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Influence of sevin, *Lactobacillus sporogenes* and essential microbes supplementation on regeneration of *Eudrilus eugeniae* kinberg

Subashini M., Divya V., Mujeebunisa M., Saravanan S. and D. Aruna

Post Graduate and Research Department of Zoology, Government Arts College, Coimbatore, TamilNadu

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ABSTRACT

Several environmental and economic problems arise due to the accumulation of domestic, agricultural and industrial wastes. Disposal of these wastes on land may cause serious contamination problems. These wastes can be converted into useful materials to improve soil structure and fertility by earthworms. Earthworms are important elements of soil biology because they contribute to mineralization and humification of the organic matter into compost on an industrial scale. Regeneration studies have focused almost exclusively on a few, very distantly related species such as hydra, planarians and amphibians. Among the invertebrates annelids are an excellent group in which to investigate the evolution of regeneration abilities. Animals show different grades of ability to replace lost body parts through the process of regeneration. They exhibit qualitative and quantitative variation in their regeneration ability. In the present study, the predigestion mixture was prepared with cow dung in the ratio of 1:1 w/w and water was sprinkled to maintain the moisture content of 65-70%. 100gms of predigestion mixture were taken into 6 containers for experimental groups. The required concentration of pesticides, L. sporogenes and essential microbes were added. Then the amputated worms were introduced in the experiment. Periodically the worms were observed under the hand lens. The number of newly regenerated segments were counted and recorded after 5, 10, 15, 20, 25 and 30 days in all experimental groups. The results are discussed in the light of enhancing regeneration by probiotic supplementation.

Key words: Regeneration, Humification, Annelids, Waste Management.

INTRODUCTION

Soil is the main sink for most toxicants which are released in the environment through industrial effluents, application of pesticides for agriculture and by dumping of civil refuse. The vermicomposting and vermi-agro production technologies maintain the global human sustainability cycle by using food wastes for the production of food for the society. Earthworms are important biocomponents of the ecosystem, contributing to maintain sustainability of soil structure and helps growth. Poisoning and other disturbances in the natural habitat of earthworms can lead to ecological imbalance (6). They are also a good source of collagen in the manufacture of pharmaceuticals, antibiotic manufacture from anti- pathogenic coelomic fluid. Soil microorganisms are essential for the growth and reproduction of earthworms (10). Microbial population of casts are enhanced due to rich nutrient concentration, microbial multiplication while passing through the gut of earthworms, optimal moisture and the large surface area of casts are better suited for feeding and multiplication (11). It helps in management of soil in complete harmony with sustainable agriculture development (12,13). Vermitechnology is an essential tool of organic farming which increases crop productivity in a sustainable manner, results in quality produce and reduces cost of agricultural inputs in addition to improving inherent capacity of soil without deleterious effects on environment.

REGENERATION

Annelids are most highly organized animals capable of complete regeneration. Among earthworms only anterior levels are able to regenerate a head and this ability ceases in the neighbourhood of the 15^{th} – 20^{th} segments. When a worm is cut into two halves, the rear half of the worm will die but the front half may live and replace the lost half of its body. This is called regeneration.

Sevin

Sevin is the registered trademark for carbaryl insecticide. Sevin was introduced in 1958. It occupies a leading position in commercial agriculture, forest and rangeland protection in home- garden pest control. Carbaryl– a synthetic organic carbamate pesticide discovered by Union Carbide, is the sole domestic powder and major world manufacturer of the chemical. Sevin ranks third domestic sales among all insecticides.

Probiotics

Probiotics are defined as live microbial adjunct which has a beneficial effect on the host by modifying the host associated microbial community, by ensuring improved use of the feed or enhancing the host response towards diseases or by improving the quality of its ambient environment. Soil probiotics including *Azotobacter*, *Azospirillium*, *Bacilli*, Cellulosic fungi, *Phosphobacter*, *Pseudomonas*, *Rhizobium*, *Streptomyces* and *Sacchromyces* increases and stimulates the natural beneficial microorganisms, resulting in massive population in the soil.

Essential Microbes (EM)

Essential Microbes is a combination of various, beneficial naturally occurring microorganisms used for or found in food. It was also introduced for animal husbandry, mainly for sick cows by Margarita Correa, (7). EM includes both aerobic and anaerobic species, which co-exist in an environment of pH below 3.5.

Lactobacillus sporogenes

L. sporogenes is a gram positive spore forming lactic acid producing bacillus. They produce spores by sporulation in which they become wrapped in a protective coat made of calcium dipicolonic acid and peptiglycan complex. On reaching the digestive tract, the spore coat imbibes water produce outgrowths and pass on to the duodenum where the outgrowths transform to vegetative cells. They multiply rapidly, produce lactic acid and bacteriocins and render the intestine non-conditional to the growth of pathogenic bacteria.

MATERIALS AND METHODS

i) Collection of earthworms and acclimatization:-

A bulk sample of the earthworm, *Eudrilus eugeniae* [Kinberg] was obtained from M/S Santhosh farms, near Pollachi [Coimbatore District], Tamilnadu. Earthworms were maintained under laboratory conditions in large cement tanks along with the mixture of leaf litter and cow dung for acclimatization before they were used in the experiments.

(ii) Preparation of predigestion mixture as feed:-

The predigestion mixture was prepared [in large plastic container] by mixing leaf litter and cow dung in the ratio 1:1 (w/w) and was sprinkled with water to maintain moisture content of 65-70%. This mixture was maintained for 21 days with sprinkling of water and turning over for pre-digestion by microbial activity. The pre-digestion of organic mixture was described by Bano and Kale, (1). Using a sharp lancet, a cut was made at the 10^{th} segment below the clitellum. The cut piece of earthworm with clitellum was maintained in small container containing pre-digested mixture. The worms were then individually transferred into control and experimental plastic containers. The cut pieces of earthworms (both the anterior and posterior pieces) were observed daily.

With this background the present study was undertaken to study the impact of pesticide sevin, probiotics and essential microbes on the regeneration capacity.

RESULTS

The control worms regenerated 31 $\pm$ 5 segments after 30 days of amputation. The sevin exposed worms regenerated 23 $\pm$ 3 segments after 30 days of amputation. The *L. sporogenes* added worms regenerated 28 $\pm$ 4 segments after 30 days of amputation. The sevin + *L. sporogenes* exposed worms regenerated 24 $\pm$ 3 segments after 30 days of

amputation. The Essential microbes exposed worms regenerated 27 ± 3 segments after 30 days of amputation. In Essential microbes + sevin exposed worms regenerated 21 ± 2 segments after 30 days of amputation in anterior portion. In posterior portion of *E. eugeniae*, the entire group shows sustained decrease, but the survival percentage differs in the various experimental groups. Survival percentage of the posterior amputated segments

The posterior amputated portions normally do not thrive. In the present study however the posterior portion survived and in some cases was also able to regenerate a head portion. The percentage of survival was 60% in control, sevin + *L. sporogenes*, and EM + sevin and 40% in sevin, *L. sporogenes* and EM exposed worms after 10 days. At 20 days there was 40% survival in all the exposures except in sevin where 20% survival was recorded. After 30 days there was no survival in *L. sporogenes* but 20% survival was noted in all the other exposures. In three of the experiments, control, sevin and sevin + *L. sporogenes* the worms were found to regenerate a head.

DISCUSSION

Our studies on regeneration in *E. eugeniae* reveals that the number of segments regenerated were 31 ± 5; 23 ± 3; 28 ± 3; 24 ± 3; 27 ± 3 and 21 ± 2 in control, sevin, *L. sporogenes*, sevin + *L. sporogenes*, EM and EM + sevin exposed worms after 30 days of amputation. The numbers of segments regenerated were the least in sevin and EM + sevin and sevin + *L. sporogenes* exposed worms. Exposure to EM and *L. sporogenes* separately showed a slight reduction from control worms which showed maximum number of segments after 30 days.

Regeneration includes the rearrangement of pre-existing tissue, the use of adult somatic stem cells and the dedifferentiation and or transdifferentiation of cells. This results in the re-establishment of appropriate tissue polarity, structure and form. Genetic induction regulates pattern formation in morphogenesis. Neural cells express growth associated proteins such as GAP-43, tubulin, actin, an array of novel neuropeptides and cytokines that induce a cellular physiological response to regenerate from damage (5). Regeneration involves coordination and organization of populations of cells into a blastema which is a mould of stem cells from which regeneration begins (9). Active cell proliferation precedes blastema differentiation occurring on the 4th day which is accompanied by a sharp decrease in DNA synthesis. Based on studies of regeneration in *Allolobophora caliginosa*, *Allolobophora terrestris*, *Eisenia fetida* and *Lumbricus rubellus*, reported that when incision were made at levels 4/5 to 30/31 the worms did not show any regeneration. Out of 84 worms of *P. excavutus* amputated, amputations from 1 to 20 segments showed a survival of 81 worms, 48 worms regenerated the exact number of segments lost (epimeric), 27 worms regenerated reduced number of segments (hypomeric), 5 worms regenerated increased number of segments (Hypermeric) and one worm failed to regenerate (3).

Gates, (3) reported that the regeneration ability of *Perionyx excavatus* where after six segments amputation, the exact number of segments lost was regenerated. Amputations beyond 26th segment showed better survival and higher percentage of successful regeneration (4). When incisions were made between 6/7 but anterior to 18/19 in *Perionyx excavatus*, the number of segments regenerated varied and a higher percentage of failure occurs (3). Heteromorphic tail regeneration in *E. foetida* has been reported by Gates (3). Heteromorphic heads may be regenerated at the posterior end of a very short anterior piece. A piece of 20 or more segments from the middle of the worm may regenerate at one end a tail and at the other end a head. The ability to regenerate segments anteriorly is widespread among the annelids, though less so than posterior regeneration ability. Several taxa that can regenerate segments posteriorly cannot do so anteriorly. In the polycheate worm *Ophryotrocha puerilis* and *Ophryotrocha notoglandulata* no anterior segments are regenerated and part of the prostomium must remain in order to elicit any regeneration. Anterior segment regeneration has been observed in the lysidice species (eunicids) *Owenia fusiformis* (Oweniid) (2).

The possible reason for the survival of the posterior portion after amputation, followed by regeneration of head as seen in some of the experiments, in the present study may perhaps be due to the already stimulated immune system on exposure to the pesticide. The stimulated immune system readily participates and contributes to the formation of a new head region which occurs at rare occasions only.

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