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Evidence of plant hormone like substances in vermiwash: An ecologically safe option of synthetic chemicals for sustainable farming

Surindra Suthar*

Environmental Biology Laboratory, Post Graduate Department of Zoology, B.R.G. Govt. Girls College, Godara College Road, Sri Ganganagar 335001, Rajasthan, India

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ABSTRACT

This study compiles the impact of vermiwash on seed germination, seedling growth and biochemistry of *Cyamopsis tertagonoloba* and *Trigonella foenum-graecum* under lab conditions. A total of four experimental solutions, i.e. 100% vermiwash, 50% vermiwash, 5% urea solution and distilled water, were used in this study. The maximum germination was in 50% vermiwash, while plant growth parameters (root length, shoot length, shoot/root ratio and leaves/plant) showed the optimum results in 100% vermiwash trial. The highest level of chlorophyll in fresh leaves was in 100% vermiwash treatment. The seedlings with 100% vermiwash foliar spray showed the maximum level of total protein, total soluble sugars and starch ($p < 0.05$) in their tissues. Thus, results clearly suggested that vermiwash may be an ecologically safe and cost-effective alternative of synthetic plant growth promoters for sustainable farming practices.

1. Introduction

Several natural and artificial chemicals are available as plant growth promoters. Plant growth promoters are not nutrients but chemicals that in small amounts promote and influence the growth, development, and differentiation of cells and tissues. These chemicals (abscisic acid, auxins, cytokinins, ethylene, gibberellins, polyamines etc.) determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruits, plant longevity and even plant death. There is increasing demand for naturally derived agro-chemicals for sustainable farming systems in the current organic era. Organic farming disallows the use of synthetic agro-chemicals and is based on the principle of nature harmony and eco-sustainability.

There is evidence that earthworms produce a considerable amount of plant hormones in their body secretions. Earthworm processed material 'casts' contain several soil nutrients in forms which are easily available to plants (Taylor et al., 2003; Suthar and Singh, 2008; Suthar, 2008, 2009, 2010). A few plant growth-promoting substances have also been reported in casts (Krishnamoorthy and Vajranabhaian, 1986; Muscolo et al., 1999). Krishnamoorthy and Vajranabhaian (1986) reported relatively higher ranges of plant nutrients such as ammonia, urea, oxidisable organic matter and exchangeable forms of some essential plant nutrients. They also reported plant hormones, e.g. cytokinins and auxins in earthworm casts. Similarly, Muscolo et al. (1999) also found an auxin-like effect of earthworm-worked humic substances on cell growth and nitrogen metabolism in *Daucus carota*. The vermiwash may contain cytokinins, auxin, amino acid, and vitamins, enzymes possibly derived from microbes associated with earthworms. After reviewing the current literature, it was found that no comprehensive study is available concerning the impact of vermiwash on biochemical aspects of seedlings. A comprehensive study is still needed to test the role of vermiwash on plant metabolic processes and biochemical synthesis of protein, sugar, starch and chlorophyll.

The aim of this study was to consider the impact of vermiwash on seed germination and seedling growth of two cereals, *Cyamopsis tertagonoloba* and *Trigonella foenum-graecum*, under laboratory conditions. The role of vermiwash in the biosynthesis of chlorophyll, sugar, protein and starch was also evaluated in this study.

2. Methods

2.1. Vermiwash preparation

Vermiwash was prepared by following method described by Karuna et al. (1999). The separated earthworms were placed in a large-sized plastic container for 15–20 min to clear the adhered casting materials. After that, earthworms were carefully removed from their casting material and than added to the glass beaker containing 500 ml warm distilled water (temperature = 40 °C). The

* Tel.: +91 946 0121182; fax: +91 154 2475960.
E-mail address: sutharss_soilbiology@yahoo.co.in.

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worms were agitated for approximately 5–7 min in warm water and after that removed immediately and added to another pre-sterilized plastic container containing water at room temperature to rinse them thoroughly to collect the exudates (body fluid and mucus and other substances) adhering to the body wall before releasing back to the stock culture containers. The light yellow contents of glass beaker and plastic container were mixed (vermiwash) to use for further experimentations. The collected vermiwash was used in two different concentrations for experimentation i.e. 100% (without further dilutions) and 50% (diluted as one part of collected exudates and one part of double-distilled water). Two other treatments comprised of distilled water and 0.05% urea solution were also run separately for comparative study. The vermiwash solutions were filled in sterilized and pre-cleaned dark-coloured glass containers and stored at 4 °C.

2.2. Seedling germination and growth experiment

Certified seeds of *C. tertagonoloba* and *T. foenum-graecum* were procured from the local Govt Seed Centre. Seeds were pretreated with 0.1% mercuric chloride solution to remove surface contaminations. The seed germination trail was performed in sterilized Petri plates containing absorbent cotton and blotting papers wetted with experimental solutions (vermiwash, urea and distilled water). Twenty uniform and healthy seeds were put into absorbent cotton. The Petri plates were kept in triplicate for each treatment and Petri plate with distilled water acted as experimental control. Seeds were allowed to germinate up to 72 h in humid and dark room conditions at a room temperature 29.1 ± 0.2 °C (SD). After 72 h of dark incubation seed germination (%) was calculated in each experimental Petri plate.

Seedling growth experiment was conducted in plastic container (2 l capacity) of sieved garden soil. Seven germinated seeds were removed carefully from Petri plates and sowed in plastic containers. Seedling growth experimental pots containers were kept in triplicate for each treatment. Each experimental container was irrigated weekly by distilled water. Germinated seeds were allowed to grow under illuminated conditions till they achieved the length of 7–10 cm. Afterwards, experimental solutions were applied as foliar spray for 15 days at 3 days interval to seedlings leaf surface using jet hand sprayer. In the control, distilled water was used as foliar spray. After 15 days, three plants were randomly harvested from each experimental container for plant growth (root length, shoot length and numbers of leaves) and biochemical parameters analysis.

2.3. Biochemical analysis

The chlorophyll in fresh leaves was estimated following method described by Maclachlam and Zalik (1963). Total sugar, starch and total protein were estimated following Loomis and Shull (1937), McCready et al. (1950) and Lowry et al. (1951), respectively. One-way ANOVA was used to analyze the differences between treatments. Duncan multiple range test was also performed to identify the homogeneous type of the data sets among different treatment sets for different plant parameters.

3. Results and discussions

3.1. Germination and seedling growth

The effect of different treatments on seed germination is described in Table 1. There was statistically significant difference among treatments for seed germination in *C. tertagonoloba* (ANOVA: $F = 23.69$, $p < 0.001$) and *T. foenum-graecum* (ANOVA: $F = 15.88$, $p < 0.001$). The maximum germination was in vermiwash treatments for both *C. tertagonoloba* and *T. foenum-graecum* seeds. Germination was the maximum in 50% vermiwash treatment followed by 100% vermiwash, urea solution (0.05%) and distilled water treatments in both cereal seeds. Comparatively, the maximum germination rate was in *C. tertagonoloba* (95%) seeds than *T. foenum-graecum* seeds. It could be due to plant species type or seed variety. Seed germination is complex process controlled by enzymes and specific chemical i.e. gibberellins. Casenave de Sanfilippo et al. (1990) suggested that microbes associated with earthworm body probably produces auxin and gibberlellin-like substances, which promotes seedling, germinates and growth in plants. Moreover, there is evidence that earthworms alter plant seed germination and seedling establishment indirectly via excreta (Eisenhauer et al., 2009). Thus, results support the concept that vermiwash contains a considerable level of plant growth promoters. Results of seedling growth are described in Table 2. In *C. tertagonoloba* seedling the maximum root length and shoot length was in 100% vermiwash treatment i.e. 8.65 ± 0.44 cm and 12.42 ± 0.91 cm, respectively that was significantly higher than other treatments (ANOVA: $p < 0.01$, for all). Root/shoot ration was the maximum in distilled water experimental seedlings followed by 100% vermiwash, 50% vermiwash and urea solution (0.05%). Statistically, root/shoot ratio did not show any significant difference (ANOVA/Duncan's multiple-ranged test; $p > 0.05$) among different treatments for both plant species (Table 2). The seedlings from 100% vermiwash treatment showed about 47.4% and 60.6% more root length and shoot length, respectively than urea 0.05% solution. On the other hand, urea 0.05% solution showed higher root as well as shoot length than 50% and distilled water treatment pots. Mean number of leaves per seedling was the maximum in 100% vermiwash pot (6.33) that was significantly higher than vermiwash (4.33) and urea solutions = distilled water (3.67) (ANOVA: $F = 14.33$, $p < 0.01$, for all). *T. foenum-graecum* seedlings showed the maximum root length (5.89 ± 0.12 cm), shoot length (11.31 ± 0.19 cm), shoot/root ratio (1.92 ± 0.14) and average leaves/seedling (11.33 ± 1.15) in 100% vermiwash experimental pot that was significantly higher than other treatments (ANOVA, $p < 0.01$), except in shoot/root ratio (ANOVA: $p > 0.01$). The root and shoot length of *T. foenum-graecum* seedlings were about 45.1% and 48.2% more than urea solution. Nonetheless, shoot length was higher in both vermiwash treatments than urea 0.05% treatments. Earlier studies indicate the presence of auxin-like impact of earthworm-derived substances. Dell'Agnola and Nardi (1987) suggested that earthworm derived substances affects plant growth by enhancing anion and cation uptake, protein synthesis and the action of nitrate metabolism enzymes. Earthworm-derived substances affect peroxidase and esterase enzymes, which are involved in organogenesis and may be indicators of somatic embryogenesis (Muscolo et al., 1996). Thus, results indicate that vermiwash may be an effective plant growth promoter for sustainable crop production at a low-input and eco-friendly basis, although further detailed study is needed to identify to active ingredients from ver-

Table 1
Seed germination in experimental plants after 72 h (mean ± SD, $n = 3$).

Treatment	*Cyamopsis tertagonoloba*	*Trigonella foenum-graecum*
50% vermiwash	90.0 ± 5.0 c	85.0 ± 5.0 a
100% vermiwash	68.3 ± 7.63 b	71.7 ± 2.88 b
Urea solution (0.05%)	61.7 ± 2.89 ab	66.7 ± 2.88 b
Distilled water	55.0 ± 5.0 a	55.0 ± 8.67 c
ANOVA		
F	23.69	15.88
P	<0.01	<0.01

Mean value followed by different letter is statistically significant (ANOVA; Duncan multiple range test, $p < 0.05$).

Table 2
Root length (cm), shoot length (cm) and root/shoot ratio of seedlings in different treatments.

Treatments	Root length	Shoot length	Shoot/root ratio	Leaves/plant
C. tertagonoloba				
50% vermiwash	5.48 ± 0.21 b	7.27 ± 0.14 b	1.32 ± 0.08 a	4.33 ± 0.58 a
100% vermiwash	8.65 ± 0.44 c	12.42 ± 0.91 c	1.43 ± 0.09 a	6.33 ± 0.58 b
Urea solution (0.05%)	5.87 ± 0.27 b	7.73 ± 0.44 b	1.31 ± 0.15 a	3.67 ± 0.58 a
Distilled water	4.19 ± 0.08 a	6.18 ± 0.29 a	1.48 ± 0.05 a	3.67 ± 0.58 a
ANOVA	**	**	NS	**
T. foenum-graecum				
50% vermiwash	4.03 ± 0.05 b	7.70 ± 0.28 a	1.91 ± 0.11 a	8.0 ± 1.0 b
100% vermiwash	5.89 ± 0.12 c	11.31 ± 0.69 b	1.92 ± 0.14 a	11.33 ± 1.15 c
Urea solution (0.05%)	4.06 ± 0.08 b	7.63 ± 0.46 a	1.88 ± 0.12 a	8.33 ± 0.58 b
Distilled water	3.30 ± 0.23 a	7.98 ± 0.44 a	2.12 ± 0.24 a	6.33 ± 0.58 a
ANOVA	**	**	NS	**

Mean value followed by different letter is statistically significant (ANOVA; Duncan multiple range test, $p < 0.05$).
** ($p < 0.05$).

miwash. The vermiwash contains a considerable amount of mucus and excretory substances (urea, ammonia and phenols), which directly promote plant growth and beneficial microbes producing plant growth promoters.

3.2. Biochemistry of seedlings tissues

Seedling biochemical parameters showed significant variations (ANOVA, $p < 0.05$, all) among different treatments. In *C. tertagonoloba* seedlings the maximum content of protein, total soluble sugar, chlor-a, chlor-b and starch was 2.16 ± 0.05 mg/g, 136.7 ± 5.36 mg/g, 1.51 ± 0.05 mg/g, 0.36 ± 0.02 mg/g and 84.8 ± 4.90 mg/g, respectively in 100% vermiwash pot. Similarly *T. foenum-graecum* seedlings also showed excellent results in vermiwash treatments than urea treatments. The maximum contents of protein, total sugar, chlor-a, chlor-b and starch were 2.52 ± 0.16 mg/g, 142.1 ± 5.36 mg/g, 1.60 ± 0.02 mg/g, 0.39 ± 0.02 mg/g and 94.2 ± 4.01 mg/g, respectively in 100% vermiwash pot. As illustrated in Fig. 1, vermiwash results showed more elaborate results than urea solution and distilled water treatment, which indicates the presence of some active ingredients in earthworm body wash. Although in *C. tertagonoloba* there was a slight difference between urea solution and 50% vermiwash treatment for protein content, it was not statistically significant

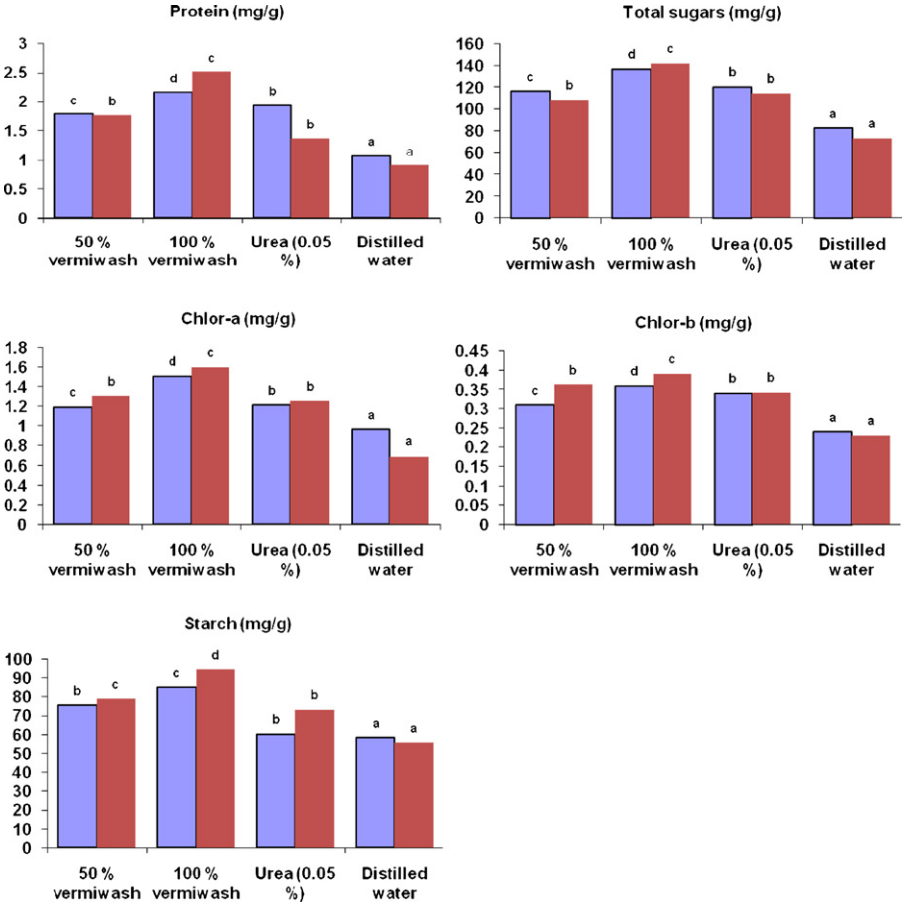

Fig. 1. Biochemical properties of seedlings of *C. tetragonoloba* (blue column) and *T. foenum-graecum* (red column). The column with different letter indicates statistically significant difference (ANOVA; Duncan multiple range test, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(ANOVA/Duncan's multiple-ranged test; $p = 0.050$). In this plant species, 100% vermiwash treatment showed about 11. 3% more protein content than urea treatment. On the other hand, in *T. foenum-graecum* seedlings the protein level was much better in both 50% and 100% vermiwash treatments (25.5% and 83.9% protein level, respectively) than urea treatment. Earlier studies suggested the hormone-like activities of earthworm-derived solids and liquids (Muscolo et al., 1999). According to Dell'Agnola and Nardi (1987), earthworm humic substances affect plant growth by increasing anion and cation uptake, protein synthesis and the action of nitrate metabolism enzymes. Muscolo et al. (1999) reported the auxin-like impact of earthworm-derived substances on growth and protein metabolism of carrot. According to them, earthworm-derived substances increase in free amino acids belonging to the oxaloacetate and a-ketoglutarate pathways. Few studies indicate the presence of vitamins and amino acids in earthworm-derived substances. For example, provitamin D (Zrazhevsky, 1957) and vitamin B (Atlavinyte et al., 1971) are reported up to a considerable level in earthworm products. Probably these act as promoters for carbohydrate and protein metabolism in vermiwash treated seedlings. The result of this study clearly indicates the presence of some growth promoters in vermiwash.

In conclusion, the earthworm body wash indicates the presence of some plant growth promoters which caused significant effect on plant growth as well as biochemistry of seedlings. This study indicates that vermiwash may be utilized effectively for sustainable plant production at low input-basis green framings. There is a great need to identify the plant growth promoter substances in vermiwash in order to determine its feasibility in crop production.

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