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Activities of *Lumbricus rubellus* and *Eudrilus eugeniae* and the Fate of *Acacia auriculiformis*, *Casurina equisetifolia* and *Dalbergia sissoo* Seeds

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ABSTRACT

Seeds at the soil surface are more vulnerable to predation. Seed burial is a potent factor in prolonging the survival of seeds. In the present study, the ability of *Lumbricus rubellus* and *Eudrilus eugeniae* to ingest seeds of *Acacia auriculiformis*, *Casurina equisetifolia* and *Dalbergia sissoo* selectively and the effect of passage through the gut on subsequent seed germination were investigated. Both *E. eugeniae* and *L. rubellus* ingested highest percentage of *A. auriculiformis* seeds (66.5% ± 1.71 and 49.6% ± 1.98, respectively) when offered alone and in mixture (46.0% ± 1.85 and 34.0% ± 1.83, respectively) with *C. equisetifolia*. Both earthworm species ingested significantly (at 0.05 level of significance) fewer seeds when offered in mixture with other seed species than when offered alone. The ability of both earthworms to distinguish between species was exhibited when seed mixture was offered. In laboratory trials, more seeds were lost in the presence of *L. rubellus* than of *E. eugeniae* and no losses in control. In field trials, losses of *A. auriculiformis* and *C. equisetifolia* from the surface of the soil containing earthworms were greater in the first five weeks after sowing. Recovery of ingested seeds was highest with *C. equisetifolia* by *L. rubellus* (92.0% ± 0.32) and *E. eugeniae* (87.88% ± 0.49). Germination of seeds lost from soil surface was higher in relation to seeds still on soil surface. Percentage increase in seed germination was highest in *D. sissoo* in the presence of *L. rubellus* (9.7times). Seed viability was slightly affected by ingestion; the reduced germination of selected plant species egested by *L. rubellus* and *E. eugeniae* were probably due to scarification and enzyme activity or delayed germination.

Keywords: gut contents, germination capability, seed burial, seedling emergence, seed ingestion, viability, worm castings

INTRODUCTION

Viable seeds which fall onto the soil surface may germinate, then be lost or passed into the seed bank. Soil seed banks have been investigated focusing on the maintenance and restoration of species-rich plant communities since they provide a source for re-establishment of species which are lost from the above-ground vegetation (Wellstein *et al*. 2007). Seeds at the soil surface are more vulnerable to predation by birds, rodents and insects and to germination in unfavourable environments (Kavian and Ghatnekar 1991; Kavian 1994; Notman and Garchov 2001). In some perennial communities, above-ground seed predation may destroy more than 95% of the seeds produced (Thompson 1987, 1992). However, seed survival prior to the germination phase is primarily driven by processes during secondary seed dispersal, including both horizontal and vertical movements (burial) and post-dispersal seed predation (Chambers and Macmohan 1994).

Seed burial is a potent factor in prolonging the survival of seeds. Zaller and Saxler (2007) demonstrated that seed transport (dispersal and burial) is an important mechanism by which earthworms selectively alter the diversity of the grassland ecosystem. Regnier *et al.* (2008) studied the impact of earthworms on seed dispersal of *Ambrosia trifida* L. (giant ragweed), indigenous to North America. The studies of Grant (1983), Ghatnekar and Kavian (1991) and Kavian (1994, 1997) have shown that a vast number of viable seeds lie buried in soil. However, on the Indian sub-continent, the mechanism by which seeds are buried has not been extensively studied from a social forestry and aforestation point of view. Darwin (1881) reported downward movement of seeds by earthworms and Kavian and Ghatnekar (1991), Kavian (1994, 1997) and others indicated that earthworm activity is likely to contribute to seed burial.

The studies of Thompson *et al.* (1993) proved that earthworms increased soil phosphates, root nodulation and enabled seedlings to establish in the presence of grass litter, by increasing soil heterogeneity. Scheu (2003) reported that shoot biomass of plants significanty increased in the presence of earthworms. Grant (1983) and Kavian and Ghatnekar (1991) demonstrated seed burial by *Lumbricus terrestris* and *Lumbricus rubellus* in wormery. Beside the direct effects of earthworms on plant seeds, there is evidence that earthworm castings can also alter seed germination and seedling establishment indirectly. However, results have been inconsistent showing that earthworm casts may break seed dormancy and increase germination and root initiation of plant seeds (Tomati *et al*. 1990; Ayanlaja *et al.* 2001; Scheu 2003).

In the present study, the activity of *L. rubellus* and *E. eugeniae* with respect to the fate of *Acacia auriculiformis*, *Casurina equisetifolia* and *Dalbergia sissoo* seeds was investigated.

MATERIALS AND METHODS

Seed ingestion recovery and germination capability

Mature individuals i.e. breeders of *L. rubellus* (minimum fresh weight 1.22 g) and *E. eugeniae* (minimum fresh weight 2.63 g) were obtained from a culture bank of the Biotechnology Resource Centre (BRC), Badlapur. Seeds of *A. auriculiformis*, *C. equisetifolia* and *D. sissoo* were obtained from the nursery of the Forest Department, Badlapur.

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Each earthworm was placed on filter paper (Whatman filter paper No. 1) moistened with 5 ml of deionized water in a Petri dish containing 20 seeds of each of the single species or 10 seeds of each of the two selected species. This was done after 24 h to allow egestion of the gut contents. The treatment was replicated 15 times, randomized and kept in the dark for 8 h at 15°C. The earthworms were then removed and the number of seeds ingested was recorded.

After removal, the earthworms were left for 48 h on moist filter paper in fresh Petri dishes to egest the gut contents. The number of seeds recovered was recorded. Sets of controlled seeds, which had not been offered to earthworms, of egested seeds and of seeds offered but not ingested, were sown on moist filter paper (Whatman No. 1) in separate Petri dishes and kept in the dark at 15°C. Germination was recorded for 21 days.

Removal of seeds from the soil surface

1. Seeds sown under laboratory conditions

In two separate trials, 45 plastic tubs (18 × 18 × 20 cm) were filled to a 15 cm depth with semi-sterilized bedding material prepared as per the composition innovated by Ghatnekar and Kavian (1991). Three mature adults of *L. rubellus* (minimum fresh weight 1.22 g) and two mature adults of *E. eugeniae* (minimum fresh weight 2.63 g) or no earthworms were added to each of the experimental tubs. Each treatment was replicated 15 times and tubs were kept moist in a refrigerator at 11-12°C.

In the first trial, 25 seeds each of *C. equisetifolia* and *A. auriculiformis* were sown alternatively 1.5 cm apart on the soil surface in a 10 × 5 grid. Seed losses from the surface were recorded for 12 days. In the second trial, seed losses of *C. equisetifolia*, *A. auriculiformis* and *D. sissoo* from the surface were recorded for 85 days.

After 5 months, the tubs were transferred to the geodesic dome-shaped greenhouse (min. temp. 24°C) at BRC for 4 months where further seedling emergence was monitored. Seeds still on the soil surface were removed and kept moist on filter paper (Whatman No. 1) in Petri dishes and their germination was recorded. The soil from each tub was emptied into seed trays and kept moist in the dark for 4 weeks.

Data collected were analyzed using statistical package described in Schaum's outline Series (Stansfield 1969). Seed losses were analyzed using *t*-tests and biometric analysis (http://www.graphpad.com/quickcalcs) was used to compare the number of seedlings that emerged.

2. Seeds sown under field conditions

Seeds of all three selected botanical species, viz. *C. equisetifolia*, *A. auriculiformis* and *D. sissoo* (25 of each) were sown on the surface of soil cores at selected areas in Ambernath and Badlapur forest reserves. The cores were placed in nylon meshed bags, inserted into plastic tubings (11 × 20 cm) and retained in their original positions. Three breeders of *L. rubellus* (minimum fresh weight 1.22 g) and 2 breeders of *E. eugeniae* (minimum fresh weight 2.63 g) or no breeders were added to each core with 15 replicates for each treatment. The number of seeds lost from the soil surface was recorded and analyzed (Kavian and Ghatnekar 1991).

Seeds and seedlings in worm castings

1. Seeds present in gut contents and castings

Surface worm casts were collected from selected areas from the farm laboratory of BRC, Badlapur and also from selected reserved forest areas of Ambernath and Badlapur.

2. Earthworm activity and seedling emergence

To each of 45 soil cores, three breeders of *L. rubellus* and two breeders of *E. eugeniae* or no earthworms were added. The surface vegetation was removed and the surface worm cast production and number of seedlings identified were recorded for 6 months.

RESULTS

Seed ingestion recovery and germination capability

E. eugeniae ingested significantly (P = 0.05) more seeds than *L. rubellus* of all three species, namely *C. equisetifolia*, *A. auriculiformis* and *D. sissoo*.

The percentage of seeds ingested varied considerably and the ability of both earthworms to distinguish between species was shown when seed mixtures were offered (**Table 1**). *C. equisetifolia* and *A. auriculiformis* seeds in particular were ingested more frequently than the seeds offered with them. For almost all three species, a 75-90% ingestion of seeds by *L. rubellus* and *E. eugeniae* was recorded. The worms egested 70-100% of the total number of seeds finally recovered (**Table 2**) in first 24 h, after detachment from the seed source.

Egestion by *E. eugeniae* had no significant effect on the number of seeds that subsequently germinated. However, the germination of *D. sissoo* was relatively lower than that of control.

Table 1 Ingestion percentage of seeds offered as single species or two species mixture

Species pair	*E. eugeniae*		*L. rubellus*	
	Single species	Two species mixture	Single species	Two species mixture
A. auriculiformis	66.50 ± 1.71	46.00 ± 1.85	49.60 ± 1.98	34.00 ± 1.83
C. equisetifolia	23.80 ± 1.93	16.00 ± 1.74	16.60 ± 1.74	11.20 ± 1.76
A. auriculiformis	55.50 ± 1.84	33.70 ± 2.13	40.50 ± 1.86	30.20 ± 2.06
D. sissoo	19.30 ± 1.81	17.60 ± 2.02	16.60 ± 1.79	14.50 ± 2.11
C. equisetifolia	22.60 ± 2.03	17.20 ± 2.15	17.55 ± 2.04	10.70 ± 1.96
D. sissoo	18.60 ± 1.91	14.90 ± 2.21	15.40 ± 2.12	12.50 ± 1.88

Values are mean ± SD of 15 replicates, SD = Standard Deviation
The mean values are significant at 0.05 level of significance.

Table 2 Recovery of ingested seeds after separation of earthworms from seed source.

	E. eugeniae			*L. rubellus*		
	№ of seeds ingested	№ of seeds egested	Egested / ingested %	№ of seeds ingested	№ of seeds egested	Egested / ingested %
A. auriculiformis	129.00 ± 2.00	111.00 ± 3.00	86.04 ± 0.99	89.00 ± 2.00	71.00 ± 3.00	79.78 ± 1.60
C. equisetifolia	99.00 ± 4.00	87.00 ± 4.00	87.88 ± 0.49	50.00 ± 2.00	46.00 ± 2.00	92.00 ± 0.32
D. sissoo	73.00 ± 2.00	56.00 ± 2.00	76.71 ± 0.64	11.00 ± 1.00	7.00 ± 1.00	63.64 ± 3.33

Values are mean ± SD of 15 replicates, SD = Standard Deviation
The mean values are significant at 0.05 level of significance.

Table 3 Effect of earthworm activity on seed germination after seven months.

	Seeds still on soil surface	Germination %	Seeds lost from soil surface	Germination %	% Increase
Control					
A. auriculiformis	199.00 ± 4.00	2.00 ± 0.18	34.00 ± 2.00	2.90 ± 0.20	45.00 ± 3.05
D. sissoo	194.00 ± 3.00	1.60 ± 0.22	39.00 ± 3.00	10.30 ± 0.17	5.40 ± 0.80 times
E. eugeniae					
A. auriculiformis	71.00 ± 2.00#	1.40 ± 0.19	156.00 ± 3.00#	3.20 ± 0.13	1.30 ± 0.21 times
D. sissoo	79.00 ± 3.00#	3.80 ± 0.21	155.00 ± 2.00#	11.60 ± 0.26	2.05 ± 0.11 times
L. rubellus					
A. auriculiformis	51.00 ± 2.00#	2.00 ± 0.24	198.00 ± 4.00#	3.00 ± 0.30	50.00 ± 3.06
D. sissoo	43.00 ± 2.00#	0.00	186.00 ± 3.00#	9.70 ± 0.28	9.70 ± 0.28 times
Total					
A. auriculiformis	321.00 ± 8.00	1.90 ± 0.20	388.00 ± 9.00	3.10 ± 0.21	63.00 ± 6.02
D. sissoo	316.00 ± 8.00	1.90 ± 0.13	380.00 ± 8.00	10.50 ± 0.23	4.50 ± 0.25 times

% Increase = % Increase in germination of seeds lost from soil surface in relation to seeds still on soil surface.
Values are mean ± SD of 15 replicates, SD = Standard Deviation
The mean values are significant at the 0.05 level of significance.

Table 4 Number of seedlings emerging in earthworm casts and gut contents.

	Worm cast		Gut content	
	Biotechnology Resource Centre [BRC]	**Reserved forests of Ambernath and Badlapur**	***E. eugeniae***	***L. rubellus***
A. auriculiformis	10.00 ± 1.21	3.00 ± 0.31	4.00 ± 0.23	1.00 ± 0.21
C. equisetifolia	22.00 ± 2.16	2.00 ± 0.20	--	--
D. sissoo	8.00 ± 1.53	2.00 ± 0.37	1.00 ± 0.14*	1.00 ± 0.17*

Values are mean ± SD of 15 replicates, SD = Standard Deviation
The mean values are significant at 0.05 level of significance.
* Values are not statistically significant.

Removal of seeds from the soil surface

1. Seeds sown under laboratory conditions

In the first trial, more seeds were lost in the presence of *L. rubellus* (44%) than *E. eugeniae* (12%). More *D. sissoo* seeds were lost than *C. equisetifolia* and *A. auriculiformis* with both earthworm species. There were no losses in the control. In the first 3 weeks of the second trial, relatively more *D. sissoo* seeds were lost in the presence of *L. rubellus* (52%) than of *E. eugeniae* (27%).

After 7 months, more *D. sissoo* seeds were found on the soil surface in the control than in either earthworm treatment. When soil was placed in trays, seedling emergence (which only occurred under greenhouse conditions) was greater in the earthworm treatments than in the control. There were relatively more *A. auriculiformis* than *D. sissoo* and *C. equisetifolia* seedlings. More of the buried seeds germinated in relation to those that remained on the surface (**Table 3**).

2. Seeds sown under field conditions

Losses of both *C. equisetifolia* and *A. auriculiformis* from the surface of soil cores which contained earthworms were greatest in the first 3 weeks after sowing. After 2 weeks, comparatively more *D. sissoo* seeds than *A. auriculiformis* seeds were lost in both earthworm treatments. In control sets, seed disappearance activity was constant throughout the experimental period. Similar constant activity was found in cores containing earthworms for first 3 weeks. Nevertheless, after 80 days, losses of *D. sissoo* and *C. equisetifolia* seeds from *E. eugeniae* cores were considerably higher than *L. rubellus*.

Seeds and seedlings in worm castings

1. Seeds present in gut contents and castings

Seedlings that emerged from the gut contents of *L. rubellus* (23% ± 0.89) and of *E. eugeniae* (30.28% ± 0.76) were collected. The worm casts from the BRC, Badlapur wormery yielded more seedlings (33% ± 0.79) than those from the Ambernath and Badlapur forest reserves (30% ± 0.99). The most common species were *A. auriculiformis* and *C. equisetifolia* (**Table 4**).

Table 5 Effect of earthworm activity on the mean number of seedlings emerging from soil cores.

	E. eugeniae	*L. rubellus*	Control
A. auriculiformis	66.00 ± 3.02	17.00 ± 2.06	9.00 ± 1.82
C. equisetifolia	16.00 ± 2.26	6.00 ± 1.88	3.00 ± 1.06
D. sissoo	19.00 ± 2.17	14.00 ± 2.08	3.00 ± 1.11

Values are mean ± SD of 15 replicates, SD = Standard Deviation
The mean values are significant at 0.05 level of significance.

2. Earthworm activity and seedling emergence

The cores containing *L. rubellus* produced more surface worm casts, while those containing *E. eugeniae* had less and the controls least. Very few seedlings emerged in any treatment during the first month, but by mid June-July, relatively more seedlings had emerged from *L. rubellus* cores than those from *E. eugeniae* or controls. At the end of the experiment, significantly (P = 0.05) more seedlings were produced in the cores of *E. eugeniae* than *L. rubellus* or control for all three selected species (**Table 5**).

DISCUSSION

The effects of an earthworm's activities on seeds are clearly important in plant population dynamics. Both *E. eugeniae* and *L. rubellus* ingested seeds offered in Petri dishes. *E. eugeniae* ingested more seeds than *L. rubellus*. Seeds of *A. auriculiformis* and *C. equisetifolia* were preferred more than those of *D. sissoo*. Grant (1983) observed that *Lumbricus terrestris* ingested more seeds than *Allolobophora longum* and his observation was in agreement to that of Satchell's (1963) in which *L. terrestris* was the more active species responding more radically to various stimuli. Experiments of Shumway and Koide (1994) showed that *L. terrestris* generally prefers seeds with smooth surfaces over seeds with rough surfaces whereas Milcu *et al.* (2006) observed small-seeded species were repressed and large-seeded species were promoted by *L. terrestris*. Zaller and Saxler (2007) demonstrated that *L. terrestris* significantly preferred leguminous herbaceous species over grass species during feeding experiments.

Some physical and chemical properties of seeds appear to influence their ingestion by earthworms. Hendriksen (1990), Kavian and Ghatnekar (1991), Marhan and Scheu (2005) described a similar mechanism for leaf litter selection by *L. terrestris*. According to Eisenhauer *et al.* (2008) anecic earthworm species function as ecosystem engineers by structuring the soil environment, incorporating large amounts of litter and seeds into soil and, thereby influence the composition of plant communities.

In the present investigation, *L. rubellus* ingested relatively less seeds than *E. eugeniae.* Grant (1983) reported 75-90% recovery of seeds ingested by *L. terrestris* and *A. longum*. The percentage of seeds ingested varied considerably and the ability of both earthworms to distinguish between species was shown when a seed mixture was offered: *A. auriculiformis* seeds were ingested more frequently.

A range of 70-100% seeds recovered was egested in the first 24 h, after removal from the seed source, which is the time it takes for material to pass through the gut of *L. terrestris* (Parle 1963). It seems likely that most of the 'lost' seeds were destroyed by the earthworms through the gizzard concentration and enzyme activity. Seed losses of 30% may not have very great effects on vegetation dynamics in relation to the effects of the much greater losses from other causes (Satchell 1983). Seed viability in the present study was only slightly affected by ingestion; the reduced germination of *C. equisetifolia*, *A. auriculiformis* and *D. sissoo* seeds egested by *E. eugeniae* may have been due to scarification and enzyme activity or due to delayed germination. Grant (1983) reported decreased and delayed germination of numerous grassland plant species after the gut passage through *L. terrestris* and *Aporrectodea longa*. Studies on germination of plant seeds after earthworm gut passage are scarce (Eisenhauer 2008). However, certain plant species might benefit from gut passage since slight damage of seeds may break seed dormancy. Studies of Eisenhauer (2008) suggest that seed germination of numerous plant species are influenced by earthworm gut passage implying that plants might have adapted to the ingestion, gut passage, and egestion by earthworms. His further studies also indicate that endogeic earthworms may strongly impact the composition of the soil seed bank and consequently, plant community assembly via direct and indirect effects on plant seeds (Eisenhauer *et al.* 2009).

Studies of Decaens *et al.* (2003) indicate that ingested seeds that survive gut transit have a greater chance to germinate than those of the soil seed bank, providing vegetation cover is sufficiently opened to enable germination processes. Thus, casts may be considered as a regeneration niche for plant species.

Satchell (1983) further suggested slower germination in the seeds egested by birds, although the proportion finally germinating was the same in the control. Germination of seeds egested by *E. eugeniae* and *L. rubellus* was delayed by 24-48 h. Earthworm casts are rich in ammonia, a condition known to induce seed dormancy or to delay germination (Kavian and Ghatnekar 1991).

Seeds of many species survive better buried than when left on the surface and for some, burial is essential (Satchell 1983). There appeared to be few natural mechanisms to explain how seeds are buried and earthworm activity may be essential for the formation of the seed bank. Seed selection by earthworms may help to explain the frequently recorded difference between species composition of buried seeds and standing vegetation. Conditions which promote germination of seeds occur at or near to the surface will therefore increase their chances of germination (Satchell 1983). Soil is moved upwards by burrowing rabbits, badgers and moles but surface-casting earthworms are wider spread and probably far more important in bringing buried seeds to the surface (Kavian and Ghatnekar 1991; Kavian 1997). The presence of worm casts in seeds of species not present in standing vegetation indicates ingestion in the soil rather than on its surface.

Scheu (2003) suggested that studies on earthworm-plant interactions may contribute significantly to a more comprehensive understanding of terrestrial ecosystems and to the development of more environmentally friendly agricultural practices. Since *L. rubellus* feeds mainly at the surface and *E. eugeniae* on sub-surface soil (Kavian and Ghatnekar 1991; Kavian 1997), they may act on different population of seeds from different depths. *L. rubellus* and *E. eugeniae* studied in present investigations positively indicate their importance in seed burial.

CONCLUSION

The present investigations reveal the positive role played by the earthworm species namely, *L. rubellus* and *E. eugeniae* in the seed dispersal *in vivo* as well as *in vitro* conditions. It is also evident that the selection of seeds of specific plant species for ingestion varies in relation to the earthworm species.

Earthworms possess the ability to control plant diversity in an ecosystem. Their gut content has been proven to be a valuable seed bank. The experimental results indicated that the selected earthworm species might have strongly influenced the composition of the soil seed bank via seed germination and plant establishment.

At a fine scale, the presence of earthworms in the ecosystem served dual roles; it maintained the soil quality and facilitated seed transport, germinability and establishment. Future research is to study earthworm-plant interrelationships as it may contribute significantly to a more comprehensive understanding of terrestrial ecosystems, in particular, in different forest types of India and evaluate its possible applications in social forestry and aforestation programmes.

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