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Full Length Research Paper

Effects of light colour and oscillator frequency on earthworm bioactivity

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An experiment was conducted at the Zoology Laboratory of the Olabisi Onabanjo University, Nigeria to evaluate the suitability of different light colours. Being generated from 15-watt, low-heat, or frequency-generated flourescent tubes as stimulants to enhance, major cast productivity of the earthworm species *Hyperiodrilus africanus*. The aim was to determine if exposure to the different light colours would improve the cast productivity of the worms and show whether *H. africanus* would show preference for any particular light colour, in terms of mass of cast produced and rates of emigration. The earthworms were kept in cylindrical plastic containers and put in different cubicles on a wooden shelf, where they are subjected to different light colours that is, white, dark (control), green, blue, and red. Results indicated that the red light colour was the most suitable in terms of cast productivity, as casting was highest (11.96 g/worm), followed by blue (10.66 g/worm), green (9.49 g/worm), dark (7.2 g/worm) and white (4.86 g/worm), respectively. Least emigration was recorded under the control (dark) , followed by red, white, blue and green respectively, which indicated that the worms were probably more 'comfortable' in the dark and the red light than the other light colours tested. Results suggest that if red light is introduced to a farm, casting activities can be increased and therefore, soil fertility can be improved.

Key words: Cast productivity, different light colour, earthworm, *Hyperiodrilus africanus.*

INTRODUCTION

Light

Within the visible spectrum, wavelengths of about 400 nm appear violet to the human eye, while wavelengths of about 700 nm appear red (Hopson and Wessels, 1990).

Light colour and plant systems

Light colours (wavelengths) have a variety of effects on the plant systems. For example, light energy from the sun is of primary importance to life processes, with particular reference to photosynthesis. Light also has a profound effect on the development of plants. It affects phototropic movements, stem and root elongation, the opening of stomata, and flowering (Roberts, 1986). In the early 1950s research workers in the United States Department of Agriculture studied systematically to find out which particular wavelengths of light were effective in bringing about germination. They discovered that red light, the range of 580 to 660 nm was the most effective, whilst far-red light between 700 and 730 nm inhibited germination (Hannay, 1976).

Light colour and animal systems

Light has been shown to also affect animal systems. When rats and mice were illuminated all night and kept in the dark all day, they changed their reproductive habits, and instead of ovulating near midnight, they ovulated near noon. Studies also indicated that continuous exposure to light resulted in an inhibition of ovulation. Such kind of phenomenon, in which periodicity of light controls the ovulatory or sexual cycle of female animals, is called 'sexual photoperiodicity'. This phenomenon has also been reported in other male animals (Verma and Agar-

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wal, 2003).

The colour of light also affects animal behaviour. For example, Riol et al. (2003) studied colour perception in fighting cattle. Using the discrimination conditioning method to determine the differentiation of seven colours that is, violet, blue, green, yellowish, orange, and red from grey samples equivalent in brightness, they concluded that these animals perceived, and differentiated perfectly, colour stimuli with long and medium wavelengths (between 550 and 700 nm), but experienced considerable difficulty in perceiving short wavelength colours (between 400 and 500 nm).

Light colours and earthworms

When Hamman et al. (2003) exposed the earthworm species, *Eisenia fetida*, to ultra-violet radiations (UV radiations), the (UV radiations) depressed the growth rate of the worm by causing a reduction in fecundity, and also decreased the cocoon fertility by as much as 70%.

Earthworms

Earthworms are ubiquitous, being among the most widely distributed invertebrates occurring over most of the world, and preferring moist soil rich in calcium and organic matter. They inhabit burrows for protection from adverse weather and predators and may penetrate far below the surface to avoid extreme heat, cold, or drought (Storer et al., 1977). They also found in both the temperate and tropical regions, occupying every soil niche of forests, woodland, shrublands and grasslands which together constitutes 80 million per sq. kilometre or 54% of the land surface of the earth. The typical earthworm would normally appear on the surface at night, especially after rains. In very dry or very hot weather, they burrow deeply and become inactive (Whittaker and Liens, 1973).

The most frequent earthworms in Nigeria have been found to be *Ephyriodrilus afroccidentalis, Eudrilus eugeniae,* and *Hyperiodrilus africanus* (Owa et al., 2003a).

Owa et al. (2003a) opined that ultimate interest is not so much in the abundance of earthworms per se, but, more importantly, in the role earthworms play in affecting soil fertility. In reviewing the classical work of Charles Darwin on earthworms, Feller et al. (2003) reported that earthworms play important roles in the physical and chemical weathering of rocks. According to them Darwin found several small stones or grains of sand in the gizzards of many earthworms which are sometimes combined with the hard calcareous concretions formed by calciferous glands. These coarser particles are apparently ingested and utilized by the worms to help triturate the ingested soil organic matter and leaves, and to facilitate digestion. These will, by particle attrition and passage through the gizzard and the gut, break up the larger particles, thereby contributing to the physical weathering of soils. Madge (1969) also found that earthworms literally eat their way through the soil, and the ingested earth is passed through the digestive tract and deposited at the surface in small mounds or 'castings'. That these activities of earthworms and many others play important roles in increasing soil fertility and productivity is now general acknowledged (Edwards and Lofty, 1977; Henry, 1978; Lee, 1983; Edwards, 1988; Dominguez et al., 2000; Ndegwa and Thompson, 2001; Ayanlaja et al, 2001; Chaoni et al, 2003; Owa et al., 2003a, 2003b, 2004a and 2004b).

Earthworms have been also shown to be useful in the rather problematic disposal of industrial sludge, so much so that the use of earthworms in sludge management has been termed "vermistabilization" (Neuhauser et al., 1988). Already, various natural and anthropogenic wastes have been converted into useful compost by different species of earthworms (Neuhauser et al., 1988; Butt, 1993; Elvira et al., 1998; Bansal and Kapoor, 2000; Gajalakshmi and Abbasi, 2004, Kaushik and Garg, 2004). Earthworms are also being used as bio-indicators of harmful chemicals in the soil (Thompson, 1971; Svendsen and Weeks, 1997). Earthworms are also now been widely used as the preferred bait in fishing throughout the world and as a good source of protein in man (Stephenson, 1930; Ljungstrom and Reinecke, 1969; McInroy, 1971; Sabine, 1978). Decaens et al. (2003) found out that seeds ingested and voided with the casts by earthworms had a greater chance of germination than those put in a soil seed bank.

Moreover, the effects of certain factors that is, soil moisture levels; pH, pesticides, animal manure and temperature on the bioactivities of earthworms have been studied by several workers (Grant, 1955; Roots, 1956; Lee, 1959: Madge, 1969; Reynolds, 1973; Barker, 1959; Wheatley and Hardman, 1968; Edwards and Lofty, 1977; Viljoen and Reinecke, 1992).

Literature revealed little information about the response of earthworms to different light wavelengths include those of Whittaker and Liens (1973) and Hamman et al (2003) with respect to sunlight and UV light on their fecundity and cocoon fertility.

Therefore, the objective of the present study is to investigate the responses of the earthworm species, *H. africanus*, to the three primary colours (red, green, and blue) in comparison white light and darkness. The performance is measured in terms of the mass of casts produced and the rates of emigration (an indication of their tolerance to each light colour

MATERIALS AND METHODS

The experiment was conducted during the period from September-November, 2004 at the Applied Zoology Laboratory on the Mini-Campus of the Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Soil preparation and treatments

Friable loamy soil was collected from the Mini-Campus of the Olabisi Onabanjo University, Ago-Iwoye. The soil was heat-sterilized, at 110°C for 2 h, to kill all present earthworms, their eggs, and other microbes in the soil. After sterilization, the soil was spread out and allowed to cool for a while before it was weighed into cylindrical plastic containers. The earthworms were cultured in these cylindrical plastic containers, each measuring 12 cm in height and 6.5 cm in diameter. There were thirty replicates per treatment.

The culture containers were kept in a wooden shelf. The shelf, 218 cm in height, had five compartments, each 90 × 75 × 35 cm. Pendent from the roof of each compartment was a 15-watt, low-heat, frequency-generated fluorescent light tube. Their different colours were red, blue, and green, (the primary colours), and white (mixed colours). The fifth compartment was left without any light and the worms placed in this compartment remained in the dark for the duration of the experiment.

Reflection of a light colour from one compartment into another was prevented by covering with opaque black septa and to cover the front faces of the compartments.

Earthworm collection

The earthworm *H. africanus* was used for this experiment because it is a turret caster and is not itinerant (Owa et al. 2003a) It is easier to handle and weigh than some other worms.

About a thousand specimens were collected from under trees and litter at the Mini-Campus of the Olabisi Onabanjo University, Ago-Iwoye. The healthy worms, dug-up and hand-sorted, were transported along with the soil in which they were collected to the laboratory for use in the experiment. Each of the cylindrical containers was inoculated with about 1 g of earthworms.

Feeding the worms

As a food source, 10 g of heat-sterilized, aged cattle dung was applied below the soil surface in each container at the beginning of each run of the experiment. The subsurface placement was a way to prevent inviting predatory insects such as the doryline ants, e.g., *Dorylus*.

The earthworm cultures

The thirty cylindrical plastic containers, each filled with 280 g of soil, were placed in each compartment on the shelf (that is, 30 replicates for each light treatment). An average of six worms, weighing an average of 1 g (0.94 - 1.21 g) was inoculated into each container - some distance below the soil surface. All cultures were left for 10 days prior to experimentation to acclimatize. The containers in each compartment were placed directly 20 cm below the different light tubes and each of the tubes was lighted throughout the period of the experiment, which lasted 21 days. To maintain the moisture level in the cultures, 6 mls of clean, well water was dispensed by a syringe into each culture, every 48 h. It was important to avoid water-logging in order to prevent anaerobiosis of the cultures. The ambient temperature in the laboratory during the period of the experiment ranged from 32 to 34°C.

Measurement

Two parameters were measured viz: mass of cast produced and the emigration rates of the worms. The casts were neatly ‘plucked’ off the top of the soil, put in a pre-weighed transparent polythene bag and weighed to 0.00 g on a top-loading electronic, Mettler balance. The number of worms in each container was recorded. The initial numbers of earthworms inoculated is noted at the beginning of each run of the experiment. Thereafter, subsequent counts of the worms were done by removing the worms manually from each container and counting to determine how many of them had emigrated from each compartment. After the measurements had been taken, the worms were returned to the containers (without the casts removed for measurement) and fresh worms were added into containers where emigration had been recorded in order to maintain the mass at about 1 g. The two measurements were taken as indicators of the physiological response of the earthworms to the light colour (emigration being a behavioural response to an unsuitable environment).

Since the energy-saving lamps used were frequency-generated, the frequency of their oscillators which generated the light colours was determined and the results correlated with the casting performance and migration of the earthworm.

Measurements of the casts and the rates emigration were taken concurrently, once every 7 days, for three consecutive weeks. At the beginning of each experimental run, the positions of the light colours were randomly decided.

Data (Statistical) analysis

The experiment was run three times. The results from the three runs were pooled together for statistical analysis. Differences in the cast produced and extents of emigration under the different lights were statistically compared using ANOVA. The statistical package SPSS, version 10, was used on a Pentium III microprocessor table-top Personal Computer.

RESULTS

Mass of cast produced under the different light colours

The analysed result (Table 1 A, B and C) shows that the earthworms’ performances decreased from under red, blue, green, control (dark) and white lights, respectively. However, the earthworms perform best in cast production under red light, and least under the white light.

Cast production per worm

Table 2 shows that the mass of cast produced per worm decrease from under the blue, red, green, control and white colours, respectively.

Rates of emigration from the different light colours

In an ascending order of emigration, the earthworms moved out from under the control (dark), red, white, green and blue lights, with values of 34.9, 36.1, 39.5, 50.48 and 50.53, respectively (Table 3)

Frequency of oscillator circuits that generate the light colours in relation to the mass of cast produced

Table 4 presents the measured frequencies of the oscillators that generated the light colours against the mean masses of the casts produced under the different light colours. The result indicates that cast production increases with the frequency of the oscillators that generated the light colours. The Pearson correlation coefficient ‘r’ is

Table 1a. Mass of cast produced under the different light colours.

Colour	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
White	80	4.8597	3.7239	0.4163	0.19	21.55
Control (Dark)	69	7.2017	3.9039	0.47	0.84	20.12
Green	74	9.4911	3.8570	0.4484	1.98	23.28
Red	80	11.9584	5.2522	0.5872	1.06	26.13
Blue	80	10.6583	4.5780	0.5118	0.96	18.61

Table 1b. ANOVA on the differences in the mass of cast produced under the different light colours.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2526.075	4	631.519	33.847	0.000
Within Groups	7052.788	378	18.658		
Total	9578.863	382			

Table 1c. Paired comparisons on the mass of cast produced under the different light colours.

(I) COLOUR	(J) COLOUR	Mean Difference (I-J)	Std. Error	Sig.
White	Control (dark)	-2.3421*	0.7097	0.001
	Green	-4.6314*	0.6967	0.000
	Red	-7.0987*	0.683	0.000
	Blue	-5.7986*	0.683	0.000
Control (dark)	White	2.3421*	0.7097	0.001
	Green	-2.2893*	0.7229	0.002
	Red	-4.7566*	0.7097	0.000
	Blue	-3.4565*	0.7097	0.000
Green	White	4.6314*	0.6967	0.000
	Control (dark)	2.2893*	0.7229	0.002
	Red	-2.4673*	0.6967	0.000
	Blue	_1.1672	0.6967	0.095
Red	White	7.0987*	0.683	0.000
	Control (dark)	4.7566*	0.7097	0.000
	Green	2.4673*	0.6967	0.000
	Blue	1.3001	0.683	0.058
Blue	White	5.7986*	0.683	0.000
	Control (dark)	3.4565*	0.7097	0.000
	Green	1.1672	0.6967	0.095
	Red	-1.3001	0.683	0.058

The mean difference is significant at the .05 level.

Table 2. Mass of cast produced per worm.

Colour	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
White light	27	6.5115a	3.0229	0.5818	0.22	12.58
Control (Dark)	27	9.4006b	2.5265	0.4862	4.09	14.92
Green light	26	12.9769c	5.0590	0.9921	5.92	25.92
Red light	28	17.8861d	7.7110	1.4572	1.16	34.55
Blue light	28	17.9524d	6.1286	1.1582	1.16	31.78

Mean values with different letters are significantly different from each other, those that carry the same letter are not. (At P = 0.05).

Table 3. Emigration rates from under the different light colours during the course of the whole experiment (pooled results)

Colour	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
White light	60	39.5275a	33.6492	4.3441	-16.67	100.00
Control (Dark)	60	34.9303a	29.3809	3.7931	-25.00	100.00
Green light	60	50.4834b	31.8727	4.1147	_0.98	100.00
Red light	60	36.1173a	26.2818	3.3930	-1.03	100.00
Blue light	60	50.5330b	28.9299	3.7348	-2.00	100.00

Mean values with different letters are significantly different from each other, those that carry the same letter are not. (P = 0.05).

Table 4. Frequency of light colour-generating oscillators against the mean masses of the cast produced under the different light colours.

Light colours	Frequency of colour-generating oscillator (KHz)	Mean mass of cast recorded under the light colours (g)
Red	44	11.96
Blue	42	10.66
Green	36	9.49
White	30	4.86

Note 1: that the light colours produced are dependent, not only on the frequencies of the generating oscillators, but also on the fluorescent coating of the fluorescent tubes.
Note 2: The correlation coefficient between the oscillator frequency and the mass of cast produced is 0.301 and is statistically significant (p = 0.01, N = 383).
Note 3: The linear regression equation between the oscillator frequency and the mass of cast produces is: Cast = -8.662 + 0.471 x (oscillator frequency).

Table 5. A comparison of the relative amounts of cast produced in the wild, and under the different light colours.

Condition of Production	Amount of Cast Produced (Ton/ ha/ yr)	Production Relative to Control in this Experiment	Production relative to Natural Production in the Wild.
The Wild (natural) (Madge, 1969; Lavelle et al., 1998, etc.)	200	0.18	1
Control (Dark)	1127.8	1	5.64
White Light	761.3	0.68	3.81
Green Light	1486.5	1.32	7.43
Blue Light	1670	1.48	8.35
Red Light	1872	1.66	9.36

is 0.301, which is significant at the 0.01 level.

DISCUSSION

Effect of light colour on cast productivity

The earthworms produced more cast under all experimental colours than in the wild (Table 5). The values of surface cast production in tropical regions range from almost zero to 200 Ton/ ha/ year (Madge 1969; Lavelle et al., 1998; Norgrove and Hauser, 1999a), which is less than the values produced under all the light treatments projected over a year.

That earthworms produce better under the experimental white soft light than in the wild suggests that it is the component (ultra-violet rays) that impedes their activities. This is further evidenced by the greater production under other light colours tested. This also proves that it is not light *per se* that inhibits their activities but the lethal component. On the other hand, the present results suggest that darkness *per se* does not provide any special casting advantage on earthworms. Therefore, the tendency exhibited by earthworms always to stay in their burrows during the daytime and only crawl out at night to form casts may not actually be an adaptation to avoid the sun, as a source of white light, but may in fact be an adaptation to avoid the sun as a source of heat energy, and UV radiation. This opinion might be supported by the findings of Hamman et al. (2003) that UV radiation lowered the fecundity of earthworms and caused a decrease in their cocoon fertility by around 70%.

Apparently, as the frequency of the light colour-generating oscillator increased, the performance of the worms also increased. That red light elicits best performance in the earthworms is similar to its effect in seed germination (Hannay 1976).

The economic importance of finding the red light to be a suitable wavelength for encouraging more cast production by the earthworms than when left in the dark cannot be overemphasized. It implies that earthworms can be made to produce more casts than before during the course of the night with an attendant increase in soil fertility. This is a potential means of increasing soil fertility and performance while minimizing the use of inorganic fertilizer and mechanization, ensuring sustainable farming that will benefit, not only the farmers and the consumers, but also, the soil and the environment. It also implies that all the advantages that earthworm casts provide for the plant would be greatly multiplied; thereby increasing both the quality and quantity of farm produce. For instance, results obtained by McInerney et al. (2001) showed that presence of earthworm casts reduced moisture fluctuations, resulted in higher organic matter content and, in the long term, helped in maintaining mineral stabilization of the soil, irrespective of the prevailing climate. Bayon and Binet (2001) found that earthworm activities, one of which is creating surface casts, act as a physical barrier reducing soil erosion. These, and many more advantages of earthworm casts (Dominguez et al., 2000; Ayanlaja et al., 2001; Ndegwa and Thompson, 2001; Owa et al., 2003a, 2003b; Chaoni et al., 2003; Feller et al., 2003), stand to be improved/ increased by the present findings

Effect of light colour on worm emigration

It was initially suspected that the rate at which the earthworms would emigrate from a particular light source, should be indicative of the tolerance of the worms for that light colour. Judging from the present findings on the emigration rates, the worms showed higher tolerance for the dark (control) and red light colour than for the other colour tested, showing the least tolerance for blue light. This, alongside with their best cast production under the red light, suggests that not only did the worms find the red light positively stimulating, in terms of cast production, they were also quite comfortable working under the light colour. Concerning the control (dark), under which the worms showed highest tolerance, it indicates that even though the earthworms were comfortable staying the dark, they were not being adequately stimulated to work at their best.

Cast productivity per worm

One implication of the results is that different light colours facilitate earthworm casting activity to varying degrees. More casting under the red light suggests more activity by the calculation of the mass of cast produced per worm. Moreover, the mass of cast produced per worm under the red and blue lights were significantly recorded higher than those of other light treatments, implying that the red and blue light had more stimulating effects on the worms, than the other lights.

Conclusion

Some conclusions that can be made from the present results include:

1. The level of performance of the experimental worms (under all the light treatments) was a lot higher; in terms of surface cast production, than that recorded for earthworms in the wild.
2. Of the four light treatments and the control used in this experiment, *Hyperiodrilus africanus* responded best under the red light treatment, in terms of cast productivity and tolerance.
3. The order of preference for the different light treatments shown by the earthworms, in terms of cast productivity, is red, blue and green in descending order. The white (mixed) light and control (dark) had significantly less stimulating effects on the productivity of the worms.
4. *H. africanus* is less likely to emigrate from an area, farm or vegetation, where they are exposed to red light or an oscillator of the frequency band about 44 KHz.

Colour as a potential "light fertilizer": The global opinion is towards finding an equally viable alternative to the use of inorganic fertilizers to enhance soil fertility, in view of the latter's attendant detrimental side effects to the soil biota and the crop consumers. The identification of a suitable light wavelength (or oscillator frequency) at which earthworms can function best is of great importance to the farmer and the world at large. When this finding is further explored and confirmed and the effective light wavelength has been discovered, not only to earthworms but also to crops and other non target organisms, we may then arrive at what may be called "light fertilizer" (use of light colour to increase soil fertility). When translated into a technology, a farmer may only need to mount the right colour lamps in the form of security floodlights, left overnights on the farm. As indicated in the present results, this will stimulate the worms to produce more casts to the benefit the plants and increase the farmer's own productivity.

This finding provides some important insight into the future development of wave-fertilizers in a bid to institute global-scale light-facilitated soil fertility awareness. The application of these results is likely also to affect the amount of farm preparative mechanization. For instance, it is now known that mechanized land preparation causes loss of soil structure. The mechanized soil, which may at first appear to be better aerated and more yielding, is actually more prone to erosion and faster loss of nutrients (Werner 1990). It is gaining opinion to minimize tillage so that earthworm populations can be sustained and engaged in the process of tilling the soil through their burrowing and cast making activities (Feller et al. 2003). When red light is mounted on such a farm, the increased earthworm casting activities will increase the rate at which both burrows and casts are made, in the soil in the process of ingesting more earth to produce more casts.

Home and industrial vermicomposting systems could also benefit from the preference shown by the earthworms for the red light, with the tubes simply mounted directly above the vermicomposting set-ups with an attendant increase in the rate at which the earthworms turn home and industrial wastes into nutrient-rich organic matter, reducing cost and increasing profit to the benefit of the farmer, the consumer and the environment. The economic gains from such an installation on a large-scale farm still needs to be investigated

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