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Effect of earthworm coelomic fluid treating on seed germination of cress (*Lepidium sativum*) and radish (*Raphanus saltivus*)

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ABSTRACT

This study analyzed the effect of supplementing soil with coelomic fluid (C.F.) of earthworm on the rate of germination of cress (Lepidium sativum) and radish (Raphanus saltivus) seed. The effects of two different concentration of coelomic fluid (1% and 5%) were compared to control conditions in which no coelomic fluid was added. C.F. administrated to the soil was found to significantly ($P<0.05$) accelerate the germination of both types of seeds compared to the control treatment, and the rate of seed germination increased as the concentration of C.F. was increased. C.F. administrated to the soil also significantly ($P<0.05$) decreased the total fungi count in the soil within four days after administration. No statistically – significant differences in soil N, K and P concentrations were detected four days after C.F. administration.

Key words: Earthworm, Coelomic fluid, Seed germination, *Lepidium sativum*, *Raphanus saltivus*.

INTRODUCTION

Earthworms (Annelida) play important roles in growth of plants (Zambare et al, 2008) such as soil aeration and the excretion of coelomic fluid (Hatti et al, 2010 a,b), which contain different enzymes including proteases, amylases and phosphatase and some of hormones like oxyn and cytokinins and essential nutrients for plant growth of like K, Ca, Mg, S, Cl, Cu, Zn, P and Na. This C.F. helps stimulate plant growth and increases diseases resistance (Umamaheswari et al, 2003 ; Suthar et al, 2005 ; Yadav et al, 2005 ; Trivedi and Bhatt, 2006), Al-Shadeedi et al, (2010) reported that earthworms significantly reduced the numbers of *Salmonella typhimurium* in contaminated soil, Furthmore earthworms reduced the numbers of total bacterial, coliform, fungi counts, increased availability of N, K and P (Al-Obaidi et al, 2011).

Cress, *Lepidium sativum* (L.) and radish, *Raphanus sativus* plants, belonging to the family Brssicaceae (Nath and Singh, 2009), are a good source of nutrients for human and animals. Cress, *Lepidium sativum* (L.) a native edible herb that is genetically related to watercress and mustard, shares their peppery, tangy flavor and aroma. Both plant and seeds contain volatile essential aromatic oils, active principle and fatty oils and carbohydrate, protein, fatty acid, Vitamin: β-carotene, riboflavin, and niacin, and ascorbic acid, Flavonoids, Isothiocynates glycoside, a literature survey reveals that cress plant and seeds extracts have bronchodilatory effects (Ravindra et al, 2008), hypoglycemic activity (Eddouks et al, 2005), antihypertensive effect (Maghrani et al, 2005) and bone fracture healing activities (Yadav et al, 2011). Radish (*Raphanus sativus*) a common vegetable in Arabic meals and recipes, is known to activate the liver, gall bladder, improves digestibility of lipids, to increase resistant against chest and skin infections and play a good role in protection from cancer, blood clotting, asthma attack and tooth decay. The raw flesh of the radish has a crisp texture and a pungent, peppery flavor, caused by glucosinolates and the enzyme myrosinase. when chewed to form allyl isothiocyanates, these chemical compounds have stop growth of bacteria which cause decay (Lewis et al, 1982 ; Nath and Singh, 2009).

The aim of this study was to determined the effect of coelomic fluid (C.F.) earthworm extract to accelerate plants seed germination of cress (*Lepidium sativum*) and radish (*Raphanus saltivus*).

MATERIALS AND METHODS

Collection of earthworm: Earthworms were collected from a forest in Zapharania south of Baghdad and transferred to the laboratory within two hours. Earthworms were immersed in clean, cool water to eliminate gastrointestinal metabolites and contaminants. They were then rinsed and rapidly dried on a filter paper, and were subsequently excited with 5V electrical stimulations to produce coelomic fluid through their epidermal dorsal pores (Roch, 1979). After centrifugation for 10 min at 4°C, 3000 rpm, the cell-free supernatant of coelomic fluid was collected and stored at – 20°C until needed (Pan et al, 2003), chemical analysis of coelomic fluid were carried out according to AOAC (1980) as showed in table 1.

Seed germination assay: Two separate experiments were carried out in same time: in experime1 and 2, seeds of *Lepidium* and *Raphidium* were planed under *InVitro* conditions. Nine middle sized of Petri dishes were used for each experiment and three treatments (3 Petri dishes treatment). For two of treatments, coelomic fluid was added once to the soil at 1% (T2) or 5% (T3). Treatment 1, in which a saline solution was added to the soil, served as the control. The germination tests were conducted at 20°C and 100 seeds were planted per petri dish. Planning involved placing the seeds on top of the peat moss soil and covering them with a little more soil. The soil was kept moist but not wet since over-watering resulted in fungal growth on the seeds which caused seed rot and reduced germination (Matthews, 2010), chemical analysis of peat moss soil was carried out according to AOAC (1980) as showed in table 1.

Chemical analyses: All analysis were carried out according to AOAC (1980). Nitrogen was determined by the method of semi-microkjeldal determination of N %**,** K was determined by automatic flame photometer PGI 2000, which give the concentration in ppm, P was determined by colorimetric methods using spectrophotometer(LKB Ultra spectronic). All measurements were done in triplicates.

Fungi counts: At sampling, Mold & yeast load in the soil of cress and radish were eluted by rinse method in which one gm soil (per replicate) was placed in sterile poly ethylene bag and carefully rinsed with 100 ml of sterile peptone water for 10 min., thereafter , several dilutions were carried out using sterile peptone water in universal 10 ml screw caped bottles, Fungus (mold & yeast) counts in the soil of cress and radish were done by culturing 1 ml of each dilution on Saubroud agar plates according to Yousef and Carlstrom (2003).

Statistical analysis: Data were analyzed by using the General Linear Model Procedure of SAS (2001). Means were compared by the Duncan's Multiple Range test at 5% probability (Steel and Torrie, 1980).

RESULTS

The data in tables 2 cleared that the addition of coelomic fluid 1% or 5% significantly ($P<0.05$) increased *Lepidium* and *Raphidium* seeds germination rate. In both cases, all of the seeds had germinated by day 4 when the concentration of coelomic fluid was 5% (Fig. 1).

Table 3 indicated that the addition of 1% or 5% coelomic fluid significantly ($P<0.05$) decreased the fungal count in the soil in which the *Lepidium* and *Raphidium* seeds were planted compared with the control lacking coelomic fluid. The differences in the fungal count observed between the two types of seeds were not significant; however, the reduction increased as the concentration of coelomic fluid was increased. The measured concentrations of soil N, K, and P were not significantly altered 4 days following the addition of C.F. (Table 4).

DISCUSSION

Earthworms perform important roles by making channels in the soil that help in aeration and water flowing and by excretion the coelomic fluid containing many important elements, enzymes and other components that aid in plant growth and increases diseases resistance (Chaudhari, 2005 ; Ansari and Sukhra, 2010). Gopal et al (2010) reported that the coelomic fluid increased the production of *Rhidium* by 20-25% due to an increase in soil organic matter. furthmore, the addition of coelomic fluid to soil used in growth of 1) *Seasamum indicum, Vinga radiate, Vinga mungo* increased plant growth rates Hatti et al, (2010 a,b) ; 2) rice, bean, corn, tobacco, oats and peanuts lead to increased there production and length of roots (Prabhu, 2006 ; Atiyeh et al, 2002). 3) *Cucumis sativum* increased the seed germination rate and plant growth rate (Hidalgo et al, 2009), 4) capsicum seeds increased germination rate, root elongation and the number of fruits per plant Arancon et al (2006). This last result was found to be due to the humic

acid content in coelomic fluid. Humic acid was found to act by increasing the amount and activity of H^+- ATPase that increased humidity within the plasmic wall of the seeds leading to accelerated germination and elongation of seedlings (Canellas et al, 2002). This result agrees with the our results that coelomic fluid accelerates seeds germination of *Lepidium* and *Raphidium*. The additions of humic acid to soil enhances the growth by increasing the uptake of micronutrient from soil and increased the rate of root growth rate that leads to increased protein, chlorophylls pigments, sugars, amino acids and auxins content. Humic acid also increases water holding capacity and the numbers of beneficial microorganism the soil (Khaled and Fawy, 2011).

There is some published evidence that coelomic fluid from earthworm inhibits fungal growth (Ansari, 2008 a,b). Elmer (2009) found that coelomic fluid inhibited some species of fungi that infects vegetables like *Fusarium oxysporum*, *F. asparagi*, *F. proliferatum*, *Verticillium dahliae*. Reiten and Salter (2002) have reported that the compost tea strongly inhibited the growth of *X. campestris pv. Carotovora* both in the laboratory as well as in the field. Our studies indicated growth inhibition of *X. campestris*, *E. carotovora* and *R. solanacearum* by the earthworm extracts following a direct administration at high concentration. This being a preliminary study, a complete biochemical characterization of the extracts was not carried out. However, coelomic fluid was found to contain the free amino acid tryptophan, which possesses antibiotic properties capable in inhibiting fungal plant diseases.

Although , earthworms perform a role in the chemical and physical properties of soil (Atiyeh etal , 2002), we did not observe any effect of C.F. on the N, K, and P content in cress and radish seedlings. Perhaps this is due to the short time period of these experiments (4 days).

Table 1: Chemical analysis of peat moss soil and coelomic fluid.

Chemical analysis (% of dry matter)	Peat moss soil	Coelomic fluid
Organic matter	97.30	99.93
Nitrogen	1.81	0.48
Potassium	2.73	0.37
Phosphorous	0.55	0.61
Ash	2.70	0.06

Table 2: Effect of coelomic fluid concentration treatments on cress and radish seeds germination rates (%) ± SE.

	cress seeds			radish seeds		
	coelomic fluid		concentration	coelomic fluid		concentration
	0 %	1 %	5 %	0 %	1 %	5 %
1 day after treatment	0 a ± 0.0	0 a ± 0.0	0 a ± 0.0	0 a ± 0.0	0 a ± 0.0	0 a ± 0.0
2 days after treatment	5 b ± 0.63	13 ab ± 1.70	44 a ± 3.52	7 b ± 1.22	15 ab ± 1.79	24 a ± 3.47
3 days after treatment	33 b ± 3.88	67 ab ± 5.16	97 a ± 6.28	30 b ± 2.41	51 a ± 3.57	63 a ± 7.22
4 days after treatment	84 b ± 4.57	98 a ± 6.37	100 a ± 6.73	79 b ± 5.24	95 a ± 7.45	100 a ± 6.59

[a,b] different superscripts in a row differ significantly (P<0.05).

Table 3: Effect of coelomic fluid concentration treatments on fungi counts in the soil of cress and radish planting seeds (cfu/gm) ± SE.

	cress seeds			radish seeds		
	coelomic fluid		concentration	coelomic fluid		concentration
	0 %	1 %	5 %	0 %	1 %	5 %
1 day after treatment	568X 10^2 b ± 26.3	352X 10^2 a ± 30.1	242 X 10^2 a ± 27.5	577 X 10^2 b ± 27.9	337 X 10^2 a ± 33.2	216 X 10^2 a ± 31.6
2 days after treatment	577X 10^2 b ± 28.8	311X 10^2 a ± 45.3	230X 10^2 a ± 32.0	589X 10^2 b ± 30.4	331 X 10^2 a ± 27.8	212 X 10^2 a ± 42.6
3 days after treatment	638X 10^2 b ± 35.1	328X 10^2 a ± 35.4	211X 10^2 a ± 33.8	710X 10^2 b ± 32.6	351X 10^2 a ± 31.1	208 X 10^2 a ± 28.2
4 days after treatment	789X 10^2 b ± 28.0	359X 10^2 a ± 34.1	247X 10^2 a ± 31.1	753X 10^2 b ± 31.3	360 X 10^2 a ± 36.1	213 X 10^2 a ± 33.2
Average	643X 10^2 b ± 31.4	338X 10^2 a ± 30.2	233X 10^2 a ± 30.5	657X 10^2 b ± 33.6	345X 10^2 a ± 34.0	212X 10^2 a ± 32.9

[a,b] different superscripts in a row differ significantly (P<0.05).

Fig. 1: pictures clarify the effect of 1% coelomic fluid concentration treatments on cress (upper) and radish (lower) seeds germination rates (A: after 2 days, B: after 3 days and C: after 4 days of planting).

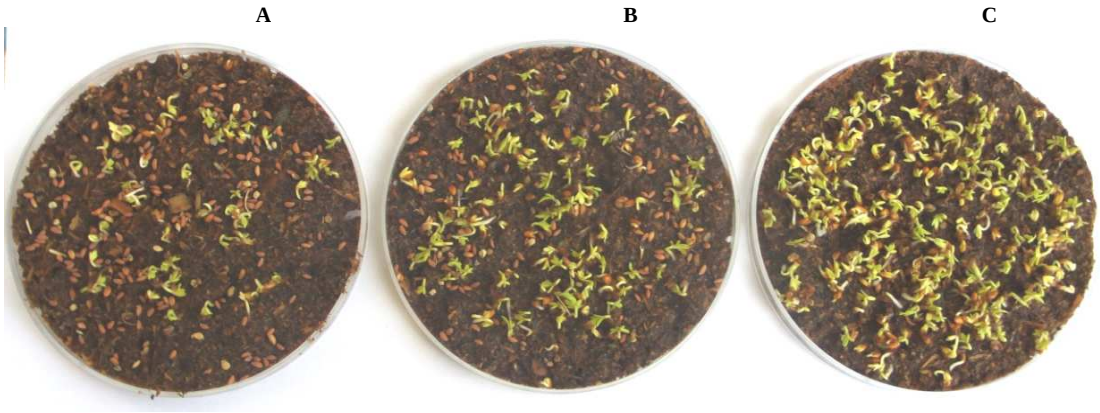

Table 4: Effect of coelomic fluid concentration treatments on N, K and P content in cress and radish seedlings after 4 days of germination (%) ± SE.

	cress seeds			radish seeds		
	coelomic fluid concentration			coelomic fluid concentration		
	0 %	**1 %**	**5 %**	**0 %**	**1 %**	**5 %**
N %	3.06 ± 0.46	3.08 ± 0.37	3.06 ± 0.34	2.94 ± 0.35	2.98 ± 0.34	2.96 ± 0.38
K %	1.79 ± 0.24	1.80 ± 0.31	1.78 ± 0.27	1.83 ± 0.33	1.83 ± 0.37	1.84 ± 0.26
P %	0.52 ± 0.11	0.54 ± 0.15	0.57 ± 0.14	0.55 ± 0.16	0.56 ± 0.12	0.56 ± 0.13

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