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International Journal of Current Research
Vol. 7, Issue, 09, pp.19936-19941, September, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOREMEDIATION OF INVASIVE WEED *PARTHENIUM HYSTEROPHORUS* USING VERMICOMPOSTING EMPLOYING EXOTIC EARTHWORM SPECIES

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ARTICLE INFO

Article History:
Received 27th June, 2015
Received in revised form 21st July, 2015
Accepted 07th August, 2015
Published online 16th September, 2015

Key words:
Parthenium hysterophorus, Eudrilus eugeniae, Vermicomposting, Cow dung.

ABSTRACT

This study reports the results of vermicomposting with *Eudrilus eugeniae* of *Parthenium hysterophorus* mixed with cow dung in different ratios (25%, 50% and 75%) in a 9 weeks experiment. In all the treatments, a decrease in pH, OC total and C: N ratio, but increase in EC, Ntotal, P aval, Ca total, K total and heavy metals was recorded. The cocoons production and earthworm biomass increased as vermicomposting progressed. The results indicated that parthenium can be a raw material for vermicomposting if mix with cow dung in appropriate quantity.

Citation: Nithya, R., Esaivani, C., Vasanthi, K. *et al.* 2015. "Bioremediation of invasive weed *parthenium hysterophorus* using vermicomposting employing exotic earthworm species", *International Journal of Current Research*, 7, (9), 19936-19941.

INTRODUCTION

Parthenium hysterophorus is an annual herbaceous terrestrial weed native to the Americas, which occurs in most of the tropical countries of the world. Owing to the absence of effective natural enemies, its allelopathic effect and photo- and thermo-insensitivity, it grows luxuriantly all through the year suppressing native vegetation, there by threatening natural diversity. It has infested millions of hectares of land including agricultural fields, wastelands, grazing lands and along highways, where it is also a human health hazard, causing allergic dermatitis and respiratory problems (Towers *et al.,* 1977). It is spreading at an alarming rate and not only compete with cultivated crops but also deplete the nutrient pool of soil in which have they grown. *P. hysterophorus* is able to colonise new areas rapidly by means of relatively high numbers of seeds, dispersal via, water, animals, farm machinery and wind, and rapid growth rate. The adverse impacts of *P. hysterophorus* on agriculture have been reviewed by several authors (Dhileepan and Senaratne, 2009). In India, *P. hysterophorus* causes a yield decline of up to 40% in agricultural crops. Tudor *et al.* (1982) also reported that *P. hysterophorus* taints the milk and meat of animals, thereby reducing the value of animal products. *P. hysterophorus* in animal feed cause's dermatitis with pronounced skin lesions and a significant amount (10–50%) of *P. hysterophorus* in the diet can kill cattle (Narasimhan *et al.,* 1977). Senthilkumari *et al.* (2013) reported that parthenium contain parthenin (0.3%), which act as a germination and radical growth inhibitor in a variety of dicot and monocot plants and it enters the soil through the decomposing leaf litter. Various attempts have been made in the yesteryears to control, utilize, or destroy, *P. hysterophorus* weed (Annapurna and Singh, 2003). It has successfully resisted eradication by chemical, biological, mechanical, or integrated methods.

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On the other hand, when viewed as a resource, it appears to be a potential raw material for vermicomposting. At present, it is one of the most troublesome weeds in India, spreading rapidly in forests, pastures and agricultural lands. Several attempts have been made for its prevention, eradication and control, but to date without success (Kavita and Nagendra, 2000). Hence, huge quantities of this weed are annually produced in India, but its economic use as food source is impaired by its toxicity. The present research was taken up to utilize a noxious and problematic weed through vermitechnology, which not only manage the weed but may provide a valuable product in the form of vermicompost. This is why vermicomposting might be a useful alternative to convert biomass from this species to a useful material that could be used as soil conditioner. The present study deals with the vermiconversion of Parthenium waste into vermicompost by using *E. eugeniae*. During the study, the waste biomass of parthenium was supplemented with cow dung (Yadav and Garg, 2009; Kaushik and Garg, 2003) for evaluation of the role of cow dung in decomposition as well as quality and quantity of the output.

MATERIALS AND METHODS

Earthworm cultures

Eudrilus eugeniae was obtained from a vermicomposting unit of Sri Parasakthi College for Women, Courtallam. The stock culture of the earthworm was maintained in plastic containers using partially decomposed biowaste and cowdung as growth medium in laboratory condition for further use in the vermicomposting experiment. Dominguez *et al.* (2001); Vasanthi *et al*. (2013b) reported that *E. eugeniae* is a fast-growing and productive earthworm in animal waste that is ideally suited as a source of animal feed protein as well as for rapid organic waste conversion.

Parthenium waste and Cowdung

Before flowering fresh *P. hysterophorus* L. were collected from in and around fallow lands of Courtallam, Tamil Nadu, India. These weeds were chopped into small pieces for the experiment. Urine free cow dung were collected separately in quantities enough for experimental use were sundried and powdered. The physico-chemical properties of Parthenium and CD are reported in the Table 1.

Experimental design

The vermibeds were prepared using *P. hysterophorus* in plastic containers and watering was done regularly to moist the medium .Three vermicomposting treatments were established having 1kg of feed mixture containing CD alone and mixed with in different ratios (25, 50 and 75% Parthenium) in plastic containers (Table 2). Each treatment was established in triplicate. Parthenium with cowdung mixture (P+CD) were precomposted for three weeks so that it becomes palatable to the earthworms (Sangwan *et al*., 2008). To each plastic container, 10 adult 40 days old earthworms of *Eudrilus eugeniae* were introduced from the stock culture after pre-composting of the raw materials. The experiment was conducted in dark room in ambient temperature. The moisture levels in the experimental containers were maintained at 70±10% (Yadav and Garg, 2009; Vasanthi *et al*., 2013a) by periodic sprinkling of distilled water. During the experimental period no extra waste mixture was added at any stage in any vermicomposter. The worms were separated from vermicomposter by hand sorting, counted, washed, dried by paper towels and weighed every 15 days and transferred back to the respective vermicomposters. Control and treatments was triplicate.

Compost analysis

About 110 g of homogenized wet samples (free from earthworms, hatchlings and cocoons) were taken out at zero day and 60th day of composting period. Sub samples were air dried, ground to pass to 0.2-mm sieve and stored for further analysis. Each sub-sample was analyzed for the following parameters: The pH and conductivity value were measured in 1:5 (w/v) water suspensions using digital pH (Elico LI 127) and conductivity meter (Elico CM 180) respectively. Total Organic Carbon was measured by Walkey and Black titration method described by Jackson (1975). Micro Kjeldhal method (Jackson, 1975) was used for measuring nitrogen.

The C: N value was calculated from the measured values of total organic carbon and nitrogen. Sulphuric acid (0.002 N) solution was used for extraction of phosphorus from the sample. Available phosphorus was determined by using the spectrophotometer (Shimadzu UV 1601) following the stannous chloride method (APHA, 1998). Total sodium and potassium was determined by acid digestion method using flame photometer with standard solution (APHA, 1998). For analysis of Ca, Mg, Mn, Cu, Zn and Fe samples were digested in microwave digester [Milestone, Ethos 900] and then analyzed by Atomic Absorption Spectrophotometer (Shimadzu AA 7000). In addition earthworm growth related parameters like earthworm biomass; and total mortality were measured at the end of the vermicomposting process.

Growth and reproduction of worm

After an undisturbed 7 days composting period, the content in the triplicates was mixed and moisture content was checked together with earthworm's activity. Every 15 days over a period of 60 days the worms were hand sorted and weighed. The weight of 10 worms were taken together (in mg) and calculated to percent of single worm weight, The Cocoons production by earthworm was measured in each worm worked compost containers, separated cocoons were counted and introduced in separate bedding containing the same material in which their parents were reared. On the basis of the obtained data about the biomass and cocoon numbers, other parameters of earthworm such as biomass increase rate (mg/day), maximum weight achieved and reproduction rate (cocoon/worm/day) were produced with the help of the recorded data for different worm worked compost treatments.

Statistical analysis

Paired sample t-test was used to analyze the differences in vermicompost production in different treatments. The same test was used to compare the mean values of different chemical parameters of the compost (control) and vermicompost.

RESULTS AND DISCUSSION

pH

The vermicompost produced from different treatments was homogeneous than the initial feed mixtures. Significant changes in physico-chemical characteristics of waste (CD and parthenium) were observed at the end (Table 2).

vermicomposting. The values of TOC content in vermicompost obtained from T_0 were significantly different from other treatments.

Table 1. The Composition of Cow dung and *Parthenium hysterophorus* in different treatments

Treatment No.	Description	CD(kg)	(*Parthenium hysterophorus* (kg)
T_0	CD(100%)*	1	0
T_1	CD(75%) + *Parthenium* (25%)	750	250
T_2	CD(50%) + *Parthenium* (50%)	500	500
T_3	CD(25%) + *Parthenium* (75%)	250	750

*The figures in parenthesis indicates the percent content in the initial substrate material

Table 2. Comparison of Physico –chemical characteristics of initial mixtures and vermicomposts obtained from different treatments (Mean ±SD, n =3)

Parameters	Control (T_0)		T_{25}		T_{50}		T_{75}	
	Initial	final	Initial	final	Initial	final	Initial	final
Ph	8.02 ±0.00	6.87±0.05	7.53±0.01	6.36±0.03	7.68±0.01	6.59±0.04	7.71±0.02	6.48±0.03
Electrical conductivity (ms/cm)	0.66±0.00	0.97±0.00	0.84 ±0.00	2.12 ±0.01	0.82 ±0.00	1.82 ±0.00	0.81 ±0.00	1.88 ±0.00
Organic Carbon	42.16 ±0.14	*34.8 ±0.36	*45.15 ±0.54	31.4 ±0.19	*46.5 ±0.24	34.75±0.04	*44.21±0.67	*32.63 ±0.24
Total Nitrogen	0.77 ±0.00	0.98 ±0.00	0.92 ±0.00	1.84 ±0.01	0.82 ±0.00	1.23 ±0.00	0.96 ±0.01	1.05 ±0.03
Total Phosphorus	0.46 ±0.015	1.07 ±0.01	0.73 ±0.00	2.15±0.01	0.74 ±0.01	1.77 ±0.00	0.65 ±0.00	1.16 ±0.01
Total Potassium	0.37 ±0.00	1.01 ±0.00	0.95 ±0.01	1.47 ±0.00	0.87 ±0.00	1.22 ±0.00	0.80 ±0.00	1.03 ±0.00
Total Calcium	0.86±0.01	1.06±0.02	1. 08 ±0.00	2.75 ±0.01	1.03±0.01	2.22 ±0.01	0.98 ±0.00	1.77 ±0.01
Total Magnesium	0.83±0.00	*1.30±0.15	0.94±0.01	1.37±0.01	0.91±0.00	1.24±0.00	0.88±0.00	1.13±0.00
Zinc	12.26±0.08	*14.7±0.13	16.30±0.01	*35.2±0.21	13.07±0.01	27.74±0.02	12.99±0.00	26.80±0.04
Iron	*595.96±1.35	869.6±1.8*	*645.4±1.45*	*1225.9±0.5	*608.20±0.5	1018.2±1.25	601.08±0.02	*928.3±4.45
Copper	144.83±0.03	*792.3±1.34	*158.6 ±0.85	*406.6±0.7	*157.20±0.5	*394.83±2.0	149.46±0.08	*328.56±2.1
C/N ratio	35.96±0.28	28.70± 0.03	38.47±0.23	16.64±0.01	*32.96±0.37	17.61±0.02	35.33±0.84	18.75±0.01

P < 0.05 - Significant * - Non – Significant

There was a decrease in pH of all the treatments (T_0 – T_{75}) relative to their initial values during vermicomposting (Table 2). Initially pH values in different treatments were in range of 8.02 ± 0.0 – 7.53 ± 0.0 and in final vermicomposts it ranged from 6.87 ± 0.05 to 6.36 ±0.03 Maximum reduction was recorded in T_0, while minimum was recorded in T_{25}. Gupta *et al*. (2007) also reported reduction in pH during vermicomposting of water hyacinth. Elvira *et al.* (1998) concluded that production of CO_2 and organic acids by the joint action of earthworms and microbial decomposition during vermicomposting lowers the pH of substrate.

Electrical Conductivity

The electrical conductivity (EC) of vermicomposts was higher than initial waste mixtures. The EC of vermicomposts ranged from 0.973 ± 0.008 (T_0) to 2.123 ± 0.01 (ms/cm) (T_{25}). The differences in EC value between vermicompost and respective controls was found statistically significant (*P<0.05*). The increased EC value during vermicomposting was also reported by many workers which may be due to loss of organic matter and release of mineral salts like phosphate, ammonium, potassium etc (Gupta and Garg 2008).

Total Organic Carbon

TOC was lesser in the vermicompost, when compared to the initial level in the treatments. The TOC loss in different treatment was in the order T_0 (34.8 ±0.3) > T_{50} (34.7 ±0.04)>T_{75} (32.63 ±0.24) > T_{25} (31.38 ±0.19) Elvira *et al* (1998) have reported that 20-43% fraction of organic matter present in the initial feed substrates is lost as CO_2 during

These results are supported by Kaur *et al.* (2010) who have reported 32.16 - 42.37% loss of TOC during vermicomposting of paper mill sludge. Suthar (2010) has reported that the digestion of carbohydrates and other polysaccharides from the substrates by inoculated earthworms may cause carbon reduction during vermicomposting of organic wastes. Some part of organic carbon may be converted to worm biomass through the assimilation process. This consequently reduces the carbon budget of waste substrate in the treatment.

Total Nitrogen

Total kjeldhal nitrogen (TKN) was higher in the final product than the initial values of the substrate. Vermicomposting resulted in significant increase in TKN in different treatments. TKN content increased in the range of 0.98±0.0 – 1.84 ± 0.01 in different treatments (Table 2). The different in the TKN content of the vermicomposts obtained from different treatments was significant. Hand *et al.* (1988) reported that *E.fetida* in cow dung increased the nitrate-nitrogen content of vermicomposting. According to Viel *et al*. (1987) losses in organic carbon might be responsible for nitrogen addition. Addition of nitrogen in the form of mucous, nitrogenous excretory substances has been reported which were not initially present in feed substrates.

Total Phosphorus

Total Phosphorus increased in the range of 1.07 ± 0.01 to 2.15 ± 0.011 in different treatments. Maximum increase in TP was in 2.15 ±0.011 (T_{50}) and minimum was in 1.07 ± 0.01 (T_0). The TP contents in the products of all the vermicomposting treatment increased significantly from their initial level and significantly different from each other. Some earlier studies indicate the highest plant available forms in vermicomposted wastes mainly due to activities of P-solubilizing bacteria and enzymatic activities of earthworm gut (Suthar, 2009c). Also, Few author suggested the role of P-solubilizing bacteria in phosphorous enhancements in deposited casts of earthworms (Prakash and Karmegum, 2010). The highest P mineralization in T_{25} suggests the suitability of waste substrate for earthworm feed and microbial propagation.

Total Potassium

Vermicompost material had higher TK concentrations in all treatments then initial level. The maximum and treatments then initial level (Table 2). The maximum and minimum level of TK was 1.47 ± 0.0 (T_{50}) and 1.01 ±0.00 (T_0), in the vermicompost. The TK content in $T_{0,}$ $T_{25,}$ T_{50} and T_{75} were significantly different from each other (P < 0.05). Suthar (2010b) reported 500-750% increase in TK during the recycling of agro-industrial sludge through Vermitechnology.

± 0.01 (T_{25}) and 1.30 ±0.15 (T_0) The difference between vermicompost and control was significant (*P<0.05*) in respect of total Mg content. Earthworm drives the mineralization process efficiently and transforms a large proportion of Mg from bind to free form (Suthar, 2008) which results higher concentration of Mg in the vermicompost (Suthar, 2009a).

Heavy Metals

The metal (Fe, Cu and Zn) concentrations of vermicompost were found higher than their initial value of the raw materials. The high concentration of metals was found in the vermicompost of (T_{50}) whereas low in (T_0) (Table 2). The initial and final iron content in the three different composting systems under investigation is given in Table 1. After 60 days of composting the (T_{25}) vermicompost compost 1225.90 ±0.52 had more iron than in the initial stage of composting. The range of iron content in final composts were 1018.2 3±1.25 in (T_{50}) compost and 928.35 ±4.4 in (T_{75}) compost). In vermicomposted material, Cu content increased in the order: T_0 > T_{25} > T_{50} > T_{75} in different treatments (Table 2).

Table 3. Growth of *Eudrilus eugeniae* in *P. hysterophorus* wastes

Animal waste	Mean Initial weight/ earthworm (mg)	Maximum weight achieved /worm(mg)	Maximum weight achieved on	Net weight gain/worm (mg)	Growth rate/worm/day/ (mg)
T_0	100±5.8	996±10.2	8th week	892±4.6	15.93±0.02
T_{25}	192±67	1294±245	6th week	1102±197	26.2±4.70
T_{50}	72±30	906±5.2	9th week	834±1.7	14.89±0.03
T_{75}	196±30	889±90	9th week	693±22	16.3±0.52

Table 4. Cocoon production by *Eudrilus eugeniae* in *P. hysterophorus* wastes

Leaf wastes	Clitellum development started in	Cocoon production started in	Total no of cocoons produced after 8 weeks	No of cocoons produced /worm	No of cocoons produced /worm/day	Juveniles
T_0	4thweek	4th week	125±3.46	23.4±3.7	0.31±0.02	8.3 ± 0.3
T_{25}	4th week	4th week	125±28.6	25.4±5.7	0.36±0.08	27.6 ± 0.8
T_{50}	4th week	5th week	94±1.73	11.3±4.6	0. 19±0.02	21.0± 0.5
T_{75}	4th week	5th week	62±23.6	12.3±4.6	0. 19±0.06	21.0 ± 0.5

Calcium (Ca)

Calcium (Ca) content in the vermicompost was also higher than initial feed substrates (Table 2). The percent increase was maximum in T_{25} (2.75±0.01) vermicompost and minimum in 1.06 ±0.02 vermicompost. The increase in calcium content was in the order $T_{25}<T_{50}<T_{75}<T_0$. The Ca concentration has increased in vermicompost as reported by Pattnaik and Reddy (2010). Calcium was observed to be increasing in the range of 6.9 – 99.0% for all treatments (Table 2). Final vermicompost obtained from the treatment of (T_{25}) showed the highest content of calcium as compared to the other treatments. A similar increase in calcium was reported by some researchers who used *E. eugeniae* in vermicomposting process (Vasanthi *et al*., 2014). The slight decrease in calcium content for (T_0) treatment could be due to leaching of captions by excess water that drained through the feed mixtures as reported by Kaushik and Garg (2003).

Magnesium (Mg)

Total Magnesium was found also higher than initial feed substrates. The maximum and minimum level of Mg was 1.37

Similarly Zn content also increased in all treatments, it was in range of 14.7 ± 0.1 – 35.2 ±0.21 in vermicomposts and in range of 12.2 ± 0.08 – 16.3 ±0.01 in initial feed mixtures. There results are supported by previous studies also, which have reported higher concentration of metals in final vermicomposts as compare to initial metal levels (Gupta and Garg, 2008) The difference in the Iron content of the vermicompost obtained from different treatments was significant (*P* <0.05). Malley *et al.,* (2006) have reported that copper accumulates within the worm tissues. The number of hatchlings decreased from the lowest to highest dosages of Cu and Zn after 10 weeks of the experiment. Similar trend has been observed in our experiments. So it may be concluded that higher concentrations of Cu and Zn may affect the growth and reproduction of *E. fetida*.

Carbon and Nitrogen Ratio (C/N)

C:N ratio is an important indicator of waste stabilization. Initial C:N ratios for different feed mixtures were in the range of 38.4 ± 0.23 to 32.9 ±0.37 The C:N ratios of vermicompost obtained were in the range of 28.7 ± 0.03 to 16.6 ±0.01 The C:N ratio decreased with time in all the feed mixtures. And a decline in C/N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes Senesi,(1989) So, in the present study, a high degree of organic matter stabilization was achieved in all the reactors. The decrease in C/N ratio over time might also be attributed to increase in the earthworm population. This led to rapid decrease in organic carbon due to enhanced oxidation of the organic matter. The release of part of the carbon as carbon dioxide (CO_2) in the process of respiration, production of mucus and N excrements, increases levels of N and lowers the C/N ratios Senapati, *et al*. (1980) A high significance ($p < 0.05$) was observed in C/N ratio for all the reactors on all the sampling days.

Earthworm population and biomass

Earthworm production is an integrated and important aspect of any vermicomposting operation (Deka *et al.,* 2011). The results covering population, biomass and cocoon production o f *E.eugeniae* are reported in Table 4. There was 6 fold increases in population of *E.eugeniae*. Similarly, biomass gain and cocoon production of the earthworm species was higher in the end of the experiment. The differences in population, biomass and cocoon production were statistically significant ($P < 0.05$). The ambient environmental variability drastically influences the survivality and reproductive activity of the earthworm. So, the variation in earthworm population, biomass and cocoon production as revealed in the present study may be due to reproductive activity of the earthworms (Vasanthi *et al*., 2011). All the vermicomposters were operated for 9 weeks and there was no mortality in any vermicomposters during this period. But earthworm's showed different behavior in terms of growth and reproduction in different vermicomposters, Table 3 shows the weekly growth of *E.eugeniae* in different vermicomposters. Mean initial biomass of earthworm in the vermicomposters T_0 was 100±5.8mg. The maximum individual live weight was in (1294±245mg/earthworm) Vermibeds (T_{25}), which was significantly higher ($p<0.05$) from all other vermicomposters. In the vermibed T_{75} minimum biomass was observed (889±90mg/earthworm).

Worm growth and fecundity

The total number of earthworms recovered and their biomass after 60 days of vermicomposting in different treatments are given in Table 3. No mortality was observed in the treatments during the study period. Table 4. describes the reproductive potential of *E.eugeniae* in different treatments. Clitellum was developed up to 4th weeks in all the treatments. The cocoon production started in 4th week in treatment T_0 and T_{25} and 5th, week in remaining treatments. Higher growth rate in T_{25}, $T_{0,}$ T_{50} and T_{75} was due to rich nutrient content and acceptability of feed by worm. Esaivani *et al*. (2015) described the higher growth rate in a particular vermibed may be due to the more palatability and acceptability of feed by worms. Total number of cocoons produced in each treatment (Table 4) also showed variation. Suthar (2007) concluded that, in general, earthworm shows better reproduction performance in beddings containing nitrogen rich substance (vegetable waste, crop residues, industrial sludge, leaf litter, etc.). Few other factors, such as easily metabolizable organic matter, non-assimilated carbohydrates, microbial population, and low concentration of growth-retarding substances are also important to enhance cocoon production rate in earthworms. The growth rate (mg biomass gained/day/worm) has been considered a good indicator of earthworm's growth in different wastes (Edwards *et al.,* 1998). The greater the earthworm population density in a culture, the slower was the growth of individual earthworms at any particular earthworm population density, up to a maximum of 16 earthworms in 100 g of separated cattle waste solids for *E. eugeniae* (Dominguez *et al.,* 2001). The feeds that provide earthworms with a sufficient amount of easily metabolizable organic matter and non assimilated carbohydrates favor the growth and reproduction of the earthworms (Edwards, 1988).

Conclusion

This work presents the utilization of in parthenium vermicomposting operation. Results thus clearly suggested that vermicomposting is significantly effective in nutrient transformations in waste mixtures. The vermicomposts were nutrient rich, odor free, more mature and stabilized than initial waste mixture. Among the Parthenium containing treatments, 25% parthenium and 75% CD containing treatment was optimum waste mixtures for the growth and reproduction of *E. eugeniae*, and hence can be recommended as feed materials in vermicomposting facilities. It is suggested that at higher concentrations, parthenium affects the vermicomposting efficiency of earthworms The results of present research indicate economic utilization of parthenium by vermicomposting technology and also a model for ecological engineering and sustainable agriculture.

Acknowledgement

Authors are thankful to Management, Principal, Sri Parasakthi College for Women, Courtallam, Tirunelveli, Tamilnadu, Lab facilities and encouragement.

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