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SHORT COMMUNICATIONS

Effect of vermiwash of *Eisenia foetida* produced by different methods on seed germination of green mung, *Vigna radiate*

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Abstract Vermiwash contains enzymes, macro and micronutrients that could stimulate the growth and yield of crops. It can be prepared by different ways. In the present study, vermiwash was produced from the earthworm, *Eisenia foetida,* under field condition and also in the laboratory under cold and heat stress methods. These washes were used in different ratios for seed germination of mung, *Vigna radiate*. The results showed that the vermiwash produced by cold stress diluted at the ratio of 1:5 showed 100 % germination of mung seed followed by 1:5 ratio vermiwash produced naturally that resulted 95 % of seed germination. Undiluted vermiwash produced naturally and by cold stress showed 90 % of germination of mung seed than the undiluted vermiwash produced by heat stress. Cold stress produced vermiwash diluted with 1:5 ratio showed a vigour index of 1650 followed by naturally produced 1:5 ratio diluted vermiwash which showed a vigour index of 1092. However, the undiluted naturally produced vermiwash showed the lowest vigour index of 450.

Keywords Vermiwash · *Eisenia foetida* · Cold stress · Heat stress · Germination · Vigour index · Seed · *Vigna radiate*

Introduction

Earthworm's role as farmer's friend is well known since time immemorial. In recent times, the commercial vermin culturists have started promoting a product, called vermiwash. This vermiwash contains enzymes, secretions of earthwarms which would stimulate the growth and yield of crops and even develop resistance in crops receiving this spray (Shield 1982; Anand et al. 1995; Suthar et al. 2005; Yadav et al. 2005). Vermiwash also has soluble plant nutrients apart from some organic acids and mucus of earthworms and microbes (Shivsubramanian and Ganeshkumar 2004). Zambare et al. (2008) showed the effectiveness of vermiwash on cowpea plant growth by laboratory-scale trial. The effect of vermiwash was observed on the growth and productivity of Marigold (Shivsubramanian and Ganeshkumar 2004). The effect of vermiwash spray significantly increased dry chilli yield (George et al. 2007). Buckerfield et al. (1999) reported that weekly applications of vermiwash increased radish yield by 7.3 %. Thangavel (2003) also showed that both growth and paddy yield increased with the application of vermiwash and vermicast extracts. The present study was carried out to evaluate the effect of vermiwash on seed germination of green mung, *Vigna radiate,* in addition to the composition of vermiwash extracted by different methods and also to find out which method was more effective in seed germination.

Materials and methods

Vermiwash was extracted by three different methods which are as follows:

Method I (heat stress method)

This method was adopted from method described by Karuna et al. (1999). Well-grown adult earthworms *Eisenia foetida* were separated from casting materials by placing

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the worms in a plastic tub for some time. Then the earthworms were removed carefully from the casting materials and transferred to a glass beaker containing 500 ml of warm (40 °C temperature) distilled water and agitated for 5–6 min with a stirrer. Around 30 g of warms were taken. They were then removed immediately and added to another pre-sterilized plastic container filled with water at room temperature. Here the worms were rinsed thoroughly to collect the remaining excretory and secretory products adhering to the body of the worms. The earthworms were then released back to the stock culture container. The light yellow straw-coloured contents of the glass beaker and plastic beaker were mixed and the solution was stored in sterilized dark colour glass bottle at 4 °C to be used for the experimental purpose.

Method II (cold stress application)

In this case, vermiwash was produced using method described by Pramanik (2010) with slight modification. Around 25 numbers having around 30 g of total weight of well-grown matured worms (*E. foetida*) of approximately equal length were applied cold stress by keeping them in chilled conditions, in ice cubes in beaker at −5 °C for 3–4 min. The earthworms were then transferred to a 500-ml glass beaker containing cold distilled (°C) water and kept for 7–8 min with occasional stirring. The worms were transferred to a sterilized beaker containing distilled water at room temperature and rinsed for around 2–3 min and then released to the stock culture container. The light yellow-coloured exudutes from the petridish, glass beaker and plastic beaker were all mixed together and the vermiwash was stored in dark-coloured sterilized glass bottle at 4 °C for experimental purpose.

Method III (field method)

Vermiwash was collected from a vermiwash unit made up of a 15-l plastic bucket with a tap fitted near the base. The lowermost part of the bucket was filled up to 20–25 cm with broken bricks and pebbles with a layer of coarse sand (20–30 cm) above it. On the top of the sand layer around 25 cm layer of moist loamy soil was laid. Finally, the unit was filled with a mixture of partially decomposed cowdung and other organic wastes. Around 1 kg (700–800 nos.) of earthworms (*E. foetida*) was released to the unit. Water was sprinkled on this unit overnight with the help of a perforated plastic container suspended above the unit. Every morning the tap was opened to collect the vermiwash, which was brought to the laboratory and stored in sterilized dark bottle at 4 °C.

Chemical analysis of vermiwash

The organic carbon of vermiwash was determined using potassium di-chromate and concentrated sulphuric acid as extractants using hot water bath, and then followed the method of Walkley and Black (1934). Total N was determined in vermiwash samples by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4 and selenium to digest sample and was estimated according to Bremner and Mulvaney (1982). Total available P was determined by the colorimetric method of Olsen et al. (1954). Total potassium, sodium and calcium were determined by flame photometer. pH was estimated by pH meter while electrical conductivity was determined by a Conductivity meter. The vermiwash was also evaluated for the plant growth hormone indole acetic acid (IAA) by modified Salkowski's method (Gordon and Paleg 1957). Chemical compositions of vermiwash produced by different methods are presented in Table 1.

Germination experiment

Each petridish was layered with Whatman no. 1 filter and 10 healthy seeds of *Vigna radiata*; green mung were placed in each petridish. Treatments were maintained in triplicates. Ten different types of treatments including control

Table 1 Chemical composition of vermiwash produced by different methods

Parameters	Vermiwash (heat stress)	Vermiwash (cold stress)	Vermiwash (field)
pH	7.98	7.40	7.52
E.C. (dS m^{-1})	0.56	0.42	1.10
Organic carbon (%)	0.042	0.036	0.009
Total nitrogen (mg L^{-1})	55.0	52.0	61.02
Total phosphorus (mg L^{-1})	19.15	17.80	18.20
Total potassium (mg L^{-1})	46.24	45.20	55.20
Sodium (mg L^{-1})	119.40	110.80	120.10
Calcium (mg L^{-1})	181.08	175.25	178.60
Magnesium (mg L^{-1})	196.34	186.90	198.00
IAA (mg L^{-1})	16.08	15.05	15.02

(with water only) were applied to the seeds. The seeds were soaked overnight in distilled water and then placed on the petridish. 5 ml of test solution was added twice daily to the petridish for 6 days. Germination percentage was calculated on the 3rd day. At the end of the experiment, root length, shoot length and vigour index were estimated. The treatments included T_1 (control, water only), T_2 (heat stress vermiwash: water in the ratio 1:1); T_3 (heat stress vermiwash: water in the ratio 1:5); T_4 (cold stress vermiwash: water in the ratio 1:1); T_5 (cold stress vermiwash: water in the ratio 1:5); T_6 (vermiwash produced normally: water in the ratio 1:1); T_7 (vermiwash produced normally: water in the ratio 1:5); T_8 (undiluted heat stress vermiwash); T_9 (undiluted cold stress vermiwash); T_{10} (undiluted normally produced vermiwash). The two dilutions 1:1 and 1:5 for all the cases were made arbitrarily. The periodic addition of vermiwash was used from the same stock solutions; only the dilutions were made freshly.

Statistical analysis

All the reported data are mean ± SD of three replicates. The one-way analysis of variance (ANOVA) was used to analyse the significant germination difference, difference in length of seedlings between vermiwash of different extraction types and dilution using statistical software SPSS (14.0 version).

Results and discussion

Germination percentage

The effect of different treatments on germination of seeds of green mung has been shown in Table 2. One-way ANOVA followed by Turkey's post hoc test showed that there was statistically significant difference ($F = 15.455$, $P < 0.05$) of germination percentage between the control (65 %) and all the other treatments, all of which showed above 80 % germination. The variance among different treatment groups was found to be homogeneous as per Levene's test of homogeneity of variance where Levene's F-statistic had a value of 0.871 which was greater than 0.05. There was 100 % germination in the seeds treated with T_5, i.e., 1:5 ratio diluted vermiwash produced from cold stress, followed by 1:5 ratio diluted vermiwash produced naturally. The vermiwash produced by cold stress was superior compared to the same produced by heat stress in seed germination. In heat stress, enzymes could be denatured. Moreover, there could be a chance of release of more amounts of micronutrients, i.e., Fe, Cu, Zn and Mn in heat stress vermiwash. Though micronutrients are helpful in seed germination, greater amounts could be toxic at the same time.

Root and shoot length

In all the cases, root and shoot length was superior over control. One-way ANOVA followed by Turkey's post hoc test revealed that there was statistical significant difference ($F = 47.122$, $P < 0.05$) of average length of seedlings between the control and all the other treatments. The variances among different treatment groups were homogeneous as per Levene's test of homogeneity of variance table where Levene's F statistic had a value of 0.456 which was greater than 0.05. The maximum root length 6.5 cm and the maximum root length 10 cm were observed at 1:5 ratio of vermiwash produced by cold stress. The minimum root length of 2–3 cm and the minimum shoot length of 3.0–3.5 cm were observed at the undiluted vermiwash

Table 2 Effect of vermiwash on seedlings, germination and vigour of green mung, *Vigna radiate*

Treatments	Root length (cm) (mean ± SD)	Shoot length (cm) (mean ± SD)	Average length of seedlings (cm) (L) (mean ± SD)	Germination (%) (G) (mean ± SD)	Vigour index (L X G)
T_1 (Control, water only)	3.0 ± 0.2	5.0 ± 0.3	8.0 ± 0.5	65 ± 5	520
T_2 (Heat stress vermiwash: water in the ratio 1:1)	3.0 ± 0.3	5.0 ± 0.3	8.0 ± 0.6	80 ± 6	640
T_3 (Heat stress vermiwash: water in the ratio 1:5)	4.0 ± 0.4	4.0 ± 0.3	8.0 ± 0.7	80 ± 5	640
T_4 (Cold stress vermiwash: water in the ratio 1:1)	4.0 ± 0.2	4.5 ± 0.3	8.5 ± 0.5	70 ± 5	595
T_5 (Cold stress vermiwash: water in the ratio 1:5)	6.5 ± 0.8	10.0 ± 0.7	16.5 ± 1.5	100	1650
T_6 (Vermiwash produced normally: water in the ratio 1:1)	4.0 ± 0.6	6.0 ± 0.6	10.0 ± 1.2	80 ± 5	800
T_7 (Vermiwash produced normally: water in the ratio 1:5)	5.5 ± 0.9	6.0 ± 0.5	11.5 ± 1.4	95 ± 5	1092.5
T_8 (Undiluted heat stress vermiwash)	2.5 ± 0.2	3.0 ± 0.3	5.5 ± 0.5	85 ± 5	467.5
T_9 (Undiluted cold stress vermiwash)	2.0 ± 0.2	3.5 ± 0.2	5.5 ± 0.4	90 ± 5	495
T_{10} (Undiluted normally produced vermiwash)	2.0 ± 0.1	3.0 ± 0.2	5.0 ± 0.3	90 ± 4	450

produced either naturally or by cold stress or by heat stress. This could be due the presence of toxicity effects of higher concentrations of nutrients in the undiluted vermiwash. The vigour index was highest at 1:5 ratio diluted vermiwash produced by cold stress followed by 1:5 ratio diluted vermiwash produced naturally. In response to vermiwash application, *V. radiate* seedlings produced a profuse growth of fine root hairs, which was not found in the seedlings that received only water. The profuse root hair growth was direct indication of involvement of IAA present in vermiwash (Gopal et al. 2010).

Provitamin D (Zrazhevsky 1957) and vitamin B (Atlavinyte et al. 1971) are reported up to a considerable level in earthworm products and probably these act as promoters for carbohydrate and protein metabolism in vermiwash-treated seedlings. Vermiwash is a collection of excretory products and excess secretions of earthworms along with micronutrients from soil organic molecules. Zambare et al. (2008) reported that boiled water extract vermiwash contained amylase and protease enzymes. They also reported that vermiwash contained nitrogen and phosphate solubilizing bacteria. Prabhu (2006) reported that vermiwash improved the germination percentage of the seeds and seedling vigour of seeds such as cowpea and paddy crops. The presence of proteases in vermiwash could help in seed germination while amylases could help for availability of simple carbon source for enhancement of plant growth and productivity (Zambare et al. 2008).

In the present study, it was evident that undiluted vermiwash was phytotoxic to *V. radiate* as it significantly reduced the percentage of germination and seedling's length of the test crop. It was also observed that in the undiluted vermiwash treatment, the roots had turned brown and had a charred appearance. The diluted vermiwash (1:1–1:5) was able to increase the seed germination percentage and the seedlings' vigour index when compared to water.

Conclusion

From the present study, it is evident that the nutrients and growth promoting substances present in the vermiwash showed its potentiality in seed germination and seedling vigour. However, the vermiwash produced by cold stress diluted at the ratio of 1:5 produced superior result followed by 1:5 ratio vermiwash produced naturally, i.e. without stress. The use and application of vermiwash could play a beneficial role in sustainable agriculture as it is environment friendly, cost effective, reliable and easily available.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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