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STIMULATORY EFFECT OF EARTHWORM BODY FLUID (VERMIWASH) ON SEED GERMINATION AND SEEDLING GROWTH OF TWO LEGUMES

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The aim of present study was to evaluate the stimulatory effect of earthworm body fluid (vermiwash) on seed germination and seedling growth of two legumes: *Vigna radiata* and *Vigna aconitifolia* under laboratory conditions. The experiment was also run using urea (0.05 %) solution and water for comparison. In this study greater seed germination in *V. radiata* and *V. aconitifolia* was recorded with T_2 treatment (100 % vermiwash) and it was statistically different ($P<0.001$) from other treatments. The greater value of root and shoot length in *V. radiata* was registered better with T_1 (50 % vermiwash) treatment. While *V. aconitifolia* showed greater values of root and shoot length in T_2 and T_1 treatment, respectively. So vermiwash could be excellent bioresource of plant nutrient/growth promoter for sustainable plant production at eco-friendly basis.

Keywords: Earthworm; Legume; *Metaphire posthuma* (Vaillant); Vermiwash; *Vigna radiata; Vigna aconitifolia.*

Introduction

Plant growth promoter plays an important role in vegetative growth and maturation of plants. There are several natural as well as artificial chemical plant stimulator/growth promoters are available. However, the application of these chemicals is not eco-friendly and when they are maintained indoors, the use of chemicals will not be accepted by environmentalists[1]. Organic agriculture is one among the broad spectrum of production methods that are supportive on the environment[2]. The plant nutrient inputs in organic farming mainly rely on biologically derived nutrients instead of using readily soluble forms of nutrients.

Most experiment on cropping cycles show significant effect of earthworm activities on plant production. These effects seem to be proportional to earthworm biomass[3]. Earthworms are one of the most useful and active agent in introducing suitable chemical, physical and microbiological changes in the soil and, thereby, directly increasing the fertility and crop producing power in the soil[4].

Vermicomposting is the biodegradation and instabilization of organic residues through the ingestion and egestion by earthworm. Much effort has been made to study the biological potential of earthworm to break down the organic residues[5-12]. Organic wastes are candidates for transformation from expensive disposal problem to suitable vermistabilized humus for use in food production and reforestation; the earthworm is uniquely designed for this role[13].

Earthworm considered a better candidate for producing phytohormones. A number of the species of plants followed significantly different growth pattern in vermicompost and that indicates a possible phytohormone effects of worm cast[10]. Krishnamoothy and Vajranrabhaian[14] demonstrated some plant growth promoters such as cytokinnis and auxins in worm cast. Similarly, Mascolo *et al*[15] reported auxin-like effects of earthworm worked humic matter on cell growth and nitrogen metabolism in *Daucus carota*. Nielson[16] stated that the presence of plant-growth-promoting compounds elaborated by earthworm promote a significant increase in plant growth and N uptake. In this paper effort has been made to evaluate the stimulatory effect of earthworm body fluids or coelomic fluid on seed germination and growth of seedlings of two legumes under laboratory condition.

Materials and Methods

The experiment was carried out in Environment Biology Lab, Department of Zoology, M. D. (PG) College, Sri Ganganagar, India, under open environmental conditions at 28.4 ± 0.1 °C temperature.

Vermiwash preparation :- For vermiwash preparation earthworms (*Metaphire posthuma* (Vaillant)) were collected from permanent sewage sludge in campus of M. D. (PG) College, Sri Ganganagar, India, by hand sorting methods.

Healthy clitellated and large sized earthworms were collected and for stock earthworms were cultured on organic rich waste resources (leaf litter + cow dung + garden soil). After acquiring appropriate population, earthworms were used for experiments. Vermiwash was prepared by using method as described by Karuna *et al*[1]. About 1 kg earthworms were separated from stock culture and placed in plastic tray for 15-20 minutes to clear out the cast material. Earthworms than carefully removed from their casting material and then added in to glass beaker containing 500 ml distilled water having 40 °C temperature. The worms were agitated for 5-7 minutes and removed and added into another plastic container containing water at room temperature to rinse them thoroughly to collect the exudates adhering to its body wall before releasing back to the stock culture tanks. The light yellow contents of glass beaker and plastic container were mixed to use for experimentation. The collected vermiwash was used in two different concentrations for experimentation i.e. 100 % (without any further dilution), and 50 % (diluted as one part of collected exudates and one part of distilled water). Two another treatments comprised of distilled water and urea solution (0.05 %) were also run for comparative study.

Seedling growth experiment design :-The seed of studied plant species were obtained from New Punjab Seed Co., Sri ganganagar. The M. H. 2 variety of *Vigna radiata* and R-MO 40 variety of *Vigna aconitifolia* were used in experimentation. For germination experiment twenty uniform and healthy seeds of each legume were separated by hand sorting. Seeds were rinsed with 0.1 % mercuric chloride solution for 3 minutes and washed trice in distilled water to remove surface fungi and other microorganisms. Seed germination trial was performed in sterilized petriplates, absorbent cotton and blotting paper were used. For each treatment including control (distilled water and urea solution), five replicates were taken and seeds were allowed to germinate up to 72 hrs in humid condition at a room temperature 31.3 ± 0.6 °C. After 72 hrs of dark incubation seed germination was counted in percentage. For further seedling growth trials germinated seed were transferred in to plastic containers of 5-liter capacity containing garden soil. Sieved garden soil was filled in these containers and germinated seed of each legume were sowed in these plastic containers. Each treatment (100 % vermiwash, 50 % vermiwash, 0.05 % urea solution, and distilled water) was replicated three times. Each experimental container was irrigated weekly by normal tap water. The germinated seeds were allowed to grow under illuminated conditions till they achieve the length up to 5-10 cm. Afterward vermiwash was used as foliar spray by using fine jet hand sprayer on leaf surface of legumes. The spray was given at 3 days interval, for about 15 days. After 15 days of spraying the plant parameters i.e. root length, shoot length, and number of leaves per plant was calculated.

Statistical analysis :- Data were subjected for analysis of variance. The significant difference between different treatments was analyzed by applying Tukey's Post-Hock test.

Results and Discussion

As data indicate percent seed germination in *Vigna radiata* ($F = 14.97$, $p<0.01$) and *Vigna aconitifolia* ($F = 8.57$, $p< 0.01$) showed statistical significant variation between different treatments. In *V. radiata* the maximum and minimum seed germination was recorded with T_2 and T_3 treatment, respectively (Table 1). T_2 treatment showed about 48 %, 91 %, and 48 % higher germination from that of T_1, T_3, and T_4 treatments, respectively. Similarly in *V. aconitifolia* the maximum and minimum seed germination was recorded with T_2 and T_3 treatment, respectively (Table 1). In *V. aconitifolia* T_2 treatment showed about 21 %, 63 %, and 27 % higher seed germination from that of T_1, T_3, and T_4, respectively. Since in *V. radiata* seed germination between T_1, T_3 and T_4 did not show statistical ($P = 0.061$) difference.

In *V. radiata* different treatment caused significant effect on root length ($F = 75.77$, $P<0.01$), and shoot length ($F = 56.01$, $P<0.01$) of seedling (Table2). Number of leaves per plant did not show statistical difference ($P = 0.752$) between different treatments. In *V. radiata* greater and lower value of root length was registered with T_1 (4.48 cm), and it was about 98 %, 107 %, and 133 % higher from that of T_2, T_3, and T_4 treatment, respectively (Table 2). Number of leaves in seedling was recorded equal in all treatments (Table 2). Similarly greater shoot value in T_1 (13.86 cm) was about 6 %, 15 %, and 35 % higher from that of T_1, T_2, and T_4 treatment, respectively (Table 2). In *V. radiata* the minimum values of both root (1.92 cm) and shoot (10.24 cm) was recorded with T_4 treatment (Table 2).

During present study *V. aconitifolia* also showed statistical significant difference for root length ($F = 92.68$, $P<0.001$) and shoot length ($F = 44.85$, $P<0.001$) with different treatments. The greater value of root length (3.54 cm) was registered with T_2 treatment, and it was about 8 %, 72 5, and 113 % higher from that of T_1, T_3, and T_4 treatment, respectively (Table 3). Nevertheless, *V. aconitifolia* showed greater shoot length (13.42 cm) with T_1 treatment, and it was about 5 %, 12 %, and 35 % higher as compared to T_1, T_3, and T_4 treatment, respectively. The lower values of both root (1.66 cm) and shoot length (9.92 cm) was registered with T_4 treatment (Table 3).

Several studies have demonstrated that worm cast can improve soil properties and increase agriculture production. Increased nutrient turnover from earthworm activities usually results in increased plant growth. Nijhawan and Kanwar[17] observed that application of

earthworm cast to wheat increased the plant height, number of tillers, number of leaves, early ear-heading, ear-head length and dry matter per plant than control. Tomati *et al*[18] reported that earthworm cast which is rich in available nutrient increased the plant growth and yield of crop. Edwards and Burrows[19] concluded that seedlings emergence of tomato, cabbage, and radish was much better in vermicompost than in thermophilically composted animal waste and as good and usually better in vermicompost than in a commercial medium. In addition, early growth of ornamental seedlings was as good or better in vermicompost/peat mixtures than in the commercial plant growth media. Grappeli *et al*[20] found that worm cast when used as manure increased height of plants, leaf area index, number of branches, stem girth and yield in respect to plants like Saliva and Aster in pots. Earthworm activated soil or worm cast apart from providing the essential nutrients to plants activates the microbial populations[21]. Earthworm activity is often credited with increased crop growth and increased root growth is often indicated as one of the reasons for the yield response. Pot studies have shown positive response of plants to earthworm inoculation at high rates and the greater increase of root growth occurred where worms are most active[22].

Earthworm increases the quality of mineralized nitrogen and makes it available for plant growth. Aldag and Graft[23] compared the growth of oat seedlings on brown podsol soil that have been treated with *E. fetida* for 8 days within same soil without worms. They recorded 7-8 % dry matter of oat seedlings in the soil with earthworms and the total protein was 21 % more. Atlavinyte *et al*[24] have reported increased microbial population and B_{12} level in the soil due to earthworm activity.

The coelome of earthworm is filled by a milky white solution termed as coelomic fluid, and this fluide poured out due to some disturbance to animal. The coelomic fluid contains excellent concentration of phosphatase, sulphates and chlorides of potassium, sodium and magnesium. Similarly a great concentration of urea as well ammonia also find in this fluid. Due to higher level of plant growth components/growth regulators vermiwash can be acted as better plant growth media. Karuna *et al*[1] reported significant effect of different concentration of vermiwash on growth and production of *Anthurium andreanum*. They concluded that soluble salts of earthworm body fluid showed more effectiveness after dilution by water due to improved

Table 1. Seed germination in different treatments (mean ± SEm, n = 5).

Treatment	*Vigna radiata*	*Vigna aconitifolia*
T_1 50 % vermiwash	58.66 ± 11.46^{a}	62.67 ± 9.98^{ab}
T_2 100 % vermiwash	86.67 ± 5.96^{b}	76.0 ± 9.97^{b}
T_3 urea (0.05 %)	45.33 ± 11.47^{a}	46.67 ± 5.97^{a}
T_4 water	58.67 ± 4.99^{a}	60.05 ± 5.97^{ab}

Mean values followed by different letters are statistically different (ANOVA, Tukey's test; $P<0.05$)

Table 2. Impact of different treatments on root and shoot length of *Vigna radiata* (mean ± SEm, n = 5).

Treatment	Root (cm)	Shoot (cm)	Number of leaves
T_1 50 % vermiwash	4.48 ± 0.12^{b}	13.86 ± 0.41^{c}	2.00 ± 0.0^{a}
T_2 100 % vermiwash	2.26 ± 0.34^{a}	13.04 ± 0.48^{c}	2.00 ± 0.0^{a}
T_3 urea (0.05 %)	2.16 ± 0.19^{a}	12.04 ± 0.21^{b}	2.00 ± 0.0^{a}
T_4 water	1.92 ± 0.36^{a}	10.24 ± 0.50^{a}	2.00 ± 0.0^{a}

Mean values followed by different letters are statistically different (ANOVA, Tukey's test; $P<0.05$)

Table 3. Impact of different treatments on root and shoot length of *Vigna aconitifolia* (mean ± SEm, n = 5).

Treatment	Root (cm)	Shoot (cm)	Number of leaves
T_1 50 % vermiwash	3.28 ± 0.19^{c}	12.78 ± 0.41^{c}	2.20 ± 0.40^{a}
T_2 100 % veriwash	3.54 ± 0.15^{c}	13.42 ± 0.41^{c}	2.00 ± 0.0^{a}
T_3 urea (0.05 %)	2.06 ± 0.27^{b}	12.00 ± 0.59^{b}	2.20 ± 0.0^{a}
T_4 water	1.66 ± 0.12^{a}	9.92 ± 0.15^{a}	2.00 ± 0.0^{a}

Mean values followed by different letters are statistically different (ANOVA, Tukey's test; $P<0.05$)

absorption efficiency of leaves on reducing the concentration of the components. In contrast to above report during present study we achieved maximum seed germination as well as growth parameters in concentrated vermiwash (100 %) solution except to *Vigna radiata* which showed greater shoot and root length in diluted vermiwash (50 %). In *Vigna aconitifolia* both root and shoot length were greater in 100 % vermiwash treatment, but it was not statistically different from that of 50 % vermiwash treatment. The seed germination in concentrated vermiwash treatment could be explained in terms of ion (Na, K, Ca, Mg) and nitrogenous compounds (NH_3 and urea) concentration in it. Besides, urea, ammonia and phenols the wormcast contained plant growth promoters such as cytokinins and auxins[14]. A number of the species of plants followed significantly different growth pattern in worm worked compounds and that indicates a possible phytoharmone effects of worms[10]. Similarly, Mascolo *et al*[15] reported auxin-like effects of earthworm worked humic matter on cell growth and nitrogen metabolism in *Daucus carota*. Nielson[16] stated that the presence of plant-growth-promoting compounds elaborated by earthworm promote a significant increase in plant growth and N uptake.

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