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Effect of Binary Combinations of Different Animal Dung with Different Agro Wastes on Reproduction and Development of The Earthworm *Eisenia fetida*

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Abstract
The aim of present study was to investigate the effect of binary combination of different animal dung with agro-wastes on the reproduction and development of earthworm *Eisenia foetida*. There was significantly increase in reproduction rate as cocoon production in binary combination of Goat dung with Rice bran 0.159±0.05 cocoons worm day^{-1} . The net weight gain by *E.fetida* was significantly highest 890.26±4.17(mg worm^{-1})in goat dung with gram bran. Food quality influences not only the size of earthworms but also their reproduction and development. After physical analysis of initial feed mixture and the final vermicompost there is an significant decrease in level of pH, C/N ratio and EC (electrical conductivity). The C/N ratios in all final vermicompost are significant decrease from initial feed mixture. The final vermicompost has significantly decreased electrical conductivity in combination of goat dung with gram bran. The chemical analysis before and after vermicomposting the TOC significantly highest in goat dung with rice bran296.23±1.06. In final vermicompost TKN, TK, TAP and TCa significantly increased in final vermicompost with respect to initial mixture. This study demonstrated that the binary combination of initial feed mixture of Goat dung with rice bran is better option for enhancement of earthworm's population as well as provides the potent vermicomposts.

Keywords
Agro wastes Binary Combination *Eisenia foetida* Vermicomposting Reproduction Development Physico-Chemical Analysis

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INTRODUCTION
Abundant uses of chemical fertilizers have made our soils sick and problematic because the preliminary basis of green revolution was chemical fertilizers and pesticides which ignored the green manure and organic farming. Addition of vermicompost enhances the soil fertility as its changes physical and chemical properties of soil [1]. Organic matter status of the soil of humid tropical country is generally low [2]. In India the livestock dung is produced annually millions of tons as the rate of cow dung 11.6 kg animal^{-1}day^{-1} and goat dung 0.70 kg animal^{-1} day^{-1}[3]. The large amounts of agricultural wastes are produced in intensive agriculture. Disposal of these large

quantities of animal and agro wastes cause a serious problem if not proper managed [2]. Vermicomposting is the best option for management of animal dung and agro waste by epigeic earthworm [4- 6]. The awareness of organic matter and concept of sustainable agriculture is gaining impetus among our farmers to produce good quality consumable agricultural products [7]. Vermicomposting is important component of organic farming without much financial involvement [8], which can convert bio wastes into nutrient rich organic manure, as well as intensify the worm populations [9].Our ancestor used a traditional composting for these which are not being fully utilized and resulted in loss of latent nutrients [2]. The epigeic earthworm *Eisenia fetida* found well suitable for vermicomposting and its product in quality is better than traditional composting [10]. Universally, unique and useful waste management is by vermicomposting as reduction of public hazards, well stabilized as well as easily available for plants and minimize the soil toxicity due to being produced at different tilth by earthworms [11, 12]. The aim of present research work to enhance the reproduction and development progenies of *E.fetida* in different binary combination and also estimate physical and chemical changes in nutrient content of vermicomposting for plants.

MATERIALS AND METHODS

Collection of earthworms

Earthworms were collected from different areas of pudukkottai and thanjavur districts in Tamilnadu, India during the year 2017-2018 which due to cropping pattern comes under sugarcane- belt region. The collected worms were washed with water, preserved in 4-10% formalin depending upon their size and were identified by Dr. A. John Paul, Assistant Professor, Department of Zoology, Arumugam Pillai Seethai Ammal College, Thirupathur, Sivagangai District, Tamilnadu, India. Earthworms were categorized according to their feeding habits before culturing in the laboratory.

Collection of Animal Dung and Agro Wastes: Animal dung of cow collected from dairy, goat from different parts of pudukottai and Thanjavur districts. The agro wastes as gram bran, rice bran collected from rice mills and gram mills and banana peel from fruit sellers of different parts of pudukkottai and Thanjavur districts.

Reproduction and Development: Biomass gains development and cocoon production recorded up to 11 weeks from each bed. Vermicomposting bed of binary combinations of animal and agro wastes were prepared for inoculation of twenty (20) young *E.fetida* of same age group. After 10 days number of clitella were be counted in each vermibed inoculated *E. foetida.* After 4th week of inoculation, the number of cocoons in vermicompost bed were counted up to 15th week. Growth (biomass) of inoculated worms measured at the end of experiment. The prepared vermicompost were used for chemical analysis.

Chemical Analysis: The pH and electrical conductivity(EC) were determined using a double distilled water suspension of each waste in the ratio of 1:10 (w/v) that has been agitated mechanically for 30 minutes and filtered through Whatsmann No.1 filter paper, Total organic carbon (TOC) was measured by the method of Nelson and Sommers [13]. Total khjeldahl nitrogen (TKN) determined after digesting the sample with conc. H_2SO_4 and conc. $HClO_4$, (9:1 v/v) according to the method of Bremner and Mulvaney, [14]. Total Available Phosphorus (TAP)was analyzed using the colorimetric method with molybdenum in sulphuric acid [9]. Total potassium (TK) determined by flame photometer after digesting the sample in diacidic mixture (ccHN03:ccHClO4 =4:1, v/v)

Statistical Analysis:

All the studies replicated at least six times to find out mean with standard error and student 't' test applied to determine significant ('t' test P <0.05) difference for reproduction and growth and two way analysis of variance (ANOVA) was applied to determine significant difference between initial feed mixture and final vermicompost [15]

RESULTS

The different binary combination of cow and goat dung with agro-wastes gram bran, rice bran and banana peels caused a significant growth of *E.fetida*as well as significantly increase in number of cocoons, initiation of clitellum development and cocoon production and weight gain. There was also significant increase in initiation of clitellum development 24±2.7worms in binary combination of goat dung with rice bran. Initiation of cocoon production was significantly earliest 34±2.8 days in goat dung with rice bran combination. The reproduction rate significantly highest 0.159±0.05 cocoons worm^{-1} day^{-1} in goat dung with rice bran (Table 1).

Table 1: Effect of binary combinations of animal dung with agro wastes on the cocoon production of *Eisenia foetida*

Binary Combinations	Initiation of clitellum development (in worms in 3rd wk)	Initiation of cocoon production (in days)	Rate of cocoon production	
			Coccons worm $^{-1}$ (90 days)	Coccons worm $^{-1}$ days^{-1}
Cow Dung	20±2.5	35±2.9	3.8±0.7	0.042±0.008
Dung +Rice Bran	15±2.0*	35±2.2*	5.7±0.48*	0.051±0.007*
Dung +Gram bran	17±3.1*	35±2.8*	4.8±0.4*	0.053±0.008*
Dung +Banana peels	16±3.4*	36±2.4	3.9±0.5	0.043±0.004
Goat Dung	24±2.7	44±3.6	4.4±0.3	0.052±0.04
Dung +Rice Bran	25±3.2*	34±2.8*	4.9±0.7*	0.159±0.05*
Dung +Gram bran	22±2.6*	42±3.9*	5.1±0.27*	0.084±0.006*
Dung +Banana peels	21±2.4*	38±3.0	4.7±0.39*	0.055±0.003*

Each value is the mean ± SE of six replicates.
***Significant growth (*P*<0.05)'t' test between treated and control group**
Cocoons production in 30.0x30.0x30.0cm3 area of vermicomposting bed.

The average weight gain 1002.52±31.69 mg worm^{-1}in goat dung with gram bran was significant in highest in *E. foetida,* whereas the inoculated earthworms were weight ranged 213.28c4.21 to 262.16±4.32 mg worm^{-1}. (Table 2).

Table 2: Growth rate of *Eisenia foetida* in different binary combinations of animal dung with agro wastes

Binary Combinations	Mean initial weight (mg worm $^{-1}$)	Maximum weight achieved (mg worm $^{-1}$)	Net weight gain (mg worm $^{-1}$)	Growth rate (mg worm $^{-1}$ day^{-1})
Cow Dung	221.65±7.94	826.24±19.42	604.15±4.84	7.62±0.22
Dung +Rice Bran	219.14±4.12	943.22±14.26	726.34±2.35	8.20±0.19*
Dung +Gram bran	213.28±4.21	882.53±38.61	745.24±3.78	8.14±0.24*
Dung +Banana peels	220.48±3.79	892.65±32.22	669.19±3.74	7.79±0.18*
Goat Dung	238.54±4.60	836.52±20.22	430.17±2.52	6.83±0.32
Dung +Rice Bran	252.14±4.12	928.03±26.42	685.1±0.27*	8.92±0.20*
Dung +Gram bran	262.16±4.32	1002.52±31.69	890.26±4.17	9.01±0.30*
Dung +Banana peels	240.62±3.26	804.37±20.52	569.38±2.04	7.02±0.18*

Each value is the mean ± SE of six replicates.
***Significant growth (*P*<0.05)'t' test between treated and control group**
Growth rate in 30.0x30.0x30.0cm3 area of vermicomposting bed.

The physical properties of initial feed mixture were changed after vermicomposting like pH, C/N ratio and electrical conductivity (EC). The pH value slightly (7.7±0.02 to 8.4±0.06) in all combination of initial feed mixture tend to decrease slightly to acidity or neutral in final vermicompost. The C/N ratio significantly decreases in all final vermicomposts than the initial feed mixtures. The significantly highest value (12.4±0.26) in the decreased levels of C/N ratio was noticed in goat dung with gram bran. The EC significantly decreased in all combination of final vermicompost in comparison to initial feed mixtures. The significantly lower EC 1.02±0.05dsm^{-1} was observed in final vermicompost of goat dung with rice bran combination (Table 3).

Table 3: Different physical parameters in initial feed mixture and the final vermicompost of different combinations of animal dung and agro wastes

Binary Combinations	pH		C/N ratio		EC (dms $^{-1}$)	
	Initial mixture	Final Vermicompost	Initial mixture	Final Vermicompost	Initial mixture	Final Vermicompost
Cow Dung	7.9±0.02	6.9±0.02	83.4±2.13	10.9±0.76	2.03±0.03	1.20±0.09
Dung +Rice Bran	8.4±0.06	7.3±0.03	50.0±0.22	10.4±0.86	2.45±0.12	1.04±0.05
Dung +Gram bran	7.7±0.02	6.8±0.04	48.1±0.32	09.2±0.13	2.20±0.04	1.05±0.08
Dung +Banana peels	7.7±0.04	6.6±0.07	49.2±0.52	10.2±0.72	2.68±0.05	1.05±0.07
Goat Dung	8.2±0.03	6.8±0.04	44.3±1.60	11.3±0.49	2.45±0.17	1.23±0.09
Dung +Rice Bran	8.4±0.05	7.8±0.07	58.6±0.75	14.5±0.53	2.81±0.09	1.02±0.05
Dung +Gram bran	8.4±0.05	7.4±0.04	92.1±0.14	12.4±0.26	2.50±0.16	1.09±0.09
Dung +Banana peels	7.9±0.04	7.0±0.06	42.6±0.89	12.1±0.81	2.80±0.14	1.05±0.08

Each value is the mean ± SE of six replicates.
Significant variance (P<0.05) two-way analysis of variance (ANOVA) was applied in between initial mixture and final vermicompost.

During vermicomposting there was significant increase in different biochemical parameters as TKN, TK, TAP and TCa in all final vermicompost than the initial feed mixtures (Table 4) other than TOC. The maximum significant decrease (178.54±1.57) was observed in level of TOC in final vermicompost of combination of cow dung with rice bran. The TKN level was significantly increasing in the range of 10.5±0.18 to 26.9±0.02(g kg $^{-1}$) in all final vermicompost than the initial feed mixtures. The maximum highest significant increase in TKN is in the vermicompost of goat dung with gram bran. The level of TK slightly increases in all final vermicompost. Total Potassium in goat dung with banana peels 8.8±0.02 g kg $^{-1}$ is significantly the highest. Due to stabilization TK may be enhanced in vermicompost as a result significant increase of 5% to 42% was noticed Total available phosphorous found significantly increased from 0.87% to 46.2% in all final vermicompost of all binary combination of different animal and agro wastes. The significantly highest TAP 8.8±0.87 g kg $^{-1}$ was in goat dung with rice bran. In all combination of different animal and agro wastes significant increase of Tca in final vermicomposts than in initial feed mixtures was noticed. Total calcium (TCa) was significantly highest 3.8±0.42 g kg $^{-1}$ in goat dung with banana peels (Table 4).

Table 4: Different physical parameters in initial feed mixture and the final vermicompost of different combinations of animal dung and agro wastes

Binary Combinations	TOC (g kg $^{-1}$)		TKN (g kg $^{-1}$)		TK (g kg $^{-1}$)		TAP (g kg $^{-1}$)		Tca (g kg $^{-1}$)	
	Initial mixture	Final Vermi compost	Initial mixture	Final Vermi compost	Initial mixture	Final Vermi compost	Initial mixture	Final Vermi compost	Initial mixture	Final Vermi compost
Cow Dung	178.54±1.57	482.14±4.24	6.1±0.03	15.8±0.42	5.2±0.08	5.9±0.05	3.6±0.04	7.2±0.82	1.4±0.	2 2.0±0.08
Dung +Rice Bran	250.42±2.42	536.12±6.26	11.2±0.41	25.2±0.04	5.9±0.41	6.4±0.08	7.0±0.06	8.6±0.86	1.4±0.4	2.7±0.10
Dung +Gram bran	261.57±1.82	532.15±1.24	12.3±0.86	25.2±0.25	7.1±0.04	7.6±0.06	4.9±0.12	7.4±0.42	2.1±0.3	2.4±0.21
Dung +Banana peels	250.64±1.96	436.51±4.21	8.2±0.12	19.4±0.25	5.1±0.12	6.5±0.06	5.4±0.04	7.7±0.89	2.2±0.1	2.9±0.32
Goat Dung	222.43±1.07	538.03±5.42	4.6±0.31	10.5±0.18	6.1±0.16	6.9±0.07	4.1±0.08	5.3±0.62	1.7±0.5	2.5±0.30
Dung +Rice Bran	296.23±1.06	567.12±6.11	10.3±0.36	25.4±0.24	7.9±0.80	8.1±0.10	7.7±0.12	8.8±0.87	1.2±0.6	3.4±0.23
Dung +Gram bran	286.05±1.98	558.69±3.02	14.5±0.14	26.9±0.20	7.2±0.18	8.6±0.04	5.8±0.18	8.1±0.50	1.6±0.4	3.2±0.31
Dung +Banana peels	241.42±1.05	510.12±3.14	8.9±0.21	12.4±0.25	6.8±0.21	8.8±0.02	8.2±0.16	6.8±0.48	2.2±0.3	3.8±0.42

Each value is the mean ± SE of six replicates.
Significant variance (P <0.05) two-way analysis of variance (ANOVA) was applied in between initial mixture and final vermicompost.

DISCUSSION

The different binary combination of goat dung with agro-wastes rice bran, gram bran and banana peels caused a significant growth of *E.fetida* as well as significantly increase in number of cocoons, clitellum development and initiation of cocoon production and weight gain. There was also significant initiation of clitellum development in the 3rd week. In binary combination of animal dung and agro wastes with the highest 25±3.2worms in goat dung with rice bran. The combination of feed material, temperature, humidity, are all very important factors for growth and reproduction, out of which food material is highly significant in deciding the growth and reproduction [16]. Elvira *et al.* [17] reported 22 to 36 folds increase the number of earthworm and also increased 2.2 to 3.9 times total biomass in the combination of paper mill sludge with goat dung. The initiation of cocoon production significantly earliest 34±2.8 days in goat dung with rice bran combination. The reproduction rate significantly highest 0.159±0.05 cocoons worm^{-1} day^{-1} in goat dung with rice bran because it may be due to presence of hemicellulose, high C/N ratio and good aeration. Hemicelluloses, lignin and cellulose are also the main components of wheat straw [18].

The average weight gain in combination of goat dung with gram bran was significantly highest in *E. foetida.* In comparison with other worms Suthar [20] studied about the change in biomass and cocoon production of *Perionyx sonsbarious* affected by different feeding material quality and. Loh *et al* [21] reported that biomass gain and cocoon production by *E.fetida*was more in cattle wastes in comparison to goat wastes. Nath *et al* [19] reported that the combination of agro and kitchen wastes with cattle dung have significant effect on growth and development of *E. fetida*.

The physical and chemical parameters were changed in all final vermicomposts with respect to initial feed mixtures. The pH value is slightly basic in all combination of initial feed mixture tend to slightly acidity or neutral in final vermicompost because the decrease in pH may be by microbial activity present in earthworms gut [22]. The higher pH in the initial mix may be due to nitrogen and during vermicomposting the elimination of nitrogen takes place as volatile ammonia [5]. Brady and Weil [23] studied that the agro wastes have low nitrogen residue due to immobilization of inorganic nitrogen of soil by microbes resulted nitrogen unavailable to plants. Three-fold earthworm's biomass was found to be increased in high C/N ratio compare to low C/N ratio [24]

Electrical conductivity significantly decreased in all combination of final vermicompost in comparison to initial feed mixtures. Garg *et al.* [3] observed that the EC was reduced by 46.0% to 28.4% from initial feed mixture. EC and pH are limiting factors for *E.fetida* growth and development [26]. The significantly lower EC in final vermicompost of goat dung with rice bran combination, it is due to the increased rate of loss of organic matter, consequently, release different mineral salts in this combination [27]. During vermicomposting there was significant increase in different biochemical parameter as TOC, TKN, TK, TAP and TCa in all final vermicompost than the initial feed mixtures. It is due to the vermicomposting mechanism by microbial degradation, organic residue assimilation and expiration of CO_2 all that are responsible for carbon losses from initial mixture. Thus, vermicomposting directly influences the TOC level in mixture which have highly TOC [21, 28,29]. The maximum significant increase 296.23±1.06 g kg 1 was observed in level of total organic carbon (TOC) in final vermicompost of combination of goat dung with rice bran because rice bran content significant amount of nitrogen, lignin, cellulose, hemicellulose, residual ash, calcium, magnesium, sodium, potassium and phosphorus. Thus, use of rice bran in binary combination with animal dung increase the nutrients in vermicomposting [30].

Total kjeldhal nitrogen level was also significantly increasing in final vermicompost than the initial feed mixtures. Because mineralization of organic matter during vermicomposting. Surplus Nitrogen was released by microorganism in the intestine as well as by earthworm which stabilized nitrogen in excreta, mucus, enzyme and certain hormones [28, 31]. Tripathi and Bhardwaj [28] reported the increase in final vermicompost may also be due to decay of worm's body as proteineous portion into ammonia and nitrogenous like substances. The maximum highest significant TKN 26.9±0.20 g kg^{-1} in vermicompost of goat dung with gram bran may be due to the decay of high organic carbon which might be responsible for nitrogen addition in the form of micro-nutrients and the excretory substances from the earthworm gut [22, 28, 32]. The level of total potassium (TK) slightly increases in all final vermicompost. The TK in goat dung with banana peels 8.8±0.02 g kg^{-1} is significantly the highest due to stabilization. TK may be enhanced in vermicompost to a significant increase ranging from 5% to 42%. Kaveiraj and Sharma [27] reported the increased TK level during vermicomposting by different earthworm species. According to Suthar

[20] increase concentration of TK is due to enhanced rate of mineralization by microbial activity during vermicomposting. Increased TK is leached from vermicomposting bed[3, 33-34] and exist in the range from 5.9± 0.05 to 8.8± 0.02 in final vermicompost. The combination of goat dung with banana peels have various organic compounds and may be due to presence of these compounds it enhance the rate of K mineralization [20].

Total available phosphorous (TAP) significantly increase in final vermicompost of all binary combination of different animal and agro wastes. It is because of *in vitro* nitrification of ammonium salt and *in vivo* phosphate solublizing by microbes and the role of worm's gut enzymes resulted in increased level of phosphorous in vermicompost as recognized [35,36]. Suthar and Lee [37] studied that the organic content when pass through gut where it is soluble and stabilizing of phosphorous occurs. The significantly highest TAP 8.8± 0.87 is in goat dung with rice bran. The rice bran has conjugated pyridoxine, like thiamine pyrophosphates may be conjugates with phosphoric acid through the 3-hydroxyl [38,39]. It is possible that break down of these organic compounds in vermicomposting enhanced the total phosphorus level. In all combination of different animal and agro wastes significant increase of TCa in final vermicomposts than initial feed mixtures were noticed. The pattern of calcium enhancement is making by unavailable calcium compound to available form in the vermicompost by organic wastes when it passes through earthworm gut [5, 20]. Total calcium (TCa) significantly highest in goat dung with banana peels because of high rate of Ca^{++} mineralization that might take place in t this combination during vermicomposting [17].

CONCLUSION

It is clear from the results that there was significant growth and development of earthworm *E.fetida* in vermibed combination of different animal dung with agro wastes. The use of earthworm *E.fetida*(goat dung + rice bran) also improve the biofertilizer by the enhance the quantity of nitrogen, phosphorus and potassium significantly and also significantly decrease the pH, electric conductivity as well as C/N ratio that are characteristics features of an ideal fertilizer. Use of epigeic earthworm *E.fetida* minimized the pollution hazard caused by organic wastes degradation. More population of earthworm is necessary for better conversion of wastes through vermicomposting

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