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Application of earthworm urine via apical bud or stem-base: Differential effects on the growth of organs of the leafy-vegetable *amaranthus*

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The present study was undertaken to determine how direct administration of earthworm urine to the apical bud or to the stem-base of the leaf-vegetable crop *amaranthus* affected the growth performance of the apical and axillary buds, stem girth and roots of the crop. Apical application significantly facilitated the growth of both apical and axillary buds, and the general growth of the whole plant. For agricultural mandates that focus on the production of apical and axillary buds (eg, production of teas), yield can be stimulated by applying low earthworm urine concentration at the stem base, or medium and high concentration of earthworm urine on the apical buds. Application of medium to high concentration of earthworm urine at stem base produces inhibitory effects on the growth of apical buds, axillary buds, stem girth and roots. This result will find commercial and technological applications if relevant farmers could: (1) have a vermin culture to aseptically produce earthworm urine, (2) mix the urine with oil to reduce evaporation when applied, and (3) air-spray by helicopter on his or her farm. Significantly improved yield is expected. Potential beneficiaries of such technology include tea farmers whose products enjoy premium value when restricted to the apical and axillary buds and their immediate neighboring young foliage leaves. Similarly, pharmacognosists and herbal practitioners could apply the principles to increase their harvest of pharmacologically active products harvestable from buds and fresh foliage leaves.

Key words: Earthworm urine, tea farming, axillary buds, apical buds, plant growth hormone, herbal extraction.

INTRODUCTION

The function of earthworms in soil development is well known (Nye, 1955; Henry, 1978; Chaoni, 2003), as is their effect on soil fertility (Dominguez et al., 2000; Ayanlaja et al., 2001; Chaoni, 2003). It is thought that the contribution of earthworms to soil fertility include their excretion of ammonia into the soil (Owa et al., 2003), creation of microenvironment in which temperature is raised (Owa et al., 2002; Owa et al., 2004a, b), creation of increasing soil porosity and aeration, (Edwards and Lofty, 1997; Lamande, 2003), bringing cations from deep in the soil up in the rhizosphere (Owa et al., 2004a). Their effect on crop performance has been demonstrated for

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many crops including rice (Owa et al., 2004b), on the leaf vegetable *Amaranthus*, and on the fruit-vegetable *Abelmoschus*. It has also been suggested that their effect on crop performance includes secretion of a growth hormone (Dynes, 2003; Owa, et al., 2004a).

MATERIALS AND METHODS

Earthworm (*Libyodrilus violaceus)* used for this study was collected from the riverside of River Ome, Ago-Iwoye, Ogun State of Nigeria. Earthworm saline (0.7% NaCl) was prepared. 1 kg live earthworm was placed in 1 L earthworm saline for 1 h. The earthworm was removed; the saline was filtered and referred to as stock (100%) earthworm urine, from which lower concentrations (75, 50 and 25%) were prepared by dilution. The ordinary saline (without earthworm urine) represented 0% dilution.

Amaranthus seeds were planted at 2.5 cm depth in uniform-sized, 3 kg loamy soil-containing plastic seedling pots. After germination, the seedlings were thinned to 2 per pot. Two weeks after the planting, earthworm urine was applied to the plants at either the apical bud or stem-base. For apical application, a small piece of cotton wool was applied to, and left in position on the apical buds. This was done for one batch of the plants. For the second batch, the cotton wool was applied to, and left in position, at the base of the stem, just above the soil. Using a dropping pipette, 1 ml earthworm urine was applied to the cotton wools either at the apical bud or at the stem base. Ten pots had 100% earthworm urine applied to their apical buds (that is, 10-replicates). Similarly, each of the lower concentrations was applied to 10-replicate pots at the apical buds. Again, each of the concentrations was applied to another set of plants at the stem base, in 10-replicates each. To avoid drying out under the sun, the applications were at evening hours, about 6 pm. Application was once daily for 11 days.

Thereafter, the soil in each pot was water-washed away to minimize damage and breakages of the roots. The entire plant was kept in a black polythene wrapper and taken to the laboratory for dissection of the organs and for weighing. The apical buds were harvested at the level of the first foliage leaves. The two apical buds from each pot were weighed together. Similarly, all axillary buds from a pot were weighed together. The average stem diameter of the two plants in a pot was taken, each being measured at the level just above the soil, using a Vernier caliper. The root of each plant was mobbed dry using paper toweling, and the two from each pot were weighed together.

RESULTS

Relative effectiveness is (result with apical application/result with stem-base application).

DISCUSSION

1. Apical bud production increased as the concentration of urine applied to the apical bud increased; and these differences were significant ($p < 0.05$, Table 1). This suggested the presence of some apical-bud stimulating substance in the earthworm urine,
2. But apical bud production decreased as the concentration of urine applied to the stem increased, ($p < 0.05$; Table 1). This suggested the presence of a substance in the earthworm urine that had apical bud-stimulating effect when applied on the apical bud, but which has an inhibitory effect when applied at the stem. This type of effect is characteristics of auxins,
3. The urine component, when applied via the apical bud also stimulated the growth of axillary buds (Table 1); but when applied via the stem-base, it tended to inhibit the growth of axillary buds,
4. With apical application, increasing earthworm urine concentration produced increasing gross mass of plant. But with stem-base application, plant gross mass decreases with increasing earthworm concentration. Again, application via the stem-base tended to produce inhibition,
5. The effect of apical application on total root mass was not steady. But with stem-base application, root production decreased with increasing concentration of earthworm urine,
6. Apical application caused slight, but significant increase in girth circumference, whereas stem-base application produced no increase in girth circumference with increasing concentration of urine.

That the point of administration of the earthworm urine to the plant (apical or stem-base), *vis a vis* the concentration of the earthworm urine, affected the growth performance of the plant organs was noteworthy. For the production of all those plant organs, at low urine concentrations application was better via the stem-base than via the apical bud; but at medium to high urine concentration, application via the apical bud became much better.

It is also noteworthy that application via the stem-base appears to produce inhibitory effect on the growth of some of the plant organs. From the table:

(i) For apical bud production, stem-base application inhibited the growth of apical bud (effect relative to control = 0.7303); but apical application stimulated apical bud production (by about twice: 1.9061) relative to control,
(ii) For axillary bud production, stem-base application of urine caused inhibition by about half (0.5522) whereas apical application stimulate growth by 4.5729 times.
(iii) For gross mass production, application via the stem-base inhibited by 0.6490 times, while apical application stimulated by about 1.4273 times,
(iv) For root mass production, both stem-base and apical applications caused inhibition which is worse via the stem-base,
(v) For girth production, stem-base application produced only a slight inhibition while apical application produced only a slight stimulation.

Why the inhibitory effect of earthworm urine when applied via stem base? That the inhibition affected the organs measured suggested that the observation may not be

Table 1. Resulting growth of organ of *Amaranthus* when different dilutions of stock earthworm urine were applied either via the apical bud or via the stem base.

	Urine application via stem			Urine application via apical bud			Resulting growth due to apical application Relative to Stem application
Urine conc. as % of stock solution	**N**	**Mass of apical bud (g)**		**N**	**Mass of apical bud (g)**	**Sig**	
0	10	0.7550 ∀ 0.20657	Sig	20	0.2665 ∀0.18397	Sig	0.35298
25	10	0.5790 ∀ 0.26010		18	0.4106 ∀ 0.30970		0.709154
50	10	0.5570 ∀ 0.25578		19	0.5200 ∀ 0.27449		0.933573
75	10	0.4610 ∀ 0.24429		18	0.6856 ∀ 0.38770		1.487202
100	10	0.4050 ∀ 0.17148		17	0.6941 ∀ 0.46184		1.713827
Total	50	0.5514 ∀ 0.25161		92	0.5080 ∀ 0.36456		0.921291
Effect relative to control		0.7303			1.90619137		
		Total mass of all axiliary buds on plant (g)			**Total mass of all axiliary buds on plant (g)**		
0	10	0.9670 ∀ 1.15835	Ns	10	0.8720 ∀ 1.04269	Sig	0.896907
25	10	0.3440 ∀ 0.36840		10	3.3800 ∀ 4.42307		9.941176
50	10	0.4600 ∀ 0.50237		10	4.1800 ∀ 3.52134		9.086957
75	10	0.4530 ∀ 0.48123		10	5.0100 ∀ 4.55531		11.13333
100	10	0.4460 ∀ 0.73864		10	6.4960 ∀ 5.62221		14.44444
Total	50	0.5340 ∀ 0.71424		50	3.9876 ∀ 4.38737		7.528302
Effect relative to control		0.5522			4.5729		
		Gross mass of plant (g)			**Gross mass of plant (g)**		
0	10	37.7770 ∀ 14.70161	Sig	10	53.3800 ∀ 29.50410	Ns	1.412917
25	10	24.4802 ∀ 10.79106		10	76.4340 ∀ 63.10137		3.122141
50	10	20.9360 ∀ 10.88751		10	76.5220 ∀ 39.67397		3.65425
75	10	21.6830 ∀ 10.12980		10	74.9420 ∀ 32.69491		3.456642
100	10	17.7240 ∀ 9.64549		10	99.6860 ∀ 61.92323		5.625847
Total	50	24.5200 ∀ 12.97503		50	76.1928 ∀ 47.96013		3.107259
Effect relative to control		0.6490			1.4273		
		Total root mass (g)			**Total root mass (g)**		
0	10	37.6450∀62.04348	Ns	10	33.2170 ∀ 17.92650	Ns	0.882337
25	10	11.0553 ∀ 4.57874		10	37.4880 ∀ 28.85400		3.389693
50	10	9.5990 ∀ 4.93396		10	30.3360 ∀ 14.38679		3.160417
75	10	9.8560 ∀ 6.16789		10	25.6540 ∀ 13.19980		2.60142
100	10	7.3500 ∀ 3.57559		10	35.0480 ∀ 20.49014		4.768707

Table 1. Contd.

Total	50	15.1011 ∀ 29.25412		50	32.3486 ∀ 19.39282		2.142384
Effect relative to control		0.4011			0.9739		
		Stem (cm) circumference			**Stem (cm) circumference**		
0	10	3.3400 ∀ 0.38064	Ns	8	2.4688 ∀ 0.578255	Sig	0.739521
25	10	3.0900 ∀ 0.39567		8	2.7438 ∀ 0.59907		0.886731
50	10	3.0600 ∀ 0.73060		9	3.1889 ∀ 0.56057		1.042484
75	10	3.1300 ∀ 0.56578		9	3.1111∀0.38388		0.99361
100	10	3.0500 ∀ 0.56421		7	3.6000 ∀ 0.34034		1.180328
Total	50	3.1340 ∀ 0.53131		41	3.0146 ∀ 0.61076		0.961661
Effect relative to control		0.9383			1.2211		

regarded as accidental. Similarly, that the inhibition affected organs both above and below the point of application (stem-base) suggested that the active component was conducted in both directions in the plant.

Implications, application and conclusion

Earthworm urine contains some active component (a hormone) that positively stimulates the growth of *Amanranthus* organs when applied apically, but inhibits them when applied via the stem base,

(i) Earthworm urine is therefore a potential source of plant growth stimulator which can be commercialized,

(ii) There will be a need to concentrate the earthworm urine to raise the concentration of the active component to a certain level to obtain the desired effect,

(iii) When prepared, better result is obtained by apical, instead of stem-base, application,

(iv) This information find application in the production of teas, which are derived from buds and fresh foliage leaves,

(v) Similarly, this information finds application in pharmacognostic studies where drugs are to be extracted from leaves and buds,

(vi) To minimize loss due to evaporation under the sun, our application of the earthworm urine was done in the evening hours. But for a commercial application on a farm, and to retain the bioactivity for a long time, the earthworm urine can be suspended in an oil-based solvent that will stick on to the plant all-day.

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