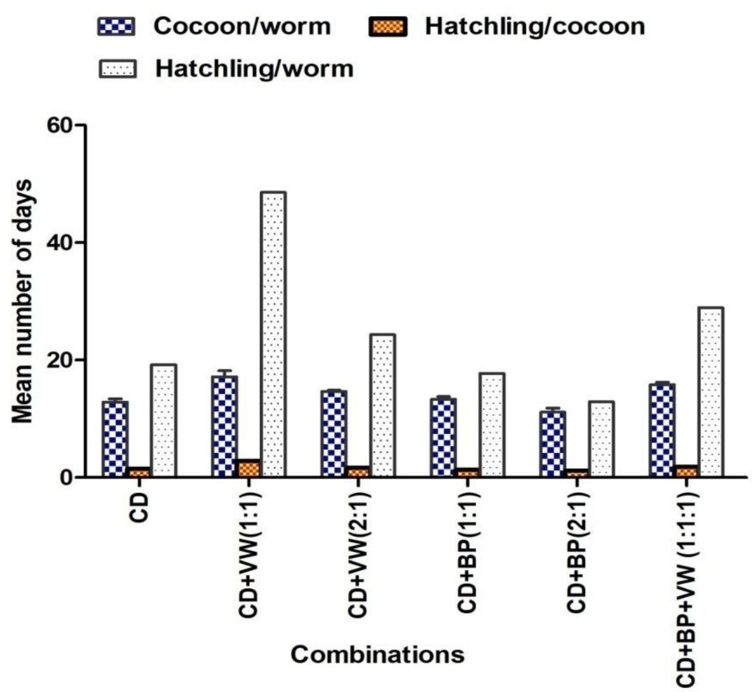

Figure 5: Effect of different combinations of cow dung with kitchen wastes on the production of cocoon and hatchling of *Eutyphoeus waltoni*. CD= Cow dung, VW= Vegetable waste, BP= Banana peel.

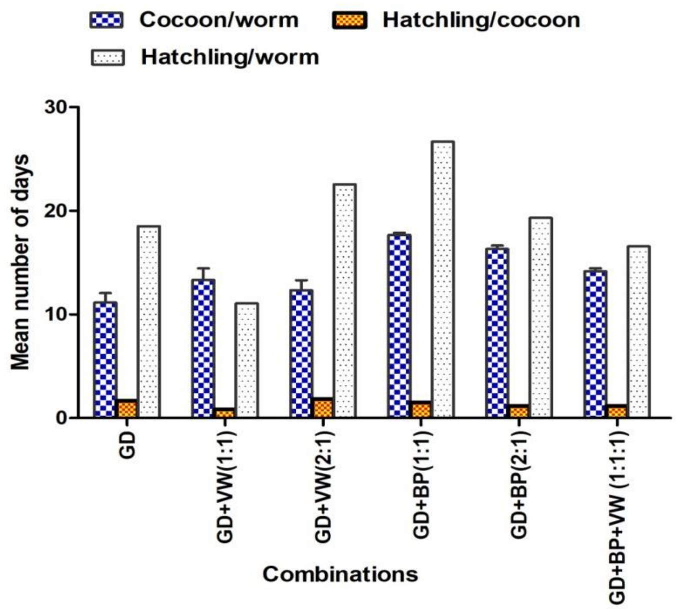

Figure 6: Effect of different combinations of goat dung with kitchen wastes on the production of cocoon and hatchling of *Eutyphoeus waltoni*. GD= Goat dung, VW= Vegetable waste, BP= Banana peel.

DISCUSSION

In the present study the reproductive potential of *Eutyphoeus waltoni* in binary and tertiary combination of animal dung (buffalo, cow, goat) with kitchen wastes (vegetable waste and banana peels) as feed material were observed which demonstrated that these feed material combinations significantly influence earthworm reproduction and vermicompost quality. Research has shown that the nutritional quality of the feed material significantly affects the reproductive biology of earthworms (Neuhauser *et al.,* 1988; Singh and Singh, 2024). The combination of animal dung with kitchen waste provides nutrient-rich environment for the growth of earthworm. Animal dung is known for its high nitrogen content, which, when combined with the carbon-rich kitchen waste, creates an optimal carbon-to-nitrogen (C/N) ratio for microbial activity (Gupta *et al.,* 2007). This balanced nutrient profile facilitates the growth of beneficial microorganisms that decompose organic matter into simpler compounds, which are more readily assimilated by earthworms (Edwards & Fletcher, 1988). It has been reported that the presence of organic carbon, high pH and electrical conductivity (EC) in the initial feed material, along with a decrease in the carbon-to-nitrogen (C/N) ratio during vermicomposting contributed to the earliest clitellum development (Siddiqui and Singh, 2023 b; c). Throughout the 90-day vermicomposting process, the organic carbon content in the feed material significantly decreased, as earthworms utilized this carbon for their growth and development (Suthar, 2007; Yadav and Garg, 2011; Chauhan and Singh, 2013; Singh and Singh, 2024).

The physical properties of the feed material, such as texture and moisture retention capacity, also influence earthworm reproduction (Butt, 1993). The addition of vegetable waste to buffalo dung improves the texture, aeration, and water-holding capacity of the feed material (Elvira *et al.,* 1996). The vermicompost produced in buffalo dung and vegetable waste combination showed higher nutrient content, with increased levels of nitrogen, phosphorus, and potassium, compared to compost of the dung alone. This is consistent with findings of Garg and Kaushik (2005), who reported that mixing animal dung with organic waste improves vermicompost quality. It has been reported that composition of the feed material act as a key factor in promoting clitellum growth in earthworms (Flack and Hartenstein, 1984; Singh and Singh 2024). Recent research has shown that incorporating vegetable waste into the feed can boost the total number of earthworms and promote better growth (Merta and Raksun, 2024). Higher concentrations of nutrients in feed material significantly decelerated degradation rates and adversely affected cocoon production in earthworms, leading to delays and reductions. Chauhan and Singh (2012) reported an increase in the cocoon production of *Eisenia fetida* when using different binary mixtures of buffalo dung and agro-wastes. The findings of increased hatchling production in this study are consistent with the work of Chauhan and Singh (2013). Studies have demonstrated that feed material with a balanced carbon-to-nitrogen ratio and sufficient organic matter content can enhance the fecundity and hatchling success of earthworms (Domínguez and Edwards, 1997; Tripathi and Bhardwaj, 2004). The combination of buffalo dung and vegetable waste in (1:1) ratio provides favourable environment for cocoon production and hatchling emergence by supplying essential nutrients and maintaining suitable moisture levels.

CONCLUSION

The (1:1) ratio of buffalo dung with vegetable waste provides the most favourable condition which increased the reproductive potential of earthworm *Eutyphoeus waltoni*. This combination offers a balanced nutrient profile, enhancing microbial activity and facilitating the decomposition process. The high nitrogen content of buffalo dung, combined with the rich organic carbon from vegetable waste, creates suitable environment for reproduction of earthworm *Eutyphoeus waltoni*. Earthworms showed higher number of hatchlings in this feed mixture. These findings suggest that using (1:1) ratio of buffalo dung and vegetable waste can be an effective strategy for managing organic waste and producing high-quality vermicompost. The study also highlights the potential of adding kitchen wastes into vermicomposting systems to enhance soil fertility and promote sustainable agricultural practices.

CONFLICT OF INTEREST

The author declares no conflicts of interest regarding the publication of this paper.

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